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Antioxidant Activity of *Psorospermum febrifugum* and *Harungana madagascariensis* (Hypericaceae) Stem Bark Ethanolic Extracts

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ABSTRACT

The phytochemical and antioxidant activity of the ethanolic extracts of stem bark of Psorospermum febrifugum and Harungana madagascariensis were carried out to assess the potential of these extracts as antioxidant agents. The antioxidant potentials of both extracts were assessed using l,l-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC). P. febrifugum extract was richer in flavonoids (898 ± 2.17 mg QE/g), tannins (44.91 ± 0.18 mg GAE/g), phenols (40.26 ± 0.05 mg GAE/g), and alkaloids (530.00 ± 13.61 mg CE/g), than H. madagascariensis extract (flavonoids, 706.38 ± 2.17 mg QE/g, tannins, 42.46 ± 0.41 mg GAE/g, phenols, 26.17 ± 0.23 mg GAE/g and alkaloids, 457.87 ± 5.05 mg CE/g). P. febrifugum extract (IC_{50} = 0.74 ± 0.02 µg/mL and 1865.42 mg GAE/g) had a better antioxidant activity than H. madagascariensis (0.82 ± 0.05 µg/mL and 1601.92 mg GAE/g) in the free radical scavenging activity and total antioxidant capacity respectively. However, H. madagascariensis extract showed greater ferric reducing antioxidant power (321.069 µmol Fe^{2+}/g) than P. febrifugum extract (266.687 µmol Fe^{2+}/g) and gallic acid standard (220.391 µmol Fe^{2+}/g) at 1000 µg/mL concentration. The extract's vitamin (A, C and E) contents showed that vitamin A is higher in P. febrifugum while vitamins C and E were higher in H. madagascariensis.

Keywords: *Psorospermum febrifugum; Harungana madagascariensis*; Antioxidant activity; *Hypericaceae*

INTRODUCTION

Many plants are known to have natural health-enhancing properties and have become sources of herbal drugs used for many years. Parts of plants such as stem barks, seeds, leaves, flowers, and roots are used for medicinal purposes. Treatment of different diseases with the use of herbal medicine have been in existence for many years forming the essential part of traditional medicine systems among which are African and Chinese traditional medicine [1]. In recent years, there has been a wide increase in the use of active biological components isolated from medicinal plants in the cure of various diseases. This is so because of the problems pertaining to synthetic drugs such as unwanted side effects, ineffectiveness, multi-drug resistance strains of bacteria and toxicity [2]. Phytochemicals

found in plants have the capacity to protect the body from several diseases [3]. Many medicinal plants contain large quantity of antioxidants in addition to vitamin C, E and carotinoids [4]. Phenols, flavonoids, tannins, and alkaloids are known to contribute to the antioxidant activities of plant extracts which are used in the management or treatment of oxidative stress diseases [5-7]. The importance of these phytochemicals as shown by their antioxidant activities have increased the medicinal uses of plants [5,8-10]. *P. febrifugum* is a plant species from the family "Hypericaceae". It grows in an open forest over a wide range of altitudes and is mostly found in tropical Africa, mostly in Zimbabwe and Mozambique. *H. madagascariensis* is also from the family Hypericaceaeis a small-to medium- sized bushy tree, about 4-7 m in height and a native of tropical Africa, Madagascar, and Mauritius. It has been established that these plants possess great medicinal properties, example, the herb tea from *P. febrifugum* leave is used in the treatment of malaria [11]. The stem bark possesses antitumor [12], anti-inflammation and anti-psoriatic properties [13], anticancer and antioxidant properties [14] and is used in the management of skin problems such as leprosy, craw-craw, eczema, scabies and insect bites [15,16]. The pulverized stem bark is used in the treatment of tuberculosis, dysentery, and whooping cough [17]. The powdered root is used on parasitic skin-diseases [17] and when mixed with oil, is a good treatment for pimples, wounds, and eruptions [17]. The root is also used as a mouth wash and gargle to treat tongue diseases and tonsillitis [16]. Besides, the root decoction of *H. madagascariensis* has been reported to be of use in the management of liver and kidney diseases [18] and as a cure for bleeding piles, dysentery, fever, trypanosomiasis, cold and cough [19]. Extract from the plant have been found to be effective against acute enteritis, jaundices, and scabies [19], psoriasis [20], urogenital infections and chest pain [21]. The stem bark has been used in the management of malaria [22], hepatitis, asthma, river blindness, ulcer, dysmenorrhea, and toothache [23].

Here we qualitatively and quantitatively examined the antioxidant contents and determine the activity of ethanol extract of *P. febrifugum* and *H. madagascariensis* stem bark as potential anti-oxidative stress agents.

GENERAL EXPERIMENTAL PROCEDURES

Collection and identification of materials

P. febrifugum and *H. madagascariensis* were collected from Edem-ani in Nsukka Local Government Area, Enugu State. They were identified by a taxonomist at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria.

Chemicals

The chemicals used in the study were of analytical grade.

Preparation of plant samples

Fresh stems of *P. febrifugum* and *H. madagascariensis* were washed with distilled water before the barks were removed. The barks were air dried and pulverized into coarse powder using mechanical grinding machine. Portions of the powdered stem barks, 400 g and 500 g respectively were extracted by cold maceration in (5.0 L) ethanol for 7 days. The mixture was filtered with Whatman filter paper and the filtrate concentrated using a rotary evaporator at 40°C to obtain crude extracts. All the extracts were subjected to qualitative screening, quantitative analyses, and antioxidant tests.

Percentage yield

Percentage yield=Mass of crude extract/mass of sample used x 100

Phytochemical analyses

Phytochemical tests for identification of the constituents in the extracts of *P. febrifugum* and *H. madagascariensis* were carried out using standard methods as described by Harborne [24], Sofowara [25] and Trease and Evans [26]. Quantitative analyses of total phenolics and tannins contents were carried out using folin-Ciocalteau's method as reported by Saeed et al, [27] and Mythili et al, [28] respectively, and were expressed as mg GAE/g. Total flavonoids content was determined using aluminium chloride colorimetric assay according to Biju et al, [29] and expressed as mg QE/g, while total alkaloids content was determined according to Harborne [24], and expressed as mg CE/g.

***In-Vitro* antioxidant analyses**

In-vitro antioxidant activities of the extracts were determined using 1, l-diphenyl-2-picrylhydrazyl (DPPH) assay [30], and the total antioxidant capacity (TAC) was determined by phosphormolybdate method [31]. The ferric reducing antioxidant power (FRAP) assay was by the method of Benzie and Strain, [32]. In all the measurements of antioxidant potentials, gallic acid and ascorbic acid were used as standards.

DPPH assay: The DPPH free radical test is based on the color change of the solution from purple to yellow when the radical is reduced by the antioxidant. In DPPH assay, I mL of varying concentration of sample solutions was mixed with 0.5 ml of 0.076 mM DPPH in ethanol. The mixture was strongly shaken and incubated in the dark for 25 mins, 1 mL of 0.076 mM. DPPH in ethanol was used as negative control whereas ascorbic acid was used as a positive control. The absorbance of the mixture and the blank (ethanol) were read at 517 nm using UV-Visible spectrophotometer. The Lower absorbance of the reaction mixture indicated higher free radical scavenging activities of the extract.

The DPPH free radical scavenging activity was calculated using the formula:

$$1 = \frac{Ao - As}{Ao}\ x\ 100$$

Where 1=percentage inhibition, A_0=absorbance of the control, A_s=absorbance of the sample

TAC assay: The total antioxidant capacity was carried out by the phosphormolydate method as used by El-Hashash et al, [31] with slight modification; precisely, 0.1 mL of varying concentrations of sample solutions was mixed with 1 mL of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate 1:1:1). The test tubes having the mixture were covered with aluminium foil and incubated in a water bath at 95°C for 90 mins. When the mixture was cooled at room temperature, the absorbance was read at 765 nm against a blank containing 1 mL of the reagent solution. Gallic acid was used as stasndard. The experiments were done in triplicates. The total antioxidant capacity (TAC) was expressed as mg gallic acid equivalent per gram of extract. The antioxidant capacity was calculated using the formula:

$$Total\ antioxidant\ capacity = \frac{Absorbance\ of\ sample - intercept\ of\ the\ standard}{Slope\ of\ the\ standard}$$

FRAP assay: The ferric reducing antioxidant power of the extract was evaluated according to method proposed by Benzie and Strain, [32]. This method is based on the reduction of ferric ions in the presence of an antioxidant which

form a blue-coloured ferrous tripyridyltriazine complex (Fe^{2+} TPTZ) at pH 3.6. Exactly, 2.0 mL of varying concentrations sample solutions was mixed with 2.0 ml of 0.2 M phosphate buffer (pH 6.6) and 2.0 ml of 10 mg/L potassium ferricyanide (0.1% w/v) solution. The mixture was incubated in a water bath at 50°C for 20 mins. After this, 2.0 ml of 100 mg/L trichloroactetic acid solution (10% w/v) was added to 2.0 ml of the mixture, 2.0 mL of distilled water and 0.4 ml of 0.1% (w/v) ferric chloride ($FeCl_3.6H_2O$) solution were added. The absorbance of the reaction mixture was determined at 700 nm after 10 min of reaction. The 1 mmol/L $FeS0_4$ was used as the standard solution. The ferric reducing antioxidant power of extract was expressed as μmole Fe^{2+}/g of extract. The ferric reducing antioxidant power was calculated using the following formula:

$$\frac{Absorbance\ of sample}{Absorbance\ of\ standard}\ x\ concenration\ of\ standard$$

Estimation of total vitamin (C, A and E) contents

The vitamin contents of the extracts of *P. febrifugum* and *H. madagascariensis* were determined using standard methods according to Pearson [33], with slight modifications.

Statistical analysis

Data obtained were analyzed for the significance of disparity using one-way using Statistical Product and Service Solution (SPSS) version 20. Values with ($p<0.05$) were regarded as significant, while values with ($p>0.05$) were regarded as non-significant.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of the ethanolic extract of *P. Fabrifugum* (400 g) and *H. Madagascariensis* (500 g) were 25 g and 28.183 g, representing 6.25 % and 5.64 % respectively.

Phytochemical screening

The results of the Phytochemical screening (Table 1) showed the presence of flavonoids, tannins, phenolics, alkaloids, glycosides, terpenoids, saponins and steroids in their relative abundance. The results obtained were in agreement with the works of Proper-cabral et al, [34], Afieroho et al, [35], Zubair et al, [36], Kisangau et al, [37], Ogungbenro et al, [38], also reported similar phytochemical constituents of *P. febrifugum*. Agbogba et al, [39], also reported a detectable amount of these phytochemicals in *P. febrifugum*. These Phytochemicals have been shown to contribute greatly to the therapeutic properties of plant and plant products [40]. Phenolics are known to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals [41,42]. The presence of such compounds could be partially responsible for the antioxidant activity found in this plant extract. The antioxidant activity depends on the method used, buttressing the fact that these extracts contain several antioxidant compounds that act in different manners. It has been stated that consumption of enough of these bioactive constituents has protective and therapeutic effects needed to prevent diseases and sustain good health [43]. The antioxidants obtained from plants are more functional towards improving the shelf life of food products and providing health promotion when compared to materials whose antioxidants have been removed during processing [44].

Table 1. Qualitative Phytochemical Screening of ethanol extracts of *P. febrifugum* and *H.madagascariensis* stem barks.

Plant Secondary Metabolites	*P. febrifugum*	*H. madagascariensis*
Phenols	++	+
Flavonoids	+++	+++
Tannins	++	++
Alkaloids	+++	++
Saponins	+	+++
Glycosides	+++	+++
Steroids	+	++
Terpenoids	+++	+++
Keys: +++ = High abundance, ++ = moderate abundance, + = low abundance		

Quantitative analyses

Quantitative analyses of total flavonoids, tannins, phenolics and alkaloids contents revealed that *P. febrifugum* extract contained higher concentrations of these constituents than *H. madagascariensis* extract (Table 2). Antia et al, [45] identified flavonoids, Anthraquinones, saponins, tannins and terpenes in the dichloromethane extract of *H. Madagacariensis.*

Table 2. Quantitative analysis of phytochemicals in ethanol extracts of *P. febrifugum* and *H. madagascariensis* stem barks.

Secondary Metabolities	Ethanolic Extract (mg/g)	
	P. febrifugum	*H. madagascariensis*
Total Flavonoids	898.88 ± 2.17*	706.38 ± 2.17
Total Alkaloid	530.00 ± 13.61*	457.87 ± 5.05
Total tannins	44.91 ± 0.18*	42.46 ± 0.41
Total Phenolics	40.26 ± 0.05*	26.17 ± 0.23
*=Significantly different at P<0.05		

The quantitative analysis of *P. Febrifugum* and *H. Madagascariensis* (Table 2) showed a total flavonoid concentration-898.88 ± 2.17 mg/g, 706.38 ± 2.17, alkaloid- 530.00 ± 13.61 mg/g, 457.87 ± 5.05 mg/g, tannin- 44.91 ± 0.18 mg/g, 42.46 ± 0.41 mg/g and phenolic- 40.26 ± 0.05 mg/g, 26.17 ± 0.23 mg/g respectively. Flavonoids are effective therapeutic plant substances, known for their anti-inflammatory, antibacterial, antifungal, and anti-allergic effects [46]. Alkaloids are physiologically active and have sedative and analgesic effects. They can reduce stress and depression symptoms. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, known to act as natural antioxidants. Additionally, they serve as anti-carcinogenic, and anti-inflammatory agents [47]. Konan et al, [48] reported a total phenolic content of 291.8 ± 6.9 mg GAE 100 g^{-1} in the leave extract of *P. Febrifugum* using methanol as the solvent. Tannins are used as diuretics against duodenal tumours, astringents against diarrhoeal, and anti-inflammory [49-51]. Antia et al, [45], reported alkaloid (0.36 ± 0.03 mg/100g) and saponins (0.37 ± 0.07 mg/100g) in *H. Madagacariensis* stem bark, using dichloromethane as extractant. The presence of different amounts of phytochemicals in the *P. febrifugum* and *H. madagascariensis* extracts explains the therapeutic claims accredited to these plants in traditional medicine.

Notwithstanding the recent modishness in antioxidant research, the deficiency of standardized assays to equate research results from different research individual or groups has been a major hitch [52]. In harmony with the

standard for evaluation of *in-vitro* antioxidant activities of natural products [53-56], we propose the following limits; (1) Extracts and compounds are considered to have high or significant capacity when IC_{50}<10 µg/mL for extract and IC_{50}<1 µg/mL for compounds, promising activity when IC_{50}=10-50 µg/mL for extract and IC_{50}=5-10 µg/mL for compounds, moderate activity when IC_{50}=50-100 µg/mL for extract and IC_{50}=5-10 µg/mL for compounds, while sample with IC_{50}>100 µg/mL for extract and >10 µg/mL for compounds were assumed to possess low antioxidant activity. (2) Antioxidants capacities of plants extracts were considered to be very high when FRAP were >20 mM/L and high when FRAP were 10-20 mM/L, good when FRAP were 5-10 mM/L and low when FRAP were 1-5 mM/L, very low when FRAP were below 1 mM/L. (3) When dealing with radical scavenging activity at a constant concentration. Plant extracts were considered to exhibit low, medium, high, and significant activities when their % RSA at 50 mg/mL were observed to be <25%, 25-50%, 50-80% and >80%, respectively. (4) When dealing with DPPH radical scavenging activities on the basis of degree of color changes extracts are considered to have high or significant capacity when showed strong intensity of yellow coloration, moderate when showed moderate intensity of yellow coloration, and low capacity when showed moderate intensity of yellow coloration.

Many antioxidant compounds have been isolated from plants including flavonoids. Flavonoids are phenolic compounds with very essential roles in scavenging free radicals and so play vital roles in preventing oxidative stress related disorders [57]. The antioxidant effects of flavonoids in biological systems are attributed to its capacity to transport electrons to free radicals, chelate metals, activate antioxidant enzymes, and reduce radicals of alphatocopherol or to inhibit oxidases while phenolic compounds exert its antioxidant activities by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [58]. Based on the aforesaid basis of standardized assays, it could be observed that *P. febrifugum* and *H. madagascariensis* stem bark extracts have high antioxidants properties.

In the antioxidant potentials determined by DPPH reagent (Table 3), the mean IC_{50} and ARP values obtained clearly showed that *P. Febrifugum* extract had more activity than *H. madagascariensis*, both of which were better antioxidants than vitamin C. Lower IC_{50} values indicate a higher radical scavenging activity [59]. T-test conducted on the IC_{50} using the ascorbic acid standard, showed that at 5 % ($p<0.05$), *P. Febrifugum* and *H. madagascariensis* extract are better antioxidants than ascorbic acid [48] in [56], reported higher value of IC_{50} (2.3 µg/ml) for *P. Febrifugum* leave extract. Chinaka et al, [60], reported higher DPPH/FRAP of 85/1.95 at 400 µg/ml concentration in root extract of *H. madagascariensis* using meOH as solvent. IC_{50} which is a measure of the effectiveness of a substance in preventing a specific biological function is also known as half maximal inhibitory concentration whereas ARP is the inverse of IC_{50}. DPPH is a free radical that accepts hydrogen atom to form a stable molecule.

Table 3. Average IC_{50} values for the extracts of *P. febrifugum* and *H. madagascariensis* with ascorbic acid standard.

Plant extract	IC_{50} value (µg/mL)	Anti-Radical Power (ARP)
P. febrifugum	0.74 ± 0.02	1.36 ± 0.02
H. madagascariensis	0.82 ± 0.05	1.22 ± 0.05
Ascorbic acid	1.54 ± 0.01	0.54 ± 0.01

Table 4 showed that both *P. febrifugum* and *H. madagascariensis* extracts varied with change in concentrations. However, at higher concentrations *H. madagascariensis* seems to become more active than *P. febrifugum* and gallic acid standard.

Table 4. Antioxidant activity of the extracts of *P. febrifugum* and *H. madagascariensis* with gallic acid standard by FRAP scavenging free radical activity.

Concentration (µg/mL)	Ethanolic extract (µmol Fe^{2+}/g)		
	P. febrifugum	***H. madagascariensis***	**Gallic acid standard**
15.63	0.714 ± 0.01^{bl}	0.663 ± 0.40^{bl}	0.845 ± 0.40^{a2}
31.25	$l.671 \pm 0.30^{b2}$	1.401 ± 0.06^{cl}	2.413 ± 0.22^{a3}
62.5	3.598 ± 0.64^{b2}	3.201 ± 0.42^{cl}	5.726 ± 0.04^{a3}
125	7.635 ± 0.10^{bl}	7.805 ± 0.13^{b1}	13.226 ± 0.11^{a2}
250	21.299 ± 0.17^{b2}	29.469 ± 0.30^{a3}	19.403 ± 0.17^{cl}
500	68.665 ± 0.78^{b2}	96.680 ± 0.51^{a3}	66.720 ± 0.26^{cl}
1000	266.687 ± 2.99^{b2}	321.069 ± 2.32^{a3}	220.391 ± 2.39^{cl}

Results expressed in means ± sem (n=3). Mean values having different letters as superscripts across the rows are significantly different at (P<0.05) while mean values having different numbers as superscripts along the column are significantly different at (P<0.05). The total antioxidant capacity (TAC) of both plant extracts (Table 5) showed higher capacity at higher concentrations, but at higher concentrations *P. febrifugum* showed higher capacity than *H. madagascariensis*. TAC is frequently used to assess the antioxidant state of a biological sample and can evaluate the antioxidant ability against the free radicals produced in each disease [61]. Low TAC could be indicative of oxidative stress or increased vulnerability to oxidative damage [62].

Table 5. Total antioxidant capacity (TAC) of the extracts of *P. febrifugum* and *H. madagascariensis* by Phosphomolybdate method.

Concentration (µg/mL)	Extract (mg GAE/g)	
	P. febrifugum	***H. madagascariensis***
15.63	311.92*	112.75
31.25	578.58*	326.08
62.5	866.92*	626.25
125	1096.08*	838.58
250	1321.08*	1068.58
500	1699.42*	1335.25
1000	1865.42*	1601.92
*=Significantly different at P<0.05		

Table 6. Estimation of Vitamin (A, C and E) Levels of the extracts of *P. febrifugum* and *H. madagascariensis.*

Micronutrients	Ethanolic extract (mg /g)	
	P. febrifugum	***H. madagascariensis***
Vitamin A	10.90 ± 0.54*	2.07 ± 0.04
Vitamin C	0.19 ± 0.03	0.44 ± 0.03*
Vitamin E	1.45 ± 0.10	1.73 ± 0.02*
* = Significantly different at P<0.05		

From the estimated vitamin (A, C and E) contents of both plant extracts (Table 6), we observed that vitamin A is higher in *P. febrifugum* while vitamins C and E were higher in *H. madagascariensis* extract.

Antia et al, [44], quantified these vitamins in the dichloromethane and methanol extract of *H. Madagascariensis* and obtained vitamin A, (2.60 ± 0.02, 4.18 ± 0.02 mg/100 gDW), vitamin C, (0.16 ± 0.01, 0.26 ± 0.01 mg/100 gDW) for dichloromethane and methanol extract respectively. Vitamin C is a very potent water-soluble antioxidant commonly found in citrus fruits and vegetables [63]. Vitamin E also has antioxidant properties and it is fat-soluble, non-polar vitamin found naturally in lipid-rich fruits and vegetables [64]. Vitamin A is a powerful antioxidant and acts as a hormone in the body, affecting the ability of genes to express itself thereby sway the phenotype [65].

CONCLUSION

P. febrifugum and *H. madagascariensis* possess substantial number of antioxidants. It was observed that the crude extract of the stembark had better antioxidant properties than the standard ascorbic acid and it will give a much better antioxidant activity. However, *P. febrifugum* stem bark contained higher quantities of phytochemicals when compared to *H. madagascariensis*. Again, *P. febrifugum* and *H. madagascariensis* exhibited strong DPPH, TAC and FRAP free radical scavenging activity and the two plants also are better antioxidant drugs than the standard gallic acid. These plants may be considered a rich source of antioxidants which control the autoxidation by interrupting the propagation of free radicals or by inhibiting the formation of free radicals and subsequently reduce oxidative stress, improve immune function, and increase healthy longevity. This justifies their ethno-medicinal uses in psoriasis treatment. Pharmacologically, the plants phytochemicals could be isolated, purified and utilized as natural antioxidants or for supplements to already-existing antioxidants in the prevention of diseases related to oxidative stress.

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