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Evaluation of antioxidant activity in fruit extracts of *Avicennia Marina* L and *Avicennia Officinalis* L

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ABSTRACT

Antioxidant activity of *Avicenni marina* L and *Avicennia officinalis* L fruit extracts in ethyl acetate, acetone, methanol and ethanol were studied by ABTS, CrO_5 and FRAP method. All the extracts possessed significant amount of the antioxidant activity, extracts in methanol and ethanol was demonstrated convincingly higher antioxidant activity by all the three methods. Whereas, the ethyl acetate exhibited less antioxidant activity. The present study reveals the potential of fruit extracts of *Avicennia marina* L and *Avicennia officinalis* L with antioxidant principles.

Keywords: *Avicenni marina* L, *Avicennia officinalis* L, Fruit, Antioxidant activity

INTRODUCTION

Since time immemorial man has been using plant extracts to protect himself against several diseases and also to improve his health and life style. The secondary metabolites present in the plants are known to be effective against various ailments. Some of the significant secondary metabolites of plants are alkaloids flavonoids, glycosides, phenolic compounds, saponins, tannins, terpenoids. It is estimated that more than 12,000 Alkaloids, 8,000 Phenolics and 25,000 Terpenoids have been identified in plants [1] These compounds are responsible for the antioxidant, antimicrobial, antiviral and anticancer activities and are the major sources of pharmaceuticals, food additives and fragrances. Most of the antioxidant compounds in a typical diet are derived from plant sources. They belong to various classes of compounds such as alkaloids flavonoids, tannins etc., The potentiality of an antioxidant depends primarily on its ability to neutralize free radicals. Antioxidant compounds scavenge free radicals such as peroxides, hydroperoxides and lipid peroxides. They inhibit the oxidative mechanism that leads to degenerate diseases. Many synthetic antioxidant compounds like butylated hydroxyanisole (BHA), butylated hydroxytoulene (BHT) propyl gallate (PG) have been used in drug compositions. However, these artificial compounds may give rise to many side effects and cause health problems. Natural source have therefore gained much significance in recent years owing to their safety. Mangroves are assorted group of plants that grow in brackish water, have always been a source of effective bioactive principles. Mangrove plants have been used in folklore medicine to treat various diseases for centuries [2]. The biochemical complexity of the plant ascribes its diverse medicinal properties [3]. Little is known of the antioxidant potentials of fruits of *Avicennia marina* (AM) and *Acicennia officinalis* (AO). These plants belong to family *Avicenniaceae*, locally called as Nalla mada and Tella mada (AM) and (AO) respectively. These plants vary from shrubby stunted individuals to tall trees with broad trunk. Their fruit is heart-shaped green, flurry and large in size. In the present study, fruits of AM and AO were selected for extraction in different organic solvents viz., ethyl acetate, acetone, methanol and ethanol to determine the antioxidant activity by ABTS, FRAP and CrO_5 method.

MATERIALS AND METHODS

Plant Material: The fruit of healthy *Avicennia marina* L and *Avicennia officinalis* L plants were collected from East Godavari mangroves at Corangi Reserved Forest, (Geographically located between 16^o 39' N longitude – 17^o N longitude and 82^o 14' E latitude -82^o 23'E lattitude) Kakinada, Andhra Pradesh, India. The fruits were collected in new polythene bags and surface sterilized with 1% mercuric chloride solution. Fruit were chopped into small pieces and shade dried at room temperature for seven days.

Extraction: The extraction of fruit was carried out with different solvents in their increasing order of polarity viz., ethyl acetate, acetone, methanol and ethanol by soaking the fruit in the respective solvents overnight at room temperature one after the other [4]. The chopped fruit exactly hundred gram (100 g) was extracted sequentially into 500 ml of the relevant solvent in a round bottom flask by initial soaking for 8 – 10 hours, followed by refluxing for about 6 – 8 hours below the boiling point of the respective solvent, in order to extract the compounds into the respective solvent. The extracts were evaporated and concentrated at reduced pressure using the rotary evaporator. The concentrated extracts were incubated at 37^oC for 3-4 days to facilitate complete evaporation of the volatile solvent leaving behind the dried plant extract [5]. The dried plant extract of100mg each was dissolved in 10ml of 1:10 diluted DMSO (in sterile distilled water) so as to obtain the final concentration of 10mg /1ml [6].

Determination of antioxidant activity by abts method: ABTS method is permitted to measure the over all antioxidant capacity of the sample [7]. The assay depends on the ability of antioxidant in the sample to inhibit the oxidation of ABTS. In this method, ABTS is converted to its radical cation by addition of sodium persulfate. It is blue in color and absorbs at 734nm. During the reaction the blue coloured ABTS radical cation is converted back to its colour less neutral form. It is best studied by spectrophotmetric method.

Procedure: Double ionized water is used as blank, 3000 µL of ABTS reagent is pipetted pipette out in a cuvette and initial absorbance is measured at 734nm. 300µl of the fruit extract (10mg /1ml) is added to ABTS reagent , the contents are mixed with vortex and the absorbance of the solution was determined at 734 nm after 3minutes. % of inhibition is calculated in accordance with the following formula.

$$\% \text{ of ABTS inhibition} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Determination of antioxidant activity by the blue cro_5 method: Chromium peroxide (CrO5) is very strong pro-oxidant produced in an acidic environment by ammonium dichromate in the presence of H_2O_2. It is a deep blue potent oxidant compound, miscible and relatively stable in polar organic solvents that can be easily measured by spectrometry [8].

Reagent preparation:
Solution1: 1a, 1b and 1c these three solutions are mixed in a 1:1:3 ratios (*v*/*v*/*v*). 1a) 10 mL of sulphuric acid (25 mM), 1b) 10 mL of 20 mM ammonium dichromate solution, 1c) 30 mL of 99.5 % DMSO (*v*/*v*).
Solution2: 1.6 M H_2O_2

Procedure*:* 3000 µl of solution 1 is mixed with 300 µl (10mg /1ml) of the fruit extract. This solution is incubated. The first absorbance is measured at the 416^{th} second (A_{416}). Subsequently, 40 µL of solution 2 is added and the solution is incubated for 192 seconds and record the second absorbance (A_{608}). Both the measurements are carried out at $\lambda = 546$ nm. Resulting absorbance was calculated in accordance with the formula $A = A_{608} - A_{416}$.

Determination of antioxidant activity by the frap method: The FRAP assay was carried out by Benzie and Strain method with some modifications [9].
The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-*s*-triazine (TPTZ) with ferric chloride,
Reagent preparation: FRAP reagent prepared freshly by mixing 25ml of acetate buffer (Ph 3.6)
With 2.5 ml of 10 mM 2,4,6 tripyridyl triazine (TPTZ) and 2.5 ml of 20mM Ferric chloride solution. The reagent was kept at 37 ^{o} C before use.

Procedure: Exactly 300 µL of the fruit extract(100mg/1000 µL) were dispensed into 2700 µL of the freshly prepared FRAP reagent and incubated at 37 ^{o} C for 30 minutes. The absorbance was recorded at 593 nm against a blank. Standard curve was prepared with 100 µM $FeSo_4$ solution. All determinations were done in triplicates and expressed as µM Fe^{2+} equivalents per 100 gram of the sample.

RESULTS

Mangroves inhabit in estuarine environment where there is high salinity, low nutrition and high solar radiation during low tide [10]. These conditions makes

the plant to produce Reactive Oxygen Species (ROS) [11]. Mangroves are bestowed with polyphenolic compounds, that acts to neutralize the effect of ROS. We studied the antioxidant activity by three different methods viz., ABTS, CrO_5 and FRAP to the fruit extracts of AO and AM in ethyl acetate, acetone, methanol and ethanol. The present study reveals a definite variation in the antioxidant compounds present in the extracts of AO and AM and also within the solvent system.

ABTS METHOD: The outcome of the antioxidant activity by the fruit extracts of AM and AO in ethyl acetate, acetone, ethanol and methanol by ABTS method is accessible in Figure-1. The Ethanol extracts showed higher antioxidant activity for fruits of both the plants compared to that in other extracts. In both the cases the antioxidant activity increased from ethyl acetate to ethanol. Fruit extracts of AM in ethyl acetate, acetone and ethanol had higher antioxidant activity compared to those of AO within the same solvents. However, the methanol extract of both the plants showed same antioxidant activity.

CrO_5 METHOD AND FRAP METHOD: Figure-2 and 3 expose the antioxidant activity of AM and AO fruit extracts by CrO_5 and FRAP methods respectively. The fruit extracts of AO in all the solvents were having superior antioxidant activity than AM by both the methods. Methanol extracts were possessed higher antioxidant activity for both the plants compared to that of the other extracts. The antioxidant activity increased from ethyl acetate to methanol. However, the activity of the fruit extracts of AM and AO in ethanol is less compared to that in the methanol extract.

DISCUSSION

Mangroves are promising source of several bioactive compounds. They are the rich source of alkaloids, flavonoids, saponins, steroids, tannins and terpenoids [2]. They are also generously gifted with polyphenolic compounds that help to fight the oxidative stress by acting as potent antioxidants[12]. Members of the family *Avicennniaceae* are rich source of tannins. Tannins acts as antibacterial and antioxidant principles[13]. The motive behind identification of antioxidant by three different methods is, to know the active principle that is present in the extract, if it is of the same nature or not. The main limitation of FRAP method is inability to detect slowly reactive phenolic compounds and thiols[14]. Our results revealed that the extracts in different solvents are of diverse nature. Packia Lincy *et.al* [15] screened root extracts of AM in benzene, ethyl acetate, ethanol, methanol and petroleum ether by ABTS, DPPH, Hydroxyl Radical Scavenging and Superoxide Radical Scavenging activity methods. They reported that all the extracts had moderate to high antioxidant activity. Thirunavukkarasu *et.al*[16] studied the antioxidant status of selected mangrove leaves in methanol by DPPH and SOD activity of Pichavaram Mangrove Forest . In 2012 Asha *et.al* [17] reported the antioxidant property by flavonoids and phenolic compounds in roots of *Rhizophora apiculata* and *Acanthus ilicifolius* by DPPH method and concluded that they are rich source of flavonoids and phenolic compounds. Varahalarao and shadrasekhar [18] reported the antioxidant potential of selected Mangrove plant in methanol extracts of leaf, bark and stem by DPPH and FRAP method. Banaerjee *et.al* [12] studied the antioxidant activity of leaves, bark and root extracts in 20% methanol of *Avicennia alba* by DPPH method and reported that the antioxidant activity is due to the total phenolic content present in *Avicennia alba*. Govindasamy *et.al*[19] evaluated the antioxidant polyphenols from selected mangroves of India. The outcome of the study is that AO is rich in polyphenols and has antioxidant activity done by DPPH method. Nutavan *et.al*[20] reported the antioxidant activity of 32 species of mangrove and mangrove associates of fruit, leaves and flowers by DPPH method. However, fruits of AM and AO were not studied. We, exclusively studied the fruit extracts of AM and AO not only in methanol and ethanol but also included ethyl acetate and Acetone solvent systems by ABTS, CrO_5 and FRAP method.

The antioxidant activity by ABTS of AM is high in ethanol. Our results did not match with that of the Packia Lincy *et.al* report. Moreover, they studied the root extracts of AM in benzene, ethyl acetate, ethanol, methanol and petroleum ether, whereas we concentrated only on the fruit extract. The chosen plant part may be the reason for the difference in the antioxidant property. However, the result of fruit extracts in ethyl acetate are in concurrence with that of their study. The effect of antioxidant activity by CrO_5 method of AM and AO differs from that of the ABTS method in particular with the methanol extract. The extracts of AO are having higher antioxidant activity than AM. This may be due the difference in the phytochemical composition of the plants. The report of Thirunavukkarasu disclose that the total phenolic content of AO is higher than that of AM. The phenolic content is responsible for the antioxidant activity. The outcome of antioxidant activity by FRAP method is high in AO than in AM. These result are not in agreement with that of the Varahalarao report . This difference may due to the presence of the active metabolite responsible for the

antioxidant activity. According to Winkel-Shirley[21] the abnormality of certain secondary metabolites may be the characteristic feature of plant stress. The antioxidant potential of the plants may be due to presence of phenolic compounds like tannins and flavonoids. This reducing activity of these extracts could serve as an indicator of potential bioactive principle present in fruits of AM and AO. Further research in the fruit of these two plants with other solvents and by new methods can pave way to the discovery of novel phytochemical compounds.

CONCLUSION

The result of the present study advocates that fruit extracts in different solvents have moderate to high antioxidant activities. Moreover detailed studies on chemical composition of the plant extracts as well as in vivo assays are essential to characterize the secondary metabolite as naturally occurring antioxidant unit.

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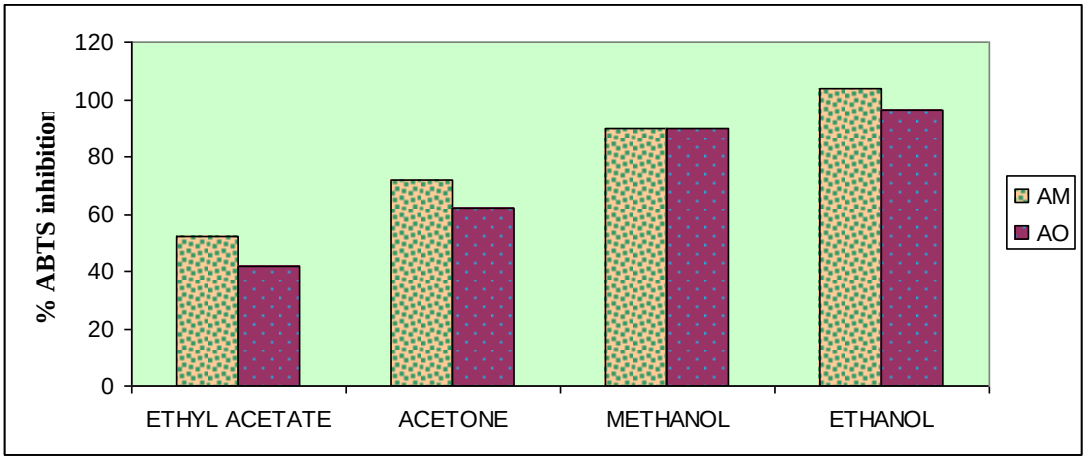

Fig-1. Anti oxidant activity by ABTS method.

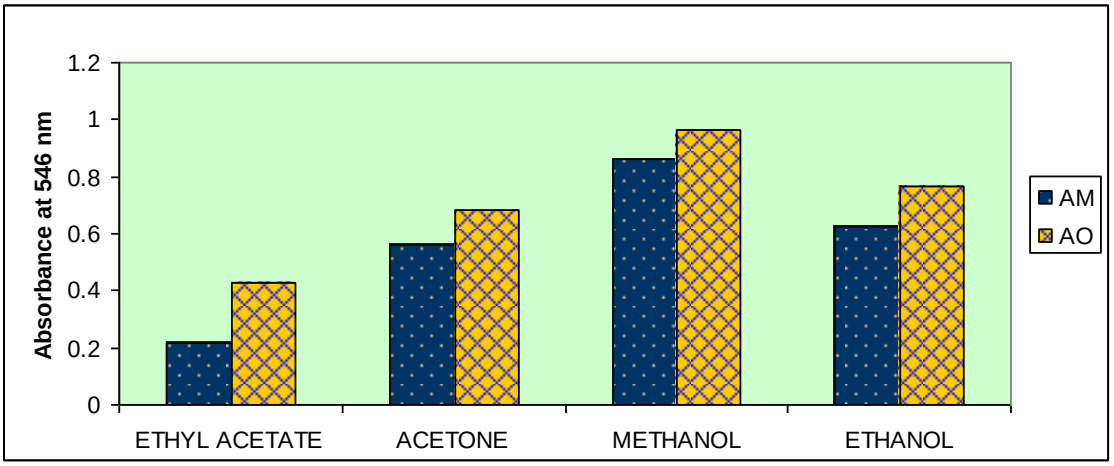

Fig-2. Anti oxidant activity by CrO_5 method.

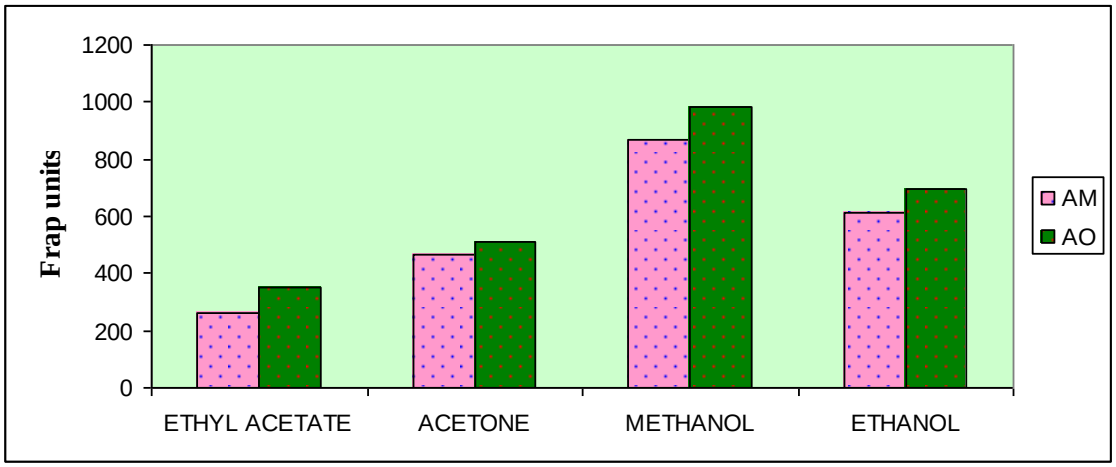

Fig-3. Anti oxidant activity by FRAP method.

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