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Research Article

QUANTIFICATION OF PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF FRUIT EXTRACT OF *AVICENNIA OFFICINALIS*

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ABSTRACT

Avicennia officinalis (AO) is an evergreen mangrove tree that finds a prominent place in folk medicine. In this study, we have quantified 2 significant phytochemicals viz., phenols and flavonoids and studied the antibacterial activity of extracts of AO fruits in ethyl acetate, acetone, methanol and ethanol against 8 pathogenic bacterial species *E. coli*, *En. aerogenes*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, *L. delbrueckii*, *S. aureus* and *Strep. pyogens*. Phenols and flavonoids were quantified Spectrophotometrically. Total phenolic content (TPC) ranged from 125-350mg of GAE/g of crude extract and the flavonoid levels were in the range of 60-192 mg of rutin equivalent/g of dry sample. The methanol and ethanol extracts showed highest levels of TPC, while acetone extract was identified to contain comparatively elevated amounts of flavonoids. Antibacterial activity of all the extracts was screened against the selected strains by agar well diffusion method. All the extracts exhibited different degrees of inhibition against the bacterial strains tested. Their MIC values ranged between 1.25-5.0mg/100µl. Methanol and Ethanol extracts showed strong inhibitory activities against both Gram negative and Gram positive bacterial strains compared to those of other extracts. However, ethyl acetate infusion restricted its activity only against the Gram positive bacterial strains tested. Strains of *E. coli*, *En. aerogenes* (Gram negative) *S. aureus* and *Strep. pyogens* (Gram positive) were inhibited by acetone extract. This study provides the necessary data for isolation and characterization of individual broad spectrum antibacterial compounds from different extracts of AO fruits. The study also supports the potentiality of AO in folk medicine.

Keywords: *Avicennia officinalis*, Phenols, Flavonoids, Antibacterial

INTRODUCTION

Mangroves are perennial plants that grow in coastal wetlands of tropical regions. They are found in the inter-tidal zones of sheltered shores, estuaries, creeks, backwaters, lagoons, marshes and mud-flats. Mangrove forests are regarded as the wetlands on earth with biological diversity. Nearly 7% of the world mangroves are distributed in India covering about 8% of India's coastline. East coast and Andaman & Nicobar Islands are known to have diverse mangrove species. The east coast is composed of about 16 species of true mangroves and 19 associated mangroves (1). Mangrove plants and plant products have been used for centuries as natural remedies in the treatment of several health disorders. Plant-derived substances have recently gained substantial interest owing to their medicinal applications. Mangroves are unique in that they produce an array of novel natural products. Mangroves and their products are known to be associated with biologically active antiviral, antibacterial and antifungal compounds (2). Chemical and biological investigations on mangrove species lead to the isolation of a variety of bioactive compounds including alkaloids, flavonoids, triterpenoids, polyphenolic compounds, xanthones, coumarins, tannins, chalcones and benzofurans (3,4). Systematic study of mangrove species has revealed that crude extracts of different plant parts in different solvents and compounds isolated from them exerted potential antibacterial, antifungal, antiviral and antioxidant activities (5,6).

Some of the species of mangroves are frequently employed in folk medicine to treat several human health disorders (7) Kokpal *et.al* 1990). Out of the 10 known species of *Avicennia,* 3 species, viz., *A. officinalis* L, *A. marina* and *A. alba* are found in the Indian coasts and Andaman Nicobar islands. *A. officinalis* is an evergreen tree plant that varies from shrubby stunted individual to tall trees with broad trunk. Its seeds are used as maturative poultices and cicatrizant of ulcers and to hasten suppuration of boils and abscesses. The roots are used as aphrodisiac, resin oozing from the bark finds use as contraceptive, bark as diuretic, in the management of skin afflictions especially scabies, rheumatism, paralysis, asthma and snake-bites. Fruits are used as plaster for tumors. Plant decoction with sugar and cumin is used in dealing with dyspepsia. Literature reveals AO exerts strong gastro- protective, antibacterial , antifungal, antiviral, antitumor and antioxidant activities (8,9). In the present study, we examined different extracts prepared from the dried fruits of AO for their TPC and flavonoid content. Antibacterial activity of the extracts was also studied against selected MTCC strains of bacterial cultures.

MATERIALS AND METHODS

Collection of Fruit Samples

The fruits of AO have a slight oval shape with a break of about 2.5cm long. They are either green or brown in colour. Fresh fruits were collected from Corangi Reserved Forest, Kakinada, East Godavari, Andhra Pradesh, India. Geographic location - between 16° 39' N longitude - 17° N longitude and 82° 14' E latitude - 82° 23'E latitude. The fruits were transported to the laboratory in new polythene bags. All the fruits were surface sterilized with 1% mercuric chloride solution and thoroughly washed with filter sterilized distilled water. The washed fruits were then chopped to small pieces and shade dried until they were suitable for extraction in the selected solvents.

Extraction

Plant extracts in ethyl acetate, acetone, ethanol and methanol were prepared according to the standard protocols (10). The chopped fruit material (100g) was initially soaked in 500ml of the respective solvent at room temperature for 24h. Subsequently, the soaked material was refluxed for 6h below the boiling point of the respective solvent. Infusions were filtered through Whatman No.1 filter paper and the residual material was re-extracted with fresh solvent. After 24h the process was repeated. Pooled extracts were individually concentrated by removing the solvent under reduced temperatures using vacuum rotator evaporator. These extracts were further concentrated by solvent evaporation using thin film method. Dried fruit extract of 100mg each was dissolved in 10ml of 1:10 diluted DMSO in sterile distilled water so as to obtain the final concentration of 10mg/ml (Nkere and Iroegbu, 2005 and Choudhury *et.al*.2005). All the extracts thus prepared were stored in a refrigerator at 4°C.

Estimation of Total Phenols

TPC in the examined plant extracts was determined by Folin-Ciocalteu method (11,12). 1ml of crude extract (100µg/ml) was mixed with 5ml Folin-Ciocalteu reagent and 4ml of 7.5% Sodium carbonate. Tubes were vortexed and incubated for 30min at room temperature for colour development. Absorbance was measured at 765nm using UV-VIS spectrophotometer. The samples were analysed in triplicates and the mean absorbance value was recorded. The standard curve was plotted using 250µg/ml of gallic acid in ethanol. TPC in each extract was expressed in terms of gallic acid equivalent (mg of GA/g of dry mass).

Estimation of flavonoids

Flavonoid content in different extracts of AO fruits was determined by spectrophotometric method (13). Flavonoid content in all the extracts was calculated from the standard curve of rutin. The samples were prepared in triplicates for each analysis and the mean value of absorbance was recorded. Results were expressed as mg of rutin equivalent /g of dry sample.

Determination of antibacterial activity

Antibacterial activity of all the extracts prepared in different solvents from dried fruit sample of AO was determined using standard agar well diffusion method (14-16). The bacterial strains used in our study were *E. coli* MTCC 7410, *En. aerogenes* MTCC 7324, *K. pneumoniae* MTCC 7028, *P. aeruginosa* MTCC 7083, *B. subtilis* MTCC 736, *L. delbrueckii* MTCC 911, *S. aureus* MTCC 737 and *Strep. pyogens* MTCC 1928. Each experiment was performed in triplicates and the average value for zone of inhibition was calculated. The zones were compared with that of Gentamicin (1mg/100µl).

Determination of MIC

Minimum Inhibitory Concentration (MIC) was determined by broth dilution assay method (14). Plant extracts were serially diluted in Mueller Hinton broth to get the concentrations of 1.25, 2.5, 5.0 and 10mg/100µl. Each experiment was repeated thrice and the mean values were tabulated.

RESULTS

Total Phenolic content

The TPC of AO dried fruits extracted with ethyl acetate, acetone, ethanol and methanol is as presented in Figure-1.

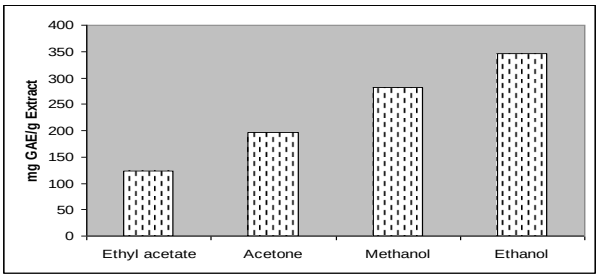

Fig.1: Total phenolic content of *avicennia officinalis* fruit extracts.

The TPC significantly varied ranging from 125–350mg of GAE/gram in the extracts of AO. Maximum levels of TPC was observed in ethanol and methanol extracts with 350 and 300mg GAE/g respectively and the minimum level was found in that of ethyl acetate with 125mg GAE/g. From these results it was understood that ethanol serves best for extraction of phenol compounds from AO fruits.

Total Flavonoid content

Total flavonoid content in all the extracts of AO fruits was determined by $AlCl_3$ method. This experimental data is presented in Figure-2.

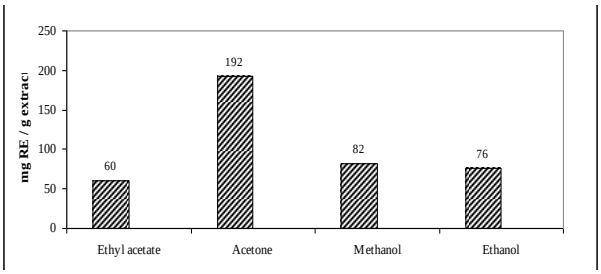

Fig.2: Flavonoid content of *avicennia officinalis* fruit extracts.

Total flavonoid content varied from solvent to solvent owing to the differences in their polarities. Acetone was found to be most effective for the extraction of total flavonoids as was found to contain maximum of 192mg of rutin equivalent/g.

Antibacterial Activity

In vitro antibacterial activity of all the extracts of AO fruit was determined by agar well diffusion method. This experimental data is presented in Figure-3.

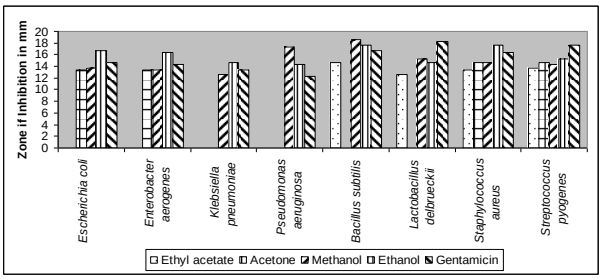

Fig.3: Antibacterial activity of fruit extracts of *avicennia officinalis* on selected bacteria.

All the extracts were found to possess various degrees of antibacterial activity against both Gram positive and Gram negative bacteria. Among the tested extracts methanol and ethanol solubles of AO fruits exhibited highest potential of antibacterial activity (12.66-18.66mm) against all the tested bacterial species irrespective of their Gram nature. However, components of AO infused into ethyl acetate were found to be active only against the Gram positive test cultures. The acetone soluble exhibited a mixed response. It inhibited both Gram negative (*E. coli* and *En. aerogenes)* and Gram positive (*S. aureus* and *Strep. pyogens*) test cultures. Further, these extracts were analyzed to determine the minimum inhibitory concentration against both Gram negative and Gram positive bacterial species. The MIC values ranged from 1.25-5mg/100µl and varied from extract to extract (Table-1).

Table 1: MIC of the *avicennia offficinalis* fruit extracts in mg/100µl

Microorganisms	Ethyl acetate	Acetone	Methanol	Ethanol
Escherichia coli	-	5	5	2.5
Enterobacter aerogenes	-	5	5	2.5
Klebsiella pneumoniae	-	-	5	2.5
Pseudomonas aeruginosa	-	-	1.25	2.5
Bacillus subtilis	2.5	-	2.5	2.5
Lactobacillus delbrueckii	5	-	2.5	2.5
Staphylococcus aureus	5	1.25	2.5	2.5
Streptococcus pyogenes	2.5	1.25	2.5	2.5

DISCUSSION

Phenols and flavonoids are amazing group of compounds virtually found in all plants. They are present in edible and non-edible plant parts and are responsible for many biological activities in both the plant and animal systems (17). Over 5000 naturally occurring phenols and flavonoids have been identified from various plants. These are known to possess antioxidant, antibacterial, antitumor and antiviral properties. Mangroves are diverse group of plants rich with many secondary metabolites like alkaloids, flavonoids, phenolics, steroids and terpenoids (18-20). They are well studied for their pharmacological and ecological importance. In folk medicine mangrove plant parts and extracts are being used in the treatment of various diseases over the centuries. In India and other tropical countries AO is being used in the treatment of several disorders (2). So far all the plant parts of AO except fruits are well studied for biological properties (21,22). In this study, we have evaluated the phenolic and flavonoid content and antibacterial potentials of AO fruit constituents infused into different solvents in the order of increased polarity. Phenols and flavonoids being associated with potent biological activities, we quantified their content in AO fruit extracts. Ethanol served best for the extraction of phenolic compounds and acetone was found to be most effective for the extraction of total flavonoids from AO fruits. The variation in the content of phenolic compounds and flavonoids in solvent extracts is attributed to the differences in polarity of the extracting solvents, number of hydroxyl groups and nature of the conjugated components with phenols and flavonoids. Our results also show that the phenolic compounds of AO fruits are more of polar in nature as their content is observed to be highest in ethanol when compared with the other solvents used. Similarly the flavonoids in AO fruits that were observed to be highest in acetone are understood to be of low polarity. The present experimental data is in accordance with that of Govindasamy *et.al* (23) who studied the chemical constituents of different mangroves in India.

The key role of phenolic compounds and flavonoids in scavenging of free radicals has been well studied (24-26). Apart from having antioxidant activity, phenols and flavonoids are also known to be associated with other biological activities (27,28). In search of potent broad spectrum antibacterial compounds, in this study we have screened for inhibitory effect of AO fruit extracts in ethyl acetate, acetone, methanol and ethanol against selected Gram negative and Gram positive pathogenic bacteria species. This data is presented in Figure 3. All the tested extracts of AO fruits possessed different levels of antibacterial activities. Among the tested extracts, methanol and ethanol extracts were found to be broad spectrum as the active constituents of AO fruits infused in methanol and ethanol suppressed the growth of both Gram positive and Gram negative test cultures. On the other hand constituents extracted in ethyl acetate are exclusively active against Gram positive test cultures. This could be due to variation in the cell wall composition of Gram negative and Gram positive bacteria. The Gram negative bacteria restrict the influx of many antibiotics (29). Multidrug efflux pumps at the transmembrane are also responsible for a higher intrinsic resistance in Gram negative bacteria (30). However, The solubles in acetone are active against Gram negative - *E.coli, Ent. aerogenes* and Gram positive - *S. aureus* and *Strep. pyogens* cultures. On correlating our results of phenolic compounds and antibacterial activities it is inferred that phenolic compounds of AO possess substantial antibacterial activity.

The MIC values of AO fruit extracts against the tested strains are shown in Table 1. The ethanol extract is active against all the Gram negative and Gram positive cultures with an MIC 2.5mg/100µl. The MIC values ranged from 1.25–5.0mg/100µl for methanol and acetone extracts and from 2.5-5.0mg/100µl for ethyl acetate extract against the test cultures. Gentamycine was used as the standard antibacterial compound.

The phytochemical constituents of AO fruits in methanol and ethanol solubles are found to be of broad spectrum nature. Thus the methanol and ethanol extracts of AO fruits will serve for further isolation and characterization of putative compounds.

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