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IN-VITRO SCREENING OF METHANOLIC LEAF EXTRACTS OF *ALBIZIA SAMAN (JACQ.)MERR,* FOR THEIR ANTIOXIDANT PROPERTY

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Abstract

The present study was designed to evaluate the *in-vitro* antioxidant potential of methanolic extract of *Albizia saman* leaves. The extraction of leaves was carried out by Soxhlet apparatus using methanol. The methanolic extract investigated for free radical scavenging activity of the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), reducing power assay and nitric oxide scavenging activity. In DPPH assay the methanolic extract at a concentration 500µg/ml was showed 79.7049±0.2158. The methanolic extracts showed maximum activity of 81.608±0.2843% at 500µg/ml in nitric oxide scavenging assay. In reducing power assay the methanolic extract showed maximum absorbance of 0.3706±0.00158 at 500µg/ml .The phytochemical screening revealed the presence of alkaloids and polyphenolic compounds. This suggest a potential utility of the plant as a source of phenolic antioxidants and may provide leads in the ongoing research for natural antioxidants form Indian medicinal plants to be used in treating diseases related to free radical reactions.

Keywords: *Albizia saman*, DPPH assay, Reducing power assay, Free radical scavenging, Antioxidant activity.

Introduction

Antioxidants are believed to quench free radicals free radicals are atom or molecule with singlet unpaired electron which make them highly reactive. Oxidative free radicals are generated by metabolic reactions. Free radicals create a chain reaction leading to membrane lipid per oxidation, DNA damage etc. Free radical oxidation has been implicated in Cancer, atherosclerosis, neuro-degenerative diseases and inflammatory bowel disease.[1] Antioxidants are added to pharmaceutical formulations as redox systems possessing higher oxidative potential than the drug that they are designed to protect, or as chain inhibitors of radical induced decomposition. In general, the effect of oxidants is to break up the chain formed during a hydrogen atom or an electron to free radical receiving the excess energy possessed by the active molecule.[2] *Samanea saman* (rain tree) is a tropically found common plant which mitigates multitude of diseases and ailments.[3] There are several folk remedies prepared from various parts of rain tree. The root decoction is used in hot baths for stomach cancer.[4] Rain tree is a folk remedy for colds, diarrhoea, headache, intestinal ailments, and stomach ache.[5] Recent evidence suggest that free radicals, which are generated in any bioorganic redox processes, may induce oxidative damage in various components of the body (e.g., lipids, proteins and nucleic acids) and may also be

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involved in processes leading to the formation of mutations.[6]

Materials and methods

Collection of Plant

The leaves of *Albizia saman* were collected from Ernakulum District in the month of march 2014 and authenticated by Dr.M.S Francis, M.Sc, M.Phil, P.hD, Associate Professor in Botany, Center for Post Graduate Studies and Research, Sacred Heart College, Kochi-13. The voucher specimen of the plant was prepared and stored in Unibiosys Biotech Research Labs; Kochi with reference number BIS 01/2014/1012.The plant material was dried, powdered and stored in airtight containers until further studies.[7]

Preparation of extracts

The Leaves of *Albizia saman* were washed with distilled water and dried in shade. About 400g of air dried powdered Leaves of *Albizia saman* was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was again packed and extracted by using methanol as solvent, till color disappeared. The temperature was maintained at 55ºC-65ºC. After that, the extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness and dry residue was obtained.

In vitro anti oxidant activity

DPPH Assay

Procedure

Methanolic extract were prepared in methanolic solution at various concentrations (100,200, 300, 400 and 500 µg/ml).To a set of test tubes, 2.9 ml of DPPH solution (100 µM/ml in methanol) and 0.1 ml of varying concentrations of test sample were added. The mixture was then shaken vigorously and allowed it to stand in dark for 30 m and absorbance was measured using a UV spectrophotometer at 517 nm.[8] A control solution was consisting of 0.1 ml of methanol and 2.9 ml of DPPH radical solution.[9] Ascorbic acid was used as standard.[10] Percentage scavenging of DPPH radical was calculated by comparing the absorbance between the test and control. All determinations were done in triplicate and the mean values were determined:

$$\text{Scavenging activity (\%)} = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Reducing power assay

Procedure

The reducing power of extracts was determined according to the method of different amounts of each extracts (100,200,300,400,500µg/ ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%).[11]The mixture was incubated at 50^{o}C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as standard. Compared the results of test and standard. All determinations were done in triplicate and the mean values were determined

Nitric oxide Scavenging Assay

Procedure

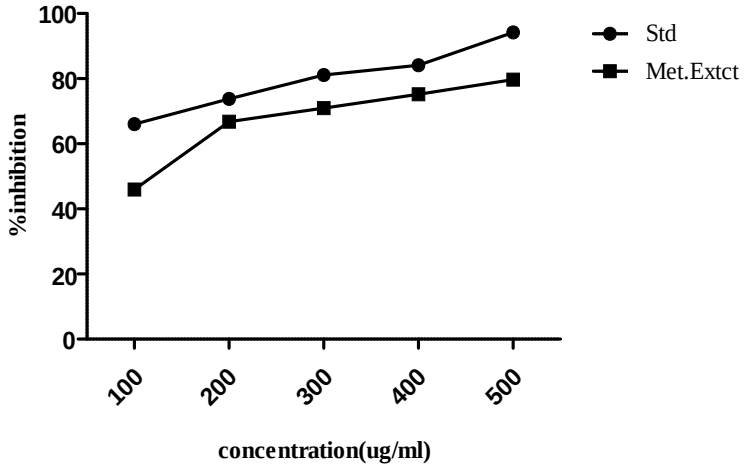
The scavenging effect of the methanolic extracts of *Albizia Saman* leaves on nitric oxide was measured according to the a reaction mixture was prepared containing: 10 mM Sodium Nitro Prusside in 0.5 M phosphate buffer, pH 7.4, and various doses (100,200,300,400,500/ml) of the test solution in a final volume of 3 ml. After incubation for 60 min at 37°C, Griess reagent (1% Sulfanilamide in 5% H3PO4 and 0.1 % Naphthyl ethylene diamine dihydrochloride) was added.[12] The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with a-napthyl-ethylene diamine was measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. All determinations were done in triplicate and the mean values were determined Nitric oxide scavenging ability (%) was calculated by using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Table No. 01: DPPH scavenger assay of methonolic extract of *Albizia saman*leaves compared with standard ascorbic acid

S.No	Concentration(µg/ml)	%inhibition of Std(Ascorbic Acid)	Ic50 (µg/ml)	%inhibition of Methanolic Extract	Ic50 (µg/ml)
1	100	66.07±0.2121***		45.93±0.0723***	
2	200	73.80±0.2121***		66.79±0.3596***	
3	300	81.13±0.2157***	75.68	70.96±0.2172***	149.72
4	400	84.90±0.3597***		75.23±0.2158***	
5	500	94.24±0.3597***		79.70±0.2158***	

Values are mean±S.D., n=3,p<0.001=*,p<0.01=**, p<0.05=* when compared to control.**

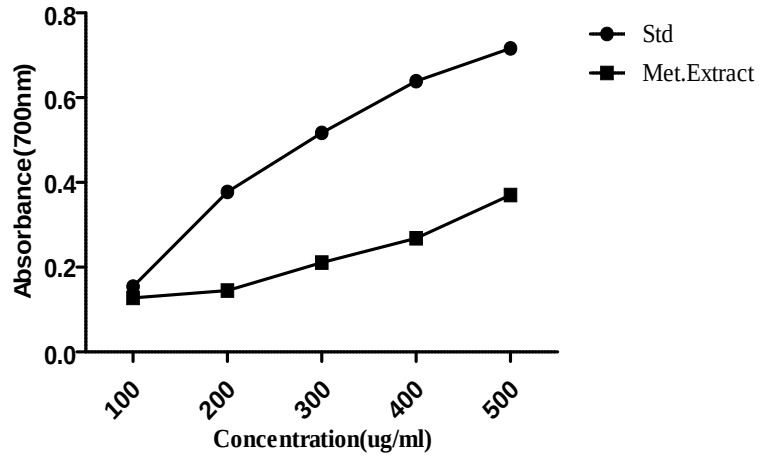


Graph 1: DPPH scavenger assay of methonolic extract of *Albizia Saman* leaves compared with standard ascorbic acid

Table No. 02: Reducing power assay of *Albizia Saman* leaves compared with standard ascorbic acid.

S. No	Concentration(µg/ml)	Absorbance of std (ascorbic acid) (700nm)	Absorbance of methanolic extract.(700nm)
1	100	0.1546±0.0032***	0.1276±0.0051***
2	200	0.3776±0.0031***	0.1453±0.0051***
3	300	0.5170±0.0020***	0.2110±0.0020***
4	400	0.6390±0.0020***	0.2686±0.0022***
5	500	0.7163±0.0031***	0.3706±0.0015***

Values are mean±S.D., n=3,p<0.001=*, p<0.01=**,p<0.05=* when compared to control.**

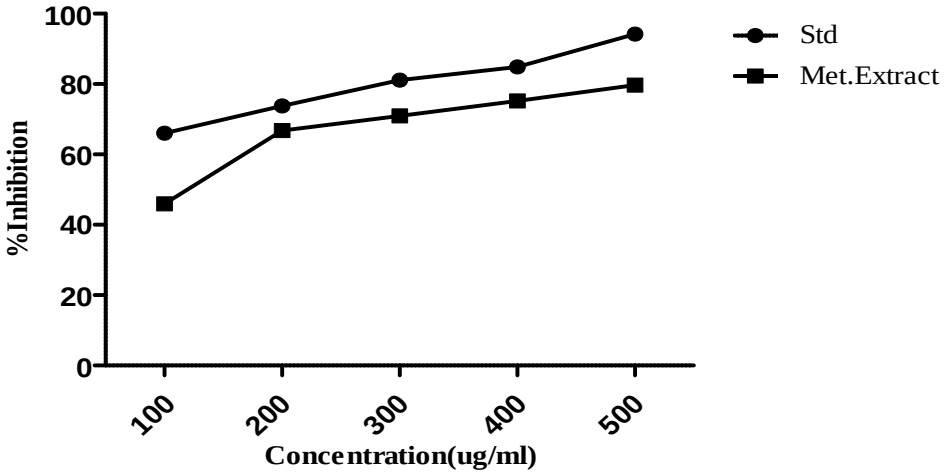


Graph 2: Reducing power assay of *Albizia Saman leaves* compared with Standard ascorbic acid.

Table No. 03: Nitric Oxide (NO) scavenging activity of *Albizia Saman* leaves compared with standard ascorbic acid.

S.No	Concentration(µg/ml)	%inhibition of Std (Ascorbic Acid)	Ic50 (µg/ml)	%inhibition of Methanolic Extract	Ic50 (µg/ml)
1	100	57.54±0.2132***		42.81±0.2843***	
2	200	66.74±0.2843***		57.54±0.2132***	
3	300	79.95±0.3554***		71.35±0.3339***	
4	400	84.12±0.1421***	86.89	77.89±0.1421***	173.79
5	500	92.46±0.5685***		81.61±0.2843***	

Values are mean±S.D., n=3,p<0.001=*,p<0.01=**,p<0.05 when compared to control.**



Graph 3: Nitric Oxide (NO) scavenging activity of *Albizia Saman* leaves compared with standard ascorbic acid

Results and discussion

DPPH Assay

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts**.** A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience.[13]

In the DPPH photometric assay method, the methanolic extract of *Albizia Saman* leaves exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested (100, 200, 300, 400,500 µg/ml). There was a dose dependent increase in the percentage antioxidant activity for all concentrations tested.

The extract at a concentration of 100µg/ml showed a percentage inhibition of 45.9303 ± 0.07255 and for 500µg/ml it was 79.7049 ± 0.2158. The concentration required for 50% inhibition Ic_{50} was found to be 149.72 µg/ml . Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied from 100 to 500µg/ml. Ascorbic acid at a concentration of 100µg/ml exhibited a percentage inhibition of 66.07±0.2121 and for500µg/ml, 94.2522±0.3597. The Ic_{50} value was found to be 75.68 µg/ml (Table 1). A graded increase in percentage of inhibition was observed for the increase in the concentration of ascorbic acid. (Graph: 1) illustrate significant decrease in the DPPH radical due to the scavenging ability of extracts and ascorbic acid.

Reducing Power Assay

In this assay the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals causes the conversion of the Fe 3+ / ferricyanide complex used in this method to the ferrous form a higher absorbance at 700nm indicates a higher reducing power. The results of the reducing power assay are given in (Table 2). Therefore by measuring the formation of pearls Prussian blue at 700nm, the Fe 2+ concentration.

The reducing power of the alcoholic extracts and standards increases with the increase in amount of sample and standard concentrations (graph: 2.Table 2). Shows increase in the absorption of methanolic extract with increase in the concentration. The methanolic extracts showed significant maximum absorbance of 0.3706±0.0015at 500 µg /ml, whereas ascorbic acid at the same concentration exhibited 0.7163±0.0031absorbance.

Nitric Oxide Scavenging Assay

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.

Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *Albizia saman leaves* decreased the amount of nitrite generated from the decomposition of SNP in vitro. The scavenging of NO by the extracts was increased in dose dependent manner (table 4). (Graph: 3) illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The methanolic extracts showed significant activity of 81.608±0.2843 % at 500 µg /ml, and Ic_{50} was 86.89 µg /ml whereas ascorbic acid at the 500 µg /ml concentration exhibited 92.4623±0.5685% inhibition and Ic_{50} was 173.79 µg /ml.

Conclusion

On the basis of the above results it can be concluded that the methanolic extract possess significant antioxidant activities studied by *in- vitro* screening methods. The phytochemical screening revealed the presence of alkaloids and polyphenolic compounds. Methanolic leaf extract was screened for antioxidant activity by performing *in-vitro* assay method namely DPPH radical scavenging, reducing power method and nitric oxide scavenging assay. In all the antioxidant assay methods, the methanolic extract of *Albizia saman* leaves exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested. There was a dose dependent increase in the percentage antioxidant activity significantly. This suggest a potential utility of the plant as a source of phenolic antioxidants and may provide leads in the ongoing research for natural antioxidants form Indian medicinal plants to be used in treating diseases related to free radical reactions.

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