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AQUEOUS EXTRACT OF *ALBIZIA STIPULATA* BOIV. BARK - *IN VITRO* ANTIOXIDANT ACTIVITY STUDIES

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ABSTRACT

The objective of the present study was to determine the *in-vitro* antioxidant activity of ac extract of *Albizia stipulate* Boiv. bark. The antioxidant properties were evaluated by determin free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-lethyl benzothiazoline-6-sulfonic acid) (ABTS), and Superoxide ion. The aqueous extract pos an IC50 value of $12.52\mu g/ml$ for DPPH radical scavenging assay on comparing to standard V C ($3.59\mu g/ml$), an IC50 value of $3.39\,\mu g/ml$ for ABST radical scavenging assay when compavitamin C ($2.32\mu g/ml$), and for Superoxide ion scavenging the IC50 value was record $13.60\mu g/ml$ on compared to standard gallic acid ($0.61\,\mu g/ml$). From the results in terms of r scavenging activity against DPPH, ABTS, and Superoxide ion, *Albizia stipulate* Boiv. bark extexcellent antioxidant activity than standards. Hence, an aqueous extract of *Albizia stipulate* babe suggested for usage as an effective natural antioxidant in pharmaceutical and nutrace products.

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INTRODUCTION

Oxidative stress in a cellular system is a biochemical abnormality produced by inequality between the absolute overload of oxidants or free radical moieties from the natural physiological and biochemical pathways and a successive diminution of antioxidant components in the body. It is engaged in the pathogenesis of a considerable number of chronic metabolic illnesses which include atherosclerosis, ischemia/reperfusion injury, chronic inflammatory diseases, renal failure, and diabetes mellitus [1]. Hence, the destructive effects of oxidative stress in several chronic metabolic disorders have obliged the scientific community to explore for antioxidative compounds that can inhibit the oxidation of oxidizable molecules in a chain reaction that could consequently be vital in the therapy and prevention of these disorders [2].

There are two important classifications of antioxidant agents to be specific synthetic, and natural. Generally, synthetic antioxidants are compounds with phenolic structures of different degrees of alkyl substitution, while natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogenous substances (alkaloids, chlorophyll subsidiaries, amino acids, and amines), or carotenoids and, also ascorbic acids [3, 4].

Unfortunately, although synthetic or chemical antioxidants, for example, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary-butylhydroxytoluene exhibit potent free radical scavenging effects but, limitations on the use of these compounds because, they cause liver damage and carcinogenesis in laboratory animals [5-7]. Therefore, there is a necessity for the progress and consumption of more efficient antioxidants of natural origin.

Therapeutic plants have been widely used for the cure of a range of chronic metabolic disorders by traditional healers, and herbalists especially in Africa, where greater than 5400 medical shrubs have been stated to have over 16,300 therapeutic uses [8]. Compounds obtained from natural products have affected the drug discovery program. This is evident in several clinically active drugs that are either natural products or have a natural product pharmacophore [9]. Thus, in the latest years, the assessment of medicinal plants with possible antioxidant properties have got considerable attention due to the increasing interest in safe and non-toxic alternative antioxidants [10]. Consistent with we have searched the number of plants for antioxidant activity finally we have selected *Albizia* species as it is easily available, has antioxidant activity and has a long history of use in traditional medicine, particularly for the treatment of asthma, allergic disorders, inflammation, and reduced ulcer effect in patients. This plant is also used in Ayurveda for the treatment of bronchitis, asthma, leprosy, eczema, pruritus, paralysis [11], and also has been reported to possess antiallergic, antioxidant, analgesic, anti-inflammatory, anticonvulsant, antiulcer, antimicrobial, and cytotoxic activities [12-17], antihistaminic, anti-spermatogenic activities [18,19], antidiabetic, various organ protective, central analgesic [20-21], anti-HIV-1 [23], memory enhancing, antidiarrheal, antispasmodic, and broncho dilatory [24] and anticancer activities [25].

Albizia is a large genus belonging to the family Leguminosae, subfamily Mimosoideae, which consists of incredibly important multipurpose tree legumes. It consists of approximately 150 species, tropical and subtropical trees, and shrubs with a short lifespan, and it is largely scattered all over Asia, including India, Mauritius, China, East Africa, South Africa, Australia, America, Mexico, West Indies, and Brazil [26].

Albizia stipulata Boiv Commonly called as nalla regi, bandi chindugu, Konda-chiragu and is a deciduous tree, up to 20m tall, crown broad, spreading, and flat-topped. The bark is pale grey or greenish-grey, smooth, horizontally furrowed. Leaves are 11-18 cm long, bipinnate, tomentose: petiole 4.5 cm, gland just below the middle. Flowers are pinkish-white, light yellow, or reddish. Albizia stipulata occurs naturally in India, Myanmar (Burma), Thailand, Indochina, China, Java, and the Lesser Sunda Island (Bali and Nusa Tenggara). Commonly seen in Anantagiri, Sunkarimetta, Borra caves, and Molachintapally. An extract of the wood has a repellent property to subterranean termites [27]. This plant was selected from the books "Flora of the presidency of Madras" Vol.1 Adlard & Sons Ltd., London. Rep. Ed.1997, Dehradun [28]. Biological and pharmacological actions of Albizia stipulata plant are anti-inflammatory, anti-ulcer activities from chloroform extract of bark [29] and antioxidant, antimicrobial activities from methanol extract of stem bark have been reported [30], which exposed that the bark has contained terpenoids as triterpenes, steroids, and flavonoids.

The objective of the present research is to study the antioxidative effects of aqueous extract of the bark of *Albizia stipulata* Boiv concerning free radical scavenging activity against DPPH, ABTS, and superoxide ion on comparing with standards, this study might help in reducing the oxidative stress-mediated chronic metabolic ailments, as well as bridging the traditional uses of this plant with the modern medicinal practices in the treatment of a vast array of chronic metabolic disorders.

MATERIALS AND METHODS MATERIALS

CHEMICALS AND REAGENTS

Analytical grade chemicals and standards were used for the study. Gallic acid, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS), 2-deoxy-D-ribose, potassium persulfate (K2S2O8), ethanol, phosphate buffer, riboflavin, EDTA, and nitro blue tetrazolium (NBT) were procured from standard companies.

COLLECTION OF PLANT MATERIAL

The fresh bark of *Albizia stipulata* was collected in March 2011 from Papikondalu, East Godavari district, Andhra Pradesh. The plant sample was identified and authenticated by Dr. K.N. Reddy, Dept. of taxonomy, Laila Impex R&D Centre, Vijayawada. The voucher specimen No. of bark material was 3318 and was deposited in the raw drug museum. The plant material was immediately cleaned with distilled water, chopped into small pieces, and air-dried completely under shade until a constant weight was attained. The dried-up materials were grounded to a fine powder utilizing a mixer and collected in air-tight bags.

METHODS

PREPARATION OF EXTRACT

The powdered bark of *Albizia stipulata* was extracted with water, concentrated to dryness in vacuum at 40°C for 25 min using Rotary Evaporator (Buchi evaporator, India), and the weight of extract was found to be 10.32g.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical screening was performed from the resultant aqueous extract of bark of A. stipulata boiv according to standard procedures [31,32] to identify the presence of various phytochemical constituents.

ANTIOXIDANT ACTIVITY

Diphenyl picrylhydrazyl radical scavenging method (DPPH)

The free radical scavenging activity of the aqueous extract was determined and compared to that of ascorbic by using a reported method [33]; To perform this assay, reaction mixtures were prepared comprising 1mL of DPPH solution (0.1 mmol/L, in 95% ethanol v/v) with various concentration of the extract. These solutions were shaken and incubated in the dark for 20 min at room temperature, and the absorbance was assessed at 517 nm against a blank, lacking the scavenger. The radical scavenging action of extract in the DPPH assay was measured as a decrease in the absorbance of DPPH and measured by applying the formula:

% Scavenging activity =
$$(Abs_{cont} - Abs_{sample}) / Abs_{cont} \times 100$$

Where Abs_{cont} is absorbance of the control, and Abs _{sample} is absorbance of the sample solution

2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) radical scavenging method (ABTS).

An ABTS radical-scavenging property of the extract was evaluated by the following reference method [34]. The ABTS + cation radical was formed by the response among 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate (K2S2O8) solution, keep aside at room temperature for 16 h. before use, this solution was diluted with ethanol to find an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract at different concentrations with 1ml of ABTS solution were homogenized, and its absorbance was read at 734nm. Ethanol was taken as blank in each test, and all estimates were done after at least 6 min. in a parallel way, the reaction mixture of the standard was recorded by combining 950 μ l of ABTS+ solution and 50 μ l of BHT. Concerning the antiradical activity, ABTS scavenging capacity was expressed as IC50 (μ g/ml). The inhibition level of ABTS radical was determined using the equation:

% Scavenging activity =
$$(Abs_{cont} - Abs_{sample}) / Abs_{cont} \times 100$$

Superoxide ion Scavenging assay

Superoxide ion scavenging activity assay was completed as per the standard procedure [35]. The activity was compared with Gallic acid, taken as a positive standard. The reaction mixture contained 50mM phosphate buffer (pH 7.6), 20µg riboflavin, 12mM EDTA and 0.1 mg/ml of NBT (nitro blue tetrazolium), all added in sequential order. The reaction was begun by illustrating the reaction mixture comprising a various concentration of sample extract for 90 sec and then measured the absorbance at 590nm.

% Scavenging activity =
$$Abs_{cont}$$
 - Abs_{contr} × 100

RESULTS AND DISCUSSION PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of an aqueous extract from bark confirmed the existence of several secondary metabolites of biological and pharmacological importance such as alkaloids, flavonoids, quinones, coumarins, resins, saponin, carbohydrates, phenols, and tannins.

ANTIOXIDANT ACTIVITY

Plant phenols preferentially Polyphenols are one of the largest and varied classes of compounds, a considerable number of which arises naturally in a wide range of nourishment and plants. The flavonoids are the major and finest examined compounds amongst polyphenols. Several polyphenols are either being effectively developed or at present marketed as dietary supplements or potential herbal derived medicine. Even though these compounds play an indistinct role in nourishment, many of them have properties including antioxidant, anti-mutagenic, anti-cancer-causing, and anti-inflammatory impacts that may be useful in preventing disease and protecting the stability of the genome. Free radical scavenging property is a measure of the effectiveness of the antioxidant(s) present in a pure compound or a mixture. The rate of scavenging and IC50 values were computed for all models.

DPPH radical scavenging assay

The reactivity of aqueous extract of *Albizia stipulate* bark was studied with DPPH, a stable free radical. As DPPH gains one electron in the presence of a free radical scrounger, the absorption diminishes, and the subsequent discoloration is stoichiometrically related to the number of electrons picked up. *Albizia stipulate* bark aqueous extract possessed an IC50 value 12.52µg/ml and standard vitamin C is 3.59µg/ml. The results are given in Table1, and radical scavenging activity is shown in Fig. 1.

Table No:1- DPPH radical scavenging assay of Albizia stipulate.

Test compound	n	% Inhibition of oxidation	IC ₅₀ (μg/ml)
	(μg/ml) 5	30.953+0.027	
ASBA	10	77.136±0.027	12.52
	25	47.346±0.029	
	1	15.546±0.033	
Vitamin-C	2.5	362.64 ± 0.029	3.59
	5	5.63 ± 0.030	

^{*%} Inhibition is expressed as mean \pm SEM (n=3).

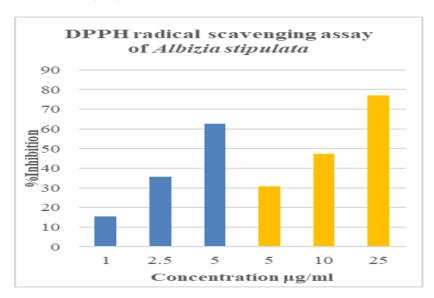


Figure No:1- DPPH radical scavenging assay of extract of Albizia stipulate.

ABTS radical scavenging assay

The ABTS+ radical scavenging method, which utilizes a specific absorbance (734 nm) at a wavelength far-off from the visible region and involves a short reaction time, can be utilized as an index that mimics the antioxidant action of the test samples.

The aqueous bark extract of *Albizia stipulate* was observed to be successful in scavenging radicals and the rise was concentration subordinate. The inhibition of the concentrate is 3.39 μ g/ml and standard vitamin C is observed to be 2.37 μ g/ml. This demonstrates *A. stipulate* bark extract introduces a good capacity to scavenging the ABTS radical. The results are displayed in Table 2 and the scavenging activity of the extract is shown in Fig. 2.

Table No:2- ABTS radical scavenging assay of Albizia stipulate.

Test compound	Concentration(μg/m l)	% Inhibition of oxidation	IC ₅₀ (μg/ml)
	5	34.453±0.037	
ASBA	10	41.24±0.029	13.60
	25	72.646±0.029	
Vitamin-C	0.25	20.543±0.049	
	0.5	38.93±0.030	2.37
	0.75	38.93±0.030	

^{*} Inhibition is expressed as mean \pm SEM (n=3).

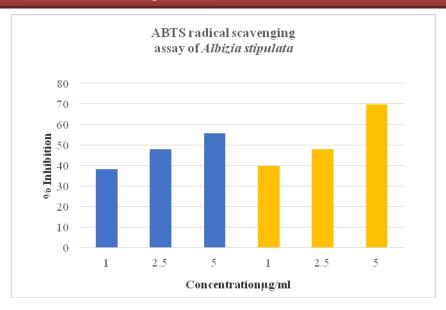


Figure No:2- ABTS radical scavenging assay of extract of Albizia stipulate.

Superoxide radical Scavenging Assay

In the phenazine methosulphate/Nicotinamide adenine dinucleotide-nitro blue tetrazolium (PMS/NADH-NBT) system, superoxide anion was produced by a nonenzymatic reaction of phenazine methosulphate in the presence of NADH and molecular oxygen.

NBT reduces into formazan by Superoxide anion at pH 7.8 at ambient temperature and formazan formation is monitored by spectrophotometry at 560 nm. The diminishing of absorbance at 560 nm with an antioxidant in this way demonstrates the utilization of superoxide anion in the reaction mixture.

The superoxide anion radical-scavenging action of the extract might be because of the presence of phenolic phytochemicals. The recorded IC50 value for aqueous extract of *Albizia stipulata* bark is $13.60\mu g/ml$ and for standard gallic acid it is found to $0.61\mu g/ml$. The increase in activity is because of an increase in the number of a phenolic hydroxyl group in the molecule. The results are depicted in Table 3 and superoxide ion scavenging activity of the extract is shown in Fig. 3.

Table No: 3- Superoxide radical scavenging assay of Albizia stipulate.

Test compound	Concentration (µg/ml)	% Inhibition of oxidation	IC ₅₀ (μg/ml)
4.675.4	5	34.453±0.037	10.10
ASBA	10	41.24 ± 0.029	13.60
	25	72.646±0.029	
Gallic acid	0.25	20.543 ± 0.049	
	0.5	38.93 ± 0.030	0.61
	0.75	38.93 ± 0.030	

^{*} Inhibition is expressed as mean \pm SEM (n=3).

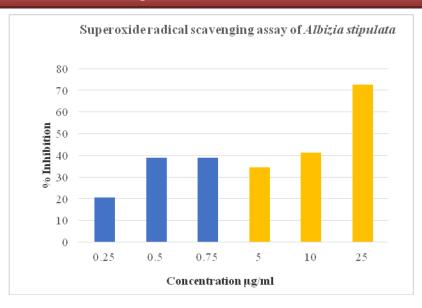


Figure No.: 3- Superoxide radical scavenging assay of extract of Albizia stipulate.

CONCLUSION

In the earlier couple of years, excitement for the discovery of new natural antioxidants has been intensified because oxidative stress has related to various autoimmune diseases. The use of synthetic antioxidants triggers issues of toxicity. Hence, Investigation for the source of natural antioxidants is gaining much importance. In this research, antioxidant activity of aqueous bark extract of *Albizia stipulata Boiv*. was screened and reported. Antioxidant property of extract was determined in the form of DPPH, superoxide, and ABTS radical scavenging activity by comparing with standard antioxidants. Aqueous bark extract possesses greater antioxidant activity compared to the standards and is due to the presence of flavonoids, glycosides, and poly hydroxy phenolic phytoconstituents. This study proves the rational origin for its use in traditional medicine for the treatment of stress-related chronic metabolic disorders such as cardiovascular diseases, atherosclerosis, aging, immune system disorders like rheumatoid joint inflammation, etc., in patients. Further phytochemical and pharmacological investigations are underway to characterize active constituents. This work, we believe, will recommend for future research.

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CONFLICTS OF INTEREST

The Laila Impex, Research and Development Centre had no association in the writing of the manuscript and the decision to submit the article for publication. The authors have indicated that they have no competing interests regarding the content of this article.

LIST OF ABBREVIATIONS

ASBA : Albizia stipulata Bark Aqueous Extract

SEM : Standard Error Mean

DPPH : 1,1-diphenyl-2-picrylhydrazyl ABTS: 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid)

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