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Evaluation of the antimicrobial activity of three medicinal plants of South India

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ABSTRACT

The present study was carried out to evaluate the antimicrobial activity of the crude methanolic extracts of *Memecylon malabaricum* Clarke. (leaves), *Cochlospermum religiosum* Linn. (leaves and flowers) and *Andrographis serpyllifolia* Vahl. (leaves) using the standard disc diffusion assay against eight strains of bacterial species, viz., *Staphylococcus aureus*, *Salmonella typhi, Enterobacter aerogenes, Pseudomonas aeruginosa, Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *malvacearum, Bacillus cereus* and *Micrococcus* sp. The extracts of the plants at a concentration of 1.25 mg/disc showed minimum to moderate activity against both Gram positive and Gram negative bacteria indicating a broad spectrum activity. A preliminary phytochemical screening was conducted on the selected plant extracts using standard qualitative procedures that revealed the presence of several secondary metabolites. The extracts failed to show antioxidant activity by reducing power assay. The result indicates the potential usefulness of these plants especially *Memecylon malabaricum* and *Cochlospermum religiosum,* in treating microbial infections in humans and plants and justifies the need for further investigations and characterization of the bioactive compounds present in the methanolic extracts of the plants.

Keywords: antibacterial activity, *Memecylon malabaricum*, *Cochlospermum religiosum*, *Andrographis serpyllifolia*, disc diffusion assay

INTRODUCTION

The development of antimicrobial agents has been undeniably one of the greatest accomplishments of modern medicine. In recent years, multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The limited life span of antibiotics, has rendered a neccessity to search for new antimicrobial substances from various sources such as medicinal plants. Plants used in traditional medicine are one of the most promising areas in the search for new biologically active compounds. Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al*., 2006). This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Pandian *et al*., 2006).

The plants *Memecylon malabaricum* (Clarke.) Cogn., *Cochlospermum religiosum* (Linn.) Alston., and *Andrographis serpyllifolia* (Vahl) Wight. were selected for this study based on their medicinal use. *M. malabaricum* (Clarke.) Cogn. (Family *Melastomataceae*) is a shrub endemic to Western Ghats- South and Central Sahyadris. It is traditionally used for the treatment of skin problems such as rashes on skin and chicken pox. The leaves are crushed and the extract is applied on skin before taking bath. It is also used in the treatment of herpes infection. The leaves are also consumed as medicine (Hullati and Rai, 2004). *C. religiosum* (Linn.) Alston. (Family *Cochlospermaceae*) is a small deciduous tree. The tree yields a gum which is known as katira. Traditionally it is used in treating cough, diarrhea, dysentery, pharyngitis, fistula, gonorrhea, trachoma and syphilis. The dried leaf and flowers are used as stimulants, antipyretic, laxative and sedative (Kirtikar and Basu, 1975). *A. serpyllifolia* (Vahl) Wight. (Family Acanthaceae) is a prostrate growing herb. The plant extract is used in treating wounds. Some practitioners claim that it is effective in treating jaundice (Manjunath *et al*., 2004).

The present study was conducted to investigate antimicrobial activity of methanolic extracts of *M. malabaricum, C. religiosum* and *A. serpyllifolia* against both clinical and plant associated bacteria which have not been evaluated in previous studies. A preliminary phytochemical screening was conducted to identify the class of compounds present in the extract and also the antioxidant activity was determined.

MATERIALS AND METHODS

Collection of plants

The leaves of *M. malabaricum* were collected from Kota region of Udupi District. The flowers and leaves of *C. religiosum* and the leaves of *A. serpyllifolia* were collected from different localities of Mysore. Collected samples were

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identified by consulting taxonomists and the herbaria of the plants were deposited in Herbarium Collection Centre, Department of Studies in Microbiology, University of Mysore and the accession number given to the herbarium specimens were *M. malabaricum* (MGMB/211/2009), *C. religiosum* (MGMB/212/2009) and *A. serpyllifolia* (MGMB/213/2009).

The plant parts were washed with water and wiped with a clean cloth. They were shade dried, ground into coarse powder and stored in airtight container at 4 ℃ until used for extraction.

Bacterial strains

The authentic culture of the test organisms were obtained from the stock culture maintained at Department of Studies in Microbiology, University of Mysore. All the microorganisms were maintained at 4 ℃ on nutrient agar slants.

Both clinical and plant pathogenic microorganisms were used as test organisms for the screening of the antimicrobial activity of the methanolic extracts of the plant parts.

The bacteria used for the study included gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus* and Gram negative bacteria viz., *Salmonella typhi*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas axonopodis* pv. *malvacearum*.

Preparation of methanolic extracts

The powdered plant parts were separately extracted in a soxhlet apparatus with methanol. For preparation of methanolic extract, 30 g of powdered plant part was weighed and placed in a thimble prepared from washed and destarched muslin cloth. It was extracted with 80% methanol for 24 h by soxhelation. The obtained extracts were evaporated at room temperature to get a crude dried extract. The yield was determined and stored in air tight container until used to prevent the loss of biological activity.

Antibacterial assay

The potency and activity of antimicrobials is usually determined by zone of inhibition they produce when they act upon bacteria grown on agar plates. Agar disc diffusion methods are the most frequently applied assay methods (Bauer *et al*., 1966). Sterile nutrient broth was prepared and inoculated with the test organisms under aseptic conditions. It was incubated for 24 h at 37 ℃ and used as inoculum. Nutrient agar was prepared, autoclaved and equal volume (15 mL) poured into sterile Petri dishes (Borosil plates having 9 cm diameter) under aseptic conditions and allowed to solidify. The plates were allowed to solidify. The microbial suspension was adjusted to have 10^6 cells/mL. Under aseptic conditions, 0.1 mL of the microbial suspension was inoculated on the plates and spread using a sterile spreader. Sterile filter paper discs of 5 mm diameter were loaded with 25 µL of the methanolic extracts (50 mg/mL) to yield a final concentration of 1.25 mg/disc. The paper discs were allowed to evaporate and then placed aseptically on the surface of the inoculated agar plates. Standard chloramphenicol (30 µg) discs were used as positive control while methanol (25 µL/disc) served as negative control. The experiment was performed in triplicates under aseptic conditions. Plates were incubated at 4 ℃ for 2 h for equilibration and then for 18 h at 37 ℃. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones (including diameter of the disc). The mean value of the diameter of the inhibition zone of the triplicates was taken as the final value.

Phytochemical screening

Qualitative phytochemical analysis of the methanolic extract was carried out using standard procedures to identify the constituent alkaloids (Mayer's test), steroids and terpenoids (Liberman- Burchard and Salkowski tests), cardiac glycosides (Keller-Kilani test), saponins (foam tests), flavonoids (Shinoda test), tannins and phenols (Ferric chloride test) as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1993).

Quantitative phytochemical analysis by estimation of total phenolic content

Folin and Ciocalteu's (FC) method was used to determine the total phenolic content in the extracts (Singleton *et al*., 1999). Total soluble phenolic compounds in the methanolic extracts were measured and expressed as gallic acid equivalents.

Determination of antioxidant activity by reducing power assay

Reducing power assay was carried out as described by Yildirim *et al*., (2001). A 500 µL of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50 ℃ for 30 min. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid and the mixture was centrifuged for 10 min at 3000 rpm. A 2.5 mL supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

The results of the antimicrobial screening assay of the crude extracts of all plants against the tested strains are shown in Table 1. The methanolic extract of the leaves of *M. malabaricum* at a concentration of 1.25 mg/disc showed significant antimicrobial activity against the plant pathogens *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *malvacearum* and the human pathogen *S. aureus*. However, the organisms *S. typhi*, *E. aerogenes*, *P. aeruginosa*, *Micrococcus* and *B. cereus* were completely

resistant to the methanolic extract at the tested concentration. It was reported by Hullatti and Rai, (2004) that on screening petroleum ether, chloroform and methanol extracts of *M. malabaricum* leaves, antimicrobial activity was shown only by methanolic extract. The present work confirms the antibacterial activity of methanolic extract of leaves of *M. malabaricum*. The methanolic extract of the leaves has shown activity against both human and plant pathogens indicating a broad spectrum activity. The potent activity against *S. aureus* justifies the extensive use of these agents for treating skin disorders. As the activity was significant against plant pathogens too, these can be used in the management of plant diseases.

The present work is the first report about the antimicrobial screening of methanolic extract of the leaves and flowers of *C. religiosum*. The screening showed significant activity against *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *malvacearum.* The organisms *S. aureus*, *S. typhi* and *E. aerogenes* were slightly inhibited. While the concentrations of the methanolic extract tested were not enough to inhibit *P. aeruginosa*, *Micrococcus* and *B. cereus.* The methanolic extracts have shown activity against a panel of bacteria such as *S. aureus*, *S. typhi*, *E. aerogenes*, *X. oryzae* pv. *oryzae and X. axonopodis* pv. *malvacearum.* This indicates that the extract of

C. religiosum has broad spectrum activity and can be used for the treatment of microbial infections. From the results it is revealed that methanolic extract of leaf and flowers have shown similar activity. Goud *et al*., (2005) reported that on *in vitro* screening of ethanol, acetone and chloroform extracts of *C. religiosum*, acetone extract showed antimicrobial activity. Considering the previous reports and current results, it is clear that the plant possesses antimicrobial property.

No work has been reported on the antimicrobial and phytochemical screening of the methanolic extract of leaves of *A. serpyllifolia*. In the present study, the methanolic extract of leaves of *A. serpyllifolia* showed moderate inhibitory action on *X. oryzae* pv *oryzae*, *S. aureus* and *S. typhi* and no activity against the other test organisms. This shows that the methanolic extract of the leaves has antimicrobial components.

All species of plants included in the present study were found to be active on at least one of the selected microbial strains. The antimicrobial activity profile of all species of plants against the tested strains indicated that *S. aureus* was the most susceptible bacterium and *P. aeruginosa* was the most insensitive strain of all the bacteria used in this study. In fact, Gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market (Alonso *et al*., 2000). Therefore, it is not surprising to learn that *P. aeruginosa* is the least responding bacterial strain to the tested plant extracts. The antibacterial activity was more pronounced on the Gram positive bacteria (*S. aureus*) than the Gram negative bacteria (*P. aeruginosa*). Being crude extracts, the overall antimicrobial activity screening results are still indicative of the potential of these herbal drugs as effective medicaments in the treatment of infectious diseases.

The result of the phytochemical screening of methanolic extract of the plant parts is given in Table 2. The phytochemical screening of methanolic extract of

Table 1: Antimicrobial activity of the methanolic extract of the different plant parts

	Diameter of inhibition zone (in mm)* of the methanolic extracts (1.25 mg/disc).					
	Memecylon malabaricum (leaves)	*Andrographis serpyllifolia* (leaves)	*Cochlospermum religiosum* (leaves)	*Cochlospermum. religiosum* (flowers)	P	N
Staphylococcus aureus	10	8	8	6	16	-
Salmonella typhi	-	8	6	8	20	-
Enterobacter aerogenes	-	-	8	9	11	-
Pseudomonas aeruginosa	-	-	-	-	10	-
Xanthomonas oryzae pv. *oryzae*	10	7	13	14	18	-
Xanthomonas axonopodis pv. *malvacearum*	11	-	12	14	15	-
Bacillus cereus	-	-	-	-	10	-
Micrococcus	-	-	-	-	12	-

* Zone of inhibition (in mm) including the diameter of the disc (5 mm) in disc diffusion assay. Assay was performed in triplicates and results are the mean of the three values. Each disc was loaded with 25 µL of the extract (1.25 mg/disc). Chloramphenicol (30 µg) was used as positive control (P) and methanol (25 µL/disc) was used as negative control (N).

Table 2: Phytochemical screening of methanolic extract of the plant parts

Secondary metabolites	Methanolic extract of the plant parts			
	Memecylon malabaricum (leaves)	*Cochlospermum religiosum* (leaves)	*Cochlospermum religiosum* (flowers)	*Andrographis serpyllifolia* (leaves)
Alkaloids	-	-	+	+
Sterols	+	+	-	+
Glycosides	+	+	-	-
Saponins	+	+	+	+
Flavonoids	+	+	+	-
Tannins and phenols	+	+	+	+

The secondary metabolites present are denoted as '+' and those not detected are denoted as '-'.

M. malabaricum leaves revealed the presence of sterols, cardiac glycosides, saponins, flavonoids, tannins and phenols. The presence of flavonoids, tannins and resins was reported earlier by Hullatti and Rai (2004). The phytochemical screening in this study has confirmed the presence of flavonoids and tannins and has also revealed the presence of glycosides and sterols. The antimicrobial activity may be attributed to the presence of these compounds. The phytochemical screening of methanolic extract of *C. religiosum* leaves and flowers revealed the presence of sterols, cardiac glycosides, saponins, flavonoids, tannins and phenols. The phytochemical screening of methanolic extract of leaves of *A. serpyllifolia* revealed the presence of sterols, cardiac glycosides, saponins, flavonoids, tannins and phenols.

The curative property of these medicinal plants may be attributed to the presence of various secondary metabolites revealed in the preliminary phytochemical analysis. The differences in the antimicrobial effects of the plant extracts might be due to the phytochemical properties. It is quite possible that some of the plants that were ineffective against certain bacteria may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective.

The amount of total phenolics varied slightly in plant materials and ranged from 19 to 49.5 mg/g of leaf extract. The highest amount was found in *A. serpyllifolia* (49.5 mg/g) and the lowest in *M. malabaricum* (19 mg/g). The total phenolic content in the methanolic extract of the leaves of *C. religiosum* was 35 mg/g of extract and that of the flowers was 45 mg/g of extract.

The antioxidant capacities of the plant extracts largely depend on the composition of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wong *et al*., 2006). The methanolic extracts of *M. malabaricum, C. religiosum* and *A. serpyllifolia* did not show antioxidant activity by reducing power assay. Antioxidant activity of these extracts can be determined by applying alternative methods.

The results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. The plants *M. malabaricum* and *C. religiosum* have shown significant activity against the tested pathogens and these can be selected for the further studies and for the determination of minimum inhibitory concentration. As the plant extracts have also shown significant activity towards the plant pathogens, they can also be used as biocontrols in plant disease management. Further research is necessary to determine the identity of the antibacterial compounds within these plants and also to determine their full spectrum of efficacy. The present study of *in vitro* antimicrobial evaluation of the selected plants forms a primary platform for further phytochemical and pharmacological studies.

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REFERENCES

Alonso, R., Fernandez-Aranguiz, A., Colom, K., Herreras, A., and Cisterna, R. (2000). Profile of bacterial isolates and antimicrobial susceptibility: Multicenter study using a one-day cut-off. *Revista Espanola de Quimioterapia* **13, 384-393.**

Bauer, A. W., Kirby, W. M. M. and Sherris, J. C. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45, 493-496.

Cos, P., Vlietinck, A. J., Vanden, B. D. and Maes, L. (2006). Anti-infective potential of natural products:

How to develop a stronger in vitro 'proof-of concept'. *Journal of Ethnopharmacology* **106, 290-302.**

Goud, P. S. P., Murthy, K. S. R., Pullaiah, T. and Babu, G. V. A. K. (2005). Screening for antibacterial and antifungal activity of some medicinal plants of Nallamalais, Andhra Pradesh, India. *Journal of Economic and Taxonomic Botany* **29, 704-708.**

Harborne, J. B. (1993). Phytochemical method. 3rd edn. Chapman and Hall, London. **pp. 135-203.**

Hullati, K. K. and Rai, V. R. (2004). Antimicrobial activity of *Memecylon malabaricum*. *Fitoterpia* **75, 409-411.**

Kirtikar, K. R. and Basu, B. D. (1975). Indian medicinal plants. 2nd edn. Jayyed Press, Delhi.

Manjunatha, B. K., Krishna, V. and Pullaiah, T. (2004). Flora of Davanagere District, Karnataka. Regency Publications, New Delhi. **pp. 300.**

Pandian, M. R., Banu, G. S. and Kumar, G. (2006). A study of antimicrobial activity of *Alangium salviifolium*. *Indian Journal of Pharmacology* **38, 203-204.**

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* **299, 152-178.**

Sofowara, A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.

Trease, G. E. and Evans, W. C. (1989). Pharmacognosy. 13th edn. Bailliere Tindal Ltd, London. **pp.176- 180.**

Wong, S. P., Leong, L. P. and Koh, J. H. W. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* **99, 775-783.**

Yildirim, A., Mavi, A., and Kara, A. A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food Chemistry* **9, 4083-4089.**