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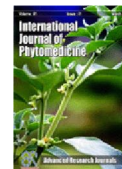

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In Vitro Antioxidant Activity of Flowers and Fruits of *Alstonia scholaris*

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ABSTRACT

The ethnobotanical and pharmacological evaluation of plant based chemicals have shown rapid strides in the last few decades. Plants have been a rich source of important therapeutic agents and form the basis of herbal systems of medicine, like ayurveda, resulting in the revival of ancient traditions of medicine. The present study was carried out to investigate the anti oxidant potential of the inflorescence and fruits of *Alstonia scholaris* using an in vitro model system like DPPH assay and Beta carotene Assay. The methanol extract of the flower showed powerful antioxidant activity by DPPH and Beta-carotene assays in comparison with the standard butylated hydroxy toluene (BHT), l- ascorbic acid. For DPPH assay the IC-50 value was also calculated and was found to have a significant correlation between benzene extract of flower and methanol extract of fruits. Overall, the methanol extracts of flower showed higher anti oxidant activity than the fruit.

Keywords: *Alstonia scholaris*, anti oxidant potential, DPPH, Beta Carotene.

Introduction

The free radicals have been found to be the cause of some of the major diseases such as heart diseases, diabetes, gout and most recently, cancer. Various pathological conditions have been found in connection with cellular necrosis because of the auto oxidation of cellular membrane lipids caused by free radicals [1]. Therefore reduction of these radicals by suitable anti oxidant molecules is crucial [2]. Nature provides answers to many ailments and is an excellent store house of remedies. Presence of flavonoids and other polyphenolic compounds in the plant parts have been reported to show their remarkable biological effects like anti oxidant property and anti cancer activity [3].

The plant *Alstonia scholaris* which belongs to Apocynaceae is an evergreen tree found in India and other parts of south Asia. Being one of the most important medicinal plant in the family, it is used against fever, malarial, abdominal disorders, dyspepsia, leprosy, skin diseases, pruritus, tumours, chronic and foul ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia and debility [4, 5]. Folkloric use of the plant parts have been known since time immemorial and is used as a bitter tonic, aphrodisiac, febrifuge, stimulant, expectorant, alterative, carminative, anti- periodic, astringent and stomachic[6]. The bark is used as a remedy for treating asthma, lung cancer, hypertension, and pneumonia while the extracts of the leaf are used to treat fever [7].

The preliminary studies on in vitro nitric oxide scavenging activity have been reported in parts of *Alstonia scholaris* plant [8]. Several natural antioxidants have been sourced from plants for treating diseases like cancer. However owing to the paucity of the clinical data available for the anti oxidant property of *Alstonia scholaris*, the present study was undertaken to explore the in vitro anti oxidant property of the flowers and fruits of *Alstonia scholaris* using DPPH assay and Beta Carotene Assay.

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Materials and Methods

Collection and Processing of Plant Sample

The *Alstonia scholaris* plant was collected from VIT University campus. The plant parts viz. leaves, stem bark, roots, flowers and fruits were then shade dried and pulverized to obtain a fine powder. Flowers and fruits were kept separate for further analysis.

Extraction

The powdered flower and fruit parts were subjected to successive extraction using different solvents in the increasing order of their polarity- hexane, benzene, methanol and water. The extracts were dried and concentrated to be used for further analysis.

Anti-Oxidant Activity

All the extracts were subjected to antioxidant activity assays using 1,1-diphenyl-2-picryl-hydrazil (DPPH) and -carotene methods. Ascorbic acid and Butylated Hydroxy Toluene (BHT) were used as the reference standards. IC50 (μg) values of the extracts for DPPH and Hydroxy radical scavenging methods were determined using Mat-lab software.

DPPH Radical Scavenging Assay

Free radical scavenging activity of the extracts was measured using the procedure described by Blois [9]. The stock solutions of crude extracts at 1mg/mL were prepared in DMSO. To each of the reaction mixtures of different concentrations, 3.0 mL of freshly prepared solution of 2, 20-diphenyl-1-picrylhydrazyl (DPPH) at concentration 19g/500ml was added. Absorbance was measured at 515 nm after 30min. The remaining amounts of DPPH-radical were calculated from calibration curve. The scavenging activity of hydroxyl radical (%) was calculated according to the equation: [(A515blank - A515sample)/A515blank]*100. IC50 values were further calculated using Mat-lab software.

Beta Carotene Bleaching Assay

10 ml of Linoleic acid solution dissolved in ethanol (2 mg/ml) and 10 ml of -carotene solution (2 mg/ml) in acetone were added to 10 ml of the molten agar (1.2% 27 solutions in boiling water). The mixture was then shaken and then poured into Petri dishes (25 ml per dish). The plates were kept away from light. Wells (8 mm diameter) were then created using sterile agar borer and 100μl of each extract (1 mg) in DMSO were transferred into the wells and the Petri dishes were incubated at 45 °C for 4 h. A zone of orange color around the well indicated positive for antioxidant activity. The diameter of the zone was measured in mm[10].

Table 1: DPPH Assay [IC50 value = Ascorbic Acid - 10.65, BHT – 32.06] of flower extracts of *Alstonia scholaris*

Extract	Concentration of Extract(μg)	Extracts			
		Hexane	Benzene	Methanol	Water
	10	0	10	26	35
	50	0	35	56	75
FLOWER	100	0	51	76	86
	250	0	61	89	87
	IC 50(μg)	NA	95	29	16

Table 2: DPPH Assay [IC50 value = Ascorbic Acid - 10.65, BHT – 32.06] of fruit extracts of *Alstonia scholaris*

Extract	Concentration of Extract(μg)	Extracts			
		Hexane	Benzene	Methanol	Water
	10	22	46	22	14
	50	43	69	63	50
FRUIT	100	62	87	90	79
	250	93	92	93	80
	IC 50(μg)	60	11.5	34.75	50

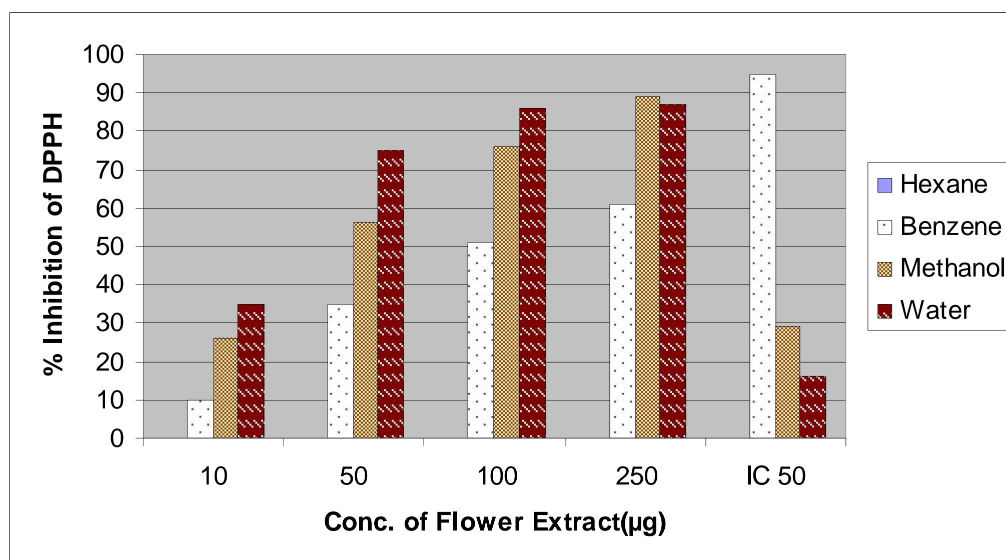

Figure 1: Inhibition of DPPH by the flower extracts of *Alstonia scholaris*
[IC50 value = Ascorbic Acid - 10.65, BHT – 32.06]

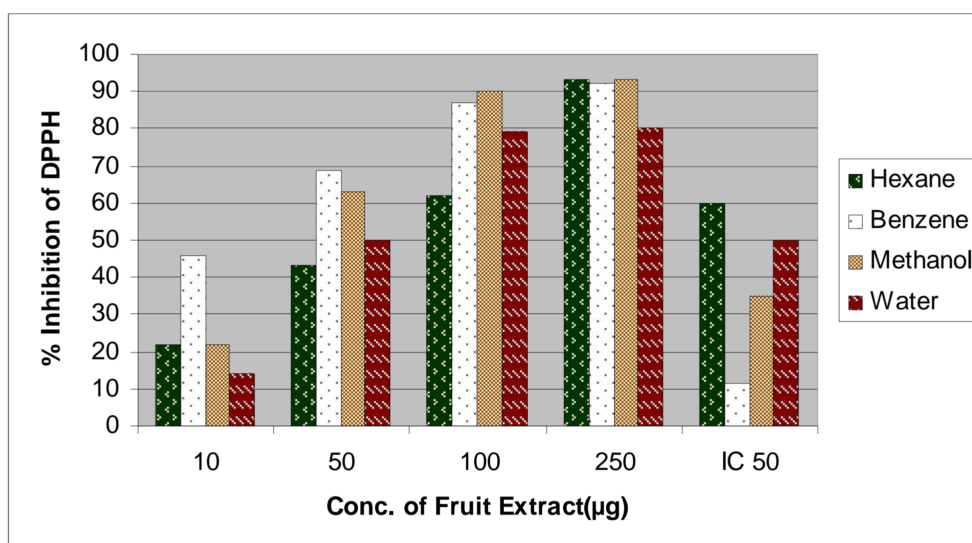

Figure 2: Inhibition of DPPH by the fruit extracts of *Alstonia scholaris*
[IC50 value = Ascorbic Acid - 10.65, BHT – 32.06]

Result and Discussion

DPPH Free Radical Scavenging Activity

DPPH, a relatively stable organic radical has been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts. The IC50 results of the DPPH scavenging activity of *Alstonia scholaris* flower and fruit extracts are presented in Table 1 and 2. The scavenging ability of all the extracts was compared with the standards ascorbic acid and BHT. Except hexane extract of flower all the other crude extracts exhibited significant DPPH scavenging efficacy as shown in Figure 1 and 2. Benzene extract of flower followed by methanol and water extract of fruit exhibited significant DPPH radical scavenging activity with IC50 values 95 µg/ml, 34.75µg/ml and 50µg/ml respectively compared to vitamin C (IC50 10.65 µg/ml). When the extracts were compared with the standards Ascorbic Acid and BHT, Benzene extract of flower and methanol extract of fruit showed significant correlation.

Table 3: -carotene Assay [BHT – 40mm (1mg) & 25mm (0.5mg)] of flower and fruits extracts of *Alstonia scholaris*

Extract	Concentration of Extract(mg)	Extracts			
		Hexane	Benzene	Methanol	Water
Flowers	0.5	8	12	15	11
	1	9	14	17	13
Fruits	0.5	9	8	14	14
	1	12	9	17	16

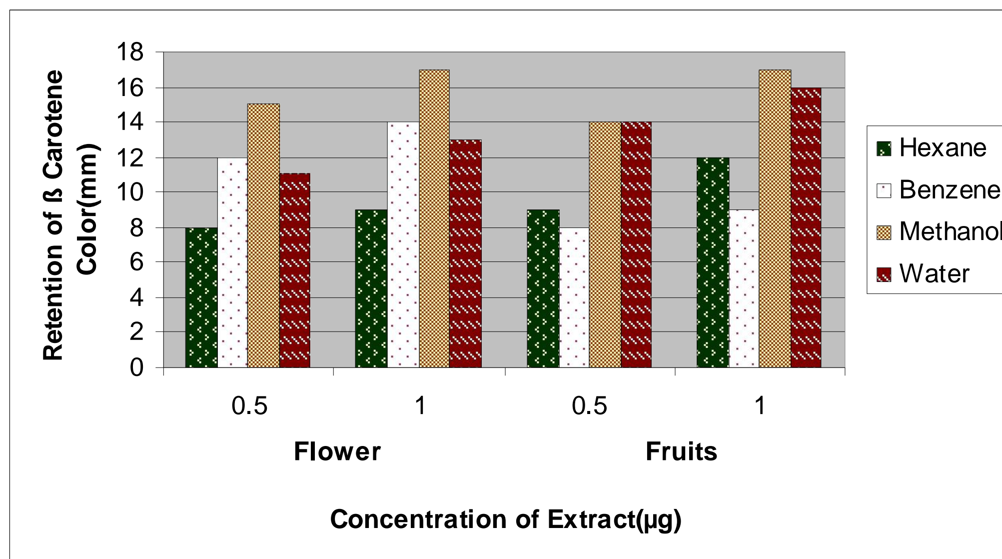

Figure 3: Antioxidant activity in terms of means of zone of Beta carotene color retention (mm) of flowers and fruits of *Alstonia scholaris*.

Beta Carotene Bleaching Assay

In a -carotene/linoleic acid model system, -carotene undergoes rapid decolorization in the absence of an antioxidant. All extracts except hexane extract of flower, were able to retain the color of beta carotene. Methanol extract of flower showed highest color retention with a mean zone of 17 mm. The values of all the extracts are shown in Table 3. It is interesting to note that the methanol and water extracts of both the flower and fruit showed equal potential as shown in Figure 3. Where as in all the other antioxidant assays methanol extract showed higher activity irrespective of the flower or fruit. In the present study, the anti oxidant potential of the flowers ad fruits are evaluated. Previous studies on *A scholaris* have suggested the presence of nitric oxide scavenging activity [11]. Further findings on the anti oxidant results of ethanolic extract of *A scholaris* [12] prompted us to evaluate the anti oxidant potential of the inflorescences by 2 methods: DPPH and Beta Carotene assays as it is impossible to evaluate the anti oxidant activity by one method due to oxidative processes involved and the variable nature of the anti oxidants[13,14]. The effect of probable anti oxidants present in plant on DPPH radical scavenging was supposed to be due to their hydrogen donating ability. DPPH being a stable free radical accepts an electron on the free hydrogen radical to become stabilised diamagnetic molecule [15]. The scavenging of the radical by hydrogen donation is caused by the anti oxidants, because of the reaction between anti oxidant molecule and free radical. The present in vitro anti oxidant study is in agreement with the findings of Arulmozhi et al. on the ethanolic extract of *A scholaris*. The presence of the flavanoids in the preliminary investigation [16] can be correlated with its potential anti oxidant activity, however further investigation is needed to validate such correlation. However the report by Juhi et al.[17] showed no correlation between

anti oxidant activity and the quantitative phytochemical screening i.e. total polyphenolic content and tannin fractions which is opposite to many published literature where anti oxidant activity is closely related to polyphenolic content[18,19].

Conclusion

The findings of the present study clearly indicate the potential antioxidant properties of the flowers and fruit. The promising results of this plant extract with antioxidant assay models proved the plant to be a potential antioxidant agent. This efficacy of the plant could be attributed to the synergistic effect of various phytochemicals like flavanoids, steroids and polyphenolic compounds. Further detailed investigation needs to be undertaken in order to evaluate the mode of action of these plant extracts at the molecular level.

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