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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Various Solvent Extracts of Some Selected Medicinal Plants Present in Jaffna Peninsula

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ABSTRACT

Medicinal plants have been very intensively screened for their bioactivity in order to treat various disease and disorders in human. In this study some common medicinal plants (*Cassia fistula*, *Heliotropium indicum*, *Mimosa pudica*, *Rhinacanthus nasutus*, *Pongamia pinnata* and *Vernonia zeylanica*) available in Jaffna peninsula, Sri Lanka, were tested for their antibacterial activity. Leaf powders of above plants were sequentially extracted with petroleum ether, ethyl acetate, ethanol and water respectively. The dried extracts were tested for their antibacterial activity against *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by agar well diffusion method. The yield percentage was relatively high in tested polar solvents than tested non polar or low polar solvents. Among the tested extracts, aqueous extract of *Mimosa pudica* and ethyl acetate extract of *Cassia fistula* revealed significantly higher inhibition against *S. aureus* and *E. coli* respectively. The test extracts of *Mimosa pudica* showed interesting results, all of its extracts were able to inhibit both *S. aureus* and *E. coli*. Most of the tested extracts could be used for further screening of specific bioactive compounds which responsible for their antibacterial activity.

Key words: Medicinal plants, sequential extraction, antibacterial activity, well diffusion method.

INTRODUCTION

In recent years plants that have been used in traditional practices are highly targeted by the scientists who involve in screening of alternative sources for western medicine. The available antimicrobials for infectious disease control have several drawbacks; they are very expensive, development of resistance in pathogens, harmful side effects.[1] In traditional practices plant based preparations are the major medicine for the treatment of infectious diseases and they have very low level risk compared to presently available synthetic antimicrobials. [2]

Cassia fistula, (common name: Kondal, Sarakkonrai) a member of Leguminosae is a deciduous tree with greenish grey bark, compound leaves, leaflets are each 5-12 cm long pairs, fruit is cylindrical pod and seeds many in black, sweet pulp separated by transverse partitions. Yellow flowers in lax racemes 30-50 cm. long; pedicels 3.8-5.7 cm long, slender, pubescent and glaborous. [3] This plant has been used in traditional medicines for different purposes; root is useful in fever, heart diseases, retained excretions and biliousness. Fruits are used as catharatic and in snake bite. Flowers and pods are used as purgative, febrifugal, biliousness and astringent. Juice of leaves is useful as dressing for ringworm, relieving irritation and relief of dropsical swelling. [4]

Heliotropium indicum (common name: Therlkodukku) (family name: Boraginaceae) is an annual, erect, branched hirsute plant, about 15 to 50 cm hight. The leaves are always opposite or alternate, ovate to oblong-ovate, somewhat hairy, acute or acuminate, base decurren along the petiole and about 3 to 8 cm long. The flowers are calyx green and about 3.5 mm in diameter. The fruits are dry 2 to 4 lobed of 2 or 4 nearly free, more or less united nutlets, 4 to 5 mm long.[5] Traditionally, leaf extracts of this plant have been found useful in ulcers, wounds, local inflammation, urticaria, ringworm infection, rheumatism, boils, psoriasis, stings and dermatitis. [6]

Mimosa pudica (common name: Thoddavadi) (family name: Fabaceae) is a diffusely spreading,

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half-woody herb, with branched stems up to one meter long, sparingly prickly with numerous deflexed, bristly hairs. The leaves are very sensitive, both pinnae and leaflets, folding when touched. Pinnae are usually four, digitately arranged at the end of each petiole, and 4 to 9 cm long. The leaflets are narrowly oblong, inequilateral, 1 to 1.5 cm long, sessile, sparingly bristly, with pointed tips. Heads are longpeduncled, solitary or 2 to 3 in each axil, about 1 cm in diameter. Pods are flat, slightly recurved, 1 to 2 cm long.[7] The roots and leaves are commonly used in treatment as bitter, astringent, acrid, cooling vulnerary, alexipharmic, diuretic antispasmodic, emetic, constipating and febrifuge.[8]

Rhinacanthus nasutus (common name: Anichchai) (family name: Acanthaceae) is a perennial shrub. The plant is 60-76cm in height, leaves are ovate to oblong, sparsely to densely pubescent, 4-6 cm long, base rounded to cordate, apex short acuminate. Flowers are violent in color.[9] Various parts of this plant have been used for the treatment in various diseases such as eczema, pulmonary tuberculosis, herpes,[10] The leaves and stems of this plant are often used for the treatment of hepatitis, diabetes, hypertension and skin disease.[11]

Pongamia pinnata (common name: Pungu) (Family name: Leguminosae) is a medium sized glabrous, perennial tree. Leaves are alternate, odd pinnately compound, 2 to 4 inches, evergreen, hairless. Flowers are lavender, pink; white, 2- 4 together, short-stalked, pea shaped, 15-18mm long. The pods are 3-6cm long and 2-3cm wide, smooth, brown, thick-walled, hard, indehiscent, 1-2 seeded. Seeds are compressed ovoid or elliptical, been-like, 10-15cm long, dark brown, oily.[12] All parts of the plant have been used as a crude drug for the treatment of tumors, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea.[13]

Vernonia zeylanica (common name: Kuppilai) (family name: Compositae) is an under shrub with many straggling, divaricate, cylindrical branches, finely tomentose when young; leaves simple, alternate, fiddle shaped, white with fine wool beneath, rather thick and stiff, venation reticulate. Flower heads small, numerous, very shortly stalked, irregularly corymbose, very pale violet. Fruit a faintly 5-ribbed pubescent achene with a yellowish white pappus, the outer row being scanty and extremely short. This plant is used as it promotes the fusion of bones. The leaves ground into a paste and applied on boils promote suppuration. Toasted with turmeric and applied for eczema of the legs with beneficial results. Internally, the juice of the leaves is used for treatment of asthma.[14]

Present study was aimed to reveal antibacterial activity of different solvent extracts of *Cassia fistula*, *Heliotropium indicum*, *Mimosa pudica*, *Rhinacanthus nasutus*, *Pongamia pinnata* and *Vernonia zeylanica* against *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922).

MATERIALS AND METHODS

Collection of plant materials

Fresh and healthy leaves of *Cassia fistula*, *Heliotropium indicum*, *Mimosa pudica*, *Rhinacanthus nasutus*, *Pongamia pinnata* and *Vernonia zeylanica* were collected from the herbal garden, Unit of Siddha Medicine, University of Jaffna, Sri Lanka. The collected samples were thoroughly washed under running tap water, dried in shade and then ground into fine powders using an electric grinder. These powders were stored in air sealed brown bottles at 4 ^{0}C until used.

Preparation of plant extracts

Each plant powder was successively extracted with different organic solvents in increasing polarity order according to Jeyaseelan *et al.*[15] Briefly, 100 g of each powder was soaked in 300 ml Petroleum ether separately with intermittent shaking for three days. They were first filtered with double layered muslin cloth and then through WhatmanNo1 filter paper. The residue was further extracted two times by using fresh Petroleum ether solvent. Then all the filtrates were pooled together. The resulting residue was air dried and used for further extraction with ethyl acetate and followed by ethanol and sterile water similar to the procedure that carried out for the Petroleum ether extraction. Finally solvents were removed from the extracts by treating at 40 ^{0}C in an oven. After complete drying, yield of the each extraction was measured separately and the extracts were stored at 4 ^{0}C until used for further study.

Test bacteria

The standard test bacteria *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) used in this study were obtained from Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka. The cultures were stored on nutrient agar slants at 4 °C temperature, and before the antibacterial testing they were sub cultured on nutrient agar medium.

Determination of Antibacterial activity

Antibacterial activity of the test extracts was tested by agar well diffusion method. Nutrient agar plates were prepared by incorporating 1 ml of test bacteria (0.5 McFarland standards) into 20 ml of molten nutrient agar. After solidification of the medium, wells were made using 8 mm diameter of sterile cork borer, and 100 µl of each of the test extracts (300 mg/ml), Streptomycin (30 mg/ml) and control (Petroleum ether, ethyl acetate, ethanol and sterile water) were added into the wells separately. Plates were incubated at 37 °C for 24 hours. The antibacterial activity of the test samples was determined by measuring the diameter of clear zone around the well [16]. Three replicates were maintained for each experiment.

Statistical analysis

The results obtained for antibacterial activity were given as mean value ± standard deviation and the data were subjected to examine by analysis of variance (ANOVA) ($P<0.05$) followed by Tukey's test ($\alpha = 0.05$) by using a software, SPSS 13.0 for Windows version.

RESULTS AND DISCUSSION

In the sequential extraction of test plant powders with Petroleum ether, ethyl acetate, ethanol and water, *Cassia fistula* and *Pongamia pinnata* powders gave their lowest yield in petroleum ether and higher yield in water extraction. Other remaining plant powders, *Heliotropium indicum, Mimosa pudica, Rhinacanthus nasutus* and *Vernonia zeylanica* showed lower yield for ethyl acetate extraction and higher yield for water extraction. In overall, the yield percentage is high in polar solvents than non polar or low polar solvents. *Cassia fistula, Heliotropium indicum* and *Vernonia zeylanica* had higher amount of polar compounds compared to other three plant powder, as well as *Mimosa pudica* and *Vernonia zeylanica* had reveled higher amount of non polar compounds than other four plant powders (**Table 1**).

In vitro antibacterial activity of sequential extracts of *Cassia fistula, Heliotropium indicum, Mimosa pudica, Rhinacanthus nasutus, Pongamia pinnata* and *Vernonia zeylanica* against *S. aureus* and *E. coli* showed antibacterial activity in various degrees. Among the tested extracts, aqueous extract of *Mimosa pudica* and ethyl acetate extract of *Cassia fistula* revealed significantly higher inhibition against *S. aureus* and *E. coli* respectively (**Table 2**).

Even though aqueous extract of *Cassia fistula, Heliotropium indicum, Pongamia pinnata* and *Vernonia zeylanica* failed to inhibit *S. aureus*, aqueous extracts of *Rhinacanthus nasutus* and *Mimosa pudica* revealed their inhibition on *S. aureus* in higher level compared to other tested extracts. On the other hand, only the *Mimosa pudica* and *Vernonia zeylanica* aqueous extracts exhibited inhibition against *E. coli*. Interestingly the aqueous extract of *Mimosa pudica* produced inhibition on both *S. auresus* and *E. coli* and there is no significant difference in their activity on both bacteria (Table 2).

Ethyl acetate and ethanol extracts of all plant powders tested were able to inhibit the growth of both *S. aureus* and *E. coli*, where *S. aureus* was more sensitive to ethanol extracts of all plant powders than ethyl acetate extracts. On the other hand, ethyl acetate extracts of *Cassia fistula* and *Pongamia pinnata* possessed significantly higher inhibitory effect on *E. coli* compared to ethanol extracts of the same plant powders. Conversely, in the other four ethanol extracts revealed significantly higher inhibition rather than ethyl acetate extracts of the same plant powder (Table 2).

All the tested petroleum ether extracts were able to inhibit the growth of *S. aureus*, but in the case of *E. coli* only *Rhinacanthus nasutus* and *Mimosa pudica* had inhibition. The diameter of clear zone produced by the petroleum ether extracts of *Rhinacanthus nasutus, Mimosa pudica, Cassia fistula* and *Heliotropium indicum* were higher than that produced by ethyl acetate extracts of same plants against *S. aureus*. But in the other plant powders investigated, ethyl acetate revealed higher inhibitory effect than petroleum ether.

The test extracts of *Mimosa pudica* showed interesting results, all of its extracts were able to inhibit both *S. aureus* and *E. coli*. The antibacterial activity of a plant extracts depends on the available bioactive compounds, secondary metabolites like Tannins, Saponins, Terpenoids, Alkaloids and Flavonoids.[17] Therefore, further isolation and identification of specific bioactive molecule or molecules from tested plant extracts could be a valuable alternative for the synthetic antimicrobial drugs.

Table 1: Yield percentage of different solvent extracts of tested plants

Test Plants	Yield Percentage (%)			
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous
Cassia fistula	2.20	4.16	6.49	11.29
Heliotropium indicum	2.98	1.95	3.91	11.23
Rhinacanthus nasutus	2.39	1.31	4.67	6.25
Mimosa pudica	4.50	1.50	3.63	9.38
Vernonia zeylanica	3.95	3.25	6.38	9.81
Pongamia pinnata	1.97	3.42	2.97	4.22

Table 2: Antibacterial activity of different solvent extracts of tested plants

Test plants	Extracts	Diameter of inhibition zone (mm)*	
		S. aureus	*E. coli*
Cassia fistula	Petroleum ether	13.8 ± 0.8^{de}	-
	Ethyl acetate	12.7 ± 0.6^{ef}	26.7 ± 0.6^{a}
	Ethanol	16.2 ± 0.3^{c}	22.5 ± 0.5^{b}
	Aqueous	-	-
Heliotropium indicum	Petroleum ether	12.8 ± 0.8^{ef}	-
	Ethyl acetate	11.0 ± 0.0^{gh}	11.7 ± 0.6^{i}
	Ethanol	12.2 ± 0.8^{fg}	13.7 ± 0.6^{gh}
	Aqueous	-	-
Rhinacanthus nasutus	Petroleum ether	16.8 ± 0.3^{c}	10.0 ± 0.0^{j}
	Ethyl acetate	12.0 ± 0.0^{fg}	10.0 ± 0.0^{j}
	Ethanol	19.3 ± 0.6^{b}	14.2 ± 0.3^{fg}
	Aqueous	20.3 ± 0.6^{b}	-
Mimosa pudica	Petroleum ether	14.5 ± 0.5^{d}	10.0 ± 0.5^{j}
	Ethyl acetate	10.0 ± 0.0^{h}	13.0 ± 0.0^{h}
	Ethanol	12.3 ± 0.6^{fg}	20.7 ± 0.6^{c}
	Aqueous	22.2 ± 0.3^{a}	22.7 ± 0.6^{b}
Vernonia zeylanica	Petroleum ether	12.0 ± 0.0^{fg}	-
	Ethyl acetate	16.2 ± 0.3^{c}	20.2 ± 0.3^{c}
	Ethanol	17.0 ± 0.0^{c}	22.3 ± 0.6^{b}
	Aqueous	-	17.3 ± 0.6^{d}
Pongamia pinnata	Petroleum ether	14.3 ± 0.6^{d}	-
	Ethyl acetate	16.0 ± 0.0^{c}	16.2 ± 0.3^{e}
	Ethanol	20.3 ± 0.6^{b}	15.0 ± 0.0^{f}
	Aqueous	-	-

**Diameter of inhibition zones includes the diameter of well (8mm); Values = mean ± SD; Values with different superscript in the same column differ significantly (P<0.05); (-) – no clear zone.*

CONCLUSION

In the present *in vitro* antibacterial study many of the tested extracts revealed greater antibacterial activity against tested bacteria. Therefore, they could be further subjected for screening and identification of active ingredients which are responsible for the antibacterial activity.

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