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Short Communication

Preliminary phytochemical analysis and Antimicrobial Activity of leaf extract of *Epiprinus Mallotiformis*

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ABSTRACT

Epiprinus mallotiformis (Muell.) is a tree belongs to the family Euphorbiaceae grows in the evergreen forests of the Western Ghats. The present study was performed to investigate the preliminary phytochemical analysis and antimicrobial activity of leaf extracts of *E. mallotiformis* the powdered leaf materials was subjected to Soxhlet extraction successively by using low polar to high polar solvents. The antimicrobial activity of leaf extracts was performed by agar well diffusion method. The preliminary phytochemical analysis shows the presence of Flavonoids, glycosides, saponins, steroids and tannins. Among the extracts methanol extract shows the significant activity when compare to all the solvent extracts. The maximum inhibition was found in *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi* fungi shows greater inhibition was found in *Microsporumgypseum, Trichophytonrubrum, Chrysosporiummerdarium.* The leaves of *E. mallotiformis* could be used in the treatment of bacterial and fungal infections; the presence of various phytochemicals might be the responsible for these activities of the extract. Further studies on isolation of constituents from the extract and their biological activities are under investigation.

Key wards: Phytochemical analysis, antimicrobial activity, leaf extract, *Epiprinus mallotiformis*.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. One of such resource is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds [1]. The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies [2]. Traditional medicine like orthodox medicine has its own methods and techniques of application which however aims at healing disease [3]. The treatment and control of diseases by the use of the available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries of the world. Nearly, all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. Due to ineffectiveness of most drugs as a result of microbial resistance to available agents mostespecially in developing countries, more patients are seen in medical centers. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or possibly eradicate the ever-increasing problems of emergence of newer diseases thought to be brought under control. In view of this, it is therefore very important to search for effective but of low cost and reliable traditional therapeutic agents, hence also the abuse of drugs for ailment is in high increase which motivated drug resistant organisms[4]. This situation forced scientists to search for new antimicrobial substances[5]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [6].

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Epiprinus mallotiformis Muell. belongs to the family Euphorbiaceae, it is distributed throughout Western Ghats and semi evergreen forests of Karnataka[7,8]. The plant is traditionally used to treat the diuretic, digestive problems, dysentery, external wounds, antimicrobial, laxative, remedy for vesical calculi, ulcers, gonorrhea etc. The present study is to carried out the Preliminary phytochemical analysis and antimicrobial activity of leaf extract of *E.mallotiformis*.

MATERIALS AND METHODS

Collection of plant materials: The leaves Sample of *Epiprinus mallotiformis* were collected from the Agumbe (13˚ 30′ N 75˚ 05′ E, 2154 Ft), Shimoga district of Karnataka. The region comes under malnad region, receives the maximum rain during the South West Monsoon. The samples were authenticated and herbarium was kept in the Department of Applied Botany, Kuvempu University Shankaraghatta, Shimoga district of Karnataka. Samples were cleaned and air dried, then powdered for future work.

Preparation of plant extracts: The powdered plant materials were subjected to the successive Soxhlet extraction method using pet-ether, chloroform, methanol and aqueous for a period of 24 hours. The extract obtained were concentrated to dryness is a rotary flash evaporator under reduced pressure and controlled temperature and stored at 4^0C in the refrigerator until further use.

Preliminary phytochemical analysis: Phyto chemical analysis of petroleum ether, chloroform, and methanol extract of the screened plants were done for the presence or absence of active secondary metabolites or different constituents such as tannins, alkaloids, flavanoids, terpenoids, steroids, glycosides and saponins. The dried extract was reconstituted in methanol and subjected to standard phytochemical analysis following the procedures of[9, 10]**.**

Source of microorganisms: The extracts were individually tested against a set of microorganisms causing infectious diseases to humans and plants *Xanthomonascampestris* (MTCC-2286), *Pseudomonassyringae* (MTCC-1604), *Agrobacteirumtumefaciens* (MTCC-431), *Klebsiellapneumonia* (MTCC-7028), *Escherichiacoli* (MTCC 1559), *Salmonellatyphi* (MTCC- 734), *Pseudomonasaeruginosa*(MTCC-1934), *Staphylococcusaureus* (MTCC-902). The tested fungi were *Candidaalbicans* (MTCC 1637), *Chrysosporiumindicum* (MTCC 2831), *Trichophytonrubrum* (MTCC 3272) and *Microsporumgypseum* (MTCC 2829), *Chrysosporiumkeratinophilum* (MTCC 1367), *Chrysosporiummerdarium* (MTCC 4608) and *Epidermophytonfloccosum (*MTCC 613) were procured from microbial type culture collection and gene bank, Chandigarh, India. The bacteria were maintained on nutrient broth (NB) at 37^0C and fungi were maintained on Sabouraud Dextrose agar (SDA) at 28^0C.

Antimicrobial activities

Antibacterial activity: The efficacy of the leaf extracts of *E. mallotiformis* was tested against some gram positive and gram-negative bacteria. Antibacterial activity was performed through agar well diffusion method by standard protocol. In this method, 24 hours old nutrient broth (HiMedia, Mumbai) cultures of the test bacteria was swabbed uniformly on solidified sterile nutrient agar (HiMedia, Mumbai) plate using a sterile cotton swab. Then, aseptically, wells of 6mm diameter were bored in the inoculation plates with the help of gel puncher.100µl of leaf extracts (100,50,25and 12.5 mg/ml of 10% DMSO), standard Ciprofloxacin, 1mg/ml and control 10%DMSO were added separately into the respective labelled wells. The plates were incubated at 37ºC for 24 hours in an upright position and the zone of inhibition formed around the well was recorded. The experiments were carried out in triplicates to get the average readings [11].

Antifungal activity: The efficacy of the leaf extracts of *E. mallotiformis* was tested against some human pathogenic fungi. Antifungal activity was performed through agar well diffusion method by standard protocol. In this method fungi were inoculated into sterile Sabouraud dextrose broth (HiMedia, Mumbai) incubated for 48 hours at 28°C. The fungi were aseptically swabbed on sterile Sabouraud dextrose agar (HiMedia, Mumbai) respectively using sterile cotton swabs followed by punching wells of 6mm diameter using sterile cork borer.100µl of leaf extracts (100,50, 25 and 12.5 mg/ml of 10% DMSO), standard fluconazole1mg/ml and control 10% DMSO were transferred into respectively labeled wells. The plates were incubated 48 hours inan upright position and the zone of inhibition formed around the well was recorded. The experiments were carried out in triplicates to get the average readings [12].

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaf extracts of *E.mallotiformis* showed the presence of Flavonoids, glycosides, saponins, steroids, and

tannins. Whereas, alkaloids was found to be absent. The results were recorded as presence and absence of zone of inhibition around the well. In this study the extract shown inhibition of antimicrobial activity is dose dependent manner. Methanol extract showed greater inhibition zone fallowed by aqueous, chloroform and pet. ether. The results of antibacterial activity of leaf extracts were summarized in the Table 2. The maximum inhibition was found in *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi.* Whereas least minimum zone of inhibition was found in *Agrobacteirumtumefaciens* fallowed by *Pseudomonas syringae.* The standard antibiotic showed the greater inhibition activity when compare to all the four different solvent extracts. No inhibition zone was observed in tested bacteria in case of control. The inhibitory activity of the leaf extracts of *E.mallotiformis* against tested fungi was revealed by the presence of zone of inhibition around the well and is depicted in Table no 3. Among the all the extracts, greater antifungal activity was found in methanol fallowed by Aquious pet. Ether and chloroform. Among the tested fungi greater inhibition was found in *Microsporumgypseum, Trichophytonrubrum, Chrysosporiummerdarium.* Whereasleast zone of inhibition was found in *Candida albicans, Chrysosporiumkeratinophilum, Epidermophytonfloccosum.* The standard antifungal shows the greater inhibitory activity when compare to all the four solvent extracts. No inhibition was observed in the control against tested fungi.

Infectious diseases caused by bacteria, fungi, viruses and parasites remain a major threat to public health despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicine and the emergence of widespread drug resistance[13]**.** Medicinal plants have been used for centuries as remedies for diseases.Antimicrobials of plant origin have enormous therapeutic potential which are due to the presence ofcertain metabolites. During the 2nd half of the 20th century, the acceptance of traditional medicine as an alternate form of primary healthcare and the drug resistance problems faced by the therapy using classical antibiotics led the researchers to investigate antimicrobial efficacy of several plants. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constitutes of plants are alkaloids, tannins, flavonoids and phenolic compounds [14].Plants-derived productions have received attention in recent years due to their diverse pharmacological activities [15]. The antimicrobial activities of and alkaloids[16], terpenoids[17], flavonoids [18], saponins[19], terpenoids[17] have been documented. In the present study, phyto-constituents namely flavonoids, glycosides, saponins, steroids and tannins were detected in the extracts, which may account for the antibacterial and antifungal activities. Dermatomycosesare the superficial fungal infections in humans and are caused by dermatophytes, a group of filamentous fungi. These fungi invade and draw nutrients from the keratinized tissues such as skin, hair and nails. Among the dermatophyte genera, *Trichophyton*, *Microsporum*and *Epidermophyton* are most important. As the dermatophytes have developed resistance to antimycotic drugs, there is an urgent need for non-toxic, safe and cost-effective antifungal agents [20,21]. In view of this, the present study highlights the possible use of plants in the treatment of fungal infections like Aspergillosis, Candidiasis and Dermatomycoses, as the test fungi were found to exhibit susceptibility to the extract.

CONCLUSION

Plants represent an unlimited source of phytochemicals. The phytochemicals present in plants consist of primary and secondary metabolites. The higher plants collectively accumulated many secondary metabolites that can be mainly classified into alkaloids, steroids, flavonoids, saponins, tanins etc., there have been widespread studies on the biological activities of higher plants relatively few studies are reported regarding isolation, purification and characterization of active compounds. In the present study an attempt has been made to study the phytochemical analysis and antimicrobial activity on leaf extract of *E. mallotiformis.* The extracts *E. mallotiformis* strong antimicrobial activity against bacteriaand fungi. Methanol extract of *E. mallotiformis* shows significant activity against gram negative bacteria and fungal activity against *Microsporumgypseum, Trichophytonrubrum*. Further isolation and purification of the extracts are required to determine the active components responsible for their activity. Although our results support the idea that *E. mallotiformis* extracts are candidate for treatment of infectious diseases, clinical trials will be required to confirm its antimicrobial action and general safety.

Table 1: showing the phytochemical analysis of leaf extract of *Epiprinus mallotiformisMuell.*

Sl no	Test extract	Petroleum extract	Chloroform extracts	Methanol extract	Water extract
1	**Alkaloids**				
a	Mayer's test	-	-	-	-
b	Wagner's test	-	-	-	-
2	**Flavonoides**				
a	Ferric chlorides test	-	-	+	+
b	Alkaline reagent test	-	-	+	+
3	**Glycosides**				
a	Keller-killiani test	-	+	-	+
b	Bromine water test	-	+	-	+
4	**Saponins**				
a	Foam test	+	+	+	-
5	**Steroides**				
a	Salkowaski test	-	+	+	+
b	Lieberman-burchard test	-	+	+	+
6	**Tannins**				
a	Ferric chloride test	-	-	+	+
b	Gelatin test	-	-	+	+

Note: '+'- Present; '-'- Absent.

Table 2: Antibacterial activity of leaf extract of *E. mallotiformis*

MO	Zone of inhibition (mm)																	
	Pet ether				Chloroform				Methanol				Water				Control	Std.
	100%	50%	25%	12.5%	100%	50%	25%	12.5%	100%	50%	25%	12.5%	100%	50%	25%	12.5%		
KP	9±0.76	8±0.57	-	-	10±0.86	8±1.0	-	-	12±1.25	8±0.50	-	-	8±0.76	7±1.5	-	-	-	29±0.5
EC	9±0.76	8±0.5	-	-	8±0.57	-	-	-	12±1.04	8±0.86	7±1.15	-	7±0.86	-	-	-	-	15±1.04
PA	10±1.04	-	-	-	10±1.25	8±0.50	7±0.86	-	12±0.76	8±0.50	7±0.57	-	9±0.50	7±0.86	-	-	-	23±0.76
ST	10±1.60	8±0.50	-	-	9±0.86	7±0.28	-	-	10±1.04	8±1.25	7±1.15	-	9±1.80	8±0.50	7±0.28	-	-	14±1.25
SA	9±0.76	8±0.57	-	-	10±1.04	7±0.86	-	-	10±0.76	9±0.57	-	-	8±1.25	-	-	-	-	17±1.25
XC	8±0.86	-	-	-	9±0.76	8±1.15	-	-	10±1.04	9±0.76	7±0.57	-	8±0.50	7±0.86	-	-	-	27±0.76
PS	9±1.52	7±0.57	-	-	8±1.15	7±1.32	-	-	9±1.52	8±1.15	7±0.76	-	8±1.73	7±1.15	-	-	-	30±1.50
AT	7±0.57	-	-	-	8±1.52	-	-	-	12±1.0	10±1.52	7±1.15	-	8±1.52	7±0.76	-	-	-	26±1.41

Note: '-'- No activity

Table 3: Antifungal activity of leaf extract of *E. mallotiformis*

MO	Zone of inhibition (mm)																	
	Pet ether				Chloroform				Methanol				Water				Control	Std.
	100%	50%	25%	12.5%	100%	50%	25%	12.5%	100%	50%	25%	12.5%	100%	50%	25%	12.5%		
CA	8±1.04	7±1.0	-	-	9±0.76	8±0.50	-	-	10±1.50	8±0.50	-	-	9±0.76	7±0.57	-	-	-	30±1.25
CM	12±0.76	9±0.50	7±0.86	-	8±0.50	7±1.0	-	-	11±0.50	9±0.76	8±1.04	-	12±1.60	11±0.86	9±0.50	8±0.57	-	20±1.25
CL	9±0.28	7±0.57	-	-	10±1.0	8±0.76	-	-	8±1.04	7±0.28	-	-	10±0.76	9±0.50	8±0.57	-	-	25±0.50
TR	10±0.86	8±0.76	-	-	8±0.50	-	-	-	12±0.57	10±1.25	9±0.76	8±0.57	9±0.50	8±0.76	-	-	-	22±0.50
MG	9±0.50	7±0.57	-	-	10±0.76	8±0.50	-	-	14±0.76	12±1.0	8±0.50	-	9±050.	-	-	-	-	28±0.76
CK	8±0.50	7±0.86	-	-	9±0.76	7±0.57	-	-	12±0.76	10±1.0	8±1.25	-	9±0.50	7±0.86	-	-	-	26±1.25
EF	10±1.0	7±0.28	-	-	8±0.76	-	-	-	10±1.0	9±0.50	7±0.28	-	9±0.76	8±0.50	-	-	-	28±1.25

Note: '-'- No activity

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