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Short Communication

Antimicrobial and Radical Scavenging Activity of *Memecylon malabaricum* (C.B. Clarke) Cogn. and *Memecylon talboltianum* Brandis

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Abstract

The present study was conducted to determine antimicrobial and radical scavenging potential of extract of two species of the genus *Memecylon* (Melastomataceae) *viz*., *M. malabaricum* (C.B. Clarke) Cogn. and *M. talboltianum* Brandis. The shade dried leaf materials of both *Memecylon* species were extracted using methanol. Antibacterial activity of leaf extracts was evaluated against five drug resistant uropathogenic bacteria by Agar well diffusion assay. Antifungal activity of leaf extracts was tested on the basis of mycelial growth inhibition of *Colletotrichum capsici* (isolated from anthracnose of chilli). Radical scavenging activity of extracts was determined by performing DPPH free radical scavenging activity. Total phenolic content of extracts was estimated by Folin-Ciocalteau reagent method. The extracts were subjected to preliminary phytochemical analysis to detect the presence of phytoconstituents. Among extracts, extract of *M. malabaricum* inhibited all test bacteria and inhibitory potential was marked against Gram positive bacteria than Gram negative bacteria. *C. capsici* was highly susceptible to extract of *M. malabaricum* when compared to extract of *M. talboltianum*. Overall, extract of *M. malabaricum* displayed marked antimicrobial activity than extract of *M. talboltianum*. Extract of *M. malabaricum* scavenged DPPH radicals more efficiently (IC_{50} 6.26µg/ml) when compared to extract of *M. talboltianum* (IC_{50} 43.80µg/ml). The content of total phenolics was also high in leaf extract of *M. malabaricum* (112µg GAE/mg) than that of *M. talboltianum* (28µg GAE/mg). Preliminary phytochemical analysis of leaf extracts revealed the presence of flavonoids, tannins, saponins and glycosides in both extracts. The antimicrobial and radical scavenging activity of leaf extracts could be ascribed to the presence of phytochemicals mainly phenolic compounds. These plants appear to be potential candidates for control of anthracnose disease of chilli and for development of agents active against drug resistant uropathogens and oxidative damage.

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INTRODUCTION

India represents rich floristic diversity accounting for about 11% of total flora in the world of which 28% are endemic to the country. Among the hotspots of floristic diversity in India, Western Ghats (mountain ranges running through five states) hosts a large number of plant species with high degree of endemism. It is a mountainous range extending from the mouth of the river Tapti in Gujarat to Kanyakumari in Tamil Nadu. The floristic diversity of the Western Ghats is significant as it accommodates various vegetation types *viz*., wet evergreen forests, moist and dry deciduous forests, montane forests, sholas, scrubs and savannas (Richard and Muthukumar, 2012; Sivu *et al*., 2013; Nampoothiri *et al*., 2013). The genus *Memecylon* L. belongs to Melastomataceae and encompasses about 250 species of shrubs and trees. The species are distributed worldwide in various types of habitats such as deciduous, semi-evergreen, evergreen and other forests with a wide range of altitude from sea level. In India, about 39 species of *Memecylon* are found, of which 21 are endemic to the country and the Western Ghats are known to host 32 species (Elavazhagan and Arunachalam, 2010; Sivu *et al*., 2013). The species *viz*., *M. lawsonii*, *M. lushingtonii*, *M. flavescens* and *M. sisparense* of Western Ghats comes under rare and endangered categories. *M. procerum*, *M. clarkeanum* and *M. parvifolium* are new records to India (Sivu *et al*., 2013). The members of the genus *Memecylon* are shown to possess several biological activities such as antioxidant (Sivu *et al*., 2013), antimicrobial (Sivu *et al*., 2013), antipsoriatic (Dhanabal *et al*., 2012), enzyme inhibitory (Sekhar *et al*., 2013), anthelmintic (Ramanjayalu *et al*., 2012), antidiabetic (Ramaiah *et al*., 2013), anti-inflammatory (Nualkaew *et al*., 2009), analgesic (Nualkaew *et al*., 2009), wound healing (Nualkaew *et al*., 2009) etc. Several species of the genus *Memecylon*are shown to possess antimicrobial activity (Elavazhagan and Arunachalam, 2010; Killedar and More, 2011; Sivu *et al*., 2013; Sekhar *et al*., 2013). In

the present study, we compared the antimicrobial potential against drug resistant urinary tract pathogens and *Colletotrichum capsici* and radical scavenging efficacy of two *Memecylon* species *viz*., *M. malabaricum* Cogn. and *M. talboltianum* Brandis from Western Ghats of Karnataka.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plants *M. malabaricum* and *M. talboltianum* were collected at a place called Haniya coming under Western Ghats area, Hosanagara Taluk of Shivamogga district, Karnataka during January 2014. The plants were identified by Dr. Vinayaka K.S, Department of Botany, Kumadvathi First Grade College, Shikaripura, Karnataka.

Extraction

The leaves were separated from plants, shade dried and powdered in a blender. A known quantity of dried powder materials of both plant species was transferred to separate beakers containing 100ml of methanol (HiMedia, Mumbai) and left for two days with intermittent stirring. The solvent extracts were filtered through Whatman No. 1, concentrated in vacuum under reduced pressure and dried in desiccator (Vinayaka*et al*., 2009).

Phytochemical Analysis of Leaf Extracts

The concentrated leaf extracts were subjected to detect phyto-constituents *viz*., tannins, saponins, steroids, flavonoids, alkaloids and glycosides by standard phytochemical tests (George *et al*., 2010; Kekuda *et al*., 2012).

Antibacterial Activity of Leaf Extracts

In order to screen antibacterial efficacy of leaf extracts, we performed Agar well diffusion assay. The assay was performed against a panel of five multidrug resistant urinary tract bacteria *viz*., *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. The test bacteria were seeded into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated at 37^{o}C for 24 hours. The broth cultures were aseptically swabbed over sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. 100µl of leaf extracts (20mg/ml of 25% Dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), standard antibiotic (Chloramphenicol, 1mg/ml) and DMSO (25%, in sterile water) were transferred into labeled wells. The plates were then incubated at 37^{0}C for 24 hours in upright position and the zone of inhibition was measured (Manasa *et al*., 2013).

Antifungal Activity of Leaf Extracts

Poisoned food technique was employed to investigate antifungal potential of leaf extracts in terms of inhibition of mycelial growth of test fungus *C. capsici* isolated previously from chilli anthracnose (Kambar *et al*., 2013). Here, sterile Potato dextrose agar medium (HiMedia, Mumbai) poisoned with leaf extracts (1mg/ml of medium) was dispensed into sterile petri dishes, allowed to solidify and inoculated at the centre with the spore suspension of *C. capsici* by point inoculation. The plates were incubated aerobically at 28^{o}C for 5 days. The colony diameter(CD) of test fungus on control and poisoned plates was measured in mutual perpendicular directions. The inhibition of mycelial growth (%) was calculated using the formula:

Inhibition of mycelia growth (%) = (C – T / C) x 100,

where C is CD in control plates and T is CD in poisoned plates (Kambar*et al*., 2013).

DPPH Radical Scavenging Activity of Leaf Extracts

The efficacy of leaf extracts to scavenge free radicals was tested on the basis of scavenging effect of extracts on DPPH radicals (Kekuda *et al*., 2012). In clean and labeled test tubes, 2ml of different concentrations of leaf extracts (2.5-50µg/ml of methanol) was mixed with 2ml of DPPH solution (0.002% in methanol). The tubes were incubated for 30 minutes in dark at room temperature. After incubation, the optical density (absorbance) was measured at 517 nm using UV-Visible spectrophotometer (ELICO, SL159). The absorbance of the DPPH control (2ml of DPPH+2ml of methanol) was noted. Ascorbic acid was used as reference standard. The scavenging potential of each concentration of both the extracts was calculated using the formula:

Scavenging activity (%) = [(A–B) /A] x100,

Where, A is absorbance of DPPH and B is absorbance of DPPH and extract/standard combination. The IC_{50} value for the extract was calculated. IC_{50} represents the concentration of extract required to scavenge 50% of DPPH free radicals.

Total Phenolic Content of Leaf Extracts

Folin-Ciocalteu reagent (FCR) method was employed to estimate total phenolic content of leaf extracts. Here, a dilute concentration of leaf extract (0.5ml) was mixed with 0.5ml diluted Folin-Ciocalteu reagent (1:1) and 2 ml of sodium carbonate (7%). The mixtures were left for 30 minutes at room temperature followed by measuring the absorbance of tubes at 765nm using UV-Visible spectrophotometer (ELICO, SL159). A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000µg/ml). The concentration of total phenolics in leaf extracts was estimated as µg Gallic acid equivalents (GAE) from the graph (Kekuda *et al*., 2012).

Statistical Analysis

The experiments were done in triplicates. Results were recorded as Mean±Standard deviation (SD).

RESULTS

Phytoconstituents in Leaf Extracts

Preliminary phytochemical analysis of extracts showed the presence of phytoconstituents *viz*., saponins, tannins, flavonoids, glycosides in extract of both *M. malabaricum* and *M. talboltianum*. Alkaloids and steroids were not detected in both extracts.

Antibacterial Activity of Leaf Extracts

The result of inhibitory activity of leaf extracts against uropathogens is shown in Table 1. The presence of zone of inhibition around the well is considered positive for antibacterial activity. Extract of *M. malabaricum* was effective in inhibiting all bacterial isolates with zone of inhibition ranging 1.2 to 1.9cm. Extract of *M. talboltianum* was found inhibitory to only *E. faecalis* and *P. aeruginosa*. *S. aureus* and *P. aeruginosa* were inhibited to higher extent by extract of *M. malabaricum* and *M. talboltianum* respectively. Extract of *M. malabaricum* and standard antibiotic were found to inhibit Gram positive bacteria to higher extent than Gram negative bacteria. DMSO did not cause inhibition of any test bacteria.

Table 1: Antibacterial activity of leaf extracts against uropathogens

Test bacteria	Zone of inhibition in cm			
	M. malabaricum	*M. talboltianum*	Antibiotic	DMSO
S. aureus	1.9±0.1	0.0±0.0	3.5±0.2	0.0±0.0
E. faecalis	1.6±0.0	0.8±0.0	3.5±0.2	0.0±0.0
E. coli	1.4±0.2	0.0±0.0	2.5±0.1	0.0±0.0
P. aeruginosa	1.3±0.0	1.1±0.1	2.5±0.1	0.0±0.0
K. pneumoniae	1.2±0.0	0.0±0.0	2.3±0.2	0.0±0.0

Antifungal Activity of Leaf Extracts

The result of antifungal effect of extracts in terms of reduction in the mycelial diameter of *C. capsici* on poisoned plates is shown in Table 2 and Figure 1. The extracts were found to inhibit the growth of fungus as indicated by reduced size of colony on poisoned plates when compared to control plate. Among extracts, extract of *M. malabaricum* inhibited the growth of fungus to higher extent (48.38%) than extract of *M. talboltianum* (32.25% inhibition).

Table 2: Colony diameter of *C. capsici* on control plates and poisoned plates

Treatment	CD (cm)	Inhibition (%)
Control	3.1±0.0	-
M. malabaricum	1.6±0.0	48.38
M. talboltianum	2.1±0.1	32.25

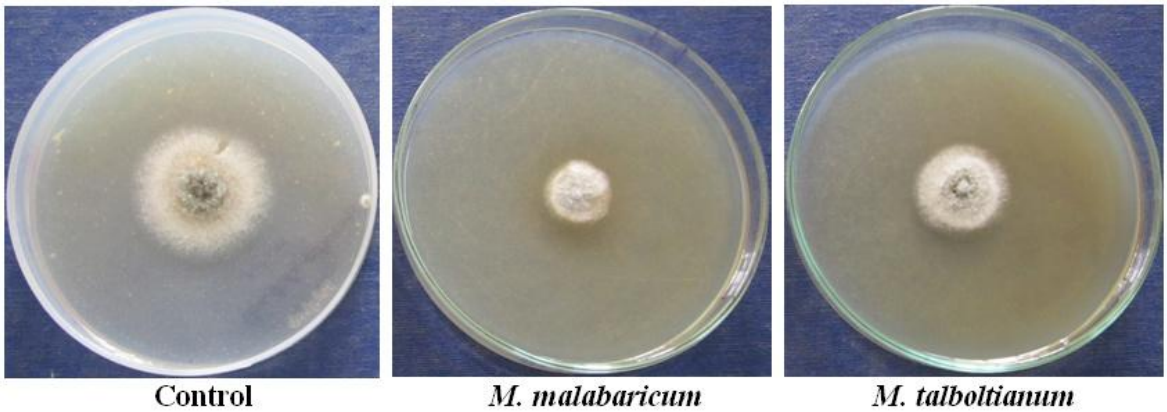

Figure 1: Growth of *C. capsici* on control and poisoned plates

Radical Scavenging Activity of Leaf Extracts

The result of free radical scavenging capacities of the leaf extracts is shown in Figure 2. The scavenging effect of extracts was dose dependent. Among leaf extracts, extract of *M. malabaricum* scavenged radicals more efficiently (IC_{50} 6.26µg/ml) than that of extract of *M. talboltianum* (IC_{50} 43.80µg/ml). Ascorbic acid scavenged radicals to higher extent (IC_{50} 2.63µg/ml) when compared to leaf extracts.

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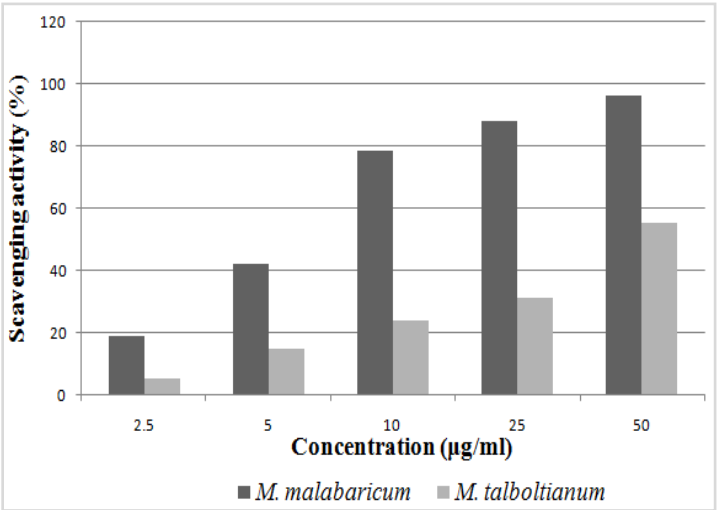

Figure 2: DPPH radical scavenging potential of leaf extracts

Total Phenolic Content of Leaf Extracts

The content of total phenolics was found to be higher in case of leaf extract of *M. malabaricum* (112µg GAE/mg) when compared to leaf extract of *M. talboltianum* (28µg GAE/mg).

DISCUSSION

Chilli (*Capsicum annuum* L.) is an important economic food crop in various parts of the world such as India, Thailand, China, Mexico etc. It is grown for domestic usage and export. Chilli is used as an important spice and vegetable crop in India. The production of chilli is greatly affected by anthracnose disease. It is a serious disease of chilli caused by several species of *Colletotrichum*. In India, *C. capsici* is one among the important pathogens causing anthracnose of chilli. The disease drastically reduces yield and deteriorates the quality of fruit, and less returns to the farmers. Typical symptoms of anthracnose on chilli fruits include sunken necrotic tissues, with concentric rings of acervuli that are often wet. Anthracnose destroys thechilli fruits during cultivation, transportation and storage. The disease accounts for 50% or higher reduction of pre-and post-harvest in chilli fruits. One of the most widely used approaches to control the anthracnose disease is to use chemical agents. However, the method is not so beneficial due to several reasons such as high cost, residual effect, toxicity to non-target organisms, emergence of resistant pathogens, and contamination in food. Therefore, search for an alternative way for a disease management is often needed and the use of resistant varieties, biological agents and natural products such as plant extracts, microbial metabolites are among the alternatives for chemical agents (Gopinath *et al*., 2006; Than *et al*., 2008; Susheela, 2012; Chaisemsaeng *et al*., 2013). In the present study, we tested antifungal effect of leaf extracts against *C. capsici* by poisoned food technique. This technique is widely employed to screen antifungal effect of crude plant extracts against several phytopathogenic fungi (Kumar *et al*., 2007; Rakesh *et al*., 2013; Dileep *et al*., 2013). We observed inhibitory potential of leaf extracts against mycelial growth of the fungus *C. capsici*. It has been observed that the extract of *M. malabaricum* exhibited stronger antifungal effect than extract of *M. talboltianum*. In an earlier study, it has been shown that methanol extract of *M. malabaricum* possess antifungal activity against *Aspergillus* species and *Fusariumoxysporum* (Hullatti and Rai, 2004).

Urinary tract infections (UTIs) caused by the invasion of genitourinary tract by microorganisms are among the most common and serious health problems in both community and hospital settings affecting each year millions of people of all age groups. These infections are the important cause of mortality and morbidity in the world. UTIs are more common in women than men (Kattel *et al*., 2008; Ahmed *et al*., 2012). UTIs are caused by a number of bacteria. These infections are caused by a single species or in some cases, the infection may be polymicrobial. Among bacteria causing UTIs, Gram negative enteric bacillus *E. coli* is isolated more commonly from majority of cases of UTIs and remains the dominant cause of UTIs. Other bacteria such as *Klebsiell apneumoniae*, *Enterococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and others are also causing UTIs (Murshidi *et al*., 2002; Taneja *et al*., 2010; Nerurkar *et al*., 2012). UTIs are commonly treated by the use of antibiotics. However, extensive use of antibiotics has resulted in development of resistance against most commonly used drugs (Sharan *et al*., 2013). Hence, there is need for development of therapeutic agents from natural sources with activity against drug resistant uropathogens. Plants have shown to be promising sources for developing novel therapeutic agents (Manasa *et al*., 2013). In the present study, the leaf extract of *M. malabaricum* and *M. talboltianum* were shown to possess inhibitory activity against clinical isolates of UTIs. It has been found that extract of *M. malabaricum* exhibited stronger inhibitory activity against bacterial isolates when compared to extract of *M. talboltianum*. In a previous study of Sivu*et al*. (2013), extracts of *M. talboltianum* and *M. malabaricum* exhibited antibacterial activity with zones of inhibition ranging 9 to 21cm and 9 to 14cm respectively. Sekhar *et al*. (2013) observed slight to moderate antimicrobial activity of *M. talboltianum* against *K. pneumoniae* and *S. aureus* when compared to activity of *M. malabaricum*.

A number of assays are used to evaluate antioxidant activity of compounds. DPPH radical scavenging assay is one among the popular *in vitro* antioxidant assays. The DPPH is a stable, organic, nitrogen centred free radical which shows maximum absorption at 517nm in alcoholic solution. On accepting an electron or hydrogen atom, the radical becomes a stable diamagnetic molecule. In the presence of an extract with ability to donate hydrogen atom, the free radical nature of DPPH is lost and the purple color of the radical changes to yellow (diphenylpicrylhydrazine). This assay is widely employed to determine radical scavenging potential of various types of samples including plant extracts (Elmastas *et al*., 2006; Chung *et al*., 2006; Kaviarasan *et al*., 2007; Kekuda *et al*., 2011; Seruga *et al*., 2011; Rekha *et al*., 2012). In this study, we have determined the radical scavenging potential of leaf extracts of *M. malabaricum* and *M. talboltianum* by DPPH free radical scavenging assay. The decrease in absorption of DPPH radical solution was monitored in the presence of varying concentrations of leaf extracts at 517nm. It is noticed that the extracts at high concentrations caused marked decrease in the absorption of DPPH radicals. Among leaf extracts, high scavenging of radical was observed in case of *M. malabaricum* when compared to *M. talboltianum* as indicated by lower IC_{50} value. The results are in justification with the earlier study of Sekhar*et al*. (2013) in which extract of *M. malabaricum* scavenged DPPH radicals to higher extent than extract of *M. talboltianum*. However, the study of Sivu *et al*. (2013) revealed potent DPPH radical scavenging activity of leaf extract of *M. talboltianum* than that of leaf extract of *M. malabaricum*. Although the scavenging abilities of leaf extracts were lesser than reference antioxidant, it was evident that the leaf extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants (Chung *et al*., 2006).

Polyphenolic compounds including flavonoids are one of the most effective antioxidant compounds of plant kingdom. It becomes important to estimate total phenolic contents of extracts in order to assess their contribution to antioxidant activity and to compare their content with the antioxidant efficacy of extracts. In the present study, we estimated the total phenolic content of leaf extracts of *M. malabaricum* and *M. talboltianum* by FCR method. The FCR method is one of the oldest and commonly used

colorimetric assays used for the estimation of total phenolic content of a variety of substances including plant extracts. The phenolic compounds react with FCR under alkaline conditions only to form blue complex with maximum absorption near 750nm. Despite the undefined chemical nature of FCR, the assay for total phenolics by FCR method is rather convenient, simple, and reproducible. It is a routinely assay for studying the phenolic antioxidants (Chung *et al*., 2006; Kekuda *et al*., 2011; Rekha *et al*., 2012; Coruh *et al*., 2007; Junaid *et al*., 2013). The total phenolic content, as estimated in this study was higher in case of leaf extract of *M. malabaricum* when compared to *M. talboltianum*. Several literatures reported direct correlation between total phenolic content of plants and their antioxidant activity (Tilak *et al*., 2004; Coruh *et al*., 2007; Rekha *et al*., 2012; Kekuda *et al*., 2012; Kekuda *et al*., 2013). In this study also, a direct correlation has been observed between total phenolic content of leaf extracts and their radical scavenging activity. Extract of *M. malabaricum* possessing high phenolic content scavenged DPPH radicals more efficiently than extract of *M. talboltianum*.

CONCLUSION

The extract of *M. malabaricum* exhibited stronger antimicrobial and radical scavenging activity when compared to extract of *M. talboltianum*. The observed inhibitory activity of extracts could be attributed to the presence of phytoconstituents mainly phenolic constituents. These plants appear promising for control of phytopathogens and for development of pharmaceutical agents active against drug resistant uropathogens and radical induced damage. To the best of our knowledge, this is the first report on inhibitory potential of *Memecylon* species against *C. capsici* and drug resistant uropathogens. Further studies concerning isolation of active principles from leaf extracts and their radical scavenging and inhibitory efficacy determinations are to be carried out.

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