WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 12, 60-75. Research Article ISSN 2277– 7105

PHARMACOGNOSTIC PROFILING AND ANTIPYRETIC POTENTIALS OF METHANOL LEAF EXTRACT OF *VERNONIA GLABERRIMA* WELW. EX O.HOFFM (COMPOSITAE)

Odoh Uchenna Estella[1*], Aniju Kenechukwu Victor[1], Chukwuma Micheal[1], Peculiar Feenna Onyekere[1], Ezugwu Obodike Christopher[1], Ezejiofor Madu[1], Vincent chukwube[1], Okonta Oboma[1], Diovu Edith[1], Udodeme Helen[1], Felix Nwafor[1] and Osuala Felix Ngozi[2]

[1]Department of Pharmacognosy & Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. 41000. Enugu State, Nigeria.

[2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Madonna University Elele, Rivers State.

Article Received on
01 July 2022,

Revised on 22 July 2022,
Accepted on 12 August 2022

DOI: 10. 20959/wjpr202212-23768

***Corresponding Author**
Odoh Uchenna Estella
Department of Pharmacognosy & Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. 41000. Enugu State, Nigeria.

ABSTRACT

Vernonia glaberrima has been traditionally used in relieving pain and inflammatory conditions as well as in treatment of feverish and malaria conditions by local people of the South-east Nigeria. Consequently, this study aims at evaluating the phytochemical content and antipyretic properties of *Vernonia glaberrima*. The leaf of *V. glaberrima* was Soxhlet extracted with methanol. Phytochemical test was conducted to ascertain the secondary metabolites present in the extract using standard procedures. Acute toxicity (LD_{50}) of the extract on laboratory mice was estimated by following protocols of Lorke. Pyrexia was induced using 20 mg/ml of 20 % turpentine and extract was given at doses of 100, 200 and 400mg/kg. Aspirin (100 mg/kg) was used as the standard (positive control), while tween was used as the negative control. Phytochemicals present include flavonoids, tannins, alkaloids glycosides, and oils which might be responsible for the pharmacological activities. There was no sign of toxicity or death recorded at the dose of 5000 mg/kg of the extract which indicates that its safe. The antipyretic study showed that the plant extract at 400 mg/kg was able to reverse pyrexia since it was able to bring back the body temperature of the test animals to normal (37 ^{O}C) by causing an average decrease in temperature of 3.3%. Comparison of the

potency of the extract and the standard drug (Aspirin) showed that the standard drug was more potent than the extract. A 3.6% decrease in temperature was observed at a dose of 100 mg/kg of the standard drug. These results demonstrate that the plant extract has antipyretic activity.

KEYWORDS: *Vernonia glaberrima,* antipyretic, phytochemical anlysis, pyrexia, acute toxicity test.

INTRODUCTION

The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life.[1] According to WHO[2], at least 80 % of people in Africa still rely on medicinal plants for their health care. In Nigeria, and indeed the entire West Africa, herbal medicine has continued to gain momentum, some of the advantages being low cost, affordability, availability, acceptability, and apparently low toxicity.[3]

Pyrexia, also referred to as fever, is an adaptive response to a physiologic stress that is tightly regulated through endogenous pyrogenic and anti-pyretic pathways, and is associated with an increase in the hypothalamic set point. As such, the elevated body temperature in patients with pyrexia responds to pharmacologic anti-pyretic therapies such as acetaminophen. On the contrary, the elevated body temperature that occurs in hyperthermia syndromes often exceeds 41.0 °C, and reflects a pathologic increase in body temperature that is not associated with an increased hypothalamic set point. This elevated temperature in hyperthermia is therefore not responsive to pharmacologic anti-pyretic therapy. Normal body temperature is between 36.0 and 37.5 °C, with intra individual variability of 0.5–1.0 °C depending on the time of day (low in early morning, peak in early afternoon/late evening).

Vernonia (Asteraceae) is the largest genus in the tribe *Vernoniae* with close to 1000 species.[4] The genus is distributed both in the New and Old Worlds although it is to be found mostly in the tropical regions. *Vernonia* species grow in a wide range of habitats of broad ecological diversity and climatic conditions including tropical forest, marshes and wet areas, dry plains, tropical savannahs, desert xeric or dry sites and even frosty regions of eastern North America.[4] The genus is morphologically made up of annuals, herbaceous perennials, lianas, shrubs, and trees.[5] Vernonia species frequently used in ethnomedicine include,

Vernonia amygdalina, Vernonia condensata, Vernonia cineria, Vernonia guineensis and *Vernonia conferta. Vernonia galamensis* is used industrially for its seed oil contents. *Vernonia amygdalina* is the most studied member of the *Vernonia* genus as well as one of the most studied plants in Africa.[5] *Vernonia glaberrima* is a medicinal plant that is used in African traditional medicine for the treatment of skin cancer. Crude methanolic leaves extract of *Vernonia glaberrima* showing significant cytotoxic activity against cancer cell lines was subjected to chromatographic separation, purification and hydrolysis to yield four compounds namely, nonacosanoic acid, lupeol, 5-methylcoumarin-4-β-glucoside and 4-hydroxy-5-methylcoumarin. Three of the isolated compounds showed significant cytotoxic activity against human malignant melanoma (A375) cell line (IC50: 59.18 ± 2.70 to 139.53 ± 10.79 µg/mL) and human caucasian colon adenocarcinoma (HT-29) cell line (IC50: 4.22 ± 0.13 to 20.0 ± 1.91 µg/mL) while only lupeol displayed significant activity against human breast adenocarcinoma (MCF7) (IC50: 34.15 ± 2.32 µg/mL) cell line. These findings reveal the therapeutic potential of *V. glaberrima* against breast cancer, skin cancer and colorectal carcinoma, respectively and further support its traditional uses in the treatment of skin cancer. Also the quantity of 5-methylcoumarin-4-β-glucoside isolated from the *V. glaberrima* leaves was found to be very large (20.068 g), representing 0.57% of the dried plant material used for the extraction, and literature search has shown that this compound has not been isolated from any genus from the entire Tribe. The n-butanol fraction of the plant have been shown to significantly inhibit the carrageenan-induced paw oedema showing that n-butanol fraction of *V. glaberrima* to possess significant analgesic and anti-inflammatory activities thereby validating its traditional use in the treatment of pain and inflammation. The aim of this study is to validate the antipyretic action of the ethnobotanical claim of methanol extract of *V. glaberrima.*

Fig 1: Habit photography of *Vernonia glaberrima.*

MATERIALS AND METHODS

Collection, Authentication and Preparation of Plant Material

The leaves of *Vernonia glaberrima* were collected from Ibagwa Nsukka in Enugu State in December, 2020 and identified by Mr Felix Nwafor, a Plant Taxonomist at the Department of Pharmacognosy and Environmental medicines, University of Nigeria, Nsukka. The plant materials were washed to make them free from any contamination and then they were air dried at room temperature (25 ^{0}C) after which they were pulverized using a milling machine, sieved to obtain fine powder and stored in an airtight container.

Study Animals

Wistar albino rats, Rattus norvegicus, and mice were utilized in this study. The animals were housed in the Animal House, Department of Pharmacology, University of Nigeria, Nsukka. They were then acclimatized for 48 h prior to the experiment. The animals were maintained under standard laboratory conditions of ambient temperature (25°C) and with 12-hour daylight. Standard rodent pellets were used to feed the experimental animals and were supplied with water *ad libitum*. The antipyretic experiments were performed on one-month-old adult male Wistar rats.

Macroscopical Analysis

Macroscopical studies of the leaves which comprised of organoleptic characters (colour, odour, appearance, taste, shape, texture) of the fresh leaves were evaluated following standard procedures as described by Trease and Evans.[6]

Chemomicroscopy

This was conducted on the powdered root to determine the presence of starch, calcium oxalate crystals and lignified vessels following the method of Odoh *et al*.,[7] A judicious quantity of the sample was dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissues.

Test for Starch

A drop of iodine was added to a little quantity of the cleared root powder on a glass slide. A drop of glycerine was added and observed under a light Olympus Tokyo (Japan No.271961) microscope at X 400 magnification.

Test for Lignin

To a little quantity of the cleared powder on a glass slide, 1:1 phloroglucinol and concentrated hydrochloric acid was dropped and a drop of glycerine was added and observed under a light Olympus Tokyo (Japan No.271961) microscope at X100 magnification.

Test for Calcium oxalate crystals

A drop of concentrated acetic acid was added to a little quantity of the cleared root powder on a glass slide. A drop of glycerine was added and observed under an Olympus (LM) light microscope at X400 magnification.

Test for Gums and Mucilage

To a small amount of cleared root powder sample, a drop of Ruthenium red was added. Appearance of pink colouration indicated the presence of gums and mucilage.

Determination of physicochemical parameters

Analyses of physicochemical constants of the powdered leaves were determined to evaluate the quality and purity of the drug.[7, 8]

Total Ash Value

A 2 g of the powdered leaves were weighted accurately into a tarred silica crucible and incinerated at 450 ^{0}C in muffle furnace until free from carbon (became grey). The crucible containing the ash was cooled in a dessicator to constant weight. The percentage of ash was calculated with reference to air-dried drug.

% Ash content = (Weight of ash)/(Weight of powder before ashing) x 100

Acid insoluble Ash

Ash obtained after determination of total ash was boiled with 25 ml of 2 M hydrochloric acid for 5 mins and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 450 ^{0}C in a muffle furnace until free from carbon. The crucible was cooled in a dessicator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried substance.

Acid insoluble ash value (%) = (Weight of acid insoluble ash)/(Weight of crude drug taken) x 100

Water soluble ash

Similarly, ash obtained after determination of the total ash was boiled with 25 ml of water for 5 min and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and was incinerated in a muffle furnace until free from carbon. To get the water soluble ash, the weight of the insoluble matter was subtracted from the weight of the ash. The percentage of water soluble ash was then calculated with reference to the air-dried drug.

Determination of Extractive Values

Alcohol soluble extractive value

A 5 g of the powdered leaves were macerated with 100 ml alcohol for 24 h, shaking frequently during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. Thereafter, it was filtered through a filter paper. 25 ml of filtrate was evaporated in a tarred dish at 105 0C and the constant weight was noted. The percentage alcohol soluble extractive value was calculated with reference to the air-dried drug.

Water soluble extractive value

A 5 g of the powdered leaves were treated with 100 ml water with frequent shaking during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. The extracts were filtered and 25 ml of filtrate was evaporated in a tarred dish at 105 0C and weighed. The percentage of water soluble extractive was calculated with reference to the air-dried drug.

Petroleum-Ether soluble extractive value

A 5 g of the powdered leaves were macerated with 100 ml pet-ether with frequent shaking during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. The extracts were filtered and 25 ml of filtrate was evaporated in a tarred dish at 105 ^{0}C and weighed. The percentage of pet-ether soluble extractive was calculated with reference to the air-dried drug.

Chloroform Soluble Extractive Value

A 5 g of the powdered leaves were macerated with 100 ml chloroform with frequent shaking during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. The extracts were filtered and 25 ml of filtrate was evaporated in a tarred dish at 105 ^{0}C and weighed. The percentage of chloroform soluble extractive was calculated with reference to the air-dried drug.

Ethylacetate Soluble Extractive Value

A 5 g of the powdered leaves were macerated with 100 ml ethylacetate with frequent shaking during the first 6 hours with a mechanical shaker and allowed to stand for 18 hours. The extracts were filtered and 25 ml of filtrate was evaporated in a tarred dish at 105 0C and weighed. The percentage of ethylacetate soluble extractive was calculated with reference to the air-dried drug.

Moisture content

A 5 g of powdered leaves were weighed into a china dish and the contents were evenly distributed to a depth not exceeding 10 mm. The loaded plate was heated at 105 0C in a hot air oven and weighed at different time intervals until a constant weight was obtained. The difference in weight after drying and before drying (initial weight) is the moisture content.

% Moisture content = (Initial weight-final weight)/(Initial weight) X 100

Extraction

A 500g of the powdered leaves were macerated in 3 litres of methanol (Analytical grade) for 72 hours. The suspension was filtered and the resulting filtrate was evaporated to dryness over a water bath to obtain a sticky extract. The percentage yield was then determined.

Qualitative and quantitative phytochemical analysis

Qualitative and quantitative phytochemical analysis of the methanol extract was performed according to the methods of Harborne[9] and Evans.[10]

Acute Toxicity Study

The method described by Lorke[11] was employed. The route of administration was intra-peritoneal. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10, 100 and 1000 mg/kg respectively. The rats were observed continuously for behavioral, neurological, autonomic and any lethality in the first 24 hours. From the result of the first phase, three mice were used for the second phase. They were given different doses 1600, 2900 and 5000 mg/kg of the extract, and were observed for any sign of toxicity and possibly death during the 24 hours. The median lethal dose was calculated using the following formula; LD50 = √ Animals and induction of diabetes mellitus.

Determination of Antipyretic Activities

The experimental animals (25 Wistar albino rats) were divided into 5 groups of five animals each and treated as shown below.

Group 1 (Negative control) - Turpentine (20%)+tween

Group II (Positive control) - Turpentine (20%) +100 mg/kg Aspirin+ tween

Group III (Experimental group A) - Turpentine (20%) +50 mg/kg extract + tween

Group IV (Experimental group B) - Turpentine (20%) +100 mg/kg extract t+ tween

Group V (Experimental group C) - Turpentine (20%) +150 mg/kg extract + tween

The thermistor probe of a digital thermometer (model YB-009) was well lubricated with glycerine and then inserted about 3 cm into the rectum to record the rectal temperatures of the rats. The digital thermometer was calibrated against a mercury thermometer. The mean body temperature measured at 15 mints intervals over the 1 h before turpentine injection was recorded as the baseline/initial temperature. The magnitude of fever as a response to intra-peritoneal injection of 20 mg/kg body weight turpentine after 1 h was defined as 100 % fever response.

After the injection of turpentine, the temperature was recorded at hourly intervals up to the fourth hours. The comparison of rectal temperature before and after treatment and the percentage change in rectal temperature was calculated.

Statistical analysis

Then one-way analysis of variance (ANOVA) was performed to compare the means of the groups, subsequently followed by Tukey's post hoc test for pair-wise mean separations and comparisons to obtain the specific significant differences among the different groups. The results were expressed as mean ± standard error of mean (SEM) for analysis and values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Macroscopic analysis of *Vernonia glaberrima*

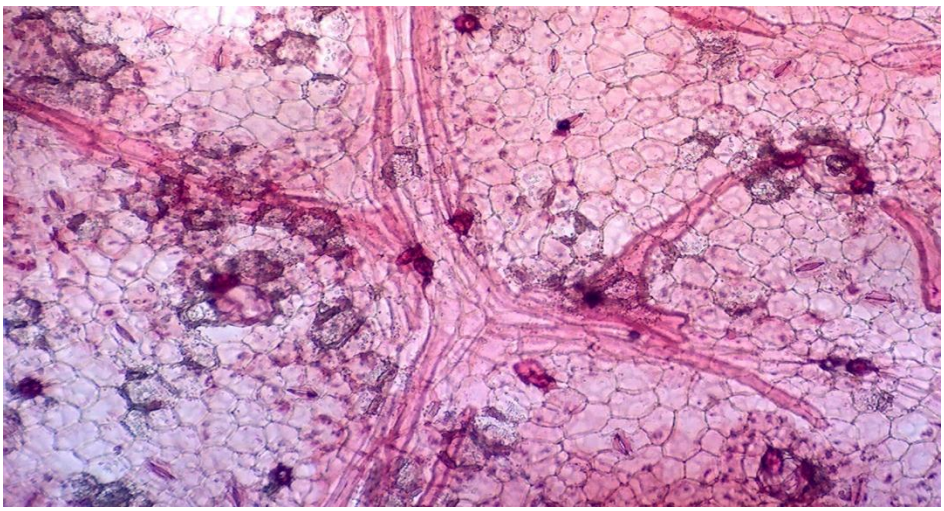
V. glaberrima is a woody under shrub. The leaves are petiolate, narrowed to base, obovate to oblanceolate, rigidly leathery, up to 10 cm. long and 3 cm. The leaf margin serrated. They are smooth with acute base and slightly acuminate apex.

Table 1: Results of the organoleptic properties of *Vernonia glaberrima.*

Character	Characteristic properties
Odour	Uncharacteristic
Colour	Green
Taste	Bitter
Texture	Smooth powder

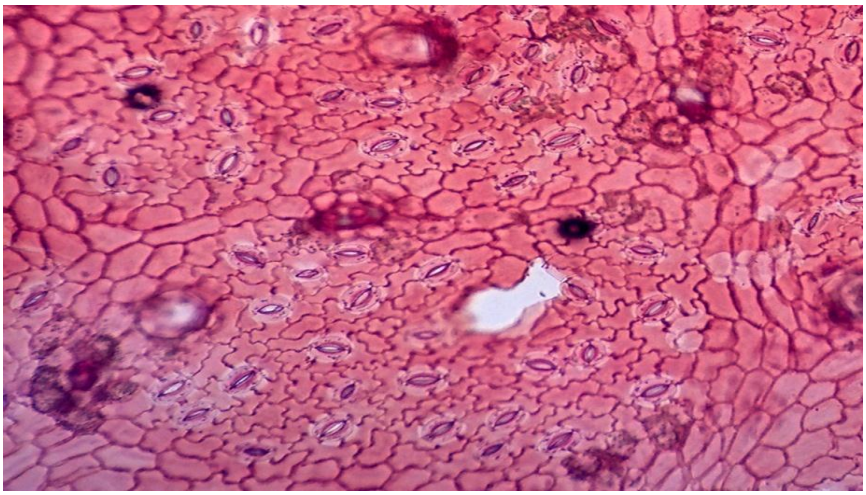
Table 2: Results of the microscopic analysis of *Vernonia glaberrima.*

Parameter	Characteristic feature
Epidermal cell	Epidermal cells are polygonal in shape on the adaxial surface and irregularly shaped with wavy/undulated anticlinal cell walls on the abaxial surfaces
Stomata type	The leaf is amphistomatic (stomata occur on both the upper and lower surface) with anomocytic type of stomata (lack of subsidiary cells; stomata surrounded directly by the epidermal cells)
Trichome	Unicellular unglandular trichomes present; scanty
Stomata density	Adaxial surface: 4.03 ± 0.11 mm^{-2} ; Abaxial surface: 14.331 ± 0.18 mm^{-2}
Stomata length	Adaxial surface: 17.90 ± 0.35 µm; Abaxial surface: 20.47 ± 0.63 µm
Stomata index	Adaxial surface: 4.17 ± 0.06 %; Abaxial surface: 12.27 ± 0.18
Stomata width	Adaxial surface: 11.27 ± 0.35 µm; Abaxial surface: 11.82 ± 0.63 µm
Stomata size	Adaxial surface: 291.00 ± 3.61 $µm^2$; Abaxial surface: 321.67 ± 1.20 $µm^2$
Palisade ratio	8.25 ± 0.01 mm^{-2}



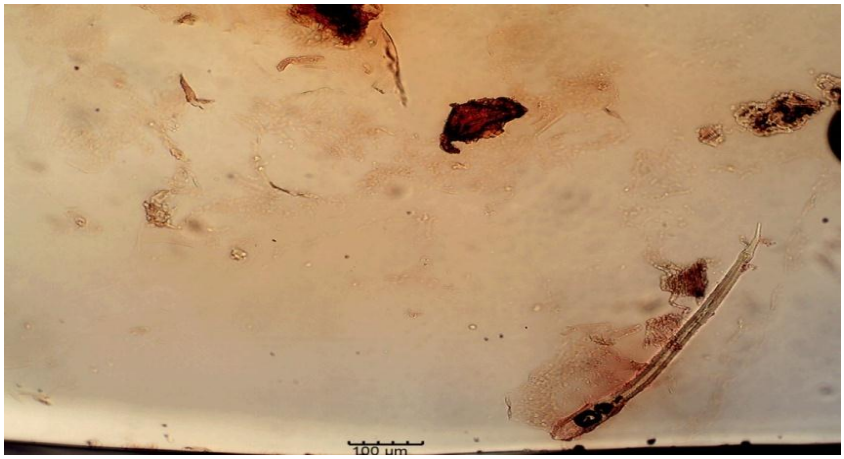
x100

Fig 2: Photomicrograph of the Adaxial surface of the leaf of *Vernonia glaberrima* showing epidermal cells and scanty stomata.



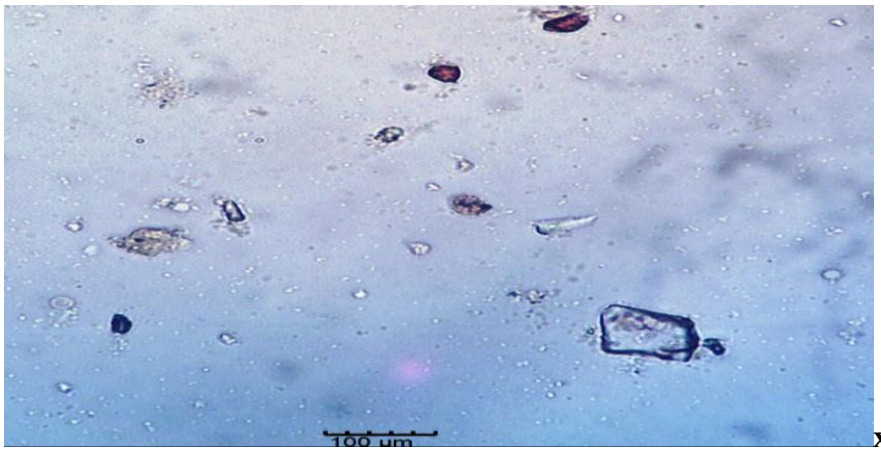
x100

Figure 3: Photomicrograph of the abaxial surface of the leaf of *Vernonia glaberrima* showing epidermal cells and abundant stomata.



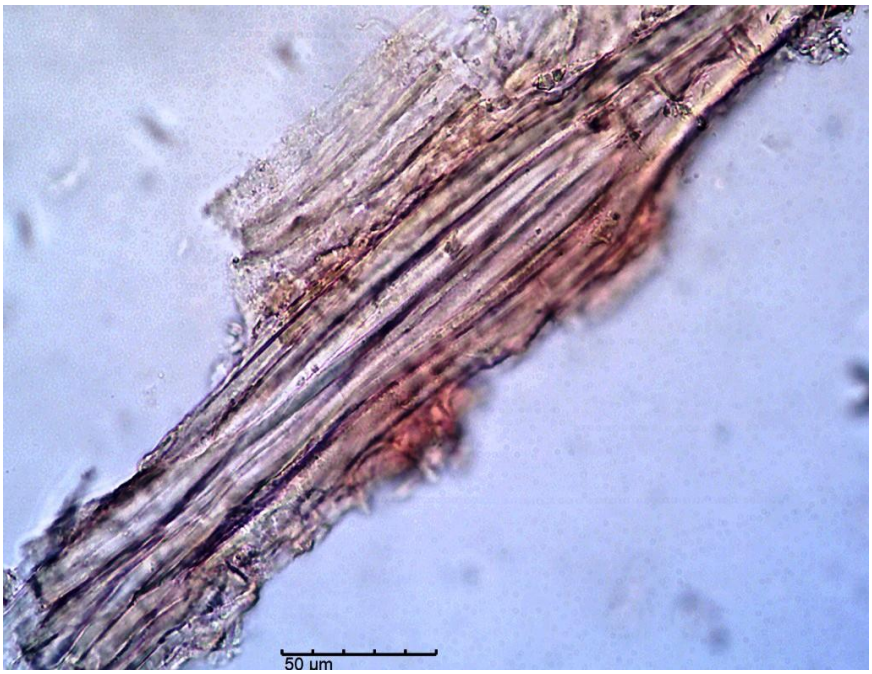
x400

Figure 4: Photomicrograph of the leaf powder of *V. glaberrima* showing unicellular trichome.



x400

Figure 5: Photomicrograph of the leaf of *Vernonia glaberrima* showing the crystals of calcium oxalate.



x400

Figure 6: Photomicrograph of the leaf of *V.glaberrima* showing lignified vessels and fiber element.

Table 3: Results of chemomicsopy of powdered leaves of *V. glaberrima.*

Parameter	Reagent(s)	Result
Starch grains	Iodine solutuion	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism and amorphous shape
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent

Table 4: Result of the physiochemical parameters of *V. glaberrima.*

Parameter	Value (%)
Moisture content	8.63 ± 0.30
Percentage yield	12.84 ± 0.25
Total ash	8.86 ± 0.01
Acid insoluble	3.44 ± 0.01
Water soluble	4.18 ± 0.25
Hexane soluble extractive	8.29 ± 0.02
Ethylacetate soluble extractive	8.74 ± 0.33
Alcohol soluble extractive	10.22 ± 0.82

Values are mean ± SEM, n = 4

Phytochemical analysis of *Vernonia glaberrima* leaves

Table 8 below shows the result of various phytochemical tests that were carried out, the qualitative and quantitative result.. Alkaloids, glycosides, terpenoids, flavonoids, saponins, reducing sugars, carbohydrates and tannins were found to be present in varying degrees.

Table 5: Results of the Phytochemical analysis of *Vernonia glaberrima* leaves.

Phytochemical	Qualitative screening	Quantitative (mg/g)
Alkaloids	+	20.5 ± 1.5
Reducing sugars	+	16.65 ± 0.22
Saponins	+	80.98 ± 2.16
Glycosides	+	9.73 ± 0.04
Flavonoids	+	26.7 ± 0.39
Tannins	+	35.00 ± 0.28
Terpenoids	+	7.9 ± 0.05
Phenolics	+	57.17 ± 0.18

Values are mean ± SEM, n=4

Acute toxicity studies

There was no death recorded for the acute toxicity test of methanol extract of the leaf *Vernonia glaberrima* at the dose of 5000 mg/kg which shows that the extract is non-toxic.

Antipyretic activity of the leaf of *Vernonia glaberrima*

There was a 3.0, 3.1, 2.7, 2.7 and 3.0 % temperature increase for groups treated with 100, 200 and 400 mg/kg, control and standard respectively, 1hour after 20 % (20 mg/kg) of turpentine was administered to the test animals (Table 6).

Table 6: Result of antipyretic activity of the methanol extract of *Vernonia glaberima.*

Treatment	Dose (mg/kg)	Pre treatment temperature	Post treatment temperature	Temperature 1 h after drug treatment	Temperature 2 h after treatment	Temperature 3 h after treatment
Extract	100	37.2200 ±o.22	38.4400 ±0.12	37.6000 ±0.28	38.2400 ±0.24	38.0200 ±0.22
Extract	200	36.9800 ±0.08	38.1400 ±0.23	37.5600 ±0.18	38.2400 ±0.24	38.0200 ±0.22
Extract	400	37.1600 ±0.24	38.1800 ±0.14	37.8000 ±0.29	37.0800 ±0.25	37.1800 ±0.22
Aspirin	100	37.1600 ±0.16	38.1800 ±0.12	36.8400 ±0.25	36.5600 ±0.18	36.8400 ± 0.22
Neg. cont	5	36.9600 ±0.21	38.6600 ±0.14	38.7000 ±0.25	39.2000 ±0.23	38.6800 ±0.18

Table 7: Result of Percentage Decrease in Temperature after drug administration.

Treatment	Dose (mg/kg)	% temperature 1 h after induction	% temperature 1 h after drug administration	% temperature 2 h after drug administration	% temperature 3 h after drug administration
	100	3.27	2.19	0.52	1.09
	200	2.74	1.52	0.26	0.31
	400	2.77	1.00	2.88	2.62
Negative control (5ml/kg)	-	2.74	3.51	4.24	3.51
Standard (Aspirin)	100	4.6	-0.1	-1.4	-0.05

DISCUSION

The identification, examination and evaluation of the leaves of *Vernonia glaberrima* were carried out and the characteristics associated with the leaves of the plant was determined by the various analysis and tests performed.

The phytochemical analysis carried out on the leaves of *Vernonia glaberrima* revealed the presence of various secondary metabolites which include alkaloids (known for a lot of physiological effects on the human body like analgesic, antimalarial, antispasmodic, psychostimulant effects), glycosides, steroids (hormonal and some effects on fertility), terpenoids, flavonoids (polyphenolic compounds known to possess antioxidant, anticarcinogenic, antimicrobial properties), saponins (known for their haemolytic, cholesterol lowering and antioxidant properties), reducing sugars, carbohydrates (which can serve as bulking agents in pharmaceutical formulations), tannins, anthocyanins and anthraquinone glycosides were present. Flavonoids, terpenoids, saponins are collectively known as phenolic compounds.[12] The presence of tannins, flavonoids, oils, terpenes is indicative of its antipyretic activity.[13] The macroscopical analysis revealed a bitter, green, smooth fine powder of the leaves of *V. glaberrima.* Chemomicroscopy may be used to distinguish cellular structures. The chemomicroscopy analysis indicated the presence of lignin, calcium oxalate crystals, starch and fibres and the absence of tannins. The microscopy of the powdered root revealed the presence of bundles of fibres, cork cells, prisms of calcium oxalate crystals, lignified tissues, oil bodies while the microscopy of the transverse section revealed.

The percentage total ash value which is the total amount of material remaining after ignition at 450 ^{0}C was calculated to be 8.86 %, this represents the ash from the plant tissue and other extraneous matter adhering to the surface of the plant, it sets a standard for the level of soil and mineral matter. The percentage water soluble ash which detects water exhausted drugs

was gotten to be 4.18 while the percentage acid insoluble ash indicating the amount of silica present, especially as sand and siliceous earth was calculated to be 3.44 %. Therefore, a sample of leaf of *Vernonia glaberrima* with percentage total ash, percentage water soluble ash and percentage acid insoluble ash more than 8.86, 4.18 and 3.44 % respectively indicates the possible presence of adulterants.

The percentage moisture content of the sample drug was gotten as 8.63. High moisture content of a drug sample results to a higher risk of deterioration of the drug sample by microorganisms; due to this lower percentage moisture contents are preferred for drug samples.

Extractive values determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. The alcohol soluble (methanol & ethanol) extractives, ethylacetate soluble extractives, water soluble extractives, and hexane soluble extractive were gotten as 10.22, 8.74, and 8.29%, respectively. These extractive values indicate that there are more polar (hydrophilic) compounds than non-polar (hydrophobic) compounds in the leaves of *Vernonia glaberrima*. The alcohol soluble extractive has the greatest value, which indicates that the constituents of the leaf of *Vernonia glaberrima* will be most extracted and soluble in alcohol. Therefore, alcohol seems to be the best solvent for the extraction of the leaf of *Vernonia glaberrima.*

The acute toxicity studies of the methanol extract of the plant shows no death at 5000 mg/kg. Although there were slight behavioral changes witnessed at doses of 2900 mg/kg and above which is indicative of some level of toxicity though not lethal.

The antipyretic activity data obtained in the present study indicated that the methanol extract at the doses used significantly ($p>0.05$) decreased the turpentine induced anal temperature in the rats. This suggests that the extract just like the standard (Aspirin) possess inhibitory activity against the arachidonic acid pathway. Inhibiting the arachidonic acid pathway, and specifically the activity of cyclo-oxygenase, may result in the reduction of temperature level within the hypothalamus. The presence of the phytochemicals or secondary metabolites such as tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, alkaloids, steroids and terpenes could be the reason for the activity of *V. glaberrima* leaf extract. These chemical agents have been implicated in so many literatures of having medicinal relevance and properties such as reducing or relieving fever or pyrexia, blocking

inflammatory acting on pain or analgesia, having antioxidant properties, antimicrobial activities.[14,15]

The result showed that the plant extract at 400 mg/kg was able to reverse pyrexia because it was able to bring back the body temperature of the test animals to normal (37^{o}C).by causing an average decrease in temperature of 3.3%. Comparison of the potency of the extract and the standard drug (Aspirin) showed that the standard drug was more potent than the extract because, a higher antipyretic action of an average of 3.6 % decrease in temperature was observed at a lower dose of 100 mg/kg of the standard drug more than the 3.3 % decrease in temperature observed in 400 mg/kg of the plant extract. These results demonstrate that the plant extract has antipyretic activity thus validating the ethnobotanical claim.

CONCLUSION

The phytochemical and antipyretic activity of *Vernonia glaberrima* leaf extract as studied in the work shows that it contains secondary metabolites and the methanol extract has a dose-dependent antipyretic effect thus validating its ethnobotanical claim.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

REFERENCES

1. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Reviews, 2012; 6(11): 1–5. https://doi.org/10.4103/0973-7847.95849.
2. WHO. Traditional Medicine Strategy 2002-2005. Geneva:: WHO Press.
3. Ivanova N, Gugleva V, Dobreva M, Pehlivanov I, Stefanov S, Andonova V. We are IntechOpen, the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech, I (tourism), 2016; 13.
4. Keeley SB, Jones SB. (1979). Distribution of pollen types in vernonia (vernonieae:Compositea). Systematic Botany, 1979; 4: 195-202.
5. Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). Journal of Ethnopharmacology, 2013; 146(3): 681–723. https://doi.org/10.1016/j.jep.2013.01.040.
6. Trease GE, Evans WC. Textbook of Pharmacognosy, Edited by Evans, W. C. 13th Edition, ELBS, Great Britain, 1989; 19-672.

7. Odoh UE, Ezugwu CO, Inya-Agha SI, Ugwuoke CEC, Ezejiofor M, Ezea SC. Techniques in Macroscopical and Microscopical Examinations of Crude Drugs. Paschal Communications, Enugu, Nigeria, 2011.
8. Kokate CK. Practical pharmacognosy. 1st ed. New Delhi: Vallabh prakashan, 1986.
9. Harbourne JB. Textbook of Phytochemical Methods and Guide to Modern Technique of Plant Analysis 2nd edn., Chapman and Hall Ltd. London, 1973; p. 279.
10. Evans WC. Trease and Evan pharmacognosy. 14th edition, London: W. B. Saunders, 2002; 33.
11. Lorke DA. new approach to practical acute toxicity testing. Arch. Toxicol, 1983; 54(4): 257-287. PMID: 6667118.
12. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Sci. World J, 2013; 2013162750. doi: 10.1155/2013/162750.
13. Shilpi JA, Uddin SJ. Analgesic and antipyretic natural products. In Annual Reports in Medicinal Chemistry (1st ed., Vol. 55). Elsevier Inc, 2020 https://doi.org/10.1016/bs.armc.2020.03.003.
14. Bouyahya A, Dakka N, Et-Touys A, Abrini J, Bakri Y. Medicinal plant products targeting quorum sensing for combating bacterial infections. Asian Pac J Trop Med, 2017; 10: 729–43.
15. Jazia S, IIhem R, Anouar F, Sana F, Wided B, Najla H, Abdelfattah E, Mohamed SA. Chemical Composition and Antioxidant, Analgesic, and Anti-Inflammatory Effects of Methanolic Extract of *Euphorbia retusa* in Mice. Pain Res Manag, 2018; 4838413, doi: 10.1155/2018/4838413.