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Research Article

SCREENING OF ANTIOXIDANT CAPACITY, TOTAL POLYPHENOLS AND PHYTOCHEMICAL ASSAY OF *OSBECKIA WYNAADENSIS*

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ABSTRACT

The total phenolic content of the plant extract is ultimately related to the antioxidant potential and other medicinal aspects of the plant extracts. The solvent extracts of plant *Osbeckiawynaadensis*was screened for antioxidant capacity & total phenolic content. The IC_{50} value for methanol & ethyl acetate extracts was calculated. The total phenolic content of the methanol and ethyl acetate extracts of the *O.wynaadensis* was quantified byFolin–Ciocalteu assay The antioxidant capacity was measured by DPPH radical scavenging activity according to the method of and ABTS radical cat-ion scavenging assay according to the method and the IC_{50} value also calculated for both ethyl acetate & methanol extracts in phytochemical assay simple qualitative assay was done and tabulated.

Keywords: Osbeckiawynaadensis, IC_{50} value, ABTS, DPPH, Folin–Ciocalteu, scavenging.

INTRODUCTION

The polyphenols, flavonoids, saponins, glycosides etc…are the major phytochemicals found in both, edible and non-edible plants, this phyto-chemicals contribute towards the biological effects like antimicrobial , antioxidant, anti-cancerous, anti-inflammatory potential [Aaby et al., 2004;Loliger, 1991.]. The polyphenols are the interesting phytochemicals in food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [Ali Aberoumand and Deokule, 2010; Balasundram et al.,2006] secondary plant metabolites and have biological properties uch as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and

Anticancer property. There are more than thousand known and many unknown phytochemicals.It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [MamtaSaxena et al]

The most commonly used methods for antioxidant screening is **ABTS** and**DPPH**free radical scavenging assay**.** Both of them are characterized by excellent reproducibility under certain assays conditions, but they also show significant differences in their response to antioxidants. The **DPPH**free radical does not require any special preparation, while the **ABTS**radical cat-ion must be generated by enzymes or chemical reactions [Arnao, 2000] there is a major difference between the **DPPH**and **ABTS** is that the **DPPH** is soluble in organic solvent, while the **ABTS** is soluble in aqueous as well as organic solvents an **ABTS** can be used forboth hydrophilic and lipophilic samples. And the **DPPH** is soluble only in organic solvents [ethanol/methanol] it cannot be used for hydrophilic antioxidant samples.

The main objective of the study is

1. To evaluate the IC_{50} value of the ethyl acetate & methanol extracts,
2. Phyto-chemical assay,

3. To evaluate the total phenolic content of the plant

MATERIALS ANDMETHODS

Hexane, ethyl-acetate, dichloromethane CH_3OH, con-H_2SO_4, tannic acid, ascorbic acid, H_2O_2, ammonium molybdate, disodium phosphate, sodium dihydrogen phosphate, NaOH, lead acetate, HCl, zinc dust, N-N dimethyl formamide, FC reagent, potassium ferric cyanide, KI, TCA, DMSO, ABTS, DPPH, etc…

Instrument used

Soxhlet apparatus, Spectrophotometer, glass wears etc…

[Chemical used for the reagents and media were produced from Hi-media laboratory Pvt ltd, and Merk India limited, Mumbai]

Sample collection

The selection of plant materials and sampling area were crucial, the plant O. wynaadensis is traditionally well known for its medicinal property. But the much of work has been not reported for this plant.

The young and old leaves and Arial tender part of O. wynaadensis were collected from the sides of wetland of Madikeri (INDIA) during the month of February 2012. The collected plant samples were thoroughly washed with tap water and washed with Sodium hypochlorite (NaOCl) and then with water and dried in shade. The dried samples were powdered using pestle and mortar.

Solvent extraction

The shade dried samples was powdered by using pestle and mortar and further subjected to extraction in soxhlet apparatus using three solvents. The samples were first extracted with hexane and followed by ethyl-acetate, & methanol. Three extracts was collected, filtered using watt man NO.1 filter paper and evaporated at 50°C to constant weight. Then the collected samples were stored at 4°C for further studies.

PHYTO CHEMICAL ASSAY

Total phenolic content

The polyphenol content of the methanol and ethyl acetate extracts of the O.wynaadensis was quantified by the method of [Taga et al., 1984] with slight modifications.10mg of standard tannic acid was dissolved in 10ml of dissolving agent (6:4 CH_3OH:H_2O+0.3M HCl). From this 0.2ml was made up to 4ml by same solvent mixture. The aliquots of the tannic acid were taken in the range 0-6 µg/ml. 20mg of extracts was dissolved in 2ml DMSO (From this 1ml+2ml DMSO). The extracted samples were taken in the range rage of 0-1000 µg/ml made up to 1ml by water. To all the samples 0.1ml of 50% Folin-Ciocalteau was added and incubated in room temperature for 30min. The colour intensity was measured at 720nm. The polyphenol content of the O.wynaadensis extracts were expressed as tannic acid equivalents.

Total reducing power

The reducing activity of O.wynaadensis extracts were evaluated according to the method of [Benzie and Strain, 1996] with slight modifications. 10mg of L-ascorbic acid is dissolved in 100ml of distilled water and used as standard for quantify reducing activity. To 1ml standard ascorbic acid solution, 2ml of phosphate buffer (pH 6.6), 2ml of **$K_3Fe(CN)_6$**were addedand incubated at 50°C in a water bath for 20min. The samples were then cooled and mixed with 2ml of 10% TCA and 2ml of aliquots removed to a fresh tube. For the final reaction, the aliquots were mixed with 2ml of water, 0.5ml of .1% $FeCl_3.6H_2O$ and left to react at room temperature for 15min. The same procedure is followed for the ethyl acetate and methanol extracts of the O.wynaadensis. The absorption was measured at 700nm. The reducing activity of the plant extracts were expressed as ascorbic acid equivalent.

Total antioxidant capacity

The total antioxidant activity of the methanolic and ethyl acetate extracts was evaluated by Phospho-molybdenum reduction assay according to the method of [Prieto et al., 1999] 10mg of the standard ascorbic acid was dissolved in 10ml of DMSO. The aliquots of standard were taken in the range 0-240µg/ml and made up to 1ml by DMSO. 20mg of the plant extracts was dissolved in 5ml of DMSO, and filtered. 0-240 µg/ml of the extracts was pipetted. To the standard and samples 2.5ml of Phospho Molybdenum **[**Ammonium-Molybdate (4mM) sodium-phosphate (28mM) and H_2SO_4(0.6M)**]*** reagent was added and incubated for 60min at 37°C. The antioxidant capacity of the plant extracts was expressed as ascorbic acid equivalents.

ANTIOXIDANT ACTIVITY

The antioxidant capacity was estimated by two commonly used methods namely

- DPPH stable free radical scavenging assay
- ABTS radical cation scavenging assay

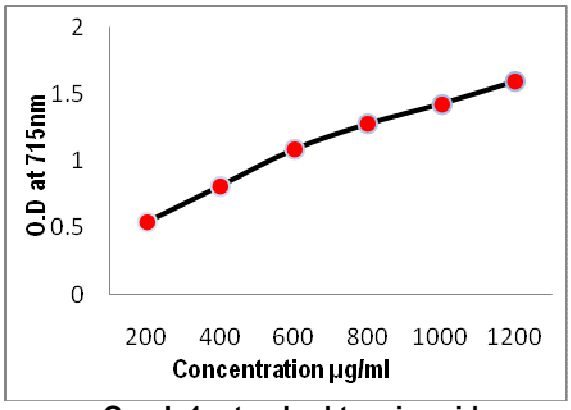
DPPH stable free radical scavenging assay:
The DPPH radical scavenging activity of O.wynaadensis extracts and ascorbic acid were determined according to the method of [Shimada et al., 1992]. The standard ascorbic acid was prepared in the range of µg/µl From this 0-350µl aliquots was taken and made up to 1ml by methanol.10mg of extracts was dissolved in 10ml of DMSO. From this 1ml was made up to 10ml using methanol. From this aliquots of samples were taken and made up to 1ml by methanol. To this 2.5ml 0.1M DPPH **[**0.010g in 100ml methanol and initial absorption was set to 1.8±0.1 at 517nm**]** was added and incubate at dark for 30min and absorbance was read at 517nm. The absorption measurement was repeated in 5 min intervals to obtain steady values. The antioxidant capacity of the extracted samples was expressed as IC_{50} value, the concentration necessary for 50% reduction of DPPH.

$ABTS^{\cdot+}$ radical cat-ion scavenging assay:
$ABTS^{\cdot+}$ radical cat-ion scavenging assay was according to the with some modification. The stock solution was prepared by incubating 7.5mM $ABTS^{\cdot+}$ solution and 3mM potassium persulfate The working solution was prepared by mixing equal volume of two stock solutions and allowing them to react for 12-16h at room temperature in dark. The stock solution was then diluted by mixing 1ml $ABTS^{\cdot+}$ solution with 60ml of methanol to obtain an absorbance of 1.1±0.02 units at 734nm using a spectrophotometer. Fresh $ABTS^{\cdot+}$ was preferred for each assay. 0.01-0.06µg/ml ascorbic acid and 0-720µg/ml plant extracts were taken for assay. 3ml of freshly prepared $ABTS^{\cdot+}$ was added and incubated in dark for 2h. Then the absorbance was read at 734nm using a spectrophotometer. The % of inhibition was calculated. The standard curve was plotted and the IC_{50} value of the standard and plant extracts was calculated & compared.

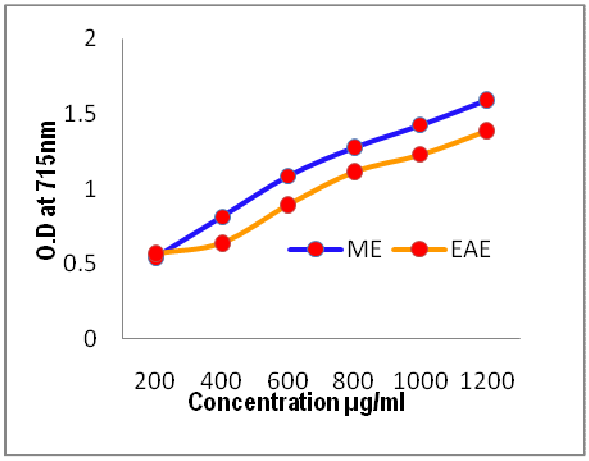
RESULT AND DISCUSSION

Solvent extraction
Aerial parts of the O. wynaadensis were successively extracted with hexane, ethyl acetate and methanol in soxhlet apparatus.100g of plant material yielded 12g hexane 8g ethyl acetate and18g methanol extracts. Hexane extract was blackish green in color with greasy nature. The ethyl acetate and methanol extracts were dark green and brownish in color respectively.

Total phenolic content
The total phenolic content of methanol and ethyl acetate extracts were estimated by the folin-ciocalteau method. The final results were expressed in terms of tannic acid equivalent. Total phenol found to be maximum in methanol extracts was little than ethyl acetate extract as shown in the below graph [NO.1&2]. Standard (Tannic acid) & extracts were showed in the graph, and the plant contains significant amount of phenols. Methanol extracts showed higher content of phenolic compounds compared to ethyl acetate extracts.



Graph 1: standard tannic acid

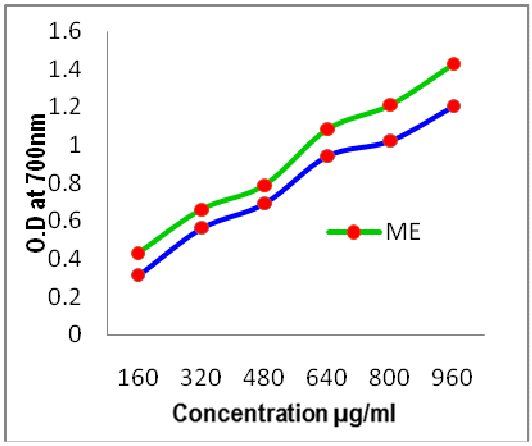


Graph 2: Total phenolic content of EAE and ME, extracts

The above graph showing the phenolic content of the EAE in (yellow) and ME in (blue) respectively.

Total reducing power:

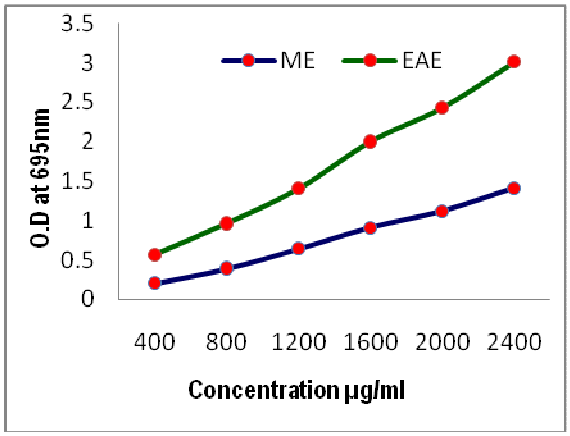
The total reducing power of the methanol and ethyl acetate extracts of Osbeckiawynaadensis was measured according to the method of [Oyaizu et al., 1986] by potassium ferric cyanide assay. The standard ascorbic acid was used as standard. Total reducing power of the extracts was expressed as ascorbic acid equivalents. The reducing power of ethyl acetate and methanol extract were found to be 32µg ascorbic acid equivalents. (Graph NO.3)



Graph 3: Reducing power of ME and EAE

Total antioxidant estimation:

The antioxidant capacity of the methanol and ethyl acetate extracts was measured by using spectrophotometer through Phospho-Molybdenum assay according to the method of [Prieto et al., 1999] Total antioxidant capacities of the plant extract were expressed in terms of Ascorbic acid equivalents. Total antioxidant activity was higher in ethyl acetate extract than the methanol extract.

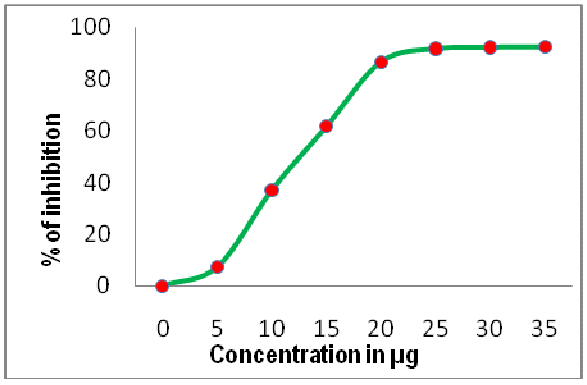


Graph 4: Total antioxidant capacity of EAE & ME

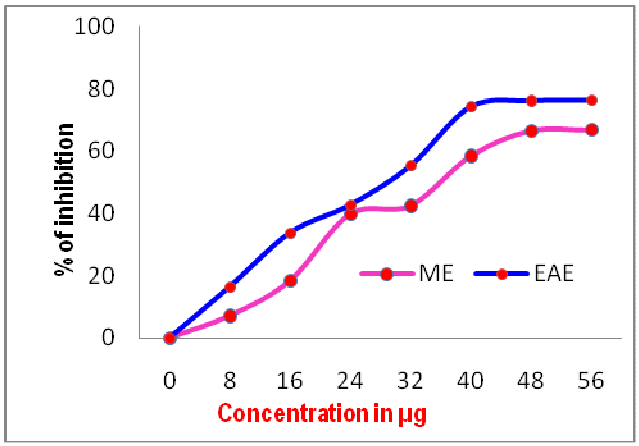
ANTIOXIDANT ACTIVITY

DPPH Radical scavenging assay:

The free radical scavenging assay was done according to the method of [Brand Williams et al., 199] 5and the IC_{50} value of standard and extracts was calculated from the graph was 7.5µg. the methanol e extract showed **IC_{50} value of 20µg** Higher than DPPH scavenging activity than ethyl acetate extract with **IC_{50} value of 22µg**



Graph 5: % inhibition of standard ascorbic acid



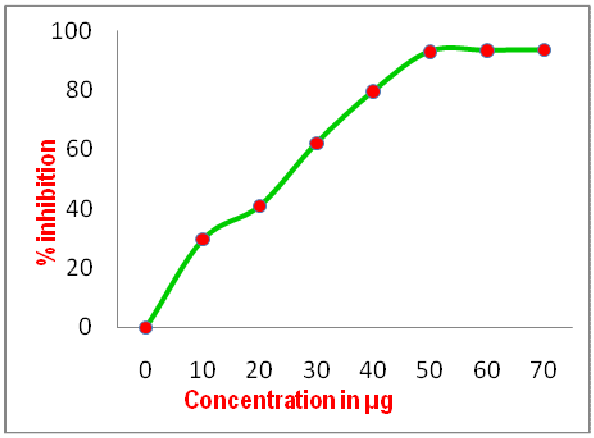
Graph 6: % of inhibition of ME and EAE extract

From the above graph IC_{50} value of the standard and extracts are as follows

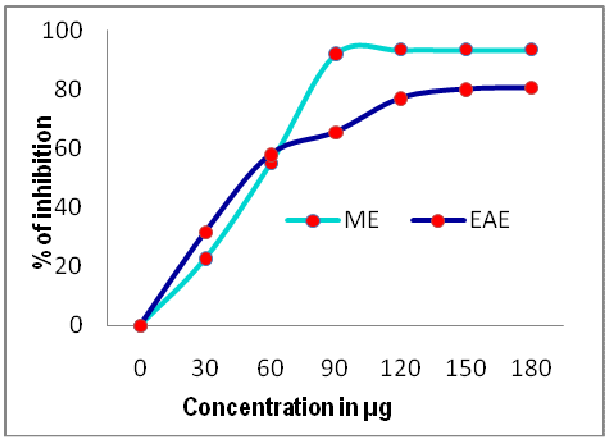
Standard ascorbic acid was **12µg**
Methanol extract was **20µg**
Ethyl acetate extracts was **22 µg**

$ABTS^{\cdot+}$ Radical scavenging assay:

The antioxidant activity of the plant extracts and the standard was assessed on the stable $ABTS^{\cdot+}$the antioxidant capacity of test compounds was expressed as EC_{50}, the concentration necessary for 50%reduction of $ABTS^{\cdot+}$ ethyl acetate extract showed better ABTS scavenging activity than the methanol extract as shown in graph no8.



Graph 7: % of inhibition of ABTS free radicals by ascorbic acid.



Graph 8: % of inhibition of ABTS free radicals by ME and EAE

From the above graphs IC_{50} value of the standard and extracts are as follows
Standard ascorbic acid was **25µg**
Methanol extract was **52µg**
Ethyl acetate extracts was **38 µg**

DISCUSSION

The phyto-chemical quantification shows the presence of the extracts showed a very good antioxidant activity. The phytochemical analysis showed the presence of flavonoids, phenolicetc. this is giving very good result forDPPH & $ABTS^{\cdot+}$ Radical scavenging assay. The total phenolic content was high in the methanol extract. After the phytochemical analysis the plant extract was studied for antioxidant activity. Natural antioxidants are considered to be multifunctional and of high interest as alternatives to synthetic antioxidants to prevent oxidation in complex food systems Wang et al., 2009 And by this I conclude that medicinal plants are used as medicine since ages. Medicines from plant origin have the advantage of availability, abundance, acceptability among the people and less side effects over synthetic drugs. Systematic study of the traditional medicine will help in the development of novel medicine for different ailments. The present study highlighted the medicinal property of the O.wynaadensis as claimed in the traditional methods. Further studies at the molecular level and in vivo may help to unravel the mechanism of action of the lead molecules present in this plant.

CONCLUSION

Medicinal plants are used as medicine since ages. Medicines from plant origin have the advantage of availability, abundance, acceptability among the people and less side effects over synthetic drugs. Systematic study of the traditional medicine will help in the development of novel medicine for different ailments. The present study highlighted the medicinal property of the O.wyanaadensis as claimed in the traditional methods. Further studies at the molecular level and in vivo may help to unravel the mechanism of action of the lead molecules present in this plant.

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