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Research Article

ANTIMICROBIAL STUDIES, BIOCHEMICAL AND IMAGE ANALYSIS IN MIRABILIS JALAPA

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ABSTRACT

Mirabilis jalapa is one of the medicinal plant, used extensively for the treatment of various diseases all over the world. Research on antimicrobial activity and Biochemical analysis specifies that the plant extracts from diethyl ether have polar compounds and shows antimicrobial properties. There are no phytochemical compounds elucidates in Thin Layer Chromatography with Ethyl acetate and methanol extracts. At 20 µg/mL, Ethyl acetate and methanol extracts did not shown any antimicrobial properties. Diethyl ether extracts of *M. jalapa* is found with antimicrobial, especially antipneumonial compounds. Better image elucidations of compounds are visualized from TLC using image analysis software.

Key words: Antipneumonia, *M.jalapa*, Antimicrobial, Image Analysis.

INTRODUCTION

Nature has been a source 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 to treat all types of diseases, all over the world. The widespread use of herbal remedies and healthcare preparations, described in ancient texts like 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 the maintenance of human health since ancient times[1]. Over 50% of all modern clinical drugs are of natural product origin[2] and play an important role in drug development programs in the pharmaceutical industry[3].

There has been a revival of interest in herbal medicines, due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine in the life process of all types of living organisms. The basic active, cellular and molecular structures for synthetic fields are provided by rich natural sources. This rapidly increasing worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims for the value of natural products in health care[4].

The relatively lower incidence of side affects to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity should be tested against microbial model 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[5,6]. Enormous amount of work has been done on phytomedicine and phytoactive compounds in India[7,8,9]. Interest in a large number of traditional natural products has increased[10] and has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[11, 12]. The selection of crude plant extracts for screening has the potential of being more successful in early steps than the screening of pure compounds isolated from natural products[13].

Mirabilis jalapa Linn. (Family: Nyctaginaceae) is a widely used in conventional medicine in many parts of the world and has antifungal, antimicrobial, antiviral, antispasmodic, antibacterial, diuretic, carminative,

cathartic, hydragogues, purgative, stomachic, tonic and vermifuge properties[14]. *Mirabilis jalapa* is said to have been exported from the Peruvian Andes in 1540. Around 1900, Carl Correns used the four o'clock as a model organism for his studies on cytoplasmic inheritance. This plant is 50-100 cm high and contains alanine, alphaamyrins, arabinose, beta-amyrins, campesterol, daucosterol and dopamine[15], and is used to treat conjunctivitis, edema, fungal infections, inflammation, pains and swellings.

MATERIALS AND METHODS

Study species

Mirabilis jalapa has tubular flowers are fragrant and vary in color among plants. The self-compatible, perfect flowers each have 5–6 stamens and a single-ovulate ovary. An individual flower opens for one night in the early evening, the exact time depending on temperature and relative humidity, and closes early the next morning. An individual plant produces between 25 and 75 flowers in one flowering season.

Plant Materials

M. jalapa (yellow, orange and pink flower cultivars) were collected from plants grown in the garden of the GITAM Institute of sciences, GITAM University, Visakhapatnam during winter season.

Sample extraction

The determined Fresh plant leaves (200g) were ground, extracted with Diethyl ether, ethylacetate and methanol separately and filtered. The plant residue was re-extracted by adding above solvents and filtered again after 48hs. Such procedure was repeated every 72hs, completing three filtration processes. The filtrate was concentrated on a rotary evaporator at 45ºC for solvent elimination, and the extracts were kept in sterile bottles under refrigerated conditions until use.

Test microorganisms

The microbial strains are identified strains and were obtained from the **MTCC**, IMTECH, Chandigarh, India. The bacterial strains studied are *Eschericia coli* **MTCC** 739, *Staphylococcus aureus* **MTCC** 737, *Klebsiella pneumoniae* **MTCC** 109, *Bacillus subtilis* **MTCC** 441 and *Aspergillus niger* **MTCC** 282.

Antimicrobial assay

The antibacterial assays were performed by the agar well diffusion method. Petri dishes (200 mm) were poured with nutrient agar (HI-Media) and allowed to solidify to make base layers. The seed layers were prepared by inoculating 10mL of test organism suspension in 100 mL Mueller-Hinton agar(for bacteria) and Sabouraud Dextrose agar (for fungi) and wells, 6 mm in diameter, were made in the agar medium with the help of a sterile steel borer. About 20 µL (20 µg/mL) of sample was added aseptically in wells. Ampicillin (20 µg/mL) and solvent (diethyl ether, ethyl acetate, and methanol 20 µg/mL) were used as antimicrobial compounds against text microorganisms. 37±1°C for 24 hours (for bacteria) and 25±1°C for 48 hours (for fungi) in the upright position. At the end of the incubation times, the diameters of the inhibition zones were measured in millimeters. The tests were conducted in triplicate.

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. The present study is performed on a sheet of glass, which is coated with a thin layer of adsorbent material, usually silica gel. This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The TLC analysis was performed on glass slides pre-coated with silica gel G/GF (E-Merck grade). Before use, glass slides were pre-washed with methanol, and dried in an oven at 105°C for 1 hour.

Plates are prepared with 5g Silica Gel G and GF in a ratio of 8:2 by using 5ml ethyl acetate as a solvent. A small spot of solution containing the sample is applied to a plate, about one centimeter from the base. The plate is then dipped in to a suitable solvent, such as hexane or ethyl acetate, and placed in a sealed container. The extracts (10 µL) were applied on the plates as bands of 7-mm width with the help of a linomat-5 sample applicator. The solvent moves up the plate by capillary action and meets the sample mixture, which is

dissolved and is carried up the plate by the solvent. Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase, and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. TLC plate is visualized under UV radiation.

TLC runned in ethylacetate and hexane are in the ratio of 1:9, 3:7 and 5:5 for 10%, 30% and 50% mobile phase.

Image analysis

Pixcavator IA - Image Analysis 2.4.1 has been developed in the last 100 years but only recently its methods became useful in practical applications.The areas of applications of Pixcavator are unlimited: medical and ecological, microscopy and satellite. Pixcavator provides new image analysis capabilities includes all standard image processing tools to scientists and researchers.

Camera used: Olympus MODEL- FE-115, 5 megepixel camara (2048X1536).

RESULTS

Mirabilis jalapa is a vulnerable medicinal plant, having wide importance based on our research. The plant is widely available in and around the regions of Visakhapatnam district. There is a huge variety and characteristic features in this plant and can be mutated easily due to the structural arrangement of pollen grain.

Table 1 provided *in vitro* antibacterial activities of the extracts of *M. jalapa* and standard antibiotic (ampicillin). The solvent controls did not show any activity against the microorganisms used in this study. The methanol and ethyl acetate extracts did not showed activity against Gram-positive, Gram-negative bacteria or fungal organisms tested at 20µg/ml. The Diethylether extracts of all varieties showed the zone of inhibition against *E.coli* (9 mm), *S.aureus* (14 mm), *B. subtilis* (20 mm), *K. pneumoniae* (13mm), which is greater than the standard antibiotic used. The Diethylether extract did not showed the zone of inhibition against *A.niger*. Hence the results are showed that all the experimented varieties of *M. jalapa* with the diethyl ether extract shown more zone of inhibition. Ampicillin did not showed any action action on Gram –ve

bacteria and fungi. The Diethyl ether extract more zone of inhibition in Gram +ve compared with Gram –ve bacteria and fungi.

TABLE 1: Antimicrobial activity of *M. jalapa* against microbes.

Solvent	**20µg/ml**	***E. coli*** **(G-)**	***S. aureus*** **(G+)**	***B. subtilis*** **(G+)**	***K. pneumoniae*** **(G-)**	***A. niger***
Diethyl ether extracts	**Orange**	9	14	20	13	---
	Pink	8	12	16	13	---
	Yellow	9	14	20	13	---
Ethyl acetate	**Orange**	---	---	---	---	---
	Pink	---	---	---	---	---
	Yellow	---	---	---	---	---
Methanol	**Orange**	---	---	---	---	---
	Pink	---	---	---	---	---
	Yellow	---	---	---	---	---
Ampicillin		---	13	13	---	---

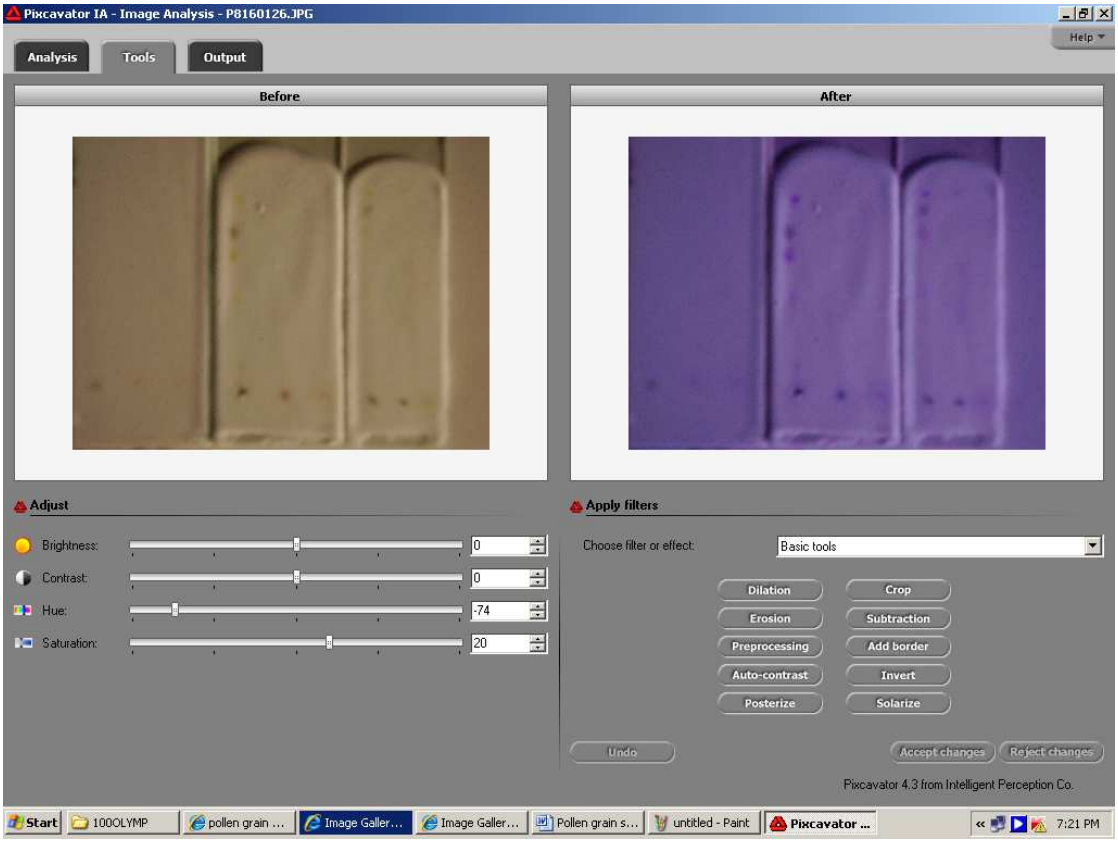
Table 2 provided results obtained in experimentation using TLC in all the 3 varieties of *M.jalapa*. The results have shown more number of compounds with diethyl ether. Only few compounds are visualized as spots on TLC plates for ethyl acetate and methanol extracts. Hence there is more number of non polar compounds than polar compounds. Based on present analysis, for diethyl ether plant extracts, 4 spots are visualized at 10% ethyl acetate (RF: 0.22, 0.39. 0.65, 0.98), 4 spots at 30% diethyl ether (RF: 0.04, 0.69, 0.89, 0.96), 1 spot at 50% diethyl ether (RF: 0.11). Based on present analysis, for ethyl acetate plant extracts, 2 spots are visualized at 10% ethyl acetate(RF: 0.04, 0.08), no spots at 30% ethyl acetate, 1 spot at 50% ethyl acetate (RF: 0.06). Based on present analysis, for methanol plant extracts, spots are not visualized at 10% Methanol, 1 spots at 30% Methanol (RF:0.02), no spots at 50% Methanol.

TABLE 2: Distance moved by extracts (in mm) in TLC.

Diethylether	**ORANGE**	**PINK**	**YELLOW**
10% ethyl acetate	Total- 4.9 1.1,1.9,3.2,4.8	Total - 4.9 1.1,1.9,3.2,4.8	Total - 4.9 1.1,1.9,3.2,4.8
30% ethyl acetate	Total -5.1 0.2, 3.5, 4.1, 4.9	Total -5.1 0.2, 3.5, 4.1, 4.9	Total -5.1 0.2, 3.5, 4.1, 4.9
50% ethyl acetate	Total -4.6 0.5	Total -4.6 0.5	Total -4.6 0.5
Ethylacetate	**ORANGE**	**PINK**	**YELLOW**
10% ethyl acetate	Total - 5.1 0.2, 0.4	Total - 5.1 0.2, 0.4	Total - 5.1 0.2, 0.4
30% ethyl acetate	Total -5.0 -	Total -5.0 -	Total -5.0 -
50% ethyl acetate	Total -4.9 0.3	Total -4.9 0.3	Total -4.9 -
Methanol	**ORANGE**	**PINK**	**YELLOW**
10% ethyl acetate	Total - 5.0 -	Total - 5.0 -	Total - 5.0 -
30% ethyl acetate	Total -4.9 0.1	Total -4.9 0.1	Total -4.9 0.1
50% ethyl acetate	Total -4.8 -	Total -4.8 -	Total -4.8 -

Figure 1 has provided the outputs of the image analysis software, pixcavator which shows clarity of the image before and after adjusting the image. Hence the software provided better visualizations after adjusting brightness, contrast, Hue and saturation.

Fig.1 Output of images using Pixcavator, an image analysis software.



DISCUSSION

In recent years 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 ethno pharmacologists.

Further acquaintance with different ethnic groups 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. For these reasons, medicinal plants are important substances 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[16]. Thus, biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development[17].

Fractions were collected from the crude solvent extracts that were effective in reducing the virus concentration on the indicator plants of *N. glutinosa*. Retardation factor (R_F)[18] of each fraction was calculated using:

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

Apart from its ornamental value, *M. jalapa* has also earned its place in herbal medicine practices around the world. Its array of biological activities continues to support its use worldwide for control of viruses, fungi and yeast[19]. According to R. Nair et al 2005,[20] the antibacterial activities of *H. rosasinensis* and *M. M. jalapa* are tested at 40 mg/0.1 ml and neither aqueous nor methanolic extracts were able to inhibit any of the tested bacterial strains.

Most of the work is done on anti viral compounds. Hence scientists taught that there are no antibacterial components from *M. jalapa*[20]. The present research provided that the antimicrobial activity is present, if the compound solvent used is diethyl ether. The Diethylether extracts of all the three varieties showed the zone of inhibition against *E.coli* (9 mm), *S.aureus* (14 mm), *B. subtilis* (20 mm), *K. pneumoniae* (13mm), which is greater than the standard antibiotic used. The Diethylether extract did not showed the zone of inhibition against *A.niger*.

The results indicated that the antimicrobial activity against Gram-positive was more pronounced than against Gram-negative bacteria. The results obtained are in agreement with the work of Nair *et al.*,[20] Parkeh and Chanda,[21]and Encarnacion *et al.*[22] The differences may be attributed to the fact that the cell wall in Gram-

positive bacteria consists of a single layer, whereas the Gram-negative bacteria it is a multilayer structure and is quite complex.

The ethanolic extract of the leaf of *Mirabilis jalapa* was tested by Oladunmoye et al., 2007 for antimicrobial activity against five pathogenic bacterial strains: *Escherichia coli, Staphylococcus aureus, Salmonella typhi,*

Bacillus cereus and Klebsiella pneumoniae. Phytochemical screening of the extract revealed the presence of tannins, saponins, alkaloids and cardiac glycosides. The toxins and the plant extract possess' antimicrobial activities comparable to conventional antibiotics; and can thus be a good source of agents for biocontrol and chemotherapy[23].

Pixcavator is an image analysis software provided good results about the picture clarity of the objects collected in TLC taken by Olympus FE-115 MODEL 5 megepixel camara (2048X1536).

CONCLUSION

The present experimentation showed the good results in application of software's along with wet lab methodologies. Diethyl ether extract of *M.jalapa* can cure the diseases such as pneumonia, pus, wounds etc. The extracts contain large quantities of non polar compounds, which act against microbes. Much research has to be conducted on *M. jalapa* in future for adaptation of these plants in past and near future against bacteria, virus, fungi and surrounding organisms.

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