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Phytochemical Analysis and Antibacterial Activity of *Mirabilis jalapa* Flower against Gastro Intestinal Pathogens

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Abstract: ***Mirabilis jalapa Linn. is widely used as a traditional medicine in many parts of the world for the treatment of various diseases viz. viral inhibitory activity, anti tumour activity. It is claimed in traditional medicine as the flower of the plant is used in the treatment of dysentery, diarrhea, muscular pain and abdominal colic. In the present study was attempted to reveal the qualitative phytochemical analysis of dried flower powder extracted in various solvents like aqueous, ethanol, methanol, chloroform and petroleum ether and their antibacterial activity against the gastrointestinal infections causing pathogens like Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeroginosa, Klebsiella pneumoniae, Bacillus cereus, Lactobacillus acidophilus, Enterococcus faecalis, Salmonella typhi and Shigella dysenteriae. TLC was carried out in all the extractions and HPLC and FT-IR were used as confirmatory test to study the nature of secondary metabolites. The antibacterial activity of the extracts of Mirabilis jalapa was examined by Disc diffusion method. Aqueous, chloroform and petroleum ether extracts displayed the minimal inhibition while all the ethanolic and methanolic extracts showed good antibacterial activity against the selected pathogens. The bacterial growth inhibition was calculated with reference to the standard.***

Keywords: Disc diffusion method, Antibacterial activity, Phytochemicals

1. Introduction

Medicinal plants are used for many centuries to treat various diseases in traditional medicine system in various countries. In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from “ethno medicinal” plants. [6]

The world health organization reported that 80% of world’s populations rely chiefly on traditional medicine[17] and plants would be the best source to obtain a variety of drugs[15] These medicinal plants evolved the ability to synthesize chemical compounds, that these compounds are toxic to plant predators, turn out to have favorable effects when used to treat human diseases. The secondary metabolic compounds include saponins, tannins, flavonoids, alkaloids, anthraquinones, and glycosides and so on used as traditional medicines are recognized as a way to learn about potential future medicines. These physiologically active principles have been utilized over the years in conventional medical practice for the treatment of various ailments such as gastrointestinal, respiratory infections etc. [1]

The human gastrointestinal infection is inflammation of the mucosa of the stomach or intestine, which may be divided into foregut, midgut and hindgut. Generally, 80-85% of the normal flora of intestine has been considered as a beneficial and 15-20% was potentially harmful. The most common gastrointestinal infections are caused by bacteria, including *Bacillus cereus, Campylobacter sp., Salmonella sp., E. coli* which are frequently acquired by intake of uncooked foods. Symptoms of gastrointestinal infections include diarrhea, fever, vomiting with abdominal cramps and loss of appetite.

Mirabilis jalapa is one of the medicinally important shrubs used in traditional medicine, belongs to the family Nyctaginaceae and commonly called as “Anthi Mandharai” in Tamil. Mirabilis jalapa plant extract contains biologically active substances that exhibit microbicidal or microstatic effects against various diseases causing microbes. The plant has high medicinal properties such that the indigenous people of Mexico use it to cure many infirmities including dysentery, diarrhea, muscular pain and abdominal colic. It is used for the treatment of various infections. Parts of the plant are used to combat dyspepsia and other digestive disorders.

Mirabilis jalapa contains a proteolytic enzyme which soothes the stomach and aids in digestion and a liquid potion has been used to reduce enlarged tonsils. It is a popular ornamental plant grown worldwide for the beauty of its flowers which can be white, red, pink, purple or multicolored and their sweet fragrance. The crude extract of *Mirabilis jalapa* flowers showed positive response against gastrointestinal disorders *in vivo*.[14] The current study attempts to examine the antibacterial activity of bioactive compounds of *Mirabilis jalapa* against gastrointestinal infections causing microbes.

2. Materials and Methods

2.1 Collection of Plant Material

The fresh plants of *Mirabilis jalapa* Linn were collected in the months of October- December, from the local areas of Kulithalai, Karur district and authenticated by the authority of the botany department of St. Joseph College, Trichy. The plant material was shade-dried for 3 days in order to make it moisture free. The dried flower sample was powdered in a mechanical grinder and kept in air tight container till use.

2.2 Preparation of Plant Extract

The shade-dried and coarsely powdered flower (100 gm) was extracted with the solvent of water, methanol, chloroform, ethanol and petroleum ether separately by using cold extraction method. All the extracts were separately filtered with a muslin cloth and then subjected to evaporate at 45°C in a hot air oven or room temperature under reduced pressure to yield a gum (6 to 10 g aqueous, 3 to 5 g ethanolic, 3 to 5 g methanolic, 3 to5 g chloroform and 4 to 6 gm of petroleum ether). The extracts were stored in labeled sterile screw capped container at -15°C for further studies.

2.3 Isolation and Maintenance of Test Organisms

Bacterial strains were procured from the Microbiology and Pathology Department of SRM Medical College and Hospital, at Trichy. Ten pathogenic bacterial species *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeroginosa, Klebsiella pneumoniae, Bacillus cereus, Lactobacillus acidophilus, Enterococcus faecalis, Salmonella typhi* and *Shigella dysenteriae* were used for the antibacterial assay. The test bacteria were maintained in slants in refrigerator.

2.4 Antibacterial Activity

By disc diffusion method antibacterial activity of all extracts was determined against all the isolates on Mueller Hinton agar plates [3]. The plates were seeded with 4 hours old fresh culture of the isolates. Tetracycline discs were used as positive control and DMSO loaded discs were used as negative control. The zone of inhibition was measured after 24 hours of incubation at 37^0C.

2.5 Phytochemical Analysis

The various solvent extract of flower of *M. jalapa* were screened for the presence of alkaloids, glycosides, carbohydrates, Phytosterol, terpenes, saponins, tannins, inulin, lignin, flavonoids , proteins and free amino acid and volatile oils using simple qualitative methods.[8,10]

2.6 Instrumentation Analysis

Fourier Transform Infra-Red Spectroscopy (FT-IR) has been used for the comparative analysis of corrosion particles. Thin layer chromatography (TLC) was performed to separate mixtures. Secondary metabolites were confirmed by using High Performance Liquid Chromatography (HPLC) technique.

3. Results and Discussion

The organoleptic characters of the powdered flower were studied and found to have a reddish brown colour with sweet odour and taste. The size ranges from 3 – 4 cm and has a smooth texture. *Mirabilis jalapa* dried flower powder was treated with various solvents like aqueous, ethanol, methanol, chloroform and petroleum ether and their quality were determined by standard pharmacopeia method.

The quantitative phytochemical analysis of various solvent extracts of *Mirabilis jalapa* has been depicted in Table-1. Tannins, alkaloids and terpenoids were present in all solvent extracts except in aqueous extracts. Volatile oil was perceived only in chloroform extract. Tannins, alkaloids, carbohydrates, terpenoids, inulin, flavonoids, glycosides, proteins and amino acids were noted in methanolic and ethanolic extracts. Protein and free aminoacids, carbohydrates and flavonoids were present in aqueous extract while petroleum ether extracts showed the presence of tannins, alkaloids, terpenoids, lignin, flavonoids, proteins and amino acids.

The presence of secondary metabolites in the plant including alkaloids, carbohydrates, glycosides, tannins, flavonoids was reported [7]. The qualitative phytochemical screening of methanolic extract of the white flowered plant also revealed the presence of trace amount of tannins, moderate amount of alkaloids, carbohydrates, terpenes and saponins. Glycosides and flavonoids were not observed as a notable amount [12].

Antibacterial activity of various solvent extracts of *M. jalapa* have been evaluated *in vitro* against test organisms like *E. coli, S. aureus, S. pneumoniae, P. aeroginosa, K. pneumoniae, B. cereus, L. acidophilus, E. faecalis, S. typhi* and *S. dysenteriae* by disc diffusion method. Tetracycline 1mg / ml was used as the reference standard and the activity was found to be concentration dependent for all different samples tested. Zone of inhibition produced by various solvent extracts of *Mirabilis jalapa* ranges from 5mm – 15mm. The antibacterial activity of aqueous extract of *Mirabilis jalapa* was highly effective against *Pseudomonas aeroginosa, E.coli* and *Staphylococcus aureus* with a zone of inhibition of 11 – 12 mm.

In general, the inhibitory activity of the ethanolic and methanolic flower extract against *Bacillus cereus, Escherichia coli and Pseudomonas aeroginosa* was greatly effective with a zone of 14 - 15mm. While petroleum ether and chloroform extract exhibited highest zone of inhibition (10mm) against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa*.

The methanolic and ethanolic extract of *Mirabilis jalapa* displayed significant activity against all organisms tested with minimum inhibitory concentration being 1mg/ml. The results of antibacterial activity are presented in Table 2.

Aqueous and ethanolic extract derived from the leaves of white flowered plant of *Mirabilis jalapa L.* showed antibacterial activities against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio cholerae* and *Bacillus subtilis*. Aqueous extracts did not display any inhibition to the test bacteria however all the ethanolic extracts showed good antibacterial activity against the selected pathogens [12]. *Mirabilis jalapa* dried flower and leaf of the plants were being used for medicinal treatment of gram negative bacterial diseases [11] whereas the flower and seeds also have antidiabetic activity [2, 4].

TLC was carried out in all extraction in crude method like aqueous, ethanol, methanol, chloroform and petroleum ether

that showed brown spot in the crude extract and various RF values (0.44, 0.50 and 0.625) were observed (Fig:1). HPLC and FT-IR was used for confirmation to identify the secondary metabolites present in the selected extract.

Table 1: Phytochemical screening of *Mirabilis jalapa* flower extract

Test	Crude method					
	P	A	E	M	C	PE
Alkaloids	+	-	+	+	+	+
Carbohydrates	+	+	-	+	+	-
Glycosides	-	-	+	-	+	-
Phytoserol	-	-	-	-	-	-
Inulin	+	-	-	+	-	+
Tannins	+	-	+	+	+	+
Terpenoids	+	-	+	+	+	+
Proteins & free amino acids	+	+	-	+	-	-
Steroids	-	-	-	-	-	-
Flavonoids	+	+	+	+	-	-
Lignin	-	-	+	-	-	+
Volatile oils	-	-	-	-	+	-

P – Powder, **A** – Aqueous, **E** – Ethanol, **M** – Methanol, **C** – Chloroform, **PE** – Petroleum Ether

Table 2: Antibacterial activity of various extract of *Mirabilis jalapa* against gastrointestinal infection causing microbes

Test organisms	Control (mm)		Extraction/ concentration (µg/disc) zone of inhibition in mm				
	+ve	-ve	E	M	C	A	PE
B. cereus	15±0.3	-	15±0.2	15±0.1	5±0.3	8±0.2	5±0.3
E. faecalis	13±0.2	-	14±0.1	13±0.2	8±0.3	7±0.2	8±0.2
L.acidophilus	12±0.3	-	13±0.4	8±0.2	8±0.2	6±0.2	10±0.2
S. aureus	12±0.3	-	12±0.4	12±0.2	10±0.2	11±0.3	8±0.2
S.pneumoniae	13±0.2	-	8±0.4	9±0.1	7±0.2	8±0.2	5±0.2
E. coli	14±0.2	-	15±0.4	15±0.2	10±0.2	11±0.3	10±0.2
K.pneumoniae	12±0.3	-	11±0.4	11±0.2	7±0.2	7±0.2	7±0.2
P.aeuroginosa	12±0.3	-	15±0.4	14±0.1	8±0.3	12±0.2	10±0.2
S. typhi	13±0.2	-	12±0.2	10±0.2	4±0.3	9±0.2	7±0.2
S. dysentriae	12±0.3	-	11±0.4	12±0.2	6±0.2	7±0.2	8±0.3

(Positive control: Tetracycline; Negative control: DMSO)

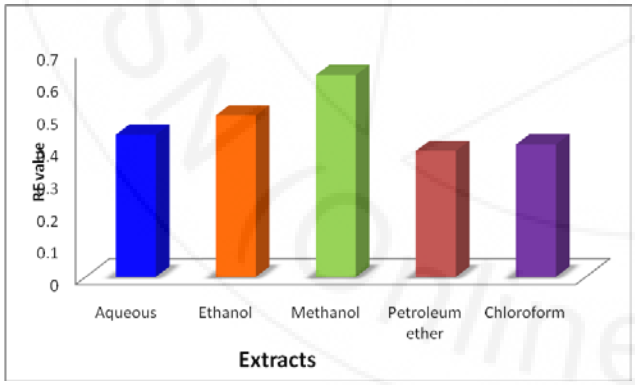

Figure 1: Thin layer chromatography of *Mirabilis jalapa*

4. Conclusion

Thus, the research has emphasized that medicinal plants are potential renewable natural resources with a beneficial role in human health care. Pharmacognostic and phytochemical parameters determined in the present work can serve as a major criterion for identity, quality and purity of a crude drug and extracts. Results revealed that the extracts of *Mirabilis jalapa* flower showed good antibacterial activity against most of the gastrointestinal pathogens. This property was attributed by the presence of bioactive compounds such as alkaloids, flavonoids, tannins and phenolic compounds. This research also declares an insight that India is enriched with valuable assets of therapeutic plants which has to be dug out.

5. Future Scope

The plant extract of *Mirabilis jalapa* contains biologically active substances that exhibit both microbicidal or microstatic effects against various microbes. *Mirabilis jalapa* have been proved to be a excellent antibacterial drug against gastro intestinal pathogens. In future, this work can be further extended *in vivo* for the discovery of new medicine for human welfare.

6. Author Profile

Dr. P. Sumithra received M.Sc., M. Phil. and Ph.D degrees in Microbiology from Bharathidasan University, Tiruchirappalli. She has published several research papers in many journals. Currently she is working as Asst. Professor in Srimad Andavan Arts and Science College, T. V. Koil, Tiruchirappalli, Tamil Nadu, India

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