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Antimicrobial studies on the extract of *Benkara malabarica* (Lam.) triveng and *Tarenna asiatica* (L.) kuntze Ex K. schum

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Abstract
Extracts of *Benkara malabarica* (Lam.) *Triveng* (BM) and *Tarenna asiatica* (L.) Kuntze ex K. Schum (TA) was screened for their *in vitro* antimicrobial activity by agar disc diffusion method. The antimicrobial activity of Petroleum ether, chloroform, methanol and acetone extracts of the leaves of these plants were studied using five bacterial cultures and the five fungal cultures as test organisms. Acetone extracts of BM and TA were found to be more effective for antibacterial against *S.paratypi* and *P.aeruginosa* and antifungal against *M.rubes*, respectively when compared to other extract of BM and TA and it reveals the presence of alkaloids and steroids respectively which suggests that these phytoconstituent may be responsible for their antimicrobial activity.

Keywords: antimicrobial activity, *Benkara malabarica*, *Tarenna asiatica*, disc diffusion method, leaves

Introduction
Rubiaceae is a family of flowering plants various called the madder family, bedstraw family or coffee family. Rubiaceae family is a large family of 630 genera and about 1300 species found worldwide, especially in tropical and warm regions. Rubiaceae species were a valuable source of new secondary metabolites for medical purposes [1]. The traditional therapeutic use of the plants has been for skin disorders and against cancer. Furthermore, the Rubiaceae family exhibit *in vivo* some interesting biological properties, such as antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic and mutagenic functions. Apart from their medicinal value, these plants are also used as natural food colourants and as natural hair dyes [2]. *Benkara malabarica* (Lam.) *Triveng* (Rubiaceae) is found in tropical and subtropical Asia. The plant species is relatively unexplored with only few reports like arthritis [3], antimicrobial [4], antimycobacterial activity[5] activity in some other plants.

Benkara malabarica (Lam.) Triveng

Tarenna asiatica (L.) Kuntze ex K. Schum

Tarenna asiatica (L.) *Kuntze ex K. Schum* (Rubiaceae). There are about 370 species distributed across the tropical world from Africa to Asia and the pacific islands. There are shrubs and trees with oppositely arranged leaves and terminal assays of whitish, greenish or yellowish flowers [6]. The antimicrobial activity is the capacity of the substance to kill or inhibit microorganism, these are several reports that plants are acting against microorganisms. Hence, in the present study the petroleum ether, ethyl acetate chloroform and acetone extracts of TA and BM belong to Rubiaceae are used against five bacterial and five fungal organisms. Since there is no report on antimicrobial activity of BM and TA an attempt was made to evaluate the antimicrobial activity of petroleum ether, chloroform, ethyl acetate and acetone extracts of leaves for both the plants by agar diffusion method.

Materials and Methods

Plant materials

BM and TA were collected from Madukkarai area, Coimbatore, Tamil Nadu and authenticated by Botanical survey of India; Voucher specimens (BSI/SRC/5/23/2014-15/Tech/1215) have been preserved in the herbarium for the future reference.

Shade dried leaves were coarsely powdered and subjected to successive solvent extraction by using Soxhlet apparatus. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80^{0}C), chloroform, ethyl acetate and acetone. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antimicrobial activity.

Preliminary Phytochemical Screening

The powder leaves of BM and TA (20g) was subjected to successive extraction with different solvents in their increasing order of polarity from Petroleum ether (PE), Chloroform (CE), Ethyl acetate (EA) and Acetone (AE). The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents.

Antibacterial activity

Plant crude extract of different solvents were tested for their *in vitro* antibacterial activity against five pathogenic bacteria used for the study were *Escherichia coli, Psedumonas aeruginosa, Salmonella paratypi, Staphylococcus aureus* and *Bacillus subtilis* using Muller-Hint agar medium by disc diffusion technique [7]. Sterile Muller-Hinton agar plates were prepared and the agar surface was inoculated with the bacteria. Extracts were dissolved in 1 ml of DMSO in various concentrations in separate tubes. Commercially available sterile discs were soaked in the preparation for half an hour. It was then placed in empty petri plates for air-drying. Using sterile forceps, the discs were placed on the surface of the agar plates and gently pressed on to the agar surface. The culture plates were inverted and incubated for 24-48 hrs at 37°C. After incubation, zone of clearance was observed and its diameter measured using microscope.

Antifungal activity

The *in vitro* antifungal activity of plant crude extract and the isolated compounds 1 and 2 was studied against the five fungal cultures were *Candida albicans, Aspergillus niger, Aspergillus fumigates, Aspergillus parasites* and *Monosus rubes* for antifungal, assays measuring inhibition of mycelia growth on agar media were used. Compounds were dissolved in 1 ml of sterile Dimethyl sulfoxide (DMSO) serving as a stock solution. Then it was transferred it to 4 ml Sabouraud dextrose agar (SDA) growth media in separate tubes and autoclaved at 121°C for 15 minutes. These tubes were allowed to cool to 50 ^{0}C and non solidified SDA of each tube was loaded with various concentration of drug solution. Tubes were then allowed to solidify at room temperature. Then each glass tube was inoculated with 4 m diameter piece of inoculums removed from 7 days old culture of fungus [8]. All these tubes were incubated at 28±1°C for 10 days. A relative humidity was maintained at 40-50% in the incubation room. Growth in the media was determined [9] by measuring linear growth (mm) of the plant crude extract and the compounds.

Result and Discussion

For *Benkara malabarica* (Lam.) Triveng the antibacterial assay, acetone extract shows higher activity against *S.aureus* and *S.paratypi,* moderate activity shows ethyl acetate and chloroform extract against *S.paratypi* and *S.aureus* and the lowest activity against Petroleum ether extract against *B.Subtilis.* (Table.1 and Fig.1). For antifungal activity acetone extract shows good activity against *M.ruber,* moderate activity showed petroleum ether against *A.fumigates* and the lowest activity against *A.parasites* (Table.2 and Fig.2). For *Tarenna asiatica* (L.) *Kuntze ex K. Schum* the antibacterial activity of acetone extract shows good activity against all the five bacterial strains, and the lowest activity showed Petroleum ether against *B.subtilis* (Table.3 and Fig.3). In antifungal activity, the highest activity against all the fungal cultures of acetone, ethyl acetate and chloroform extract and the lowest activity against *A.parasites* of petroleum ether extract (Table. 4 and Fig. 4).

Table 1: Antibacterial activity of different extracts of *Benkara malabarica*

Organisms	Zone of inhibition of extract in mm				
	STD	**PEE**	**EAE**	**CE**	**AE**
Escherichia coli	40±0.54	09±0.1	13±0.8	14±0.4	22±0.8
Psedumonas aeruginosa	36±0.72	09±0.5	13±0.27	14±0.11	22±0.56
Salmonella paratypi	41±0.64	12±0.3	22±0.74	17±0.21	37±0.43
Staphylococcus aureus	35±0.42	11±0.4	20±0.62	16±0.45	38±0.47
Bacillus subtilis	44±0.52	0	18±0.4	07±0.52	36±0.72

PEE-Petroleum ether extract, EAE-Ethylacetate extract, CE-Chloroform extract and AE-Acetone extract

Table 2: Antifungal activity of different extracts of *Benkara malabarica*

Organisms	Zone of inhibition of extract in mm				
	STD	**PEE**	**EAE**	**CE**	**AE**
Candida albicans	13±0.45	10±0.06	11±0.72	09±0.42	13±0.42
Aspergillus niger	15±0.65	14±0.04	11±0.67	07±0.64	13±0.12
Aspergillus fumigates	14±0.72	17±0.21	21±0.03	12±0.47	12±0.16
Aspergillus parasites	11±0.41	10±0.43	13±0.01	05±0.36	16±0.76
Monosus rubes	21±0.52	24±0.47	17±0.51	18±0.75	40±0.73

PEE-Petroleum ether extract, EAE-Ethylacetate extract, CE-Chloroform extract and AE-Acetone extract

Table 3: Antibacterial activity of different extracts of *Tarenna asiatica*

Organisms	Zone of inhibition of extract in mm				
	STD	PEE	EAE	CE	AE
Escherichia coli	40±0.54	15±0.75	20±0.04	20±0.52	32±0.82
Psedumonas aeruginosa	36±0.72	20±0.21	29±0.56	29±0.64	39±0.84
Salmonella paratypi	41±0.64	16±0.02	32±0.08	32±0.72	40±0.75
Staphylococcus aureus	35±0.42	18±0.61	25±0.10	25±0.61	32±72
Bacillus subtilis	44±0.52	13±0.31	21±0.46	21±0.82	42±0.95

PEE-Petroleum ether extract, EAE-Ethylacetate extract, CE-Chloroform extract and AE-Acetone extract

Table 4: Antifungal activity of different extracts of *Tarenna asiatica*

Organisms	Zone of inhibition of extract in mm				
	STD	PEE	EAE	CE	AE
Candida albicans	13±0.45	09±0.46	10±0.07	11±0.21	13±0.35
Aspergillus niger	15±0.65	07±0.02	11±0.42	14±0.35	15±0.43
Aspergillus fumigates	14±0.72	12±0.06	14±0.51	17±0.42	21±0.52
Aspergillus parasites	11±0.41	05±0.47	10±0.61	11±0.47	13±0.61
Monosus rubes	21±0.52	17±0.24	20±0.21	25±0.52	32±0.78

PEE-Petroleum ether extract, EAE-Ethylacetate extract, CE-Chloroform extract and AE-Acetone extract

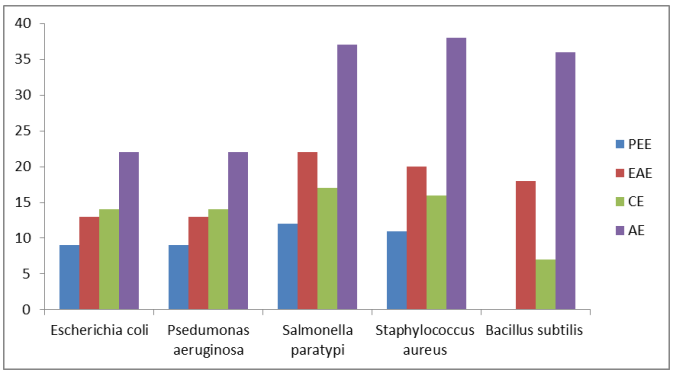

Fig 1: Antibacterial activity of different extracts of *Benkara malabarica*

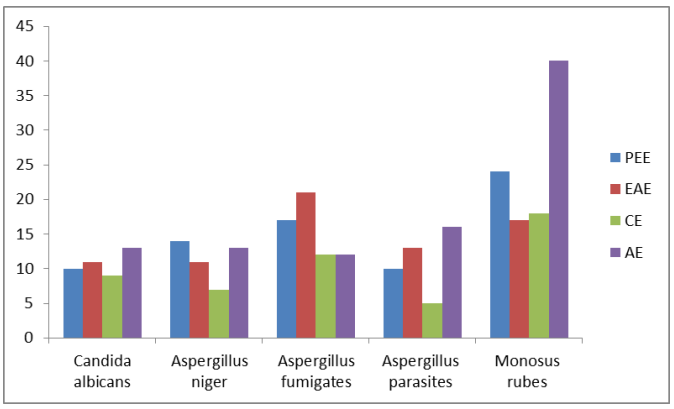

Fig 2: Antifungal activity of different extracts of *Benkara malabarica*

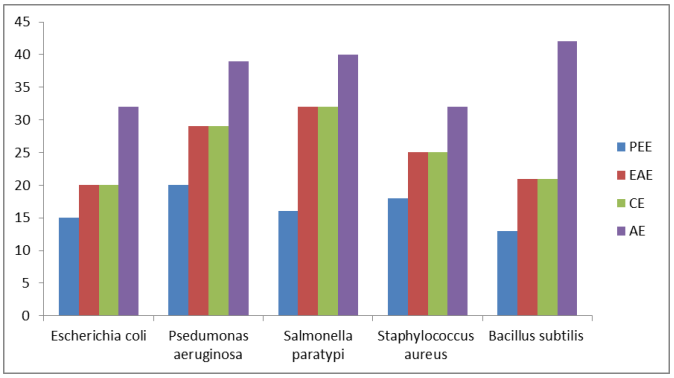

Fig 3: Antibacterial activity of different extracts of *Tarenna asiatica*

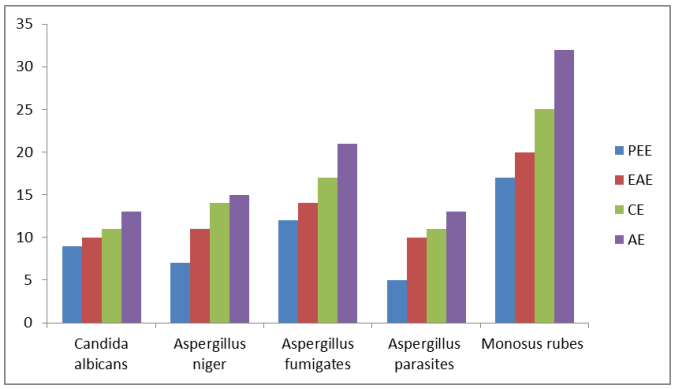

Fig 4: Antifungal activity of different extracts of *Tarenna asiatica*

Table 4: Phytochemical screening of different extracts of both the plants

	Benkara malabarica				*Tarenna asiatica*			
Phytoconstituents	**PEE**	**EAE**	**CE**	**AE**	**PEE**	**EAE**	**CE**	**AE**
Alkaloids	+	+	+	+	+	+	+	+
Carbohydrates	-	-	-	+	-	+	-	+
Glycosides	-	-	-	+	-	-	-	+
Saponins	-	-	-	+	-	-	-	+
Steroids	+	-	-	+	-	+	-	+
Flavanoids	-	-	+	+	-	-	+	+
Tannins	-	-	+	+	-	-	+	+

PEE-Petroleum ether extract, EAE-Ethylacetate extract, CE-Chloroform extract and AE-Acetone Extract

Phytochemical screening of the different extract of *B.malabarica* and *T.asiatica* showed the presence of alkaloids, tannins, saponins, flavonoids, coumarins and sugar (Table. 4). Phytochemical screening of the petroleum ether, ethylacetate, chloroform and acetone extract of the BM and TA revealed the presence of alkaloids and steroids. Further studies are needed to isolate and characterize the bioactive compounds.

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