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Research article

ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF SOME TRADITIONAL MEDICINAL PLANTS FROM TAMIL NADU, INDIA

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ABSTRACT

Objective: We assessed the antimicrobial activity of methanol extracts of 4 medicinal plants against human pathogenic Gram positive and Gram negative bacteria and fungi. Four medicinal plants namely *Terminalia arjuna*(bark), *Butea monosperma*(bark), *Mollugo nudicaulis*(whole plant), *Evolvulus alsinoides*(whole plant) were collected from different places in Tamil Nadu, India.

Methods: Methanol extracts of these plants were assessed for antimicrobial activity using disc diffusion method at 5mg/disc and 10mg/disc. Minimum inhibitory concentration (MIC) and phytochemical analysis were also determined.

Results: Methanol extract inhibited the growth of bacterial and fungal strains. Minimum inhibitory concentration (MIC) of *T.arjuna* (bark) methanol extract was 500µg/ml against Gram positive and Gram negative bacteria. The MIC of *B.monosperma* (bark) methanol extract was 250µg/ml against fungi. The phytochemical analysis of methanol extracts of *T.arjuna* and *B.monosperma* showed the presence of glycosides, flavinoids, steroids and tannins. *T.arjuna* and *B.monosperma* showed good antimicrobial activity.

Conclusion: It can be concluded that the methanol extract possesses potent bactericidal and fungicidal activity which in turn may be due to the presence of biologically active ingredients with antimicrobial activity in the medicinal plants.

Keywords: Antibacterial, Antifungal, *Terminalia arjuna, Butea monosperma,* Minimum inhibitory concentration (MIC).

INTRODUCTION

Nature has been a source of medicinal agents for many years and an impressive number of modern drugs has been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [1]. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since the discovery of penicillin (1929) and its use in chemotherapy in 1941 as a response to the great fatalities in the Second World War, a great number of important antibiotics have been found [2]. The investigation of certain local plants for their biological properties may yield useful results. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloid constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds. These substances emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus [3].

Commonly *Butea monosperma* is used as a tonic, astringent, aphrodisiac and diuretics [4]. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours (Mengi and [5]. It is reported to possess antifertility, aphrodisiac and analgesic activities [6]. Flowers are useful in diarrhoea, astringent, diuretic, depurative and tonic [7]. The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite. Besides medicinal uses, it is also having the economic use such as leaves are used for making platters, cups, bowls and beedi wrappers. Bark fibres are used for making cordage. Wood is used for well curbs and water scoop. It is a cheap board, wood.Wood pulp is suitable for newsprint manufacturing [8]. Butea is also a host to the Lac insect, which produces natural lacquer [9]. The novel pharmacological action of *B. monosperma* indicates a potential therapeutic value for the treatment of inflammatory and other diseases and these compounds given as a drug to play a lead role [10].

T.arjuna bark contains calcium salts and a little amount of tannin, magnesium and is astringent, diuretic, prostaglandin enhancer and anti-oxidant. It is mainly used for both prevention and treatment of heart diseases, including angina, heart failure and hyper cholesterolemic. It is also used in treating asthma, impotence and to help bones regain their strength. The juice made of the leaves was also once used as a cure for dysentery and earache. Although hundreds of plant species have been tested for the antimicrobial properties, the vast majority of them has not been adequately evaluated [11]. Considering the vast potentiality of plants as sources for antimicrobial drugs a systematic investigation is needed to screen the local flora [12]. 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 [13]. 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 [14]. The present study was conducted to investigate the antimicrobial properties of four medicinal plants namely, *Terminalia arjuna, Butea monosperma, Mollugo nudicaulis* and *Evolvulus alsinoides.*

Materials and methods

Chemicals and media

Dimethyl sulphoxide (DMSO), Mueller Hinton Agar (MHA), Sabouraud dextrose agar (SDA), the antibacterial agent ciprofloxacin and antifungal agent fluconazole were obtained from Himedia, Mumbai, India. Methanol was obtained from Rankem company, India.

Collection of plants and identification

The plants *Terminalia arjuna, Butea monosperma, Mollugo nudicaulis* and *Evolvulus alsinoides* were collected from different places in

Tamil Nadu and authenticated by a taxonomist Dr. P. Pandikumar from Entomology Research Institute, Loyola College, Chennai. The voucher specimens (No.ERI/ETHPH/TA/240-243) were deposited in the herbarium of the Entomology Research Institute, Loyola College, Chennai.

Preparation of Plant extracts

The plants were collected and shade dried at room temperature and ground in a manual mill. The powder was extracted with methanol for a period of 48h. The extract was filtered through Buchner funnel with Whatmann No.1 filter paper. The filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The remaining residue of the plant material was extracted with the same methanol in a similar manner to get more extract. The extract was stored at 4˚C until further use.

Preparation of Inoculum

Bacterial inoculums were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37˚C. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28˚C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB) at 28˚C for 48 h.

Antibacterial activity

The following bacteria were used for the experiments; *Enterobacter aerogens* (MTCC 111), Methicillin resistant *Staphylococcus aureus* (MRSA), *Vibrio parahaemolyticus* (MTCC 451), *Klebsiella pneumoniae* (MTCC 109), *Micrococcus luteus* (MTCC 106), *Yersinia enterocolitica* (MTCC 840), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 3615), *Staphylococcus aureus* (ATCC 25923).The methanol solvent bark extracts of *Terminalia arjuna* and *Butea monosperma* and whole plant extracts of *Mollugo nudicaulis* and *Evolvulus alsinoides* were tested by disc diffusion method [15]. Different concentrations of the extracts (10mg/disc and 5mg/disc) were applied on the sterile discs. 25µl of each extract was added to the sterile discs and placed on the agar plate. Ciprofloxacin was used as an antibacterial agent for positive control. The plates were incubated at 37˚C for 24h. The diameters of the inhibition zones were measured in mm.

Antifungal activity

The antifungal activity was screened by disc diffusion method [16]. The following fungal pathogens were used for the experiments: *Scopulariopsis* sp, *Candida albicans, Malassezia pachydermatis*, *Candida parapsilosis, Botyritis cinerea, Aspergillus flavus, Candida krusei, Trichophyton mentagrophytes.* The sterile discs were treated with 25µl of each extract containing different (10mg/disc, 5mg/disc) concentrations. Fluconazole was used as an antifungal agent for positive control. The activity was determined after 72h of incubation at 28˚C. The diameters of the zones of inhibition were measured in mm.

Phytochemical analysis

The active methanol extracts of *T.arjuna* and *B.monosperma* were analyzed for alkaloids flavanoids, glycosides, tannins, steroids and carbohydrates etc [17-19]. About 50 mg of the solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows: Mayer's and dragendorff's test [20, 21] was adopted. To a 1 ml of filtrate, few drops of Mayer's reagent were added by the side of the test tube. The white creamy precipitate indicated test as positive. To a 1 ml of filtrate, 2 ml of Dragendorff's reagent was added and the result was observed carefully. A prominent yellow precipitate confirmed the test as positive for the presence of alkaloids. For flavonoids test the aqueous solution of the methanol extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids. For glycosides, chloroform (3ml) and ammonia solution (10%) were added to 2ml plant extract. Formation of pink color indicated the presence of glycosides. For tannins test [22] the extract (5 mg) was dissolved in 5 ml of distilled water and a few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicated the presence of tannins. For steroids, to 0.5 ml of the plant extract equal volume of chloroform was added along with a few drops of concentrated sulfuric acid. Appearance of brown ring indicated the presence of steroids. For carbohydrates, one ml of the extract was boiled on water bath with 1 ml each of Fehling solutions A and B. The color change was observed. A red precipitates indicated the presence of sugar (Fehlings test). To 0.5 ml of extract, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes and the result was observed. A red precipitates indicated the presence of sugar (Benedict's test).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations were assessed for the plant extracts using standard reference methods for bacteria [23] and for filamentous fungi [24]. 20mg of the crude extract was dissolved in 1ml DMSO and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100µl from each well was inoculated. The antifungal agents fluconazole for fungi and streptomycin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48–72 hrs at 28°C and for bacteria the plates were incubated for 24 h at 37˚C. The MIC for fungi was defined as the lowest extract concentration showing no visible fungal growth after incubation time. 5 µl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.

RESULTS

The methanol extracts of four medicinal plants, namely bark extracts of *Terminalia arjuna* and *Butea monosperma* and whole plant extracts of *Mollugo nudicaulis* and *Evolvulus alsinoides* exhibited promising activity against bacteria and fungi. The activities of methanol extract against bacteria are given in Tables 1and 2. *Terminalia arjuna and Butea monosperma* showed appreciable activity against Gram positive bacteria: *S.aureus25923* (12 mm), *M. luteus* (11 mm), *S. epidermidis 3615* (11 mm) and *S. aureus* MRSA (10 mm), *S.aureus96* (11mm) and Gram negative bacteria: *E.aerogens111* (11mm), *V.parahaemolyticus 451* (11mm), *K.pneumoniae109* (10mm) and *Y.enterocolitica 840* (10mm). The activites of methanol extract against fungi are given in Tables 3 and 4. The methanol extract of *T.arjuna* and *B.monosperma* showed promising activity against all the fungal pathogens except *Trichophyton mentagrophytes* at 5mg/disc concentration. The phytochemical tests showed the presence of substances like Alkaloids, Flavonoids, Glycosides, steroids, tannins and carbohydrates in *T.arjuna* and *B.monosperma* (Table 5). Minimum inhibitory concentrations (MIC) varied. *T.arjuna* (bark) methanol extract showed the following MIC values: *S.aureus* (500µg/ml), *S.aureus 25923* (500µg/ml), *K.pneumoniae* (1000µg/ml), *S.aureus* MRSA (1000µg/ml), *V.parahaemolyticus* (1000µg/ml), *M.luteus* (1000µg/ml) and *S. epidermidis* (1000µg/ml); no activity was seen against *E.aerogens, Y.enterocolitica* (Table 6). The methanol extract showed the following MIC values: *C.albicans* (250µg/ml), *Scopulariopsis* sp.(500µg/ml), *A.flavus* (500µg/ml), *M. pachydermatis* (1000µg/ml), *C. parapsilosis* (1000µg/ml), *B.cinerea* (1000µg/ml) and *C.krusei* (1000µg/ml); no activity was noted against *T.mentagrophytes* (Table 7).

Table 1: Antibacterial activity of methanol extracts of medicinal plants (zone of inhibition in mm)

5mg/disc	*T.arjuna* (bark)	*B.monosperma* (bark)	*M.nudicaulis* (whole plant)	*E.alsinoides* (whole plant)	C
E.aerogens (MTCC 111)	11	10	-	-	21
S.aureus (MRSA)	11	10	10	-	16
V.parahaemolyticus(MTCC 451)	10	-	-	-	29
K.pneumoniae (MTCC 109)	10	10	10	-	29
M.luteus (MTCC 106)	10	-	-	-	14
Y.enterocolitica (MTCC 840)	-	11	-	-	17
S.aureus(MTCC 96)	10	-	-	-	24
S. epidermidis (MTCC 3615)	11	10	-	-	29
S.aureus (ATCC 25923)	11	10	-	-	14

-: no activity ; C-Control (Ciprofloxacin)

Table 2: Antibacterial activity of methanol extracts of medicinal plants (zone of inhibition in mm)

10mg/disc	*T.arjuna* (bark)	*B.monosperma* (bark)	*M.nudicaulis* (whole plant)	*E.alsinoides* (whole plant)	C
E.aerogens (MTCC 111)	10	10	-	-	21
S.aureus (MRSA)	10	10	10	-	16
V.parahaemolyticus (MTCC 451)	11	10	-	-	29
K. Pneumonia (MTCC 109)	11	10	10	10	29
M.luteus(MTCC 106)	11	10	-	-	14
Y.enterocolitica (MTCC 840)	10	10	11	-	17
S.aureus(MTCC 96)	12	10	-	-	17
S. epidermidis (MTCC 3615)	11	10	-	11	24
S.aureus (ATCC 25923)	11	10	10	-	29

-: no activity ; C-Control (Ciprofloxacin)

Table 3: Antifungal activity of methanol extracts of medicinal plants (zone of inhibition in mm)

5mg/disc	*T.arjuna* (bark)	*B.monosperma* (bark)	*M.nudicaulis* (whole plant)	*E.alsinoides* (whole plant)	FLU
Scopulariopsis sp.	11	13	10	11	20
C.albicans	11	13	10	10	18
M. pachydermatis	10	12	11	10	16
C. parapsilosis	12	11	10	11	22
B.cinerea	11	12	12	10	24
A.flavus	12	11	11	11	29
C.krusei	13	12	10	11	19
T.mentagrophytes	11	12	-	11	18

-: no activity ; FLU- Fluconazole (Antifungal agent)

Table 4: Antifungal activity of methanol extracts of medicinal plants (zone of inhibition in mm)

10mg/disc	*T.arjuna* (bark)	*B.monosperma* (bark)	*M.nudicaulis* (whole plant)	*E.alsinoides* (whole plant)	FLU
Scopulariopsis sp.	11	13	10	11	20
C.albicans	10	13	10	11	18
M. pachydermatis	10	11	11	12	16
C. parapsilosis	11	11	10	10	22
B.cinerea	11	12	11	12	24
A.flavus	10	11	12	12	29
C.krusei	10	11	10	11	19
T.mentagrophytes	11	12	10	10	18

Note: Flu- Fluconazole (Antifungal agent)

Table 5: Phytochemical analysis of *T.arjuna* and *B.monosperma* (methanol extract)

Phytochemicals	*T.arjuna* (met)	*B.monosperma* (met)
Alkaloids	+	-
Flavonoids	+	+
Glycosides	++	-
Steroids	+	-
Tannins	++	+
Carbohydrates	+	-

Note: (++)-High concentration, (+)-moderate concentration, (-)-absent

Table 6: Minimum inhibitory concentration of methanol extract of *T.arjuna* against tested bacteria

Organism	*T.arjuna* (bark)	Streptomycin
E.aerogens (MTCC 111)	-	>25µg/ml
S.aureus (MRSA)	1000µg/ml	>6.25µg/ml
V.parahaemolyticus (MTCC 451)	1000µg/ml	>6.25µg/ml
K.pneumoniae (MTCC 109)	1000µg/ml	>25µg/ml
M.luteus(MTCC 106)	1000µg/ml	>6.25µg/ml
Y.enterocolitica (MTCC 840)	-	>30µg/ml
S.aureus(MTCC 96)	500µg/ml	>6.25µg/ml
S. epidermidis (MTCC 3615)	1000µg/ml	>25µg/ml
S.aureus (ATCC 25923)	500µg/ml	>6.25µg/ml

Table 7: Minimum inhibitory concentration of methanol extract from *B.monosperma* against tested fungi

Organism	*B.monosperma* (bark)	Fluconazole
Scopulariopsis sp.	500µg/ml	<12.5 µg/ml
C. albicans	250µg/ml	>100 µg/ml
M. pachydermatis	1000µg/ml	>12.5 µg/ml
C. parapsilosis	1000µg/ml	>100 µg/ml
B. cinerea	1000µg/ml	-
A. flavus	500µg/ml	>25 µg/ml
C. krusei	1000µg/ml	>100 µg/ml
T. mentagrophytes	-	>25µg/ml

DISCUSSION

In this communication we report the antimicrobial activity of methanol extracts of four traditional medicinal plants from Tamil Nadu, India. The methanol extracts of four medicinal plants, namely bark extracts of *Terminalia arjuna* and *Butea monosperma* and whole plant extracts of *Mollugo nudicaulis* and *Evolvulus alsinoides* exhibited promising activity against bacteria and fungi. The activities of methanol extract against bacteria are given in Tables 1and 2. *Terminalia arjuna and Butea monosperma* showed appreciable activity against Gram positive bacteria: *S.aureus25923* (12 mm), *M. luteus* (11 mm), *S. epidermidis 3615* (11 mm) and *S. aureus* MRSA (10 mm), *S.aureus96* (11mm) and Gram negative bacteria: *E.aerogens111* (11mm), *V.parahaemolyticus 450* (11mm), *K.pneumoniae109* (10mm) and *Y.enterocolitica 840* (10mm). Earlier, [25] reported that the aqueous extract of *T. avicemoides* inhibited all the *Shigella* isolates and *E.coli*. [26] reported similiar antimicrobial activity of methanol extract for *P. pterocarpum* flowers against *B. subtilis, S. aureus, S. epidermidis, E. faecalis, E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris,* and *C. albicans*. [27] reported that the extracts of *B.monosperma* inhibited the Gram positive bacteria better than gram negative bacteria.

The results of methanol extract against fungi are given in Tables 3 and 4. The methanol extract of *T.arjuna* and *B.monosperma* showed promising activity against all the fungal pathogens except *Trichophyton mentagrophytes* at 5mg/disc concentration. [28] also reported antifungal activity. *B.monosperma* showed promising results against all the fungal pathogens with maximum zone inhibition of 14mm against *Candida albicans.* The phytochemical tests showed the presence of substances like Alkaloids, Flavonoids, Glycosides, steroids, tannins and carbohydrates in *T.arjuna* and *B.monosperma* (Table 5). [29] reported that the phytochemical screening of *P.dulce pod pulp* extract indicated the presence of phytosterols, flavonoids, terpenes, triterpenoid, saponins in total ethanolic extracts.

Minimum inhibitory concentrations (MIC) varied. *T.arjuna* (bark) methanol extract showed the following MIC values: *S.aureus* (500µg/ml), *S.aureus 25923* (500µg/ml), *K.pneumoniae* (1000µg/ml), *S.aureus* MRSA (1000µg/ml), *V.parahaemolyticus* (1000µg/ml), *M.luteus* (1000µg/ml) and *S. epidermidis* (1000µg/ml); no activity was seen against *E.aerogens, Y.enterocolitica* (Table 6). [25] reported that *T. avicennoides* had the lowest MIC value and provided a range of 243.8–431.3 µg/mL against *shigellae* isolates. *B.monosperma* (bark) methanol extract showed the following MIC values: *C.albicans* (250µg/ml), *Scopulariopsis* sp.(500µg/ml), *A.flavus* (500µg/ml), *M. pachydermatis* (1000µg/ml), *C. parapsilosis* (1000µg/ml), *B.cinerea* (1000µg/ml) and *C.krusei* (1000µg/ml); no activity was noted against *T.mentagrophytes* (Table 7). [28] reported similiar MIC values against *Bacillus subtilis* and *Bacillus cereus*. Future investigations of phytochemical research on these plants may produce novel compounds for the treatment of various diseases.

CONCLUSION

Methanol extracts of *Terminalia arjuna* (Bark), *Butea monosperma* (Bark), *Mollugo nudicaulis* (Whole plant) and *Evolvulus alsinoides* (Whole plant) showed a broad-spectrum of activity against both Gram-positive and Gram-negative bacteria and fungi.

Conflict of interest statement

We declare that we have no conflict of interest.

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