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PHYTOCHEMICAL PROFILE AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *BAUHINIA TOMENTOSA* LINN. LEAVES

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ABSTRACT

Natural products have endless potential. Most important of such molecules are flavonoids, alkaloids, tannins, terpenoids, saponins and other phenolic compounds. These compounds have been reported to quench or decompose free radicals and its products. *Bauhinia tomentosa* is one such medicinal plant belonging to Caesalpinaceae family. Various parts of this medicinal plant has been used to treat various ailments in traditional systems of medicine. The purpose of the present study is to demonstrate the phytochemical content, phytonutrients and mineral contents of ethanol extract of *B. tomentosa* leaves. The study also investigates the free radical scavenging potential of *B.tomentosa* leaf extract. The collected leaf samples was subjected to physiochemical studies and safety studies like total microbial load assay, aflatoxins assay, pesticides analysis and heavy metal analysis as per WHO guidelines. Total polyphenols, flavonoids, tannins, alkaloids, total protein sugars, fibres, fats, macro minerals (Ca, Mg, P&K) and micro minerals (Fe, Cu, Mn &Zn) were determined using standard procedures. Ethanol extract of *B. tomentosa* (EBT) was then subjected to DPPH, nitric oxide and superoxide radical scavenging assays and FRAP assay. From the results, it was observed that the collected leaf sample was found to be free from pesticides, aflatoxins, heavy metals and microbial contamination. The water and alcohol extractive values of *B.tomentosa* leaves were 9.1 ± 0.8% and 8.1 ± 0.4 % respectively. The total ash value, water soluble ash and acid insoluble ash were found to be 12.19 ± 1.1%, 3.1 ± 0.3 % and 8.5 ± 0.7% respectively. The moisture content was found to be 10.13 ± 0.9%. The results have also demonstrated the presence of appreciable amounts of phytochemicals, phytonutrients and minerals in EBT. The IC_{50} value of EBT for DPPH radical was found to be 59.54 µg/ml and it also exhibited appreciable activity towards other radicals as compared with the standards like BHT and curcumin. Thus, the present study justifies the traditional usage of *B. tomentosa* for the treatment of various ailments. From the study it can be concluded that the *B. tomentosa* possess significant amounts of phytochemicals and also has appreciable activity towards free radicals. Future work may be carried out to identify the active principle for the effective treatment against various diseases which involves oxidative stress in its pathogenesis.

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INTRODUCTION

Plants have always played a central role in traditional systems of medicine for the prevention and treatment of disease worldwide [1]. It is important to understand that for thousands of years the traditional medicine in all countries exclusively employed naturally occurring plant products because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body [2]. In the last years, interest in the antioxidant activity of plant extracts, or isolated substances from plants, has grown, especially in countries possessing great biodiversity.

Reactive oxygen species(ROS) that are produced as a result of cellular metabolism are highly toxic and are involved in the etiology of many chronic diseases due to oxidative damage to lipids, nucleic acids and proteins. Free radicals are responsible for causing a large number of diseases including cancer , cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis [3,4]. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention.

There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Although an internal system of antioxidant exists in our body but to get rid of excessive free radicals, exogenous antioxidants are recommended [5]. Antioxidants can be natural and synthetic, but due to toxic and carcinogenic effects, synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG), and tertiary butyl hydroxytoluene exhibit potent free radical scavenging effects but they induce liver and kidney dysfunction and have also been reported to be carcinogenic in laboratory animals [6]. Thus, there is a need to identify and utilize more antioxidants of natural origin, which can relieve the deleterious effects of free radicals and other biological oxidants [7].

The use and ingestion of natural antioxidants present in different parts of plants are due to their phytochemical constituents [8]. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. These compounds have been reported in several studies to quench free radicals or decompose formation of peroxides owing to the presence of conjugated rings or carboxylic acids [9]. Furthermore, some plant constituents such as saponins, alkaloids, glycosides and tannins have also been documented to exhibit various biological activities including anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic,antibacterial and antiviral activities [10].

Bauhinia tomentosa commonly known as Yellow bell orchid tree belongs to *Fabaceae* family and is one of the best, versatile and most commonly used household remedy for many manifestations . *Tomentosa* derived from tomentose, meaning with dense, interwoven hairs. It is commonly known as 'Kanchini' in Tamil and 'Phalgu' in Sanskrit [11]. The decoction of the plant extract is used for the treatment of liver conditions and abdominal problems. Fruit is used as a diuretic. Flowers, buds, and dried leaves are used for dysentery treatment. Root bark is used for inflammation of liver . Seeds are tonic and aphrodisiac. Infusion of stem bark is useful as an astringent gargle . Leaves have anti-diabetic potential [12]. It is used for snake bite and scorpion sting [13].

From thorough literature search, it was known that there was no scientific report to give credence to the ethnomedicinal usage of this plant for the management of various ailments.

The present study was carried out to provide information on the quantitative composition of the phytochemicals and antioxidant activities of the ethanol extract of *B.tomentosa* leaf in order to provide scientific basis to justify its therapeutic usage.

MATERIALS AND METHODS

Sample Collection and Authentication

The leaves of *Bauhinia tomentosa* Linn were collected from Villivakkam, Chennai and authenticated by Dr.S. Jayaraman, Director of Plant and Anatomy Research Centre, West Tambaram, Chennai (PARC/2014/2294)

Sample preparation

The leaves were washed with water, shade dried and powdered coarsely. Crude extract was obtained after maceration with 96% ethanol at room temperature for 72 hrs, and repeated till exhaustion of the material. Thereafter, the crude ethanol extract was distilled, evaporated and dried under reduced pressure to yield ethanol extract of *Bauhinia tomentosa leaves,*EBT (yield 8%).

Phytochemical profile of B.tomentosa

Physiochemical analysis

Physicochemical values such as percentage of ash values and extraction values were performed according to official methods prescribed I.P (1996)[14] and WHO Guidelines on Quality Control methods for medicinal plant materials (1998)[15]. The results were expressed as mean ± SD.

Microbial Load

This was performed by the method described by WHO (1998), using pour plate method [15]. The number of microorganisms in the sample is determined by multiplying the average number of colonies by the dilution used. The results are expressed as no. of Colony Forming Unit (CFU) per gram of plant material.

Toxicity Studies – Pesticides and aflatoxins analysis

Pesticidal residues were analysed using GC-MS Shimadzu instrument equipped with Electron capture detector as per the method of AOAC, 2005[16]. Aflatoxins were also detected by the method of ASTA, 1997[17].

Heavy Metals

Estimation of heavy metals was done by the method described by WHO (1998) and AOAC (2005) [15,16].

Quantitative Analysis of Phytochemicals

Total Phenols were estimated by the method of Mcdonald, *et al (*2001) [18]. Total Flavanoids were estimated by following the method of Chang, *et al (*2002) [19]. Total tannins were determined by using Peri and Pompi (1971) [20] method and total alkaloids were estimated by the method of Harborne, (1973) [21]. The results were expressed as mg/g.

Nutrients Composition

Total protein in EBT was estimated by the method of Lowry *et al* (1951) [22]. The amount of Carbohydrates (total sugars) was estimated using the anthrone method of Morales *et al* (1973) [23]. Total fibre and Total fat were determined by the method of Chopra and kanwar (1991) [24]. The results were expressed as mg/g

Macro and Micro minerals

The major elements, comprising calcium, phosphorus, sodium, potassium, magnesium and trace elements (Copper, Iron, Manganese and Zinc) were determined according to the method of Shahidi *et al* (1999) [25]. Calcium, Potassium, Phosphorus and Magnesium were done on dry matter basis. Copper, Iron, Manganese and Zinc contents were determined from the ash samples. For each analysis triplicate samples were used.Phosphorus, magnesium, zinc, manganese and iron was determined using absorption mode and potassium in emission mode of atomic absorption spectrophotometer.

In vitro Free Radical Scavenging activity of *B.tomentosa*

DPPH radical scavanging activity

The free radical scavenging activity of EBT was measured using DPPH by the method of Blois, (1958) [26]**.** BHT was used as a reference compound. Percentage inhibition was calculated by comparing the absorbance values of control and samples.

$$\% \text{ inhibition} = (A_{control} - A_{test} / A_{control}) \times 100$$

Super oxide anion radical scavenging activity

The superoxide anion scavenging activity of EBT was determined by the method described by Nishimiki *et al.,* (1972) [27]. Quercetin were used as reference compound. Percent inhibition was calculated by comparing the results of control and test samples.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction **(**Green *et al.,* 1982**)** [28] . Percentage inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations. Curcumin was used as a positive control.

Ferric Reducing Assay

The reducing power of EBT was determined by the method of Oyaizu(1986) [29]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

All the tests were performed in triplicates and the graph was plotted with the average of the three determinations.

RESULTS AND DISCUSSION

Phytochemical Profile of *B.tomentosa*

Physiochemical Characteristics of *B.tomentosa*.

The herbal drugs can be used as a therapeutic agent only if they are genuine and their standard and quality are up to the mark. From the time of collection of the drug to its storage and up to the production of medicine, chances of deterioration in quality are quite frequent, resulting in the decline of the efficacy of drug. To overcome these problems, it is almost inevitable to standardize the drugs for their rational therapeutic use. Hence, physicochemical studies of the *B.tomentosa* leaf was carried out.

The physicochemical parameters like extractive value, ash value and moisture content are depicted in the Table 1. These values help in the determination of the adulteration and are an index of the purity of the drug.

Table 1. Physiochemical Parameters of *B.tomentosa* Linn.Leaves.

Physiochemical Parameters	Value in % (w/w)
Alcohol soluble extractive value	8.1 ± 0.4
Water soluble extractive value	9.1 ± 0.8
Total ash	12.19 ± 1.1
Water soluble ash	3.1 ± 0.3
Acid insoluble ash	8.5 ± 0.7
Moisture content	10.13 ± 0.9

Values were expressed as mean ± SD.

The water and alcohol extractive values of *B.tomentosa* leaves were 9.1 ± 0.8% and 8.1 ± 0.4 % respectively. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. Comparing the water-soluble and alcohol-soluble extractive values of the drug, it was concluded that the percent water-soluble extractive values were higher than the alcohol extractive value. This indicates the presence of more amounts of water-soluble constituents in the leaf of *B.tomentosa*. The variation in the extractive values may be possible due to the presence of specific compound, according to the solubility, soil condition, atmospheric condition and water content of the sample.

Ash value is useful in determining authenticity and purity of drug. These values are also important quantitative standards. Ash value can also detect the nature of the material added to the drug for the purpose of adulteration. The total ash value, water soluble ash and acid insoluble ash were found to be 12.19 ± 1.1%, 3.1 ± 0.3 % and 8.5 ± 0.7% respectively. The total ash value was relatively high which may be due to high content of carbonates, phosphates, silicates and silica.

Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. The moisture content is a good parameter for detecting the quality of crude drugs. Low moisture content is always desirable for higher stability of drugs. Even high values compromise the quality of drug and affect its efficacy. The less value of moisture content could prevent bacterial, fungal or yeast growth. It is an important parameter particularly for the dried powdered drugs which face environment rich in moisture contents, then the factor of deterioration would be more apparent. The risk of microbial attack would also increase. Therefore the establishment of moisture content may assist in the prediction of its preferable storage condition and may help general characterization of the powdered drug. *B.tomentosa* leaves have a moisture content 10.13 ± 0.9%. Hence this drug (*B.tomentosa* leaves) may have least chance for deterioration during its storage.

Total Microbial Load Analysis

Table 2 represents the level of total microbial load in 1g of *B.tomentosa* leaves. The WHO permissible limit is also depicted in table 2. The total aerobic bacterial count was 1.0×10^4 / g of *B.tomentosa* leaves. The fungal count was $<10^3$. *Enterobacteriaceae* level was <10 and the pathogens like *Salmonella, E.coli, S.aureus* were absent. All these levels were found to be within the WHO permissible limits. Thus, the drug was found to have permissible microbial load and hence it may be considered as standard drug.

Table 2. Total Microbial Load of *B.tomentosa* leaves.

S.No.	Parameter	Organisms level in 1g of *B.tomentosa* leaves	Permissible limit WHO (1998)
1	Total aerobic bacterial count	1.0×10^4	$<10^5$
2	Total fungal count	$<10^3$	$<10^4$
3	*Enterobacteriaceae*	$<10^3$	$<10^3$
4	*Salmonella*	Absent	Absent
5	*Staphylococcus aureus*	Absent	Absent
6	*Escherichia coli*	Absent	Absent

Pesticides Residue and aflatoxins analysis

Table 3 shows the level of various pesticides in the leaves of *B.tomentosa*. From the table, it was clear that all the pesticides studied were below the detection limit (BDL). As recommended by WHO [30], *B.tomentosa* leaves were free from pesticides and hence the leaves could be used as a drug for the treatment of many diseases.

Table 3. Pesticide Residues in *B.tomentosa* leaves.

S.no	Parameter	Result
1	O,p-DDT	Not detected
2	P,p-DDT	Not detected
3	O,p-DDE	Not detected
4	P,p-DDE	Not detected
5	O,p-DDD	Not detected
6	P,p-DDD	Not detected
7	alpha-Endosulphan	Not detected
8	Beta-Enosulphan	Not detected
9	Endosulphan sulphate	Not detected
10	Alpha HCH	Not detected
11	Beta HCH	Not detected
12	Delta HCH	Not detected
13	GammaHCH	Not detected

Table 4 shows the level of aflatoxins in 1g of *B.tomentosa* leaves. Aflatoxins are secondary metabolites produced by filamentous fungi *Aspergillus flavus* and *Aspergillus para-siticus* [31].There are four naturally occurring aflatoxins, designated B1,B2, G1and G2 with aflatoxins B1 the most common and toxic. The carcinogenic, mutagenic and immuno-suppressive effects of aflatoxins on several animals have been fully documented [32].

Table 4. Level of Aflatoxins in *B.tomentosa* leaves.

S.no	Parameter	Result
1	Aflatoxin B1	Not detected
2	Aflatoxin B2	Not detected
3	Aflatoxin G1	Not detected
4	Aflatoxin G2	Not detected

Moulds are widely distributed as environmental contaminants , in fact, under favourable conditions of temperature and humidity, *moulds* grow on many commodities including cereals, oil seeds, nuts, herbs and spices [33]. Furthermore, previous work show that aflatoxins levels were not reduced by domestic cooking with either microwave or conventional gas oven heating [34] and that aflatoxins do not decompose at the temperature of boiling water during the preparation of the drink . Hence any standard drug should not contain aflatoxins as it causes serious hazards. From table 4, it was evident that *B.tomentosa* leaves do not contain any traces of all these four aflatoxins (B1, B2, G1 and G2). Thus *B.tomentosa* leaves may be considered as a standard drug.

Heavy Metal Analysis

The contributions of medicinal plants in the traditional system of medicine for curing diseases has been documented. Even trace of toxic metals may cause serious effects on human health. WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like lead, cadmium, mercury and arsenic which amount to 10, 0.3, 1.0 and 3 ppm, respectively. Medicinal herbs are easily contaminated during growth, development and processing. After collection and transformation into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different types of cancers.

Table 5 depicts the level of heavy metals in *B.tomentosa* leaves. It contains 2.17 ppm of lead, < 0.12 ppm of Cadmium, 0.05ppm of Mercury and 0.30 ppm of Arsenic. All th heavy metals level were below the WHO permissible limit. Thus, *B.tomentosa* leaves may be good candidate for the drug development.

Table 5.Heavy Metals level in *B.tomentosa* leaves.

S.no	Parameter	Result (in ppm)	Permissible Limit
1	Lead	2.17	10 ppm (WHO,1998)
2	Cadmium	< 0.12	0.3 ppm (WHO, 1998)
3	Mercury	0.05	1 ppm (API,2008)
4	Arsenic	0.30	3 ppm (API,2008)

Quantitative Phytochemical Analysis

Table 6 shows the quantitative analysis of total phenols, flavonoids tannins and alkaloids of EBT. The total phenol content of EBT was found to contain 0.552 ± 0.08 mg of GAE/g of leaf.

Phenolic compounds are effective hydrogen donors which makes them good antioxidants. Current research has shown that polyphenols contribute to the prevention of cardiovascular disease, cancer and osteoporosis and have a role in the prevention of neurodegenerative disease and diabetes mellitus [35]. The presence of phenolic compounds in the plants indicates that these plants may be antimicrobial agent. They exhibit marked physiological activity when administered to animals.

The beneficial effects of fruits, vegetables and tea or even red wine have been attributed to flavonoid compounds rather than to known nutrients and vitamins. Various researchers have also reported that phenols and flavonoids are potent inhibitors of LDL oxidation [36] and also have strong protective effects against major disease risks, including cancer and cardiovascular diseases . Flavonoid has been recognized as a very important phytochemical mainly for their antioxidant activity and metal chelating properties. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [37]. Flavonoids have redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. The total flavonoid content was found to be 0.483 ± 0.09 mg of QE/g of leaf. This may be the reason that *B.tomentosa* has been used for the treatment of diseases in herbal medicine.

The ethanol extract of *B.tomentosa* was found to contain 0.03 ± 0.01 mg of GAE/g of leaf of tannins. Tannins are a group of natural products which are recognized as health protecting antioxidants. It has been reported by Beninger and Hosfield (2003) [38]that tannin extract from *P.vulgaris* was more active and efficient in scavenging free radicals than pure flavonoid compounds. Tannins have stringent properties, hasten the healing of wounds and inflammed mucous membranes. Besides tannins are found to have antioxidant [39], antiallergic [40], anticancer and antidiabetic properties [41].

Table 6. Quantitative Analysis of Phytochemicals in Ethanol Extract of *B.tomentosa (EBT).*

Phytochemicals	EBT
Total Poly Phenols mg of GAE/g	0.552 ± 0.05
Total Flavanoids mg of QE/g	0.483 ± 0.09
Total Tannins mg of TAE/g	0.03 ± 0.01
Alkaloids mg/g	18 ± 1.2

The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory, analgesic, antispasmodic [42] and pharmacological effects [43]. EBT contains 18 ± 1.2 mg/g of alkaloids. This accounts for the use of *B.tomentosa* in the treatment of various diseases by traditional medical practitioners.

Nutritional Evaluation of EBT

Table 7 shows the level of total protein, total sugars, fibre content and fat present in the ethanol extract of *B.tomentosa*. The protein content was found to be 16.01 ± 1.6 mg/g of leaf. Protein is a nutrient needed by the human body for growth and maintenance. When broken down into amino acids, they are used as precursors for nucleic acid, co-enzymes, hormones, immune response, cellular repair and molecules essential for life. Since *B.tomentosa* contains a good amount of proteins, it has greater nutritional value. The total sugar content of *B.tomentosa* was found to be 60.02 ± 5.9 mg/g of leaf. Sugars are the major energy reserve and they play important role in metabolism. They can be stored in liver and muscle in the form of glycogen for future use. They are the important constituent of coenzymes and nucleic acids. As *B.tomentosa* contains appreciable amount of sugars, it has great nutritional value.

Table 7. Level of Nutrients in Ethanol Extract of *B.tomentosa.*

Nutrients	EBT
Total Proteins (mg/g)	16.01 ± 1.6
Total Carbohydrates (mg/g)	60.02 ± 5.9
Fibre Content (mg/g)	10.82 ± 1.0
Fat (mg/g)	1.65 ± 0.08

The level of fibre and fat in *B.tomentosa* leaves were found to be 10.82 ± 1.0 mg/g and 1.65 ± 0.08 mg/g respectively.

Mineral Analysis

Minerals are required for both the plants and animals in critical amounts of balance. The mineral content of a plant is influenced by so many factors, in particular physical and chemical properties of the soil, water supply, climate, plant species and stages of development [44]. Leaf is almost invariably higher in minerals than the stem.

Table 8 shows the amount of macro minerals like calcium, phosphorus, potassium, magnesium and sodium present in *B.tomentosa* leaves. 100g of *B.tomentosa* leaves contains 4.03 ± 0.3 mg/100g of calcium, 215.07 ± 10.7 mg/100g of phosphorus, 1.20 ± 0.06 mg/100g of potassium and 0.30 ± 0.01 mg/100g of magnesium.

Table 8. Analysis of Macro minerals in *B.tomentosa* leaves.

S.no	Parameter	Result
1	Calcium (mg/100g)	4.03 ± 0.3
2	Phosphorous (mg/100g)	215.07 ± 10.7
3	Potassium (mg/100g)	1.20 ± 0.06
4	Magnesium (mg/100g)	0.30 ± 0.01

Calcium is the most abundant macro element in the plants. The calcium contents in plant increases in dry weather and decreases under conditions of high humidity. Normal extra cellular calcium concentrations are necessary for blood coagulation and for its integrity and also for intracellular cement substances. Magnesium, a ubiquitous element that plays a fundamental role in many cellular reactions, is involved in >300 enzymatic reactions in which food is catabolized and new chemical products are formed. The presence of Mg in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases [45]. Potassium is essential to all the organisms except blue green algae. It is a major cation and is important in nerve action. This cation is present in extracellular as well as in intracellular fluid. It plays a very important role in acid base balance, osmotic pressure and water retention. When present in extracellular fluid it influences muscle activities.

Concentration of trace minerals viz. Cu, Fe, Mn and Zn present in the leaves of *B.tomentosa* has been presented in table 9. EBT contains 0.99 ± 0.06 mg/Kg, 91.32 ± 6.2 mg/Kg, 3.84 ± 0.2 mg/Kg and 4.87 ± 0.3 mg/Kg of Cu, Fe, Mn and Zn respectively.

Table 9. Analysis of Micro Minerals in *B.tomentosa* leaves.

S.no	Parameter	Result
1	Copper (mg/Kg)	0.99 ± 0.06
2	Iron (mg/Kg)	91.32 ± 6.2
3	Manganese (mg/Kg)	3.84 ± 0.2
4	Zinc (mg/Kg)	4.87 ± 0.3

The importance of these micronutrients is revealed by the diversity of metabolic processes they help to regulate. Copper is the third most abundant trace element in the body. It is essential in the biosynthesis of hemoglobin and for iron absorption. Its deficiency may be a risk factor for cardio vascular diseases [46]. Iron has important role in the formation of hemoglobin and certain enzymes and its deficiency leads to anaemia. It is needed for healthy immune system and for energy production [47]. Manganese is essential for bone development, reproduction and the normal functioning of the central nervous system [48]. The presence of zinc in the plants could mean that the plants can play valuable roles in the management of diabetes, which result from insulin malfunction. Thus the medicinal plant *B.tomentosa* was rich with P, Fe, Mn, Ca and Zn and it was expected that this plant with high contents of the above mentioned macro and micronutrients, might play an important role in maintenance of human health. Also, all of the detected values for metallic elements in plants studied in this present investigation were below the WHO permissible levels and may not constitute a health hazard for consumers.

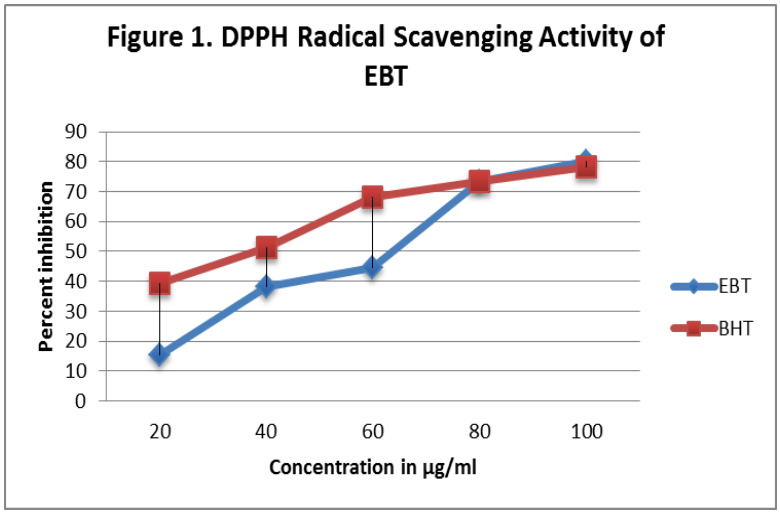
In vitro* antioxidant analysis of *B.tomentosa

***In vitro* Antioxidant Analysis**

Different concentrations of EBT were tested for their antioxidant activity using different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a dose dependent manner by various methods.

DPPH Radical Scavenging Activity

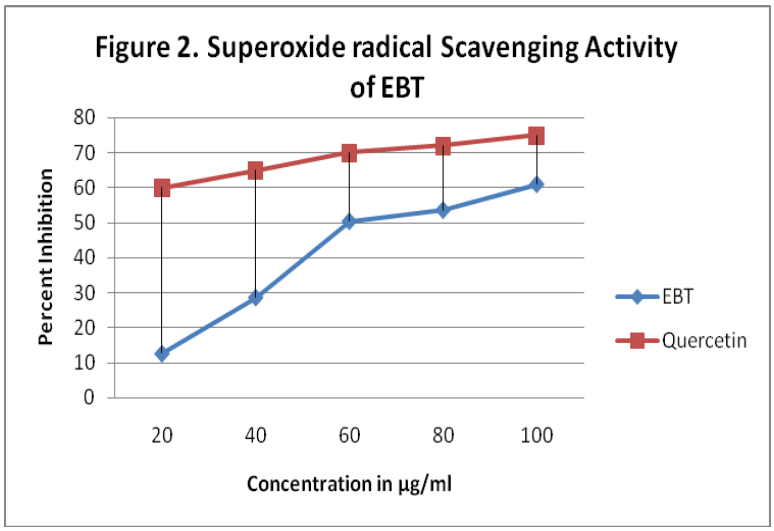
DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The radical scavenging activity of EBT was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. The positive DPPH test suggests that the samples are free radical scavengers. The scavenging effects of EBT and BHT on the DPPH radical were illustrated in figure 1. EBT had significant scavenging effects on the DPPH radical which increased with increasing concentration from 20 - 100 µg/ml. The IC_{50} value of EBT and for standard BHT was 59.54 µg/ml and 38.5 µg/ml respectively.



IC $_{50}$ value for EBT – 59.54 µg/ml; BHT – 38.5 µg/ml.

Superoxide Anion Radical Scavenging Activity

Superoxide anion was produced from molecular oxygen due to oxidative enzymes in the body by nonenzymatic reaction such as auto oxidation by catechol amines [49]. The scavenging activity towards the superoxide radical was measured in terms of inhibition of generation of O_2^-. Superoxide anions indirectly initiate lipid oxidation as a result of superoxide and hydrogen peroxide, serving as precursors of singlet oxygen and hydroxyl radicals. Robak and Glyglewski (1988) [50] reported that the antioxidant properties of flavonoids were effective in scavenging of superoxide anion. From figure 2, it was found that EBT possess good scavenging activity on superoxide anions at all concentrations tested. The IC_{50} value of EBT on superoxide radical scavenging activity was found to be 60.00 µg/ml, whereas the IC_{50} value of quercetin was 16.66 µg/ml, respectively.

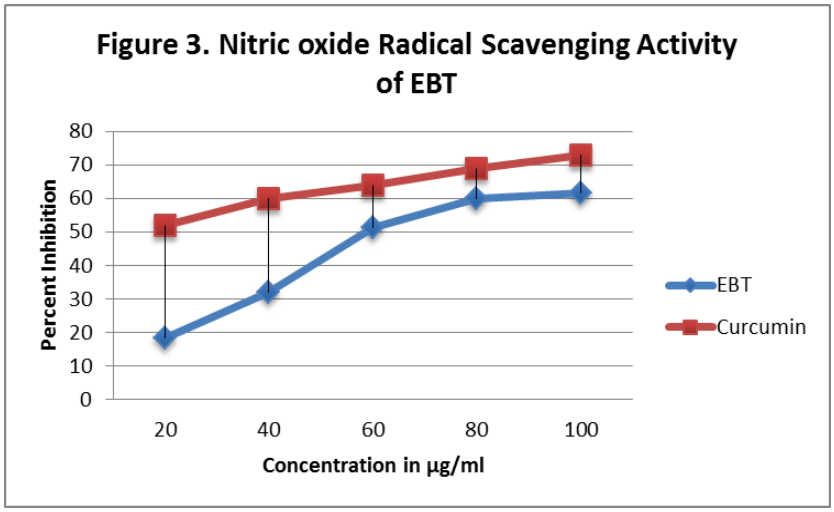


IC_{50} value for EBT – 60.00 µg/ml; Quercetin – 16.66 µg/ml.

Nitric Oxide Radical Scavenging Activity

NO is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction [51]. Excess concentration of NO is associated with several diseases, Oxygen reacts with nitric oxide to generate nitrite and peroxynitrite anions which acts as free radicals. Nitric oxide can react rapidly in the intracellular environment to form nitrate, nitrite and *S*-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. NO causes DNA damage via peroxynitrite.

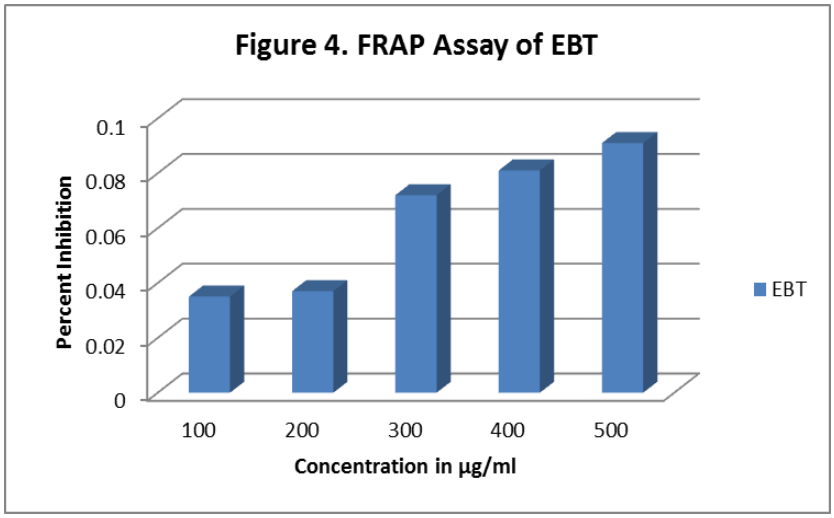
In the present study, the ethanol leaf extract was checked for its inhibitory effect on nitric oxide production. Figure 3 illustrates the percent inhibition of nitric oxide generation by EBT. Curcumin was used as a reference compound. The concentration of EBT needed for 50% inhibition was 58.36µg/ml, whereas 20.4 µg/ml w as needed for an equal weight of curcumin.



IC $_{50}$ value for EBT – 58.36 µg/ml; Curcumin – 20.4 µg/ml.

Ferric Reducing Power Assay

Figure 4 shows the reducing capacity of EBT. Antioxidant activity has been reported to be concomitant with the development of reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [52]. With the tested concentration, the EBT has exhibited good reducing activity.



CONCLUSION

From the results of present study it was observed that EBT has appreciable amounts of phytochemicals, phytonutrients and minerals. EBT also acts as a significant antioxidant. Thus this study has provided the biochemical basis for the ethnomedical use of extract from leaves of *B.tomentosa* for the treatment of various ailments and also in the prevention of infections.

Abbreviations

EBT – Ethanol Extract of *Bauhinia tomentosa*
DPPH – 2,2-Diphenyl-1-Picryl Hydrazyl
BHT – Butylated Hydroxy Toluene
IC – Inhibitory Concentration
NO – Nitric Oxide

Competing Interests

The authors declare no conflict of interest.

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