RESEARCH ARTICLE

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The antimicrobial activity of *Hibiscus* and *Murraya* leaf extracts against a variety of multidrug-resistant samples

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ABSTRACT

This research examined the antimicrobial properties of leaf extracts from *Hibiscus rosa sinensis* and *Murraya koenigii* against various human pathogenic bacteria, including *E. coli, K. pneumoniae, P. aeruginosa*, and *S. aureus*. The study utilized the disc diffusion method to assess the antimicrobial effects of benzene, acetone, and methanol extracts. Results showed that the extracts displayed broad-spectrum antibacterial activity against all pathogens tested. The most significant zone of inhibition was observed for *P. aeruginosa, E. coli*, and *S. aureus* in the benzene, acetone, and methanol extracts, respectively. Phytochemical screening identified several compounds, including terpenoids, tannins, alkaloids, and flavonoids, which may contribute to the antimicrobial activity of the plants. In conclusion, *Hibiscus rosa sinensis* and *Murraya koenigii* could be valuable sources of antibacterial agents against *K. pneumoniae, E. coli, P. aeruginosa*, and *S. aureus*.

Key words: antibacterial, *Hibiscus rosa-sinensis*, *Murraya koenigii*, medicinal plants, human pathogens, disc diffusion

Introduction

Medicinal plants represent an upscale source of antimicrobial agents. Plants are used medicinally in several countries and are a source of many potent and powerful drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesised in the secondary metabolism of the plant (Medina et al., 2005). Plants remain an extremely significant source of medicine. The use of these plants as medicines predated the ancient period. In India, the references to the curative properties of some herbs in the Rig-Veda seem to be the earliest records of the use of plants in medicine. Medicinal plants are extensively used throughout the planet in two distinct areas of health management; traditional systems of drugs and modern systems of drugs. The traditional system of medicine mainly functions through two distinct streams: (Holley & Cherla, 1998) local or folk or tribal stream and (Antibiotic/antimicrobial resistance: action plan, Atlanta: US Department of Health and Human Services Centres for Disease Control and Prevention, 1999) a codified and organised Indian system of medicine like Ayurveda, Siddha, Unani, etc. The Rigveda (3700 B.C.) mentions the utilisation of medicinal plants. Traditional systems of medicine, especially Ayurveda, Unani, Siddha, Homeopathy, etc., benefit from herbs for treatment. It is estimated that 40% of the world population depends directly on plant-based medicine for their health care (World Health Organization. Guidelines for the Assessment of Herbal Medicine Programme on Traditional Medicine, 2003). The natural compounds of various plants, full of phytochemicals, protect the human body against various diseases. Primary metabolites consist of carbohydrates, amino acids, proteins, and chlorophyll, while secondary metabolites consist of alkaloids, saponins, flavonoids, etc. (Prabha et al., 2013). Thus, the most important factor needed is to derive the utmost enjoyment from the normal system of drugs for providing adequate healthcare service to rural people (Ghani, 1990). Considering the vast potentiality of plants as sources for antimicrobial drugs with regard to antibacterial agents, a scientific investigation was undertaken to screen the local flora for antibacterial activity of *Hibiscus rosa sinensis* and *Murraya koenigii*. *Murraya koenigii* is commonly known as curry leaf in Indian accents, and it also belongs to the family Rutaceae (Satyavati et al., 1987). It has been used in Indian cookery for centuries and has a versatile role to play in traditional medicine. Bark and roots are worn as stimulants and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for the cure of dysentery, diarrhoea, and to check vomiting. Leaves and roots are used traditionally as anthelmintic, analgesic, for curing piles, inflammation, itching, and are useful in leukoderma and blood disorders (Nadkarni, 1976; Kirtikar & Basu, 1981). Hibiscus belongs to the family *Malvaceae*, and according to previous studies, its extract affects male fertility (Sachdewa & Khemani, 2003). The treatment of inflammatory diseases and spermatogenesis (Sachdewa et al., 2001). The crude extract of the aerial part of *H. rosa sinensis* clearly showed the presence of two

components that have cholinomimetic and calcium antagonist activities. So, the possible pharmacological rationale for the use of this plant for constipation and diarrhoea was suggested (Nade et al., 2011).

Materials and Methods

Fresh leaves of two different plants were collected from the local area of Aligarh Muslim University Campus. The leaves were washed thoroughly 2-3 times with running water and once with sterile water and then air dried on sterile blotters under shade.

Solvent extraction

When the moisture content of the leaves of two plants was totally evaporated, they were crushed to a coarse powder with the help of a mixer. 3 gm of leaf powder of acetone, benzene, and methanol were taken separately and then extracted at room temperature in 15 ml each of three different solvents, i.e., 80% (v/v) of acetone, benzene, and methanol. The overnight extracts were filtered through sterilised Whatmann No. 1 filter paper before being concentrated to dryness in a 40°C oven and stored at 4°C in a refrigerator until further testing against bacterial isolates (Raman, 2006). The obtained extracts were stored in the refrigerator and were dissociated in dimethyl sulfoxide prior to use. A total of four microorganisms were used to assess the antibacterial properties. They included one gram–positive bacteria, *Staphylococcus aureus*; and three gram–negative bacteria, *Klebsiella pneumoniae, E. coli*, and *Pseudomonas aeruginosa*. The microorganisms were originally obtained from the microbial collection centre at Ram Manohar Lohia Hospital, Delhi, India.

Phytochemical screening

The phytochemical screening of plant extracts was done to identify the main groups of chemical constituents in Benzene, Acetone, and Methanol extracts *Hibiscus rosa sinensis* and *Murraya koenigii* by Raman and Harborne (Harborne, 1998).

Preparation of inoculum

The microorganisms were pre-cultured in nutrient broth and stored at 4°C in the refrigerator until use. Incubate the bacterial slant culture at 37°C for 24 hours.

Antimicrobial activity

Seeds Methanol, acetone, and benzene leaf extracts were extracted using three different solvents. *Hibiscus-rosa-sinensis* and *M. Koenigii* were tested by disc diffusion method (Anonymous, 1996). Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol, acetone, and benzene respectively. The test microorganisms were implanted into the respective medium by spread plate method. 10ul with the 24h cultures of bacteria growth in nutrient broth. After solidification, the filter paper discs (5 mm in diameter) impregnated with the extract were placed on test organism-implanted plates. Gentamycin (10 µg/ml) was used as the positive control and methanol, acetone, and benzene solvent (100 µg/ml) were used as negative control. The antibacterial assay plates were incubated at 370°C for 24h. The diameter of the inhibition zones was measured in millimetres (mm).

Results

Phytochemical screenings

The extractive value of Benzene, Acetone, and Methanol extract was revealed to contain the following phytochemicals (Table 1).

Table1. *Different groups of phytochemicals are present in Benzene, acetone, and methanolic extract of H. rosa-sinensis and M. koenigii plant leaves*.

S. No	Phytochemicals	*Hibiscus rosa sinensis*			*Murraya koenigii*		
		BE	AE	ME	BE	AE	ME
1.	Alkaloids	+	+	-	-	+	+
2.	Carbohydrate	+	-	+	-	+	-
3.	Saponins	-	-	+	-	+	-
4.	Amino acid	+	+	-	-	+	+
5.	Flavonoids	+	+	-	+	+	+
6.	Terpenoids	-	+	+	+	+	+

BE- Benzene extract, AE-acetone extract, ME-methanol extract.

Antimicrobial activity

Results obtained in the present study revealed that the two tested medicinal plant extracts possess potential antibacterial activity against *E. coli, K. pneumonia, P. aeruginosa*, and *S. aureus*. When tested using the disc diffusion method, the results of this study revealed that two medicinal plant extracts have potential antibacterial activity against *E. coli, K. pneumonia, P. aeruginosa*, and *S. aureus*. The benzene and acetone leaf extracts of plants, viz., *H. rosa sinensis* and *M. koenigii*, showed significant activity against all the tested microorganisms when compared to Methanol extracts (Table 2). The highest antibacterial activity was recorded in *S. pneumoniae* (9.47mm), *E. coli* (12.73mm), and *S. Aureus* (12.90mm) in benzene, acetone, and methanol, respectively, from *H. rosa sinensis* leaf extracts. A leaf extract of *M. koenigii* showed the highest antimicrobial activity against *S. aureus* (11.20mm) benzene, (13.03mm) acetone, and methanol (2.73mm) in comparison to other microbial strains (Table 2).

Discussion

Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganisms (Aqil et al., 2005) and many components of

Table 2. *Antimicrobial activity of benzene, acetone, and methanolic extracts (100g/ml) of H. rosa-sinensis and M. koenigii by disc diffusion assay.*

S.No	Human pathogenes	Zone of inhibition (in mm)						Gentamycine (10µg/ml)
		Hibiscus rosa sinensis			*Murraya koenigii*			
		Benzene Extract	Acetone Extract	Methanol Extract	Benzene Extract	Acetone Extract	Methanol Extract	
1.	*E. coli*	9.30	12.73	0.00	6.83	6.07	0.00	18.36
2.	*K. pneumoniae*	9.40	10.97	1.77	4.53	1.50	1.60	19.49
3.	*K. pneumoniae*	9.47	7.67	11.00	10.33	12.77	1.73	17.32
4.	*S. aureus*	9.30	11.00	12.90	11.20	13.03	2.73	19.62

plant products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro et al., 2006). The emergence of multidrug-resistant strains of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most chemotherapeutic agents is unbearable to the public, especially in developing countries like India (Gopalkrishnan et al., 2010). Therefore, attempts must be directed towards the development of effective natural, non-toxic drugs for treatment. The present work was initially to explore the antimicrobial properties of *H. rosa-sinensis* and *M. koenigii*.

Many reports are accessible on the antimicrobial, antifungal, anticancer, anti-inflammatory, hypoglycaemic, dental caries, pancreatic lipase inhibitory effects, and antidiarrheal properties of plants (Mahesh & Satish, 2008). The benzene leaf extract of *H. rosa-sinensis* and *M. koenigii* showed activity against all four tested microorganisms but the activity was very significant against *S. pneumoniae*. Acetone leaf extracts of *H. rosa-sinensis* and *M. koenigii* both plants showed activity against *E. coli* and *K. pneumoniae* respectively. The methanolic leaf extract showed activity against three of four tested microorganisms and was highly active against *S. aureus*. The plant-based products have been effectively proven for their utilization as source for antimicrobial compounds. For instance, methanol extracts of A. ferox and *W. somnifera* exhibited inhibitory activity against all strains of N. *gonorrhoea* (Kambizi & Afolayan, 2008). The essential oil was found to be effective against *Rhizoctania batiticola* (ED 500.112%) and *Helminthosporium oryza* (0.1214%), and the effects are possibly due to the presence of β-caryophyllene and gurjunene (Kambizi & Afolayan, 2008). Essential oil and aqueous extract of leaf were found active against *Staphylococcus epidermidis, S. aureus,* and *Streptococcus* species. Crude extract and chloroform soluble fraction and petroleum ether soluble fraction showed promising antibacterial activity against all tested bacteria (Akerel & Ayinde, 1998; Shimomura & Hattori, 2001; Srivastava & Singh, 2001). The present study showed that all three extracts of leaves of *H. rosa-sinensis* and *M. koenigii* have more phytochemical constituents studied with slight variation. Thus, the significant activity against *K. pneumoniae* and *S. aureus* may be due to their phytochemical or secondary metabolites.

Conclusion

The study aimed to investigate the antibacterial potential of *Hibiscus rosa sinensis* and *Murraya koenigii* leaf extracts against human pathogenic bacteria including *E. coli, K. pneumoniae, P. aeruginosa,* and *S. aureus*. The researchers used benzene, acetone, and methanol extracts to examine the antimicrobial activities of these plants. The study showed that these plants had a wide spectrum of antibacterial activities against all bacterial pathogens studied. Phytochemical screening revealed the presence of different compounds such as terpenoids, tannins, alkaloids, and flavonoids that may contribute to the antimicrobial action of these plants. The study suggests that *Hibiscus rosa sinensis* and *Murraya koenigii* could be potential sources of antibacterials against *E. coli, P. aeruginosa, S. aureus,* and *K. pneumoniae*. The study also discussed the significance of medicinal plants in traditional systems of medicine and their potential use in modern medicine.

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