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Antimicrobial Activity of Methanolic Extract of *Merrimea gagentica* and *Litsea glutinosa* Leaves

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Methanolic extract of leaves of *Merremia gagentica* (MEMG) and *Litsea glutinosa* (MELG) were investigated for their *in vitro* antimicrobial properties by agar disc diffusion method. The crude methanolic extracts of MEMG and MELG inhibited the growth of both Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). The Gram positive bacteria tested appeared to be more susceptible to the extracts than the Gram negative bacteria. Both the extracts at the concentration range between 250 and 1000 µg/mL showed inhibitory activity against all tested bacteria except MEMG which did not show activity against *S. typhimurium* at 250 µg/mL concentration. At 100 µg/mL concentration of MEMG was found neutral against *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* while MELG was neutral against *Escherichia coli* and *Pseudomonas aeruginosa* at same concentration. The extracts also showed significant antifungal activity against *Aspergillus niger* and *Candida albicans*. All tested microorganisms showed dose dependent susceptibility towards the methanolic extracts. The antibacterial and antifungal activity of the extracts and standard drugs were statistically significant. Based on the current findings, it can be concluded that both the plants possess potent antimicrobial activity.

Key Words: *Merremia gagentica*, *Litsea glutinosa*, Antimicrobial activity, Agar disc diffusion method.

INTRODUCTION

In recent years there has been a growing interest to evaluate plants possessing antimicrobial activities for various diseases[1]. Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and morality worldwide[2]. During the past several years there has been an increasing incidence of bacterial and fungal infections due to a growth in immuno compromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment

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with several antimicrobial drugs[3] has been the reason for an extended search for newer drugs to treat opportunistic microbial infec-tions[4]. Synthetic antifungal/antibacterial drugs widely used at present are sometimes causing toxicity and adverse drug reactions. Further more herbal medicines and supplementation are considered less toxic than the synthetic compounds[5].

Merremia gagentica; (Family: *Convolvulaceae* (Morning glory family). This is a slender, prostates, creeping, smooth or somewhat hairy herb. The stems root at the nodes and are 10-80 cm in length. The leaves are small, kidney-shaped to somewhat heart-shaped, 6-15 mm long, often wider than long and irregularly toothed. One to three flowers occur on short stalks in the axils of the leaves. The sepals are rounded and about 4 mm long, with few to many white, weak hairs. The corolla is yellow and nearly twice as long as the calyx. The capsule is rounded and about 5 mm in diameter. The decocted leaves and tops are sometimes employed as a diuretic[6-9]. In India, the leaves are useful as a diuretic and an alterative and used in rheumatism and neuralgia.

Litsea glutinosa (Lour.) C.B. Rob., Lauraceae is moderately-sized tree; bark thin, grey or pale brown; live bark 3 mm thick, pale brown, very slimy. Branchlets rather slender, stiff, minutely tomentellous towards apex, hairs very slender; terminal with dense layer of sub-appressed, glossy, long hairs. Leaves 7-15 × 3-7 cm, spirally arranged, variable in size, usually oblong-oval or elliptic, shortly acuminate or obtuse, base acute, chartaceous to stiffly chartaceous, very densely, finely areolate-reticulate above, glabrous; midrib and slender lateral veins prominent, basal part of midrib often pilose, slightly impressed; paler beneath, minutely reticulate, very sparsely, minutely pilose, soon glabrous, midrib and lateral veins prominent, lateral veins slender, erect-patent, c. 8-12 pairs, secondary veins parallel, not horizontal. Petiole 1.5-3 cm long, slender, pilose. Flower umbels numerous, densely grey-tomentellous, 4-5 mm diameter; peduncles up to 5 mm long, slender, densely pilose on slender, short branches up to 14 mm long; perianth tube silky, funnel-shaped; tepals 0; stamens up to 20 with slender, very hairy filaments; glands on long stalks. Fruit c. 6 mm diameter, globose, purplish black, on flat, 4 mm diameter thin disc; fruiting pedicel slender[10-14]. The seeds contain an aromatic oil which has been used to make candles and soap. The roots yield fibres used in Thailand for rope manufacture and for paper pulp. The fruits have a sweet creamy edible pulp. The young leaves are eaten by livestock. The pounded seeds are also applied medicinally against boils. The leaves and the mucilage in the gum from the bark have been used for poultices. The bark also acts as a demulcent and mild astringent in diarrhoea and dysentery.

However, no work has been done so far on the antimicrobial properties of these two plants keeping this in view the present study was undertaken to investigate the antimicrobial activity of the methanol extracts of leaves of *Merremia gagentica* (MEMG) and *Litsea glutinosa* (MELG) against various strains of bacteria and fungi.

EXPERIMENTAL

The entire herb of *Litsea glutinosa* has been collected in the month of December from Mangulam village near Madurai and authenticated. The plant was shade dried and pulverized. The plant materials *Merremia gagentica* were collected in the month of April from Narasingampatti, Madurai district of Tamilnadu. The plant materials were taxonomically identified by taxanomist and the voucher specimens have been preserved in our laboratory for future reference.

Extraction: The plant materials were dried under shade with occasional shifiting and then powdered with a mechanical grinder and stored in air-tight containers. The dried materials were defatted separately with petroleum ether (60-80 ºC) in a Soxhlet apparatus.The defatted powdered thus obtained was further extracted separately with methanol (80 %) in Soxhlet apparatus for 72 h. The solvent was completely removed by distillation under suction and the resultant semi solid mass was dried using the rotary flash evaporator to yield a solid residue. The dried MEMG and MELG were dissolved in dimethyl sulfoxide and used for the present study.

Preliminary phytochemical screening: Preliminary phytochemical screening of the MEMG and MELG showed the presence of flavanoids, saponins, terpenoids, fatty acids and phenolic acids.

Microorganisms and media: The following bacterial strains used were *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 10240) *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 7853) and *Salmonella typhi* (ATCC 43579). The fungal species used were *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), for the present study. The bacterial and fungal cultures were maintained on Muller Hinton Agar Medium and Sabouraud dextrose agar slants, respectively, which were stored at 4 ºC. Eight microorganisms maintained on nutrient agar base were used to assess the antimicrobial activity of the plant extracts. The fungi were maintained on Sabouraud dextrose agar, which is often used with antibiotics for the isolation of pathogenic fungi.

Antimicrobial screening: Agar cultures of the test microorganisms were prepared as described by several workers[15-17]. Three to five similar colonies were selected and transferred to 5 mL broth with a loop and the broth cultures were incubated for 24 h at 37 ºC. The MEMG and MELG were dissolved in dimethyl sulfoxide with a magnetic stirrer. For screening sterile 6 mm diameter filter paper discs were impregnated with 100-1000 µg of the MEMG and MELG and then placed in Muller Hinton Agar Medium. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was 1×10^5 colony forming units/mL. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity. All data regarding antimicrobial activity are the average of triplicate anaylsis. The antibacterial amikacin (10 µg/mL) and antifungal griseofulvin (20 µg/mL) was used as reference as standards as recommended by National Committee for clinical laboratories standards.

Statistical analysis: Data are reported as the mean ± SD of three measurements. Statistical analysis was performed by student's t-test[18].

RESULTS AND DISCUSSION

It was absorbed (Table-1) that both the extracts showed antibacterial activity against all tested organisms. However no activity was seen against *E. coli* and *S. typhimurium* at 100 µg/mL concentration. It was also observed that the methanol extracts exhibited antifungal activity against *A. niger* and *C. albicans* in a dose dependent manner (Table-2). Tested extracts at higher concentration exhibits comparable antimicrobial activity with that of the standard drugs.

TABLE-1
ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *Merrimea gagentica* (MEMG) *Litsea glutinosa* (MELG) AND STANDARD ANTIBIOTIC AMIKACIN

Samples	Conc. (µg/mL)	Diameter of zone of inhibition (mm)					
		BS	SA	ML	EC	PA	ST
MEMG	100	9.7 ± 1.7	10.6 ± 0.7	12.3 ±0.3	–	9.8 ± 2.3	–
	250	9.8 ± 0.5	12.3 ± 1.8	6.9 ± 0.1	9.2 ± 0.6	8.2 ± 1.7	–
	500	15.8 ± 1.0	16.7 ± 0.7*	9.2 ±1.8	11.0 ±1.4	11.9 ± 1.0	7.8 ± 1.0
	1000	18.3 ± 1.2	20.8 ± 1.0	13.5 ±0.6	16.8 ± 0.4	17.9 ± 0.9	10.7 ± 1.2
MELG	100	9.20 ± 1.2	7.34 ± 1.4	8.56 ± 0.7	–	–	8.02 ± 0.6
	250	10.83 ± 0.3	9.15 ± 0.5*	9.98 ± 0.3	7.56 ± 1.1	7.34 ±1.2	9.58 ± 0.4
	500	12.58 ± 1.0	11.87 ± 1.0	12.49 ± 1.5	11.20 ±1.4	8.75 ± 1.1	10.9 ± 1.0
	1000	15.56 ± 1.4	14.34 ± 1.2	14.89 ± 0.6	13.10 ± 0.4	9.86 ±1.1	12.45 ± 1.4
Amikacin	10	22.2 ± 0.6	21.9 ± 1.0	16.5 ± 1.0	18.7 ± 0.6	22.7 ± 1.4	21.6 ± 0.6

– = No inhibition zone; BS = *Bacillus subtilis*, SA = *Staphylococcus aureus*, ML = *Micrococcuc luteus*, EC = *Escherichia coli*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhimurium*; Values are mean ± SD (mm) of three separate experiments Statistical value *p < 0.05 when compared to standard.

TABLE-2
ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF *Merrimea gagentica*, (MEMG) *Litsea glutinosa* (MELG) AND STANDARD ANTIBIOTIC GRISEOFULVIN

Samples	Conc. (µg/mL)	Diameter of zone of inhibition (mm)	
		Aspergillus niger	*Candida albicans*
MEMG	100	6.30 ± 0.8	12.70 ± 1.2
	250	9.80 ± 1.3	15.80 ± 1.7
	500	11.60 ± 0.4	19.70 ± 0.3
	1000	14.70 ± 1.1	20.20 ± 0.9
MELG	100	7.87 ± 0.6	12.23 ± 1.3
	250	10.36 ± 0.9	14.87 ± 1.5
	500	12.65 ± 0.7	17.59 ± 0.5
	1000	16.78 ± 1.2	20.87 ± 1.1
Griseofulvin	20	20.80 ± 0.8	21.37 ± 1.1

Values are mean ± SD (mm) of three separate experiments.

Disc diffusion methods are used extensively to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of the substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. Due to limited capacity of discs, holes or cylinders are preferably used[19].

Most of the bacterial species and the fungal species were inhibited by the plant extract as shown in Tables 1 and 2. In this study 8 different bacterial and fungal species were used to screen the possible antimicrobial activites of the MEMG and MELG. The MEMG and MELG showed a broad spectrum of activity against all the bacterial strains at the tested concentration of 100-1000 µg/mL. Amikacin (10 µg/mL) and griseofulvin (20 µg/mL) were used as positive controls for bacteria and fungi, respectively. As reported earlier secondary metabolites like flavonoids, saponins are likely responsible for the observed antibacterial activity of plants[20-22].

Conclusion

As evident from the results antibacterial activities extract are more pronounced on Gram positive than on Gram negative bacteria and these findings correlate with the observation of the various screenings of medicinal plants for antibacterial activity where the most of the active plants showed activity against Gram positive strains only[23]. The antimicrobial activities of these plants may be due to the presence of active principles present in their leaves. In addition the results confirmed the evidence in previous studies which reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, ethanol and hexane[24-26].

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