Screening for Anti-Microbial and Phyto Chemical Properties of Different Solvents Extracts of Leafs of Pongamia Pinnata

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***Abstract*-** Plants based natural products have been widely used as curative agents for variety of ailments since immemorial time. In this study, powered plant material, leaves of Pongamia pinnata sequentially extracted using petroleum ether, ethyl acetate, ethanol, methanol and water and the extracts were concentrated to dryness by evaporating the solvent at 40oC. In vitro antibacterial activities of these extracts were studied by agar well diffusion method against gram positive Staphylococcus aureus and gram negative Escherichia coli. Streptomycin and different solvent extracts (petroleum ether, ethyl acetate, ethanol, methanol and water) were used as standard and control respectively. Phytochemical analysis also done to report the presence of biomolecules. This study demontratrated that the ethyl acetate extracts was higher antibacterial activity against tested bacterial pathogens than other extracts. All these extracts were able to inhibit the growth of S.aureus except aqueous extract. Phytochemical screening of the methanol, ethanol and aqueous plant leaf extracts revealed the presence of saponins and terpinods. The tannin was present in methanol extract and flavonoids was also present in methanol and ethanol extracts. The results also indicated the less antibacterial activity of different solvent extracts to standard, streptomycin and control also rather than didn't reveal any antibacterial activity against the pathogens. This present study concluded that the antibacterial activity of various extracts of leaves of P.pinnata was carried in attempt to support the use by medicinal practitioner for the treatment of various diseases. However, further studies should be needed for the isolation and characterization of these active compounds.

***Index Terms*-** Atibacterial activity, P.pinnata, different solvent extracts.

I. INTRODUCTION

Various medicinal plants have been used for many years in daily life to treat disease in all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Natural products play an important role in drug development programs in the pharmaceutical [1].

Pongamia pinnata (L.) [syn. *Pongamia glabra*(Vent); *Derris indica*(Lamk.)] belongs to family Leguminosae. It is a medium sized glabrous, perennial tree grows in the littoral regions of South eastern Asia, especially it is an exotic species to Sri Lanka. This plant contains alternate, odd pinnately compound, 2 to 4 inches, evergreen, hairless leaves. All parts of this plants have medicinal properties and traditionally used as medicinal plants. They have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers [2]. Besides this, the plant posssess antiflammatory, anti plasmodial, anti nonciceptive, anti- lipidperoxidative, anti- diarrhoeal, anti-ulcer, anti-hyperammonic and anti oxidant activity.[3]. Particularly, leaves have anthelminthitic, digestive and laxative used for inflammations, piles and wounds [4], and juice of the leaves is taken for cold, cough, diarrhoea, dyspepsia, flatulence, gonorrhea and leprosy [5].

Today there is wide spread interest in drugs obtained from natural plant products for their potential antibacterial activity. Therefore in the present study an attempt was made to findout the phytochemical constitutes and antibacterial effects of leaves of *P.pinnata* (L.), against *E.coli* and *S.aureus*.

II. MATERIALS AND METHODS

Preparation of plant extracts

The healthy plant leaves were collected from Unit of Siddha medicine, University of Jaffna, Sri Lanka and Fresh leaves were washed under running tap water and dried in shade. Dried leaves were ground into fine powder using electric blender. The powder was successively extracted using solvents of increasing polarity according to Arokiyaraj *et al* (2009) with some modifications. 15 g powder was initially soaked in 60ml of petroleum ether in air tight conical flask for two days and then it was first filtered through double layered muslin cloth and then filtered through Whatman no 1 filter paper and filtrate was collected into sterile air tight bottle. Similar process was repeated twice with fresh petroleum ether and the filtrate was collected together. After all, petroleum ether was removed from the filtrate at 40^{o}C using oven and the extract was stored at the refrigerator for further studies. Likewise, the above dried residue was used for sequential extraction of ethyl acetate, ethanol, methanol and water.

Phytochemical analysis

The extracts were subjected to phytochemical analysis to detected the presence of following biomolecules using the standard qualitative procedures as described by Trease and Evans (1989) [6].

a) Test for tannins

To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue black coloration.

b) Test for terpenoids

5 ml of extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed for the presence of terpenoids.

c) Test for saponins

5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

d) Test for flavonoids

A few drops of 1% NH_3 solution was added to the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

e) Test for cardiac glycosides

1 ml of concentrated H_2SO_4 was taken in a test tube. 5 ml of extract was mixed with 2 ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$.The above mixture was carefully added to the 1 ml of concentrated H_2SO_4. Presence of cardiac glycosides was detected by the formation of a brown ring.

f) Test for phlobatannins

10 ml of extract was boiled with 1% HCl in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins.

g) Test for Alkaloids

1ml of 1% HCl was added to 3ml of extract in a test tube and was treated with few drop of Meyer's reagent. A creamy white precipitate indicted the presence of alkaloids.

h) Test for Resins

5ml of copper was added to 5ml of extract. The resulting solution was shaken vigorously and allowed to separate. A green colored precipitate indicated the presence of resin.

i) Test for Glycosides

10ml of 50% H_2SO_4 was added to 1ml of extract in a boiling tube. The mixture was heated in boiling water for 5min. 10ml of Fehling's solution (5ml of each solution A and B) was added and boiled. A brick red precipitate indicated presence of glycosides.

j) Test for Anthraquinones

Extract was mixed well with benzene, and then half of its own volume of 10% ammonia solution was added. Presence of a pink, red or violet coloration in the ammonial phase indicated the anthraquinones.

III. TEST MICROORAGANISMS

Test bacteria Gram negative *E.coli*, and Gram positive *Staphylococcus aureus* were procured from bacterial culture collection, Department of Botany, University of Jaffna. The bacteria were rejuvenated on nutrient agar medium at 37°C for 24 hours and stocked at 4oC on nutrient agar slant. Sub culture were prepared from the stock before the bioassay.

Determination of antibacterial activity by agar well diffusion method

20 ml molten nutrient agar media were mixed with 1 ml of (10^6 cfu/ml) each test bacteria and poured in sterile Petri dishes separately. After complete solidification, 8mm diameter wells were made using sterile cork borer and filled with 100µl of (30mg) petroleum ether; (30mg) ethyl acetate, (30mg) ethanol, (30mg) methanol extract and aqueous extracts of *Pongamia pinnata.* (30mg) streptomycin and different solvents (100µl of petroleum ether, acetone, ethanol, ethylacetate, methanol and water) were used as standard and control respectively.

Then the plates were incubated at 37°C for 24 hours and antibacterial activity was determined by measuring the diameter of clear zone around the well [7].

IV. RESULTS AND DISCUSSION

Table 1: Phytochemicals constituents of different leaf extract of *Pongamia glabra*

Phyto chemicals	Methanol	Ethanol	water
Glycosides	-	-	-
Alkaloids	-	-	-
Saponins	+	+	+
Cardiac glycosides	-	-	-
Tannins	+	-	-
Phlobatannins	-	-	-
Resins	-	-	-
Flavonoids	+	+	-
Terpenoids	+	+	+
Anthra-quinones	-	-	-

(+) – Presence (-) – Absence

The qualitative test for the presence of phytochemicals revealed that the methanol, ethanol and aqueous extracts of *P.pinnata* posses few type of phytochemicals.

Terpenoids and saponins were present in all three solvent extracts. Among these three solvent extracts, methanol and ethanol extracts revealed the presence of flavonoids and tannin was present only in the methanol extracts.

Unfortunately, cardiac glycosides, alkaloids, Resins, glycosides, phlobatannins, anthraquinones were not present in any of the tested extracts.

Table 2: Yield percentage of different solvent extracts of *P.pinnata*

Extracts	Yield(g)	Yield percentage
Petroleum ether	0.2959	1.97
Ethyl acetate	0.5135	3.42
Ethanol	0.4450	2.97
Methanol	0.7294	4.86
Water	0.6337	4.22

Table 3: Antibacterial activity of different form of solvent extracts of *P.pinnata* leaf.

Test extract	Diameter of inhibition zone (mm)*	
	E.coli	*S.aureus*
Petroleum ether	-	14
Ethyl acetate	16	16
Ethanol	15	13
methanol	12	12
water	-	-
streptomycin	28	-

- No activity, *Zone of inhibition includes the diameter of well (8mm)

High percentage of yield was obtained from methanol extracts followed by aqueous, ethyl acetate, ethanol and petroleum ether extract (Table 2). But methanol extracts revealed lower inhibitory effects on tested organisms compared to other extracts. It indicates that the inhibitory effect doesn't depend on the yield percentage.

The ethyl acetate, ethanol, methanol and petroleum ether were able to inhibit the growth of both while the petroleum ether extract failed to inhibit the growth of *E.coli*.

Out of five extracts, four extracts showed better inhibitory effect on *S.aureus* where ethanol extract produced better inhibitory effect on *E.coli* rather than *S.aureus* .But *P.pinnata* petroleum ether and aqueous extracts haven't any antibacterial activity against *E.coli* .

Interestingly ethyl acetate and methanol extracts contributed in equal antibacterial against *S.aureus* and *E.coli* which were found to be 12mm and 16mm respectively.

Both organisms were highly inhibited by ethyl acetate leaf extract of *P.pinnata* compared to other extracts. Ethanol extract was slightly higher antibacterial against bacterial pathogen than methanol extract.

The standard antibiotic streptomycin exhibited excellent inhibitory effect on tested organisms. Aqueous extract of *P.pinnata* and controls were revealed no inhibitory effect on both of the organisms.

V. Conclusion

This study has proved that leaf extracts of *P.pinnata* shows antibacterial activity and phytochemical constituents. Further studies should be need to isolation of bioactive compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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