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Phytochemical analysis, analgesic and antipyretic properties of ethanolic leaf extract of *Vernonia amygdalina* Del.

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ABSTRACT

Introduction: *Vernonia amygdalina* Del. has been traditionally used in relieving pain and inflammatory conditions as well as in treatment of feverish conditions by local people of the North-east Nigeria. Consequently this study aims at evaluating the phytochemical content, antipyretic and analgesic properties of *V. amygdalina* (biter leaf).
Methods: The leaf of *V. amygdalina* was soxhlet extracted with ethanol and sequentially partitioned using solvent of different polarities. 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 rats was estimated by following protocols of Lorke. The antinociceptive activity of the ethanolic extract was also evaluated using acetic acid induced pain and hot plate method.
Results: The results revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, alkaloids, steroids and terpenes. Anthraquinones were absent in the extract. The intraperitoneal LD_{50} was found to be 3721 mg/kg. On administration of 5000 mg/kg dose of the extract via oral route, there was no dead. The extract demonstrated significant antinociceptive activities as 36.0 ± 0.81, 43.8 ± 0.11 and 52.8±0.37 (Mean number of writhings) respectively for the doses 600, 400 and 200 mg/kg i.p.) as compared to the control (60.0 ± 0.11). High dose of 400 mg/kg significantly reduced rectal temperature ($P < 0.05$)
Conclusion: These results demonstrated the medicinal potentiality of *V. amygdalina* and might be used as analgesic, and antipyretic agent. Phytochemicals found in such as flavonoids, tannins, alkaloids and steroids seem to be implicated in having such pharmacological activities.

Implication for health policy/practice/research/medical education:
The ethanolic extract from *V. amygdalina* has phytochemicals such as flavonoids, tannins, alkaloids and steroids and the extract is non-toxic. It has pharmacological properties such as analgesic and antipyretic activities which could be due to the presence of secondary metabolites.

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Introduction

For long, plants have been utilized by man in treatment of various ailments and diseases. Medicinal plants are important source of pharmacological and toxicological value. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (1). Biter leaf (*Vernonia amygdalina*) is an indigenous African plant species, which grows in most parts of sub-Saharan Africa. *Vernonia* is a genus of about 1000 species of forbs and shrubs of which *V. amygdalina* is the most prominent specie and one of the pantropical tribes of the family Asteraceae (2). It grows predominantly in tropical areas of Africa, especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa (3). Traditional medicinal plants play an important role in the medical system in Nigeria; however, plant materials remain an important resource to combat serious diseases in the world. It has been reported that *V. amygdalina* has many medicinal properties such as antihelminth and laxative properties and possess cytotoxic effects towards human carcinoma cells, it is effective against

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gastrointestinal disorders and has antimicrobial and antiparasitic activities.

Vernonia amygdalina has been reported to be effective against amoebic dysentery, gastrointestinal disorders, hepatotoxicities and diabetes mellitus (4). The reported bioactive compounds of *V. amygdalina* are saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones (5), edotides (4), tannins (6) and sesquiterpene lactone (7). These compounds have been shown to be responsible for the observed biological activities. For example, the antiplasmodial (antimalarial) activity of its extracts has been related to the presence of flavonoids, saponins and alkaloids (7).

Also, there has been report that *V. amygdalina* was used in relieving pain and inflammatory conditions as well as in treatment of feverish conditions by the local people, Numan in Adamawa state and Bali in Taraba state of the North-east Nigeria. Consequently, scientific basis for the folkloric claimed is needed to be established. So the aim of this research was to verify some pharmacological properties of *V. amygdalina* (obtained from Adamawa state of Nigeria), such as analgesic and antipyretic activities as well evaluating its phytochemical composition.

Materials and Methods

Sample collection and identification

The leaves of *V. amygdalina* (biter leaf) was collected in Numan Local Government Area of Borno state of Nigeria. The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S. S. Sanusi of the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The herbarium specimen with a voucher number 666b was deposited at the Post Graduate Research Laboratory, Department of Chemistry, University of Maiduguri, Nigeria. The leaf of *V. amygdalina* (biter leaf) was cleaned and air-dried in the laboratory. 800 g of the sample was pulverized into a coarse powder using mortar and pestle.

Extraction and phytochemical analysis

The weighed powdered air-dried sample (800 g) was soxhlet extracted with ethanol and partitioned sequentially with chloroform, ethyl acetate, and n-butanol. The extract obtained was concentrated to dryness in vacuo at 40°C using a rotary evaporator. The extract concentrate was labeled and the percentage yields were calculated in $^{w}/_{w}$. The ethanolic extract was subjected to qualitative chemical screening for identification of phytochemicals such as flavonoids, alkaloids, sterols, triterpenes, saponins, anthracenosides, tannins, polyuronides, emodol, etc as described by previous studies (8-10).

Pharmacological evaluations

Animals

Eighty Albino rats of both sexes weighing 100-150 g, thirty mice of both sexes weighing 20-30 g, and 2 male rabbits were used for the experiments. They were obtained from a colony of rats maintained at the animal house of the Institute of Trypanosomiasis Research Vom, Nigeria. They were housed in clean cages and had access to feeds (ECWA Feeds) and water *ad libitum*. They were allowed to acclimatize for 2 weeks in the Veterinary Physiology, Pharmacology and Biochemistry Laboratory before the commencement of the study. All the animals were handled according to the guiding principles of biomedical research involving animals (11) as certified (VPPB, 069) by the ethics committee of Faculty of Veterinary Medicine, University of Maiduguri.

Acute toxicity evaluation (LD_{50})

The acute toxicity (LD_{50}) value of the crude ethanolic leaf extract was determined using standard procedures (12). In this study two different routes of administration were considered, that were oral and intra-peritoneal. In phase I the rats were divided into 3 groups of three rats for each route and then treated with crude ethanolic extract at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally and orally and observed for 24 hours for mortality. In the phase II, 4 rats for each route were divided into 4 groups of one rat each and the ethanolic extract was administered at various doses to be determined after the phase one. The final LD_{50} value was calculated as the geometric mean of the surviving group and the death group in the second phase.

Effect of extract on acetic acid-induced writhing in Mice

Adult mice weighing 20-30 g were used for the experiment. The abdominal constriction resulting from i.p. injection of acetic acid (0.6% v/v) consisting of a contraction of abdominal muscle, together with a stretching of hind limbs, was carried out according to the procedure previously described (13-16). The animals were divided into 4 groups of 6 mice per cage. Group was served as control while the groups 2-4 were pretreated with graded doses (mg/kg) of the extract (i.p.). Thirty minutes later, acetic acid was administered. The number of writhing movements was counted for 30 minutes.

Effect of extract on thermally-induced pain in mice

The effect of the extract on hot plate-induced test was investigated in mice (20-30 g). In this experiment, the electric hot plate was maintained at 45±1°C (as it was connected to the thermostatically controlled water bath). Animals were placed into a glass beaker of 50 cm^3 diameter on the heated surface and the time(s) between placement and the shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30 seconds cut-off was used to prevent tissue damage. The animals were divided into 4 groups of 5 mice per cage. Group I was served as control and received only water. Groups 2-4 were pretreated with graded doses (g/kg) (i.p.) of the extract. Ten minutes prior to their being placed on the hot plate (13,14)

Antipyretic evaluation

The procedure described by Besra et al (17) was

adopted. Thirty-five rats of both sexes were used for this experiment. The rats were given a solution of 20% Brewer's yeast (1 mL) subcutaneously at the neck region and fasted to induce fever. About 18 hours thereafter, their body weight and temperature were recorded. Twenty rats with elevated temperature were selected and divided into 4 groups of 5 rats each. Group A served as the control and was given distilled water only; while groups B, C and D were given various doses of 100 mg/kg, 200 mg/kg and 300 mg/kg of the methanolic extract. The body temperature of the rats in various groups was determined at 1 hour interval following the administration of the ethanolic extract using a probe thermometer.

Statistical analysis

Data were expressed as mean ± SD and mean ± SEM. Test of significance between control and treatment means was carried out by analysis of variance (ANOVA) using GraphPad software.

Results

The extracts concentrate yield of ethanol was 17% $^{w}/_{w}$. Phytochemical analysis of the ethanolic leaves extract of *V. amygdalina* (biter leaf) revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, alkaloids, steroids and terpenes. Anthraquinones were absent in the extract (Table 1).

Acute toxicity

The intraperitoneal LD_{50} was found to be 3721 mg/kg. On administration of 5000 mg/kg dose of the extract via oral route, there was no dead, hence the extract was considered safe to be administered.

Analgesic evaluations

All the doses were found to show significant ($P<0.05$) analgesic activities (Table 2). The ethanolic extract demonstrated significant antinociceptive activities as 36.0 ± 0.81, 43.8 ± 0.11 and 52.8 ± 0.37°C (Mean number of writhings) respectively for the doses of 600, 400 and 200 mg/kg i.p. as compared to the control (60.0 ± 0.12) ($P<0.05$). The activity was more pronounced at a high dose of 600 mg/kg which give the highness percentage of 43.2% inhibition of the abdominal constriction induced by acetic. Also, the extract appeared to induce an increase in pain threshold to Eddy's hot plate when compared to control (Table 3) ($P<0.05$).

Antipyretic evaluations

The ethanolic leaf extract of *V. amygdalina* possessed antipyretic properties (Table 4). High dose of 400 mg/kg significantly reduced rectal temperature from 40.22 ± 0.03°C after 18 hours of induction of fever by yeast to 38.10 ± 0.12°C after one hour on administration of the extract. Four hours later the rectal temperature reduced to 37.21 ± 0.23°C. Also aspirin (60 mg/kg) reduced the rectal temperature from 40.80 ± 0.13 on 18 hours of fever induction to 38.09 ± 0.10°C after just one hour and subsequently to 37.20 ± 0.21 after 5 hours. The effects of the aspirin (60 mg/kg) was high as compared to the extract.

Table 1. Phytochemical analysis of ethanolic leaf extract of *Vernonia amygdalina*

Class of chemical components	Results
Test of Alkaloids	
Dragendorff reagent	+
Mayer's reagent	+
Test for flavonoid	
Shinoda test	+
Lead acetate test	+
Sodium hydroxide test	+
Ferric chlorides test	+
Test for carbohydrate	
General Test (Molish test)	+
Test of monosaccharide	–
Test for reducing sugar (Fehling test)	+
Combine reducing sugar test	+
Test for ketoses	+
Test for pentose	–
Test for tannins	
Ferric chloride	+
Lead acetate	+
Hydrochloric acid test	+
Test for free Anthraquinones (Bontrase)	–
Test for combined anthraquinone	–
Test for cardio active glycoside	
Salkowski test	+
Liebermann Burchard test	+
Terpenoid test	+
Test for soluble starch	+
Test for phlobatannins	+
Test for saponins	
Frothing test	+
Fehling test	+

– Absen; + Present in low concentration.

Table 2. Effects of stem bark ethanolic extract of *Vernonia amygdalina* of acetic acid (0.6 %) induced writhes in mice

Treatment	Dose (mg/kg)	No. of Writhes Mean ± SEM	Protection (%)
Control (DW)		52.8 ± 0.37	0.00
Extract + AA	200	55.8 ± 0.37*	15.0
Extract + AA	400	43.8 ± 0.11*	30.1
Extract + AA	600	36.0 ±0.81*	40.9
Pentazocine + AA	60	4.60 ± 0.40*	93.0

Abbreviations: DW, distilled water; AA, acetic acid.
*P < 0.05 is significantly different from the control.

Discussion

The presence of the phytochemicals or phytonutrients or secondary metabolites such as tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside,

Table 3. Effects of ethanolic extract of *Vernonia amygdalina* of thermal nociception in mice

Treatment	Dose (mg/kg)	Time of pad licking or jumping (s)	No. of rats used
Control (DW)		1.90 ± 0.09	5
Extract	100	4.40 ± 0.55*	5
Extract	200	6.80 ± 0.44*	5
Extract	400	8.60 ± 0.55**	5
Pentazocine	20	10.0 ±0.54**	5

Abbreviation: DW, distilled water.
*P < 0.05 is significantly different from the control.
**P < 0.001 is significantly different from the control.

flavonoids, alkaloids, steroids and terpenes could be the reason why *V. amygdalina* leaf has pharmacological activities. These chemical agents have been implicated in so many literatures of having medicinal relevance and properties such and reducing or relieving fever or pyrexia, blocking inflammatory acting on pain or analgesia, having antioxidant properties, antimicrobial activities, etc (13). As shown in Table 1, *V. amygdalina* has all the phytochemicals that have such activities listed and this might be the reason why it is used in local pharmacopoeia. According the classification of Clarke and Clarke (18), substances that have an intraperitoneal LD_{50} between 50 and 500 mg/kg are considered toxic. The fact that no dead was obtained with high doses tested and hence we could not get LD_{50}, is might be indicated that the extract could be administered with some degree of safety both on the oral and intraperitoneal routes.

The ethanolic extract demonstrated significant antinociceptive activities as 36.0 ± 0.81, 43.8 ± 0.11 and 52.8±0.37 (Mean number of writhings), respectively for the doses 600, 400 and 200 mg/kg i.p. as compared to the control (60.0 ± 0.12). The activity was more pronounced at a high dose of 600 mg/kg which give the highness percentage of 43.2% inhibition of the abdominal constriction induced by acetic acid. This was found to be significantly lower than pentazocine (20 mg/kg) in the extent to which the writhing or stretching induced by acetic acid was reduced.

All the doses were found to show significant (P < 0.05) analgesic activity**.** The analgesic activity may be due to presence of bioactive compounds such as flavonoids and tannins that are present in the extract. Flavonoids and tannins possessed analgesic and/or anti-inflammatory activities (19). Also, the extract appeared to induce an increase in pain threshold to Eddy's hot plate when compared to control group. The various extract doses (200, 400 and 600 mg/kg) significantly increased the time of pad licking. Also pentazocine significantly increased the time of pad licking with superior effect compared to the extract.

The ethanolic leaf extract of *V. amygdalina* possessed antipyretic properties. Also aspirin (60 mg/kg) reduced the rectal temperature. Also, the extract efficacy in rectal temperature reduction was higher than the control. This observation supports the claim of local people in folkloric treatment of fever. Fever may be a result of infection or one of the consequence of tissue damage or other diseases state (16). Reduction of elevated body temperature may be due to presence of flavonoid compounds in the extract as some flavonoidal compounds are predominant inhibitors of cyclooxygenase and lipoxygenase (10).

Conclusion

Extraction of *V. amygdalina* yielded 17% $^{w}/_{w}$ which is relatively high. Phytochemical analysis of the ethanolic leaf extract of revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, alkaloids, steroids and terpenes. The LD_{50} showed that the extract could be administered with some degree of safety. The extract has analgesic and antipyretic activities when tested on laboratory animals. These pharmacological properties observed could be due the presence of the secondary metabolites present in the extract and thus *V. amygdalina* leaf might be used as an analgesic and antipyretic remedy.

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Table 4. Effect of ethanolic extract of *Vernonia amygdalina* against Brewer's yeast induced hyperpyrexia in rats

Treatment groups (mg/kg)	Rectal temperature (man ±SD)					
	After yeast injection		After drugs administration			
i.p.	0 (min)		60 (min)	120 (min)	180 (min)	240 (min)
Control (DW)	37.0	39.3 ± 0.11	39.4 ± 0.12	38.1 ± 0.44	38.00 ± 0.22	37.9 ± 0.01
100	37.1	40.81 ± 0.34	40.11 ± 0.42	39.72 ± 0.12	38.90 ± 0.11	37.7 ± 0.24*
200	37.5	40.47 ± 0.55	39.12 ± 0.19*	38.80 ± 0.13	37.92 ± 0.12	37.50 ± 0.15*
300	37.5	39.91 ± 0.23	38.56 ± 0.17*	37.11 ± 0.24 *	37.14 ± 21*	37.0 2 ± 0.10*
400	37.5	40.22 ± 0.03	38.10 ± 0.13	38.00 ± 0.80*	37.9 ± 0.23 *	37.2 ± 0.23*
Aspirin (60 mg/kg)	37.5	40.80 ± 0.13	38.09 ± 0.10*	37.82 ± 0.14*	37.52 ± 0.67*	37.21 ± 0.23*

*P < 0.05 significant compared to control.

Authors' contributions

This research was carried out in collaboration with four authors. MAT is the principal author, conceived the idea, designed the methodology and the literature. GTM and YTA assisted in carrying out the pharmacological evaluations. TBA collected the sample, played key role in extraction of the samples and the phytochemical test. FIA assisted in interpretation of results and supervision of the research. All the authors have gone through the manuscript and approved it.

Conflict of interests

None.

Ethical considerations

Ethical issues such as plagiarism, falsification and fabrication of results, double publication etc were observed and strictly adhered to government.

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