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IN VITRO COMPARATIVE STUDY OF THE ANTIOXIDANT ACTIVITY OF METHANOLIC LEAF AND STEM BARK EXTRACTS OF *Bombax buonopozense*

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ABSTRACT

Medicinal plants with antioxidant activities have been reported to be useful for the prevention of diseases. *Bombax buonopozense* is a wild tree found in Nigeria mainly in Nigeria. This study was aimed to determine *in-vitro* antioxidant activities of the methanolic extracts of leaf and stem bark of *Bombax buonopozense* (P. bauve). The phytochemical components of the methanolic leaf and stem bark extracts were qualitatively determined using standard methods. The antioxidant properties of the extracts specifically the DPPH free radical scavenging, reducing power and total antioxidant capacity were also carried by the method described by Dehshahri *et al*.,(2012) and preito *et al.*,(1999). The result of the phytochemical screening revealed the presence of alkaloids, carbohydrates, **c**ardiac glycosides, saponins, terpenoids, tannins and phenolics in the leaf extracts.The extract of the stem bark contains carbohydrates, **c**ardiac glycosides, saponins, terpenoids, steroids, tannins and phenolics. At the lowest concentration of 0.2 mg/ml the leaf and stem bark extracts of the plant had DPPH free radical scavenging capacity of 88.9 and 88.9 % respectively. A significant difference ($p < 0.05$) was observed when the results of the samples was compared with that of ascorbic acid. The antioxidant potential of *Bombax buonopozense* could be due to the phenols content, phenol donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells. Therefore, methanol extracts of the leaf and stem bark of *Bombax buonopozense* posses antioxidant activity and are rich in phenolics.

Keywords: *Bombax buonopozense*, Phytochemicals, DPPH, Free radical

INTRODUCTION

Medicinal plants used for maintaining good health are getting attention worldwide. Plants with medicinal properties are used for the treatment of diseases (Sofowora *et al*., 2013). They produce several secondary metabolites with many important biological activities (Guerriero *et al*., 2018). Some of these plant have anticoagulant and antioxidant activities (Felix, 2014). Oxidation is a critical metabolic process that aid living functions. Metabolism of oxidation produces reactive oxygen species (ROS) that causes oxidative stress which leads to cell structure damages and contribute to pathogenesis of most inflammatory diseases, neurodegenerative cardiovascular diseases, and cancers by attacking biological molecules (Do Hoang *et al*., 2018).Free radicals are unstable chemical species, which tend to trap electrons from the molecules in the immediate surroundings (Phaniendra *et al*., 2015). These radicals may damage lipids, proteins and DNA leading to disease condition if not scavenged (Lobo *et al*., 2010). Antioxidant plays a central role in the termination of oxidative chain reactions by removing the free radical intermediates. Plant antioxidants not only restrain ROS production by scavenging free radicals, but also help boost endogenous antioxidant defenses of the body (Kasote *et al*., 2015). Naturally occurring polyphenols are present in fruits, vegetables, and other nutrient-rich plant foods and are widely used for prevention of oxidative stress-related diseases due to their antioxidant and anti-inflammatory activities (Byung, 2013). Natural antioxidants have an important role in the prevention of many age-related diseases and promotion of health. Among natural antioxidants from plants, flavonoids and other phenolic compounds are potent antioxidants and chelating agents (Tungmunnithum et al., 2018) .Mechanism of action of antioxidants includes the suppression of ROS formation, the inhibition of enzymes or chelating of elements involved in free-radical production. Furthermore, antioxidants scavenge reactive species and upregulate antioxidant defences (Shadab *et al.*, 2012). Researchers have been focused on protecting the human body from oxidative damage caused by free radicals with the use of natural antioxidants (Godwin *et al.*, 2017). Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits and seeds. Any part of the plant body may contain active components (Kalyani and Bandita, 2014)

Bombax buonopozense P. beauv (Bombacacea) is a large tropical tree that grows up to 40 metres in height with large buttress roots that can spread 6 metres. Many parts of the plant are used for medicinal purposes, as food, building material, as cotton wool and as dye. The fruits are eaten by animals such as water chevrotain. Decoction of the leaves is used for feverish conditions, diarrhea, pains and muscle aches. Root decoction is used as antimicrobial and in treatment of stomach aches (Godwin *et al*., 2011).Therefore this research is aimed at

evaluating the antioxidant activity of methanolic leaf and stem bark extracts of *Bombax buonopozense.*

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The samples, being the leaves and stem bark of *Bombax buonopozense* were collected from National Institute for Pharmaceutical Research and Development (NIPRD). The plant was identified and authenticated by Dr Jamila Aliyu Ibrahim in the herbarium unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Preparation of Plant Extract

The fresh leaves and stem of *Bombax buonopozense* were air dried at room temperature and pulverized into a dry powder, and macerated with 70% methanol in water for 72h with constant shaking. The resultant mixture was filtered using whatman (No 1) filter paper and the filtrate concentrated to dryness using water bath. The extracts were reconstituted at various experiments conducted.

Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening according to standard methods described by Trease and Evans (1989), and Sofowora (1998).

Estimation of Parameters

The methanolic extract of stem and leaf of *Bombax buonopozense* were subjected to *in vitro* antioxidant analysis, DPPH was evaluated by the method describe by Dehshahri *et al*.,(2012). Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) estimations in *% reduction* was carried out using the method described by Oyaizu and total antioxidant activity (TAA) evaluations in *mg of ascorbic acid equivalent/g* (*mgAAE/g*) was done using the phosphomolybdenum method described by preito *et al*.,(1999). All the estimations were carried out at both leaf and stem extracts.

Statistical Analysis

Each experimental parameter was measured in triplicate and was presented as mean ± standard deviation. Data were statistically analysed by one-way ANOVA with Tukey multiple comparison post-test using GraphPadInStat software (2000), version 3.05 (32 bit for Win 95/NT) from GraphPad Incorporated and statistical limit of significance was set at $P < 0.05$. Percentage differences of methanolic extracts of stem and leaf of *Bombax buonopozense w*ere calculated as:

$$PercentageDifferences\ (\%) = \left(\frac{(ethanolicextractvalue - methanolicextractvalue)}{methanolicextractvalue}\right) \times 100$$

RESULTS

Table 1: Result of phytochemical screening of methanolic extracts of the leaves of *Bombax buonopozense*

Phytochemical Test	Chemical Test	Methanolic Extract	Plant Powder
Alkaloids	Dragendorff's	+++	+++
	Mayer's	+++	+++
Carbohydrates	Molisch's Test	+++	+++
Anthraquinone	Bontrager's Test	-	-
Tannins and Phenolics	Ferric Chloride Test	++	++
Cardiac Glycosides	Killer Kilani Test	++	++
Saponins	Froth Test	+++	+++
Flavonoids	Shinoda's Test	-	++
	Lead Ethanoate	-	-
Terpenoids	Salkowski Test	++	++
Steroids	Lieberman-Burchard Test	-	-

Key: +++ = Highly present ++ = Moderately Present + = Slightly present -= Nil

Table 1 shows the result of the phytochemical screening of the stem bark. The crude powder and methanol extract of the stem bark contains carbohydrates, **c**ardiac glycosides, saponins, terpenoids and steroids. While alkaloids, flavonoids, anthraquinones tannins and phenolics were absent in both crude plant and methanol extract of the stem bark of plant.

Table 2: Result of phytochemical screening of Methanolic Extracts of The Stem Bark of *Bombax buonopozense*

Phytochemical Constituent	Chemical Test	Methanolic Extract	Plant Powder
Alkaloids	Dragendorff's	-	-
	Mayer's	-	-
Carbohydrates	Molisch's Test	+++	+++
Anthraquinone	Bontrager's Test	-	-
Tannins and Phenolics	Ferric Chloride Test	++	++
Cardiac Glycosides	Killer Kilani Test	+	+
Saponins	Froth Test	+++	+++
Flavonoids	Shinoda's Test	-	-
	Lead Ethanoate	-	-
	Salkowski Test		
Terpenoids and	Lieberman-	++	++
Steroids	Burchard Test	++	++
		++	++

Key: +++ = Highly present ++ = Moderately Present + = Slightly present -= Nil

Table 3: **Result of DPPH free radical scavenging capacity of leaf and stem bark of *Bombax buonopozense e*xtract**

Samples	**Percentage antioxidant activity**				
Concentration	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1.0mg/ml
BBL	88.9±0.16^{b}	89.4±01.5^{b}	92.1±0.47^{b}	92.3±0.73^{b}	95.29±0.68^{b}
BBSB	88.9±0.35^{b}	91.3±0.74^{b}	92.1±0.68^{b}	93.3±0.22^{b}	94.4±0.68 ^{b}
Ascorbic acid	95.3±0.45^{a}	95.4±1.4 ^{a}	96.9±0.27^{a}	96.9±0.41^{a}	97.4±0.11 ^{a}

Values are means of triplicate determinations and are expressed as mean ± standard deviation; Means with different letters Superscripts within each column differ significantly ($p < 0.05$) from one another.BBL= *Bombax buonopozense* leaf extracts, BBSB= *Bombax buonopozense* stem bark extracts

Table 4: Result of reducing power capacity of leaf and stem bark of *Bombax buonopozense e*xtract

Samples	**Absorbance at 700nm**				
Concentration	0.2mg/ml	0.4mg/ml	0.6mg/ml	0.8mg/ml	1mg/ml
BBL	1.21±0.31^{b}	1.25±0.04^{b}	1.38±0.03^{a}	1.38±0.005^{ab}	1.48±0.3^{a}
BBSB	1.07±0.02^{c}	1.17±0.03^{c}	1.19±0.05^{b}	1.31±0.05^{b}	1.37±0.07^{ab}
Ascorbic acid	1.33±0.04^{a}	1.36±0.02^{a}	1.38±0.03^{a}	1.48±0.8^{a}	1.49±0.08^{a}

Values are means of triplicate determinations and are expressed as mean ± standard deviation; Means with different letters superscripts within each column differ significantly ($p < 0.05$) from one another.

Table 5: Result of the total antioxidant capacity of leaf and stem bark of *Bombax buonopozense* extract

Samples	**Absorbance at 695nm**				
Concentration	0.2mg/ml	0.2mg/ml	0.2mg/ml	0.2mg/ml	0.2mg/ml
BBL	0.18±0.0^{b}	0.57±0.05^{b}	0.65±0.05^{c}	0.87±0.0^{c}	1.25±0.0^{c}
BBSB	0.02±0.005^{b}	0.31±0.005^{b}	3.14±0.01^{b}	3.62±0.0^{b}	4.44±0.005^{b}
Ascorbic acid	4.19±0.02^{a}	5.27±0.01^{a}	5.56±0.04^{a}	6.40±0.01^{a}	8.55±0.01^{a}

Values are means of triplicate determinations and are expressed as mean ± standard deviation; Means with different letters superscripts within each column differ significantly ($p < 0.05$) from one another.

DISCUSSION

Medicinal plants with antioxidant activities have been reported to be useful for the prevention of diseases such as atherosclerosis, diabetes, cardiovascular diseases and others by reducing lipids peroxidation and free radicals(Lobo *et al.*, 2010) .The results of the phytochemical analysis of the crude methanolic plant extracts of of *Bombax buonopozense p.* revealed the presence of flavonoids, tannins saponins, alkaloids, terpenoids, steroids and carbohydrates but the tannins and carbohydrates were only present in the leaf extracts of the plant. This result showed some similarity in the compounds present in both the leaf and the stem bark extract. In this study, at lowest concentration the leaf and stem bark methanol extract of the plant have DPPH free radicals scavenging capability. A significant difference was observed when the free radical scavenging ability of the methanol extract of the leaf and stem bark of *Bombax buonopozense* when compared with that of ascorbic acid. While the free radical scavenging ability of the methanol extract of the leaf and stem bark of *Bombax buonopozense* are similar. This is due to the ability of DPPH to accept an electron donated by an antioxidant compound. DPPH is a stable free radical. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically, depending on the number of electrons taken up. This may be due to ability of the extract to reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles (Kamaludden *et al*.,2017). The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability involvement of free radicals, especially their increased production leads to the development of cardiovascular diseases and cancer. Thus, the consumption of *Bombax buonopozense* can be beneficial in preventing oxidative stress related numerous chronic diseases.

In this study the Reducing powers capacity of the extracts were however significantly lower than those of ascorbic acid. Reducing powers of all the samples responded to

concentrations, with higher concentrations resulting in higher reducing powers. Furthermore, there is significant difference between the reducing power of methanol extracts of the leaf, stem bark and ascorbic acid. The reducing properties are generally associated with the presence of reduced tones which have been shown to exert antioxidant action by breaking the free radical chain through electron donation (Duhand yen, 1999). The presence of antioxidants in the samples result in the reduction of Fe^{3+} to its lower valence state, Fe^{2+}, by donating an electron. Increasing absorbance at 700 nm indicates an increase in reductive ability (Rao *et al*., 2011).The observed reducing power of the leaves and stem bark was in agreement with the chemical constituents in the extracts (Das *et al.*, 2014). In this study, significant difference was observed between the total antioxidant capacity of the standard (ascorbic acid) and the two extracts. Reduction of metal ions is an important mechanism of antioxidant action and a potent antioxidant often acts as a potent reductant (Niki, 2010). The ability of the extracts to reduce Mo (VI) to Mo (V) complex, however confirms the presence of reductants in them and proves that the methanol extract of the leaf and stem bark of *Bombax buonopozense* could serve as electron donors, terminating the free radical chain reactions (Ebrahim *et al.*, 2010). The antioxidant activity shown by the leaves and stem bark may be due to the presence of tannins, terpenoids, steroids and flavonoids (Das *et al.*, 2014). Phenolics, flavonoids and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier hence, these may be responsible for the observed activity in both of these species (Kamaludden *et al*., 2017).

CONCLUSION

This study shows that the leave and stem bark extracts of *Bombax buonopozense* showed antioxidant activity at different levels. This shows the potency of *Bombax buonopozense* as a medicinal plant and can be use in the treatment of diseases. The presence of phenols in synergy with other phytochemicals present in the plant accounts for its antioxidant activity

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