Original Research Article

Antimicrobial and antioxidant properties of *Hedranthera barteri* leaf and root extracts

Abstract

Due to the increasing use of plants for therapeutic purposes, there is a need to evaluate their medicinal constituents. This study assessed the phytochemical potential, antioxidant, and antimicrobial proprieties of *Hedranthera barteri* extracts. Qualitative and quantitative procedures were used to evaluate the phytochemical constituents. 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picryl/hydrazine (DPPH) radical scavenging properties of *Hedranthera barteri* extracts. The scavenging activities of nitric oxide (NO) radical, and its total reducing capacity methods, were used to analyze the antioxidant potentials of *Hedranthera barteri* extracts. Serial dilution and disc diffusion methods were used to assess the minimum inhibitory concentration and antimicrobial activity of *Hedranthera barteri* extracts. The result of this study revealed that extracts of *Hedranthera barteri* possess abundant phytochemical constituents as well as high antioxidant potential. The data also showed that the root and leaf extracts of *Hedranthera barteri* possess very strong antibacterial potential against *Staphylococcus spp, Streptococcus spp, Neisseria spp, Escherichia coli, Pseudomonas spp,* and *Enterobacter spp*. The root extract exhibited an inhibition zone of 11.00±1.00 to 22.00±1.00 mm while the leaf exhibited an inhibition zone of 8.00±1.00 to 18.67±1.53 mm. This study disclosed the antimicrobial and antioxidant properties of *Hedranthera barteri* extracts. For further studies, we will explore the characterization of bioactive constituents, a critical step forward to determine the potential therapeutics of these extracts.

Keywords: *Hedranthera barteri*, Antioxidant, Secondary metabolite, Antimicrobial

UNDER PEER REVIEW

INTRODUCTION

For ages, plants have been used for medicinal purposes and have been an important part of human tradition and culture. World Health Organization (WHO) reported that about 80% of the world population rely on traditional or alternative medicine for their primary health care (Sumeet and Lantana 2008). Several benefits, such as treating some diseases, have been associated with traditional medicinal plants. Because of poverty, unavailability of modern health facilities, ignorance, and poor communication mean many individuals all over the world, particularly in Africa, are still using traditional medicines for therapeutic purposes. Reports of plants used against various illnesses may be documented in areas where the plant's use is of great importance (Hidayat, 2011). Bacteria have the genetic ability to transmit and have resistance to therapeutic agents such as drugs. One of the ways to prevent antibiotic resistance is through the use of natural compounds that are not based on synthetic antimicrobial agents (Shah, 2005). According to Zahid *et al*., 2010, antimicrobials extract from plants have enormous therapeutic potential, as they are more effective than synthetic components in the treatment of infectious diseases. In addition, these natural components have the advantage of not exhibiting many side effects, which are often observed with synthetic antimicrobial drugs.

There have been several research works on the uses of medicinal plants and plant parts that have afforded models for 25-50% of drugs made in the western world (Khan, 2009). The importance of plant-derived drugs includes their safety, practical therapeutic benefits, and affordable treatment of various illnesses (Manju *et al*., 2011). Many medicinal plants contain phytochemicals that have shown antimicrobial activities because of their different structures. There is a correlation between the phytochemical constituents of a medicinal plants with its pharmacological actions (Prachayasittikul et al., 2008).

Hedranthera barteri (Hook.F) Pichon, belongs to the family of Apocynaceae. It is a shrub that is found in the dump situation of Ghana, Western Cameroon, North and South Nigeria, and Zaire (Congo Brazzaville) (Burkill, 1985). The shrub grows up to 2m high. The flowers are large, tubular, and white with a fragrant scent. The plants have been reported to be effective in preventing miscarriage (Burkill, 1985. This plant has been described as effective against gonorrhea. It is also used as a vermifuge and the leaf is used in the management of painful tumors (Ainslie, 1937). The leaf decoction is used to treat dizziness by the Igbos of South-

UNDER PEER REVIEW

Eastern Nigeria. It is used in the treatment of convulsion. Several reports (Duru and Mbata; Onasanwo et al., 2010; Onasanwo, 2006) have described the anxiolytic, antimicrobial, antinociceptive, anti-inflammatory, and antidepressant properties of the plant. In addition, antioxidant and antiulcer activities of the root have also been reported.

This study aims to determine the antimicrobial and antioxidant activity, and phytochemical composition of root and leaf extracts of *Hedranthera barteri*.

MATERIAL AND METHODS

The root and leaves of *Hedranthera barteri* were harvested at Alihame, Agbor, Delta state Nigeria. The plant was identified by Prof Obadoni, a Professor of botany, Ambrose Ali University Ekpoma, Edo State. The root and leaves were collected, chopped into smaller sizes, and washed to remove all foliar contaminants. Both the root and leaves were allowed to sundry. After drying, plant materials were blended into fine powder. 250g of the powdered material was macerated into 1200ml of 96% ethanol for 48 hours and stirred at intervals. After which, the extracts were filtered using a muslin cloth. The extracts were concentrated using a rotary vacuum evaporator (Re-52A, lab science, England), at 37°C.

PHYTOCHEMICAL SCREENING

Qualitative and quantitative phytochemical analysis was used to evaluate the presence of secondary metabolites such as flavonoids, phenols, tannins, saponins, anthraquinones, cardiac glycosides, cyanogenic glycosides, and alkaloids in the extracts using standard procedures:

Test for proteins: Million test

0.2ml of the ethanol extracts was mixed with 2ml of million's reagent, and a white precipitate was observed, which turn red on gentle heating, indicating protein presence (Trease and Evan, 1996).

Test for carbohydrates: Fehling's test

2ml of Fehling A and Fehling B reagents were mixed, and 2ml of the mixture was allowed to react with 0.2ml of the extracts and gently heated. The presence of brick red precipitate at the test tube bottom indicates the presence of reducing sugar (Trease and Evan, 1996).

UNDER PEER REVIEW

Iodine test

0.2ml of the crude extracts was allowed to react with 2ml of iodine solution. The presence of purple or dark blue precipitate indicates carbohydrate presence (Trease and Evan, 1996).

Test for phenols and tannins

0.2ml of crude extracts reacted with a 2% solution of $FeCl_3$. The precipitate of blue-green colour indicates the presence of tannins and phenols (Harborne, 1992).

Test for flavonoids: Shinoda test

Magnesium ribbon fragments were allowed to react with 0.2ml of the crude extracts, and concentrated hydrochloric acid was added dropwise. Appearances of pink, scarlet precipitate indicate flavonoid's presence (Harborne, 1992).

Alkaline reagent test

0.2ml of extracts was mixed with 2ml of 2% of NaOH. A yellow precipitate was formed, which, on the addition of dilute acid in drops, turned colourless, indicating flavonoid's presence (Harborne, 1992).

Test for triterpenoids

Extracts of 10mg was dissolved in 1ml of chloroform followed by the addition of 1ml of acetic anhydride, after which 2ml of concentrated H_2SO_4 was added. Formation of a reddish-violet precipitate indicates triterpenoids presence (Harborne, 1992).

Test for phlobatannins

2ml of 1% HCl was added to 2ml of each extract. The mixture was boiled. The presence of red precipitate indicates phlobatannins presence (Brain and Turner, 1975).

Test for saponins

Extracts of 0.2ml were mixed with 5ml of distilling water and shaken vigorously. The development of stable foam indicates saponin's presence (Harborne, 1992).

Test for glycosides: Liebermann's test

UNDER PEER REVIEW

Extracts of 0.2ml were mixed with 2ml of chloroform followed by the addition of 2ml of acetic acid. The reacting mixture was ice-cooled and concentrated H_2SO_4 was added to the reacting mixture. The precipitate formed changes to blue to green from violet, which indicates the steroidal nucleus, which is the glycone portion of the glycoside (Brain and Turner, 1975).

Keller- Kiljani test

Extracts of 0.2ml were mixed with glacial acetic containing 1-2 drops of 2% of $FeCl_3$ solution. The reacting mixture was allowed to react with 2ml of concentrated H_2SO_4. Brown ring formation at the interphase indicates cardiac glycosides presence (Brain and Turner, 1975).

Test for steroids

0.2ml of the extract was allowed to react with chloroform and concentrated H_2SO_4. Formation of red precipitate at the lower chloroform layer indicating steroids presence (Ciulei, 1981).

Test for terpenoids

Extract of 0.2ml was mixed with 2ml of chloroform and allowed to evaporate to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for 2 minutes. The presence of greyish precipitate indicates terpenoids' presence (Ciulei, 1981).

Test for alkaloids

Extracts of 0.2ml were mixed with 2ml of 1%HCl and allowed to heat. Mayer's and Wagner's reagents were added to the reacting solution. The presence of turbidity indicates alkaloids presence (Harborne, 1992).

Estimation of total phenolic content (TPC)

The total phenolic content of the plant extracts was estimated by mixing 500 µl of diluted Folins-phenol reagent (1:1 ratio with water) with the appropriate dilution of plant extracts, and 2.5 ml of 20% sodium carbonate was added, to the reacting solution. The reacting solution was shaken and incubated in the dark for 40 minutes for colour development. After incubating, absorbance was read at 725nm using a UV spectrophotometer (Jenaway, Germany). The plant extracts TPC was

UNDER PEER REVIEW

expressed as mg of gallic acid equivalent (mg GAE/g extract) using a standard curve (Siddhuraju *et al*., 2003).

Estimation of total saponin content (TSC)

Total saponins determination involves mixing 250 µl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5% ethanol) with the appropriate extract dilution. 2.5 ml of 72% sulfuric acid was added and mixed well. The reacting solution was kept in the water bath at 60 °C for 10 minutes and then the reacting solution was cooled in ice-cold water, and absorbance was spectrophotometrically read at 544nm. The value was expressed as quinine equivalent (mg QU/g extract) (Makkar *et al*., 2007).

Estimation of total flavonoid content (TFC)

Total flavonoids were estimated by treating appropriate dilution of the extracts with 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M sodium acetate and left to stand for 30minutes and absorbance was read at 415nm using. The TFC in the extracts was determined using quercetin as standard (mg QE/g extract) (Jan et al., 2011).

Estimation of total tannin content (TTC)

The total tannin content of the extracts was estimated according to the method reported by Polshettiwar *et al*., 2007. 100 µl of extracts and standard solution of tannic acid was taken and made up to 1 ml with distilled water, then 0.5 ml of Follin-Denis reagent with 1 ml of Na_2CO_3 solution was added to the reacting solution. The volume of the reacting solution was made up to 10 ml with distilled water and absorbance was spectrophotometrically measured at 700nm. The total tannin content was expressed as tannic acid equivalent (mg TAN/g extract).

Antioxidant Assay

Determination of DPPH (2,2 diphenyl-1-picry/hydrazine) free radical scavenging ability

Different concentrations of plant extracts were added to equal volumes of methanolic solution of DPPH. The reacting mixtures were allowed to react at room temperature in the dark for 30 minutes and absorbance was read at 517nm. A lower absorbance value indicates higher radical scavenging activity. DPPH radical scavenging activity was calculated using DPPH scavenging effect (% inhibition) (Makkar et al., 2007).

UNDER PEER REVIEW

DPPH radical scavenging activity (%) = $\frac{A_0 - A_1}{A_0} x100$

Where A0 is absorbance of control and A1 is absorbance of sample extract.

Determination of ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical scavenging ability

ABTS scavenging activity of the extracts were determined by method reported by (Re et al., 1999). The stock solution which consisted of 7mM ABTS solution and 2.4mM potassium persulfate (1:1) was allowed to react in the dark for 16 hours at room temperature. The radical generated wasQ mixed with methanol to obtain an absorbance of 0.702±0.001 unit at 734nm. Exactly 2ml of the resulting solution was added to the extracts or vitamin C and absorbance was measured at 734nm after 7minutes. The percentage scavenging ability was calculated using the formula

ABTS radical scavenging activity (%) = $\frac{\text{Abs control - Abs sample}}{\text{Abs control}} x100$

Where Abs control is absorbance of control and Abs sample is absorbance of sample extract.

Determination of Nitric Oxide Radical Scavenging Ability

A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline was mixed with 1 mL of the different concentrations of the ethanol extracts (100−1000 μg/mL) and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. The absorbance was measured at 546 nm. Ascorbic acid was used as the positive control (Siddhuraju et al., 2003).

Nitric oxide radical scavenging ability (%) = $\frac{\text{Abs control - Abs sample}}{\text{Abs control}} x100$

Where Abs control is absorbance of control and Abs sample is absorbance of sample extract.

UNDER PEER REVIEW

Isolation, Identification, and Antimicrobial Assay

The following microorganisms, *Staphylococcus spp.*, *Streptococcus spp.*, *Neisseria spp.*, *Escherichia coli*, *Pseudomonas spp.*, and *Enterobacter spp.*, were cultured and identified, using appropriate media and reagents.

Isolation of microorganism

The collected samples were inoculated into the various agars and incubated at 37^{o}C for 24 hours. After 24 hours subcultures were carried out on the plates with growths using the nutrient agar, which was incubated at 37^{o}C for 24 hours. The colonies in the various plates were picked and subjected to gram staining and a series of biochemical tests for identification.

Identification of microorganism

Biochemical tests such as gram stain test, motility test, catalase test, oxidase test, citrate test, indole test, and triple sugar ion (TSI) test were carried out, using the method reported by Cheese Brough, 2006 on the colonies of the microorganisms for proper identification.

Antimicrobial Assay

The disc diffusion method was used. Each of the organisms was stricken uniformly on the surface of the solidified nutrient media. The disc was made from Whitman's No1 filter paper using a paper puncher, and the sterile disc was soaked in the extracts. Extracts of the concentrated disc were placed on the Petri-dishes containing the nutrient agar and the test organism using sterile forceps. The Petri dishes were incubated for 24 hours at 37°C. The control was done using a sensitivity disc containing the following antibiotics: Gentamycin 10ug, Taravid 30ug, and Zinnacef 20ug. The Petri dishes were incubated at 37°C for 24 hours. The Petri dishes were observed for growth inhibition zone, and inhibition zones were measured in millimeters.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration is the concentration with the lowest inhibition of microorganisms. The serial dilution method was used to determine MIC. The inoculated bacteria

UNDER PEER REVIEW

were prepared from 24hours of broth culture and standardized suspensions were adjusted to 0.5 Mc Farland turbidity standard. Four different test tubes were sterilized and placed on a rack, 10ml of already prepared nutrient broth was added to each test tube and 2ml of the extract was added to the first test tube swirled properly. 0.1ml of the standard inoculums prepared earlier was collected with a pipette and dropped into each test tube which was then sealed properly to prevent loss by evaporation and incubated at 37^{o}C for 24 hours. The presence or absence of growth was then observed. The concentration with no growth was recorded as minimum inhibitory concentration (MIC).

Statistical analysis of the research work was carried out using Graph Pad Prism 8.0.

RESULTS

Table 1: Phytochemical constituents of ethanol root and leaf extracts of *Hedranthera barteri*.

Test	**Root**	**Leaf**
Reducing Sugars	-	-
Proteins	+	-
Carbohydrates	-	-
Phenols & Tannins	+	+
Flavonoids	+	+
Triterpenoids	+	-
Phlobatannins	+	-
Saponins	+	+
Cardiac Glycosides	+	+
Steroids	+	+
Terpenoids	-	-

UNDER PEER REVIEW

Alkaloids	+	+

Key: (+) positive and (-) negative

Table 2: Quantitative phytochemical content of ethanol extract of the root and leaf of *Hedranthera barteri*

Plant part	**TPC (mg/GAE/g)**	**TFC (mg/QUE/g)**	**TSC (mg/QE/g)**	**TTC (mg/TAN/g)**	**TAC (mg/QUN/g)**	**% yield**
Leaf	185.70±2.7	213.00±2.62	131.90±1.49	50.81±2.22	170.90±2.23	36.67
Root	151.60±2.03	201.60±2.23	117.42±1.80	35.12±1.45	144.80±2.10	30

TPC – total phenolic content, TFC – total flavonoid content, TSC – total saponins content, TTC – total tannin content, TAC – total alkaloids content, Values represent mean ± standard deviation (n=3).

Table 3: Biochemical investigation of microorganisms

Catalase	**Oxidase**	**Citrate**	**Motility**	**Gram stain**	**Shape**	**Indole test**	**Gas bubble**	**Lactose**	**Hydrogen sulfide**	**Crack**	**Microorganism**
+	-	+	-	+	Cocci chain	+	+	+	-	+	*Streptococcus spp.*
+	-	+	+	+	Cocci	+	-	+	-	+	*Staphylococcus spp.*
+	+	-	-	-	Cocci	+	+	+	-	+	*Neisseria spp.*
+	+	+	+	-	Rod	-	+	+	-	+	*Pseudomonas spp.*
+	-	+	+	-	Rod	+	+	-	+	+	*Escherichia coli*
+	-	-	+	-	Rod	-	-	+	-	+	*Enterobacter spp.*

Key: (+) Positive (-) Negative

UNDER PEER REVIEW

Table 4: Minimum inhibitory concentration (MIC) of ethanol extracts of *Hedranthera barteri* root (R) and leaves (L) on the microorganism.

Microorganism	**Concentration of extracts (mg/dl)**							
	500		**250**		**125**		**62.5**	
	R	**L**	**R**	**L**	**R**	**L**	**R**	**L**
Streptococcus spp.	-	-	-	-	-	+	+	+
Staphylococcus spp.	-	-	-	-	+	-	+	+
Neisseria spp.	-	-	+	-	+	-	+	+
Pseudomonas spp.	-	-	-	-	-	-	+	+
Escherichia coli	-	-	-	-	+	+	+	+
Enterobacter spp.	-	-	-	-	-	-	+	+

Key: (+) Positive (-) Negative

UNDER PEER REVIEW

Table 5: Minimum inhibitory concentration (MIC) of ethanol extract of *Hedranthera barteri* root on the microorganism.

Microorganism	**Concentration of extracts**			
	500mg/dl	**250mg/dl**	**125mg/dl**	**62.5mg/dl**
Streptococcus spp.	-	-	-	+
Staphylococcus	-	-	+	+

spp.				
Neisseria spp.	-	+	+	+
Pseudomonas spp.	-	-	-	+
Escherichia coli	-	-	+	+
Enterobacter spp.	-	-	-	+

Key: (+) Positive (-) Negative

Table 6: Minimum inhibitory concentration (MIC) of ethanol extracts of *Hedranthera barteri* leaves on the microorganism.

	Concentration of extracts			
Microorganism	**500mg/dl**	**250mg/dl**	**125mg/dl**	**62.5mg/dl**
Streptococcus spp	-	-	+	+
Staphylococcus	-	-	-	+

spp				
Neisseria spp	-	-	-	+
Pseudomonas spp	-	-	-	+
Escherichia coli spp	-	-	+	+
Enterobacterspp	-	-	-	+

Key: (+) Positive (-) Negative

Table 7: Effect of ethanol extracts of *Hedranthera barteri* leaves, root, and the standard antibiotic on tested microorganisms.

	Inhibition	Zone (mm)			
Microorganism	**leaf**	**root**	**Gentamycin**	**Tarivid**	**Zinnacef**
Streptococcus spp.	18.67±1.53	22.00±1.00	8.66±0.58	13.00±1.00	8.66±0.58

UNDER PEER REVIEW

Staphylococcus spp.	11.67±1.56	14.00±1.53	8.67±1.16	11.33±1.53	9.67±1.16
Neisseria spp.	9.67±1.53	12.67±1.55	10.33±6.57	9.67±0.57	6.33±1.15
Pseudomonas spp.	8.00±1.00	21.68±1.67	6.33±1.15	7.00±1.00	8.00±1.00
Escherichia coli	12.67±1.58	11.33±0.58	12.33±0.58	13.33±1.53	12.33±0.58
Enterobacter spp.	14.33±1.16	11.00±1.00	10.33±1.14	8.67±0.58	5.66±0.58

Values represent mean ± standard deviation (n=3).

UNDER PEER REVIEW

Table 8: IC_{50} values for the inhibitory effect of *Hedranthera barteri* root and leaf extracts on DPPH, ABTS, and nitric oxide activity.

	IC_{50} (mg/ml)		
Assays	**Ascorbic acid**	**leaf**	**Root**
DPPH	0.098±0.002	0.117±0.004	0.143±0.007
ABTS	0.105±0.002	0.141±0.006	0.171±0.009

NITRIC OXIDE	0.098±0.03	0.127±0.004	0.145±0.005

Values represent mean ± standard deviation (n=3).

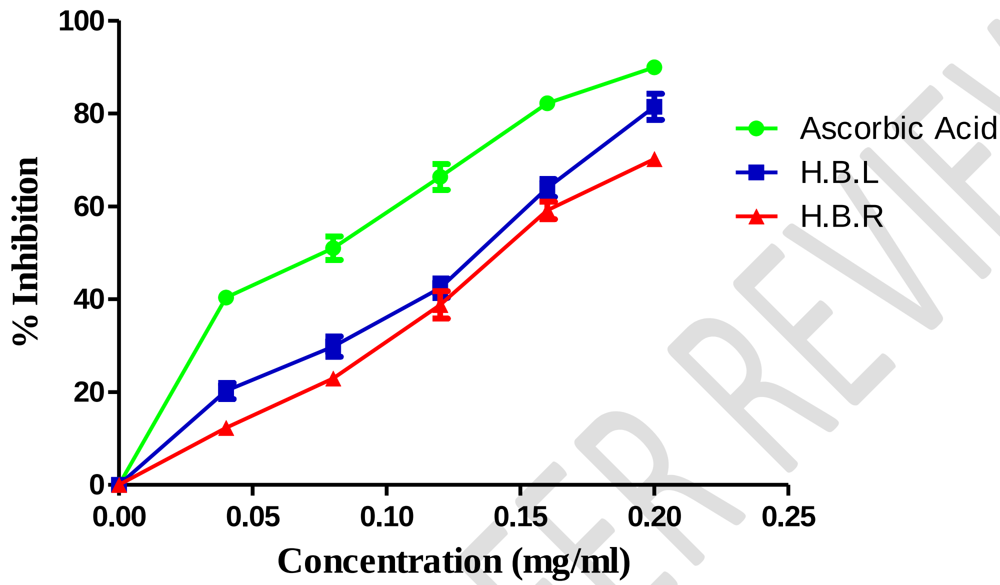

Fig.1: Nitric oxide activity of the *H. Barteri* leaf and root extracts. H.B.L for leaf extract and H.B.R for root extract.

UNDER PEER REVIEW

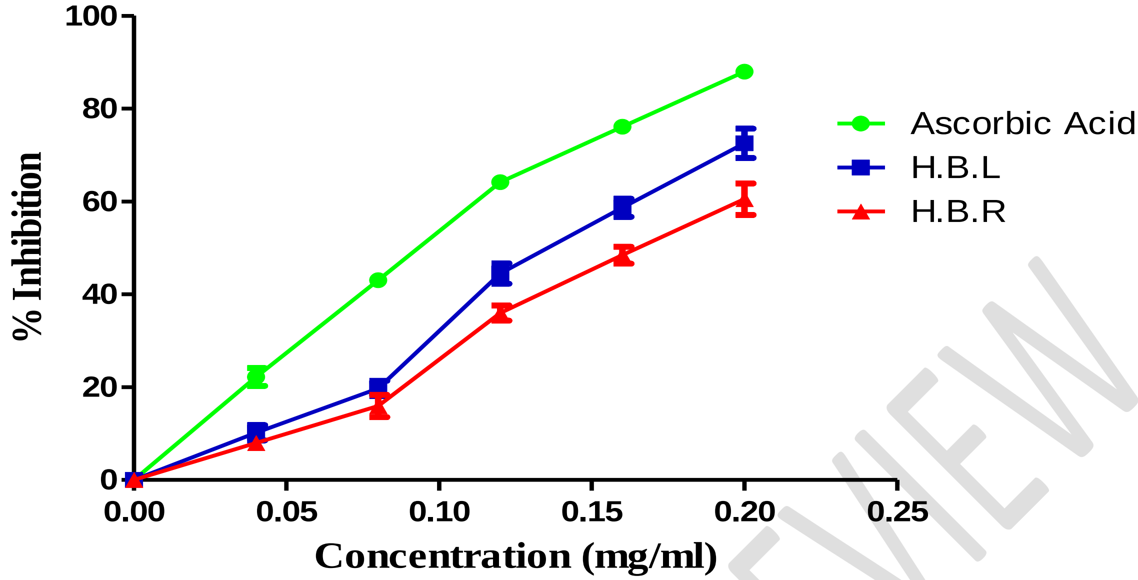

Fig 2: ABTS activity of the analyzed *H. Barteri* leaf and root extracts plant extracts. H.B.L for leaf extract and H.B.R for root extract.

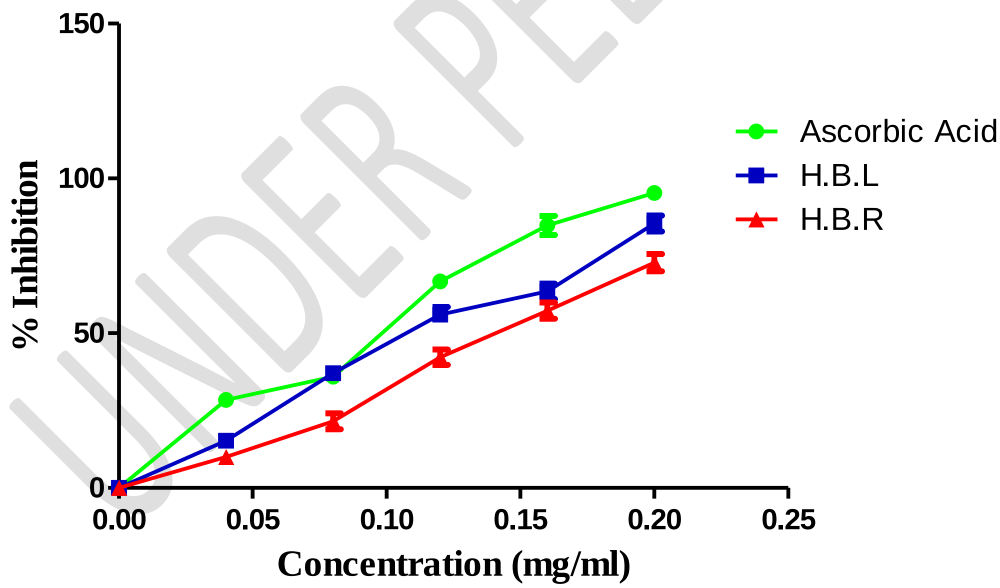

Fig 3: DPPH activity of the investigated *H. Barteri* leaf and root extracts. H.B.L for leaf extract and H.B.R for root extract.

UNDER PEER REVIEW

Discussion

The phytochemical analysis of ethanol extracts of *Hedranthera Barteri* (H.B) root and leaves revealed the presence of proteins, phenols and tannins, flavonoids, triterpenoids, saponins, phlobatannins, cardiac glycosides, steroids, and alkaloids, as shown in Table 1. The presence of these phytochemical components has also been reported by previous investigations (Duru and Mbata, 2010). Research has attributed the medicinal activities of plants to the presence of several phytochemical components (Fabricant and Farnsworth, 2001; Akiyama *et al*., 2001). Saponins have been reported to be useful in managing inflammation (Chukwujekwu *et al*., 2005; Liu *et al*., 2009). Flavonoids have also been described to exhibit useful properties which include anti-inflammatory, antimicrobial, antioxidant, antiallergic, vascular and cytotoxic antitumor activities (Bagchi et al., 1999). The quantitative analysis of the plant extracts revealed that both extracts (leaves and root of H. B) contain abundant secondary metabolites as shown in table 2. Flavonoid is the most abundant of the phytochemicals present in the extracts. Obtained results in this study confirmed published data of Onasanwo *et al*., 2010.

In vitro analysis of H.B extracts revealed its antioxidant activity and DPPH, ABTS, and nitric oxide assays were measured in a dose-dependent manner. DPPH is one of the widely used assays to assess antioxidant activity. Plant extracts are known to exert an effect on DPPH due to their ability to donate hydrogen atoms (Panda, 2012). DPPH is a stable free radical that can accept hydrogen radicals to form a stable diamagnetic molecule (Lin *et al*., 2004). Discolouration of the DPPH reaction from purple to yellow can be attributed to the reaction between the antioxidant molecules and the radicals. Our data showed that the leaf extract had the highest DPPH inhibitory activity with an IC_{50} value of 0.117±0.004 compared to the root extract with an IC_{50} value of 0.143±0.007. The leaf extracts were able to scavenge DPPH due to their polyphenols content. It is also known that ABTS can be oxidized by potassium persulfate to create a radical $ABTS^{+}$. The inhibitory test using the ABTS test showed that the H.B leaf extract had the highest ABTS scavenging potential with an IC_{50} value of 0.141±0.006 compared to the root with an IC_{50} value of 0.171±0.009. This ABTS scavenging ability of the extracts is due to the flavonoids, tannin, and phenol content. Nitric oxide is a mediator generated by type I and pro-inflammatory macrophages, endothelial cells, and neurons are involved in various biological processes. The increased production of NO leads to the development of several diseases (Kolodziej and

UNDER PEER REVIEW

Kiderlen, 2005; Samuel *et al*., 2010). In this study, the extracts of H. B. were able to react with oxygen and inhibit the generation of anions. The scavenging activity of these extracts can be attributed to their phenolic composition. It has been reported that the phenolic acid antioxidant activity can be due to the quantity, number, and position of the hydroxyl group in the molecule (Funatogawa *et al*., 2004). Flavonoids have also been reported to possess antioxidant properties due to their hydroxyl/group that act as the reducing agent, metal chelation, and free radical scavengers (Agati *et al*., 2012).

Ethanol extracts of *Hedranthera barteri* (H.B) root and leaves inhibited all microorganisms tested. The root extract had an inhibition zone ranging between 11.00±1.00 to 22.00±1.00 mm while the leaf extract possesses an inhibition zone that ranges between 8.00±1.00 to 18.67±1.53 mm. Both extracts were effective in inhibiting gram-positive bacteria more than gram-negative bacteria which corroborates with the similar report of Wei *et al*., 2008. The difference in the gram-positive and gram-negative sensitivity is due to the difference in their morphological makeup. Gram-negative bacteria possess an outer phospholipid membrane containing lipopolysaccharide components. While the gram-positive bacteria are susceptible due to their outer peptidoglycan layer which is not an effective permeability barrier (Kaushik et al., 2015; Lamikanra, 2010). The antimicrobial activity of these extracts can be attributed to their phytochemical constituents. Phenols, flavonoids, and tannins have been reported to possess antimicrobial activity. Antimicrobial mechanisms of phenolic compounds include permeation and destabilization of the bacterial membrane, which result in the disturbance of electron flow, proton motive force, coagulation, and leakage of its cellular content resulting in a bactericidal effect (Cushnie, 2011; Ahmad et al., 2016; Saritha et al., 2015).

In conclusion, the result of this study revealed that H.B leaf and root extracts possess high phytochemical content and antioxidant potentials which are responsible for their biological activities. Little or no research work has been carried out to separate and identify the various components of *Hedranthera barteri* secondary metabolites. Further study is recommended aiming for the isolation and identification of *Hedranthera barteri* bioactive compounds for more therapeutic studies.

UNDER PEER REVIEW

ETHICS APPROVAL AND CONSENT OF PARTICIPATE

The research work does not contain any human subject participants.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available from the corresponding author, upon request.

REFERENCE

Agati G, Azzarello E, Pollastri S and Tattini M, 2012. Flavonoids as antioxidants in plants: Location and functional significance. Plt. Sci. 196: 67-76

Ainslie JR, 1937. A listof plants used in native medicine in Nigeria.Imperial Forestry Institute, Oxford paper 7 (monograph), p. 30

Ahmad M, Khan MPZ, Mukhtar A, Zafar M, Sultana S and Jahan S, 2016. 'Ethnopharmacological survey on medicinal plants used in herbal drinks among the traditional communities of Pakistan'. Jour. Ethnopharmacol. 184: 154–186.

Akiyama H, Kazuyasu F, Yamasaki O, Oono T and Iwatsuki K. 2001. Antibacterial action of several tannins against Staphylococcus aureus. J. Antimicrob.Chemother. 48: 487-491

Bagchi M, Mimes M, Williams C, Balmoori J, Ye X, Stohs S and Bagchi D. 1999. Acute and chronic stress-induced oxidative gastrointestinal injury inratsand the protective ability of a novel grape seed proanthocyanidin extract. Nutr Res. 19: 1189–1199.

Brain, K.R. and Turner, T.D. (1975). Practical Evaluation of PhytoPharmacenticals. Wright-Scientechnica Pp. 90 – 121.

Burkill HM, 1985. The Useful Plants of West Tropical Africa. Vol. 1. Royal Botanic Gardens. Kew.Pp.16.

Cheesbrough, M. (2006). Medical laboratory manual for tropical countries. London: Tropical Health Technology.

UNDER PEER REVIEW

Chukwujekwu J, Staden CJV and Smith P. 2005. Antibacterial, anti-inflammatory, and antimalarial activities of some Nigerian medicinal plants. South Afr. J. Bot. 71 (3 & 4): 316-325.

Ciulei I. (1981). Methodology for analysis of vegetable drugs. Romania: United Nations Industrial Development Organization. pp. 17–25.

Cushnie TPT and Lamb AJ, 2011. Recent advances in understanding the antibacterial properties of flavonoids. Internat. Jour. Antimicrob. Agents. 38 (2): 99-107.

Duru CM and Mbata TI, 2010. The antimicrobial activities and phytochemical screening of ethanolic leaf extracts of *Hedrantherabarteri Hook* and *TabernaemontanapachysiphonStapf*. J Dev. Bio.Tiss. Eng. 2: 1–4.

Fabricant D.S. and Farnsworth NR. 2001. The value of plants used in traditional medicine for drug discovery. Envi. Healt. Persp. 109: 69–75.

Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano, T and Ito H. 2004. Antibacterial activity of hydrolysable tannins derived from medicinal plants against Helicobacterpylori. Microbiol. Immunol. 48(4):251-261

Harborne, J.B. (1992). Phytochemical methods. Chapman and Hall Publications, London, 7-8.

Hidayat H, Javid H, Ahmed A.H, and Zabtakhan S. 2011. Chemistry of Some species-genus Lantana. Pak. J. 43: 51-62.

Jan FA, Ishaq M, Khan S, Shakirullah M, Asim SM, Ahmad I, Mabood F. (2011). Bioaccumulation of metals in human blood in industrially contaminated area. J Environ Sci China. 23(12): 2069–2077.

Kaushik, G, Spenlehauer A, Sessions AO, Trujillo AS, Fuhrmann A, Fu Z, Venkatraman V, Pohl D, Tuler J, Wang M, Lakatta EG, Ocorr K,Bodmer R, Bernstein SI, Van EJE, Cammarato A, and Engler AJ, 2015. Vinculin network-mediated cytoskeletal remodeling regulates contractile function in the aging heart. Sci. Transl. Med. 7(292). 292 - 299.

Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M, and Khan AU. 2009. Antimicrobial activity of five herbal extracts against Multi-Drug Resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules. 14: 586-597.

UNDER PEER REVIEW

Kolodziej, H and Kiderlen A.F. 2005. Antileishmanial activity and immune-modulatory effects of tanninsandrelated compounds on Leishmania parasitized RAW 264.7 cells. Phytochemi. 66:2056–2071.

Lamikanra A, 2010. Essential Microbiology for students and practitioners of pharmacy, medicine, and microbiology. 2nd ed. Amkra books.

Lin LU, Shu-wen L, Shi-bo J and Shu-guang W. 2004. Tannin inhibits HIV-1 entry by targeting gp 41. ActaPharmacol Sin. 25(2):213-218

Liu YZ, Cao YG, Ye JQ, Wang WG, Song KJ, Wang XL, Wang CH, Li RT and Deng XM. 2009. Immunomodulatory effects of proanthocyanidin A-1 derived invitro from Rhododendronspiciferum. Fitoterapi. 81: 108–114.

Makkar HP, Siddhuraju P, Becker K. (2007). Methods in molecular biology: plant secondary metabolites. Totowa: Human Press. pp. 93–100.

Manju P, Vivek K and Jaya PY, 2011. In vitro antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann. Clin. Microbiol. Antimicrob. 10: 21.

OnasanwoSA, Chatterjee M and Palit G, 2010. Antidepressantand anxiolytic potentials of dichloromethane fraction from *Hedrantherabarteri*. Afr. J. Biomed. Res. 13: 76–81.

Onasanwo SA and Elegbe RA, 2006. Anti-nociceptive and anti-inflammatory properties of the leaf extracts of Hedrantheribarteriin rats and mice. Afr. J. Biomed. Res. 9: 109–117.

Panda S, (2012). Assay-guided comparison for enzymatic and non-enzymatic antioxidant Activities with special reference to medicinal plants. InTech. 14:382–400

Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeole PG (2007). Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian J. Pharm. Sci. 69:574-576.

Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S and Prachayasittikul V, 2008. Antimicrobial and antioxidant activity of bioreactive constituents from *Hydnophytumformicarum* Jack. Molecules. 13: 904–921.

UNDER PEER REVIEW

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26(9/10), 1231–1237.

Samuel OA, Neetu S, Samuel OB,Vaibhav M, and Gautam P, 2010. Anti-ulcer and antioxidant activities of *Hedranthera barteri*{(Hook F.) Pichon} with possible involvement of H+, K+ ATPase inhibitory activity. Indian J. Med. Res. 132: 442-449

Saritha K, Rajesh A, Manjulatha K, Setty OH and Yenugu S, 2015. Mechanism of antibacterial action of the alcoholic extracts of *Hemidesmusindicus* (L.) R. Br. ex *Schult*, *Leucasaspera* (Wild.), *Plumbagozeylanica* L., and *Tridaxprocumbens* (L.) R. Br. ex Schult. Front. Microbiol. 6:577.

Shah PM, 2005. The need for new therapeutic agents: what is in pipeline. ClinMicrobiol Infect. 11: 36-42

Siddhuraju P, Becker K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. J Agric Food Chem. 51(8):2144–2155.

Sumeet D and Lantana CL, 2008. A Noxious weed having Pivotal importance in therapeutics, Pharmainfo. 6.

Trease, G. E. and Evans, W. C. (1996). Phenols and Phenolic Glycosides in Trease and Evans Pharmacology and Bikere. Tindall, London. Pp. 832-836.

Wei LS, Musa N, Seng C, Wee W, and Shazili AM, 2008. Antimicrobial properties of tropical plants against 12 pathogenic bacteria isolated from aquatic organisms. Afri. Jour. biotech. 7(13):2257-2278

Zahid Z, Khan SW, Patel KA, Konale AG, and Lokre SS, 2010. Antimicrobial activity of essential oil of flowers of *Plumeria alba linn* (Apocynaceae). Internat. Jour. Pharm. Pharmac. Sci. 2: 155-157.

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