Evaluation of Antimicrobial and Antioxidant Activity Of Aqueous Flower, Bark Extract And Hydrodistilled Oil From Seeds Of Karanja Plant (*Pongamia Pinnata*) Linn.

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**ABSTRACT**

*Pongamia pinnata* L commonly known as Karanja,Jatropha or Indian beach tree belongs to family of Fabaceae is a flower and fruit generating angiosperm. This study is to evaluate the biological activities of Aqueous Flower, Bark extract and oil obtained from seeds. Oil was obtained by conventional distillation method. Phytochemical Screening for chemical constituents and Biological assay for antimicrobial activity was carried out by the micro dilution method and the antioxidant potential was assessed by the DPPH scavenging method. The Results indicates that plant extracts contains various Phytochemical and antimicrobial activity was tested against three pathogenic bacterial strains (*Bacillus subtilis*, *Escherichia coli* and *Aspergillus niger*) by agar well diffusion method. The seed oil exhibited maximum zone of inhibition against *Bacillus subtilis* (17 ± 1.67mm) followed by *Aspergillus niger* (12 ± 1.20mm) and showed least activity against *E.coli*. The antimicrobial assay indicated MIC > 500 µg/mL against tested microorganisms. Comparatively the Antioxidant activity was also observed to be excellent for bark extract. This study suggests that *pongamia pinnata* extracts exhibits a great potential for antimicrobial, antioxidant activity and may be useful for nutritional and medicinal functions.

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INTRODUCTION:
Plants have great significance due to their nutritive value and are a major source of not only nutrition but also medicines. Plants have served mankind throughout the history of human civilization [1]. 30 to 40% of the currently available drugs are based on the medicinal & curative properties of various plants and are in use as herbal supplements, botanicals and nutraceuticals [2,3] Wild edible plants, many of which are potentially valuable as alternative food for human beings, can play an important role to strike a balance between population explosion and limited agricultural productivity, especially in developing countries[4]. Most of the developing countries depend on starch-based food products as primary source of energy and proteins. This fact partly accounts for the prevailing protein deficiency in such countries as recognized by Food and Agricultural Organization [5].

Medicinal plants are important not only for their biologically active secondary metabolites but also for their primary metabolites like carbohydrates, proteins and lipids. These primary metabolites are centrally important for the growth and development of a plant and are carriers of chemical energy to the subsequent tropic levels of the food chain. The carbohydrates, proteins and fats are referred to as the proximate principles and form the major portion of diet of herbivores and omnivores [6]. On the other hand, secondary metabolites are produced as by-products of metabolic pathways and though not essential for the survival of the plant, are important in their defence system. A variety of secondary metabolites has been isolated from plants and includes alkaloids, tannins, polyphenols, quinines,flavonoids, coumarins, terpenoids and saponins. These major groups represent classes of structurally and chemically diverse groups of compounds that exert strong physiological effects in humans. Their therapeutic properties have been utilized since long and research is still in progress to explore their applications as medicines [7].

2. Experimental Section
2.1. Materials and Reagents
The part of the plants were collected from the mangrove region and examined for the healthiness of flower, bark and seed materials, subjected to shade drying for further processing. Gallic acid was purchased from Sigma-Aldrich. Ascorbic acid, were a kind donation from the National Agricultural Research Foundation (Puducherry, Yanam).The Folin-Ciocalteu reagent was purchased from Merck. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich. All the other chemicals and solvents were of Laboratory grade.

2.2. Extraction Procedure
The extraction method used for dried samples are as follows: 100 ml pure distilled water was added to 1g of dried sample. The extraction mixture was refluxed in a water bath at 70°C for 1 h. The mixture was then filtered and placed in a test tube in the fridge. The essential oils were prepared by hydro distillation, 30 g of plant materials were added in 300 mL water, for 3 h using a Clevenger-type apparatus. The supernatant was separated by decantation, dried over anhydrous Na₂CO₃ and kept in sterile flasks. To prevent oxidation, all steps were carried out in dark with the flasks covered with aluminum foil. Moreover, it is essential that the waste (due to evaporation) of the solvent was kept at the lowest minimum [8].

2.3 Antimicrobial assay
The well diffusion method was employed for the determination of anti-microbial activity. The activity of aqueous flower, bark extract and distilled oil was examined against different Gram-positive (Bacillus subtilis) Gram negative (Escherichia coli) and fungal strains Aspergillus niger by measuring the zone of inhibition. The antimicrobial activity was performed by Agar well diffusion method at different concentration level of 100, 500, 1000 µg/ml against blank. Ciprofloxacin and ketoconazole were used as standard drug at a concentration of 100µg/ml. Nutrient agar was used as culture media for antibacterial activity and Sabouraud dextrose agar was used as culture media for antifungal activity and DMSO as control [9].The results of the antimicrobial activity are shown in Table 1.The plates were incubated for 24±2h.
at 37°C under anaerobic conditions. The results were evaluated by measuring the areas around the well with no bacterial growth. The experiments were made in triplicates. The readings were taken in duplicates and its average values were documented.

2.4 Determination of total Phenolics

The total phenol content (TPC) was measured using the Folin-Ciocalteu assay [10]. A volume of 0.2mL of the extract was introduced into test tubes followed by 0.5 mL Folin-Ciocalteu reagent (diluted 10 times with water). The solution was then kept at dark for 5 min and then 1mL sodium carbonate (7.5% w/v) was added. The tubes were covered with parafilm and kept again in the dark for 1 h. Absorption at 765 nm was measured with a spectrophotometer UV-Vis (Lab-India and compared to a Gallic acid calibration curve. The results were expressed as mg of gallic acid/g dried sample. Assay was carried out in triplicate.

2.5 Antioxidant Activity (DPPH) Radical Scavenging Method

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method of Blois [11] with a slight modification [12]. The reduction of the radical is followed by a decrease in the absorbance at 517 nm. A volume of 2 mL of aqueous methanolic stock solution of the extracts was put into test tubes and 2 mL of 1 mM DPPH solution was added. The tubes were covered with parafilm and kept again in the dark for 1 h. Absorbance at 517 nm was measured with a spectrophotometer UV-Vis (Lab-India) and compared to an ascorbic acid calibration curve. The results were expressed as mg ascorbic acid/g dried sample. Assay was carried out in triplicate.

RESULTS AND DISCUSSION

Flower, bark and seeds of *pongamia pinnata* plant were macerated by demineralised water at room temperature, and extracted successfully. The maceration extract of the plant yielded 2.35gm, 1.80gm and 8ml of oil extracts respectively on the basis of sample dry weight of plant material used. Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. The antioxidant potential of extracts derived from *pongamia pinnata* was investigated in the present study. It became clear that *pongamia pinnata* plant leaves, bark and hydrodistilled oil exhibit the activity. The bark extract revealed the highest antioxidant activity compared with reference antioxidant Vitamin C for DPPH scavenging activity. Phenols were found in all the samples and in the following order: Bark > Seed oil > Flower. Aqueous Bark extract of *Pongamia pinnata* showed strongest antioxidative effects causing 79% DPPH inhibition at the concentration of 50 ppm. The obtained results for DPPH are in good agreement with standard. Plant polyphenols act as reducing agents and antioxidants by the hydrogen donating property of their hydroxyl groups [13]. Hence, we could conclude that these polyphenols are responsible for the observed antioxidant activity in this study.

Total Phenolics

The results of the determination of total Phenolics are demonstrated as follows. The bark extract demonstrated the highest total phenol content with more than 15 ± 0.02 mg gallic acid/g dried sample flower extract seemed to have the lowest antioxidant capacity with less than 7.1 ± 0.03 mg gallic acid/g dried sample the seed oil expressed to have 9.6 ± 0.02mg gallic acid/g dried. These results indicated that the Phenolic compounds had a major contribution to the antioxidant capacity of herbal extracts.

Antimicrobial assay

The experimental results obtained from the present study illustrates that hydrodistilled oil extract from seeds of pongamia found to be more effective to control the pathogens growth compared to less effective inhibition by flower and bark extract as
shown in Table 1. Infectious diseases have become the major cause and serious concern in public health issues. The occurrence of drug resistant strains with less susceptibility to antibiotics due to mutation is a tedious challenge amongst the researcher to invent newer drugs. At this scenario, evaluation of antimicrobial substances from various sources of medicinal plants is considered to be a pivotal role. Few studies states that Pongamia pinnata L seeds have antimicrobial properties and thus being used in bronchitis, leprosy and chronic skin disease \[14,15\]. In field of agriculture, Pongamia pinnata seeds are used as fertilizer to enhance the soil fertility.

The earlier phytochemical investigation of Pongamia pinnata also indicated the seeds contain a flavones derivative called pongal. The structures of Karangin and pongal of Pongamia pinnata L were also elucidated \[16,17\] which have antimicrobial activity. However, in the present study results also exhibited the confirmation of the antimicrobial property that showed bactericidal action on the pathogens commonly encountered. Even though, further studies are required to exploring the mechanism of biochemical active principles of the isolated extract for the inhibitory action on various pathogens selected in the study.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Flower</th>
<th>Bark</th>
<th>Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC µg/ml</td>
<td>Zone mm</td>
<td>MIC µg/ml</td>
</tr>
<tr>
<td>Bacillus subtilis,</td>
<td>100</td>
<td>10 ± 0.58</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>500</td>
<td>7 ± 0.33</td>
<td>500</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>1000</td>
<td>8 ± 0.35</td>
<td>1000</td>
</tr>
<tr>
<td>Control</td>
<td>DMSO</td>
<td>1 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>Ketoconazole</td>
<td>28 ± 0.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>30 ± 0.33</td>
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</tbody>
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Table 1: Antimicrobial Activity of extracts derived from Pongamia pinnata Aqueous Extracts

Fig: 1 Zone of Inhibition by Seed oil  
Fig: 2 Zone of Inhibition by Bark
CONCLUSION

The present work has proved that the extracts of leaves, bark, and seed oil of *pongamia pinnata* possessed strong antimicrobial and antioxidant properties. The results serve as a scientific basis to further develop the extracts into medicinal products.

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