



# Recent Research Transition in Life Sciences

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**India | UAE | Nigeria | Uzbekistan | Montenegro**

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## **PREFACE**

Life Sciences is the scientific study of living things. It involves many levels of exploration: from the study of the interactions of organic molecules to the interactions of animals and plants with their environment. In this book we aimed to assemble a comprehensive collection of papers covering all core aspects of Life sciences and is to provide you with an overview of the skills that you will need to develop as a Life Scientist. In this book you will learn how to gather evidence using the scientific method. This book is written to satisfy the needs of all students, research scholars and academicians, this book thoroughly explains the fundamentals of the research in life science. The insights related to the future aspects of life sciences have been provided to the readers with the help of this book.

## **ACKNOWLEDGEMENT**

We would like to express our gratitude to God almighty and many people who saw us through this book; to all those who provided support, talked things over, read, wrote, offered comments and assisted in the editing, proofreading and design of this book. We would like thank to all the authors who provided their chapters to this book. We would thank to our parent institutions. We thank our family members who supported and encouraged us in spite of all the time it took us away from them. Thanks to our publisher Empyreal Publishing House for constant guidance and support without which this book would never find its way to the world and to the students, Research Scholars and faculties.

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## STUDYING THE EFFECT OF AGE AND GENDER ON METACOGNITION

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### ABSTRACT

*Demographics work as identification status for an individual and for “how you think” there is always these small identifications that influence otherwise a greater objective. For advancement in psychopathology and understanding behaviour that fuel behavioral irregularities, there have been lack of research that have examined demographic correlates that affect the metacognition. In this perspective, 350 young adults took part in a study in the city of Ahmedabad, the present investigation was undertaken to investigate the influence of specifically two demographic factors, that is, age and gender. The Metacognition Questionnaire (MCQ-30) has been used as a measure of metacognition. Mean, standard deviation, ‘t’ test and Analysis of Variance (ANOVA) have been employed for the analysis of data. The results of the study reveal significant impact of demographics on the metacognition of young adults. This study suggests understanding and regulation of one’s own unhelpful metacognitions and treads path for future research in using the above understanding in the field of psychopathology.*

*Keywords: Demographic factors, Age, Gender, Metacognition, Young adults, psychopathology.*

### INTRODUCTION

Metacognition is a very new concept in the field of psychology. As much as it is there to understand metacognition as a concept, equal capacity of It is there to put to use in the field of research. Metacognition, by definition, is any knowledge or cognitive process that has involvement in the appraisal, monitoring or control of cognition. By concept, it is multifaceted. It is a high order organized system that makes a person recognize events in their own mind and manage them in a changeable manner. An essential role in functional and adaptational operation of an individual’s cognitive process is played by metacognition. Deteriorations or inconsistencies in the metacognitive system is thought to have psychopathology trails. (Corcoran KM, 2008). One of the models of metacognition, the S-REF model, stresses on the essentiality of metacognition in the maintenance of psychopathology of various psychological disorder, mainly emotional disorders. The concept of metacognition suggests that there are three levels within which cognitive processes are divided, these are: processing which is automatic and involuntary, the low-level processing; processing which is voluntary and within one’s conscious awareness, the online conscious processing; and lastly, the self-knowledge, that constitutes declarative and procedural knowledge.

Metacognition has found to have been showing compelling relations with various psychological processes such as emotional processes, cognitive processes and behaviour (Brune, 2006), therefore many studies have understood the role of metacognition as one of the aspects of information processing process that incorporates four mechanisms of content processing- monitoring, interpreting, evaluating and regulating. On basis of this one study by (Mathews, 1996) stated that dysfunctional metacognitive beliefs possibly

form the basis for developing and also has a key role in the maintenance of psychological dysfunction. Research pool is huge contributing to the link between metacognition and prediction of development of psychological symptoms. Apart from the evident presence in clinical samples, metacognition in non-clinical samples have shown significant relations with perceived stress, negative emotions, anxiety and depression. (Wells, 2007). Negative beliefs about worry, worry concerning uncontrollability and danger, cognitive confidence, need to control thoughts and cognitive self-consciousness are the sub variables governing one's metacognition, and all of these have significant relationships with depression and anxiety spectrum disorders. (Spada, 2008). Therefore, the ideology of this study lies in understanding differential metacognition through varied demographics in order to understand and manage the effects of metacognition on one's psychological health. This study seeks to understand the effect of metacognition at a very preliminary level so as to give scope in future research to understand the prevalence of psychological disorders through varied demographics.

## OBJECTIVES

1. To investigate if there is any significant relationship between metacognition and age of an individual.
2. To investigate if there is any significant relationship between metacognition and Family income

## METHODOLOGY

The current study was conducted in the city of Ahmedabad, Gujarat on a population of 350 young adults on basis on random sampling technique. Demographics details (Age and Gender) were taken for this study and the Metacognition Questionnaire (MCQ-30) by Wells & Cartwright-Hatton 2004, was incorporated. MCQ-30 is a 30-item self-report questionnaire which are supposed to be rated on a 4- point Likert scale ranging from 1 (do not agree) to 4 (completely agree). This tool measures metacognitive beliefs relevant to vulnerability and maintenance of psychological disorders and items are grouped in 5 sub scales- cognitive confidence, cognitive self-consciousness, positive beliefs about worry, negative beliefs about worry concerning uncontrollability and danger and beliefs about need to control thoughts. Correlation and ANOVA were used for statistical analysis.

## FINDINGS

### A. Metacognition and Age

**H<sub>0</sub>**- there is no statistically significant relationship between metacognition and age of an individual

**H<sub>1</sub>**- there is statistically significant relationship between metacognition and age of an individual

Correlations							
		Age	lack of cognitive confidence	positive beliefs of worry	cognitive self-consciousness	negative beliefs about uncontrollability	need to control thoughts
Age	Pearson	1	-.069	-.081	.037	.074	-.014

	Correlation						
	Sig. (2-tailed)		.200	.130	.491	.165	.787
	N	350	350	350	350	350	350
	Sig. (2-tailed)	.130	.001		.000	.006	.020
	N	350	350	350	350	350	350

**Table 1:** shows correlation between metacognition and age at 10% confidence (0.01)

Table 1 shows the correlation between age and metacognition suggesting as whether facets of metacognition increase or decrease with increasing or decreasing age. Firstly, it has shown significant results between age and all sub variables of metacognition, which confirms that as age increase, there are some changes in the metacognition of a person. Age and lack of cognitive confidence showed a weak negative correlation, that is as age increased, confidence about their own memory of event increased. Age and positive beliefs about worry showed a weak negative correlation too, suggesting that, as age increased, positive metacognitive beliefs decreased. Age and cognitive self-consciousness showed a weak positive correlation, suggesting that, as age increased, tendency to monitor their own thinking and be aware about their thinking increased. Lastly, age and need to control thoughts showed weak negative correlation, suggesting that, as age increased, need to exercise their power of their thoughts in an attempt to control them decreases.

We reject the null hypothesis and accept alternate hypothesis stating that, there is a significant relationship between metacognition and age.

## B. Metacognition and Gender

**H<sub>0</sub>**- there is no statistically significant relationship between metacognition and gender of an individual

**H<sub>1</sub>**- there is statistically significant relationship between metacognition and gender of an individual

Group Statistics					
	Gender	N	Mean	Std. Deviation	Std. Error Mean
lack of cognitive confidence	Male	81	12.8395	3.54421	.39380
	Female	269	11.7175	3.55056	.21648
positive beliefs of worry	Male	81	14.3704	3.72641	.41405
	Female	269	12.6766	3.95160	.24093
cognitive self-consciousness	Male	81	16.8025	3.43300	.38144
	Female	269	16.6320	3.95335	.24104
negative beliefs about uncontrollability	Male	81	13.5432	3.97822	.44202
	Female	269	15.0260	4.60484	.28076
need to control thoughts	Male	81	13.0000	2.86356	.31817
	Female	269	12.5762	4.11390	.25083

**Table 2:** Descriptive for metacognition and gender

Independent Samples Test				
t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference
lack of cognitive	2.497	132.057	.014	1.12203

confidence					
positive beliefs of worry		3.536	138.606	.001	1.69379
cognitive self-consciousness		.378	149.529	.706	.17050
negative beliefs about uncontrollability		-2.832	150.271	.005	-1.48281
need to control thoughts		1.046	188.592	.297	.42379

**Table 3:** T-test for table 2 for significance @ 10% confidence

Table 2 shows the difference between the metacognitions of male and female. Ones highlighted are on the basis of table 3, that is t-test table, that was run in order to understand which differences were significant. Lack of cognitive confidence was seen to be significantly more in males than females. Positive beliefs of worry was again seen to be more in males than females, and lastly, negative beliefs about uncontrollability was seen to be more in females than in males.

Therefore, we reject the null hypothesis and accept the alternate, stating that there is a significant relationship between metacognition and gender of an individual.

## DISCUSSION

Many studies and researches have shown the role of these five dimensions of metacognition as vulnerability factors that predict development of psychological symptoms. However, no studies have explored in depth the role of socio-demographic aspects and family demographics aspects. Regarding the socio-demographics details, first we start with discussing age. Through the analysis, it is seen that age had a significant relationship with metacognition, that means, metacognition of individuals have a lot to do with what age group they belong to. A weak negative correlation was found between age and three metacognition facets- lack of cognitive confidence, positive beliefs about worry and need to control thoughts. This suggests that, individuals who are older have involvement in disadvantageous and harmful engagement in cognitive activities, they develop higher cognitive confidence resulting in low self-doubts and ultimately positive coping behaviour, unsatiating need to exercise their power of their thoughts in an attempt to control them is way less than the younger individuals, enhanced tendency of focusing on their own cognition making them vulnerable to fixations is higher. Individuals coming from higher or lower age group will have significant effect on the metacognitions of either. Second demographic for the study was gender. While males have higher lack of cognitive confidence and positive beliefs of worry, females scored more on negative beliefs about controllability. This suggests that more than females, males involve themselves in advantageous and beneficial engagement of cognitive activities and have low cognitive confidence resulting in self-doubts and ultimately negative coping behaviour. While, more than males, females have threatening reviews and negative interpretation of mental events and uncontrollable thoughts.

Studying metacognition and understanding how evidently unhelpful metacognition fuels psychological disorders, it is the call of the hour to also understand its correlates to better understand what all social and basic correlates energises unhelpful metacognitions. Therefore, through this study, the richness of understanding metacognition from base level was attempted.

## BIBLIOGRAPHY

- Beate Sodian, C. T. (2012). Metacognition in infants and young children. *Foundations of Metacognition*.
- Brune. (2006). La “Teoria della Mente” nella schizofrenia: Una rassegna della letteratura esistente. *Psychotherapy Research*, 123-177.
- Corcoran KM, S. Z. (2008). Metacognition in depressive and anxiety disorders: current directions. *international journal of cognitive therapy*, 33-44.
- Downing, K. (2012). *The Impact of Moving Away from Home on Undergraduate Metacognitive Development*. USA.
- Dr. Indu.H, G. V. (2015). Metacognitive awareness among adolescents. *Indian Journal of Research*, 32-34.
- Filiz Ozsoy, I. T. (2020). Cognitive distortions in epilepsy patients: metacognitive functions, automatic thoughts, and dysfunctional attitudes. *The Journal of Psychiatry and Neurological Sciences*, 261-269.
- jenaabadi, h. (2016). The Relationship of Metacognitive Strategies and Creativity with Learning Styles of Students Who Have Siblings with Internalizing, Externalizing, and Emotional Disorders. *Biquarterly Journal of Cognitive Strategies in Learning*, 1-15.
- Kaur, M. K. (2017). Influence of Demographic Factors on Metacognition and its Relationship with Critical Thinking of Higher Secondary Students: Foundations for learning. *IJRASET*, 358-366.
- Massimo Mucciardi, V. L. (2016). Metacognition by gender: A pilot Study based on canonical correlation analysis. *The European Proceedings of Social and Behavioural Sciences*, 41-52.
- Mathews, W. &. (1996). Modelling cognition in emotional disorder: The S-REF model. *Behaviour Research and Therapy*, 881-888.
- Rekha Rani, P. G. (2013). METACOGNITION AND ITS CORRELATES: A STUDY. *International Journal of Advancement in Education and Social Sciences*, 20-25.
- Spada. (2008). Metacognition, perceived stress, and negative emotion. *Personality and Individual Differences*, 1172-1181.
- Wells. (2007). Cognition about cognition: Metacognitive therapy and change in Generalized Anxiety Disorder and Social Phobia. *Cognitive and Behavioural Practice*, 18-25.

## CONVOLUTIONAL NEURAL NETWORK BASED RECOGNITION TECHNIQUE FOR PLANT LEAF CLASSIFICATION

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### ABSTRACT

*The form of a plant's leaves remains constant regardless of its age; because of their diversity, plants with seeds are typically the most difficult to identify. One of a plant's most important qualities is its leaf diversity. The form and size of leaves from the same species can vary substantially depending on the plant's environment. In the practice of plant classification, the ability of computer vision professionals to encode morphological features that are predefined by botanists is more important. However, the distinct feature that each plant has as demonstrated by its leaves can be automatically learned based on the end-to-end advantage of Convolutional Neural Network (CNN) or ConvNet. Therefore, CNN based plant leaf recognition technique for plant leaf classification is an important approach nowadays. In this article we are applying CNN for efficient classification of plant species. It automatically detects and classifies the plant leaves without human intervention. The method was tested with the wealth of plant leaves dataset. The input image data is pre-processed, to convert it into meaningful floating-point tensors for feeding into Convolutional Neural Networks. We have used the CNN effectively in classifying the plants using their leaf images with better accuracy.*

*Index terms: Classification, Convolution Neural Network (CNN) or ConvNet, feature extraction, leaf images, overfitting.*

### 1) INTRODUCTION

A detailed knowledge of plants is fundamental to human life on earth. Plants underpin all the aspects of life—from the food we eat, to the clothes we wear, the materials we use, the air we breathe, the medicine we take much more. According to the State of Plants report[1], there exist about 3,90,000 plant species known to the modern science. It is highly difficult for the botanists or experts to identify and classify all the species of plants using leaves. So classification of plant leaves is vital to prevent plants being given different names by different botanists at different times. There are more species of plants which are not discovered yet. However, scientists are finding new species all the time. Recently, researchers from the M.S. Swaminathan Research Foundation, and the Payyanur College have reported two new plant species from the biodiversity-rich Western Ghats regions in Thiruvananthapuram and Wayanad districts in Kerala, South Indian state. They have been christened *Fimbristylis sunilii*[2] and *Neanotis prabhuii*[3].

Plant leaves contain a significant amount of information about the species, and a lot may be learned about a plant from its leaves. They are very important to a plant's overall health and survival. All the living organisms on our planet depend on the plants. They maintain the atmosphere, at the same time their existence is highly challenging due to crucial environmental conditions and unexpected natural calamities. Plants are more valuable, they provide us oxygen to breathe, food to eat, medicinal herbs for healthcare, maintains the climate, helps us to handle emotional and physical well being and also provides clothing and shelter. So it's more important to identify the plant species and preserve them as it is a loan given by nature for our future generations. Therefore there is a need for adequate knowledge on plant species for both botanists and non-experts. Hence, there is a need to develop an

automated or computerized system to identify and classify plants in an efficient manner. The existing traditional artificial methods are based on plant morphology, which though having its form of success is subjective, costlier in terms of manpower and proves inefficient in the long run. Fig. 1 shows the general leaf features used in the classification of plant species.



**Figure 1:** Sample leaf images of the data set

Plant leaves are naturally two-dimensional. As a result, using image-processing techniques, it will be feasible to identify plant leaves automatically. In recent times, Deep learning and in particular the use of Convolutional Neural Networks (CNNs) has proven well suited for computer vision problems. The features that are widely used in classification are shape, color and texture for generic identification of leaves.

This paper proposes a method for efficient plant classification applied on plant leaf images. When executing Deep Learning tasks, a conditional Generative Adversarial Network is used to address the problem of insufficient training data or uneven class balance that can be discovered inside datasets. This helps to supplement datasets of leaf images that aren't large enough, as this field still requires a significant number of datasets for adequate training for greater generalization.

The structure of the paper is organized as follows: Section II reviews previous literature with related work done on plant classification, Section III details the various datasets we used, describes our approach; Section IV gives the discussion of results, and also provides a comparison with conventional solutions. Finally, conclusions are drawn in Section V.

## 2) RELATED WORK

In Machine Learning, there are a variety of strategies for classification. Plant classification is no different in that training the computer to sufficiently and correctly identify items is a significant problem that artificial intelligence practitioners have been working on for many years. Because leaves are easily viewable, accessible, and describable compared to other plant parts, variations on leaf traits are preferred in automatic plant identification systems using computer vision technologies. *Kadir et al. [4], as well as Cope et al. [5], and Ahmed et al. [6],*

*K. Pankaja and G. Thippeswamy [7], P. G. M. Sobha and P. A. Thomas [8]* gives comprehensive surveys on methods for automated plant identification. Reference [9] The proposed technique is simple and computationally efficient. It is based on a combination of two types of texture features, named Bag-of-features (BOF) and Local Binary Pattern (LBP). These features are utilized as inputs to a decision-making model that is based on a multiclass Support Vector Machine (SVM) classifier.

*Wilf et al. [10]* applied their approach to the vein features of leaves and reported a classification accuracy of 72.14% for 19 leaf families. However, the images are taken from cleared specimens that are prepared laboriously and there is still a limitation to a restricted class of features. However, the images are taken from cleared specimens that are prepared laboriously and there is still a limitation to a restricted class of features. *Kumar et al. [11],* proposed a mobile app, called LeafSnap, to enable users to identify trees from leaf photographs. It achieves a top-1 recognition rate of about 73% for 184 tree species, however, a higher accuracy could still be achieved. Another study by [12] attempted deep learning in

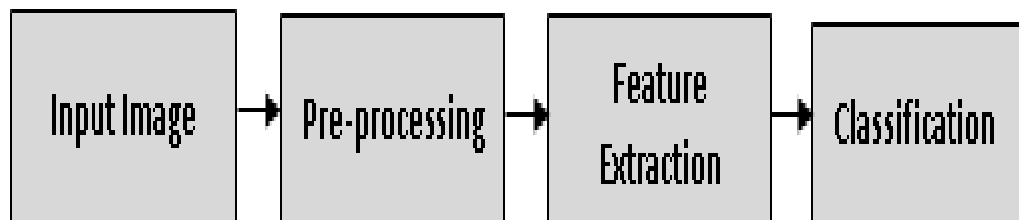
plant identification using vein morphological patterns. They first extracted the vein patterns using UHMT, and then trained a CNN to recognize them using a central patch of leaf images, yet, accuracy and complexity are still problems to combat, and having the model generalize well is still a great need. Therefore, in the follow-up study, we should focus on how to improve identification accuracy. If the number of available images is less than the number of images required for state-of-the-art based models such as GoogleNet [13], AlexNet [14], and so on, we will be challenged to train the network having only a handful of leaf images in our datasets. This is because CNNs on a small dataset surely get overfitted. To overcome this problem, the number of training data should be increased. But providing a large amount of training data is very difficult and costly.

Reference [15] applied a CNN classifier learns the features of plants such as classification of leafs by using hidden layers like convolutional layer, max pooling layer, dropout layers and fully connected layers. The model acquires a knowledge related to features of the Swedish leaf dataset

in which 15 tree classes are available, that helps to predict the correct category of unknown plant with accuracy of 97% and minimum losses. Result is slightly better than the previous work that analyses 93.75% of accuracy. C.Zhang.et.al [16] a seven-layer ConvNet using data augmentation is proposed for leaves recognition and implemented multiform transformations (e.g., rotation and translation etc.) to enlarge the dataset without changing their labels. Data augmentation is used as a secret sauce in nearly every state-of-the-art model for image classification, and is becoming increasingly common in other modalities such as natural language understanding as well [17]. S. Anubha Pearlina [18] a plant species recognition is carried out using two approaches, namely, traditional method and deep learning approach. In traditional method, feature extraction is carried out using Hu moments (shape features), Haralick texture, local binary pattern (LBP) (texture features) and color channel statistics (color features). The extracted features are classified using different classifiers (linear discriminant analysis, logistic regression, classification and regression tree, naive Bayes, k-nearest neighbor, random forest and bagging classifier). Also, different deep learning architectures are tested in the context of plant species recognition.

### 3) MODEL IMPLEMENTATION AND METHODS

This section introduces the leaf dataset, which is utilized in training and pre-processing of leaf images, as well as the CNN model of plant identification system, which has been used to identify and classify plant species using a single leaf image. Using image pre-processing techniques, images are first transformed into a suitable format for feature extraction, after which features are extracted utilizing different layers of CNN, and ultimately, using these features, it classifies plants.

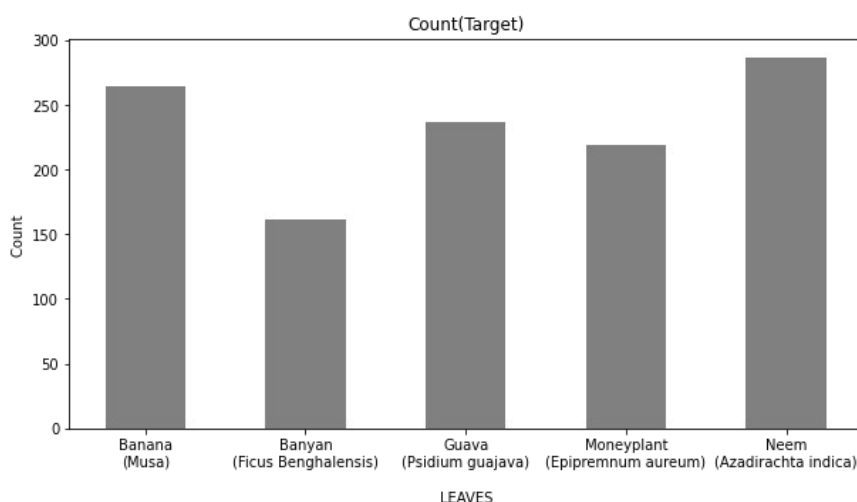


**Figure 2:** Steps involved in image based plant leaf identification

#### a) Dataset

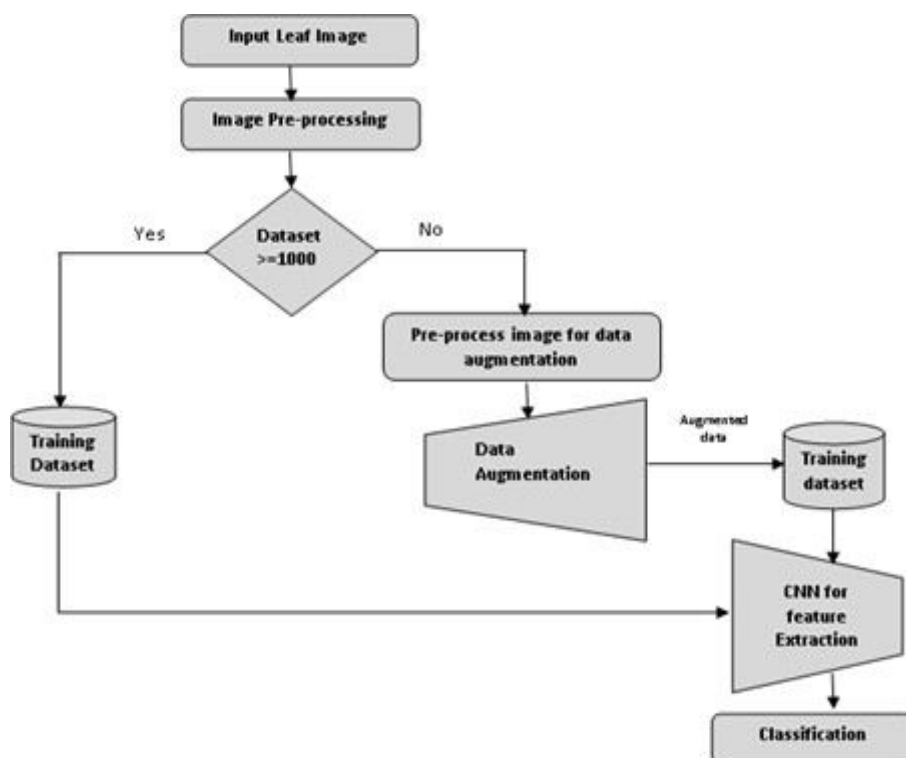
Dataset used: [own dataset : Source - web] 1168 plant leaf photos, 372 of which were obtained from fresh leaves for this study. All of the images collected were categorized into five classes in a local directory based on their family. Guava leaf (*Psidium Guajava*), Banyan leaf (*Ficus Benghalensis*), Banana leaf (*Musa*), Money Plant Leaf (*Epipremnum aureum*), and Neem Leaf are the classes noticed (*Azadirachta Indica*)





**Figure 3:** Chart showing the total number of leaves in each class

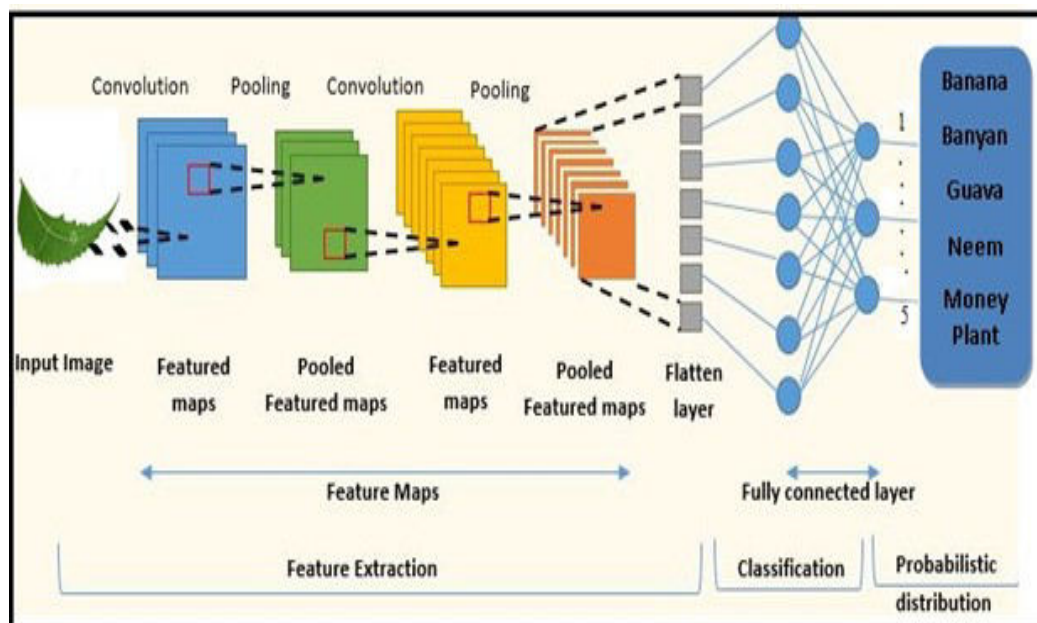
- b) Image Preprocessing:** Images collected from different sources are of different shapes and sizes. The process encompasses transformation and noise removal, which is applied to the images before feeding them into the model. Image rotation, centering, resizing, normalization, rescaling i.e. gray scaling and shear are the operations which are included under pre-processing
- c) Data Augmentation** The size of the database has a direct impact on CNN's performance. A huge amount of data is usually necessary because a small size can quickly overfit the training set. Data augmentation technique helps to address this issue by enlarging the training set. Data augmentation techniques include horizontal and vertical flips, noise, color jittering, and rotation.



**FIGURE:** shows the process flow in the proposed system

#### d) Model Selection : Convolutional Neural Network

The CNN (Convolutional Neural Network) is a deep neural network used in computer vision and image processing. The network is built on biological mechanisms in the human brain that can recognise different items depending on prior observations.



**Figure 4:** Overview of the proposed model

**Convolutional Layer:** The main component of a CNN is convolution. It is used to calculate feature extraction and to establish the convolution kernel size, stride values, bias, and paddings. The picture location feature cannot be reflected if the convolution kernel is too tiny. The intricacy of the feature, on the other hand, may exceed the expressiveness of the larger convolution kernel.

**Max-Pooling Layer:** The max-pooling layer's purpose is to lower the spatial size of the representation in order to reduce the number of parameters and network data memory. The second purpose is to prevent overfitting. The maximum of each zone is computed using a max-pooling layer, which accomplishes downsampling.

**Activation Function:** The output of a node is defined by its activation function given an input or group of inputs. Nonlinear activation functions can help networks solve nontrivial issues with a small number of nodes. The Sigmoid, ReLU, and Tanh functions are all frequent activation functions. The ReLU activation function converges quickly and eliminates the problem of overfitting.

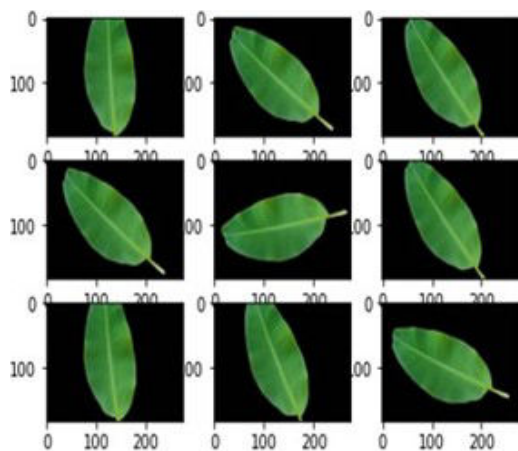
At the fully connected layers, all features retrieved from the convolutional and pooling layers are flattened together. A classification job is then performed using an activation function in the fully connected layer. TanH, ReLU, Softmax, and Sigmoid are a few examples of techniques. Dropout layers can also be used to avoid overfitting by removing some neuron connections. When classifying an item, the output layer commonly utilizes the Softmax function to generate probability-based values.

## 4) RESULTS AND DISCUSSION

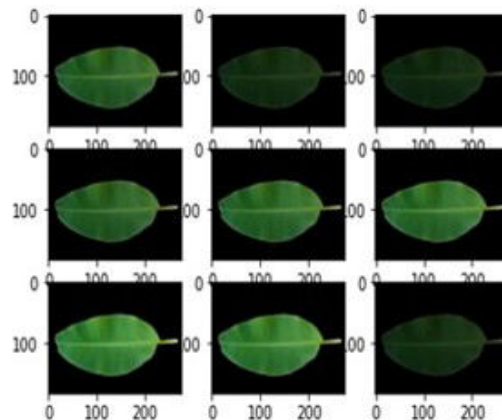
### a) Data Augmentation Results

Each epoch took an average of 3 seconds to complete. By employing augmentation techniques such as Geometric transformations — randomly flipping, cropping, rotating, or translating photos, the model's performance was improved. Change RGB colour channels, amplify any

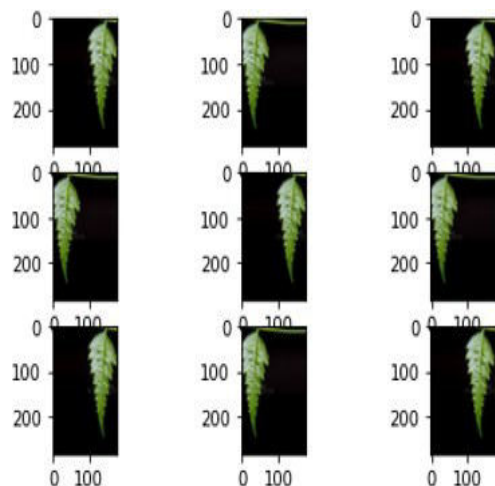
colour, etc. Color space transformations Kernel filters are used to sharpen or blur an image, while Random Erasing is used to destroy a portion of the original image. The results are displayed in Figure 5-10.



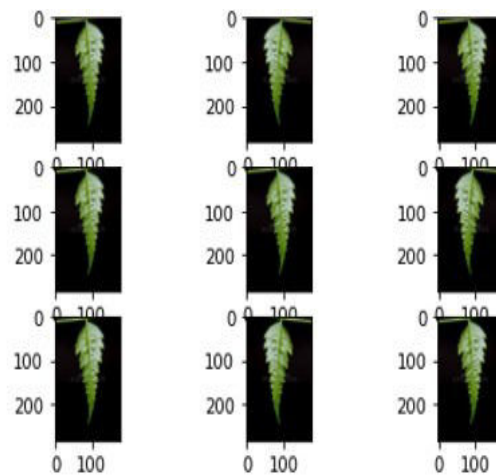
**Figure 5. Leaf Rotation**



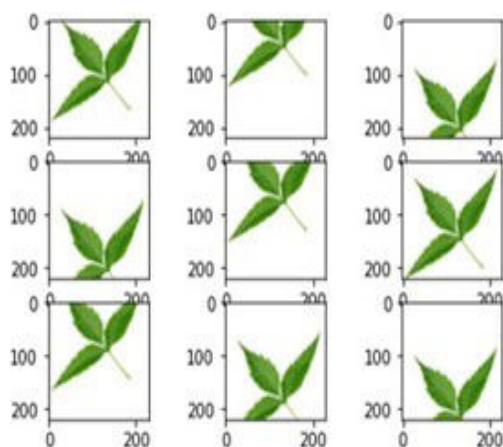
**Figure 8. Random brightness**



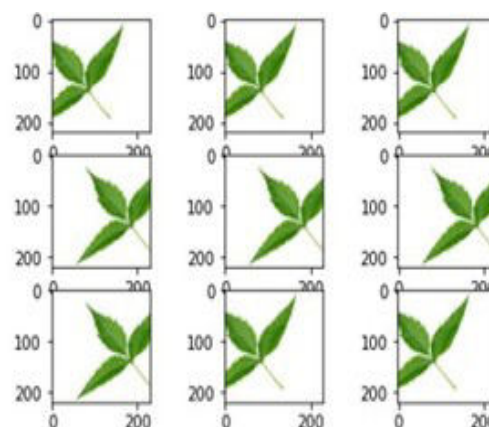
**Figure 6. Horizontal shift**



**Figure 9. Horizontal flip**

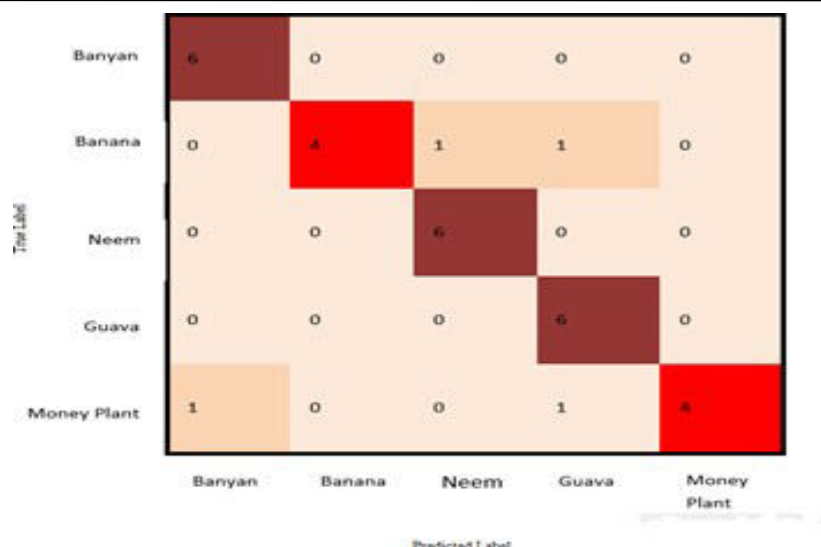


**Figure 7. Height shift**



**Figure 10. Width Shift**

**Figure 5-10:** Shows the result of the data augmentation on the leaf dataset



**Figure 11: Confusion matrix**

Experiments show that our system works on five different plants with an accuracy of more than 87.4 percent. In comparison to previous methods, our algorithm is simple to change, recognise, and implement in a wide range of mature deep learning structures. Our ConvNet-based deep learning system exceeds other traditional approaches in classification accuracy. Without a doubt, our work has opened a new door for leaf classification, which will help to safeguard endangered species significantly. It is being considered for future work to improve it.

## 5) CONCLUSION

CNN has been widely used for different purpose classification tasks for plant leaves like disease classification. We have proposed a 5 layer CNN network in order to classify the augmented leaf dataset into 5 different classes. The results in this paper suggest that data augmentation can be used to tackle the problems of insufficient training data or uneven class balance that could be found within datasets in Deep Learning tasks. Many image segmentation approaches can be used in the future to extract areas of interest within the leaf, and various color factors can be used to fine-grained the classification task and increase performance more precisely and accurately. Furthermore, the proposed network structure can be modified by adding additional layers to reduce losses and hence improve performance.

## 6) REFERENCES

- [1] K. J. Willis, "State of the world's plants 2017. Report," Roy. Botanic Gardens, U.K., Tech. Rep., 2017.
- [2] Fimbristylis sunilii (Cyperaceae): A new species from Southern Western Ghats, Kerala, India, DOI: 10.11646/PHYTOTAXA.527.1.9, PUBLISHED: 2021-11-26
- [3] Neanotis prabhuii, a new species of Rubiaceae from Western Ghats, India, the plant taxonomy journal Phytotaxa . DOI: 10.11646/PHYTOTAXA.527.3.8, PUBLISHED: 2021-12-03
- [4] A. Kadir, L. E. Nugroho, A. Susanto, and P. I. Santosa, "Leaf classification using shape, color, and texture features," Int. J. Comput. Trends Technol., vol. abs/1401.4447, pp. 226229, Nov. 2013.
- [5] J. S. Cope, D. Corney, J. Y. Clark, P. Remagnino, and P. Wilkin, "Plant species identification using digital morphometrics: A review," Expert Syst. Appl., vol. 39, no. 8, pp. 75627573, 2012, doi:10.1016/j.eswa.2012.01.073.
- [6] N. Ahmed, U. G. Khan, and S. Asif, "An automatic leaf based plant identification system,"

- Sci. Int., vol. 28, no. 1, pp. 14, Jan. 2016.
- [7] K. Pankaja and G. Thippeswamy, "Survey on leaf recognition and classification," 2017 International Conference on Innovative Mechanisms for Industry Applications (ICIMIA), 2017, pp. 442-450, doi: 10.1109/ICIMIA.2017.7975654.
- [8] P. G. M. Sobha and P. A. Thomas, "Deep Learning for Plant Species Classification Survey," 2019 International Conference on Advances in Computing, Communication and Control (ICAC3), 2019, pp. 1-6, doi: 10.1109/ICAC347590.2019.9036796.
- [9] R. Ali, R. Hardie and A. Essa, "A Leaf Recognition Approach to Plant Classification Using Machine Learning," NAECON 2018 - IEEE National Aerospace and Electronics Conference, 2018, pp. 431-434, doi: 10.1109/NAECON.2018.8556785.
- [10] P. Wilf, S. Zhang, S. Chikkerur, S. A. Little, S. L. Wing, and T. Serre, "Computer vision cracks the leaf code," Proc. Nat. Acad. Sci. USA, vol. 113, no. 12, pp. 33053310, Mar. 2016, doi: 10.1073/pnas.1524473113.
- [11] N. Kumar, P. N. Belhumeur, A. Biswas, and D. W. Jacobs, "LeafSnap: A computer vision system for automatic plant species identification," in Proc. Eur. Conf. Comput. Vis., Oct. 2012, pp. 502516, doi: 10.1007/978-3-642-33709-3\_36.
- [12] G. L. Grinblat, L. C. Uzal, M. G. Larese, and P. M. Granitto, "Deep learning for plant identification using vein morphological patterns," Comput. Electron. Agricult., vol. 127, pp. 418424, Sep. 2016, doi:10.1016/j.compag.2016.07.003.
- [13] C. Szegedy, W. Liu, Y. Jia, P. Sermanet, S. Reed, D. Anguelov, D. Erhan, V. Vanhoucke, and A. Rabinovich, "Going deeper with convolutions," in Proc. IEEE Conf. Comput. Vis. Pattern Recognit., Jun. 2015, pp. 19, doi:10.1109/CVPR.2015.7298594.
- [14] A. Krizhevsky, I. Sutskever, and G. E. Hinton, "ImageNet classification with deep convolutional neural networks," in Proc. Adv. Neural Inf. Process. Syst., 2012, pp. 10971105, doi: 10.1061/(ASCE)GT.1943-5606.0001284.
- [15] Ref: D. Bisen, "Deep convolutional neural network based plant species recognition through features of leaf," Multimedia Tools Appl., vol. 80, no. 4, pp. 6443\_6456, Feb. 2021, doi: 10.1007/s11042-020-10038-w
- [16] C. Zhang, P. Zhou, C. Li, and L. Liu, "A convolutional neural network for leaves recognition using data augmentation," in Proc. IEEE Int. Conf. Comput. Inf. Technol., Oct. 2015, pp. 2143\_2150, doi:10.1109/CIT/IUCC/DASC/PICOM.2015.318.
- [17] L. Sharon, Automating Data Augmentation: Practice, Theory and New Direction. Stanford, CA, USA: Stanford Univ., 2020. Accessed: May 25, 2021. [Online]. Available: <https://ai.stanford.edu/blog/dataaugmentation/>
- [19] Liu, Bin, et al. "Identification of Apple Leaf Diseases Based on Deep Convolutional Neural Networks." Symmetry 10.1(2017):.
- [20] X. Zhang, W. Zhao, H. Luo, L. Chen, J. Peng, and J. Fan, "Plant recognition via leaf shape and margin features," Multimedia Tools Appl., vol. 78, no. 19, pp. 2746327489, Oct. 2019, doi: 10.1007/s11042-019-07846-0.
- [21] D. Bisen, "Deep convolutional neural network based plant species recognition through features of leaf," Multimedia Tools Appl., vol. 80, no. 4, pp. 64436456, Feb. 2021, doi: 10.1007/s11042-020-10038-w.
- [22] A. Sujith and R. Neethu, "Classification of plant leaf using shape and texture features," in

- Inventive Communication and Computational Technologies (Lecture Notes in Networks and Systems), vol. 145. Singapore: Springer, 2021, pp. 269282, doi: 10.1007/978-981-15-7345-3\_22.
- [23] N. Goyal, N. Kumar, and Kapil, "On solving leaf classification using linear regression," *Multimedia Tools Appl.*, vol. 80, no. 3, pp. 45334551, Sep. 2020, doi: 10.1007/s11042-020-09899-y.
- [24] Y. Zhang, J. Cui, Z. Wang, J. Kang, and Y. Min, "Leaf image recognition based on bag of features," *Appl. Sci.*, vol. 10, no. 15, p. 5177, Jul. 2020, doi: 10.3390/app10155177.
- [25] M. Turkoglu and D. Hanbay, "Leaf-based plant species recognition based on improved local binary pattern and extreme learning machine," *Phys. A, Stat. Mech. Appl.*, vol. 527, Aug. 2019, Art. no. 121297, doi: 10.1016/j.physa.2019.121297.
- [26] P. Pawara, E. Okafor, L. Schomaker, and M. Wiering, "Data augmentation for plant classification," in *Advanced Concepts for Intelligent Vision Systems (Lecture Notes in Computer Science)*, vol. 10617. Cham, Switzerland: Springer, 2017, pp. 615626, doi: 10.1007/978-3-319-70353-4\_52.

## NEEM (AZADIRACHTA INDICA A. JUSS.) MICROBIOTA: ENDOPHYTIC BACTERIA AND RHIZOSPHERE MICROORGANISMS

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### ABSTRACT

*Azadirachta indica* A. Juss., (neem), which is native to India, is globally well known for its medicinal properties. Neem products are known to have antibacterial, antifungal, insecticidal and other versatile biological activities. Since times immemorial, neem plant has been used for the treatment of several human ailments. Plants provide an important biological niche for the growth of a wide variety of microorganisms and neem plant is no exception to this. Many endophytes and rhizosphere microorganisms with numerous biological properties and functions have been reported associated with neem. Endophytic microbes are the storehouse of new bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant, etc. The rhizosphere, the narrow zone surrounding and influenced by plant roots, is a hot spot for numerous microorganisms with multifarious bioactivities.

**Keywords:** *Neem, Azadirachta indica, microbiota, endophytes, bioprospecting, rhizosphere microorganisms*

### INTRODUCTION

*Azadirachta indica* A. Juss., commonly known as *neem*, is a tree that grows in tropical regions such as India. *Neem* has been extensively used in Ayurveda, Unani and Homoeopathic medicine. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Its role as health-promoting component is attributed to its rich source of phytochemicals (Girish and Shankara Bhat, 2008).

Endophytes constitute plant-colonizing microorganisms in a mutualistic symbiosis relationship. They live in apparently healthy internal plant tissues, without causing disease (Schulz and Boyle, 2006). Endophytes are found in plants of most ecosystems and are of importance since they help to improve yields, by stimulating plants growth and immune response, excluding plant pathogens by niche competition, as well as actively participating in antioxidant activities (Pandey *et al.*, 2018).

The root and its surrounding region or rhizosphere is a novel ecosystem which is the harbor of wide variety of microorganisms including bacteria, fungi, algae etc. (Prashar *et al.*, 2014). The rhizospheric microbial population has immense role in agriculture and crop improvement. Many studies have reported that these rhizosphere microorganisms can have profound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases, and productivity (Rodrigo Mendes *et al.*, 2013)

### Endophytic Bacteria from *Azadirachta Indica*

Among plant microbiota, endophytic bacteria can be found in most plant species and be recovered from roots, leaves, stems, and a few from flowers, fruits, and seeds (Lodewyckx *et al.*, 2002). Many studies have emphasized endophytic bacteria from

neem plant and their application in different areas

Verma *et al.* (2009) isolated 55 different isolates of endophytic actinomycetes from neem plant. They reported *Streptomyces* to be the dominant species followed by *Streptosporangium*, *Microbispora*, *Streptoverticillium*, *Saccharomonospora* sp., and *Nocardia*. Actinomycetes were recovered more from roots (54.5%), followed by stems (23.6%), and leaves (21.8%). A *Streptomyces* strain was isolated from the neem. The isolate was closely related to the type strain of *Streptomyces plicatus* sharing a 16S rRNA gene sequence similarity of 96% and this new strain was named as *Streptomyces* sp. mrinalini7 (Singh and Padmavathy, 2014). Seven novel endophytic bacterial species viz., *Bacillus amyloliquefaciens* (JNU-001), *Burkholderia denitrificans* (JNU-002), *Pseudomonas aeruginosa* (JNU-003), *Xanthomonas campestris* (JNU-004), *Azotobacter tropicalis* (JNU-005), *Acetobacter xylinum* (JNU-006) and *Azospirillum lipoferum* (JNU-007) were recovered from native neem varieties of Rajasthan state, India. Among these endophytic bacterial isolates obtained, *Bacillus amyloliquefaciens* (JNU-001) was dominant (Tiwari and Thakur, 2014).

An actinomycetes strain was isolated from neem leaves and named as NEK5 (Vijayan *et al.*, 2014). Endophytic actinomycetes were isolated from healthy leaves, stem and root samples of *A. indica*, with highest species richness. *Streptomyces* species was the predominant actinomycetes isolated, while other actinomycetes isolated were *Kocuria*, *Microbispora*, *Micrococcus*, *Micromonospora*, and *Timonella* (Gohain *et al.*, 2015). The endophytic *Streptomyces coelicolor* strain AZRA 37 was isolated from the surface sterilized root of neem plant (Kumar *et al.*, 2016). An actinomycete closely related to *Micromonospora costii* and *Micromonospora avicenniae* (98.75% similarity in 16S rRNA gene sequences) was isolated from the roots of neem and was named *Micromonospora azadirachtae* sp. nov. (Kuncharoen *et al.*, 2019). Seven endophytic bacterial strains (6 Gram positive and 1 Gram-negative bacteria) were isolated from 12 neem samples collected in Phan Thiet and Lagi (Binh Thuan province), Vung Tau (Ba Ria - Vung Tau province), Vietnam (Linh *et al.*, 2020).

### **Bioprospecting of Neem Endophytic Bacteria**

Endophytes are a source of large number of bioactive secondary metabolites with unique structures including alkaloids, benzopyranones, flavonoids, phenolic acids, quinines, steroids, terpenoids, tetralones, xanthenes and others (Tan and Zou, 2001). Such bioactive metabolites find wide range of applications such as agrochemicals, antibiotics, immunosuppressants, antiparasitic, antioxidant and anticancer agents (Gunatilika, 2006). The bioactive compounds found in the host plant tissues might be due to the associated endophytes. A few reports in the recent years show that the endophytic bacteria from *A. indica* produce bioactive compounds (Verma *et al.*, 2011b; Arun Kumar *et al.* 2015).

### **Antimicrobial Activity**

Actinomycetes isolated from neem plant were screened for their antibacterial and antifungal activities. *Streptomyces* had acute activity against *Pseudomonas fluorescens* and *Escherichia coli*, while an isolate of *Nocardia* sp., from leaves showed antagonism against *Bacillus subtilis*. A few isolates of *Streptomyces*, *Nocardia* sp., and *Streptosporangium* sp., also showed significant antagonistic activity against root pathogens, including *Pythium* sp., and *Phytophthora* sp. (Verma *et al.*, 2009). Endophytic actinomycetes species isolated from neem effectively inhibited the growth



of the *Alternaria alternata* causing early blight disease in tomato (Verma *et al.*, 2011).

Actinomycetes strain NEK5 isolated from neem leaves showed good antifungal activity. The ethyl acetate extract of culture filtrate of NEK5 isolate inhibited the growth of *Fusarium* sp., *Pythium* sp., *Curvularia* sp. and *Cercospora* sp. (Vijayan *et al.*, 2014). Methanol and ethanol extracts of endophytic bacterial (*Bacillus cereus* NRL2) cells isolated from neem were screened for antibacterial activity by agar well diffusion assay. Methanol and ethanol extracts showed significant antibacterial activity against *S. aureus* with IZ of 33 mm and 29 mm, respectively. Four major compounds having antimicrobial activity were obtained from this bacteria such as Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, Methyl-2-O-methyl- $\alpha$ -arabinopyranoside, Propionylfilicinic acid and Benzene carboxylic acid (Arun Kumar *et al.* 2015).

The crude extract from *Streptomyces* species isolated from neem, grown in Casein-starch peptone-yeast (CSPY) broth, showed significant inhibition of bacteria *Pseudomonas syringae* (MTCC 673) and *S. aureus* (MTCC 96), and fungi *C. albicans* (MTCC 3017), and *Rhizoctonia solani* (MTCC 4634) (Gohain *et al.*, 2015). The *Streptomyces coelicolor* was treated with different concentrations of 5-azacytidine and evaluated for its antibacterial potential against five human pathogenic bacteria (*Aeromonas hydrophila* IMS/GN11, *Enterococcus faecalis* IMS/GN7, *Salmonella typhi* MTCC 3216, *Shigella flexneri* ATCC 12022 and *S. aureus* ATCC 25923). The crude extract obtained from cultures treated with 25  $\mu$ M concentration of 5-azacytidine, was found effective against all the five pathogenic bacteria tested (Kumar *et al.*, 2016).

A total of 25 endophytic bacterial isolates were obtained from neem fresh and healthy leaves. Maximum isolates were Gram-positive and rod shaped. In disk diffusion assay using culture broth of endophytic bacteria, significant antibacterial activity was observed against *Bacillus cereus*, *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *Salmonella typhimurium*, and *Streptococcus pyogenes*. Out of 25 isolates 2 isolates were effective against *B. cereus*, 23 against *E. coli*, 22 against *K. pneumoniae*, 20 against *S. aureus*, 22 against *S. typhimurium*, and 21 against *S. pyogenes* (Singh *et al.*, 2017). A total of 80 bacterial endophytes were isolated from various parts of neem plant such as leaf, flower, seed, bark, cortex and root using novel neem-based media. Out of them, only three bacterial isolates showed antifungal potential by inhibiting the growth of *Magnaporthe oryzae* causing blast of rice (Agasimundin *et al.*, 2019).

Antifungal and antibacterial activities of endophytic bacteria from *A. indica* were studied by well diffusion agar method. KT2 strain inhibited *Salmonella typhi* and *Staphylococcus aureus*, KT3 strain showed inhibitory activity against three human pathogenic fungi such as *Candida albicans*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*, while KT1 and KT2 strains showed antifungal activity against *C. albicans* and *T. rubrum*. By biochemical tests, KT2 strain was identified as the *Bacillus subtilis* (Linh *et al.*, 2020).

### Other Activities

Endophytic actinomycetes species isolated from *A. indica*, significantly improved the growth of tomato plant by producing the phytohormone indole acetic acid (IAA) and siderophores. *Streptomyces* strain AzR-051 produced the highest amount of IAA at 13.73  $\mu$ mol / ml, compared to strains AzR-049 and AzR-010 that produced 9.22  $\mu$ mol / ml and 10.43  $\mu$ mol / ml respectively. The *Streptomyces* strain also produced siderophores (Verma *et al.*, 2011). The *Streptomyces* sp. mrinalini7 isolate when inoculated into model tomato plants significantly enhanced the biomass production of the plant and seed germination (Singh and Padmavathy, 2014).

### Rhizosphere Microorganisms of Neem

The rhizosphere is a narrow zone adjacent to and influenced by living plant roots (Kennedy, 1999). It is a site of high microbial activity in and around roots in soil

(Sorenson, 1997). It harbors a great diversity of microorganisms affecting plant growth and health (Campbell and Greaves, 1990; Boehm *et al.*, 1993). The diversity and composition of microbial taxa in the rhizosphere can be affected by several factors including plant species (Miller *et al.*, 1989). The composition of microbial community in the rhizosphere is important for the performances of the plant, as microbial species can have beneficial, neutral or harmful relationships with the roots (Buchenauer, 1998; Atkinson and Watson, 2000; Sylvia and Chellemi, 2001). Microorganisms in the rhizosphere are found to be more in population and are having high metabolic rate than in non rhizosphere soil (Tamilarasi *et al.*, 2008). There are many reports of neem rhizosphere microorganisms and their associated bioactivities.

Field investigation was carried out to determine the arbuscular mycorrhizal fungi (AM) population and their diversity in neem-based agroforestry fields. *Glomus*, *Gigaspora* and *Sclerocystis* were the genera of AM present in the neem-based agroforestry system. Among the three genera, *Glomus* occurred most frequently with 15 species, three species were of *Gigaspora* and two were of *Sclerocystis*. *Glomus fasciculatum* was the predominant AM fungus infecting neem (Pande and Tarafdar, 2004). Arbuscular mycorrhizal (AM) fungi are recognized as an essential component of sustainable agricultural ecosystems (Jefferies, 1987; Barea, 1991).

Rhizosphere microflora of medicinal plants including *A. indica* was estimated. The total number of heterotrophic bacteria in the neem rhizosphere was  $41 \times 10^4$  cfu / g, actinomycetes population was  $17 \times 10^2$  cfu / g and fungal population was  $18 \times 10^2$  cfu / g. The predominant bacterial genus was *Bacillus* followed by *Pseudomonas*, *Enterobacter*, *Corynebacterium*, *Micrococcus* and *Serratia*. Among the fungus the most dominant genus was *Rhizopus* followed by *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium*. Among the actinomycetes, isolates of *Streptomyces* was found to be maximum followed by *Frankia* sp. (Tamilarasi *et al.*, 2008).

Both Vesicular-arbuscular mycorrhizal fungi (VAM) and soil fungal diversity and frequency were studied in the neem rhizosphere from five ecogeographically different regions. Mycofloral diversity included *Aspergillus niger*, *A. flavus*, *A. nidulans*, *A. versicolor*, *A. fumigatus*, *Alternaria tenuis*, *A. alternata*, *Cladosporium* sp., *Cephalospora* sp., *C. albicans*, *Fusarium oxysporum*, *Pestalotia monorhinca*, *Paecilomyces*, *Monilia sitophila*, *Nigrospora oryzae* and *Rhizopus nigricans*. Saline-arid-parched soil exhibited three unique fungal species namely, *Monilia sitophila*, *Aspergillus versicolor* and *Paecilomyces fusisporus*, whereas, the delta-wet region exhibited *Rhizopus nigricans* as its unique species. Overall, in the five regions studied, three VAM genera with nine species were observed, with *Glomus* being the predominant genus viz., *Glomus mosseae*, *Glomus microcarpum*, *Glomus macrocarpum*, *Glomus constrictum*, *Glomus fasciculatum*, *Glomus multisubstance*, *Glomus deserticola*, *Gigaspora albida*, *Gigaspora margarita* and *Acaulospora* sp. (Chary, 2011).

Phosphate solubilizing bacteria (PSB) isolates namely N-B (col-1) and N-C (col-2), were isolated from neem rhizosphere (Shankarrao, 2012). Neem rhizosphere soil can be a rich source for the isolation of phosphate solubilizing microorganisms, due to high phosphate requirements of neem tree and other medicinal plants (Phavaphutanon *et al.*, 1996), or due to long term association and interaction between neem root and microorganisms found in the rhizosphere environments (Lucas Garcia *et al.*, 2001). The bacterial phosphate solubilization activity is due to secretion of organic acids such as

oxalic, citric, formic, acetic, propionic, lactic, succinic and gluconic acid which chelate the cation bound to phosphate and convert it to soluble forms through their hydroxyl and carboxyl groups and production of acid /alkaline phosphatase enzyme (Chen *et al.*, 2006).

Rhizospheric bacteria were isolated from neem and identified as *E. coli*, *Lactobacillus fermentum*, *Micrococcus luteus*, *Neisseria sicca*, *Sporosarcina* sp., *Streptococcus* sp. and *Streptococcus faecalis* (Pandey and Singh, 2013). Phosphate-solubilizing rhizosphere fungus, *Talaromyces funiculosus* SLS8, was isolated from neem (*A. indica*) on saline soil. The fungus was tolerant to environmental stressors, salinity and agricultural systemic fungicides (Kanase *et al.*, 2015). The rhizosphere soil of neem showed maximum population density of AM fungi, PSB, *Azotobacter* sp. and *Azospirillum* sp. (Maohan and Saranya Devi, 2015). A total of 27 bacterial cultures were isolated from the neem rhizosphere samples. Then three cultures were characterized and found to be *Micrococcus luteus*, *Sporosarcina* sp., and *Staphylococcus epidermidis* (Mhatre and Nanoty, 2015). Bacterial strains named RHSAN-1 to 6 were isolated from neem rhizosphere of North 24 Parganas district of West Bengal (Biswas *et al.*, 2016).

A total of six morphologically different fungal isolates (NS1, NS2, NS3, NS4, NS5, NS6) were isolated from the rhizospheric soil of neem and were identified as *Aspergillus* sp. (NS1, NS3, NS4, and NS6), *Fusarium* sp. (NS2) and *Penicillium* sp. (NS5) based on colony characterization (colony color, colony growth) and sporulating structures. One isolate was subjected for molecular characterization and found to be *Aspergillus niger* (Nisha Rani *et al.*, 2017). Arbuscular mycorrhizal fungi (AMF) species belonging to five genera (*Glomus*, *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora*) were isolated from the rhizosphere soil of *A. indica*. *Glomus* species was dominant followed by *Gigaspora* and *Acaulopsora* (Anusha Duvva *et al.*, 2018). Eleven bacterial isolates that produced indole acetic acid (IAA) were obtained from the rhizosphere of *A. indica* (Damle and Kulkarni, 2018). N<sub>2</sub>-fixing bacteria *Azotobacter paspali*, *Azotobacter vinelandii* and *Actinomyces* sp., were isolated from the rhizosphere of neem (Hala and Ali, 2019).

Microorganisms are also intentionally introduced into the rhizosphere environments to enhance certain agriculturally beneficial activities mainly aiming at plant growth promotion (Tamilarasi *et al.*, 2008). Inoculation of neem rhizosphere with AM fungi (Habte *et al.*, 1993; Phavaphutanon *et al.*, 1996) reduced fertilizer requirement in plant production. The effect of inoculation of neem with VA-mycorrhizal fungi (*Glomus fasciculatum*) and PSB was examined under nursery conditions to understand the compatibility between phosphate solubilizing and phosphate mobilizing organisms in the neem rhizosphere. The results clearly indicated that combined inoculation markedly increased the plant growth of the neem seedlings when compared to individual inoculants or uninoculated control, showing the synergistic effect (Kalavathi *et al.*, 2000).

Neem seedlings were inoculated with arbuscular mycorrhizal (AM) fungi, *Glomus intraradices*, *Azospirillum brasilense* and PSB. Microbial inoculation resulted in greater plant height, increased mycorrhizal colonization, leaf area and number, root collar diameter, biomass, phosphorus, nitrogen and potassium content, and seedling quality. Microbial inoculation effects were greatest when seedlings were inoculated with a

combination of microbes rather than individually. This clearly indicated that these microorganisms act synergistically (Muthukumar *et al.*, 2001).

### Bioprospecting of Rhizosphere Microorganisms

The isolates of bacteria, actinomycetes and fungi isolated from neem rhizosphere were evaluated for IAA production. Among them 62.5% of fungal isolates produced IAA followed by 52.17% of actinomycetes and 23.7% of bacterial isolates (Tamilarasi *et al.*, 2008). The microbial diversity of VAM and soil fungi observed in neem rhizosphere could be correlated with the azadirachtin-A content of the neem trees (Chary, 2011).

PSB isolates from neem rhizosphere N-B (col-1), N-C (col-2) showed potent antifungal activity against *Helminthosporium gramineum* and *Rhizopus oryzae*. Isolate N-B (col-1) also showed good antifungal activity against *Aspergillus niger* and *Ustilago maydis*. Comparitively, *R. oryzae*, *H. gramineum*, *A. niger* and *U. maydis* showed more sensitiveness to tested isolates than *Alternaria brassicicola*, *A. solani* and *Sclerotium rolfsii*. Both the isolates exhibited maximum antibacterial activity against *S. aureus*, followed by *Ps. aeruginosa* and *S. typhimurium* (Shankarrao, 2012). N-B (col-1) showed more than one PGPR trait such as phosphate solubilization, antifungal and antibacterial activity and phytohormone production. This isolate might promote plant growth directly, indirectly or synergistically in the soil environment (Shankarrao, 2012).

Rhizospheric bacteria isolated from neem were subjected for extraction of intracellular secondary metabolites using methanol and extracellular secondary metabolites using chloroform. These extracts exhibited significant antibacterial activity against *E. coli*, *Ps. aeruginosa* and *S. aureus*. *Sporosarcina* sp., *M. luteus* and *N. sicca* inhibited all the tree bacteria viz., *E. coli* (IZ 17.5, 12.5 and 13 mm), *Ps. aeruginosa* (21, 17.5 and 28.5 mm) and *S. aureus* (11.5, 13 and 27.5 mm) respectively. *Streptococcus* sp., and *S. faecalis* inhibited *E. coli* (36.5 and 17 mm) and *S. aureus* (11 and 10 mm) respectively. *Lactobacillus fermentum* inhibited only *E. coli* (13.5 mm) and rhizosphere *E. coli* inhibited only *Ps. aeruginosa* (12.5 mm) (Pandey and Singh, 2013).

Phosphate solubilization under different nutritional conditions was investigated by culturing *T. funiculosus* SLS8 in Pikovskaya liquid medium. The highest concentration of solubilised phosphate (187 mg / l) was achieved after 5 days of incubation in the medium with glucose and ammonium sulphate (Kanase *et al.*, 2015). The soil fungi have been reported to solubilize insoluble phosphates by secreting weak organic acids (Maliha *et al.*, 2004). Three bacterial cultures *M. luteus*, *Sporosarcina* sp., and *S. epidermis* obtained from neem rhizosphere were screened for antibacterial activity using culture broth extract by agar well diffusion method. All the bacteria tested showed significant antibacterial activity. *M. luteus* exhibited IZ of 14, 13 and 13 mm, *Sporosarcina* sp. showed IZ of 16, 16.5 and 16 mm, and *S. epidermis* exhibited IZ of 14, 15 and 14 mm against *E. coli*, *Ps. aeruginosa* and *S. aureus*, respectively (Mhatre and Nanoty, 2015).

The culture filtrates of three fungal strains NS1, NS4 (*Aspergillus* sp.) and NS5 (*Penicillium* sp.) isolated from neem rhizosphere were screened for antibacterial activity by agar well diffusion assay. All the three isolates showed significant antibacterial activity against *E. coli* MTCC 40 with IZ of 20.33, 14.66 and 17.0 mm, respectively. NS1 that showed the best activity was identified as *A. niger* and studied for optimization of fermentation conditions to maximize antibiotic production. Process using glucose as

carbon source, ammonium nitrate as nitrogen source, at pH 5 and 25°C resulted in maximum yield of antibiotic (Nisha Rani *et al.*, 2017). Good amount of IAA was produced by bacterial isolates obtained from neem rhizosphere. The range of IAA of the isolates ranged from 10.425 to 43.404 µg/ml. The highest amount of IAA was produced by the isolate 6 (Damle and Kulkarni, 2018).

## CONCLUSION

Endophytic bacteria of neem are an under-investigated group of microorganisms that represent an abundant and renewable source of bioactive and chemically new compounds with potential for exploitation in a wide variety of fields including medicine, agriculture, and industry. The microbial population of the rhizosphere of this plant also has not been studied so far in detail. Further studies are required in this direction to explore and evaluate the enormous microbial population in these ecosystems. Endophytic bacteria with their peculiar potential compounds might provide a range of bioactive compounds catering to the need of novel drugs. The rhizosphere isolates of neem plant might have sufficient bioprospective potentiality like biofertilizer formulations for better crop production as well as therapeutic drugs for human diseases.

## REFERENCES

1. Agasimundin VB, Rangiah K, Sheetal A, Gowda M. 2019. Neem microbiome. In: The Neem Genome, Compendium of Plant Genomes (Eds. Gowda M, Sheetal A, Kole C). Springer, Cham. pp 111-123. ISBN 978-3-030-16122-4
2. Anusha Duvva, Dayakar Govindu, Sharat Bellamkonda, Srinivas Podeti. 2018. Diversity of arbuscular mycorrhizal fungi in the rhizosphere soils of four agroforestry tree species of North Telangana. Asian J. Microbiol. Biotechnol. Environ. Sci., 20(2): 277-283.
3. Arun Kumar G, Robert Antony A, Rajesh Kannan V. 2015. Exploration of endophytic microorganisms from selected medicinal plants and their control potential to multi drug resistant pathogens. J. Med. Plants Studies 3(2): 49-57.
4. Atkinson D, Watson CA. 2000. The beneficial rhizosphere: A dynamic entity. Appl. Soil Ecol., 15: 99-104.
5. Barea JM. 1991. Vesicular arbuscular mycorrhizae as modifiers of soil fertility. Adv. Soil Sci., 15: 1-40.
6. Biswas K, Basu J, Ghosh A, Giri P. 2016. Study of rhizospheric bacterial population of *Azadirachta indica* (neem) of North 24 Parganas district of West Bengal for bioprospective consideration. Int. J. Exp. Res. Rev., 6: 62-66.
7. Boehm M, Madden V, Hoitink HAJ. 1993. Effect of organic matter decomposition level on bacterial species diversity and composition in relation to *Pythium* damping off severity. Appl. Environ. Microbiol., 59: 4171-4179.
8. Buchenauer H. 1998. Biological control of soil borne diseases by rhizobacteria. J. Plant Dis. Protect., 105: 329-348.
9. Campbell R, Greaves MP. 1990. Anatomy and community structure of the rhizosphere. In: The Rhizosphere (Ed. Lynch JM). Wiley, New York. pp. 11-34.
10. Chary P. 2011. A comprehensive study on characterization of elite neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-A as a biomarker. Physiol. Mol. Biol. Plants 17(1): 49-64.
11. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. 2006. Phosphate solubilizing

- bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil. Ecol.*, 34: 33–41.
12. Damle N, Kulkarni S. 2018. In Vitro screening of indole acetic acid (IAA) producing rhizobacteria from some medicinal plants. *DAV Int. J. Sci.*, 7(1): 1-5. <http://davijs.com/2018/DAVIJS-Vol-7-1-4.pdf>
  13. Girish K, Shankara Bhat S. 2008a. Neem – A green treasure. *EJBio.*, 4(3): 102-111.
  14. Gohain A, Gogoi A, Debnath R, Yadav A, Singh BP, Gupta VK, Sharma R, Saikia R. 2015. Antimicrobial biosynthetic potential and genetic diversity of endophytic actinomycetes associated with medicinal plants. *FEMS Microbiol. Lett.*, 362(19): <https://doi.org/10.1093/femsle/fnv158>
  15. Gunatilaka AAL. 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. *J. Nat. Prod.*, 69: 509-526.
  16. Habte M, Muruleedhara BN, Ikawa H. 1993. Response of neem (*Azadirachta indica*) to soil P concentration and mycorrhizal colonization. *Arid Land Res. Manage.*, 7: 327–333.
  17. Hala Y, Ali A. Isolation and characterization of *Azotobacter* from neem rhizosphere. *J. Phys.: Conf. Ser.* 1244 012019. doi:10.1088/1742-6596/1244/1/012019
  18. Jeffries P. 1987. Use of mycorrhizae in agriculture. *Crit. Rev. Biotechnol.*, 5: 319–357.
  19. Kalavathi BP, Santhanakrishnan P, Divya MP. 2000. Effect of VA-mycorrhizal fungus and phosphorus solubilising bacterium in neem. *Indian For.*, 126(1): 67-70.
  20. Kanse OS, Whitelaw-Wecker, M, Kadam TA, Bhosale HJ. 2015. Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the neem rhizosphere. *Ann. Microbiol.*, 65(1): 85–93.
  21. Kennedy AC. 1999. The rhizosphere and spermosphere. In: *Principles and Applications of Soil Microbiology* (Eds. Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA). Prentice Hall, New Jersey.
  22. Kumar J, Sharma VK, Singh DK, Mishra A, Gond SK, Verma SK, Kumar A, Kharwar RN. 2016. Epigenetic activation of antibacterial property of an endophytic *Streptomyces coelicolor* strain AZRA 37 and identification of the induced protein using MALDI TOF MS/MS. *PLoS ONE* 11(2): e0147876. doi:10.1371/journal.pone.0147876
  23. Kuncharoen N, Kudo T, Ohkuma M, Tanasupawat S. 2019. *Micromonospora azadirachtae* sp. nov., isolated from roots of *Azadirachta indica* A. Juss. var. *siamensis* Valetton. *Antonie Van Leeuwenhoek* 112: 253–262.
  24. Linh DN, Thien BV, A Ni TT, Duy NT, Minh NV, Dong TC. 2020. Studies on antimicrobial activities of endophytic bacteria isolated from Neem tree (*Azadirachta indica*). *Ho Chi Minh City Open Univ. J. Sci.*, 10(1): 72-80.
  25. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M., et al. (2002). Endophytic bacteria and their potential applications. *Crit. Rev. Plant Sci.*, 21: 583–606. doi: 10.1080/0735-260291044377
  26. Lucas Garcia JA, Probanza A, Ramos B, Gutierrez Manero FJ. 2001. Genetic variability of rhizobacteria from wild populations of four *Lupinus* species based on PCR-RAPDs. *J. Plant Nutr. Soil Sci.*, 164: 1–7.

27. Maliha R, Samina K, Najma A, Sadia A, Farooq L. 2004. Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. *Pak. J. Biol. Sci.*, 7: 187–196.
28. Mhatre SR, Nanoty VD. 2015. Antimicrobial activity of rhizospheric bacteria of *Azadirachta indica* producing metabolites against human bacterial pathogens. *Int. J. of Life Sci.*, Special issue A3: 21-25.
29. Miller HJ, Henken G, Van Veen JA. 1989. Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. *Can. J. Microbiol.*, 35(6): 656-660.
30. Mohan V, Saranya Devi K. 2015. Study on the status of beneficial microbes from afforested textile and urban waste water polluted sites in Tirupur district, Tamil Nadu, South India. *ENVIS Centre Newsletter* 13(1). Available from: [www.envismadrasuniv.org](http://www.envismadrasuniv.org)
31. Muthukumar T, Udaiyan K, Rajeshkannan V. 2001. Response of neem (*Azadirachta indica* A. Juss) to indigenous arbuscular mycorrhizal fungi, phosphate-solubilizing and asymbiotic nitrogen-fixing bacteria under tropical nursery conditions. *Biol. Fertil. Soils* 34: 417–426.
32. Nisha Rani, Pranay Jain and Geetanjali. 2017. Isolation of antimicrobial compound producing fungi from the rhizospheric soil of the medicinal plant *Azadirachta indica*. *J. Chem. Pharma. Res.*, 9(9): 265-270.
33. Pande M, Tarafdar JC. 2004. Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. *Appl. Soil Ecol.*, 26: 233–241.
34. Pandey A, Singh A. 2013. A comparative study on secondary metabolites producing microbes isolated from rhizospheric and non-rhizospheric region of *Azadirachta indica* and *Oscimum tenuiflorum*. *Int. J. Pharm. Res. Allied. Sci.*, 2: 36-48.
35. Pandey PK, Singh S, Singh MC, Singh AK, Yadav SK, Pandey AK., et al. (2018). Diversity, ecology, and conservation of fungal and bacterial endophytes. In: *Microbial Resource Conservation* (Eds. Sharma SK, Varma A.). Springer International Publishing, New York. Pp. 393–430.
36. Phavaphutanon L, Davies FTJr, Duray SA. 1996. Growth, root alteration, and nutrient uptake of neem tree (*Azadirachta indica* A. Juss) seedlings in response to vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition. *Int. Tree Crops J.*, 9: 59-67.
37. Prashar P, Kapoor N, Sachdeva S. 2014. Rhizosphere: its structure, bacterial diversity and significance. *Rev. Environ. Sci. Biotechnol.*, 13: 63-77. <https://doi.org/10.1007/s11157-013-9317-z>
38. Rodrigo Mendes, Paolina Garbeva, Jos M. Raaijmakers. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.*, 37(5): 634–663. <https://doi.org/10.1111/1574-6976.12028>
39. Schulz B, Boyle C. 2006. What are endophytes? *Soil Biol.*, 9: 1-14. doi: 10.1007/s1540-33526-9\_1
40. Shankarrao O. 2012. Isolation and characterization of phosphate solubilizing bacteria from rhizospheric soil samples. *Online Int. Interdiscip. Res, J.*, 2(4): 28-39.
41. Singh AK, Sharma RK, Sharma V, Singh T, Kumar R, Kumari D. 2017. Isolation, morphological identification and in vitro antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves. *Veterinary World* 10(5): 510-516.

42. Singh MJ, Padmavathy S. 2014. Isolation, screening and characterization of endophytic PGPR actinomycetes present commonly in neem and tulsi leaves - in vitro study (tomato). *Int. J. Recent. Sci. Res.*, 5(3): 574-579.
43. Sorensen J. 1997. The rhizosphere as a habitat for soil microorganisms. In: *Modern soil Microbiology* (Eds. Van Elas JD, Trevors JT, Wellington EMH). Marcel Dekker, New York. pp. 21-45.
44. Sylvia DM, Chellemi DO. 2001. Interactions among root-inhabiting fungi and their implications for biological control of root pathogens. *Adv. Agron.*, 73: 1-33.
45. Tamilarasi S, Nanthakumar K, Karthikeyan K, Lakshmanaperumalsamy. 2008. Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum*. *J. Environ. Biol.*, 29(1): 127-134.
46. Tan RX, Zou WX. 2001. Endophytes: A rich source of functional metabolites. *Nat. Prod. Rep.*, 18: 448-459.
47. Tiwari K, Thakur HK. 2014. Diversity and molecular characterization of dominant *Bacillus amyloliquefaciens* (JNU-001) endophytic bacterial strains isolated from native neem varieties of Sanganer region of Rajasthan. *J. Biodivers. Biopros. Dev.*, 1: 115. doi:10.4172/2376-0214.1000115
48. Verma VC, Gond SK, Kumar A, Mishra A, Kharwar RN, Gange AC. 2009. Endophytic actinomycetes from *Azadirachta indica* A. Juss.: Isolation, diversity, and anti-microbial activity. *Microb. Ecol.*, 57: 749–756.
49. Verma VC, Singh SK, Prakash S. 2011. Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J. Basic Microbiol.*, 51: 550–556.
50. Vijayan VM, Radhakrishnan M, Balagurunathan R. 2014. Bioprospecting of endophytic actinomycetes for antiphytofungus activity. *Int. J. Chem. Tech. Res.*, 6(5): 2689-2694.



## GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES BY USING MEYER LEMON

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### ABSTRACT

*In modern era of technology significant efforts have been made by researchers for the synthesis of nanoparticles for various fields such as water cleaning, sensing, energy storage, environmental remediation, cosmetics, pharma, communications, and many others. Green synthesis of nanoparticles using plant derived materials has attracted attention for its low cost, eco-friendly nature, and simplicity. In this study, we have discussed the green method for synthesis of nanoparticles of silver oxide. The green synthesis of nano particles is also an environmentally friendly method. Recently, researchers have focussed on the green or biological synthesis of metal and metal oxide nanoparticles using the extract from different parts of plants and other bioorganic materials. Among the inorganic metal oxide, silver nanoparticles have been efficiently synthesised from extract of Meyer lemon. Silver nano particles find wide application in various fields such as pharma, cosmetics and in food and packaging. Silver oxide nanoparticles are better used for their thermal stability, photo-catalytic activity & microbial resistance electrical properties.*

*Keywords: Nanoparticles, Green synthesis, Silver NPs, Meyer Lemon*

### INTRODUCTION

In the last few years, a lot of people have been working on nanotechnology with the goal of making life better for everyone. Because nanotechnology deals with things that are smaller than 100 nm in size, this is what it is called. It is the branch of science that is done at the nanoscale, which is about 1–100 nm. It's becoming more common to make metal nanoparticles, which have unique shapes and sizes. They also have very different properties from bulk metals, which is why this is a growing field of study. It's now common for metal nanoparticles to be used in fields such as medicine, physics, chemistry, and materials science [1-2].

Nanoparticles that are made from plants or come from any part of a plant are thought to be safe and eco-friendly. The plants are very easy to find, safe to handle, and have a wide range of metabolites [3]. Green nanotechnology has been made possible using plants to make nanoparticles. This method has been found to be better than other methods of making nanoparticles, such as other biological processes. These methods for making nanoparticles use things like sugars, biodegradable polymers like chitosan, plant extracts, and more [4-5].

Ocimum Sanctum, Petroselinum crispum, Murraya koenigii, and Coriandrum Sativum are some of the plant extracts that can be used to make metal nanoparticles. They were not toxic, bigger, or dangerous [6-8]. Green methods also made particles that were more stable and had better dimensions. Nanoparticles made from plants or plant extracts can be used to replace chemical reduction processes and are better for the environment because they don't use a lot of energy.

In terms of metal nanoparticles, silver nanoparticles (AgNPs) are very important and are used the most. They can be made by a variety of physical and chemical processes, such as electrochemical reduction, photochemical reduction, and the process of evaporation, to name a few. Most of these methods are not only costly, but they also use toxic and harmful chemicals that could harm the environment and animals [9]. Silver nanoparticles (Ag-NPs or nano silver) are used in a lot of different industries and fields because of their unique chemical, physical, and biological properties. Silver nanoparticles have been used as an antimicrobial agent for a long time, but they have also been used for other things. Antibacterial and fungicidal effects are also known to be strong with it. It can also be used in biomolecular detection, catalysis, and biosensors, and it can also be used in medicine [10].

The nanoparticles that are made through chemical reduction have toxic residues that are not good for use in any kind of biomedical application. This means that the medical applications of AgNPs are limited because of this. Since silver nanoparticles can be made with green technology, this process is becoming more popular. Silver nanoparticles are usually made by reducing silver ions to silver atoms that are not charged. This is done by reducing silver ions with a reducing agent, which is how it works. In the old-fashioned way of making things, synthetic reducing agents like ethanol, sodium borohydrate, formaldehyde, ethylene glycol, and so on are used to make things go down. The same can be said for biological reducing agents like flavonoids, tannins, and vitamin C. They can be used to solve problems like toxic pollution and cost [11]. The use of plants or their extracts to make nanoparticles has been getting a lot of attention recently because of how easy it is to do. Also, the costs of the processes are less. Plant extracts are both reducing agents and stabilising agents when they are used to make nanoparticles, which are small pieces of matter. In the literature, there are many examples of plants extracts being used to make AgNPs. In their paper, Sathyavathi, R. et al. have talked about different plant extracts that have been used to make silver nanoparticles [12].

## **MATERIAL AND METHODS**

All AR grade reagents of Rankem (India) were used for synthesis and analysis. Fruits of Meyer lemon were collected from local Dehradun region.

## **SYNTHESIS OF NANOPARTICLES**

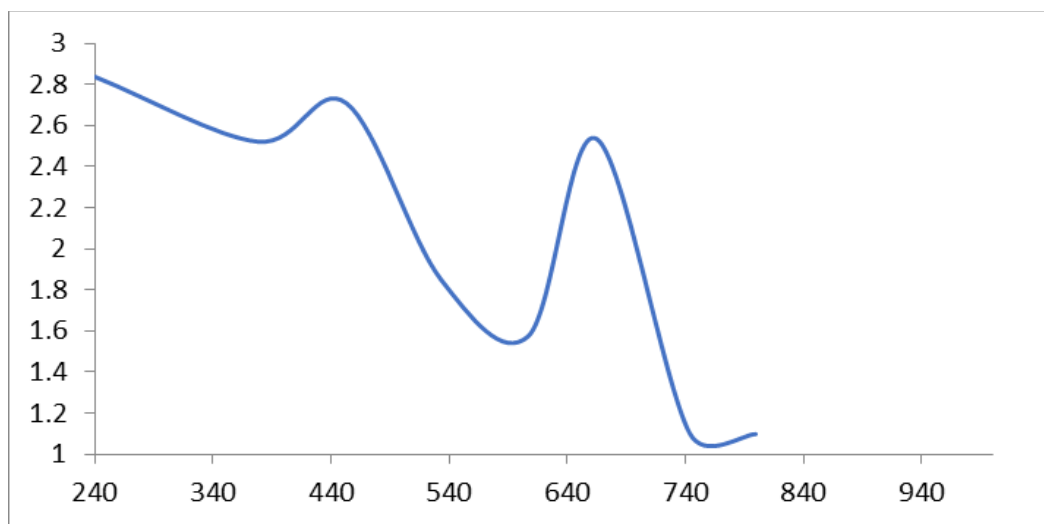
AgO-NPs can efficiently be synthesised by using the extract obtained from any part of the plants. The different parts of the plant such as leaves stem or root can be washed and cut into small pieces and then boiled with distilled water for a specific time.

In this, fruits of Meyer lemon were cut into small pieces, squeezed, and then boiled with distilled water mixed with propanol for 3-4 hrs. After that, the content was filtered, and filtrate was used as plant extract. Then, a requisite amount of extract was treated with the solution of salts containing cerium for a specific time at a fixed rate. Then, added alkali base in the content and mixed it again till all the AgO-NPs came out in the form of precipitate. This formed AgO-NPs precipitate was completely dried and calcinated for 1 hr and was then cooled and weighed.

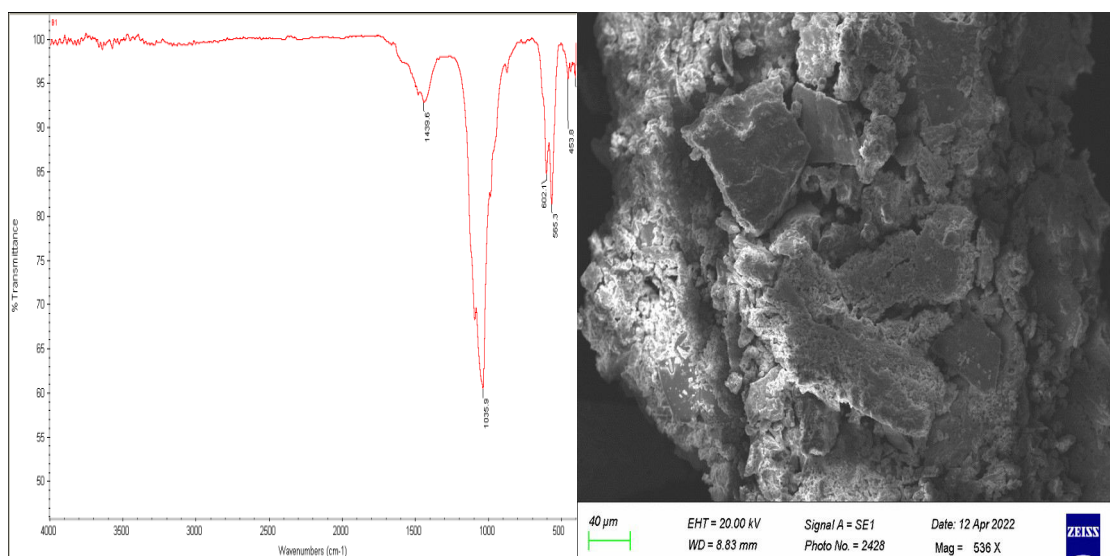
## **RESULTS AND DISCUSSION**

Formed AgO-NPs were characterised by UV, FTIR, SEM & EDX as shown in Fig-1-4. FTIR (Fourier Transform Infrared Spectroscopy) is used to determine the types of bonds

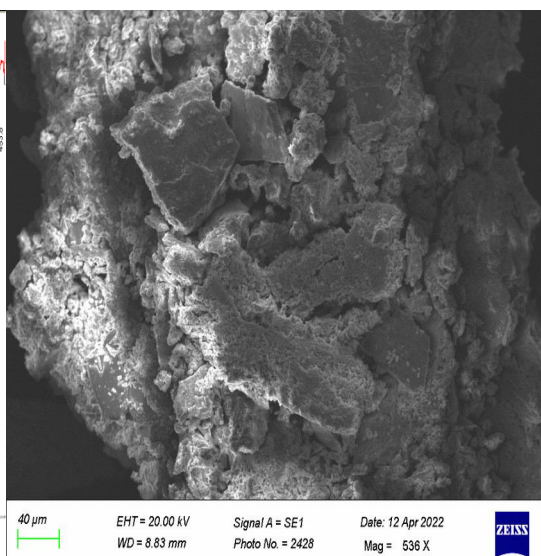
between the elements of biologically synthesised nanoparticles. This spectroscopy is interference based and highly sensitive [13]. The FTIR spectra is represented in figure 2 and SEM analysis in figure 3 for biologically synthesised AgO NPs by using the extract of *Meyer Lemon* plant. The characteristic peaks are obtained which indicate the presence of O-H, C=O, C=C, C-C, C-Silver and Silver-Oxide bonds in NPs. SEM (scanning electron microscope) technique is used to observe the morphological characters of nanoparticles [14]. AgO-NPs have been applied as antibacterial agents for the *Escherichia coli* and *Staphylococcus aureus* by using well diffusion method. The experimental results show that these silver oxide NPs are good antibacterial agents. Antibacterial activity is due to the smallest size; they can easily enter the bacterial cells and destroy the cell membranes and also due to its stable electronic structure. After incubation period, the zones of inhibition have been found as 23 mm and 19 mm for *Escherichia coli* and *Staphylococcus aureus* respectively. The formed nanoparticles were also examined for their anti-oxidant activity by the DPPH method. It showed scavenging activity of 89.43%.



**Fig.1: UV Analysis**



**Fig.2: FTIR Analysis**



**Fig.3: SEM Analysis**

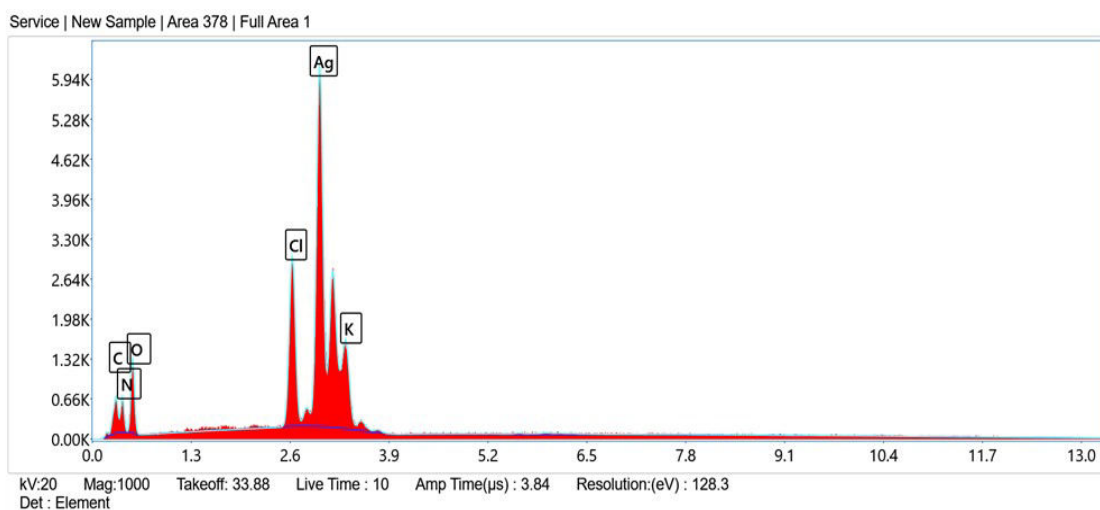


Fig.4: EDX

## CONCLUSIONS

The development of reliable and environmentally friendly technologies for the amalgamation of metallic nanoparticles is a new trend in nanotechnology. The goal of this research is to devise a simple method for producing 'Green' silver nanoparticles. By reducing silver nitrate solution with Meyer Lemon, we attempted to establish a simple, rapid, eco-friendly, and cost-effective process for creating stable silver nanoparticles. UV-Visible spectroscopy, FTIR, SEM, and antibacterial activity were used to evaluate the properties of the produced silver nanoparticles. In the experiments, the created silver nanoparticles with an average size of about 10-30 nm were determined to be stable.

The use of Meyer Lemon for the creation of silver nanoparticles is supported by the findings of this study, and it may also be used as an antioxidant and antibacterial agent. As a result, this environmentally friendly method of manufacturing Ag NPs is both cost-effective and efficient, and it may pave the way for future Meyer Lemon research in the medicinal and nanotechnology industries.

## REFERENCES

1. Ahmad, W., & Kalra, D. (2020). Green synthesis, characterization and anti microbial activities of ZnO nanoparticles using Euphorbia hirta leaf extract. *Journal of King Saud University-Science*, 32(4), 2358-2364
2. Kandwal, A., Purohit, M. C., Khajuria, A. K., & Joshi, R. K. (2019). Green synthesis, characterization and antimicrobial activity of silver nanoparticles using leaf extract of Ajuva Parviflora Benth. *Plant Archives*, 19(2), 762-768.
3. Ahmad, W., Jaiswal, K. K., & Soni, S. (2020). Green synthesis of titanium dioxide (TiO<sub>2</sub>) nanoparticles by using Mentha arvensis leaves extract and its antimicrobial properties. *Inorganic and Nano-Metal Chemistry*, 50(10), 1032-1038.
4. Ahmad, W., Singh, A., Jaiswal, K. K., & Gupta, P. (2021). Green Synthesis of Photocatalytic TiO<sub>2</sub> Nanoparticles for Potential Application in Photochemical Degradation of Ornidazole. *Journal of Inorganic and Organometallic Polymers and Materials*, 31(2), 614-623.
5. Singh, A., Singh, G., & Gupta, A. K. (2018). Development of new Nanocomposite by using bagasse dust and polyaniline for removal of heavy metal ions from Pharma industry effluent. *Indian Journal of Pharmaceutical and Biological Research*, 6(04), 43-47.

6. Negi, P., Pandey, S., Rawat, B. S., Ramola, B., Kalra, G., & Thakur, R. (2020). Effect of Altitude on Essential Oil composition, Antifeedant and Antimicrobial potential of *Murraya koenigii* Isolated from different regions of North Himalaya. *Research Journal of Pharmacy and Technology*, 13(12), 5953-5957.
7. Pandey, S., Kumar, V., Vlaskin, M. S., & Nanda, M. (2021). Sustainability of *Ageratum conyzoides* (billy goat weed) for bioethanol and recycling of residues for gaseous fuel production. *Engineering Reports*, 3(1), e12284.
8. Kumar, V., Arora, N., Pandey, S., Jaiswal, K. K., Nanda, M., Vlaskin, M. S., & Chauhan, P. K. (2020). Microwave-assisted pretreatment of harmful algal blooms for microbial oil-centered biorefinery approach. *Biomass Conversion and Biorefinery*, 1-9.
9. Smetana AB, Klabunde KJ, Sorensen CM. Synthesis of spherical silver nanoparticles by digestive ripening, stabilization with various agents, and their 3-D and 2-D superlattice formation. *Journal of colloid and interface science*. 2005 Apr 15; 284(2):521-6.
10. Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: nanotechnology, biology and medicine*, 6(2), 257-262.
11. Sahoo, P. K., Kamal, S. S., Kumar, T. J., Sreedhar, B., Singh, A. K., & Srivastava, S. K. (2009). Synthesis of Silver Nanoparticles using Facile Wet Chemical Route. *Defence Science Journal*, 59(4).
12. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances*, 31(2), 346-356.
13. Roy, K., Sarkar, C. K., & Ghosh, C. K. (2015). Plant-mediated synthesis of silver nanoparticles using parsley (*Petroselinum crispum*) leaf extract: spectral analysis of the particles and antibacterial study. *Applied Nanoscience*, 5(8), 945-951.
14. Sathyavathi, R., Krishna, M. B., Rao, S. V., Saritha, R., & Rao, D. N. (2010). Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. *Advanced science letters*, 3(2), 138-143.

**STUDY OF BRONCHIAL PATTERN OF GOAT LUNGS BY CORROSION CASTING****Mrs. S. Sangeetha\***

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**ABSTRACT**

**Introduction:** The corrosion casting technique by using resin is usual technique to demonstrate luminal patterns of bronchus, blood vessels, etc... in life science. A corrosion cast of a lung can give a detailed study of the bronchial patterns. Corrosion cast is done using various resin solutions. The aim of the present work is to study the bronchial tree pattern of a goat lung using corrosion casting method.

**Materials and Methods:** Deflated lungs along with intact trachea of a goat were collected from the slaughterhouse. Commercially available rubber silicone has been injected into the lungs through the trachea so that the bronchial patterns can be easily appreciable. After complete filling, the specimen is kept undisturbed for the next 24–48 h. The tissue surrounding the cast is alone removed away and washed with plain water to remove the bloodstains.

**Results:** After dissecting out the tissues using forceps, the cast was washed in plain water. According to the observation of the specimen, it is found that the trachea is branched into the right and left primary bronchi. The secondary bronchi are further branched to form the tertiary bronchi which resemble like fine bristles. In the right lung, it is observed that before the branching of primary bronchus from the trachea, it is known as the apical bronchus supplying the apical lobe of the right lung.

**Conclusion:** Demonstration of bronchial pattern with its various branches is not easy without a corrosion casting preparation. This study can be a valuable teaching aid and museum specimen.

**Keywords:** Bronchial tree, Goat lung, Rubber silicone

**INTRODUCTION**

A corrosion cast is a resin-made cast of the lumen of the passages in real organs.<sup>[1]</sup> Gross anatomy of the lungs can be studied easily with the wet specimen or a dry plastinated lung, but the bronchial patterns cannot be studied by handling a gross lung specimen.<sup>[2]</sup> The lung is the major organ in the respiratory system that is located near the heart. It is a very soft tissue enclosed within the pleural sac in the thorax.<sup>[3]</sup> They play an important role in gas exchange. Each lung is divided into many lobes where the right lung consists of four lobes and the left lung consists of two lobes.<sup>[2]</sup> In most of the vertebrates, there are two lungs, a right lung and a left lung where mostly the right lung is larger than the left lung. The lungs together usually weigh around 1.3 kg where the right one is heavier than the left one.<sup>[4]</sup>

A corrosion cast is a resin-made cast of the lumen of the passages in real organs.<sup>[5]</sup> Gross anatomy of the lungs can be studied easily with the wet specimen or a dry plastinated lung, but the bronchial patterns cannot be studied by handling a gross lung specimen.<sup>[6]</sup> A corrosion cast of a lung can give a detailed study of the bronchial patterns. Corrosion cast is done using various resin solutions.

The most common resin solution used for cast preparations is an epoxy resin which makes the cast hard, firm, and brittle.<sup>[7]</sup> Hence, the resin implied in this study is rubber silicone. This resin

has a unique advantage which can withstand extreme conditions at the same time keeps the cast softer.

### MATERIALS AND METHOD

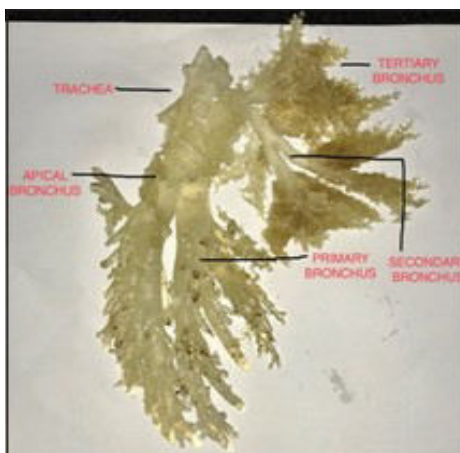
Deflated lungs along with intact trachea of a goat were collected from the slaughterhouse [Figure 1].<sup>[8]</sup> Commercially available rubber silicone has been injected into the lungs through the trachea so that the bronchial patterns can be easily appreciable. After complete filling [Figure 2], the specimen is kept undisturbed for the next 24–48 h. The tissue surrounding the cast is alone removed away and washed with plain water to remove the bloodstains



**Figure: 1** Fresh Goat Lung



**Figure: 2** Injecting Rubber silicone into trachea



**Figure: 3** Corrosion cast of Bronchial pattern

## RESULTS

After dissecting out the tissues using forceps, the cast was washed in plain water. According to the observation of the specimen, it is found that the trachea is branched into the right and left primary bronchi.<sup>[5]</sup> The secondary bronchi are further branched to form the tertiary bronchi which resemble like fine bristles.<sup>[9]</sup> In the right lung, it is observed that before the branching of primary bronchus from the trachea, it is known as the apical bronchus supplying the apical lobe of the right lung [Figure 3].

## DISCUSSION

Demonstration of bronchial tree pattern with its various branching is not that easy without a corrosion cast preparation.<sup>[1]</sup> With the help of such casts, the caliber of the lumen of trachea, primary bronchi, secondary bronchi, and tertiary bronchi is easily explained to the students.<sup>[2]</sup> The branching pattern varies for each animal; hence, it can be explained and differed only with the help of such corrosion casts.<sup>[10]</sup> Corrosion casting is done using various resin solutions. The most common resin solution used for cast preparation is an epoxy resin which makes the cast hard, firm, and brittle. Hence, the resin implied in this study is rubber silicone. This resin has a unique advantage which can withstand extreme conditions at the same time keeps the cast softer. Rubber silicone cast is more continuous and flexible; hence, the integrity of very fine branches could be maintained.<sup>[6]</sup> After dissecting out the tissues using forceps, the cast was washed in plain water. According to the observation of the specimen, it is found that the trachea is branched into the right and left primary bronchi. The secondary bronchi are further branched to form the tertiary bronchi which resemble like fine bristles.<sup>[5]</sup> In the right lung, it is observed that before the branching of primary bronchus from the trachea, it is known as the apical bronchus supplying the apical lobe of the right lung.<sup>[2,3]</sup> From the results, the study concluded that the compare to other casting materials rubber silicon resin may be good substance to study the patterns of luminal structures and it could be the best teaching aid in the field of life science and medicine to study branching pattern of luminal structures.

## CONCLUSION

Demonstration of bronchial pattern with its various branches is not easy without a corrosion casting preparation. This study can be a valuable teaching aid and a museum specimen.

## Author Contributions

Mrs.S.Sangeetha: Study design, data verification, manuscript drafting and correcting.

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## Conflict of Interest:

None to declare

## REFERENCES

1. Sivagnanam S, Sathyamoorthy OR, Paramasivan S. Tracheo bronchial corrosion cast in a calf lung. *Shanlax Int J Vet Sci* 2014;1:18-20.
2. Tamilselvan S, Sivagnanam S, Lavanya C, Iniyah K, Jayachitra A, Balasundaram K. Demonstration of bronchial tree in sheep with silicone resin cast. *Indian Vet J* 2017;94:73-4.
3. Menaka R, Thanvi P, Joshi H. Luminal corrosion cast of tracheo-bronchial tree of equine lung. *Vet Res Int* 2015;3:68-70.
4. Autifi MA, EL-Banna AK, Ebaid AS. Morphological study of rabbit lung, bronchial tree and pulmonary vessels using corrosion cast technique. *Al Azhar Assiut Med J* 2015;13:41-5.



5. Plopper CG, Mariassy AT, Lollini LO. Structure as revealed by airway dissection. A comparison of mammalian lungs. *Am Rev Respir Dis* 1983;128:S4-7.
6. Streck P, Nowogrodzka-Zagórska M, Litwin JA, Miodoński AJ. The lung in closeview: Acorrosion casting study on the vascular system of human foetal trachea. *Eur Respir J* 1994; 7:1669-72.
7. Ishaq M. A morphological study of the lungs and bronchial tree of the dog: With a suggested system of nomenclature for bronchi. *J Anat* 1980;131:589-610.
8. Phalen RF, Oldham MJ. Tracheobronchial airway structure as revealed by casting techniques. *Am Rev Respir Dis* 1983; 128:S1-4.
9. Lorentziadis M, Chamogeorgakis T, Toumpoulis IK, Karayannacos P, Dosios T. Topographic anatomy of bronchial arteries in the pig: A corrosion cast study. *J Anat* 2005; 207:427-32.
10. Schlesinger RB, McFadden LA. Comparative morphometry of the upper bronchial tree in six mammalian species. *Anat Rec* 1981;199:99-108.

## DEVELOPMENT AND EVALUATION OF TOPICAL LIPOSOMAL GEL OF LEMON OIL AND ORANGE OIL FOR SYNERGISTIC EFFECT TO ENHANCE SKIN BEAUTY

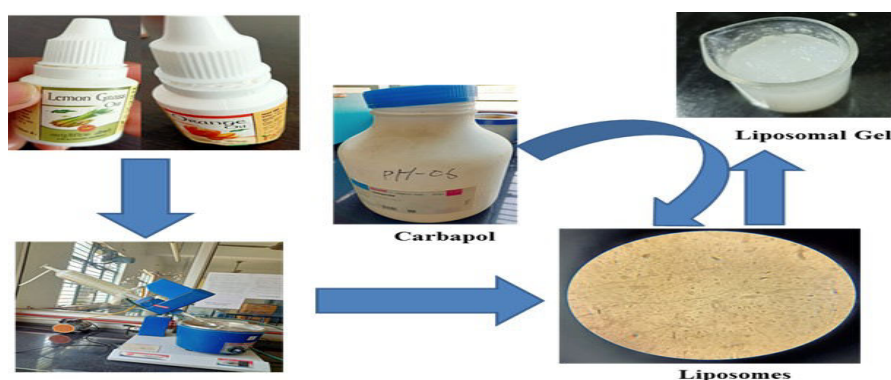
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### Graphical Abstract



### ABSTRACT

**Background:** In the development of cosmeceuticals for skin care, nanotechnology is critical. Essential oils are complex mixtures of numerous derivatives, including terpenoids, aliphatic chemicals, and phenol derived compounds. Due to their numerous qualities such as virucidal, bactericidal, fungicidal, local anaesthetic, anti-inflammatory, analgesic, and so on, they have been widely utilised for a long time. Essential oils are chosen over synthetically manufactured medications because they are safer for the skin. To improve the penetration of essential oils via the skin, novel nanosystems such as liposomes, niosomes, nanoemulsions, solid lipid nanoparticles, dendrimers, and nanospheres are used. These nanocarriers allow for controlled release of natural components while also improving their stability. Furthermore, nanotechnology-based skin care products have a pleasing appearance.

**Study Aim:** The aim of the study is to develop lemon oil and orange oil based liposomal gel formulation for skin care

**Study Design:** The research objective was achieved by focusing following studies. Firstly, lemon oil and orange oil is used to synthesize the liposomes. Secondly, the liposomes were converted in to liposomal gel to find an alternative skin care. Thirdly, the characteristics of were liposomal gel studied.

**Purpose:** To develop another alternative and herbal option for customers.

**Results:** The study predicament that statistical analysis multiple comparisons were made by using Unpaired Student's t-test the statistical significance was considered at  $*p < 0.05$  i.e.  $p$  value summary  $*P < 0.01$ .

**Conclusion:** The sensational study indicates that lemon oil and orange oil were successfully entrapped in a lipoosomes to formulate gel.

**Keywords:** *Lemon oil, Orange oil, Entrapped, Liposomes, Gel, Sensational.*

## INTRODUCTION

Herbaceutical outcomes and herbal based medicaments are a magnificent source of modish therapeutic agents and for the build out of complementary and alternative medicines over traditional drug regimen [1]. Over half of the populace of globe depends on classical medicine for healthcare aid, more than 80% of the less developed countries [2-4]. Orange oil is a markable medicinal plant which defines a wide range of biological activities, and Sensational vesicular drug carrier term was highlighted in 1991 by Gregor Cevc the aqueous core surrounded by a complex of lipid bilayer. Greek word soma clarifies the body [5, 6]. Leptosomes have enomorous advantages towards biocompatibility and biodegradability as they are made from natural phospholipids similar to liposome so protect the encapsulated drug from metabolic degradation with excessive permeation of the drugs through the skin [7]. Transfersomes fetch phospholipids, surfactants, solvent, buffering agent, dyes [8, 9]. Transfersomes formulation is the process of administration of active herbaria pharmaceutical dosage form through epidermal route [10]. Cosmetics are a broad category that includes both external and internal goods. They are commonly used to give off a pleasant odour or to alter or correct the look of the body part to which they are applied. The use of cosmetic items has skyrocketed as people become more conscious about their appearance and live longer. Cosmetics are no longer restricted to simple soaps and creams; instead, more complicated goods such as anti-ageing, skin whitening, anti-acne, and soon have taken over the market. It's no surprise that the worldwide cosmetics sector is valued USD 532 billion, with forecasted growth reaching USD 716 billion by 2025 [11]. The cosmetic and skincare industries are a huge and growing part of our society. Cosmetics are defined by the FDA as "items intended to be applied to any part of or to the human body for beautifying, cleansing, increasing attractiveness, or making a striking appearance." The term "cosmeceutical" is derived from the words "cosmetics" and "pharmaceutical medicament." Although these items have both cosmetic and biological effects on the skin, they are promoted as cosmetic or skincare products because they purport to improve the appearance of the skin [12]. Due to consumer demand to improve the appearance of their skin, hair, and nails, cosmeceutical treatments incorporate chemicals that can prevent wrinkles, UV damage, and other ailments. Nano-cosmeceuticals is defined as a cosmetic formulation that uses nanotechnology as a delivery mechanism to improve the performance of bioactive ingredients [13-17].

## MATERIALS AND METHODS

### Collection of Materials

Lemon oil, Orange Oil, was procured from local drug store, Lucknow, India. Soya lecithin, cholesterol, Carbopol 934, betadine was procured from Transgene Biotech, Lucknow, India.

### METHOD

As per Table 1, Liposomal formulation was prepared. Method of Assembly operation: Soyalecithin and cholesterol was dissolved in chloroform 10ML by using individual beaker by obtained ratio respectively, Orange oil and Lemon oil was also dissolved in a separate beaker by using solvent chloroform. Taken four RBF of 1000ML loaded with respective ratio and coded with S1, S2, S3, and S4 by the 1ml addition of dissolved

preparation of Orange oil and Lemon oil individually, and the RBF attached with working instrument (Rotary Vacuum Evaporator) and allowed to rotate for one hour with temperature equilibrium 60°C. When complete solvent was evaporated than a visualized thin film was succeed at the wall of RBF and the obtained thin film was hydrated by using phosphate buffer pH6.8 [18]. On the basis of observation table 1 the formulations S1 was selected on the basis of thin film around the RBF (figure) for the gel preparation and further studied.

**Table 1:** Selection of formulation by the help of Rotary Vacuum Evaporator *via* thin film method

S.No	Cholesterol and Soyalecthin chloroform	Orange oil + Lemon oil	Obtained [Thin film formed on the surface of RBF]
S1	1:1	1:1	√
S2	2:1	1:1	×
S3	2:1	2:1	×
S4	2:2	1:1	×

### Preparation of Gel

In this method the 2 mg Carbopol 934 was dissolved in 50 ml of deionized water with constant stirring and heat for 30 min after heating left for cooling. When gel was stable add the prepared formulation (Figure 2) [19-20].

### Drug Content

The uniformity of drug content was determined using a UV spectrophotometric method. 500 mg of gel was dissolved in 50 mL of methanol. The volumetric flask was maintained for 2 hours and then thoroughly mixed in a shaker. The solution was filtered after passing through the filter paper. The drug concentration was determined using spectrophotometry with methanol as a blank [21-24].

### Measurement of Density

The bulk density of transfersomes was calculated by dividing its weight by its volume. The final density of a transfersomes was estimated by using an average of three specimens with a volume of  $1 \times 1 \times 1 \text{ cm}^3$  [25-28].

### Particle Size Analysis by Malvern

The Malvern is a particle size analyzer that uses laser diffraction to measure particle sizes ranging from 0.1  $\mu\text{m}$  to 3 mm. For analysis, only a little sample (0.25g) is required, and findings can be obtained in as little as 10 minutes per sample. The fluctuation in angular scattered light intensity is measured using a laser beam passing through a dispersed sample [29-32]. (The scattering angle of small particles is modest, whereas the scattering angle of large particles is huge).

### Zeta Potential Analysis by Malvern

For the measurement of zeta potential, or electrophoretic mobility, Malvern Analytical offers leading zeta potential analyzers. The Zetasizer range offers a simple, fast, and accurate approach to measure zeta potential in a number of cell types, including inexpensive disposable folded capillary cells, in both laboratory and process environments. It improve formulation stability and shelf life while reducing formulation

time and expense with zeta potential measurements. One of the fundamental characteristics known to affect stability is zeta potential, which is a measure of the level of electrostatic or charge repulsion/attraction between particles [33-36].

### **pH of Liposomes**

The pH of the formulated liposomes was checked by using buffer solution pH 6.8. Firstly 1ml of liposomes was weight and kept in 50ml of buffer for 1 hrs. Then with the help of calibrated pH meter the pH of the obtained liposomes were identified [37].

### **In Vitro Drug Release Study**

The release studies will be carried out in 500 ml beaker containing 100 ml of phosphate buffer pH 5.8. The beaker will be assembled on a magnetic stirrer and the medium was equilibrated at  $37 \pm 50^\circ\text{C}$ . Dialysis membrane will be taken and one end of the membrane was sealed. After separation of non-entrapped transfersomes dispersion was filled in the dialysis membrane and other end will closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (1 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium pH 5.8. [38].

### **Drug Entrapment Efficiency Study**

The transfersomes were formed and immersed in 100 ml of phosphate buffer with a pH of 7.4 overnight at  $25^\circ\text{C}$ . The sample leach ate was then filtered using a 0.2 mm membrane filter after being sonicated for 10 minutes. The concentration of drug in the solution was evaluated using the UV spectrophotometric (Shimadzu, Japan) method, at 228-554nm and the percentage drug entrapment was estimated by:

Entrapment Efficiency= Amount of drug entrapped / Theoretical weight of drug $\times 100$

### **pH of the gel**

pH meter was calibrated by the help of standard buffer solution. About 1 gm of gel mixed with 100 ml of distilled water and store for 2 hours for the measurement of pH.

### **Microbial Study**

The formulated gel was inoculated on the plates of agar media. The plate was controlled by formulated transfersomes gel at  $37^\circ\text{C}$  for 24 hours under incubator. After duration of time period the growth rate checked by visually [39].

### **Stability Study**

A stability study is a set of tests used to ensure that a pharmacological product will remain stable over time. The optimum gel formulation was kept at  $5^\circ\text{C} \pm 3^\circ\text{C}$  and  $30^\circ\text{C} \pm 2^\circ\text{C}$  in a well-sealed stoppard glass container. For one month, data was collected by physical appearance and with digicam microscope at regular intervals.

### **Statistical Analysis**

Graph Pad Prism was used to do the statistical analysis. Multiple comparisons were made using Unpaired Student's t-test and ANOVA which was considered significance at  $p < 0.05$ .

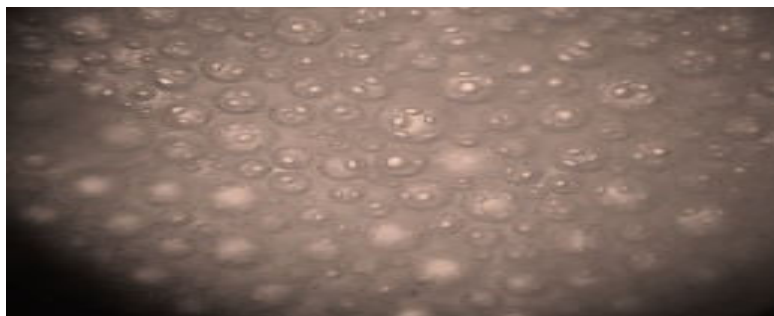
## **RESULT**

Lemon oil and Orange oil based liposomal gel was formulated by mentioned the above technique and further it was optimized on the basis on gel with hydrophilic. A table 1 shown the loading efficacy of formulation and batch S1 was selected on the basis of optimization because it has highest entrapment among them, which making it ideal for

use in gel formulations. In gel preparations, carbopol 934 was frequently used as a gelling agent.

#### **Formation of Transferosomes by Rotatory Vacuum Evaporator method**

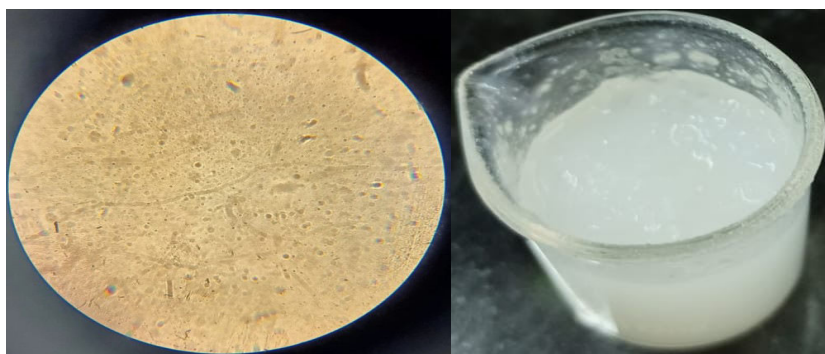
As per Table 1, the S1 selection shows the prepared formulation having a thin layer around the round bottom flask and it was hydrated by buffer with constant hand shake for 15 minute then it was observed under digicam microscope, the tiny vacuoles was visible which provide the conformation regarding the presence of transferosomes as is shown in Figure1.



**Figure 1:** Microscopic image of liposomes at 45x by using Digicam microscope it seems that the prepared formulations were spherical in shape. The Liposomes was formed successfully at a surface of RBF with a smooth and creamy texture and hydrated by buffer pH 6.8 and microscopically seemed spherical vesicles were formed.

#### **Formation of Transferosomes Gel**

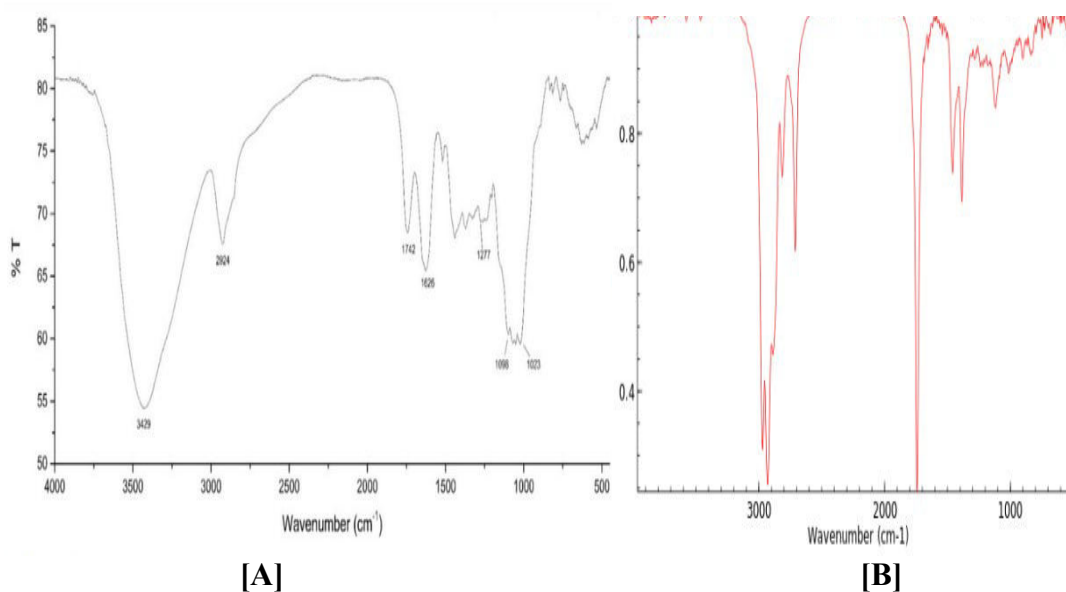
Incorporated carbopol 934 was assure the formation of curcumin based transferosomes gel which was seemed under projection microscope as is shown in Figure2.



**Figure 2:** The Macroscopic view of gel was thick and clear, highly viscous appearance and the Microscopic view of gel under projection microscope that shows the vesicles of drug entrapped in a spherical shape.

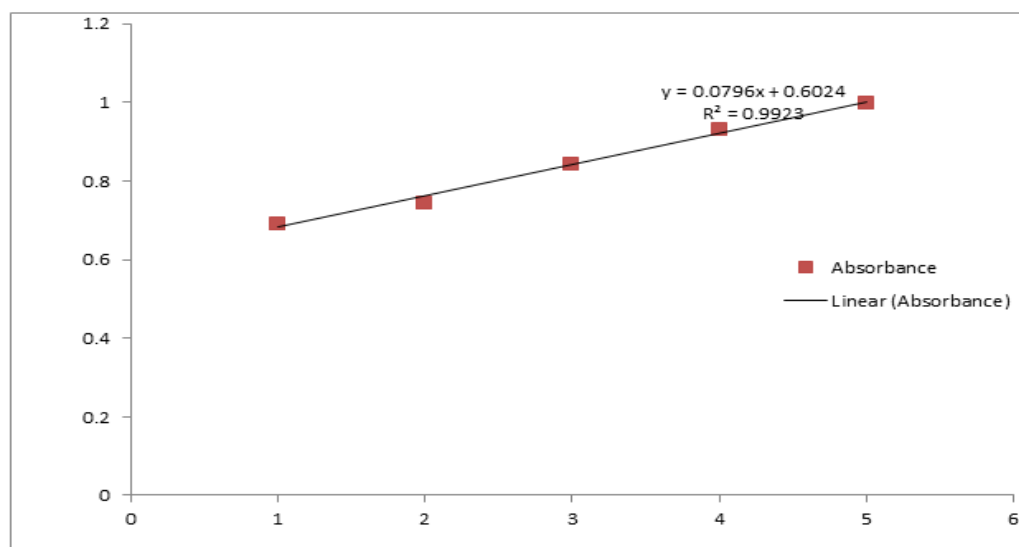
#### **Fourier Transform Infrared Spectroscopy**

FTIR identifies the chemical bonds in a molecule by producing an infrared absorption spectrum. Here the curcumin has a peak around  $1500\text{ cm}^{-1}$  assigned to  $\text{C}=\text{C}$  and a peak around  $1300\text{ cm}^{-1}$  ascribed to  $\text{C}-\text{O}$  in phenol groups present at curcumin as is shown in Figure 3.



**Figure 3:** FTIR of Orange oil and Lemon oil

## Drug Content



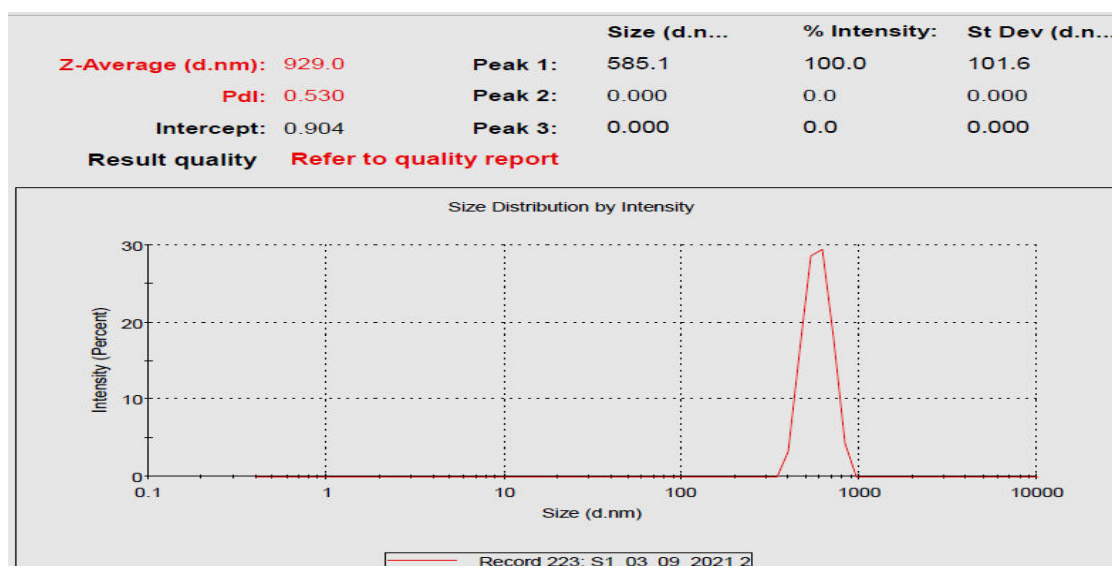
**Figure 4:** The clear supernatant solution was collected and drug concentration was evaluated using a Calibration curve of in methanol.

## Density

The density of formulation at 2% w/v is 0.063 gm/cm<sup>3</sup>. According to the findings of the density study, the developed formulation has a high degree of porosity, as evidenced by its ultralow density. Additionally, the created preparation's high porosity assures that it

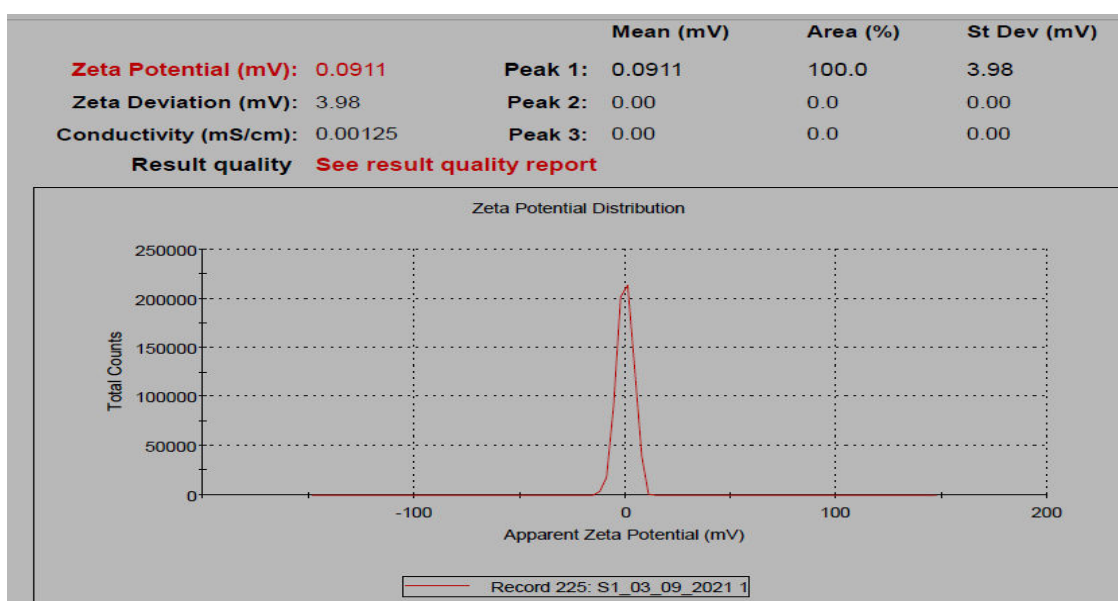
has a high adsorption capacity for wound secretion absorption.

### Particle Size Analysis by Malvern



**Figure 5:** Liposomes appeared as spherical, well identified, unilamellar nanovesicles at peak 585.1 and the numerical value of ideal PDI ranges from 0.0 to 1 which represents perfectly uniform sample with respect to the particle size and the polymer chains are of same molecular weight, so this graph have PDI: 0.530 which is less than 1 which means the prepared formulation are uniformed with respect to particle size. Result summary, Malvern based on Laser diffraction measure the particle size distribution here, Record 223; S1 represent the liposomes and at 585.1 shows the peak with 100 % intensity, PDI: 0.530.

### Zeta Potential



**Figure 6:** The zeta potential represents the measurement of the surface potential of suspended particles. Particles with a zeta potential greater than  $\pm 30$  mV are considered to be stable, because the repulsive force of the same charge can avoid particle

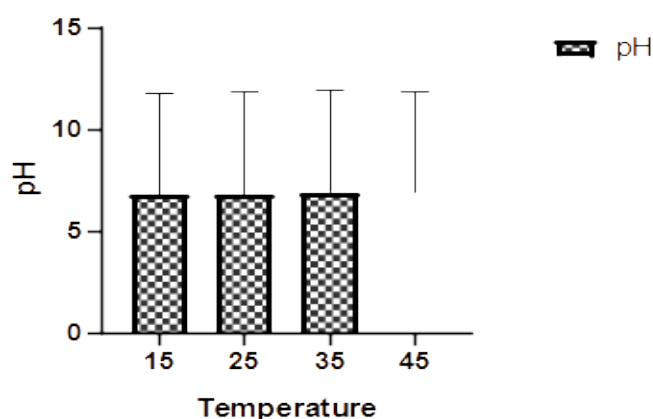


aggregation. So The results of liposomes showed that the best formulation was obtained as per mentioned graph the obtained value is  $\pm 3.98$  mV, which means the formulation has stability because of no aggregation between the charged particles. Result summary

Malvern based on Laser diffraction measure the Zeta potential at 0.0911 mV, with 0.00125 ms/cm conductivity and zeta deviation 3.98 mV, and Record 225; S1 represent the liposomes.

### pH

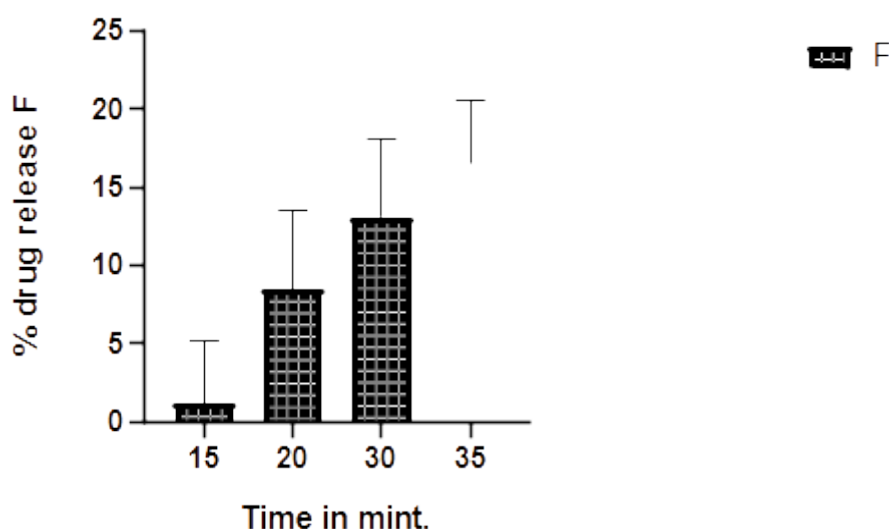
The pH of the liposomes was determined successfully with respect to temperature up to 45°C and it was seemed that pH was similar at different time of interval.



**Figure 7:** pH of liposomes at different temperature was found 5.8 which is good for topical purpose by applying student “t-test” and all the values are expressed as Mean  $\pm$  SD calculated by ANOVA summary i.e. p value summary \*, (significant diff. among means [ $P < 0.01$ ]).

### In Vitro DR

*In vitro* DR of Liposomes was increased by the increase of time period.

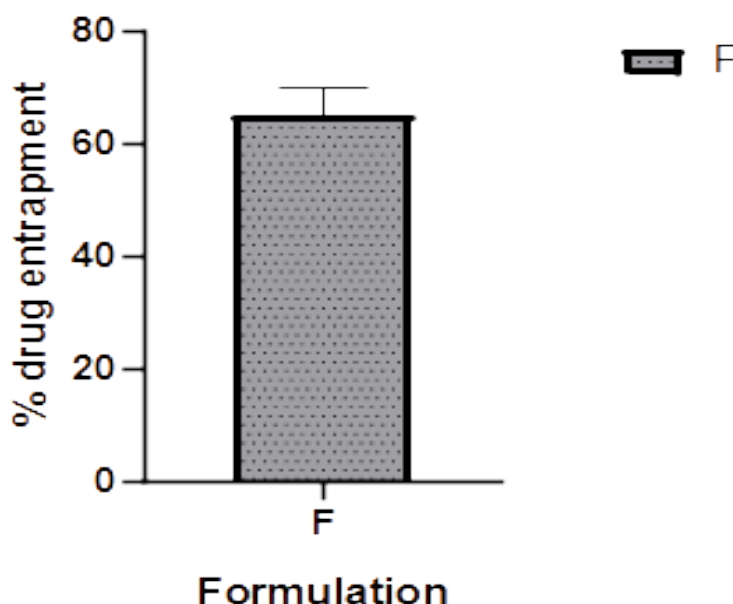


**Figure 8:** Graph representing *In vitro* DR of liposomes by increasing the rotation time the release property was increased values are expressed as Mean  $\pm$  SD calculated by

ANOVA summary i.e. p value summary \*, (significant diff. among means [ $P < 0.01$ ]).

### DEE of Leptosomes of Formulation

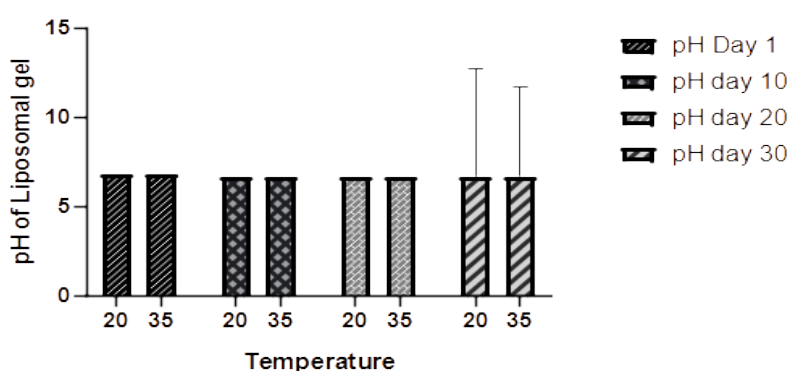
DEE of liposomes shows that the how much amount of drug was entrapped here the result reveals that the maximum amount of drug was entrapped but less than 100 %.



**Figure 9:** Graph representing DEE of formulation. Values are expressed as Mean  $\pm$  SD calculated by ANOVA summary i.e. p value summary \*, (significant diff. among means [ $P < 0.01$ ]).

### Ph of Gel

The resultant values reveal that the pH was slightly increased but the pH values approx 5.8 which is good for skin which in not acidic nor basic.

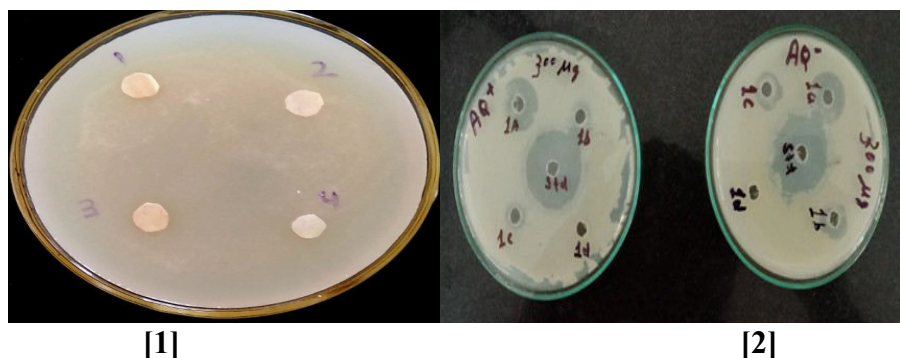


**Figure 10:** Graph representing the pH was slightly increased of prepared gel at 20°C-35°C at different days of interval up to 30<sup>th</sup> days. All values are expressed as Mean  $\pm$  SD calculated by ANOVA summary i.e. p value summary \*, (significant diff. among means [ $P < 0.05$ ]).

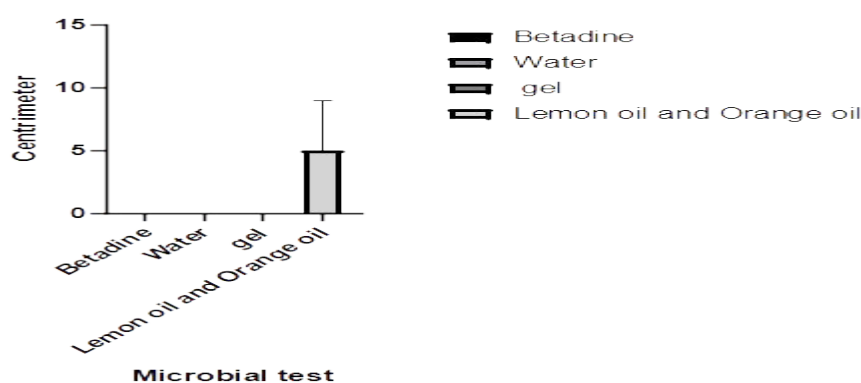
### Microbial Study

During microbial test the distilled water, Betadine, and drug extract was compared by

gel and the resultant values reveals that gel reject the null hypothesis.



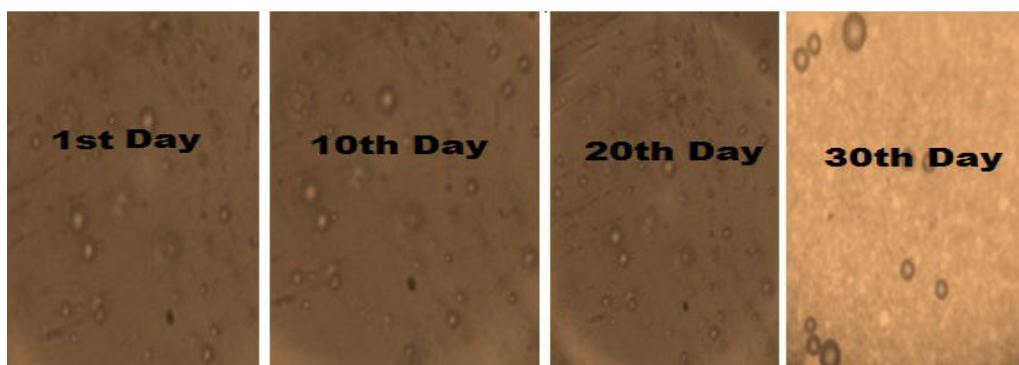
**Figure 11:** [1] Microbial Study of gel was done by using agar media and result was achieved by using whatsmann filter paper [2] Microbial Study of gel was done by using agar media and result was achieved by using B.subtilis (gram positive bacteria), and E.coli (gram negative bacteria).



**Figure 12:** Graph representing the microbial study of gel in this graph the comparative study was done with each other. All values are expressed as Mean  $\pm$  SD calculated by ANOVA summary i.e. p value summary \*, (significant diff. among means [ $P < 0.01$ ]).

### Stability Study

After one month, the physical appearance of the optimized formulation S1 was found to be unchanged in all of the situations to which it was subjected. Even One month later, the medication content was confirmed to be satisfactory. The formulation was observed under digicam microscope at different time interval there was no noticeable reduction.



**Figure 18:** Microscopic image of gel by using Digicam microscope at different time interval for one month which shows no noticeable changes occur during one month of storage which ensures the product stability.

## DISCUSSION

### Aspects of Formulation

The current work of based liposomal gel for topical formulation was studied on different parameters. Firstly, the formation development which was prepared by using cholesterol and soyalecthin in chloroform that pay a significant relief on selecting a designable development of transferosomes because they are phospholipids and they have the property of self-assembling and biodegradable in nature with biocompatibility. The drug which is the main ingredient of the formulation with recognized pharmacological actions. Figure 1 given the assurity of formulated vesicles of microscopic structure and then the pH of the liposomes was determined successfully as per Figure 7 with respect to temperature up to 45°C and it was seemed that pH was similar at different time of interval. The density at 2% w/v was found to be 0.063 gm/cm<sup>3</sup>. Figure 5 showed the particle size distribution by Malvern based on laser diffraction at the record 223; S1 the peak was found at 585.1 with 100 % intensity and have PDI: 0.530 which is less than 1 which means the prepared liposomes are uniformed with respect to particle size. Figure 6 showed zeta potential at 0.0911 mV, with conductivity 0.00125 ms/cm and zeta deviation 3.98 mV, formulation means particles with a zeta potential greater than  $\pm 30$  mV are considered to be stable, because the repulsive force of the same charge can avoid particle aggregation so the results of liposomes showed that the best formulation was obtained as per mentioned graph of the mentioned value is  $\pm 3.98$  mV, which means the formulation has stability because of no aggregation between the charged particles. Figure 8 showed the graph representing *In vitro* release of drug by increasing the rotation time the release property was increased. Figure 9 showed the drug entrapment efficiency of formulation which is less than 100%. Then step, towards the nurtured of transferosomes gel which was obtained by the addition of gelling agent carbopol and Figure 2 showed the Microscopic view of Transferosomes gel under projection microscope with small vesicles of encapsulated drug inside. Figure 10 representing the pH was slightly increased of prepared gel at 20°C-35°C at different days of interval. Figure 3 showed the FTIR and Figure 4 showed the drug content studies by using UV Spectroscopy. Figure 11 showed the microbial study of gel which was done by using agar media and result was achieved by using whatsmann filter paper and Figure 12 showed the graph representing the microbial study of gel with the comparative study.

## CONCLUSION

The present study indicates that liposomes were successfully entrapped in a formulation and. The developed formulation was evaluated for pH, particle size, drug entrapment efficiency, and percentage drug release and animal study. Summarizing all the results we can conclude that nature has provided us the best healing product than the synthetic. So need to explore herbal system for more health benefits in human subjects. They are promising carriers for because they have antioxidant properties and they can protect and stable at 4-5°C for 6 months at least. Solubility was shows in chloroform the pH of the formulated gel was found to be 5 which confirming it is suitable for topical application because in the range of 4.8 to 5.60 which is good recommended pH for the skin. All values are expressed as Mean  $\pm$  SEM calculated by ANOVA summary i.e. p value summary \*, (significant diff. among them). The therapeutic potential of transferosomes gel of curcumin explores the proposed research in the management of wound healing.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

## REFERENCES

1. Cordell GA and Colvard MD (2012). Natural products and traditional medicine: turning on a paradigm. *Journal of Natural Products*; 75(3):514-525.
2. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD and Guo Z (1985). Medicinal plants in therapy. *Bulletin of the World Health Organization*; 63(6):965-981. 7.
3. FAO (2004). Trade in medicinal plants, Economic and Social Department, Food, and Agriculture Organization of the United Nations, Rome, 2-3.
4. Nabi NG and Shrivastava M (2015). In vitro propagation of *Psoralea corylifolia* L.-an important endangered medicinal plant. *Indian Journal of Applied and Pure Biology*; 30(2):201-205.
5. Modi CD, Bharadia PD. Transfersomes: A newdominants for transdermal drug delivery. *Am J PharmTech Res* 2012; 2:71-91
6. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. *Biochim Biophys Acta* 2009;1788:2362-73.
7. Honeywell-Nguyen PL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. *Drug Discov Today Technol* 2005; 2:67-74.
8. Kombath RV, Minumula SK, Sockalingam A, Subadhra S, Parre S, Reddy TR, et al. Criticalissues related to transfersomes - Novel vesicular system. *Acta Sci Pol Technol Aliment* 2012; 11:67-82.
9. Ashish Y. Pawar, Khanderao R. Jadhav, Laxmikant H. Chaudhari Transfersome: A Novel Technique Which Improves Transdermal Permeability. *Asian Journal of Pharmaceutics* • 2016; 10(4)(Suppl):S425.
10. Kaul, S., Gulati, N., Verma, D., Mukherjee, S., & Nagaich, U. (2018). Role of nanotechnology in cosmeceuticals: a review of recent advances. *Journal of pharmaceutics*, 2018.
11. Verma, D., Gulati, N., Kaul, S., Mukherjee, S., & Nagaich, U. (2018). Protein based nanostructures for drug delivery. *Journal of pharmaceutics*, 2018.
12. Enhanced Transdermal delivery of indinavir sulfate via transfersomes, Sheo DM, Shweta A, Vijay KT, Ram CD, Aklavya S, Ghanshyam M, *Pharmacie Globale (IJCP)* 2010; 1(06):1-7.

13. Swarnlata Saraf, Gunjan Jeswani, Chanchal Deep Kaur, Shailendra Saraf. Development of novel herbal cosmetic cream with Curcuma longa extract loaded transfersomes for antiwrinkle effect. *African J Pharm Pharmacol* 2011; 5(8):1054- 1062.
14. Woo HJ, Carraro C, Chandler D. Assembly of extended interfaces and micelles: Charge frustrated models of amphiphilic mixtures. *Faraday Discuss.* 1996; 104:183-191.
15. Strittmatter P, Enoch HG. Formation and properties of 1000-Å diameter, single-bilayer phospholipid vesicles. *Methods Enzymol.* 1978; 52:188-193.
16. Brannon Peppas L. Controlled Release in the Food and Cosmetics Industries in Polymeric. Delivery Systems American Chemical Society. 1993; 8(3):42-52.
17. Kirby C, Gregoriadis G. Dehydration Rehydration Vesicles. A Simple Method for High Yield Drug Entrapment in Liposomes. 1984; 2(7): 979-984.
18. Herris V. Interactive Inc. Anti-ageing medicine, vitamins, minerals and food supplements. A public opinion survey conducted for the International Longevity Center. *Journal of Anti-Aging Medicine.* 2003; 6: 83-90.
19. Thornefeldt C. Cosmeceuticals containing herb. Facts, fiction and future. *Dermatologic Surgery.* 2005; 37: 873-80.
20. Grove GL. Physiologic changes in older skin. *Clin Geriatr Med* 1989; 5:115-25.
21. Gupta MA, Gupta AK. Photodamaged skin and quality of life: reason for therapy. *J Dermatolog Treat* 1996; 7:161-4.
22. García-Varela, R.; Ramírez, O.R.F.; Serna-Saldivar, S.O.; Altamirano, J.; Cardineau, G.A. Cancer cell specific cytotoxic effect of Rhoecydis discolor extracts and solvent fractions. *J. Ethnopharmacol.* 190, 46-58 (2016) .
23. Shanmugam, M.; Rane, G.; Kanchi, M.; Arfuso, F.; Chinnathambi, A.; Zayed, M.; Sethi, G. The multifaceted role of curcumin in cancer prevention and treatment. *Molecules* 20, 2728-2769 (2015) .
24. Ni, H.; Jin, W.; Zhu, T.; Wang, J.; Yuan, B.; Jiang, J.; Liang, W.; Ma, Z. Curcumin modulates TLR4/NF-κB inflammatory signaling pathway following traumatic spinal cord injury in rats. *J. Spinal Cord Med.* 38, 199- 206 (2015) .
25. Bhatia, A.; Singh, B.; Raza, K.; Wadhwa, S.; Katare, O.P. Tamoxifen-loaded lecithin organogel (LO) for topical application: Development, optimization and characterization. *Int. J. Pharm.* 444, 47-59 (2013) .
26. Chaudhary, H.; Kohli, K.; Amin, S.; Rathee, P.; Kumar, V. Optimization and formulation design of gels of Diclofenac and curcumin for transdermal drug delivery by Box-Behnken statistical design. *J. Pharm. Sci.* 100, 580-593 (2011) .
27. Trelle, S.; Reichenbach, S.; Wandel, S.; Hildebrand, P.; Tschannen, B.; Villiger, P.M.; Egge, M.; Jüni, P. Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. *Br. Med. J.* 342, c7086 (2011) .
28. Paul, S.; Kang, S.C. Natural polyamine inhibits mouse skin inflammation and macrophage activation. *Inflamm. Res.* 62, 681-688 (2013) .
29. Esposito, E.; Ravani, L.; Mariani, P.; Huang, N.; Boldrini, P.; Drechsler, M.; Puglia, C. Effect of nanostructured lipid vehicles on percutaneous absorption of curcumin. *Eur. J. Pharm. Biopharm.* 86, 121-132 (2012) .
30. Wang, X.; Jiang, Y.; Wang, Y.W.; Huang, M.T.; Ho, C.T.; Huang, Q. Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. *Food Chem.* 108, 419-

- 424 (2008) .
31. Huang, M.-T.; Robertson, F.M.; Lysz, T.; Ferraro, T.; Wang, Z.Y.; Georgiadis, C.A.; Conney, A.H. Inhibitory effects of curcumin on in vitro lpx and cox activities in mouse epidermis. *Cancer Res.* 507, 338-349 (1992) .
  32. Yadav, K.S.; Maan, P.; Bhatia, S.; Yadav, N.P. Exploration of anti-inflammatory activity of turmeric and onion combination on phorbol ester induced ear inflammation in mice. *Ann. Phytomed.* 3, 50-54 (2014) .
  33. Nazari-Vanani, R.; Moezi, L.; Heli, H. In vivo evaluation of a self-nanoemulsifying drug delivery system for curcumin. *Biomed. Pharmacother.* 88, 715-720 (2017) .
  34. Yuan, J.; Liu, R.; Ma, Y.; Zhang, Z.; Xie, Z. Curcumin attenuates airway inflammation and airway remodeling by inhibiting NF- $\kappa$ B signaling and COX-2 in cigarette smoke-induced COPD Mice. *Inflammation* 41, 1804- 1814 (2018).
  35. Kaur R, Garg T, Das Gupta U, et al. (2014d). Preparation and characterization of spray-dried inhalable powders containing nanoaggregates for pulmonary delivery of anti-tubercular drugs. *Artif Cells Nanomed Biotechnol* 1–6.
  36. Chaudhary S, Garg T, Murthy RS, et al. (2014). Recent approaches of lipid-based delivery system for lymphatic targeting via oral route. *J Drug Target* 1–12.
  37. Goyal G, Garg T, Rath G, et al. (2014). Current nanotechnological strategies for an effective delivery of drugs in treatment of periodontal disease. *Crit Rev Ther Drug Carrier Syst* 31:89–119.
  38. Goll DE, Bray R, Hoekstra W. (1963). Age-associated changes in muscle composition. The isolation and properties of a collagenous residue from bovine musclea. *J Food Sci* 28:503–09.
  39. Parks KL, Beckman EJ. (1996). Generation of microcellular polyurethane foams via polymerization in carbon dioxide. II: foam formation and characterization. *Polym Eng Sci* 36:2417–31.

## STUDY OF A METHODOICAL APPROACH FOR TREATING MYOCARDIAL BRIDGE

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### ABSTRACT

*Myocardial bridge (MB) is a congenital heart defect in which one of the coronary arteries tunnels through the heart muscle itself (myocardium). In normal patients, the coronary arteries rest on top of the heart muscle and feed blood down into smaller vessels. Most MBs are asymptomatic, but a small portion of patients develop chest pain, as well as exertional dyspnea. This means that the coronary artery is partially covered. It is a benign condition. Patients with myocardial bridge may have sudden cardiac arrest or even sudden death.*

*In this study, we propose a muscle coordination method in which various muscles (monoarticular muscles and a polyarticular muscle) are unroofed asymmetrically. First, muscle coordination control models for the musculoskeletal robot are built using virtual antagonistic muscle structures with a virtually symmetric muscle arrangement. Finally, position control experiments are conducted, and the effectiveness of the proposed muscle coordination control and the virtual antagonistic muscle structure is evaluated. The ultrasonic and infrared sensors are actualized to distinguish obstacles on the robot's way by interfacing a microcontroller. The miniature regulator is obtained by inciting the motors in request to keep away from the distinguished obstacle.*

**Keywords:** Myocardial bridge, muscle, heart, IoT, Robot, Arduino, Ultrasonic sensor, Obstacle,

### I INTRODUCTION

Typically, the coronary arteries lie on the surface of the heart. However, in people with myocardial bridges, one or more of the arteries goes through the heart muscle for a short distance. The flap of heart muscle that goes over this small section of artery forms a "bridge." It means, Myocardial bridging occurs when the heart is malformed. The bridge of muscle fibers overlying a section of a coronary artery, usually the left anterior descending (LAD) artery. When the heart beats, the artery is squeezed and normal blood flow is disrupted during both the pumping and relaxed cycles.

### II How is it Treated?

In most patients, a myocardial bridge is not treated if it is not causing any symptoms. Myocardial bridges have traditionally been considered a benign condition, but recent studies have demonstrated that the clinical complications can be dangerous. These complications include acute coronary syndromes. Middle segment of the left anterior descending coronary artery was indicated as the most frequent location of myocardial bridging. However bridging on the circumflex branch of the left coronary artery, and right coronary arteries have been also reported (3,7,13). In our case left anterior descending coronary artery penetrated into myocardial muscle 2 cm proximal to its origin, and coursed intramural for 2 cm.

### III Medication

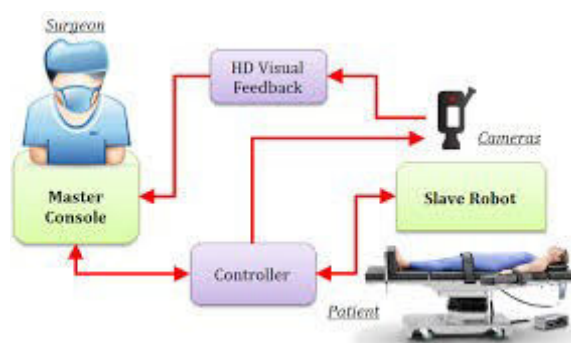


1. **Beta Blockers:** Drugs that slow the heart rate and help ease the amount of force with which the heart squeezes
2. **Calcium Channel Blockers:** Medications that help to relax and widen blood vessels
3. **Statins:** These reduce cholesterol levels in the blood, which helps to prevent plaque build-up.
4. **Aspirin:** Aspirin may help prevent blood clots or artery blockages.

#### IV Unroofing

Some people with myocardial bridging may still experience intolerable chest pain and other symptoms, despite taking medication. In those cases, surgical unroofing is the best option.

During this procedure, the surgeon removes the myocardial bridge and frees the constricted artery underneath. The surgeon uses a detailed map, created during a cardiac catheterization that shows the precise location of the bridge.

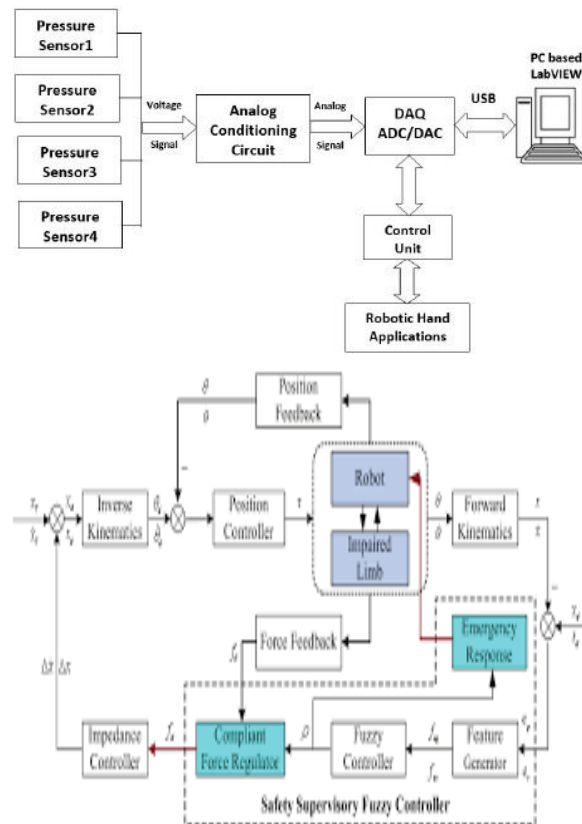
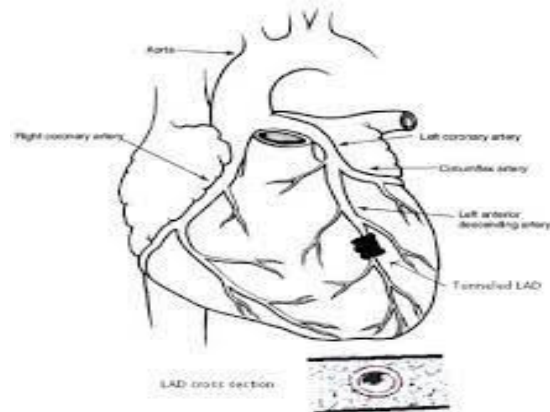


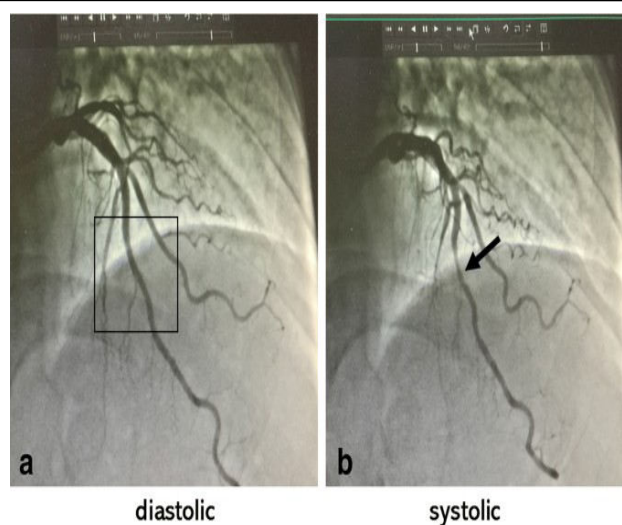
#### V CT and Angiography

Both angiography and coronary artery MDCT can detect LAD myocardial bridging

Coronary artery MDCT directly shows a portion or all of the artery in an intramyocardial location - the length and depth of the tunneled segment along with the location (proximal/mid or distal segment) can be determined on CTCA

- A retrospective gating technique was used to synchronize data reconstruction with the ECG signal. A mono-segment reconstruction algorithm that uses the data from a quarter rotation from both detectors was used for image reconstruction.
- In each patient, images were previewed automatically, and the optimal phase was selected for post-processing. Whenever necessary, additional images were reconstructed in 5% steps of the R-R interval within the full tube current window. For CTCA images reconstructed from the contrast-enhanced DSCT scan with a slice thickness of 0.75 mm, the reconstruction increment was set at 0.4 mm using a medium-soft tissue convolution kernel. The reconstructed field-of-view was adjusted to exactly encompass the heart (image matrix 512×512 pixels), based on individual anatomy.





## VI DATA ANALYSIS

All CT images were analyzed by consensus of two radiologists who were blinded to the CCA findings. The image quality of the LAD was assessed semi-quantitatively with a previously described four-point ranking scale (4, excellent image quality; 3, good image quality; 2, fair image quality; 1, poor image quality). Excellent image quality indicated a clear delineation of the coronary artery without motion artifact. Good image quality was classified as having minor motion artifact and mild blurring of vessel margins. Fair image quality was defined as having moderate motion artifacts and moderately blurred vessel without structure discontinuity. Poor image quality was classified as having severe motion artifact and severely blurred vessel with structure doubling or discontinuity. Image evaluation was performed on the workstation.

Myocardial bridging was diagnosed and evaluated when part of the LAD was located in the interventricular gorge and completely or incompletely surrounded by the left ventricular myocardium. For each tunneled segment, the following parameters were recorded: the location of the LAD (proximal, middle, and distal), the length and depth of the tunneled segment, and the presence of atherosclerosis in the tunneled segment and in a 2-cm-long segment proximal to the entry of the tunneled segment. The depth and length of the tunneled segment of the LAD were analyzed using dedicated vessel analysis software, particularly measured using an electronic caliper. The length of the tunneled segment was measured as the length of the myocardial bridge covering the distance between the entrance and the exit of the tunneled segment. The depth of the tunneled segment was measured as the widest part between the surface of the myocardial bridge and the tunneled segment.

The tunneled segments of the LAD were divided as being superficial or deep based on the depth of the tunneled segment ( $\leq 1$  or  $> 1$  mm). We also subdivided superficial MB into complete and incomplete based on the full or partial encasement of the LAD within the left ventricular myocardium.

A computer system causes the robot arms to mimic the surgeon's hand motions that are applied through the surgical console while also enabling the features of tremor elimination, motion scaling and motion indexing.

Despite the reduced invasiveness of the robotically assisted cardiac procedures, there is still a need for the use of CPB and its inherent risks, albeit low, for cardiac and noncardiac complications [13]. Furthermore, since in most of these procedures, bypass is achieved by peripheral vessel cannulation, the small size of children's vessels with respect to cannula size introduces the added risk of permanent vessel damage and its impact on limb growth [14].

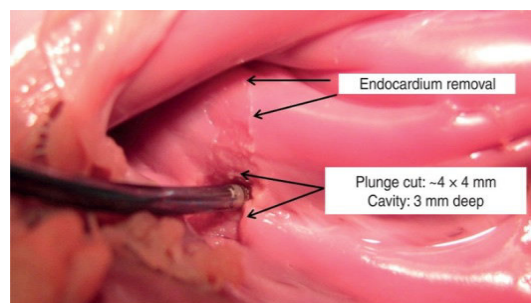
The potential value of robotics in beating-heart intracardiac surgery is to provide a tool delivery platform that possesses the stability, stiffness and dexterity necessary to perform beating-heart interventions with the efficacy of open-heart procedures. For intracardiac surgery, however, the workspace available to a robot consists of the comparatively small internal volumes of the cardiac chambers. In addition, navigation through the heart, while avoiding damage to delicate fast-moving structures, such as valves and valvar apparatus, favors the use of robots comprised of curved rather than straight components. Thus, the challenges of intracardiac surgery suggest that new approaches are needed.

## VII RESULTS

One hundred consecutive autopsied MI hearts either with MBs [MI(+)MB(+) group; n=46] or without MBs (n=54) were obtained, as were 200 normal hearts, 100 with MBs [MI(-)MB(+) group] and 100 without MBs. By microscopy on LADs that were consecutively cross-sectioned at 5-mm intervals, the extent and distribution of LAD atherosclerosis were investigated histomorphometrically in conjunction with the anatomic properties of the MB, such as its thickness, length, and location and the MB muscle index (MB thickness multiplied by MB length), according to MI and MB status. In the MI(+)MB(+) group, the MB showed a significantly greater thickness and greater MB muscle index ( $P<0.05$ ) than in the MI(-)MB(+) group. The intima-media ratio (intimal area/medial area) within 1.0 cm of the left coronary ostium was also greater ( $P<0.05$ ) in the MI(+)MB(+) group than in the other

The robotic platform should enter the heart and provide ergonomic control of both tools and imaging. Essential imaging features include high-quality real-time views of the entire heart volume as well as high-fidelity tool-tip views for visualizing the tool–tissue interaction. In combination with imaging, the incorporation of touch sensing, to monitor forces applied to the tissue, will be critical for safe and effective pediatric intracardiac interventions.

The two tissue layers are first pierced at the location where approximation is desired. During robotic deployment, a sharpened stylet inserted through the robot was used in place of the cannula. The robot produces appropriate overlap of the septum secundum and primum by first piercing the secundum and then dragging it laterally to achieve the desired overlap with the septum primum. The robot then punctures the second layer and proceeds with device deployment.



## VIII CONCLUSION

This procedure snap into a motorized drive system (common to all procedures) in a manner similar to existing robotic catheters. These tube sets can be designed to be either disposable or sterilizable. For the purpose of designing robots for new procedures inside the heart, design algorithms have been created that utilize anatomical images to define workspace constraints, while the steps of the surgical task provide the set of tip configurations (positions and orientations) that the robot must reach [12]. A total of 536 (226 males, 310 females; mean age,  $58.6 \pm 11.8$  years; age range 24-90 years) out of 1,275 (42%) patients showed 557 cases of MB. Of the 557 tunneled segments, 529 (95%) were located in the mid LAD, 19 (3%) were located in the distal LAD, 2 (0.4%)

were located in the proximal LAD, four (0.7%) were located in the proximal to mid LAD, and three (0.5%) were located in the mid to distal LAD. Nineteen patients had two areas of MB, whereas one patient had three areas of MB in the LAD. The superficial MB type ( $\leq 1$  mm in depth) was seen in 368 of 557 (66%) of all of the tunneled LAD segments. Of the superficial forms of MB, the complete type was seen in 128 of 368 (35%) of cases, whereas the incomplete type was seen in 240 of 368 (65%) cases. The deep MB type ( $> 1$  mm in depth) was seen in 189 of 557 (34%) tunneled LAD segment. The mean length of a tunneled segment was  $21.0 \pm 11.6$  mm. Superficial MB length as determined by CT was  $16.4 \pm 8.6$  mm and deep MB length was  $27.6 \pm 12.8$  mm. The mean length of the complete type ( $17.4 \pm 8.3$  mm) and incomplete type ( $16.0 \pm 8.7$  mm) were also determined.

## REFERENCES

- [1] Angelini P, Trivellato M, Donis J, et al. Myocardial bridges: a review. *Prog Cardiovasc Dis.* 1983;26:75–88.
- [2] Alegria JR, Herrmann J, Holmes DR, et al. - Myocardial bridging. *Eur Heart J.* 2005;26:1159–68.
- [3] Polacek P, Kralovec H - Relation of myocardial bridges and loops on the coronary arteries to coronary occlusions. *Am Heart J.* 1961;61:44–52.
- [4] Angelini P, Velasco JA, Flamm S - Coronary anomalies. incidence, pathophysiology and clinical relevance. *Circulation.* 2002;105:2449–54.
- [5] Reyman HC *Disertatio de vasis cordis propriis* (dissertation). Göttingen: Med Diss Univ. 1737.
- [6] Cranicianu A *Anatomische Studien über die Coronararterien und experimentelle Untersuchungen über ihre Durchgängigkeit.* *Virchows Arch A Pathol Anat.* 1922;238:1–8.
- [7] Mohlenkamp S, Hort W, Ge J, et al. Update on myocardial bridging. *Circulation.* 2002;106:2616–22.
- [8] Ge J, Erbel R, Rupprecht HJ, et al. Comparison of intravascular ultrasound and angiography in the assessment of myocardial bridging. *Circulation.* 1994;89:1725–32.
- [9] Schwarz ER, Klues HG, vom DJ, et al. Functional, angiographic and intracoronary Doppler flow characteristics in symptomatic patients with myocardial bridging: Effect of shortterm intravenous beta-blocker medication. *J Am Coll Cardiol.* 1996;27:1637–1645.
- [9] Çay S, Oztürk S, Cihan G, et al. Angiographic prevalence of myocardial bridging. *Anadolu Kardiyol Derg.* 2006;6:9–12.
- [10] Portmann W, Iwig J *Die intramurale Koronarie im Angiogramm.* *Fortschr Röntgenstr.* 1960;92:129–32.
- [11] Ferreira AG Jr, Trotter SE, König B Jr, et al. Myocardial bridges: morphological and functional aspects. *Br Heart J.* 1991;66:364–7.
- [12] Arjomand H, AlSalman J, Azain J, et al. Myocardial bridging of left circumflex coronary artery associated with acute myocardial infarction. *Invasive Cardiol.*

- 2000;12:431–4.
- [13] Mazzu A, Di Tano G, Cogode R, et al. Myocardial bridging involving more than one site of the left anterior descending coronary artery: An uncommon cause of acute ischemic syndrome. *Cathet Cardiovasc Diagn*. 1995;34:329–32.
- [14] Berry JF, von Mering GO, Schmalfuss C, et al. Systolic compression of the left anterior descending coronary artery: A case series, review of the literature, and therapeutic options including stenting. *Catheter Cardiovasc Interv*. 2002;56:58–63.
- [15] Tio RA, Ebels T Ventricular septal rupture caused by myocardial bridging. *Ann Thorac Surg*. 2001;72:1369–70.
- [16] Feld H, Guadanino V, Hollander G, et al. Exercise-induced ventricular tachycardia in association with a myocardial bridge. *Chest*. 1991;99:1295–6.
- [17] den Dulk K, Brugada P, Braat S, et al. Myocardial bridging as a cause of paroxysmal atrioventricular block. *J Am Coll Cardiol*. 1983;1:965–9.
- [18] Galli M, Politi A, Zerboni S “Functional myocardial bridging” and “hyperkinetic state”: A rare association as a cause of acute myocardial infarction. *G Ital Cardiol*. 1997;27:1286–9.
- [19] Cutler D, Wallace JM Myocardial bridging in a young patient with sudden death. *Clin Cardiol*. 1997;20:581–3.
- [20] Yetman AT, McCrindle BW, MacDonald C, et al. Myocardial bridging in children with hypertrophic cardiomyopathy— a risk factor for sudden death. *N Engl J Med*. 1998;339:1201–1209.
- [21] Channer KS, Bukis E, Hartnell G, et al. Myocardial bridging of the coronary arteries. *Clin Radiol*. 1989;40:355–9.
- [22] Michels R, Brueren G, van Dantzig, et al. ACS, myocardial bridging, Tako-tsubo syndrome and mitral regurgitation. *Neth Heart J*. 2005;13:57–61.
- [23] Kracoff OH, Ovsyshcher I, Gueron M Malignant course of a benign anomaly: myocardial bridging. *Chest*. 1987;92:1113–5.
- [24] Haswani L, M L HK, Kiran J A heart with multiple coronaries anomalies: myocardial bridging, left dominance and high take off of ostia-an autopsy case. *J Clin Diagn Res*. 2014;8:143.
- [25] Peters S Unusual case of myocardial bridging of the circumflex artery and initially intractable chest pain. *Int J Cardiol*. 2015;179:25–6.
- [26] Kang HY, Seo DY, Chung JY, et al. Variant angina associated with myocardial bridging and obstructive sleep apnea syndrome after lumbar spine surgery. *Korean J Anesthesiol*. 2014;67(Suppl):S27–9.

## ORAL HEALTH STATUS AND UTILIZATION OF DENTAL CARE SERVICES AMONG DIFFERENTLY ABLED POPULATION IN CHENNAI –A CROSS SECTIONAL SURVEY

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### ABSTRACT

**Background:** Special children are more susceptible for oral diseases due to poor oral hygiene, differential oral health treatment, specialized knowledge, increased awareness, attention, adaptation, and accommodative measures beyond what are considered routine is required to provide healthcare for individuals with special needs.

**Aim:** The aim of this study was to assess the oral health status of differently abled population and oral health literacy status among the parents and guardians of challenged individuals who were the residents of Chennai city including the determination of inequalities in oral health and its impact on utilization of dental care services.

**Materials and Methods:** A cross-sectional study was conducted among 200 differently abled individuals using suitable standardized forms, namely, the “World Health Organization (WHO) oral health assessment form for children (surface), 2013” for recording clinical oral health status surface wise and a self-administered “WHO oral health questionnaire for adults” with a self-prepared questionnaire.

**Results:** About 82 (58.99%) had dental decay in their permanent teeth. Majority of the caregivers 80 (40%) perceived financial constraint as the barriers for utilization of dental care services. About 53 (26.5%) of them felt that lack of assistance was the barrier to approach dentists while nearly 33 (16.5%) had reported that non-availability of dental professionals at nearby locations was the barrier to undergo dental care.

**Conclusion:** In the present study, with the observations of the findings, it can be concluded that there is high prevalence of dental caries and gingival diseases among differently abled individuals and the parents or caregivers have inadequate knowledge of oral health.

**Keywords:** Differently abled, Oral Health Status, Unmet Treatment needs, Early Diagnosis, Prompt Treatment, Oral Health Policy

### INTRODUCTION

It is estimated that there are about 500 million people worldwide with disabilities. As per the World Health Organization (WHO) report, it is estimated that there is around 10% of the population in developed and 12% in the developing countries belonging to groups with disabilities <sup>[1]</sup>. They have a comparatively higher prevalence and severity of

oral disease when compared to the general population. The indicators of poor oral health in adults with disabilities are high rates of dental caries, missing teeth, periodontal disease, and prolonged retention of primary teeth, misaligned or supernumerary teeth, and malocclusion <sup>[2, 3]</sup>.

Special children are more susceptible for oral diseases due to poor oral hygiene due to the fact that they are partially or wholly dependent on someone else to perform their daily care activities creating situation which may cause difficulties for their families <sup>[4, 5]</sup>. It is great challenge to maintain oral health among special population because inaccessibility could be a barrier for physically challenged individuals while involuntary behaviours such as lip biting, tongue thrusting, finger sucking, and those involved in mastication such as excessive swallowing, food pocketing, bruxism, and drooling of saliva might be reasons for the same among mentally compromised persons <sup>[6]</sup>.

Due to the fact that, the contributing factors for poor oral health of individuals with disabilities could be under diagnosis and differential oral health treatment, specialized knowledge, increased awareness, attention, adaptation, and accommodative measures beyond what are considered routine is thus required to provide healthcare for individuals with special needs <sup>[7,9]</sup>

Health is one of the basic human and social rights of an individual. It is the duty of every respective government to provide oral health service and increase health-care awareness. Effective utilization of services is a concept of expressing the enhanced interaction extent between the service provider and the people for whom it is intended <sup>[10, 11]</sup>.

The role of dentists is imperative since they play a crucial role in bridging the gap between various sections of people and the health sector. Provision of “appropriate services in the right place and at the right time” is the true meaning of creating good access to health services <sup>[12]</sup>.

Hence, the aim of this study was to conduct the assessment of dentition status among children and adults with disabilities. It also assessed the oral health literacy status among the parents and guardians of special children and physically challenged individuals who were the residents of Chennai city including the determination of inequalities in oral health and its impact on utilization of dental care services.

## **MATERIALS AND METHODS**

A cross-sectional study was conducted in Chennai, the capital of Tamil Nadu state of India and the 4th largest metropolitan city with a population of about 4,681,087 people according to the 2011 census report in individuals with disabilities and their caretakers who were residents.

Children and adults with physical and mental disabilities who are residents having association with a special school within the city limit were included as participants for oral health assessment. The parent's/guardian's/caretakers of children and adults with mental disabilities who are residents were also included for oral health knowledge assessment. Unconscious residents of Chennai and those who did not give consent were excluded from the study. Ethical approval was obtained from the Institutional Scientific Review Board. The sample size was calculated using the formula,  $N = Z\alpha^2 PQ/L2$  and approximated to 200. Simple random sampling technique was adopted.



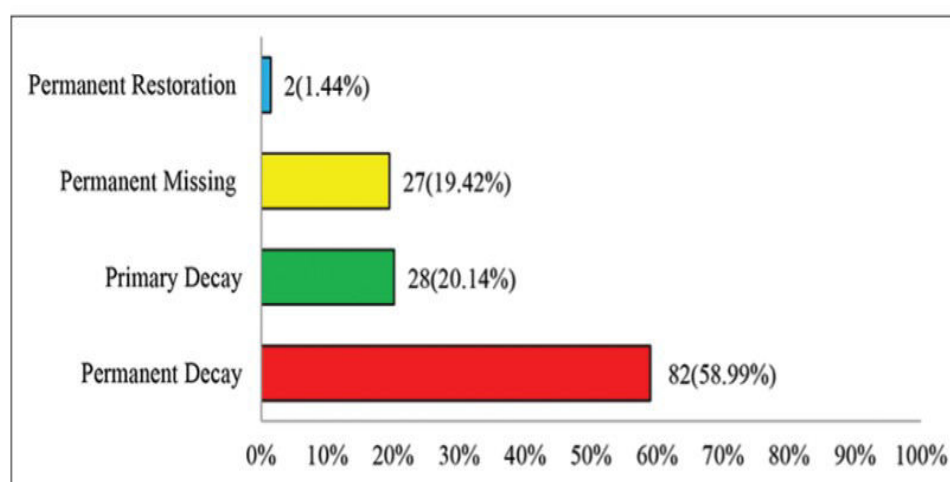
The survey instruments used were WHO oral health assessment form for children (2013) tooth surface wise, WHO oral health assessment form for adults (2013) tooth surface wise and WHO oral health knowledge form of adults. Self-prepared and administered questionnaire that was validated by a pilot study was used to determine the inequalities in oral health and its impact on utilization of dental care services. The armamentarium used consisted of disposable mouth mirrors, probes, No. 5 explorer, tweezers, kidney tray, sterilized cotton, disposable gloves, disposable mask and head cap.

After a brief introduction on the purpose of the study, oral examination was conducted by a single examiner who had been trained through a series of clinical training sessions. Demographic information was collected followed by clinical examination in accordance with the WHO criteria and method <sup>[13]</sup>. Completed filled forms were considered for analysis. A single examiner conducted intraoral examination under torch light using mouth mirror and explorer (No. 5) and assessed the oral health status.

The data collected were analyzed and tested for significance using statistical software packages, SPSS software version 17.0. Frequency tables were computed. Chi-square test was done to test whether the difference in the various parameters is statistically significant. For all the tests,  $P < 0.05$  is considered statistically significant.

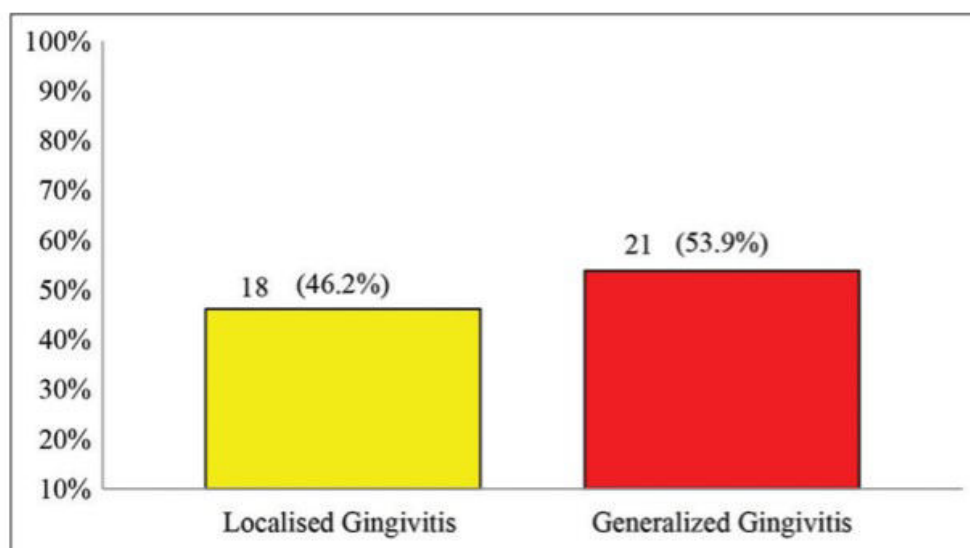
## RESULTS

Majority of the study participants 82 (58.99%) had dental decay in their permanent teeth while 28 (20.14%) of them were having primary decayed teeth. It also shows that edentulousness was found to be seen among 27 (19.42%) of the study subjects involving only permanent dentition. The graph also shows that only two individuals in this study have undergone restorations on their permanent dentition.



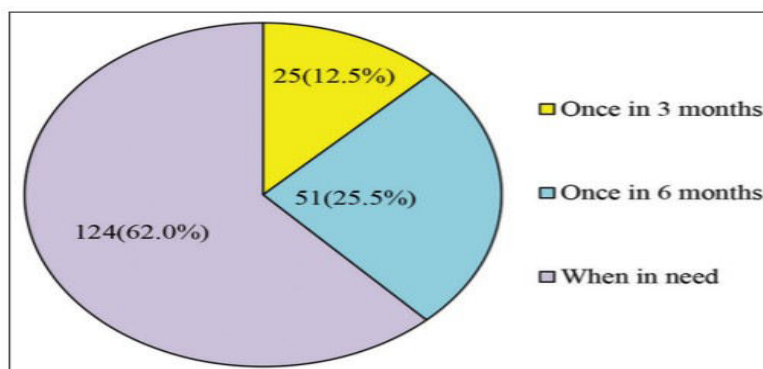
**Graph-I** shows the distribution of study participants based on decay, missing and filled status

Majority of them 21 (53.9%) had generalized form of gingivitis while 18 (46.2%) of them had the localized form of gingivitis.



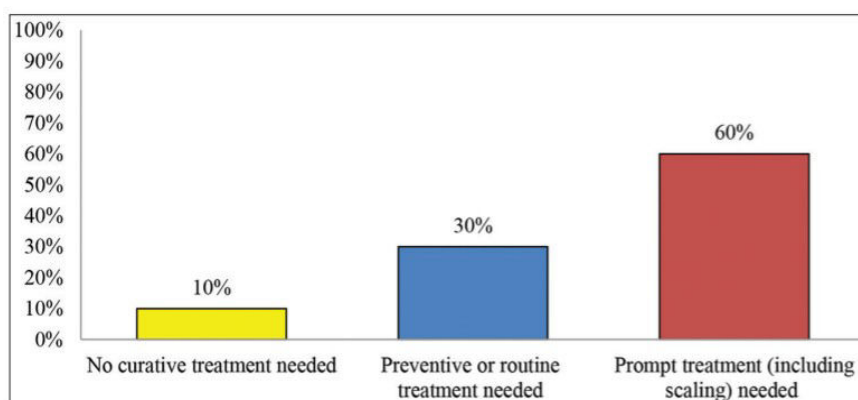
**Graph-II** shows the distribution of study participants based on decay, missing and filled status

Majority of the participants, 124 (62%) out of the total 200 had reported that they visit the dentist. Around 51 (25.5%) of them had stated that they see their dentist once in every 6 months while only 25(12.5%) had reported that they take a dental visit once in every 3 months.



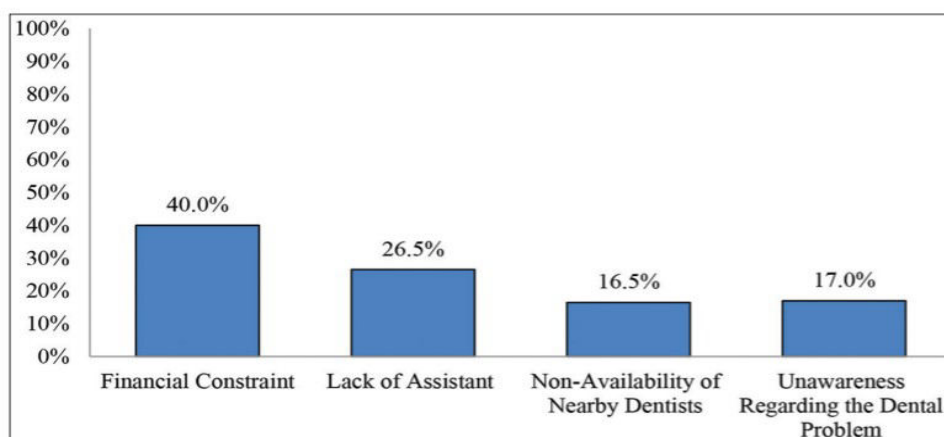
**Figure 1:** shows the distribution of study subjects based on the frequency of their dental visit.

About 136 (60%) of them required prompt dental treatments including scaling followed by 61 (30%) of them in need of preventive or routine dental treatments. Dental treatments were not indicated for nearly 3 (10%) of the total population.



**Graph-III** shows the distribution of study participants based on their dental treatment needs

Majority of the caregivers 80(40%) perceived financial constraint as the barriers for utilization of dental care services. Around 53 (26.5%) of them felt that lack of assistant was the barrier to approach dentists while nearly 33(16.5%) had reported that non-availability of dental professionals at nearby locations was the barrier to undergo dental care. About 34 (17%) of them were unaware regarding the status of their oral cavity.



**Graph IV** shows the distribution of the study subjects based on the perceived barriers for utilization of dental care services among residents in Chennai.

## DISCUSSION

Oral disease represents a major health problem among individuals with disabilities. The recent National Sample Survey Organization report suggests that the number of disabled persons in the country is estimated to be 18.49 million which forms to about 1.8% of the total population and the mentally challenged population accounts to 0.44 million individuals.

Majority of the study participants 82 (58.99%) had dental decay in their permanent teeth while 28 (20.14%) of them were having primary decayed teeth. High prevalence of dental caries in this study could be due to the difficulty in accessing dental care, noncooperation, or communication problems and this finding is supported by a similar study conducted by Jongh et al. in 2012, in which it is reported that a significantly higher proportion of children with disabilities in their study did not receive any routine dental care in comparison to healthy controls <sup>[14]</sup>.

Caries prevalence was higher in children with special health-care needs than in the healthy controls. Similar findings have been reported by other authors where the prevalence of dental caries ranged from 78.3% to 89.6% in different types of children with special health-care needs.

Studies by other authors have reported higher rates of DMFT than in this study, with values ranging from 3.5 to 12.51 in children with SHCN. Ivancic et al reported the mean DMFT in disabled and healthy children to be 6.39 and 4.76 respectively <sup>[15]</sup>. Other studies by Kamatchy reported a mean DMFT of 1.06, 0.8, 2.0 and  $1 \pm 1.42$  respectively in children with various forms of SHCN, which was lower than our study <sup>[16]</sup>. The 2002–2003 National Oral Health Survey also reported a lower mean DMFT of 1.87 compared with this study <sup>[17]</sup>.

About 39 (19.5%) of them in this study were found to be having gingivitis that could progress to periodontal diseases as observed in other studies, in which a significantly greater prevalence of periodontal disease in children with special health-care needs compared with healthy controls is reported.

## CONCLUSION

It is evident from this survey that differently abled individuals high prevalence of dental caries and gingival diseases and the parents or caregivers have inadequate knowledge of oral health.

## RECOMMENDATION

Provision of prompt treatments following early diagnosis is the key for delivery of successful special care dentistry. The need of the hour is offering professional dental health professional's support with focus on preventive approach and dental education to parents of disabled individuals. In addition, the oral hygiene habits of individuals with disabilities can be improved by close monitoring and periodic dental check-ups.

## REFERENCES

1. **Baykan Z.** Causes and prevention of disabilities, handicaps and defects. *Journal of Contemporary Medical Education* 2003; 9:336-8.
2. **Beange H.** Caring for a vulnerable population: Who will take responsibility for those getting a raw deal from the health care system? *The Medical Journal of Australia* 1996; 164:159-60.
3. **Gordon SM, Dionne RA, Snyder J.** Dental fear and anxiety as a barrier to accessing oral health care among patients with special health care needs. *Special Care in Dentistry* 1998; 18:88-92.
4. **Siklos S, Kerns KA.** Assessing the diagnostic experiences of a small sample of parents of children with autism spectrum disorders. *Research in Developmental Disabilities* 2007; 28:9-22.
5. **Faulks D, Hennequin M.** Evaluation of a long-term oral health program by carers of children and adults with intellectual disabilities. *Special Care in Dentistry* 2000; 20:199-208.
6. **Winter K, Baccaglini L, Tomar S.** A review of malocclusion among individuals with mental and physical disabilities. *Special Care in Dentistry* 2008; 28:19-26.
7. American Academy of Pediatric Dentistry. Guideline on management of dental

- patients with special health care needs. *Paediatric Dentistry Journal* 2010; 32:132-6.
8. **Cumella S, Ransford N, Lyons J, Burnham H.** Needs for oral care among people with intellectual disability not in contact with community dental services. *Journal of Applied Research in Intellectual Disabilities* 2000; 44:45-52.
  9. **Reichard A, Turnbull HR, Turnbull AP.** Perspectives of dentists, families, and case managers on dental care for individuals with developmental disabilities in Kansas. *Mental Retardation and Developmental Disabilities Research Reviews* 2001; 39:268-85.
  10. **Shi L, Lebrun LA, Tsai J.** Access to medical care, dental care and prescription drugs: The roles of race/ethnicity, health insurance, and income. *The Southern Medical Journal* 2010; 103:509-16.
  11. **Wellstood K, Wilson K, Eyles J.** Reasonable accesses to primary care: Assessing the role of individual and system characteristics. *Health Place* 2006; 12:121-30.
  12. **Gulliford M, Morgan M.** *Access to Health Care*. London: Psychology Press; 2003.
  13. World Health Organization. *Oral Health Surveys: Basic Methods*. 4th ed. Geneva: World Health Organization; 1997. p. 39-44.
  14. **Ad de Jongh, Caroline van Houtem, Marielle van der Schoof, Gail Resida, Dyonne Broers.** Oral health status, treatment needs and obstacles to dental care among non institutionalized children with severe mental disabilities in The Netherlands. *Special Care Dentistry* 2008;28(3), 111-115.
  15. **Jokic, Natasa & Majstorovic, Martina & Bakarcic, Danko & Katalinic, Andrej & Szirovicza, Lajos.** Dental caries in disabled children. *Collegium antropologicum* 2007; 31(1):321-4.
  16. **K R Kamatchy, J Joseph, C G Krishnan.** Dental caries prevalence and experience among the group of institutionalized hearing impaired individuals in Pondicherry- a descriptive study. *Indian Journal of dental research: official publication of Indian Society for Dental Research* 2003; 14(1):29-32
  17. **R.K. Bali,V.B. Mathur, P.P. Talwar, H.B. Channa.** National Oral Health Survey & Fluoride Mapping 2002-2003 India. <https://dcindia.gov.in/Download/Books/NOHSBOOK.pdf>

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## MANAGEMENT OF ACUTE FISSURE IN ANO WITH AN AYURVEDIC ANAL SUPPOSITORY – CASE REPORT

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### ABSTRACT

**Introduction:** Parikartika is most common ano-rectal disorder. The text of parikartika is mentioned in Samhitas. The word Parikartika means Parikartanavatvedana around Guda region. In this disease there are symptoms such as bleeding per rectum, hard stools, burning sensation around anal region. In this case study, a 38 yrs male complaining of excruciating pain during and after defecation, bleeding per rectum, hards stools since last 2 months. After clinical examination he was diagnosed with acute fissure in ano. He had taken allopathic medicines for this but didn't get relief.

**Materials and Methods:** Different types of ayurvedic texts, samhitas, Internet source, Journals, Research articles etc. Patient was selected from Shalya Tantra OPD of Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod, Wardha.

**Discussion:** He was treated with gudavarti which give effective in management of acute fissure in ano. Gandharva haritaki choorna acts as laxative and helps to relieve constipation and sitz bath was given to quickly relieve burning pain. Changing in lifestyle and healthy diet also plays important role in the management of Fissure in ano.

**Conclusion:** The treatment given was found effective relief from pain, bleeding, itching at perianal region.

**Keywords:** Parikartika, fissure in ano, anal suppository, gudavarti

### INTRODUCTION

Parikartika is considered as fissure in ano. There are different causative factors of Parikartika such as Vamana-Virecana Vypada, Basti Karma vypada and Upadrava of Atisara, Grahani, Arsha, Udāvarta<sup>1</sup>. In this regard Acharya Sushruta explained the pathogenesis of disease<sup>2</sup>

The word Parikartika means Parikartanavatvedana around guda i.e. cutting type of pain. Parikartika also has symptoms such as pain in the penis, anus, neck of the urinary bladder and umbilical region with flatus cessation<sup>3</sup>. References about Parikartika are available from all Bruhatrayi and also mentioned in kashyap samhita and later authors of Ayurveda<sup>4</sup>

Anal Fissure is one of the major causes for pain at anal region<sup>5</sup>. The fissure-in-ano is categorized into two types depending on the clinical symptoms & durations of the disease; viz. Acute and Chronic<sup>6</sup>. The two primary signs of this disorder are, bleeding and pain; pain is often unbearable<sup>7</sup>. In long-standing instances, sentinel tag and

haemorrhoids can be associated with this. Pruritus ani can be another symptom of this disorder<sup>8</sup>. In male's anal fissure typically occurs in the midline posterior- 90 percent and 10 percent much less frequently. Subsequently, female fissures on the anterior midline are somewhat more common than before<sup>9</sup>.

Analgesics, antibiotics, laxatives, ointment, and anal dilatation, sphincterotomy, and fissurectomy are some of common treatments advised in fissure in ano. Fissure-in-ano surgeries are costly and involve a lengthy stay in the hospital. All of these procedures come with their own set of risks<sup>10</sup>. There are numerous preparations and surgical procedures in Ayurveda. Fissure-in-ano, which is widely seen in ano-rectal practise, has comparable location, pathology, and clinical symptoms to parikartika, such as anal pain, burning feeling at anal, constipation, blood-streaked faeces, and so on.

### **A Case Report**

The patient of age 38 year male was having chief complaint of pain during and after defecation, burning sensation at anal region, hard stools on and off, blood streaks coming with stools since 2 months.

### **History of Present Illness**

The patient of age 38 years complaining of pain during and after defecation, burning sensation at anal region, hard stools on and off, blood streaks coming with stools since 2 months. He had taken allopathic medicine for this but no relief. Presently, the patient vitals were as follows pulse was 78/min, Blood pressure 124/70 mm hg, respiratory rate 17/min, temperature 98.5F. His cardio-vascular examination and Respiratory examination was normal. On per rectum examination fissure wound was present at 6 o'clocks, spasm Grade 1, with no active bleeding was present. The patient was diagnosed with acute fissure in ano. Hence for ayurvedic treatment patient came to shalya tantra OPD of mahatma Gandhi Ayurveda College hospital and research center, salod, wardha.

### **Past History**

No history DM/HTN/ Asthma/thyroid.

### **Personal History**

Marital status - married

Smoker - NAD

Tobacco - No history

Alcohol - NAD

### **Family history**

Father - HTN

Mother - NAD

### **On Examination**

Pulse rate -78/min

BP- 124/70 mmhg

Urine- normal

Stool- hard stools, blood streaks with stools

Tongue- Saam  
 Speech- normal  
 Agni – Mandya  
 Akruti- krusha  
 Bala- madhyama

### Local examination

#### Per Rectal Digital Examination

Fissure present at 6 o clock position  
 Tenderness present  
 Mild Spasm present (Grade 1)  
 No active bleeding present  
 No sentinel tag present

### MATERIAL AND METHOD

#### Method

Center of study: Shalya Tantra OPD of mahatma Gandhi Ayurveda College hospital and research center, salod, wardha, simple Random Single Case Study.

Research Drug – Palashadigudavarti was prepared by mixture of ghruta kalpana and suppository base with moulding method.

#### Material

- 1) Advise Sitz bath with lukewarm water twice a day
- 2) Advise pathya
- 3) Palashadigudavarti once a day for local application
- 4) Gandharva Haritaki Choorna 1 tablespoon at night with lukewarm water

#### Investigation

Hb-10.8gm%. TLC: White blood count (WBC) 7500/mm<sup>3</sup>.

KFT- With normal limits

LFT- With normal limits

Human immunodeficiency virus-1 (HIV-1) test was negative

Hepatitis B surface antigen, non reactive

#### Intervention

In this case report patient is treated by using palashgudavarti for the management of acute fissure in ano. Palashgudavarti was used for local application along with sitz bath. The effectiveness of Palashvartivarti was assessed by pain, bleeding, and healing. Assessment was done on 1st day, 7th day, 14thday and follow up was taken on 28th day.

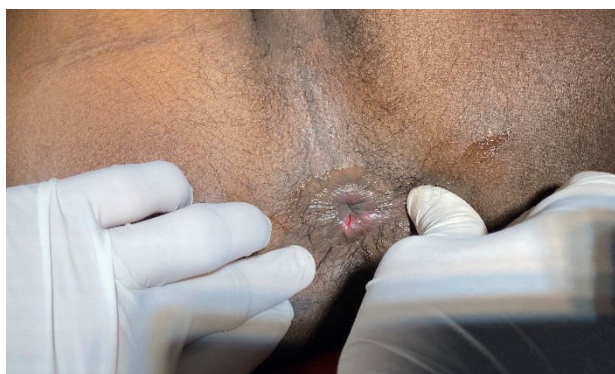
### RESULT

Sr No	Symptoms	Day 0	Day 7	Day 14	Day 28
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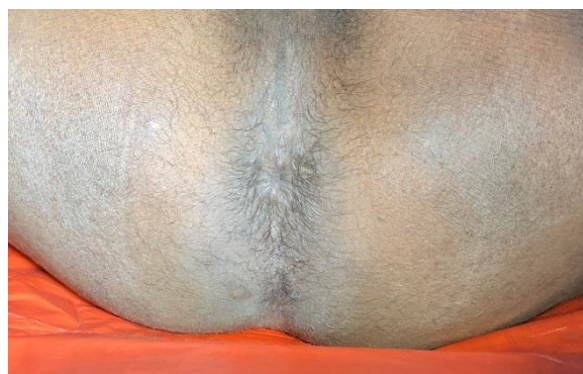


1	Tenderness	3	2	1	0
2	Bleeding per rectum	2	1	0	0
3	Fissure wound healing	3	1	0	0

### Before Treatment



### After Treatment



## DISCUSSION

In Ayurveda, palash is used for treatment of Vrana, Vatakaphahara and Gudaja vikara Rasapanchaka of palash is Rasa-katu, tikta, Kashaya, Guna- Laghu, snigdha , virya-ushna, vipaka- katu, doshaghnata- vatakaphahara<sup>11,12</sup>. As palash (*Butea Monosperma*) was highly responsible for wound healing, anti-inflammatory, analgesic, antifungal and anti-ulcer activity.

Fissure in ano is the tear or considered as Sadhya vrana in guda pradesh. In contemporary sciences surgical treatment available for fissure in ano are sphincterotomy, lord's dilatation, fissurectomy, but these surgical procedures having adverse effects such as bleeding, infection, incontinence. Palashadigudavarti is effective in subsiding the symptom of Fissure in ano such as pain & bleeding per rectum, itching. On day 14 the fissure wound was completely healed and no other

complain was observed. Gandharva Haritaki choorna acts as laxative and helps to relieve constipation and sitz bath was given to quickly relieve Burning Pain. . To sort out these problem we need treatment which is cost effective and non-surgical.

## CONCLUSION

The treatment given was found effective relief from pain, bleeding, itching at perianal region using which has anti-inflammatory activity, antibacterial, antifungal scavenging activity, antiulcer, anti-proliferative activity, antioxidant activity. Changing in lifestyle and healthy diet also plays important role in the management of Fissure in ano

The outcome of this management is that (ayurvedic anal suppository) palashadigudavarti is effective in the management of Fissure in ano. Patients who take all follow-up after treatment will have less chance of symptom reoccurrence.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for this study.

## Funding Support

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## REFERENCES

1. Shastri A.D. Sushruta Samhita. Vol I. 2013.Chikitsasthan.34/16.Chaukhambha Sanskrit sansthan: Varanasi. p.155
2. Yadavaji T.A. SushrutSamhita.Vol II. 2002. Chikitsasthan. Chaukhambaorientalia: Varanasi. pp.207-8
3. Yadavaji T. SushrutSamhita. Vol II. 2002. ChikitsaSthan. ChaukhambaOrientalia Publishers: Varanasi; p288.
4. Sharma P.H. KashyapaSamhita. 2013. KhilvaSthana. 10/102-5.ChaukhambhaOrientalia Publishers: Varanasi; p.299
5. Shashidharan, M., Beaty, J. 2016. Anal Fissure. Clinics in Colon and Rectal Surgery, 29(01):030–037.
6. Zaghiyan, K., Fleshner, P. 2011. Anal Fissure. Clinics in Colon and Rectal Surgery, 24(01):022–030.
7. Villalba, H. 2007. Anal Fissure: A Common Cause of Anal Pain. The Permanente Journal, 11(4):62–67.
8. Siddiqi, S., Vijay, V., Ward, M., Mahendran, R., Warren, S. 2008. Pruritus Ani. The Annals of the Royal College of Surgeons of England, 90(6):457–463.
9. Jonas, M., Scholefield, J. H. 2001. Surgical Treatment: Evidence-Based and Problem-Oriented. Zuckschwerdt.
10. Das S.A concise Text book of Surgery. 9thed. Kolkata: Somen Das Publisher; 2016. p 1083-1085.
11. Shashtri J.L.N. Dravyaguna vijnana Vol. II 2003 Chaukhambha Orientalia Publishers: Varanasi.p.145-148
12. Paranjape Prakash, Indian medicinal plants, forgotten healers, Chaukhamba Sanskrit Prakashan, Delhi, reprint 2012, pp192-193



## HEADACHE TYPES, CAUSES AND TREATMENT

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### ABSTRACT

*Headache is pain that arises in the head, scalp, or neck, and the main cause of headaches of all kinds is unknown, and most people can improve if a quiet lifestyle is changed either by getting used to and adapting to relaxation or by taking medications for it. Headaches may be caused organically as a result of a light blow to the head or due to fever, tumors, sudden rise in pressure and eye disorders such as conjunctivitis and others, or caused by inflammation of the sinuses, and otitis media may cause headaches, and perhaps problems Teeth, constipation, and premenstrual symptoms are causes of headaches, and each case is treatable according to the diagnosis and supervision of the specialist doctor.*

*Keyword: (Headache types, main causes, medications).*

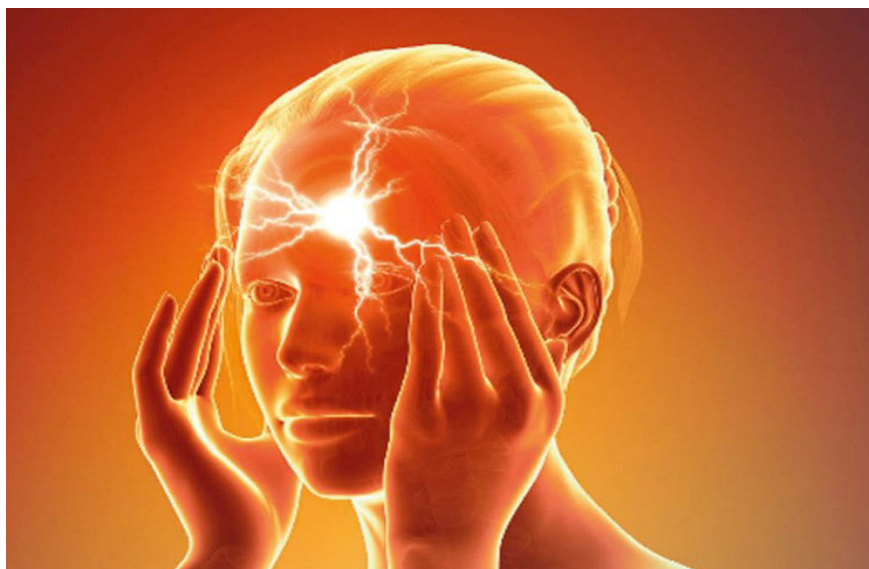
### INTRODUCTION

Headache is one of the common and very important symptoms that all people face frequently in their lives, and it can be described as a feeling of pain that leads to discomfort in the head or scalp area, and may extend to the neck area. The headache pain is often severe, in which the individual may lose his focus or ability to perform most of the usual daily tasks, which forces the individual to use all methods to treat it. About (95 %) of the general population have experienced headache at several stages in their life with a (1 year) dispersals of nearly one in two adults (Stovner L, et al. 2007). Headaches are classified into several types, including tension headaches, which are caused by stiffness that occurs in the muscles in the shoulders, neck, scalp or jaw. And among the causes of headaches are stress, depression and anxiety, and headaches may be caused by a lot of work or due to lack of sleep, and sometimes it is due to eating or drinking alcohol or excessive use of narcotic pills. Eating tyramine in chocolate, cheese and nuts, as well as monosodium glutamate, which is placed in canned meat, are all harmful substances that may generate headaches. And that people who take caffeine with tea, coffee and cola feel headaches, especially when they take daily doses they are accustomed to taking. Exposing the sensitive areas of the head to external and internal influences such as the brain, eyes, and ears, as well as the bones of the skull, facial muscles, sinuses, arteries, and others lead to headaches. Patients often adopt for using several approaches at various times and being able to employ this flexibility resorts to progress treatment outcomes (Stovner L, et al. 2007, MacGregor EA, et al, 2010, Ferrari MD, et al. 2002). All of these organs, if exposed to a certain effect, will be a cause of headache, and there are some organs far from the head that can provoke a headache attack if they are exposed to some stimuli. On this basis, headaches are classified into forms and types. Headaches may be intermittent, generate and then go away, or persistent, always present. A person may experience headaches frequently, monthly, weekly, or daily. The headache may remain for several hours, varying in intensity

between mild pain or moderate pain to feeling severe and harmful pain. The pain may be in the forehead area or near the eyes, and at other times it may be in the back of the head, and it may be generated in a fissure of the face or in both. The headache may be accompanied by nausea and vomiting, as well as confusion or lack of vision and bad mood. Some patients shown perfection with vestibular rehabilitation physical therapy, affecting they can afford it (Fife TD. 2009). The treatments for headaches varied according to its type, severity, and the amount of pain generated by it. In most cases, the reason is to take paracetamol, and sometimes aspirin is the appropriate treatment to get rid of headache pain, while it has spread in the present time to take panadol in severe cases by the patient who He suffers from headaches, and there are other cases of headaches for which he uses other treatments according to the diagnosis of the specialist doctor. In the event that the headache has been born for some reason, the body can be eliminated as soon as the cause is treated.

### Headache Types

Headaches are classified into two main types, primary headaches and secondary headaches, and each type is classified into different subtypes according to the different causative factors. Headache related directly with central nervous system happens at any part of head some time completely or migraines and other times in the neck as shown in **figure no-1**.



**Figure-No. 1:** to show the net of headache by white color in head (complete and migraine), and neck.

Patients taking simple analgesic medicines are few likely for responding to sharp\ preventive treatments, and hence stopped of the over used medicine is the first-priority in all cases (Hagen K, et al. 2009).

Below is a breakdown of the diversity of these different types of headaches:

#### 1- Primary Headache:

This type of headache occurs as a result of the excessive activity of the muscles in the head area, as well as the neck, blood vessels, or another specific area in the brain, and sometimes due to a change or disturbance of the chemical substances and elements in the brain. Its types include:

- **Tension Headache:** Tension headache is pain that occurs in the head as a result of muscle tension in the neck or scalp area, and this type of headache is one of the most common types, and it often occurs as a result of wrong head positions or due to tension.
- **Migraines Headache:** Migraine headaches cause severe pain to the patient and this pain is throbbing, and usually focused on one side of the head, and migraine headaches occur in the form of attacks of varying duration and intensity. The effect on an individual's fineness of life is hard to quantify with (75 %) of patients reported functional disabilities through a migraine attacked, and (50 %) required the helping of all family\ friends with a major impact on their social-life (Ferrari MD, et al. 2002). Migraines are also divided into several types according to their causes, and they can affect children and adults. Studies and research are still underway to accurately search for the causes of left and right migraines, but it is not known exactly now, but there are a number of factors that directly contribute to stimulating a migraine attack in a patient, including fluctuating hormone levels in the body, which is a common cause. Women have more than men.
- **Cluster Headache:** Cluster headaches cause attacks of pain for the patient, and these attacks are repeated many times a day for a period of a week or more, and then followed by a long period of time that may last for years in which the patient does not suffer from pain in the head throughout this period. An intense cluster offense reply's quite for injectable or nasal-sumatriptan, or nasal-zolmitriptan unless contraindicated (Solomon GD, et al. 1989). As for the causes of cluster headaches, the main cause of cluster headaches is still unknown, but it is believed that the hypothalamus gland located at the base of the brain may be responsible for its occurrence, due to its sudden release of histamine and serotonin in the brain.

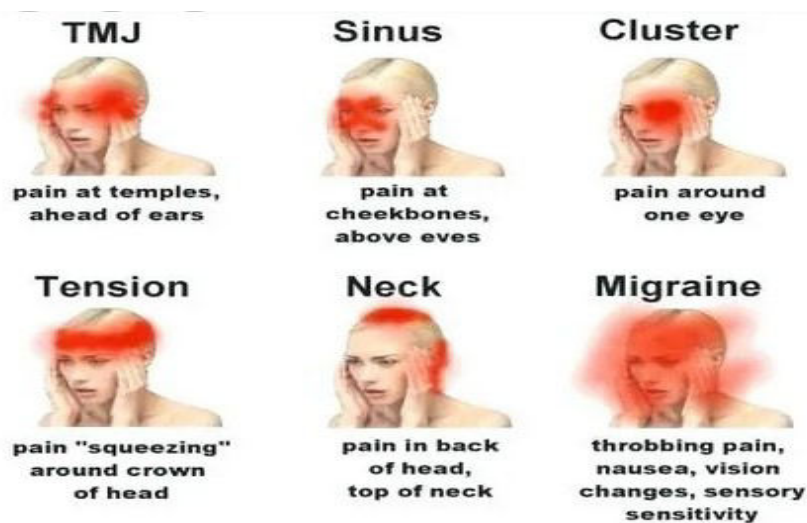
## 2- Secondary Headache:

Secondary headaches are associated with a number of medical conditions that cause headaches, as a result of exposure to it, causing the following headaches, and they include several types, including:

- **Sinus Headache:** It is a headache that causes pain whose main source is inflammation or swelling of the tissues lining the sinuses.
- **Spinal Headache:** This type of headache occurs as a result of slowly leaking cerebrospinal fluid, and most often it occurs during a spinal tap, or during spinal or epidural anesthesia.
- **Headache of Tumor and Cerebral Hemorrhage:** There is not enough space inside the skull to accommodate tumors or bleeding, so the growth of tumors, both benign and malignant, causes, and as is the case in the case of blood pooling when a fake inside the skull creates pressure on the brain tissue, which causes a feeling Headache.
- **Cervical Headache:** This type of headache is caused by damage to the discs between the vertebrae of the spine, which causes abnormal pressure on the spine, and this degeneration of the discs usually causes pain in the neck and head area.
- **Sudden Thunder Headache:** It is a severe pain that occurs suddenly without warning and continues for a short period of up to five minutes, and this type of headache can indicate the presence of a specific problem that has been exposed to the cerebral blood vessels, and this requires the need for medical care, the necessary emergency and monitoring the case after presenting it to the specialist doctor in order to control and treat it by giving the appropriate treatment to the patient who has this type of headache.
- **Meningitis Headache:** Meningitis occurs as a result of infection of the membranes surrounding the spinal cord and brain and lining the skull. These membranes are known as meningitis.

- **Headache or Neck Trauma:** This type of headache is caused by head and neck injuries. And these injuries are caused by accidents such as falling from a high place, or car accidents, which leads to headaches.
- **Rebound Headache:** It is a headache that can occur due to the long-term daily use of pain relievers such as acetaminophen, aspirin, as well as ibuprofen, leading to headaches as a reaction to reducing or stopping the use of these drugs.

Each type of headache leads to pain in a specific area of the head or neck, according to the severity and the extent of the impact, so **figure no. 2**; illustrates this, and the reason is due to the type of factor that led to the headache.



**Figure no. 2:** to show headache types and location.

### Causes of Headaches

The causes of headaches and the risk factors resulting from them vary according to the type of pain experienced by the affected individual, and a person may suffer from more than one type of headache at the same time, and what should be noted is that the brain does not contain nerve fibers, This means that the pain is not in the brain itself, but rather occurs as a result of a certain defect in the body, for example, a migraine occurs due to irritation or inflammation of the structure surrounding the brain itself, thus affecting the most important functions performed by the brain, causing headaches. It might increase pains starting by regulated the secretions of endogenous-opioids that act as analgesic effects. It has been limited that endogenous-opioids, such as enkephalins, endorphins, and dynorphins, which binds to opiate receptors in brain, and neuron terminals for causing the analgesic effectives, and regulated human emotions (Guyton AC, et al. 2001, Takeshige C, et al. 1992). The causes of headaches vary between simple causes that can pass and end, and severe causes that are dangerous that require immediate medical intervention in order to treat and get rid of them. Possible headache causes include the following:

- **Systemic Diseases:** These include a group of medical conditions, changes in blood circulation, an imbalance in the blood flow process, or exposure to shock that leads to headaches.
- **Chemical Changes:** Occur as a result of excessive and irregular use of medicines without consulting doctors, or drug abuse and abrupt cessation of its use and quitting.

- **Viral Infection:** Viral infection is a major cause of headache as it leads to cumulative symptoms that affect all body systems, the most important of which is the immune system, for example, HIV infection (AIDS).
- **Encephalitis:** or what is known as stroke, arterio-venous malformation, subarachnoid hemorrhage, and carotid arteritis, in which headaches occur as a result of idiopathic intracranial pressure.
- **High Blood Pressure:** High blood pressure is one of the most important causes of headaches, as it is linked to the circulatory system on the one hand and the central nervous system on the other.
- **Hypothyroidism:** It is known that any defect in the secretory functions related to hormones and enzymes, the glands that secrete them, leads to headaches as a result.
- **Dehydration:** Dehydration is one of the causes of headaches due to the absorption disturbance in cells and the imbalance of their osmosis, as well as the imbalance that may occur in the acid function.
- **Ear, Nose and Throat Problems:** They are considered one of the direct causes of headaches as they are part of the head. Any problem that you get will generate headaches from it.
- **Dental pain:** being directly related to the skull bone, head nerves, and muscles and tissues of the face and jaws.
- **Eye problems:** the other ones that are directly related to the head, from which one feels any defect that occurs in both eyes or one of them, such as iritis and glaucoma.

#### - Headache Symptoms.

Headache symptoms vary according to the different type of head pain, and the differences between the symptoms of different types of headaches help to identify the type of headache and determine the most important causes, and the following is to identify the headache symptoms that occur in the most common types of headaches:

- **Symptoms of Tension Headache:** It includes mild or moderate pain on both sides of the head, and the neck also includes pain, in addition to the feeling that there is heaviness around the head.
- **Symptoms of Cluster Headache:** These symptoms include pain in the cluster head and pain around or behind one eye, redness and excessive watering of the eyes, stuffy and runny nose, as well as narrowing of the pupil in one of the eyes, eyelid drooping, and facial sweating.
- **Migraine Symptoms:** These symptoms include pain accompanying a migraine attack with sensitivity to light and sound, changes in vision, nausea accompanied by vomiting in some cases, as well as dizziness, and a feeling of high pressure in the eye area or the area above the eyebrow.
- **Rebound Headache Symptoms:** These symptoms are congestion in the nasal passages, with insomnia, pain in the neck area, and a feeling of fatigue.

#### Symptoms of Secondary Headache:

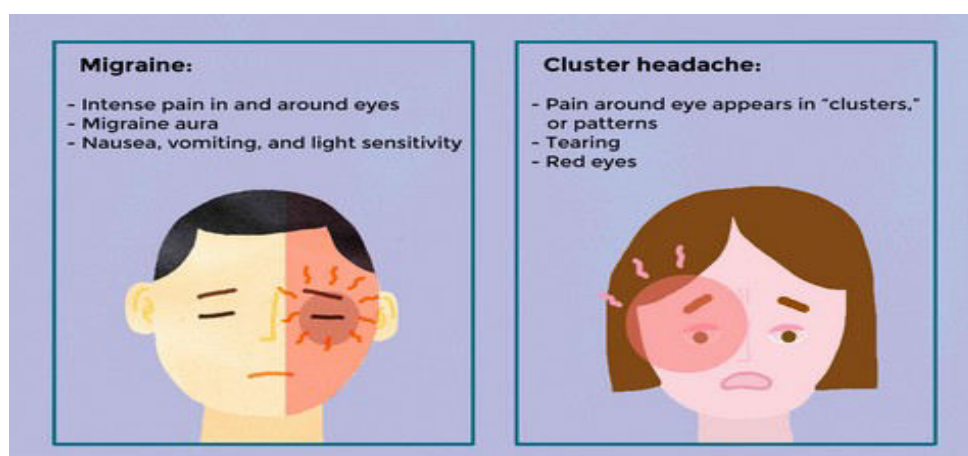
Secondary headaches are accompanied by many uncountable symptoms, and vary according to the number of cases that caused them. It is good to understand if this is a modern headache to patients, or if they have had a same headache in the previous. The follow step is to eliminate every potential red-flag symptoms (Oxford: Oxford University Press, 2008.). These accompanying symptoms that may indicate the presence



of a medical emergency, such as subarachnoid hemorrhage or stroke, are as follows:

- **Headache:** It occurs suddenly, which may worsen when doing some activities such as bending over, exerting physical effort, or the occurrence of some pathological conditions such as coughing.
- **Some changes** in vision, or disturbance in perception, behavior, or even speech.
- **A headache different** from what was used to it in the past.
- **Pain that appears** after a fall or head trauma.
- **Head pain** is associated with convulsions or muscle spasms, and seizures such as epileptic fits.
- **Persistent** nausea or vomiting.
- **Feeling weak and numb** on one side of the body, which is a symptom of a stroke.
- **Pain generated by a change** in a person's quality of life and ability to perform daily tasks.
- **Pain arising** due to not responding to treatment, or that worsens after taking some kind of inappropriate treatment.
- **Pain that is generated** by taking more than the recommended dose of pain reliever treatments that can be obtained without consulting and going to specialist doctors.

There are a simple example some symptoms of headache, in **figure no. 3**; showing the symptoms of headache that affects vision as compare between migraine and cluster.



**Figure no. 3:** to show the symptoms of headache that affects vision.

### Headache Treatment

The methods of treating headaches differed from one patient to another according to the type of headache that the patient suffers from. It is very important for recognition relevant symptom blends that propose common primary headaches, or medicament overuse headache (Headache Classification Committee of the International Headache Society, 2004, Headache Classification Committee of the International Headache Society, 2013). It is possible to control most headaches, both primary and secondary, including headaches that occur in the back of the head or pain in the area above the head with rest, and the use of appropriate non-prescription pain-control treatments, such as acetaminophen or paracetamol and ibuprofen. The medical condition that caused the

secondary headache must be treated in order to get rid of the headache completely, such as headache caused by sinusitis. In cases of severe primary headaches, the appropriate treatment for a headache depends on knowing some details such as the patient's medical history, pain patterns, in addition to a complete physical examination and also laboratory in order to determine the type of headache that most people suffer from, and then choose the appropriate treatment path and the necessary medications for treatment. Headache treatments have been prevention and sharp, pharmacological, and non-pharmacological too (Lipton R, et al. 2007).

### Headache Treatment at Home

There was slightly differences ( $P < 0.05$ ) between treatment-group, and control-group (Zhang CY, et al. 2008). It is possible to control mild and moderate headaches by practicing some necessary steps at home, examples of which are the following:

- Doing light and moderate exercise at regular times, which helps in the production of some basic chemicals in the brain that have a relaxing effect and a feeling of happiness.
- Use hot compresses or take warm baths for (5 to 10) minutes, especially in cases of headaches caused by muscle spasms, but it is necessary to avoid using warm compresses in cases of headaches that are considered severe, and that require consulting a specialist doctor in order not to be it has a negative effect instead of a positive.

### CONCLUSION

Headache is one of the serious diseases that most people suffer from, and the study around it is renewed and continuous, as it is caused by a specific disease in the body or a group of diseases that the individual is exposed to through the exercise of his activities in daily life. Therefore, light was shed on the types of headaches and the most important causes that result from them and ways to treat them.

### REFERENCES

1. Ferrari MD, et al. Triptans (serotonin, 5-HT<sub>1B/1D</sub> agonists) in migraine: detailed results and methods of a meta-analysis of 53 trials. *Cephalalgia* 2002;22:633–58.
2. Fife TD. Migraine associated vertigo: A common but difficult-to-define disorder. *Pract Neurol* 2009;Sept:27–33.
3. Guyton AC, Hall JE (2001) Textbook of medical physiology. WB Saunders, Philadelphia MacGregor A, Rigmor Jensen R, eds. Migraine and other primary headaches.
4. Hagen K, Albrechtsen C, Vilming ST, et al. Management of medication overuse headache. 1-year randomised multicentre open-label trial. *Cephalalgia* 2009; 29: 221–232.
5. Headache Classification Committee of the International Headache Society. The international classification of headache disorders, 3rd edn (beta version). *Cephalalgia* 2013;33 (9):629–808.
6. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders, 2nd edn. *Cephalalgia* 2004;24(Suppl 1):9–160.
7. Lipton R, Bigal M, Diamond M, Freitag F, Reed M, Stewart W (2007) Migraine prevalence, disease burden, and the need for preventive therapy. *Neurology* 68(5):343
8. MacGregor EA, et al, for the British Association for the Study of Headache. Guidelines for all healthcare professionals in the diagnosis and management of migraine, tension-type headache, cluster headache and medication-overuse headache. 3rd edn, 2010.

- [http://www.bash.org.uk/wp-content/uploads/2012/07/10102-BASH-Guidelines-update-2\\_v5-1-indd.pdf](http://www.bash.org.uk/wp-content/uploads/2012/07/10102-BASH-Guidelines-update-2_v5-1-indd.pdf).
9. Oxford: Oxford University Press, 2008. MacGregor A, Rigmor Jensen R, eds. Migraine and other primary headaches. Oxford: Oxford University Press, 2008.
  10. Solomon GD, Price KL. Burden of migraine: a review of its socioeconomic impact. *Pharmacoeconomics* 1989; 11: 1–10.
  11. Stovner L, Hagen K, Jensen R, et al. The global burden of headache: a documentation of headache prevalence and disability worldwide. *Cephalalgia* 2007; 27: 193–210.
  12. Takeshige C, Nakamura A, Asamoto S, Arai T (1992) Positive feed-back action of pituitary beta endorphin on acupuncture analgesia afferent pathway. *Brain Res Bull* 27(1):37–44
  13. Zhang CY, Mao CJ, Wen ZM (2008) Observation on the effects of Eprisome and Tian shu capsule for the patients of tension headache. *Chin J Pract Nerv Dis* 11(4):42–43.

## IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF SPINDLE CELL CARCINOMA OF THE ORAL CAVITY- A REVIEW

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### ABSTRACT

*Spindle Cell Carcinoma is also known as sarcomatoid carcinoma, is a rare and peculiar biphasic malignant neoplasm that occurs mainly in the upper aerodigestive tract. It is most frequently encountered in the larynx, and also occurs in the nasal cavity, hypopharynx, oral cavity, esophagus, trachea, and skin. It is a potentially aggressive variant of squamous cell carcinoma which has spindled or pleomorphic tumor cells which simulate a true sarcoma but are epithelial in origin. It consists of sarcomatoid proliferation of pleomorphic spindle cells and squamous cell carcinoma. It frequently recurs and metastasizes, reinforcing the pattern of its correct diagnosis. In this review, we present a brief outline of the general characteristics of the neoplasm and emphasize the use of immunohistochemistry in its diagnosis.*

**Keywords:** *Spindle cell carcinoma, histogenesis, immunohistochemistry, keratin, vimentin.*

### INTRODUCTION

Spindle cell carcinoma is a rare biphasic malignant neoplasm that mainly occurs in the upper aerodigestive tract.[1] The tumor has alternative names including sarcomatoid carcinoma, carcinosarcoma, pseudosarcoma, pleomorphic carcinoma, and polypoid carcinoma due to its microscopic peculiarities.[2,3] WHO classification has categorized this tumor under a highly malignant variant of squamous cell carcinoma.[1,2] Its incidence amongst the tumors of the regions is less than 1%.[2,4] Four factors are considered predisposing for this condition: alcohol abuse, tobacco abuse, poor oral health, and previous irradiation to the area of the tumor.[5] Site predilection for spindle cell carcinoma is the lower lip, tongue, maxillary sinus, alveolar ridge, or Gingiva although most tumors occur in the larynx.[6] The mean age of diagnosis is the sixth decade of life and shows male predilection.[7] The tumor has spindle or pleomorphic tumor cells which simulate a true sarcoma but of epithelial origin.[4] Since this tumor frequently recurs and metastasizes, diagnosis is critical.[8] Immunohistochemistry along with routine histopathology is essential in establishing the diagnosis.[5]

### CLINICAL FEATURES

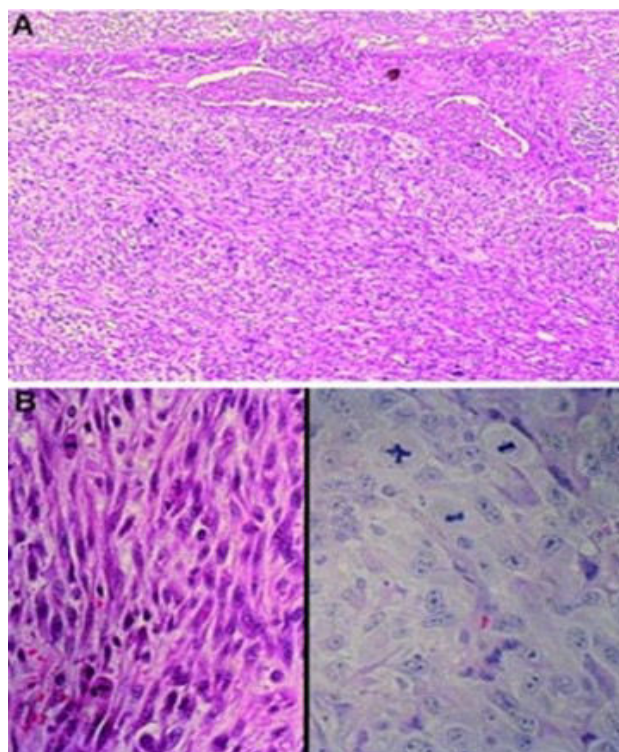
Clinical presentation of oral Spindle cell carcinoma (Spcc) varied from an exophytic polypoid mass with an ulcerated surface to a completely infiltrative ulcer, that mainly occurs in the alveolar ridge.[9] However, it may also involve predominantly lips, tongue, and mandibular alveolar ridge with reports of very few lesions in the maxilla. [10] Clinically polypoid growth with ulcerated surface involving unusual location like the Maxillary alveolar ridge is exhibited in this neoplasm. It most commonly affects the glottis in (70%) of cases, and the majority of patients present with symptoms of hoarseness, dyspnea, cough, and dysphagia for less than 1-year duration.[11] The tumor being polypoid or pedunculated (98.9%) tumors that are often less than 2 cm in size.

[12]

### **HISTOGENESIS**

Numerous hypotheses regarding the histogenesis of SPCC have been proposed. Three such dominant pathogenesis are: the tumor 1) represents a "collision tumor" (carcinosarcoma), 2) is a squamous cell carcinoma with an atypical reactive stroma (pseudosarcoma), or 3) is of epithelial origin, with "dedifferentiation" or transformation to a spindle cell morphology (sarcomatoid carcinoma). [13] The first hypothesis in this context indicates tumor differentiation, meaning it does not imply a tumor the tumor arises from two separate clones of cells. It is generally accepted that the majority of the tumors originate from a single clone. If a clone should develop along divergent lines into epithelial and mesenchymal elements, the term carcinosarcoma would seem to be appropriate. It is believed that the SPCC, encompasses four different categories of tumors that are potentially separable only by the use of immunohistochemistry or electron microscopy. These include squamous cell carcinoma with a reactive or desmoplastic stroma, SPCC (carcinosarcoma and collision tumor). It has been suggested, however, that Spindle cell squamous carcinoma and carcinosarcoma may represent a spectrum of a single entity and that the two share a similar prognosis.[14,15] The finding of individual tumor cells that co-express both epithelial and mesenchymal Immuno markers does add credence to this hypothesis.[16] However, to suggest that they have a similar prognosis is undoubtedly premature and based on a very limited sample. The third hypothesis has been supported by the following evidence: (1)occurrence in the exact sites that normally have squamous epithelium and a preponderance of carcinomas rather than sarcomas; (2) the direct continuity and the smooth transition of the spindle cells with areas of the squamous epithelium; (3) immunoreactivity with epithelial antigens; (4) a dual expression of epithelial and mesenchymal differentiation with double-labeling techniques in some neoplastic spindle cells; (5)and the presence of only epithelial, only sarcomatous, or duality of expression in metastatic deposits from laryngeal sarcomatoid carcinoma [17]

### **HISTOPATHOLOGICAL FEATURES:**



**A** laryngeal spindle cell squamous carcinoma showing a differentiated squamous cell carcinoma (**top**) associated with a malignant (undifferentiated) spindle cell proliferation. **B**, the malignant cell component of spindle cell squamous carcinoma may include (**left**) spindle-shaped cells and (**right**) epithelioid cells. Increased mitoses, including atypical forms, are seen. \***Bruce M Wenig M.D; Squamous Cell Carcinoma of the Upper Aerodigestive Tract: Precursors and Problematic Variants, Mod Pathol 2002;15(3):229–254.**

Spindle cell carcinoma can demonstrate varied histopathological appearance from case to case or within different areas of the same tumor tissue. The cells may appear like epithelial cells or may appear as atypical mesenchymal cells to add to the difficulty in diagnosis.[18] Histopathologically the microscopic features of this neoplasm included the presence of two distinct epithelial-derived components: a carcinomatous or SCC component and a sarcomatoid or dysplastic spindle cell component.[19] The former component may be very scant or limited to noninvasive areas of epithelial dysplasia or carcinoma in situ located at the surface of the tumor, and its identification may require extensive sampling for histological examination. Often, the overlying epithelium may be ulcerated, and because of this, the squamous component may not be seen. The pleomorphic spindle cells usually form the bulk of the lesion; they are arranged fascicles or whorls, storiform, myxoid, microcystic, or giant cell areas may also be present.[20] moreover, foci of osteoblastic or chondroblasts differentiation(both benign and malignant) sometimes are observed. There may be sharp borders between SCC areas and the spindle cell component, but a gradual transition, SCC- cell“drooping off” from the epithelial nests or overlying squamous epithelium into the pleomorphic spindle cell areas may also be frequently observed.[21,22]

The bulk of the tumor was mainly composed of bizarre, basophilic, hyperchromatic, pleomorphic spindle cells accompanying small areas of squamous cell carcinoma.

Invasive spindle shape cells with frequent prominent mitotic figures arranged irregularly with fascicle patterns in some areas resembling fibrosarcoma histopathologically. Spindle-shaped cells revealed pleomorphism, hyperchromatism, and abnormal mitotic figures. Atypical mitoses were abundant. Together with the spindle-shaped tumor cells, the proliferation of polygonal epithelial cells was seen at the periphery. The amount of collagen in the sarcomatoid zones varied from almost none to abundant.[23]

#### **USE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF SPCC**

Histological and immunohistochemistry demonstration of both squamous cell components and the spindle cells with sarcomatous appearance is essential in establishing the diagnosis.[7] Immunohistochemical studies are useful to know the histogenesis of the spindle cells within these tumors and the nature of SPCC.[24] Immunohistochemical characterization of tumor cells using antibodies to keratin, vimentin, and S-100 protein is very helpful in differentiating Spindle cell carcinoma from true spindle cell sarcoma, melanoma, and malignant myoepithelioma.[15] This was performed using a Polymer HRP IHC detection system. Immunohistochemistry revealed that the SCC component was strongly positive for cytokeratin. The spindle cells were focally positive for cytokeratin and epithelial membrane antigen (EMA) Moreover, the spindle-cell component was strongly positive for vimentin. No immune activity was observed for desmin. Immunoreactivity for Ki-67 was detected in up to 60-70% in the carcinomatous region.[25] The spindle cell in SPCC is a variant growth pattern of Squamous cell carcinoma(SCC), neither a non-neoplastic mesenchymal reaction nor a malignant admixture of epithelial and mesenchymal neoplasms.[25] The epithelial and spindle components share a common pathway of tumorigenesis despite their conspicuous divergence at the phenotypic level.[26] Histological studies alone cannot explain the spindle cell components. However, recent immunohistochemical studies have attempted to address the histogenesis of the spindle cells within these tumors and the concept that spindle cell elements are epithelial in origin is now proven by positive keratin immunostaining and demonstrating of desmosomes and tonofilaments in the cells strongly supported.[27,28] Immunohistochemically the most sensitive and reliable epithelial markers to be used for demonstration of the epithelial phenotype is keratin (AE1/AE3) and epithelial membrane antigen.[29]

Keratin expression forms the bulk of the tumor definitive decreases whereas vimentin expression increases in spindle cell of spindle cell carcinoma.[35] There was severely reduced expression of E-cad & the heterogeneous expression of alpha-cat or beta-cat is responsible for the morphologic shift from conventional. squamous cell carcinoma to sarcomatoid a component in spindle cell carcinoma. According to Shibuya et al the expression of cadherin- catenin complex was regarded as the hallmark of epithelial cells. It is believed that dysfunctional cadherin catenin complex causes cells to shift in morphology from squamoid to a more spindled type and permits a more infiltrative & diffuse pattern of growth.[35] The concept that the sarcomatous portion arises from the transformation of squamous cells was proposed as early as 1900 by Krompecher[36] and was later supported by other light microscopists. Battifora[37] has reported that the sarcomatous portion of this aspect of these tumors represents actual mesenchymal metaplasia. The malignant epithelial cell malignancies undergoing alteration resulting in loss of keratin and acquiring vimentin as the cytoskeletal protein. [37] Recently, p63 has been reported as a useful marker for spindle cell carcinoma.[38]

## HISTOPATHOLOGICAL DIFFERENTIAL DIAGNOSIS

Differential diagnosis includes various benign and malignant tumors, such as fibromatosis, nodular fasciitis, reactive epithelial proliferation, squamous cell carcinoma, malignant fibrous histiocytoma, leiomyosarcoma, fibrosarcoma, rhabdomyosarcoma, malignant peripheral nerve sheath tumor, mesenchymal chondrosarcoma, and malignant melanoma.[39] nodular fasciitis may exhibit mitotic figures, but they are not atypical and show no cellular pleomorphism, therefore discerning this lesion from SPCC and does not pose any difficulty. Distinguishing between monophasic SPCC and spindle cell sarcomas such as fibrosarcoma and leiomyosarcoma may become more difficult. However sarcomas in the head and neck are extremely rare and when they occur, an intervening fibrous layer usually separates the lesion from the overlying epithelium. Also, differentiating SPCC from the inflammatory myofibroblastic tumor is the absence of dysplastic or carcinomatous epithelial components and no atypical mitotic figures.[22] sometimes, UADT mucosal melanomas may present as polypoid masses and in such cases, immunohistochemistry with the appropriate antibodies( S100, HMB45) serves to confirm or rule out this diagnosis if melanin is not found in the primary tumor. In the IHC, it is important to remember that SPCC should not be ruled out of the differential diagnosis by a positive reaction for vimentin in sarcomatoid tumor cells. The absence of staining for keratin in the sarcomatoid tumor cells does not always exclude SPCC because some cases of SPCC show immunoreactivity of keratin in their sarcomatoid components only with some anti-keratin antibodies. Different kinds of anti-keratin antibodies should be applied in the differential diagnosis of SCC.[40] The differential diagnosis based on epithelial markers are stated in (figure1)[41]

## TREATMENT AND PROGNOSIS

The treatment in the case of SPCC has been debated. Many are in the opinion that wide excision which includes radical dissection along with metastasis is adequate. However, Some believe that surgery alone is not sufficient and that it should be followed by Radiotherapy that is regarded as an acceptable alternative for inoperable patients.[42] Although it is difficult to predict biological behavior in every case, patients whose tumors are deeply invasive tend to have a poor prognosis, whereas those with early-stage tumors usually have an excellent prognosis.[43] Distant metastases and depth of tumor invasion into underlying structures were found to be reliable prognostic features, together with their polypoid configuration. Thus, metastasis usually contains SCC or both SCC and spindle cell component, and rarely only just the spindle cell component.

## CONCLUSION

Spindle cell carcinoma of the head and neck is a rare and unique variant of squamous cell notably carcinoma. It is of epithelial origin. The gripping aspect of this tumor is that it mimics other connective tissue sarcomas and spindle cell malignancies under light microscopy. Immunohistochemistry is helpful to know the histogenesis and nature of SPCC and is thereby useful in diagnosing the condition along with histopathological features of the neoplasm. It is an aggressive tumor and tends to recur and metastasize easily. The most widely approved treatment includes surgery followed by radiotherapy although the treatment regimen is quite controversial. The prognosis depends upon the depth of the tumor invasion.

## REFERENCES

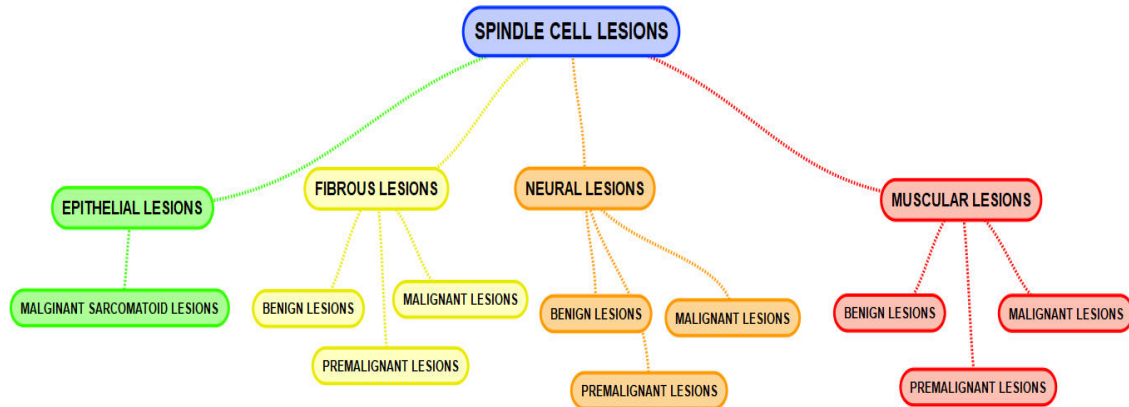


1. Boamah H, Ballard B (2012) A case report of spindle cell (sarcomatoid) carcinoma of the larynx. *Case Rep Med* 2012: 370204.
2. Ravindran R, Mohan V, Saji AM (2013) Spindle Cell Carcinoma of Maxilla: Case report of a rare entity and review of literature. *Oral & Maxillofacial Pathology Journal*: 4.
3. Lewis JS Jr (2008) Spindle cell lesions--neoplastic or non-neoplastic: spindle cell carcinoma and other atypical spindle cell lesions of the head and neck. *Head Neck Pathol* 2:103-110.
4. Sarma A, Das R, Sharma JD, Kataki AC (2012) A Clinicopathological and Immunohistochemical Study of 40 Cases. *Journal of Cancer Therapy* 3: 1055-1059.
5. Parikh N, Desai N (2011) Spindle Cell Carcinoma of the oral cavity: A case report of a rare entity and review of literature. *J Academy Adv Dental Research* 2: 2.
6. C. Rizzardi, C. Frezzini, M. Maglione, G. Tirelli and M. Melato, "A Look at the Biology of Spindle Cell Squamous Carcinoma of the Oral Cavity: Report of a Case," *Journal of Oral and Maxillofacial Surgery*, Vol. 61, No. 2, 2003, pp. 264-268.
7. Thompson LD, Wieneke JA, Miettinen M, Heffner DK (2002) Spindle cell (sarcomatoid) carcinomas of the larynx: a clinicopathologic study of 187 cases. *Am J Surg Pathol* 26: 153-170.
8. Leventon GS, Evans HL. Sarcomatoid Squamous Cell Carcinoma of the Mucous Membranes of the Head and Neck: A Clinicopathologic Study of 20 Cases. *Cancer* 1981; 48:994-1003,
9. Mario Jose Romanach, Rebeca Souza Azevedo, Roman Carlos, Oslei Paes de Almeida, Fabio Ramoa Pires. Clinicopathological and immunohistochemical features of oral spindle cell carcinoma *J Oral Pathol Med* 2010; 39: 335-41.
10. Ellis GL and Corio RL. Spindle cell carcinoma of the oral cavity: a clinicopathologic assessment of fifty-nine cases. *Oral Surg Oral Med Oral Pathol*. 1980; 50: 523-33.
11. H. U. Völker, M. Scheich, S. Höller et al., "Differential diagnosis of laryngeal spindle cell carcinoma and inflammatory myofibroblastic tumor—report of two cases with similar morphology," *Diagnostic Pathology*, vol. 2, no. 1, article 1, 2007.
12. L. D. R. Thompson, J. A. Wieneke, M. Miettinen, and D. K. Heffner, "Spindle cell (sarcomatoid) carcinomas of the larynx: a clinicopathologic study of 187 cases," *American Journal of Surgical Pathology*, vol. 26, no. 2, pp. 153-170, 2002.
13. S. F. Huang, I. H. Chen, C. T. Liao, T. M. Chen and K. F. Lee, "Sarcomatoid Carcinoma of the Parotid Gland with Apparent Metastasis of Epidermoid Elements to Cervical Lymph Nodes," *Acta Oto-Laryngologica*, Vol. 126, No. 6, 2006, pp. 667-671.
14. Humphrey PA, scroggs MW, Roggli VL, Shelburne JD. Pulmonary carcinomas with a sarcomatoid element: an immunocytochemical and ultrastructural analysis. *Human pathol* 19:155-165, 1988.

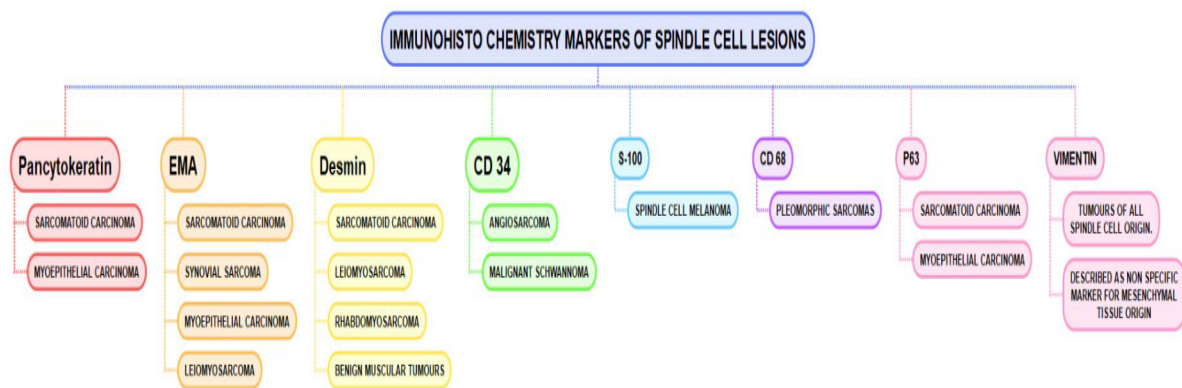
15. Wick MR, Swanson PE. Carcinosarcomas: current perspectives and a historical review of nosological concepts. *Semin DIAGN pathol* 10:118-127, 1993
16. Zarbo RJ, Crissman JD, Venkat H, Weiss MA. Spindle cell carcinoma of the upper aerodigestive tract mucosa: an immunohistologic and ultrastructure study of 18 biphasic tumours and comparison with seven monophasic spindle cell tumours. *Am J Surg Pathol* 10:741-753, 1986.
17. L. D. Thompson, J. A. Wieneke, M. Miettinen and D. K. Heffner, "Spindle Cell (Sarcomatoid) Carcinomas of the Larynx: A Clinicopathologic Study of 187 Cases," *American Journal of Surgical Pathology*, Vol. 26, No. 2, 002, pp. 153-170.
18. Anupam Sarma<sup>1</sup>, Rajjyoti Das<sup>2</sup>, J. D. Sharma<sup>1</sup>, A. C. Kataki : Spindle Cell Carcinoma of the Head and Neck: A Clinicopathological and Immunohistochemical Study of 40 Cases, *Journal of Cancer Therapy*, DECEMBER 2012. Pg 1055-1059.
19. Cardesa A and Zidar N. World Health Organization Classification of Tumors. Head and Neck Tumours: Oral Cavity and Oropharynx. IARC Press, Lyon. 2005; 127-28.
20. Ansari MA Hoque M, Califano J, Westra WH. Immunohistochemical p53 expression patterns in sarcomatoid carcinoma of the upper respiratory tract. *Am J Surg Pathol*. 2002; 26:1024-31.
21. Takata T, Ito H, Ogawa I, Miyauchi M, Ijuhin N, Nikai H. Spindle cell squamous carcinoma of the oral region. An immunohistochemical and ultrastructural study on the histogenesis and differential diagnosis with a clinicopathological analysis of six cases. *Virchows Arch A Pathol Anat* 1991; 419: 177-82.
22. Shibuya Y, Umeda M, Yokoo S, et al. Spindle cell squamous carcinoma of the maxilla: report of a case with immunohistochemical analysis. *J Oral Maxillofac Surg* 2000; 58: 1164-69.
23. Neelampari Parikh, Nimisha Desai; Spindle Cell Carcinoma of the oral cavity: A case report of a rare entity and review of literature *Journal of Academy of Advanced Dental Research*, Vol 2; Issue 2: May 2011,pg 32.
24. Ezulia T, Saim L, Sha PP, Kenali MS (2015) Spindle Cell Carcinoma of the Oral Cavity: A Case Report. *Clin Med Rev Case Rep* 2:015
25. Neelampari Parikh, Nimisha Desai; Spindle Cell Carcinoma of the oral cavity: A case report of a rare entity and review of literature *Journal of Academy of Advanced Dental Research*, Vol 2; Issue 2: May 2011,pg 33-34.
26. Ansari MA Hoque M, Califano J, Westra WH. Immunohistochemical p53 expression patterns in sarcomatoid carcinoma of the upper respiratory tract. *Am J Surg Pathol*. 2002; 26:1024-31.
27. Takata T, Ito H, Ogawa I, Miyauchi M, Ijuhin N, Nikai H. Spindle cell squamous carcinoma of the oral region. An immunohistochemical and ultrastructural study on the histogenesis and differential diagnosis with a clinicopathological analysis of six cases. *Virchows Arch A Pathol Anat* 1991; 419: 177-82.
28. Shibuya Y, Umeda M, Yokoo S, et al. Spindle cell squamous carcinoma of the maxilla: report of a case with immunohistochemical analysis. *J Oral Maxillofac Surg* 2000; 58: 1164- 69.

29. Thompson LDR. Squamous cell carcinoma: variants of the head and neck. *Curr Diagn Pathol*. 2003; 9: 384-96.
30. Ellis GL, Langloss JM, Heffner DK, et al. Spindle cell carcinoma of the aerodigestive tract: an immunohistochemistry analysis of 21 cases. *Am J Surg Pathol* 1987; 11: 335-39.
31. Gnepp DR. Diagnostic surgical pathology of the head and neck. Saunders; Philadelphia 2009; 78-79.
32. Munakata R, Cheng J, Nakajima T, Saku T: Spindle cell carcinoma of the gingiva: report of an autopsy case. *J Oral Pathol Med* 1998; 27: 180–84.
33. Sisilia F Fifita, Kayo Kuyama, Akira Suzuki, Yoshio Tamaki, Takashi Matsumoto and Hirotugu Yamamoto A Case of Spindle Cell Carcinoma of the Oral Cavity: With Special Reference to Cytopathological Features and Review of Literature *Oral Med Pathol*. 2006; 11: 127- 32.
34. Zarbo RJ, Crissman JD, Venkat H, Weiss MA. Spindle-cell carcinoma of the upper aerodigestive tract mucosa: an immunohistologic and ultrastructural study of 18 biphasic tumors and comparison with seven monophasic spindle-cell tumors. *Am J Surg Pathol* 1986; 10:741–53.
35. Rathy Ravindran Vishnu Mohan Ajish M. Saji Oral & Maxillofacial Pathology Journal [ OMPJ ] Vol. 4 No. 2 July - Dec. 2013,pg382.
36. Shibuya Y, Umeda M, Yokoo S, Komori T, Lubsen H. Spindle cell carcinoma of the oral Spindle cell squamous carcinoma of the maxilla:Report of a case with immunohistochemical Analysis. *J oral Maxillofac surg* 2000; 58:1164-69.
37. Munakata R, Cheng JMR, Nakajima T, Saku T. Spindle Cell carcinoma of the gingiva: report of an autopsy case. *J oral pathol med variants of upper aero digestive tracts*. *Am J*1998; 27:180-4.
38. Lewis JS, Ritter JH, El-Mofty-S. Alternative epithelial markers in sarcomatoid carcinomas of head and neck, lung, and bladder- p63, MOC-31 and TTF-1. *Mod Pathol* 2005; 18:1471-81.
39. L. D. Thompson, J. A. Wieneke, M. Miettinen and D. K. Heffner, “Spindle Cell (Sarcomatoid) Carcinomas of the Larynx: A Clinicopathologic Study of 187 Cases,” *Amer-ican Journal of Surgical Pathology*, Vol. 26, No. 2, 002, pp. 153-170.
40. T. Takata, H. Ito and I. Ogawa, “Spindle Cell Squamous Carcinoma of the Oral Region.An Immunohistochemical and Ultrastructural Study on Histogenesis and Differential Diagnosis with Clinicopathological Analysis of Six Cases,” *Virchows Archiv A, Pathological Anatomy and Histology*, Vol. 419, 1991, pp. 177-182.
41. Rachna rath, bijay k das, SN das, Manas baisakh; Spindle cell carcinoma of maxilla: Histomorphological and immunohistochemical analysis of a case, sept 2014.
42. Thompson LDR, Squamous cell Carcinoma. variants of the head and neck. *CurrentDiagnostic Pathology* 2003; 9:384-96.
43. Su HH, Chu ST, Hou YY, Cjang KP, Chen CJ. Spindle cell carcinoma of the oral

cavity and oropharynx: factors affecting outcome. J Chin Med Assoc. 2006; 69: 478–83



**FIGURE 1:** Major classification of Spindle cell lesions



**FIGURE 2:** Specific IHC markers of spindle cell lesions in common use.

## MAINTAINING CONTROL OVER BLOOD SUGAR THROUGH YOGA – A SELF-STUDY

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### ABSTRACT

*Diabetes is a common disease in India and is making a huge impact on the health of an individual. People suffering from diabetes often are scared and afraid that they are not going to survive. On the positive side diabetes makes you disciplined and makes your routine quite systematic. The intake of foods is systematic and time to time, secondly regular exercise is also followed resulting into people getting into healthy mode. The major diabetes in India is either generic or stress. Life has become so stressful that people of age 35 – 45 are having blood sugar problem on a regular basis. Secondly life is so hectic that people are unable to take time out from their regular to do exercise resulting health hazards.*

*In this research paper, I will be addressing certain Yoga postures which will help people suffering from diabetes to have a systematic and health life. In addition these yoga postures will keep him fit and also energetic. As you know diabetes is an ailment where there is lot of restrictions with respect to food intake which reduces the overall energy of the individual. These yoga postures will allow them to regain this energy.*

*Keywords: Diabetes, Yoga, disease, stress and generic.*

### INTRODUCTION

In my family my grandfather and father had diabetes so it has been a regular practice on my part to get my blood test on a regular basis. The reason is many people say diabetes is generic. I live in lot of stress thinking that in future I may have the same problem. I have seen my father living a much disciplined life after blood sugar was detected. He used to take medicine regularly and also do his walking or exercise very systematically. Expecting the same from me at this moment is very difficult as life is very hectic and taking time out to doing exercise is not easy.

### Asanas

#### 1. Dhanurasana (Bow Pose)<sup>1</sup>



This asana can be done by lying down in your stomach. Keep your hands on the side and then lift both the legs upward. Now hold the ankle with your hands and lift yourself upwards. Keep a smile on your face and keep yourself in the same position for 15 seconds. In the meantime inhale and exhale, come in normal lying position and relax.

**2. Balasana<sup>1</sup> or Child Pose is a reclining back-bending asana in hatha yoga and modern yoga as exercise.**



Sit on your knees and try to touch your heel to the hips. Now stretch your hands and bend down as much as you can. Remain in this position for 15 seconds. Keep inhaling and exhaling during the entire asana.

**3. Bhujangasana (Upward facing Dog Pose)<sup>1</sup>**



Lie down on the floor on your stomach. Keep your hands with palm down on the side of your neck and then lift yourself upwards. The pressure should be given to the legs in order to lift. Face should be upwards and keep inhaling and exhaling slowly. Remain in this position for 15 seconds. You can repeat it 10 – 20 times depending upon your energy.

**4. Shavasana (Corpse Pose)<sup>1</sup>**



Lie down straight on your back and keep your eyes closed. This asana must be done for at least 30 minutes. You must remain calm slowly inhaling and exhaling during the asana. It is a type of meditation in a lying position. You must relax stress slowly during this asana.

#### 5. Viparita Karani (legs up the wall)<sup>1</sup>



Lie down beside the wall and keep a soft pillow under your neck. Raise your legs alongside the wall keeping at 90 degree. Remaining in this position for 5 to 10 minutes. Your neck, chin and throat must be relaxed during this asana.

#### 6. Tadasana (Mountain Pose)<sup>1</sup>



Stand straight on the flat ground and keep your arms on the sides of your body. Keep your palm in the upward direction. Slowly breathe in and extend your arms up and down to the sides of your body. Hold this position for a while. Repeat this position ten times. Exhale slowly and bring down your arms back in the starting position.

#### 7. Mandukasana (Frog Pose)<sup>1</sup>



Fold your knees backward and sit down on the ground with the assist of your knees. Make a fist and put your hand on your stomach. Keep your fist in such a way that the joint of your fists comes at the navel. Place your fist firmly and press your stomach. Bend forward in this position and try to touch the ground with your forehead. Hold this position for 15-20 seconds then, exhale and relax

#### 8. Chakrasana (Wheel Pose)<sup>1</sup>



Lie down on your back. Bend your knees and bring your legs close to your hips. Bring your palm under your shoulder such that your fingers point towards your shoulders. Keep your elbows in shoulder-width apart. Press your palm firmly on the ground and inhale while lifting your shoulders, elbows, and hips. Straighten your arms and legs so that your hips and shoulder feel the upward push. Hold this pose for few seconds then, bend your elbows and shoulders first to bring your head down. After that, bend your knees to lower your hips and spine to the ground.

- Practice yoga under qualified professional
- Vigorous exercise and fast-paced yoga are done in hot temperature conditions. These are not recommending for patients with diabetes and heart diseases.
- Beginners to yoga should avoid hard yoga practices.
- Diabetic patients should regularly monitor their body reactions after every physical activity.
- Any sign or symptom or pain should not be overlooked if you are diabetic.
- Don't do yoga beyond your capabilities.
- Generally, yoga requires an empty stomach, but diabetic patients should take light snacks to avoid hypoglycemia.



- Any sign of dizziness, headache, etc. should be reported to the practitioner.

### **Benefits of Yoga in Diabetes**

1. It tones your digestive organs and heart.
2. Stretches chests and lungs, open the heart and lungs.
3. It relaxes your body completely and keeps you rejuvenated.
4. It calms the brain, increase awareness and attentiveness.
5. It helps to lower blood pressure, calms the brain and helps relieve stress and mild depression, lastly relaxes the body.
6. Strengthens the abdominal muscle, massages the intestine and internal organs of digestive system, and improves digestion.

### **CONCLUSION**

The research on diabetes disease and its implication concludes that YOGA plays a vital role in maintaining your health. It also gives an impetus to your body to keep moving. Any disease basically sends a negative signal and to counter it YOGA plays a major role. The study gave an opportunity to understand the reasons for diabetes and subsequently how YOGA can provide benefits to a common man. Everybody should take out time from their regimen to focus on YOGA as it will give an added energy to your vital organs and keep you healthy and fit. Secondly the postures recommended by the researcher is very simple and can be done by any age group person. The various YOGA poses will help you in leading a happy and stress free life. Keep doing it and stay healthy and keep your blood sugar under control.

### **LEAVE A HEALTHY LIFE.**

### **REFERENCE**

1. Picture taken from <https://www.breathewellbeing.in/blog/best-yoga-asanas-for-diabetes-to-control-at-home-naturally/>
2. <https://www.artofliving.org/in-en/health-and-wellness/yoga-diabetes>
3. <https://www.webmd.com/diabetes/yoga-diabetes-poses>
4. <https://www.healthifyme.com/blog/7-effective-yoga-asanas-that-will-help-with-diabetes/>
5. <https://www.arhantayoga.org/blog/can-yoga-help-cure-diabetes/>

## NATURAL ANTIOXIDANTS IN COSMETICS: A REVIEW

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### ABSTRACT

1. Numerous cosmetics that are retailed currently frequently contain antioxidants as the active constituents.
2. It's known that oxidation responses could produce free revolutionaries, which can start chain responses that will damage skin cells.
3. Adding the quantum of free revolutionaries could initiate the wrinkling, photoaging, elastosis, drying, and saturation of the skin.
4. Topical antioxidants could terminate the chain responses by removing the free revolutionary interceders and inhibit other oxidation responses by being oxidized themselves; this could defend the skin against free revolutionaries.
5. The general importance and relations between the various cell reinforcements is an authentically complicated question, with the vivid cancer prevention agent composites and anti-cancer agent catalyst frameworks having synergistic and reliant merchandise on each other.
6. The activity of one cancer prevention agent rely on the appropriate capacity of different individuals from the cell reinforcement framework.
7. The quantum of insurance gave by any one cancer prevention agent will likewise rely upon its consideration, its reactivity towards the specific responsive oxygen species being thought of, and the cell reinforcements with which it connects.
8. Treatment with active phytomolecules has lately gained important interest in pharmaceutical healthcare system.
9. The operation of nanotechnology has enhanced the cosmetics field in recent times.
10. Numerous kinds of nanoparticles, similar as polymeric nanoparticles, nanosuspensions, nanoemulsions, liposomes, niosomes, dendrimers, over ornamental phrasings.
11. The use of nanoformulation helped to overcome poor bioavailability; reduced hematological poisonous goods; and drop other side goods, similar as alopecia, nausea, puking, diarrhea, fatigue, and skin rash.
12. The use of finagled nanomaterials has garnered important attention in cosmetics manufacturers to gather the eventuality of nanocosmetics in their phrasings.

**Keywords:** Anti-oxidant, Cell reinforcements, free radicals, nanoparticles.

### 1. INTRODUCTION

Antioxidants<sup>(1)</sup> are composites that inhibit oxidation, a chemical response for revolutionaries and chain responses that damage cells of organisms. Antioxidants similar as thiols or ascorbic acid may act to inhibit these responses. To balance oxidative stress, shops and creatures maintain complex systems of lapping antioxidants, similar as

glutathione.

The only salutary antioxidants are vitamin A, C, as well as E. The term antioxidant is also used for artificial chemicals added during manufacturing to help oxidation in synthetic rubber, plastics, and energies.

The general importance and relations between these various cell reinforcements is an authentically complicated question, with the vivid cancer prevention agent composites and anti-cancer agent catalyst frameworks having synergistic and reliant merchandise on each other. The activity of one cancer prevention agent rely on the appropriate capacity of different individuals from the cell reinforcement framework. The quantum of insurance gave by any one cancer prevention agent will likewise rely upon its consideration, its reactivity towards the specific responsive oxygen species being thought of, and the cell reinforcements with which it connects.

### **1.1. Polyphenols**

Polyphenols<sup>(1)</sup> are micronutrients that naturally do in shops. They 're included in multitudinous supplements, though they 're also easy to get in your diet from foods like fruits, vegetables, teas, and spices. Polyphenols can reduce and control blood sugar situations. They also stimulate your body's release of insulin, a hormone that signals your body to use sugars efficiently. These goods can lower your insulin resistance — a condition where your body doesn't respond properly to the hormone. Maintaining low insulin resistance and healthy blood sugar situations reduces your trouble of conditions like obesity and diabetes.

### **1.2. Flavonoids**

Flavonoids<sup>(1)</sup> are phenolic substances protected from a wide scope of vascular shops, with more than 8000 individual composites known. Various examinations have proposed that flavonoids march regular molding, including antiallergenic, antiviral, mitigating, and vasodilating conduct. In any case, most extreme interest has been dedicated to the cell reinforcement effort of flavonoids, which is because of their ability to diminish free progressive adaptation and to search free progressives.

### **1.3. Terpenes<sup>(1)</sup>**

Responsive Oxygen Species are engaged with the obsessive improvement of various significant human circumstances comparative as neurodegenerative circumstances, cardiovascular cycles, diabetes and various others. The most encouraging methodology to help from the oxidative harm brought about by these receptive species is the utilization of cancer prevention agent bits. These composites can go about as immediate cancer prevention agents through free revolutionary searching instruments as well as roundabout cell reinforcements by improving the cell reinforcement status (enzymatic and non-enzymatic).

### **1.4. Vitamins<sup>(1)</sup>**

The knowledge of free revolutionaries and ROS in biology has given a new understanding of the pathogenesis of conditions, and it promises new perceptivity into health as well as complaint operation. The presence of unmatched electron results in specific common parcels that are participated by utmost revolutionaries. Numerous chemical species are unstable and largely reactive; they can either contribute an electron to another patch or accept an electron, thus carrying as oxidants or reducing agents.

## **2. Natural Antioxidants in cosmetics<sup>(2)</sup>**

"Antioxidant" is a any emulsion that can offset unstable moles called free revolutionaries that damage DNA, cell membranes, and other corridor of cells. Because free revolutionaries warrant a full complement of electrons, they get electrons from other moles and damage those moles in the process. Antioxidants neutralize<sup>2</sup> free revolutionaries by giving up some of their own electrons.

While free revolutionaries<sup>2</sup> are damaging by their veritably nature, they're an necessary part of life. The body generates free revolutionaries in response to environmental cuts, similar as tobacco bank, ultraviolet shafts, and air pollution, but they're also a natural derivate of normal processes in cells. When the vulnerable system musters to fight interferers, for illustration, the oxygen it uses spins off an army of free revolutionaries that destroy contagions, bacteria, and damaged body cells in an oxidative burst.

Numerous cosmetics that are retailed currently frequently contain antioxidants as the active constituents. It's known that oxidation responses could produce free revolutionaries, which can start chain responses that will damage skin cells. Adding the quantum of free revolutionaries could initiate the wrinkling, photoaging, elastosis, drying, and saturation of the skin. Topical antioxidants could terminate the chain responses by removing the free revolutionary interceders and inhibit other oxidation responses by being oxidized themselves; this could defend the skin against free revolutionaries.

### Examples of Natural Antioxidants

#### 1. Tocopherols (E-306)

Tocopherols<sup>(2)</sup> were one of the first liposoluble antioxidants insulated from shops. They have high attention and habitual presence in vegetable canvases, similar as soybean or sunflower canvas,

#### 2. Ascorbic Acid (E-300)

Ascorbic Acid<sup>(2)</sup> is white crystalline greasepaint used to stabilise potables, fruits and vegetables. This cumulative acts as an antioxidant through the extermination of oxygen, the reduction of free revolutionaries and the rejuvenescence of primary antioxidants. It's considered to be safe as a food cumulative and has no use limits.

#### 3. Rosemary Extract (E-392)

This antioxidant is an excerpt attained from the Rosemary<sup>(2)</sup> factory using detergents suitable for mortal consumption. Its most important composites from the antioxidant point of view are Rosmarinic Acid, Carnosol and Carnosic Acid.

**Table2:** Natural ingredients, antioxidants produced as well as their role in cosmetics

Ingredients	Bioactive Molecules	Bioactivity	Relevance
Green Tea	Catechin derivatives	Free radical scavengers.	Separates have a delayed saturating impact, improve microrelief, lessen harshness, sebum creation, forestall and treat skin break out.
Coffee Arabica	Proanthocyanidins	Anti-oxidant	Extricates skin-easing up specialist upgrade

			wrinkle, scarcely discernible difference, and pigmentation in patients with actinic harm.
<b>Vitus vinifera</b>	Stilbenes	Antioxidant properties	Vitis vinifera extricates hinder UV light-intervened skin maturing.
<b>Pomegranete</b>	Ellagic acid	Anti-oxidant, anti-fungal, and anti-inflammatory properties.	Pomegranate extricates decline wrinkles
<b>Glycine max</b>	Isoflavones	Antioxidant properties.	Glycine max separates diminish UV-prompted oxidative DNA harm and skin photodamage.
<b>Aloe vera</b>	Aloesin	Antioxidant, anti-inflammatory, and water-retention properties.	Aloe separates have a skin-easing up impact, further develop skin flexibility, and lessen wrinkles.
<b>Citrus limon</b>	Flavanones	Anti-oxidant	Separates have antiaging and depigmenting impacts, and lessen skin inflammation and hair issues.
<b>Opuntia ficus indica.</b>	Linoleic acid	Cell renewal, supporting skin moisturizing as well as collagen production.	Anti-aging properties

### 3. Effect of Natural Antioxidants on Skin

The skin cells continuously produce, through cellular respiration, metabolic processes or under external onslaughts, largely reactive moles oxidation products, generally called free revolutionaries. These moles are incontinently annulled by enzymatic and non-enzymatic systems in a physiological and dynamic balance. In situations where this balance is broken, colorful cellular structures, driving or worsening skin conditions.

#### 3.1. Photodamage<sup>(3)</sup>

As of now, there's sufficient validation to affirm that all sun based diapason leans toward the age of free progressives; this age possibly leans toward, to a lesser or lower degree, photoaging, photoimmuniosuppression and photocarcinogenesis. Bright (UV) radiation, in its UVB range (290-320 nm), is answerable for the quick harms of sunlight based radiation, acting considerably on keratinocytes; UVA band (320-400 nm), which initiates cell changes, especially compromises melanocytes and fibroblasts.

#### 3.2. Aging<sup>(3)</sup>

Chaperon with sun oriented radiation and other natural elements liable for oxidative wonders, skin maturing, as well as of all organs, is joined by the downfall of the endogenous cell reinforcement components. Clinically, the discoveries of photoaging are the overwhelming, and it's fragile and as often as possible unwarranted by and by to recognize the effect of exogenous variables on the sequential interaction, however it's realized that the fundamental finding of regular maturing is cutaneous decay, by the decrease of epidermis, at the same time, considerably, by the drop in the collagen content and other dermal basics.

### 3.3. Melasma<sup>(3)</sup>

Incited UV melanogenesis that happens in melasma is enhanced by adding the oxidation of dopaquinone; cell reinforcements comparable as L-ascorbic acid, which decrease dopaquinone (DOPA), help the adaptation of free progressives. The persuaded UV rebellious cycle likewise inclines toward the increment of melanogenesis. A clinical preliminary to concentrate on the endogenous the endogenous cancer prevention agent frameworks in cases with melasma exhibiting a critical utilization of superoxide dismutase (SOD) and glutathione peroxidase. This outcome shows the break of the redox balance.

### 3.4. Non-melanoma skin cancer<sup>(3)</sup>

Age of UV persuaded free progressives in the skin creates oxidative pressure when it surpasses the capacity of regular guard the main skin insurance frameworks are cancer prevention agent compounds and melanin, the principal line of safeguard against DNA harm. DNA retains bright light, whose energy can break its sub-atomic bonds; most extreme of these breaks are fixed by catalysts present in the actual nexus, still the excess harms instigate transformations that lead to neoplasia. The two primary lead of safeguards that cancer prevention agents can give are comparable to blocking the compliance of free progressives or negating the progressives previously produced.

## 4. Recent Applications

It's believed that using vitamins and antioxidants in cosmetics on a topical base can help to cover from and conceivably repair the damage caused by free revolutionaries. Likewise, some vitamins may be salutary due to their goods, similar as reduction in saturation and bruising, activation of collagen product, keratinization refinement, and anti-inflammatory goods.

**Table 2:** Vitamins and their benefits in skincare

Vitamin	Benefit
Vitamin A	Improves Acne Prevents skin aging
Vitamin B complex	Offers anti-inflammatory benefits Hydrates skin
Vitamin C	Reduces cell damage Prevents wear and tear
Vitamin D	Treats dry skin Prevents skin aging
Vitamin E	Moisturizes skin Reduces sun damage
Vitamin K	Evens skin tone

	Reduces bruises and swelling
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Clinical enhancement in the visible signs of skin aging has been linked to reductions in both skin wrinkling and skin excrescence conformation. Tocopherol and its acetyl ester outgrowth, tocopherol acetate, have been studied considerably. While tocopherol is the most active form of vitamin E, topically applied vitamin E esters epidermis.

Polyphenols have been considerably studied and are reported to have antioxidant and anti-inflammatory parcels. Polyphenolic composites are plant in colorful shops, including tea leaves, grape seeds, blueberries, almond seeds, and pomegranate excerpt.

Flavonoids deduced from green-tea leaves/ seeds and wine grape leaves, as well as oligomers of these composites plant in Mediterranean pine dinghy (Pycnogenol), are considered effective for guarding the skin from free revolutionaries.

Treatment with active phytomolecules has lately gained important interest pharmaceutical healthcare system. The operation of nanotechnology has enhanced the cosmetics field in recent times. Numerous kinds of nano-particles<sup>(5)</sup>, similar as polymeric nano-particles, nano-suspensions<sup>(5)</sup>, nano-emulsions<sup>(5)</sup>, liposomes<sup>(5)</sup>, niosomes<sup>(5)</sup>, dendrimers<sup>(5)</sup>, over ornamental phrasings. The use of nanoformulation helped to overcome poor bioavailability; reduced hematological poisonous goods; and drop other side goods, similar as alopecia, nausea, puking, diarrhea, fatigue, and skin rash. The use of finagled nanomaterials has garnered important attention in cosmetics manufacturers to gather the eventuality of nanocosmetics in their phrasings. Also, important disquisition is necessary to determine their efficacy in delivering active constituents into the skin. The new regulation formed by European Union has passed emendations in its cosmetics directory for safer nanocosmetics to enter into the request, securing the beauty and health of consumers.

## 5. Challenges

Various normal items with fancy possibility are unsuitable to get to the skin, are shaky to the territory, debase are insufficiently bioavailable and responsible, and have a quick fire digestion and unbridled-discharge; in this manner, they can not be utilized in decorative phrasings since they're ill suited to complete their regular effort.

All things considered, various tests, albeit utilized regularly, warrant a vigorous affirmation technique. It would be important to approve sensible styles to permit right capsule and information recognizability, remaining for severe regulation on the utilization of dynamic constituents of regular beginning in beauty care products. The coherent strategies for measuring cell reinforcements include two starting way birth from the natural lattice and evaluation.

We expect to peel light on the nutricosmetic demand remaining for a particular guideline for green beauty care products to assist purchasers with settling on informed decisions.

## 6. Innovations:

The lipid-based nano-exemplification<sup>(5)</sup> frameworks are extensively utilized since they're steady, control discharge, and support discharge profiles

### 6.1. Liposomes

The liposomes are cell-like spherical bilayer vesicles with unilamellar or multilamellar structures which will protect and encapsulate lipophilic and hydrophilic compounds. they're generally made with phosphatidylcholine and have a hydrophobic tail and hydrophilic head.

### 6.2. Niosomes

Niosomes are cell-like spherical bilayer nano-vesicles with unilamellar or multilamellar structures. they're made from self-assembly of hydrated nonionic surfactants (e.g., spans, brijs, tweens, sorbitan ester, alkyl amides, crown ester, steroid-linked surfactants, and polyoxyethylene alkyl ether), with or without cholesterol or lipids.

### 6.3. Nanoemulsions

The nanoemulsions are a dispersion of liquids during which a surfactant combines the oil phase and water phase stably. There are three sorts of nanoemulsions (water in oil, oil in water, and bicontinuous nanoemulsion) with variable sizes from 50 nm to 200 nm. they typically have low viscosity, high interfacial area, high solubilization capacity, and high kinetic stability.

### 6.4. Nanoparticles

The nanoparticles differ in chemical compositions and morphologies. Nevertheless, they're utilized in sunscreen preparations (e.g., TiO<sub>2</sub>-nanoparticles, ZnO-nanoparticles, CeO<sub>2</sub>-nanoparticle, and ZrO<sub>2</sub>-nanoparticles) and physical UV filters.

### 6.5. Silicone Matrices and Vesicles

Silicones, in association with various active ingredients (e.g., aluminum, zirconium, and tetrachlorohydrate), can act as delivery vesicles for cosmetic actives. The silicon vesicles reduce stickiness and defend the actives from hydrolysis.

### 6.6. Multi-Walled Delivery Systems

The multi-walled delivery system (MDS) mixes structured vesicle-forming ingredients and high-shear processing to offer long-term stability to cosmetic formulations. Amphiphilic molecules (e.g., derivatives of polyglycerols, monounsaturated fatty acid, and aminoalkanoic acid residues) make MDS. As a result, MDS gives stability to liposomes and sustains and defends the skin, optimizing cosmetic product performance.

## 7. CONCLUSION

Antioxidants are composites that inhibit oxidation, a chemical response for free revolutionaries and chain responses damaging the cells of organisms. Antioxidants similar as thiols may act to inhibit these responses. To balance oxidative stress, shops and creatures maintain complex systems of lapping antioxidants, similar as glutathione.

The only salutary antioxidants are vitamins A, C, and E. The term antioxidant is also used for artificial chemicals added during manufacturing to help oxidation in synthetic rubber, plastics, and energies.

Salutary supplements<sup>(7)</sup> retailed as antioxidants haven't been shown to ameliorate health or help complaint in humans. According to some studies, supplements of beta-carotene, vitamin A, and vitamin E have negative effect on mortality rate or cancer threat. Also, supplementation with selenium or vitamin E doesn't reduce the threat of cardiovascular complaint.

Treatment with active phytomolecules has lately gained important interest in pharmaceutical healthcare system. The operation of nanotechnology has enhanced the



cosmetics field in recent times.

## REFERENCES

1. Idha Kusumawati, Gunawan Indrayanto, Chapter 15 - Natural Antioxidants in Cosmetics, Studies in Natural Products Chemistry, Elsevier, Volume 40, 2013, Pages 485-505, ISSN 1572-5995, ISBN 9780444596031, <https://doi.org/10.1016/B978-0-444-59603-1.00015-1>.
2. Elham H. Fini, Shakiba Ayat, Farideh Pahlavan, Phenolic Compounds in the Built Environment, Phenolic Compounds - Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications, 10.5772/intechopen.98757, (2022).
3. Mengyang Liu, Shuo Chen, Zhiwen Zhang, Hongyu Li, Guiju Sun, Naibo Yin, Jingyuan Wen, Anti-ageing peptides and proteins for topical applications: a review, Pharmaceutical Development and Technology, 10.1080/10837450.2021.2023569, 27, 1, (108-125), (2022).
4. Marijan Marijan, Anamarija Mitar, Lejsa Jakupović, Jasna Prlić Kardum, Marijana Zovko Končić, Optimization of Bioactive Phenolics Extraction and Cosmeceutical Activity of Eco-Friendly Polypropylene-Glycol-Lactic-Acid-Based Extracts of Olive Leaf, Molecules, 10.3390/molecules27020529, 27, 2, (529), (2022).
5. Sanda Vladimir-Knežević, Marijana Perković, Kristina Zagajski Kučan, Mateja Mervić, Marko Rogošić, Green extraction of flavonoids and phenolic acids from elderberry (*Sambucus nigra* L.) and rosemary (*Rosmarinus officinalis* L.) using deep eutectic solvents, Chemical Papers, 10.1007/s11696-021-01862-x, 76, 1, (341-349), (2021).
6. Hyde, K.D., Bahkali, A.H. & Moslem, M.A. Fungi—an unusual source for cosmetics. Fungal Diversity 43, 1–9 (2010). <https://doi.org/10.1007/s13225-010-0043-3>
7. Alesandra R. Nunes, Ícaro G. P. Vieira, Dinalva B. Queiroz, Antonio Linkoln Alves Borges Leal, Selene Maia Moraes, Débora Feitosa Muniz, João Tavares Calixto-Junior, Henrique Douglas Melo Coutinho, "Use of Flavonoids and Cinnamates, the Main Photoprotectors with Natural Origin", Advances in Pharmacological and Pharmaceutical Sciences, vol. 2018, Article ID 5341487, 9 pages, 2018. <https://doi.org/10.1155/2018/5341487>
8. V. A. Kostyuk, A. I. Potapovich, A. R. Albuhaydar, W. Mayer, C. De Luca, and L. G. Korkina, "Natural substances for prevention of skin photo-ageing: screening systems in the development of sunscreen and rejuvenation cosmetics," Rejuvenation Research, vol. 21, no. 2, pp. 91–101, 2017
9. A. N. Panche, A. D. Diwan, and S. R. Chandra, "Flavonoids: an overview," Journal of Nutritional Science, vol. 5, no. 47, pp. 10–19, 2016.
10. Sun, LC., Li, SY., Wang, FZ., Xin, FG. Research progresses in the synthetic biology of terpenoids. Biotechnol Bull. 2017;33(1):64-75.
11. Zhang, JH., Liu, WJ., Luo, HM. The research of progress of the medicinal plant terpenoids. World Science and Technology/Modernization of Traditional Chinese Medicine and Materia Medica. 2018;20(3):419-430.
12. Mônica Manela-Azulay, Ediléia Bagatin, Cosmeceuticals vitamins, Clinics in Dermatology, Volume 27, Issue 5, 2009, Pages 469-474, ISSN 0738-081X, <https://doi.org/10.1016/j.clindermatol.2009.05.010>.
13. Andrés Felipe Alzate Arbeláez, Eva Dorta Pérez, Camilo López-Alarcón, Farid B.

- Cortés, Benjamín A. Rojano, Immobilization of Andean berry (*Vaccinium meridionale*) polyphenols on nanocellulose isolated from banana residues: a natural food additive with antioxidant properties, *Food Chemistry*, 10.1016/j.foodchem.2019.05.085, (2019).
14. Jeong Hun Kim, Gyeong Han Jeong, Yong-Ha Jeong, Tae Hoon Kim, Free radical scavenging and  $\alpha$ -glucosidase inhibitory activities of the extracts of *Dystaenia takesimana* from Ulleung Island, *Korean J. Food Preserv.*, 10.11002/kjfp.2019.26.2.246, 26, 2, (246-252), (2019).
  15. Shuang Ni, Fangjie Han, Wei Wang, Dongfang Han, Yu Bao, Dongxue Han, Haoyu Wang, Li Niu, Innovations upon antioxidant capacity evaluation for cosmetics: A photoelectrochemical sensor exploitation based on N-doped graphene/TiO<sub>2</sub> nanocomposite *Sensors and Actuators B: Chemical*, Volume 259, 2018, Pages 963-971, ISSN 0925-4005, <https://doi.org/10.1016/j.snb.2017.12.154>.
  16. Mu, L., Sprando, R.L. Application of Nanotechnology in Cosmetics. *Pharm Res* 27, 1746–1749 (2010). <https://doi.org/10.1007/s11095-010-0139-1>

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## ABOUT THE BOOK

Life Sciences have always been a elemental area of science. The exponential augment in the capacity of scientific information and the rate, at which new inventions are made, need detailed, interdisciplinary and latest information and their perceptive. Improved thoughtful of biological phenomenon integrated with interdisciplinary approaches has resulted in key products for betterment of society. To continue the outlook in mind we are contented to publish our book entitled "Recent Research Transitions in Life Sciences Volume I".

This book is the collection of good articles of acknowledged experts in the fields of basic and applied life science. This book is published in the wishes of giving out the new research and findings in the field of life science theme. We developed this book with the purpose of helping people attain that sensation of achievement.

The articles in the book have been contributed by PG students, research scholars and academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Empyreal Publishing House, India for taking effort in bringing this book.

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