PHYTOCHEMICAL AND ANTIOXIDANT EVALUATION OF ETHANOLIC EXTRACTS OF *HEDRANTHERA BARTERI* ON FEMALE ALBINO WISTER RATS

ABSTRACT

Phytochemical and antioxidant evaluation of ethanolic extracts of *Hedranthera barteri* leaves is an anti-inflammatory agent which exhibits both hyposecretive and cytoprotective effects. The aim of this study is to evaluate the phytochemical screening and antioxidant capacity of *Hedranthera barteri* leaves using female white albino rats. Ethanolic extraction of *Hedranthera Barteri* leaves was done using 70% ethanol. The sub-acute toxicity of the extract to the female albino Wister rats were monitored The results of Glutathion (GSH) concentration revealed that ($p<0.05$) there was no significant difference between different concentrations of *Hedranthera barteri* and chlomphene citrate compared to the control group. Results of superoxide dismutase (SOD) showed ($p>0.05$) that there was significant difference between 100mg/kg *Hedranthera barteri* and 200mg/kg *Hedranthera Barteri* compared with the control group. Also, the result of catalase (CAT) revealed that $p>0.05$ there was a significant difference between 100mg/kg *Hedranthera Barteri* compared to the control group. However, there was $p<0.05$ no significant difference between different concentrations of *Hedranthera Barteri* and clomiphene citrate compared to the control group of malonaldehyde (MDA). The results above indicated that *Hedranthera Barteri* leaves extracts is capable of scavenging free radicals. The female albino Wister rats did not show signs of toxicity or mortality. The presence of phenols, alkaloids, tannins, flavonoids, phlobatanin and terpenoids may be responsible for the antioxidant activities of the extracts.

KEYWORDS: *Hedranthera Barteri*, phytochemicals, sub-acute toxicity, antioxidant

INTRODUCTION

"Medicinal plants from time immemorial have been used in virtually all cultures as a source of medicine. They are considered to be the backbone of traditional medicine and are widely used to treat acute and chronic diseases". (Crags and Newman, 2001). "The World Health Organization estimated that perhaps eighty percent of the inhabitants of the world rely chiefly on traditional medicines. It, therefore, approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of herbal products" (Thanabhorn *et al*., 2008). "Natural products, including plants, animals, and minerals have been the basis of the treatment of human diseases. The history of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years through scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies" (Patwardhan*et al*., 2004). "The recent experience of Covid-19 has also created an emphasize on reusability and using natural products" (Al-Amin *et al*, 2023) "Whenever we administer a chemical substance to a biological system, different types of interactions can occur and a series of dose-related responses result. In most cases, these responses are desired and useful, but there are a number of other effects which are not advantageous. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute, and chronic toxicity. Moreover, different environmental agencies are routinely observing the effects of this harmful toxic substance to phase out their uses gradually" (Al-Amin *et al*., 2020, 2021; Al-Amin, 2020).

“Additionally, Acute toxicity is involved in the estimation of LD50 the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds”. (Akhila *et al.*, 2007). “*Hedranthera barteri* (HB) pichon is a shrub of 2m high found in damp situations of the closed forest in South and eastern Nigeria, Ghana, North/West Cameroon, Congo Brazzaville, and other parts of the world. It has large white tubular flowers, with a fragrant scent and contains white latex that does not coagulate. The shape of the free bi-carpellate fruits evokes the bawdy Ijaw name meaning "goat testicles" and also the Yoruba name "dog's penis" in Nigeria” (Dalziel, 1995). HB is used as a medicinal infusion for children as a laxative (Schmelzer and gurib ,2008); the leaf decoction for dizziness (Schmelzer and gurib ,2008); the fruit for treating gonorrhoea, as a femifuge and the exudates from the leaf for treating painful tumor and inflammation and to prevent miscarriage in women. Its leaf extract has been reported to have anti-inflammatory, anti-malarial and anti-bacterial activities; *in vitro*(Chukwujekwu *et al*., 2005)and *in vivo* studies reported its anti-nociceptive and anti-inflammatory potentials(Onasanwo and elegbe, 2006). “Beta-sitosterol was suggested to be partly responsible for its anti-inflammatory pain relief” (Onasanwo *et al*., 2008). “Screening of some Nigerian medicinal plants reported HB to have antibacterial and antimalarial activities” (Chukwujekwu *et al*, 2005). “The leaf has been demonstrated to have analgesic and anti-inflammatory properties, and contain some secondary metabolites like flavonoids, alkaloids, saponins” (Onasanwo and Elegbe, 2006). *Hedranthera barteri* has been established scientifically for the management of certain nervous system challenges like dizziness (Thomas, 1967), pain and inflammation with COX-2 inhibiting activity, in vitro (Ainslie, 1937; Chukwujekwu *et al*, 2005; Onasanwo and Elegbe, 2006; Onasanwo *et al.*, 2008). “Phytochemical investigations of the HBdemonstrated the presence of pure compounds namely β-sitosterol, amataine and vobstusine. The β- sitosterol has been reported as anti-ulcerative agents in cold stress and acetic acid induced ulcer models” (Xioa *et al*., 1992). “The leaf has been demonstrated to have analgesic and anti-inflammatory properties, and contain some secondary metabolites like flavonoids, alkaloids, saponins” (Onasanwo and Elegbe, 2006).

“HB fruits have been implicated in herbal remedies against gonorrhoea, as a vermifuge and the exudates from the leaf used to suppress painful tumor and prevent miscarriages” (Ainslie, 1937). This stimulated interest to further investigate the plant to determine the Fertility properties and phytochemical composition of the leaf extracts of the plants.

MATERIALS AND METHODS

Apparatus/Laboratory Equipment

The equipment used for this study is of a measurable standard. Test tubes, Centrifuge, weighing balance, micropipette, dissecting sets and board, measuring cylinder, homogenizers, cotton wool, spatula, UV spectrophotometer, UV- visible, timer, conical flask, rotary vapor water bath, water distiller.

Materials and Disposables.

Clomiphene citrate, Methylated spirits, Lithium heparin and Fluoride oxalate vacuum blood sample bottles, plain bottles, Eppendorf bottles, cotton wool, latex gloves, vacuum blood sample bottle holder, test tubes, conical flasks, beakers, measuring cylinders, micro/automatic pipettes, petri dish, spatula, test tube racks, thermometer, Disposable pipette tips, Distilled or deionized water, Vortex mixer or equivalent, Absorbent paper, Microliter plate reader.

Sample collection

The leaves of *Hedranthera barteri* leaves used for this study were collected from Oko town in Orumba-north L.G.A, a rural suburb in Anambra state, Nigeria and authenticated at the department of Botany, University of Lagos, Akoka by a Botanist, Mr. O. O. Oyebanji, with voucher specimen (LUH-6865) and a sample was deposited in the herbarium.

Extraction of Plant Materials

Dry leaves of *Hedranthera barteri*, were grounded to a fine powder using a motor laboratory plant mill and it was extracted exhaustively with 5L of ethanol through soxhlet extractor. At the end of the extraction, ethanol was evaporated at 520C under reduced pressure in a rotary vapor. The solid samples of the extract with a percentage yield 0f 11.51% were stored in the refrigerator and the fresh extract was prepared each day of the experiment as suspensions with 5% Tween-80 as described by Azwanda,. (2015).

Determination of plant extracts (LD_{50})). The median LD_{50} was determined by a modification of Lorke's method, (1983).

Phase 1: 12 mice were groups of three animals each. Each group of animals is administered different doses (1.0, 10.0, 100.0 and 1000.0 mg/kg) of extract. Immediately after administration, aggressive behavior and fast breathing were seen in the 1000mg/kg, and two deaths occurred subsequently. After 24 hours of observation, all the mice died in the 1000mg/kg group, while no death occurred in the other group.

Phase 2: This phase involves the use of 2 animals, which are distributed into 6 groups of two animals each. The animals are administered doses within the ranges of 400- 900 mg/kg i.e. (400, 500, 600,700,800, and 900 mg/kg) of *Hedranthera barteri* leaf extract and then observed for 24 hours for behavior as well as mortality (Lorke, 1983)**.**

Table 1: Determination of the toxicity of plant extract on albino Wister rats using LD50

Groups	400	500	600	700	800	900
No. of Death	--	--	--	2	2	2

Then the LD_{50} is calculated by the formula D_0 = Highest dose that gave no mortality D_{100} = Lowest dose that produced mortality

Median $LD_{50} = \frac{D_0 + D_{100}}{} = \frac{700+600}{} = 650$mg/kg 2 2

Experimental animals

Wistar rats weighing between 120-150g were purchased from the animal house, College of Medicine, University of Lagos. They were acclimatized for 2 weeks before the experiment commenced and exposed to 12h light and 12h dark cycle. All administrations were done daily between the hours of 9.00–10.00am. The animals were grouped according to their body weight into five groups respectively. They were allowed free access to pellets and distilled water *ad libitum*.

Subacute Toxicity Studies

Experimental Design

The animals will be divided in five groups (n=5) each, all administrations will be carried out orally using graduated plastic syringes/gavages, every day for a period of 4 weeks.

Group I: Control (normal saline).

Group II: 100 mg/kg b.wt of HB Leaf extract.

Group III: 200mg/kg b.wt of HB leaf extract

Group IV: 300mg/kg b.wt of HB leaf extract

Group V: clomifiene citrate (0.0035 mg/kg b.wt).

After 4 weeks of treatment, the rats were fasted overnight and blood sample collected by ocular puncture. A portion was dispensed into potassium ethylenediaminetetraacectic acid (K+EDTA) bottle for estimation of antioxidant parameters.

PHYTOCHEMICAL SCREENING

PROCEDURE

Test for glucose

1ml of the filtrate was poured into a test tube and 2ml of fehlings solution A &B was added in a ratio 1:1. The presence of red precipitate when boiled indicated the presence of glucose.

Test for tannins

The analysis used was a method reported by Ejikeme *et al*. (2014).1ml of the filtrate plus 0.3ml drops of 1% ferric chloride was mixed together in a beaker, the appearance of grey or ash indicated the presence of tannins.

Determination of total phenols by the spectrophotometric method:

Phenol was determined according to the method described by Mac, M.D. (1963). 1ml of the filtrate plus 0.3ml drops of 1% ferric chloride was mixed together in a beaker, and the appearance of grey or ash indicated the presence of tannins.

Flavonoids

Flavonoids were determined as adopted by Sofowora. (1993) and Harborne, J.B. (1973).1ml of the filtrate and 1ml of aluminum chloride are mixed together in a beaker, the appearance of a yellow color indicated the presence of flavonoids.

Saponins

Saponin was determined as described by Ejikeme *et al*. (2014). Some quantity of the filtrate was poured into the test tube and shaken, the presence of foams which persisted for a long time indicated the presence of saponins.

Phlobatannins

It was determined according to Ejikeme *et al*. (2014). 1ml of the sample of 1% HCL in a test tube was boiled in a water bath, the appearance of a reddish precipitate indicates the presence of phlobatannins

Test for alkaloids

Alkaloids were determined as reported by Hikino *et al*.(1984). From the final result of phlobatannins, 2ml of Mayer's reagents was added. the presence of turbidity or cloudiness indicated the alkaloids.

Test for cardiac glycosides

Cardiac glycosides were determined as described by Hikino *et al*. (1984). 2ml of the filtrate was treated with 2ml of glacial acetic acid containing 1 drop of 0.1% ferric chloride and shaken. 2ml of conc. H2SSO4 was then added. The appearance of a brown ring on the interface indicates the presence of cardiac glycosides.

Test for steroids:

Steroids are determined as described by Ejikeme *et al.* (2014)**.** 2 ml of acetic anhydride was added to 1 ml of the filtrate with 2 ml H2S04. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test):

Terpenoids are carried out as described by Ejikeme *et al.* 92014). Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Determination of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increased absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3ml) contained 2.95ml 0.05 M sodium carbonate buffer pH 10.2, 0.02ml of the plasma, and 0.03ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95ml buffer 0.03ml of the substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5min, $\sum$= $4020M^{-1}$ cm^{-1}.

Determination of Catalase Activity

Catalase activity was determined according to Sinha *et al.* (1972). It was assayed colorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25°C. The reaction mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of plasma, and 0.4ml of 2M H_20_2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic were mixed in a 1:3 ratio). The specific activity of catalase was expressed as moles of H_2O_2 produced per minute per mg protein. $\sum$ = $40M^{-1}$ cm**Reduced Glutathione Determination**

The reduced glutathione (GSH) content in the plasma a non-protein sulphydryl, was estimated according to the method described by Sedlak and Lindsay. (1968). 1.0ml of the was treated with 0.5ml of Elman's reagent (19.8 of 5.5 dithiobisnitro benzo acid (DTNB mg) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M. pH 8.0). the absorbance was read at 412nm, $\sum$=1.34X10 $4M^{-1}$

$^{-1}$ cm**Determination of Malondialdehyde Activity (Lipid Peroxidation)**

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Ausi. (1978) 1.0ml of the plasma was added to 2ml of (1:1:1 ratio) TCA-TBA-HCI reagent (thiobarbituric acid 0.37%, 0.24N HCI and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at l00°C for 15mins and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for l0min. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA - complex of 1.56 x 10 5 M^{-1}

$^{-1}CM^{-1}$.

Statistical Analysis.

Results were expressed as using SPSS software and their differences was tested using mean±standard error of mean (S.E.M). The data were subjected to one-way analysis of variance (ANOVA) and $p<0.05$ was considered significant.

RESULT

Table 2: Phytochemical composition of ethanolic extract *of Hedranthera barteri* leaf

Qualitative

TEST	INDICATION
Phenol	+
Alkaloid	+
Terpenoid	+
Phlobatanin	+
Tannin	+
Saponin	-
Flavonoid	+
Cardiac Glycoside	+
Sterols	+

KEYS

+ Indicates positive

- Indicates negative

Sub-acute toxicity studies

In the sub-acute toxicity test, the extract of *Hedranthra barteri* leaves was found to be non-toxic at all the dose levels (100, 200, 300mg/ kg body weight). All the animals were free of intoxicating signs throughout the dosing period of 4 weeks. No physical changes were observed throughout the dosing period. All rats showed a significant increase in body weight compared to their initial values. However, there was no significant difference between the different treatment groups, the control and clomiphene citrate group, indicating that it did not has any adverse effects on the body weight, which is used to assess the response to the therapy of drug as shown in **(Table3)**. No mortality was observed during the whole experimental period. During the dosing period and in the last day, the quantity of food and water intake by different dose groups was found to be comparable with the control group. No abnormal deviations were observed.

Table 3: Changes in body weight of rats during the experimental period in control,clomiphene citrate and different doses of *Hedranthera barteri* leaf extract.

Groups	Day1	Day8	Day12	Day20	Day 25	Day 29
control	105.4±2.07[a]	127.00±11.25[a]	127.20±11.52[a]	125.40±12.03[a]	129.60±10.71[a]	129.90±13.32$_a$
Clo.Cit.	130.00±~~10.65s~~	137.20±19.11	142.20±14.13	138.20±15.51	141.00±15.89	140.00±16.84

100H.B	127.60±5.00	136.40±8.53	140.50±14.55	147.80±18.87	157.50±16.28	157.00±14.17
200HB	141.40±1.67	154..40±5.55	160.40±10.31	155.80±10.08	157.00±10.98	160.80±13.68
300HB	152.40±1.51	165.40±7.40	169.00±5.00	167.80±5.40	168.20±4.43	171.80±6.41

KEY:
HB= ***Hedranthera barteri***

The data were expressed as mean ±S.E.M of 5 animals. **(a)** Represents comparison with control group.

Table 4 Effect of ethanolic extract of *Hedranthera barteri leaf* on the relative organ weight of female albino wistar rats. Values were expressed as mean ± SEM (N=5) p <0.05 when compared with the control. (Sub acute toxicity studies).

Groups	**Organ Weights (g)**				
	Heart	Kidney	Liver	Ovary	Uterus
Control	0.42 ± 0.04[a]	0.63 ± 0.62 [a]	3.56 ± 0.46 [a]	0.11 ± 0.00 [a]	0.25 ± 0.02 [a]
Clomiphene citrate	0.39 ± 0.03[b]	0.56 ± 0.08 [b]	3.24 ± 0.19 [b a]	0.06 ± 0.01[b]	0.23 ± 0.00 [b]
100mg/kg (HB)	0.34 ± 0.02	0.59 ± 0.07	3.26 ± 0.42 [a]	0.10 ± 0.02	0.23 ± 0.07
200mg/kg (HB)	0.35 ± 0.01	0.54 ± 0.03	3. 5 ± 0.34 [b]	0.08 ± 0.02	0.20 ± 0.02
300mg/kg (HB)	0.32 ± 0.02	0.51 ± 0.03	3.24 ± 0.14 [a]	0.09 ± 0.01	0.23 ± 0.05

KEY:
HB=***Hedranthera barteri***
Values are expressed as mean ± SD of 5 animals.(a) represents comparison with control group and **(b)** represents comparison with clomiphene citate group.

KEY:

GSH - Reduced glutathione

1. **The effect of *Hedranthera barteri* leaf extract on reduced glutathione concentration in the liver. There is no significant difference in all groups when compared with control.**

KEY:

SOD – superoxide dismutase,

2. **The effect of *Hedranthera barteri* leaf extract on superoxide dismutase concentration in the liver. There is significant increase when compared with Control to 100mg/kg (HB) and 200mg/kg (HB)**

KEY:

CAT – catalase

3. **The effect of *Hedranthera barteri* leaf extract on catalase concentration in the liver. There is significant increase in Control group compared to 100mg/kg (HB).**

KEY:

MDA - Malonaldehyde

4. **The effect of *Hedranthera barteri* leaf extract on Malonaldehyde concentration in the liver.There is no significant difference in all groups when compared with control**

DISCUSSION

The phytochemical analysis conducted on Hedranthera *barteri* plant extract reveals (table 1) the presence of phenol, alkaloids, terpenoid, phlobatanin, tannin, flavonoids, cardiac glycoside, sterols and tannins. They are known to show medicinal activity as well as exhibiting physiological activity (Sofowora, 1993).

In the acute toxicity study, the rats did not show signs of toxicity, less behavioral changes, and mortality in the test groups as compared to the controls and clomphene citrate group when observed during 4 weeks of the sub-acute toxicity experimental period. These results showed that a single oral dose of the extract caused no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.

The body weights of control and *Hedranthera barteri* leaf-treated rats at various dose levels as presented in Table 3 did not affect the weight significantly. No physical changes were observed throughout the dosing period. All rats showed a significant increase in body weight compared to their initial values. However, there was no significant difference between the different treatment groups and the control, indicating that it did not have any adverse effects on the body weight, which is used to assess the response to the therapy of the drug and thus does not impair the normal metabolic process of the body.

The antioxidant assessment of the HB extract on the liver shows no significant difference in reduced glutathione (GSH) and malonaldehyde (MDA) levels, but a significant increase in the level of catalase and superoxide dismutase when compared with the control group, this indicates that HB plant extracts are capable of scavenging free radicals which may be as a result of alkaloids and flavonoids (Okwu, 2001), present in the plants extract.

CONCLUSION

The results of the sub-acute toxicity revealed that the leaf extracts of Hedranthera Barteri is safe for medical application since there were no significant changes in the hypertoxicity and phytochemical contents of the leaf extracts.

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