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Phylogenetic Studies and Image Analysis of Pollen Grains in Some Herbal Plants from Eastern Ghats

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Abstract* — Present research paper provides the information of some medicinal herbs available at Rushikonda, Visakhapatnam, India. Pollen biology is an important subject where the hidden information in the pollen architecture can be reveled using image analysis softwares. Phylogenetic analysis provides relationship of *Oldenlandia umbellate* with *Merimia tridantata* and *Anisomeles ovata*, *Justicia procumbens* with *Datura inxora*, *Pavonia hastata* with *Datura stramonium* and *Evolvulus alsinoides*, and unrelated species as *Vernonia crinita* and *Tridax procumbens

***Keywords* — Image analysis, Phylogeny, Pollen grains.**

I. INTRODUCTION

Medicinal plants since ancient times have been using virtually in all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations are described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants. This was possibly due to the occurrence of natural products with medicinal properties. The practice of traditional medicine is widespread in various countries such as China, India, Japan, Pakistan, Sri Lanka and Thailand.

Rudolf et al, 2002 proposed that about eighty percent of the world population depends on herbal based alternative systems of medicine [1]. An estimated 70,000 plants are presently in use in diverse forms for treating ailments. Herbal medicine in the present decades is expanding its base at a faster rate due to the great inputs from ethno medicinal practices being pooled from all over the world.
Nature is constructing various biological sources of medicinal agents for thousands of years with various energy sources, many based on their use in traditional and conventional medicine. Various medicinal plants have been used for years in daily life in the treatment of all types of diseases from all over the world. The widespread use of herbal remedies and healthcare preparations, described in ancient cultures and texts like Indian and Chinese cultures, the Bible and the Vedas, has been traced to the occurrence of natural products and energies with medicinal properties. In fact, plants produce a diverse range of bioactive compounds, making them a rich source of different types of medicines. Medicinal compounds have continued to play a dominant role in maintenance of human health since ancient times. Over 50% of all modern clinical drugs are from natural product origin and play an important role in drug designing and development programs in the pharmaceutical industry [2].

There has been a renewal interest in herbal medicines, due to increased knowledge of the partial aptitude of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures from the plant kingdom. Plants are the basic source of information to modern medicine in the life process of all types of living organisms [3]. The basic active, cellular and molecular structures for synthetic fields are presented by rich natural sources. This rapidly increasing worldwide interest in phytomedicine reflects recognition of the validity of many traditional claims for the value of natural products in health care.

The relatively lower rate of side effects to plant preparations compared to modern conventional pharmaceuticals, together with their reduced cost, is encouraging both the consuming public and national health care to consider plant medicines as alternatives to synthetic drugs [4]. Plants with possible antimicrobial activity should be examined against microbial models to confirm the activity and to discover the parameters associated with it. The affect of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Enormous amount of research has been done on phytomedicine and phytoactive compounds in India. Concentration on large number of traditional natural products has increased and has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [5]. The selection of crude plant extracts for screening has the potential of being more thriving in early steps than the screening of pure compounds isolated from natural products.

In recent decades, the preservation of local knowledge, the promotion of indigenous medical systems in primary health care, and the conservation of biodiversity have become even more of a concern to all scientists working at the interface of social and natural sciences but especially to ethnopharmacologists [6]. Further acquaintance with different ethnic collaborators has contributed to the development of research on natural products, to the increase in knowledge about the close relationship between the chemical structure of a certain compound and its biological properties, and to the understanding of the animal/insect-plant interrelation. Hence medicinal plants are important organisms for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development [7]–[14].

Since the arrival of information technology, Bioinformatics has evolved as an interdisciplinary subject to becoming a new generation of interdisciplinary course

with diverged fields such as systems biology, immunoinformatics, genomics, proteomics, molecular modeling and drug designing [15]. Biochemical principles as well as the skill of using application programs for storing, retrieving, analysis, and management of biochemical data with microcomputers is the new focus in finding biological questions [16].

The analysis of the captured image was performed on a PC using image analysis softwares. The microscopy image sent to computer-assisted image analyzers such as GSA Image Analyzer provided changes in staining of pollen. The GSA Image Analyzer v 1.32 is a program for the scientific evaluation of 2D images of Photographic objects [12].

Research on pollen architecture provides the properties of aggregates, such as size and shape, in searching valuable information for pollen characterization and the production optimization. For the last twenty years, image analysis techniques have been used for aggregate particle measurement where the stability of measurement methods is very important. There are a number of image analysis methods for measuring aggregate particle size and shape, which increases speed and accuracy of analysis [16].

II. Materials and Methods

A. Plant Materials

Herbal plants were collected from Rushikonda of Eastern Ghats during winter season.

B. Micrographs of pollen grains

Photographs of the most copious pollen grains in the pollen masses were taken with an optical light microscope (Olympus BH-2). A standard magnification of 1000x has been used for most of the light micrographs. The pixel size of 2048X1536 has been adjusted in Olympus MODEL-FE-115, 5 megepixel cameras, at a distance of 25cm from object.

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D. Staining

Select the appropriate flower buds of different size from the inflorescence. Fix them in Tween 80 fluid, which is used as fixative agent. Take a preserved flower bud and place it on a glass slide. Separate the anthers and discard the other parts of the bud. Put one or two drops of acetocarmine, and squash the anthers. Leave the material in the stain for five minutes. Place a cover slip over the squashed anthers and tap it gently with a needle or pencil. Warm it slightly over the flame of a spirit lamp for 1 minute. Put a piece of blotting paper on the cover slip and apply little and uniform pressure with the thumb. Observe the slide under the light microscope at 100X magnification

E. Image analysis

Image analyzer has provided the outputs of visualization of pollen grain by image analysis softwares, which shows clarity of the image before and after adjusting the image. The softwares provided better visualizations after adjusting brightness, contrast, Hue and saturation.

F. Phylogenetic analysis

Phylogenitic tree construction is done using MEGA (Molecular Evolutionary Genetics Analysis) software (UPGMA). MEGA 5.04 is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses

III. Results and Discussion

The first written records detailing the use of herbs in the treatment of illness are the Mesopotamian clay tablet writings and the Egyptian papyrus during 2000 B.C. Today United States Pharmacopoeia contains 250 herbal drugs (including garlic, still a favorite of herbal doctors) [1], [17]. The Ebers Papyrus, the most important of the preserved Egyptian manuscripts, was written around 1500 B.C. includes much earlier information with 876 prescriptions made up of more than 500 different substances.

Since the writing of the Classic of the Materia Medica almost 2,000 years ago, the traditional Chinese materia medica is steadily increasing in number. This increase is resulted from the integration into the authorized tradition of substances from China's folk medicine as well as from other parts of the world [18]. Many substances and formulations is being used in traditional Chinese medicine originate in places such as Southeast Asia, India, the Middle East, and the Americas [19]. The compilation of Chinese materia medica was published in 1977 contains information on medicinal plants [20].

In most developed countries, pharmaceutical drugs are increasingly using over prescribed, expensive, and even dangerous allopathic medicines [21]. Herbal remedies are seen as less expensive and less toxic [22]. Exposure to exotic foreign foods prepared with non-European culinary herbs has guided many Australians and Americans to examine and often consider using medicinal herbs that were brought to the United States along with ethnic culinary herbs.

People are increasing medical needs by investigating and using herbs and herbal preparations [19], [23]. Many Americans, especially those with chronic illnesses such as arthritis, diabetes, cancer, and AIDS, are turning to herbs as adjuncts to other treatments.

Various medicinal herbs are located in Rushikonda with a wide range of variation. The biodiversity of these plants presents the orthologous and paralogous features. The present results isolated 10 different varieties of plants (see Fig. 1). Image analyses of pollen grains are provided in Fig. 2, showed good clarity of image compared with normal image data. Phylogenetic sequence provides relationship of Oldenlandia with Merrimia and

Anisomeles, Justicia with Datura, Pavonia with Datura and Evolvulus, and unrelated species as vemonia and tridax (see Fig. 3).

Fig.1. Plants selected in the present studies

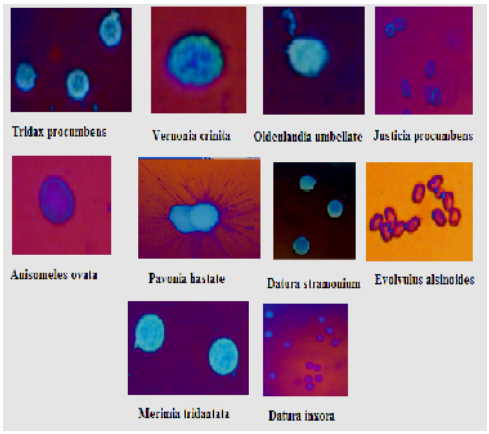

Fig.2. Pollen studies of herbal plants through image analysis

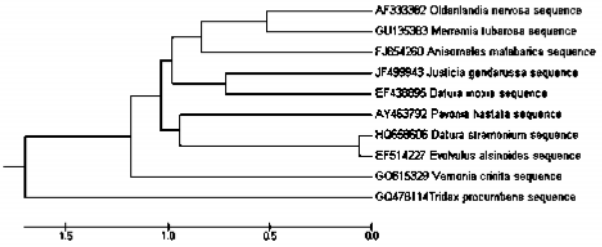

Fig.3. Phylogenetic tree construction using UPGMA method

IV. Conclusion

Biodiversity in pollen studies is utmost important in the present day research. Pollen studies using image analysis can provide the better architecture compared with the normal images. Phylogenetic studies on these herbs are needed to be understood with the biological components for the treatment and cure of various microbial, metabolic and aging diseases. Further work is need for better comparison of pollen architecture for understanding the survival of pollen to climate changes.

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