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Research Article

A COMPARATIVE STUDY OF *IN VITRO* ANTIMICROBIAL ACTIVITY AND TLC STUDIES OF PETALS OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT

Objective: The present study was to evaluate the *in vitro* antibacterial activity, and thin-layer chromatography (TLC) studies from the petals of four different Indian medicinal plants (*Punica granatum, Hibiscus rosa-sinensis, Cassia auriculata,* and *Moringa oleifera*).

Methods: The phytochemical screening of the methanol extract of petals of four different Indian medicinal plants was performed using standard procedures. The antimicrobial activity was tested against various test organisms using the agar disc diffusion method.

Results: The preliminary phytochemical screening for petals of four different medicinal plants revealed the presence of flavonoids, alkaloids, tannins, and saponins. From the above study, the results indicated that the methanol extract of *M. oleifera* petals showed the highest antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* with zone of inhibition 17.93 and 23.40, respectively, at the concentration of 20 µl/ml and also showed the maximum inhibitory activity at the highest concentration (20 µl/ml) than the lowest concentration (5 µl/ml) against Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris, Pseudomonas aeruginosa,* and Gram-positive *B. subtilis* and *S. aureus.* TLC studies of methanol extracts of petals of Indian medicinal plants revealed the presence of different phytoconstituents as evidenced by separated compounds with different Rf values.

Conclusion: The results obtained in the present study indicate that the petals of four different Indian medicinal plants showed the highest antibacterial activity and can be used as an antibacterial agent against bacterial diseases.

Keywords: Phytochemicals, Antibacterial activity, Thin-layer chromatography.

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INTRODUCTION

Phytomedicines play a major role in human health-care system. Plants are also known to contain enormous biological active compounds such as flavonoids, glycosides, phenols, carotenoids, alkaloids, and terpenoids [1] which possess antibacterial properties. According to World Health Organization, 65-80% of the world populations rely on traditional medicine to treat various diseases [2]. In recent decades, many antimicrobial drugs have been discovered, developed, and widely used, but it was found that microorganisms build resistance to drugs [3,4]. Hence, development of new drugs without any side effects is the urgent need of the society. Plants have been a therapeutic source for a long time, and plant products played an essential role in ancient medicine. Plants are the cheapest and safest alternative sources of antimicrobials [5-7].

Hibiscus (Malvaceae) is a genus of herbs, shrubs, and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to possess various medicinal properties. Studies have shown that the plants of the Hibiscus genus have the potential to provide biologically active compounds that act as antioxidants and cardioprotective agents. Hence, *Hibiscus* genus may be a great natural source for the development of new drugs and may provide a cost-effective mean of treatment for cancer and other diseases in the developing world [8]. The ancient Indian medicinal literature reported that the flowers of *Hibiscus rosa-sinensis* have beneficial effects in heart diseases, mainly in myocardial ischemic disease and without producing any cytotoxic effects [9]. Recently, [10] suggested that *H. rosa-sinensis* had a protective role against age and scopolamine-induced amnesia, indicating its utility in the management of cognitive disorders.

Cassia auriculata commonly known as "avaram" and belonging to the family Caesalpiniaceae. It is a common plant in Asia, profoundly used in Ayurvedic medicine as a tonic, astringent, and as a remedy for diabetes, conjunctivitis, ulcers, leprosy, skin, and liver diseases [11]. There are many therapeutic uses, and medicinal properties are reported in different parts (leaves, stem, seeds, flowers, fruits, stem bark, etc.,) of the plants. They are known for antihyperglycemic activity [12], hepatoprotective activity [13], antihelmintic activity [14], antimicrobial activity [15], and antioxidant activity [16]. The flowers of the plant are used in the preparation of tea, which is prescribed in diabetes. The seeds are used in diabetes, ophthalamia, and chylous urine [17]. The plant has been reported to antibacterial and microbicidal activity [18,19].

Moringa oleifera (Moringaceae) is commonly known as a drumstick tree or horseradish tree. Traditionally, its roots are applied as plaster to reduce the swelling and rheumatism. Almost all parts of the plant are used culturally for its nutritional value, medicinal properties and for taste and flavor as a vegetable and seed [20]. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers, and immature pods act as cardiac and circulatory stimulants and possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer [21]. The whole *M. oleifera* plant is used in the treatment of psychosis, eye diseases, and fever, other important medicinal properties of the plant include antispasmodic [22], diuretic [23], antihypertensive [24], cholesterol lowering [25], antioxidant, antidiabetic, hepatoprotective [26], antibacterial, and antifungal activities [27].

The pomegranate (*Punica granatum* L., *Punicaceae* family) is a shrub, and its fruit is a rich source of bioactive phytochemicals such as tannins and other phenolics. Pomegranate fruit products have been

used for centuries since ancient civilizations for medicinal purposes. In Ayurvedic and Siddha medicine, the pomegranate is considered "a pharmacy into itself" and is used as an antiparasitic agent [28] a "blood tonic" [29] and to heal diarrhea and ulcers. The potential therapeutic properties of pomegranate are wide ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation [30].

The present investigation is to study the *in vitro* antimicrobial activity and to assess the phytocompounds by thin-layer chromatography (TLC) of petals of four different medicinal plants.

METHODS

Plant material

The petals of four medicinal plants (*H. rosa-sinensis, P. granatum, C. auriculata,* and *M. oleifera*) were collected from the Government Siddha Medical College, Herbal garden, Tamil Nadu, India. The petals of medicinal plants were washed with distilled water and shade dried. The shade dried petals were powdered and stored in air tight containers for further studies.

Preparation of plant extract

About 20 g of the finely grounded petals of four medicinal plants were soaked in 70% methanol at room temperature for 24 hrs. The extract was filtered using Whatman filter paper No. 1 and then concentrated in vacuum at 40°C-50°C (overnight) using a rotary evaporator. The residue was mixed with methanol and used for further studies.

Phytochemical screening

The extracts were subjected to phytochemical analysis to ascertain the presence metabolites such as alkaloids, tannins, flavonoids, terpenoids, and saponins using standard procedures [31].

TLC

TLC was performed using standard methods [32]. About 15 µl of extract was applied to the precoated aluminum silica gel 60 F, Merck F 254. Developing solvent system used was toluene, acetone, and formic acid (6:6:1). All plates were visualized directly after drying and with the help of UV at 240 nm and 360 nm in UV TLC viewer. The Rf value of the different spots that were observed was calculated.

Bacterial cultures

Bacterial strains used were *Staphylococcus aureus* MTCC 29213, *Bacillus subtilis* MTCC441, *Escherichia coli* MTCC 25922, *Pseudomonas aeruginosa* MTCC 2488, and *Proteus vulgaris* MTCC 1771. All bacterial strains were obtained from microbial type culture collection and gene Bank, Institute of Microbial Technology, Chandigarh, India. All bacterial strains were stored and maintained at 4°C for further study.

Antibacterial activity assay

The antibacterial assay was performed by disc diffusion technique [33]. The disc diffusion technique is highly effective to determine the antibacterial activities for methanol extracts of 4 different medicinal plants. About 25 ml of Mueller-Hinton agar was poured into each Petri plate. Once the agar solidified, the bacteria were inoculated on the surface of the plates. The methanol extract impregnated discs (Whatman No. 1 filter paper) were prepared and air dried well. The test was conducted at four different concentrations of the crude extract (5, 10, 15, and 20 µl/ml) with 3 replicates. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 hrs. After 24 hrs incubation at 37°C, all plates were observed for zones of inhibition, and the diameter of these zones was measured in millimeters. All tests were performed in triplicate and the antibacterial activity was expressed as the mean ± standard deviation.

Determination of minimum inhibitory concentration (MIC)

The effect of MIC of the methanol extracts was carried out using the method of [34]. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. To 0.5 ml of bacterial cultures, varying concentrations of the extracts (5, 10, 15, and 20 µl/ml) were added to test tubes. The culture tubes were then incubated at 37°C for 24 hrs. After incubation, the tubes were then examined for microbial growth by observing for turbidity, and OD was measured spectrophotometrically at 580 nm.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of petals of four different medicinal plants (*H. rosa-sinensis, P. granatum, C. auriculata,* and *M. oleifera)* revealed the presence of flavonoids, alkaloid, saponins, and tannins (Table 1).

TLC studies of petals of four different Indian medicinal plants

TLC studies of methanol extract of petals of four medicinal plants were carried out using mobile phase toluene:acetone:formic acid (6:6:1). The spots were visualized under 240 nm and 360 nm (Figs. 1-4).

Antibacterial activity

The antibacterial potential of petals of four medicinal plants was compared according to their zone of inhibition against several pathogenic organisms by disc diffusion method (Table 2). *M. oleifera* showed the highest antimicrobial activity against *S. aureus* and *B. subtilis* with zone of inhibition 17.93 and 23.40, respectively, at the concentration of 20 µl/ml. Among the four medicinal plants, the methanol extract of *M. oleifera* petals showed the maximum inhibitory activity at the highest concentration (20 µl/ml) than the lowest concentration (5 µl/ml) against Gram-negative bacteria such as *E. coli*, *P. vulgaris, P. aeruginosa,* and Gram-positive *B. subtilis* and *S. aureus* (Table 3). In the present investigation, it shows that the methanol extracts of petals of four medicinal plants can inhibit the growth of pathogenic organisms.

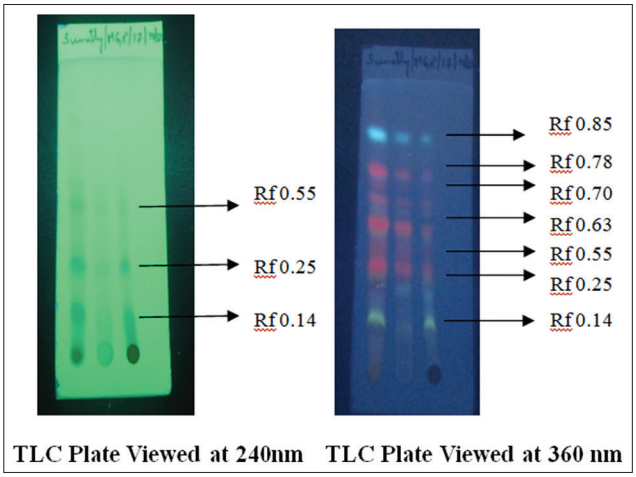

Fig. 1: Thin-layer chromatography profile of methanol extract from the petals of *Punica granatum*. Thin-layer chromatography plate viewed at 240 nm. Thin-layer chromatography plate viewed at 360 nm

Table 1: Phytochemical screening of petals of four Indian medicinal plants

Phytochemical constituent	*Hibiscus rosa-sinensis*	*Punica granatum*	*Cassia auriculata*	*Moringa oleifera*
Flavonoid	+	+	+	+
Alkaloid	+	+	+	+
Saponin	+	+	+	+
Tannins	+	+	+	+

Hibiscus rosa-sinensis: H. rosa-sinensis, Punica granatum: P. granatum, Cassia auriculata: C. auriculata, Moringa oleifera: M. oleifera

Table 2: Antibacterial activity of methanol extract of petals of four different Indian medicinal plants

Medicinal plants	Methanol extract (µl/ml)	Zone of inhibition [in mm diameter]a				
		S. aureus	*E. coli*	*B. subtilis*	*P. vulgaris*	*P. aeruginosa*
Punica granatum	5	9.4±0.65	-	6.43±0.60	-	7.4±0.6
	10	11.33±0.83	7.56±0.40	9.10±0.45	6.43±0.60	9.36±0.77
	15	14.06±0.60	7.46±0.92	10.96±0.45	9.06±0.40	11.36±0.70
	20	16.13±0.41	8.36±0.90	13.06±0.60	11±0.40	14.03±0.45
Hibiscus rosa-sinensis	5	9.03±0.45	10.3±0.70	7.4±0.65	11.36±0.77	9.36±0.70
	10	11.06±0.40	12.43±0.66	10.06±0.40	15.10±0.45	11.43±0.66
	15	12.96±0.45	14.33±0.76	11.96±0.55	17.13±0.41	13.33±0.76
	20	14.46±0.61	17.06±0.40	14±0.4	18.96±0.45	16.2±0.43
Moringa oleifera	5	11.36±0.77	7.43±0.60	11.4±0.79	7.06±0.40	6.43±0.60
	10	13.43±0.73	11.53±0.72	16.1±0.45	8.46±0.61	7.3±0.75
	15	15.3±0.75	13.96±0.45	19.3±0.81	10.43±0.60	9.3±0.81
	20	17.93±0.60	15.43±0.60	23.4±0.79	12.4±0.79	11.43±0.81
Cassia auriculata	5	10.3±0.75	11.3±0.75	9.4±0.65	12.2±0.91	8.3±0.70
	10	12.9±0.45	14±0.40	12.06±0.40	14.43±0.60	10.3±0.77
	15	15.1±0.47	16.06±0.60	13.9±0.45	16.4±0.79	12.3±0.81
	20	16.8±0.41	18.03±0.55	16.06±0.50	19.06±0.40	14.4±0.65

Punica granatum: P. granatum, Hibiscus rosa-sinensis: H. rosa-sinensis, Moringa oleifera: M. oleifera, Cassia auriculata: C. auriculata

Table 3: Minimum inhibitory concentration of methanol extract of petals of four different Indian medicinal plants

Medicinal plants	Methanol extract (µl/ml)	Zone of inhibition [in mm diameter]a				
		S. aureus	*E. coli*	*B. subtilis*	*P. vulgaris*	*P. aeruginosa*
Punica granatum	Positive control	0.652±0.010	0.527±0.009	0.572±0.009	0.576±0.011	0.653±0.009
	5	0.557±0.011	0.447±0.008	0.490±0.005	0.471±0.010	0.538±0.009
	10	0.463±0.010	0.336±0.008	0.353±0.010	0.353±0.011	0.456±0.009
	15	0.370±0.011	0.242±0.009	0.242±0.011	0.275±0.005	0.340±0.011
	20	0.276±0.009	0.120±0.011	0.154±0.010	0.151±0.010	0.255±0.008
Hibiscus rosasinensis	Positive control	0.655±0.012	0.715±0.008	0.628±0.008	0.759±0.011	0.666±0.011
	5	0.510±0.011	0.621±0.010	0.521±0.010	0.533±0.009	0.567±0.007
	10	0.443±0.008	0.491±0.011	0.434±0.009	0.421±0.10	0.448±0.007
	15	0.315±0.015	0.38±0.005	0.343±0.009	0.302±0.010	0.354±0.008
	20	0.220±0.008	0.232±0.010	0.234±0.008	0.249±0.013	0.232±0.009
Moringa oleifera	Positive control	0.642±0.010	0.666±0.012	0.759±0.011	0.609±0.011	0.513±0.009
	5	0.56±0.006	0.502±0.010	0.652±0.010	0.495±0.011	0.426±0.008
	10	0.523±0.010	0.416±0.009	0.523±0.011	0.403±0.010	0.305±0.011
	15	0.472±0.010	0.313±0.010	0.432±0.010	0.272±0.010	0.222±0.010
	20	0.312±0.010	0.180±0.011	0.206±0.010	0.168±0.012	0.162±0.010
Cassia auriculata	Positive control	0.58±0.006	0.62±0.06	0.57±0.01	0.61±0.009	0.533±0.01
	5	0.46±0.008	0.49±0.011	0.50±0.008	0.57±0.011	0.42±0.008
	10	0.37±0.012	0.43±0.008	0.42±0.008	0.52±0.008	0.33±0.011
	15	0.30±0.008	0.36±0.008	0.36±0.005	0.484±0.005	0.28±0.011
	20	0.23±0.009	0.25±0.011	0.27±0.008	0.37±0.008	0.230±0.009

Punica granatum: P. granatum, Hibiscus rosa-sinensis: H. rosa-sinensis, Moringa oleifera: M. oleifera, Cassia auriculata: C. auriculata

The minimal inhibitory concentration was determined by measuring the turbidity of the bacterial culture that is the mean±SD of three replicates.

DISCUSSION

According to the present study, the preliminary phytochemical screening of methanol extract of petals of four different medicinal plants (*H. rosa-sinensis, P. granatum, C. auriculata,* and *M. oleifera)* showed the presence of flavonoids, alkaloids, saponins and tannins. Phytoconstituents have been found to inhibit bacteria [35]. The four medicinal plants screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health [36].

The methanol extract of petals of different medicinal plants showed varying degree of antibacterial activities against the test organisms (Table 2). Among the four medicinal plants, the methanol extract of *M. oleifera* at the concentration of 20 µl/ml showed maximum zone of inhibition percentage of 23.4 mm against *B. subtilis*. This is followed by 17.93, 15.43, 12.40, and 11.43 mm zone of inhibition against *S. aureus, E. coli, P. vulgaris, and P. aeruginosa,* respectively. The MIC of the methanol extract for different organisms ranged between 5-20 µl/ml. TLC results showed the presence of phytoconstituents and it is active against multidrug-resistant organisms. The results of this study showed that the methanolic extract was more effective, and this may be due to the better solubility of the active components in organic solvents [37]. It supports the earlier investigation that the phytoconstituents isolated from the flower of *M. oleifera* possess remarkable toxic activity against bacteria and may assume pharmacological importance [38]. The traditional method of treating a bacterial infection, decoction of the plant parts, or boiling the plant in water is employed, whereas according to the present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by the previous literature [39].

CONCLUSION

From our study, it clearly indicates that the methanol extract of petals of four different medicinal plants is rich in phytochemicals which have potent antibacterial activity against pathogenic organisms. The result

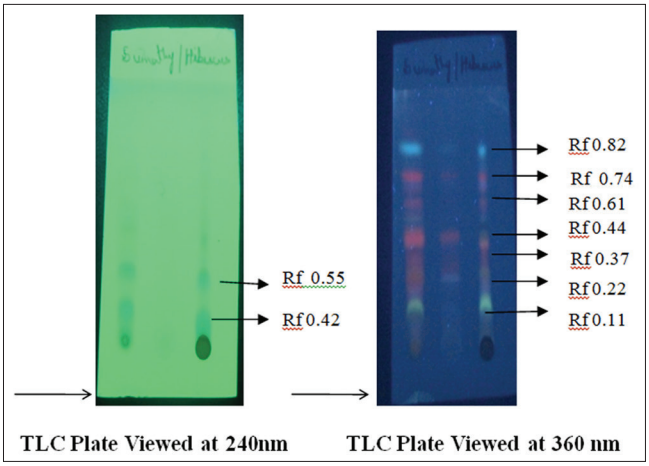

Fig. 2: Thin-layer chromatography profile of methanol extract from the petals of *Hibiscus rosasinensis*. Thin-layer chromatography plate viewed at 240 nm. Thin-layer chromatography plate viewed at 360 nm

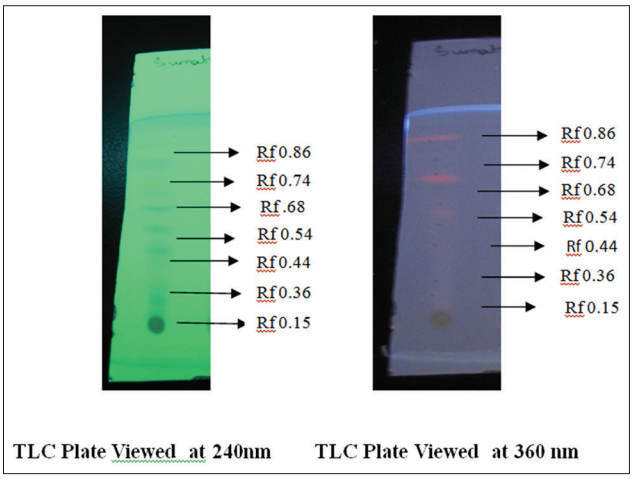

Fig. 3: Thin-layer chromatography profile of methanol extract from the petals of *Moringa oleifera*. Thin-layer chromatography plate viewed at 240 nm. Thin-layer chromatography plate viewed at 360 nm

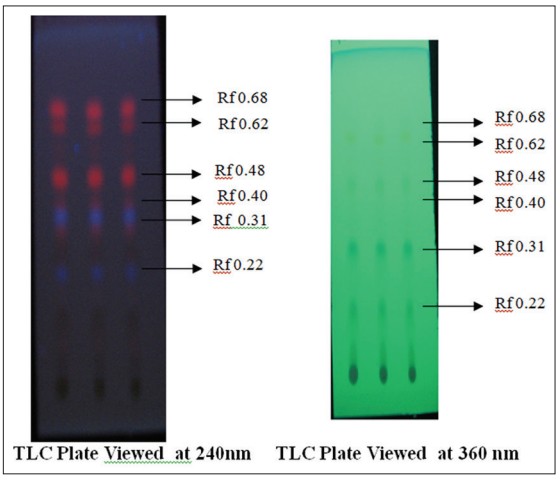

Fig. 4: Thin-layer chromatography profile of methanol extract from the petals of *Cassia auriculata*. Thin-layer chromatography plate viewed at 240 nm. Thin-layer chromatography plate viewed at 360 nm

of the study supports the traditional application of the plants and suggests that the methanol extract of four medicinal plants possess phytocompounds and can be used as antibacterial agents in novel drugs for the treatment of bacterial diseases. The use of these plants in traditional medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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