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# ANTIOXIDANT AND PHYTOCHEMICAL STUDIES OF THE LEAF OF *BREONADIA SALICINA* HEPPER AND J. R. I. WOOD (RUBIACEAE)

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## ABSTRACT

**Aim:** This research aims to carry out phytochemical screening and to evaluate the antioxidant potential of the leaf of *Breonardia salicina*

**Study design:** The study include collection and identification of the plant, phytochemical screening of the leaf sample collected, extraction and fractionation of the sample then antioxidant evaluation using DPPH and ABTS methods.

**Place and Duration of Study:** The study was carried out in Kaduna State University, Kaduna-Nigeria and Universiti Putra Malaysia (UPM), Selangor-Malaysia. The study last for six months.

**Methodology:** The plant was collected around Kudingi Village, Giwa Local Government Area, Kaduna state, Nigeria. Sample was then identified in the Department of Botany, Ahmadu Bello University, Zaria. Standard methods of preliminary phytochemical screening followed in screening the powdered leaf sample. The dried powdered leaf macerated with 95% ethanol using mechanical shaker, dried using rotary evaporator then fractionated using ethyl acetate and n-butanol. The antioxidant activity of the extracts and fractions of the plant leaf were evaluated using Trolox as standard antioxidant and the Trolox equivalence of the extract /fractions were determined.

**Results:** Preliminary phytochemical screening of the sample shows the presence of carbohydrates, cardiac glycosides, saponins, triterpenes and steroids, tannins, flavonoids and alkaloid and anthraquinones. Antioxidant equivalence of the extracts/fractions of the plant at $R^2$ value of 0.9938 and standard equation ($y = 0.9891x - 1.996$) was found to be highest at 205.4 ± 0.6 mg Trolox Equivalent and lowest at 118.7 ± 2.7 mg Trolox Equivalent.

**Conclusion:** The good antioxidant values obatained revealed possible justificstion for the use of the plant in the treatment of many diseases that are known to respond to antioxidantion.

**Keywords**: *Breonadia salicina*, Breonadia, Rubiaceae, Antioxidant, DPPH, ABTS

## INTRODUCTION

Antioxidants are natural or synthetic central basics that intercept or mitigates damage to cells caused by free radicals or unstable molecules that the body manufactures in response to environment or pressures [1]. These free radicals, which are also referred to as reactive oxygen species (ROS) perhaps, are the major cause of degenerative diseases such as cancer and neurodegenerative diseases [2]. They can be managed using a synthetic or natural approach. The synthetic antioxidants such as vianol and embanox have been useful in managing degenerative free radical complication. However, due to their associated side effect and attendant problems such as accumulation in tissues, people are forced to look for an alternative approach. This issue makes natural antioxidants more popular, natural antioxidants are metabolites that are mostly from plant origin [3], and are often phenolic and organic acids [4]; [5]. DPPH assay was based on the measurement of the scavenging capacity of antioxidants towards a stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH). Rubiaceae is rank the number four most abundant flowering plant family [6], consisting of 13,000 species, 620 genera, 40 tribes and three subfamilies [7]. Members of this family grow all over the globe including Antarctic environment with some species such as *Coprosma*, *Galium* and *Sherardia* [8]. A great diversity of little form was observed in this plant family, ranging from higher plants, small woody plants to large rainforest trees [7]. Some researches suggestively divided the family into three subfamilies:Rubioideae, Cinchonoideae and Ixoroideae which are further subdivided into 43 tribes [9]. *Breonadia salicina* is an evergreen plant, it growth ranges from medium to large in both size and height, reaching up to 20 m in height and 80 cm in diameter. It can grow between 500 to 2000 m above sea level along riverbanks, streams and river tributaries [10]. According to [11], the stembark of *B.salicina* contain triterpenes, saponins in abundance and tannins while the wood is rich in polyphenols and quinines. Isolation of Ursolic acid from the acetone leaf extract of the plant was reported by [12], which shown profound antifungal activity. Six chemical compounds were isolated by [13] from the stem bark of the plant. The compounds isolated and elucidated are; 7-(*β*-Dapiofuranosyl (1 -6)-*β*-D glucopyranosyl) umbelliferone, *α*-amyrin, stigmasterol, 7-hydroxycoumarin and 6- hydroxy-7-methoxy coumarin. Ethno medically, *Breonadia salicina* is shown to be use in the treatment of various diseases, which include; treatment of cancer, headache, and arthritis, gastrointestinal illness, fevers, diabetes, inflamed wounds, ulcers, bacterial and fungal infections. However, only few biological studies reported to have been conducted on the plant in spite of its tremendous usage by the rural dwellers and herbalists especially in tropical and subtropical regions. Pharmacological studies conducted on the plant include antibacterial studies by [10] showed potent activity against the tested bacteria pathogens. Similarly, [12] reported antifungal activity. Anti trypanasomal activity of the plant was found to be potent at concentration and time dependent manner [14]. This current study aims to study phytochemical and antioxidant potentials of the plant.

## MATERIALS AND METHODS

### Identification, Collection and Preparation of the Leaves of *Breonadia salicina*

*B. salicina* was first identified on the field using its morphological features around Kudingi Village, Giwa Local Government Area, Kaduna state, Nigeria. Sample of the plant was then collected and transported to Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria for proper identification and authentication. A voucher

specimen number of ABU900383 was given and deposited in the Herbarium of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria-Nigeria. After authenticating the identity of the *Breonadia salicina*, sufficient quantities of the leaf was obtained for further studies. The leaves were cleaned and all foreign matters removed. It was then, air-dried under shade, communited to powder form using pestle and mortar, and then stored in an airtight container.

**Phytochemical Screening of the Leaves of *Breonadia salicina***

Standard methods of preliminary phytochemical screening followed in screening the powdered leaf sample for the presence or absence of carbohydrates, cardiac glycosides, saponins, triterpenes and steroids, Tannins, flavonoids and alkaloid and anthraquinones as described by [15].

**Extraction of the Leaves of *Breonadia salicina***

The dried powdered leaf sample of the *B. salicina* (300 g) each was macerated with 1L of 95% ethanol using mechanical shaker (Stuart Scientific Flask Shaker, Great Britain) at 25$^{0}$C, 200 rpm for 6 hours. The extract obtained was filtered with a whatman filter paper No 1, and then evaporated to dryness using rotary evaporator (Buchi Labortechnik) at 50$^{0}$C and reduced pressure. The dried extract was then weighed and the percentage yield calculated. It was transferred into an airtight container and kept properly in a dessicator for further use.

**Fractionation of the Aqueous Ethanolic Extract of the Leaf of *B. salicina***

The extract of the leaf (2.5 g) was suspended in 500 mL of water and sonicated at 20 $^{0}$C for 10 minutes. Thereafter, n-Hexane (300 mL) was added and then shaken using the mechanical shaker (Stuart Scientific Flask Shaker, Great Britain) at 20 $^{0}$C, 200 rpm for 30 minutes. The mixture was transferred to a separating funnel, allowed to stand and the hexane fraction was collected, and then evaporated to dryness using rotary evaporator (Buchi Labortechnik) at 50 $^{0}$C. The aqueous portion was then extracted with ethyl acetate (300 mL) as described above. The same procedure was repeated using n-butanol. The Ethyl acetate, n-butanol and aqueous fractions were concentrated over a water bath, transferred into sample bottles and kept for further use.

**Determination of the antioxidant activities of *B. salicina* Leaf**

**a) DPPH radical scavenging assay**

The DPPH radical scavenging activity of the ethyl acetate leaf fraction (EAL), the n-Butanol leaf fraction (NBL) and the aqueous leaf fraction (AQL) were determined using the method described by [16], modified by [17]. A solution of 40 uL of the samples and the standard (Gallic acid) at different concentrations were completed with 160 uL of the DPPH in methanol (0.1mM). DPPH reagent in methanol was used as a blank. After 30 min., the absorbance of each solution was measured at 517 nm using a microplate reader. Gallic acid was used as the standard (positive control). The percentage of radical scavenging activity was calculated as follows: % inhibition = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$, where $A_{control}$ is the absorbance of the control and $A_{sample}$ is the absorbance of the test extracts. The result was expressed as $IC_{50}$ value (µg/mL) corresponding of sample concentration that inhibits 50% DPPH radical

**b) ABTS radical cation scavenging assay**

The ABTS radical cation scavenging of the extract the ethyl acetate leaf fraction (EAL), the n-Butanol leaf fraction (NBL) and the aqueous leaf fraction (AQL) were determined using method described by [18]. The ABTS (7 mM) and potassium persulfate solutions (2.45 mM) were prepared and mixed together, incubated

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for 8-hours in the dark. The stock solution was then diluted with methanol and its absorbance adjusted to 0.900 (± 0.02) at 745 nm at 30°C. 300 uL (125- 2000ug/mL in methanol ) for each of the sample was mixed with the ABTS working solution and measured the absorbance. The percentage scavenging property of the samples and the standard were calculated thus: Scavenging effect (%) = [(control absorbance (ABTS) – sample absorbance / (control absorbance)] × 100

## RESULTS

### Preliminary Phytochemical screening of the Leaf of *Breonardia salicina*

The preliminary phytochemical screening of the powdered leaf, stem bark and the root of the plant indicated the presence of carbohydrates, cardiac glycosides, saponins, triterpenes and steroids, Tannins, flavonoids and alkaloid and anthraquinones as shown in (Table 1) below:

**Table 1: Phytochemical Screening of the Leaf of *Breonardia salicina***

| Tests | Expected Reactions | Inference |
|---|---|---|
| **Anthraquinones** | | |
| Bontrager's test | A bright pink colour in the upper aqueous layer observed | + |
| Modified Bontrager's test | A rose pink at the lower layer observed | + |
| **Cardiac glycosides** | | |
| Keller-Killiani test | A purple-brown ring at the interface and a pale green colour in the upper acetic acid layer observed | + |
| Legal test | A deep red colour observed | + |
| Kedde test | A purple-blue colour observed | + |
| Saponin glycosides | | |
| Frothing test | A honey comb froth that persisted for 15 minutes observed | + |
| **Flavonoids** | | |
| Ferric Chloride test | A greenish black colour observed | + |
| Sodium hydroxide test | A yellow solution observed | + |
| Shinoda's test | A pink colour solution observed | + |
| **Steroids/Triterpenoids** | | |
| Libermann-Burchard test | A purple colour observed | + |
| Salkowski test | A cherry red colour at the interface observed | + |
| **Tannins** | | |
| Ferric Chloride test | A green precipitate observed | + |
| Lead sub-acetate | A colour precipitate observed | + |
| Bromine water test | A buff colour precipitate observed | + |
| **Alkaloids** | | |
| Meyer's test | A cream coloured precipitate observed | + |
| Dragendoff's test | An orange-red precipitate observed | + |
| Wagner's test | A reddish brown precipitate observed | + |
| 1% Picric acid test | A yellow precipitate observed | + |
| 10% Tannic acid test | A buff coloured precipitate observed | + |

**Key**: + present, - absent

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**Extraction of the powdered leaf of *Breonadia salicina***

The yield of the extract in 95% ethanol is 14.14%.

**Fractionation of ethanol extracts of the Leaf of *B. salicina***

Four (4) fractions were obtained, after successive fractionation with three solvents, these are; Hexane (HX), ethyl acetate (EA), and n-butanol (NB). The fractions were HXL, EAL, NBL and AQL.

**Free radical scavenging activities of the leaves of *Breonadia salicina* by DPPH**
Free radical scavenging power (Antioxidant property) of the extract/fractions (LEE, EAL, and NBL) of the *B. salicina* were determined using DPPH method with Trolox as the standard antioxidant. Trolox antioxidant equivalence were calculated of the extract/fraction using a standard regression curve of Trolox with $R^2$ value of 0.9938 and standard equation ($y = 0.9891x - 1.996$).

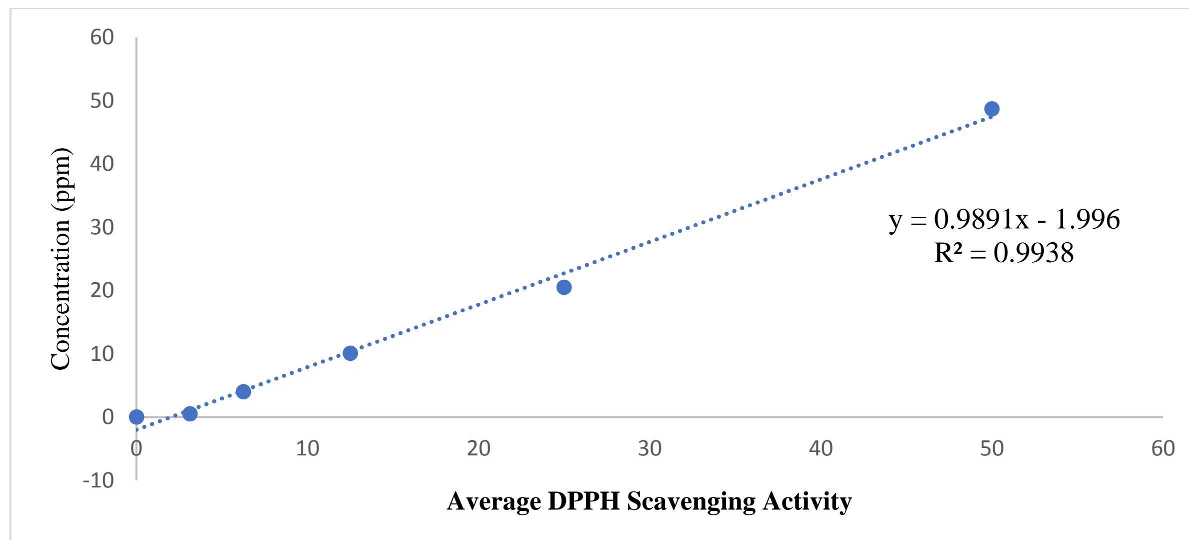


**Figure 1: Standard curve of Trolox for the determination of mg equivalence of scavenging activities of the extracts and fractions of the leaf of *Breonadia salicina***

**Table 2: Antioxidant of the extracts/fractions of Leaf of *Breonadia salicina* express as Trolox mg/g equivalence**

| Plant part | Fraction | mg Trolox Equivalent ± SD |
|---|---|---|
| **Leaf** | LEE | 205.4 ± 0.6 |
| | EAL | 281.7 ± 0.8 |
| | NBL | 176.3 ± 2.8 |
| | AQL | 118.7 ± 2.7 |

**Key:** Values are means ± SEM of 3 replicates. LEE = 95% Leaf Ethanol Extract, EAL = Leaf Ethyl acetate Fraction, NBL = n-Butanol Leaf Fraction, AQL = Aqueous Leaf Fraction

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### Free radical scavenging activities of the Leaves of *Breonadia salicina* Using ABTS

Free radical scavenging power (antioxidant property) of the extracts/fractions (LEE, EAL, NBL and AQL) of the *B. salicina* were also determined using ABTS method with Trolox as the standard antioxidant. Trolox antioxidant equivalence were calculated for the extract/fraction using a standard regression curve of Trolox with $R^2$ value of 0.9996 and standard equation ($y = 0.8039x + 0.2045$).

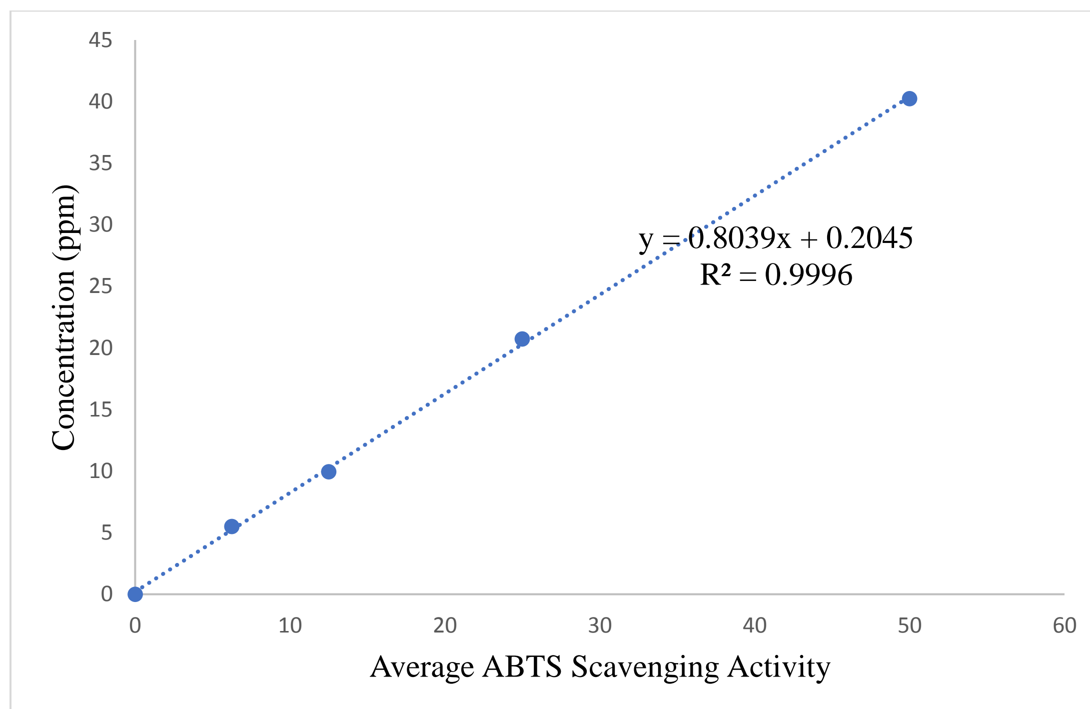


**Figure II: Standard curve of Trolox for the determination of scavenging activities of the extracts and fractions of the leaf of *B. salicina***

**Table 3: Antioxidant of the extracts/fractions for the Leaf of *Breonadia salicina* express as mg/g Trolox equivalence**

| Plant part | Fraction | mg Trolox Equivalent ± SD |
|---|---|---|
| **Leaf** | LEE | 195.4 ± 0.8 |
| | EAL | 275.8 ± 0.6 |
| | NBL | 196.5 ± 1.1 |
| | AQL | 143.1 ± 1.8 |

**Key:** Values are means ± SEM of 3 replicates. LEE = 95% Leaf Ethanol Extract, EAL = Leaf Ethyl acetate Fraction, NBL = n-Butanol Leaf Fraction and AQL = Aqueous Leaf Fraction

## DISCUSSION

Phytochemical screening is a technique that is use to carry out simple chemical tests on drug materials in order to establish the presence or absence of a particular bioactive chemical constituent. The test is useful in the evaluation and identification of samples of drugs.

The phytochemical screening of the powdered leaf of the *Breonardia salicina* reveals the presence of anthraquinones, saponins, cardiac glycosides, triterpenoids, flavonoids, tannins and alkaloids as shown on (Table 1). This is consistent with the findings of [9], in which several secondary metabolites that are of the above phytochemical groups.DPPH assay was based on the measurement of the scavenging capacity of antioxidants towards a stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH). The odd electron of the nitrogen atom from antioxidants to the corresponding hydrazine. The DPPH and ABTS results were presented as percent scavenging activity comparable to the Trolox (standard), used. The scavenging activities of the ethyl acetate fraction was found to be highest while the aqueous fraction had the least scavenging activity, see (table 2 and 3). This is possibly because of the removal of some vital phytochemicals due to the fractionation. Same trend of scavenging activity on the family was observed by [19]. The activity of the extracts and fractions of the plant using the two antioxidants assays shows strong positive correlation, which is in agreement with that of [20]

## CONCLUSION

The present study suggests that *Breonardia salicina* leaf possesses potent antioxidant activity. Thus, it shows that the leaf could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing down the progress of oxidative stress related degenerative diseases.

## RECOMMENDATION

Further studies should be conducted on the leaves of the plant aiming at isolating chemical compound(s) which may be responsible for its the antioxidant activity.

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