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Phytochemical, anti-inflammatory, analgesic and antipyretic properties of leaf extracts of *Amaranthus viridis* L. (Amaranthaceae)

Daniel Christopher KAGARU[1*], Dalen Gwatau DAFAM[1], Taiwo Emmanuel ALEMIKA[2]

[1]*Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, P.M.B. 2084, Jos, Nigeria.* [2]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, P.M.B. 2084, Jos, Nigeria.*

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Abstract

Inflammation, pain and fever are prevalent and debilitating conditions associated with diseases, infections and injuries, affecting a significant global population. Orthodox medicines used for treatments are known to induce toxic adverse effects, including gastrointestinal ulcers, and dependence. Problems of adulteration, substitution and false claims also exist for medicinal plants like *Amaranthus viridis* L., due to insufficient scientific data. Previously, crude extracts of the plant were used to demonstrate the anti-inflammatory, analgesic and antipyretic properties, without room to understand the probable responsible constituents. The plant material was successively extracted with hexane, ethyl acetate, methanol and water, followed by determinations of their yields and phytochemical constituents. The extracts were tested for acute toxicity prior to preliminary *in vivo* testing of their anti-inflammatory, analgesic and antipyretic properties in mice and rats, following standard procedures. Yields of extracts from 1000 mg of the leaf powder were highest in water, followed by hexane, methanol, and ethyl acetate, while the phytochemical constituents included steroids, cardiac glycosides, flavonoids, alkaloids, saponins, tannins, and carbohydrates. The extracts were safe and significantly active compared to standards. The results provide further scientific data for the traditional uses of *A. viridis* leaf in the treatment of inflammation, pain, and fever.

Keywords: *Amaranthus viridis*; Analgesic activity; Anti-inflammatory activity; Antipyretic activity; Extracts

INTRODUCTION

From time immemorial people have depended on the traditional medicinal system, which mostly utilise medicinal plants for the cure of various ailments. These practices have remained very relevant even now, especially in the developing and underdeveloped worlds like Nigeria and parts of Africa, Asia and the Americas. In these places such practices serve as substitutes for orthodox health care services due to the relative safety, accessibility and cost-effectiveness of herbal medications. Certainly, local practice of the use of medicinal plants is unrestricted in developing countries, but it is obligatory to ascertain the pharmaceutically vital agents responsible for protection against fatal diseases. Currently the developed world is also inclined towards complementary and alternative medicines, specifically derived from natural sources. At

*Correspondence. *E-mail*: christopherk@unijos.edu.ng *Tel*: +234-*8035460415*. ISSN 0189-8442

present, varieties of medicinal plants have been extensively used as curative agents for various diseases globally. It is anticipated that about one quarter of approved modern medicines have been derived from botanicals and globally efforts are ongoing to introduce novel medicinal plants to develop effective, economic and innocuous drugs [1]. Screening of plants for bioactive agents, remains one of the most intensive areas of natural product research today, as only around 10% of all plants had been investigated in detail for bioactive agents creating so much room for further research [2]. Also, there is need to overcome the challenge of insufficient data on very useful medicinal plants including *Amaranthus viridis,* which gives room for adulteration, substitution and false claims [2-4].

Inflammation is the primary physiologic defensive response that helps the body to protect itself against infections, burns, toxic chemicals, allergens, injuries etc, but when uncontrolled and persistent can lead to severe diseases of pain, fever and so on, which are sometimes chronic [5]. Inflammation, pain and pyrexia (fever) underlie several pathological conditions. These debilitating, disabling and morbid conditions are important since they occur in most, if not all, diseases [6]. Most diseases and injuries are associated with debilitating, morbid and even fatal symptoms of inflammations, pain and fever, such that 20% of adults suffer from pain globally and 10% (about 60 million people) are newly diagnosed with pain each year [7, 8]. Majority of the orthodox medicines used for the treatments, including non-steroidal anti-inflammatory analgesics (NSAIDs), opioids and corticosteroids are imported and so expensive, as well as induce toxic side effects like gastrointestinal ulcers, bleeding, renal disorders, dependence, immunosuppression etc, thereby necessitating research into safer, effective and cheaper potential natural sources [1].

Amaranthus viridis Linn. is an annual herb nearly 1m high, pan-tropical and present throughout the West African region, on wastelands, around habitations and as a weed of cultivation, where it is not normally cultivated, but occasionally so as in Zaire (D. R. Congo) where it is called Congo spinach. It is a palatable vegetable in parts of Africa and South India, where it is known as "Kuppacheera [9]. It has been known to have various traditional medicinal applications. The leaf is used for its diuretic and emollient properties, and also used as a poultice in the treatment of inflammations, boils, abscesses, gonorrhoea and orchitis, and in enemas and suppositories for piles. The preparations of the leaves are given to persons suffering from fever in parts of West Africa and the Congo. An infusion of the whole plant is used in Nigeria to purify the blood and as a cooling medicine of value in eye complaints. The plant is known as Ewe Tete to the Yoruba in West Africa and is used for medicinal and spiritual purposes [9]. Agunu, Dafam and Kagaru [10] also reported the use of *A. viridis* leaf, among other medicinal plants used in pain management around the Jos area in Plateau state, North Central Nigeria. Other traditional uses include its use as an anti-inflammatory agent of the urinary tract, for the treatment of venereal diseases, as vermifuge, diuretic, anti-rheumatic, antiulcer, analgesic, antiemetic, laxative and antileprotic, as well as the treatment of respiratory diseases like asthma and eye problems [11]. It has also been reported to be utilised as an antipyretic agent in traditional Ayurvedic medicine, as well as for the treatment of inflammation, ulcer, diabetes, asthma and hyperlipidemia [12].

The anti-inflammatory and analgesic activities were reported by [13], where crude ethanol and water extracts (not derived from successive extractions beginning with non-polar solvents), of *Amaranthus viridis* leaf were observed to significantly reduce the oedema induced by carrageenan better than

Petroleum Ether extract in a dose dependent manner; an indication of anti-inflammatory effect of the extracts. Ezekiel [14] also reported same activities for 70% Aqueous Ethanol leaf extract of the plant. The report by Ashok Kumar et al. [15] indicates that methanolic extract of the plant was screened for anti-nociceptive activity using acetic acid induced writhing, hot plate and tail immersion tests in mice and the antipyretic potential of the extract using yeast induced pyrexia method in rats at the doses of 200 and 400 mg/kg body weight, respectively. The report demonstrated that methanolic extract of *A. viridis* had significant (p<0.01) dose dependent antinociceptive and antipyretic properties at 200 and 400 mg/kg. All these have been able to confirm the ethnomedicinal uses of the plant. More refined extracts of *A. viridis* leaf with fewer and more specific constituents need to be tested for their biological activities to provide a lead to the possible specific bioactive constituents.

This study involved the determination of the anti-inflammatory, analgesic and antipyretic activities of four (4) successive extracts of *A. viridis* leaf, using hexane, ethyl acetate, methanol, and water, in order of increasing polarity, from most non-polar to most polar, in rats and mice. The extraction method here differs from the earlier reported works, and the water extract resulted from a fractionation process due to successive extraction, instead of single solvent crude extraction. The animals were housed under standard environmental conditions, with feed and water provided *ad libitum*. Proper handling and using of the animals were in accordance with the guidelines and regulations, monitored and approved by the Ethical Committee on Animal use, Department of Pharmacology, University of Jos, Nigeria, with the issuance of the Certificate of Ethical Clearance.

EXPERIMENTAL METHODS

Plant collection and preparation. The plant material was collected in Zaria, Kaduna State, Northern Nigeria, and authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The leaves were then separated from the other plant parts and dried in the shade for about two weeks and afterwards pulverized into powder with a blender (Philips®) and stored in an airtight container. The leaf powder **(**1 kg) was successively extracted by cold maceration with constant stirring in 5 litres each of *n*-hexane, ethyl acetate, methanol and water in the following order: *n*-hexane – ethyl acetate – methanol – water, ranging from the most non-polar to the most polar, thereby generating four (4) different extracts. In each case, the plant material was soaked with constant stirring for about 72 hours, followed by filtration and drying or concentration of the extract with the aid of a rotary evaporator and then the drying cabinet at 30 – 40ºC. But the water extract was dried or concentrated on the hot water bath at 40 – 60ºC, and then the drying cabinet. Also, after each extraction, the remaining plant material (marc) was sufficiently dried of the previous solvent, by evaporation in the drying cabinet, before applying the next solvent. Each of the concentrated extracts (Hexane, Ethyl acetate, Methanol and Water) was then, weighed and the percentage yield determined based on the 1000 g of the *A. viridis* leaf powder, according to the formula:

$$\text{Yield}(\%) = \frac{\text{Weight of Extract}}{\text{Weight of Powder}} \times 100$$

The concentrated extracts were then stored in the refrigerator for further use [16, 17].

Phytochemical screening of extracts. The four leaf extracts: *n*-hexane, ethyl acetate, methanol and water were each subjected to preliminary phytochemical tests for the presence of various metabolites, including carbohydrates, alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, and

cardiac glycosides, using standard procedures [2, 17-20].

Acute toxicity (LD_{50}) test. LD_{50} determination was conducted using the Lorke's method. Nine mice were divided into three groups of three mice each. The extracts were orally administered to the first group at a dose of 10 mg/Kg; group 2 received the extract at a dose of 100 mg/Kg, while the last group received the extract at the dose of 1000 mg/Kg body weight. The animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours. In the second phase, three mice were divided into 3 groups of one mouse each. The extracts were administered at the doses of 1500, 2900 and 5000 mg/Kg to groups 1, 2 and 3, respectively based on the result of the first phase. The final LD_{50} was calculated as the square root of the geometrical mean of the highest non-lethal dose and the lowest lethal dose [21].

Test for analgesic activity. The hot plate method was used. The animals (mice) were divided into 14 groups of 5 mice each. Group 1 (Negative control) received 5mg/kg of normal saline intraperitoneally, Group 2 were administered pentazocine (Standard/Positive control) (intraperitoneally) at 10mg/kg, while the remaining groups were administered the extracts orally at 250mg/kg, 500mg/kg and 1000mg/kg, as follows: Groups 3, 4 and 5 (*n*-hexane), 6, 7 and 8 (ethyl acetate), 9, 10 and 11 (methanol) and 12, 13 and 14 (water). After 1 hour pretreatment, each animal was gently placed inside a 100 mL beaker on a hot plate at 55°C. The beaker was to prevent the animals from jumping out before reaction time. The time at which each animal jumped or licked its paw was recorded as the reaction time. The responses were measured at 30, 60 and 90 minutes, and the latency or nociceptive thresholds between control groups and extract treated or test groups were compared [17, 22, 23].

Test for anti-inflammatory activity. The method using egg albumin to induce inflammation (swelling) in Albino rats was used. The animals were divided into 14 groups of 5 rats each. Group 1 (Negative control) received 10 mg/kg of normal saline intraperitoneally (i.p.), Group 2 were administered Diclofenac (Standard/Positive control) (i.p.) at 20mg/kg, while the remaining groups were administered the extracts orally at 250mg/kg, 500mg/kg and 1000mg/kg, as follows: Groups 3, 4 and 5 (*n*-hexane), 6, 7 and 8 (ethyl acetate), 9, 10 and 11 (methanol) and 12, 13 and 14 (water). One hour after administration, animals were injected with egg albumin (0.1mL) at the left hind paw to induce inflammation. The paw diameter was measured before and after administering the egg albumin using electronic Vernier calliper for each animal in each group. Results were recorded at 30 minutes interval for 3 hours and used to determine the degree of inflammation and percentage inhibition of oedema according to the formula [17, 24, 25]:

$$\text{Inhibition (\%)} = \frac{\text{Mean paw diameter (control)} - \text{Mean paw diameter (test)}}{\text{Mean paw diameter (control)}} \times 100$$

Test for antipyretic activity. The antipyretic activity was evaluated with fever induced by Brewer's yeast. Methods by Subedi, Abdur Rahman and Akbar [26] and Amagon et al. [27] in mice were adapted. At zero hour, the basal rectal temperature of each mouse was recorded using clinical digital thermometer. Pyrexia was induced by subcutaneous injection of 20% w/v suspension of Brewer's yeast in distilled water at a dose of 1 mg/kg body weight. After 18 hours of Brewer's yeast injection the rise in rectal temperature was recorded and only animals showing an increase in temperature of at least 0.6°C were selected for the study. The animals were randomly divided into 14 groups, each group containing five mice. Group 1 received normal saline orally at 5 ml/kg. Group 2 was given standard drug paracetamol at the dose of 15 mg/kg per oral. The remaining groups were administered

the extracts orally at 250mg/kg, 500mg/kg and 1000mg/kg, as follows: Groups 3, 4 and 5 (*n*-hexane), 6, 7 and 8 (ethyl acetate), 9, 10 and 11 (methanol) and 12, 13 and 14 (water). The temperatures of all the mice in each group were recorded 1 hour post treatment.

Data analysis. Data were recorded as mean ± SEM. The statistical significance of differences between the groups was determined by analysis of variance (ANOVA) (one-way ANOVA) using the IBM SPSS Statistical tool (version 20). Tukey's test was used for post hoc analysis. Differences of $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Yields of extracts. The quantitative determinations of the yields of the respective extracts of 1000 g of *A. viridis* leaf powder: hexane, ethyl acetate, methanol and water, were as follows, based on the quantities obtained, from the highest to the lowest: water – 67.7g (6.77%) > *n*-hexane – 41.5g (4.15%) > methanol – 22.0g (2.20%) > ethyl acetate - 11.7g (1.17%). It can be seen from the result that water extract had the highest percentage yield followed by hexane, then methanol and ethyl acetate. This implies that the *A. viridis* leaf contains mostly polar or hydrophilic compounds, as well as a lot of some nonpolar or hydrophobic compounds, because extractive yields are strongly determined by the associative relationships between the type of solvent and the nature of compounds, as confirmed by Abdel-Aal, Haroon & Mofeed [28]. The high yield of the water extract can be explained by the known abundance of polar phenolics and glycosides like tannins, flavonoids and saponins in members of the amaranthaceae family, such as *A. viridis* [29, 30] and higher water extractive value (2.17 %) than alcohol extractive value (1.71 %) in *A. viridis*, discovered in a previous work [31]. Also, the high yield of the hexane extract can be attributable to the great presence of steroids, triterpenes and betalain pigments in the members of the amaranthaceae family like *A. viridis* as explained by other workers [29, 30].

Phytochemical constituents of the extracts. The phytochemical screening of the *n*-hexane, ethyl acetate, methanol and water extracts of *A. viridis* leaf revealed the presence of phytoconstituents including alkaloids, tannins, flavonoids, carbohydrates, saponins, steroids and cardiac glycosides. However, anthraquinones were found to be absent as presented in Table 1. The presence of many varieties of phytochemical constituents in all the extracts of *A. viridis* leaf is expected for members of the Amaranthaceae family, deriving from the works of Muller and Borsch [29] and Salvador et al. [30]. The various classes of constituents found in the leaf including: Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrates, Steroids, and Cardiac glycosides agree with the findings of previous workers who used crude extracts of the leaf of *A. viridis* [31-33]. Furthermore, as expected the polar phenolics, carbohydrates (free sugars) and glycosidal constituents, as well as alkaloidal salts were present in the polar extracts: methanol and water and traces in ethyl acetate being in the mid polar zone, while nonpolar steroids and aglycones of cardiac glycosides were in the hexane extract and traces in the ethyl acetate extract.

Acute toxicity (LD_{50}). Acute toxicity testing showed that all the extracts did not cause any mortality even at 5000 mg/kg (Table 2). This implies that the hexane, ethyl acetate, methanol and water extracts contain mixtures of constituents that are very safe since there were no morbidity and mortality at a high dose of 5000 mg/kg in mice, *in vivo*, as reflected by other authors [21,25,27]. The crude extracts have also been shown to be safe by previous studies [32,34]. This could justify the wide use of *A.viridis* leaf for food, as a palatable vegetable, animal forage, and in folkloric medicine as documented by Burkill [9] and Chopra et al. [35].

Anti-inflammatory effects of the extracts. The effects of the various extracts in relation to the controls (positive and negative) on egg albumin-induced inflammation in rats at 30, 60, 90, 120, 150 and 180 minutes post treatment were as shown in Table 3. The percentage inhibitions of oedema were also determined for all the test extracts and controls with respect to the negative control (normal saline) as shown in Table 4. All the extracts showed some relative dose dependent anti-inflammatory activities compared to the controls (negative (normal saline) and positive (Diclofenac)) by rat paw size reductions. This is similar to the significant anti-inflammatory activities recorded for the crude extracts by previous researchers [13-15]. The degree of the significance ($p \leq 0.05$) of the anti-inflammatory activities of the various extracts are as follows: methanol > hexane > water > ethyl acetate. The activities of the extracts could be attributable to the abundant presence of phenolics (tannins, flavonoids) and steroidal triterpenoids, which are known to have anti-inflammatory properties [36]. These findings could justify the traditional uses of this plant to treat various skin inflammations and piles [9, 35].

Table 1: Phytochemical constituents in leaf extracts of *A. viridis*

Constituents	Hexane	Ethyl acetate	Methanol	Water
Alkaloids	-	-	+	+
Saponins	-	-	+	+
Tannins	-	-	+	+
Flavonoids	-	+	+	+
Carbohydrates	-	+	+	+
Steroids	+	+	-	+
Anthraquinones	-	-	-	-
Cardiac glycosides	+	+	+	+

Key: + = Presence; - = Absence

Table 2: Acute toxicity (LD_{50}) for hexane, ethyl acetate, methanol and water extracts of *A. viridis* leaf

Phase	Dose (mg/kg)	No. of animals	Mortality from Extracts			
			Hexane	Ethyl acetate	Methanol	Water
1	10	3	0	0	0	0
1	100	3	0	0	0	0
1	1000	3	0	0	0	0
2	1600	1	0	0	0	0
2	2900	1	0	0	0	0
2	5000	1	0	0	0	0

Table 3: Effects of the extracts of *A. viridis* on egg albumin-induced inflammation in rats

Group/Treatment	Dose (mg/kg)	Basal (mm)	30 min	60 min	90 min	120 min	150 min	180 min
1) Normal Saline	10 ml/kg	3.69 ± 0.11	5.80 ± 0.13	6.95 ± 0.20	7.31 ± 0.25	7.42 ± 0.31	7.34 ± 0.25	7.34 ± 0.17
2) Diclofenac	20	3.60 ± 0.14	6.00 ± 0.16	6.85 ±0.20^{a}	6.61 ±0.12^{a}	6.44 ±0.11^{a}	6.26 ±0.14^{a}	6.01 ± 0.14^{a}
3) Hexane	250	3.90 ± 0.05	6.69 ± 0.48	6.61±0.46^{ab}	6.30±0.45^{ab}	6.21±0.46^{ab}	6.16 ±0.44^{a}	6.05 ± 0.58^{a}
4) Hexane	500	3.57 ± 0.17	5.84 ±0.23^{b}	6.50±0.17^{ab}	6.65 ±0.13^{a}	6.31±0.15^{ab}	5.97±0.10^{ab}	5.83 ± 0.09^{ab}
5) Hexane	1000	4.07 ± 0.10	6.13 ± 0.45	6.27±0.44^{ab}	5.87±0.45^{ab}	5.72±0.45^{ab}	5.65±0.43^{ab}	5.48 ± 0.45^{ab}
6) Ethyl acetate	250	3.65 ± 0.13	5.29±0.15^{ab}	6.45±0.35^{ab}	6.56±0.36^{ab}	6.52 ±0.40^{a}	6.05±0.28^{ab}	6.05 ± 0.28^{a}
7) Ethyl acetate	500	3.40 ± 0.06	4.92±0.09^{ab}	7.05 ± 0.24	7.00 ±0.20^{a}	6.74 ±0.13^{a}	6.41 ±0.10^{a}	6.37 ± 0.13^{a}
8) Ethyl acetate	1000	3.41 ± 0.15	5.10±0.05^{ab}	6.81 ± 0.16	6.67 ±0.21^{a}	6.37 ±0.23^{a}	6.44 ±0.09^{a}	6.30 ± 0.23^{a}
9) Methanol	250	3.58 ± 0.16	6.79 ± 0.11	6.65±0.18^{ab}	6.50±0.31^{ab}	6.28±0.20^{ab}	5.87±0.23^{ab}	5.16 ± 0.42^{ab}
10) Methanol	500	3.27 ± 0.06	6.17 ± 0.19	6.16±0.16^{ab}	6.04±0.24^{ab}	5.95±0.17^{ab}	5.74±0.16^{ab}	5.04 ± 0.31^{ab}
11) Methanol	1000	3.51 ± 0.19	6.41 ± 0.33	6.49±0.34^{ab}	6.44±0.32^{ab}	6.29±0.33^{ab}	6.04±0.25^{ab}	4.86 ± 0.29^{ab}
12) Water	250	3.49 ± 0.23	6.24 ± 0.18	6.51±0.12^{ab}	6.43±0.13^{ab}	6.19±0.18^{ab}	5.98±0.21^{ab}	5.71 ± 0.22^{ab}
13) Water	500	3.60 ± 0.10	6.32 ± 0.31	6.58±0.19^{ab}	6.38±0.14^{ab}	6.06±0.15^{ab}	5.89±0.15^{ab}	5.89 ± 0.11^{ab}
14) Water	1000	3.84 ± 0.15	6.43 ± 0.29	6.64±0.20^{ab}	5.94±0.18^{ab}	5.80±0.17^{ab}	5.58±0.16^{ab}	5.52 ± 0.20^{ab}

n=5; $^{a}P<0.05$ compared to Normal saline; $^{b}P<0.05$ compared to Diclofenac; Data presented as mean ± SEM; min= minutes

Table 4: Percentage inhibition of extracts of *A. viridis* leaf of paw oedema in rats

Group/Treatment	Dose (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
1) Normal Saline	10 ml/kg	-	-	-	-	-	-
2) Diclofenac	20	0%	1.4%[a]	9.6%[a]	13.2%[a]	14.7%[a]	18.1%[a]
3) Hexane	250	0%	4.9%[ab]	13.8%[ab]	16.3%[ab]	16.1%[ab]	17.6%[a]
4) Hexane	500	0%	6.5%[ab]	9.0%[a]	15.0%[ab]	18.7%[ab]	20.6%[ab]
5) Hexane	1000	0%	9.8%[ab]	19.7%[ab]	22.9%[ab]	23.0%[ab]	25.3%[ab]
6) Ethyl acetate	250	8.8%[ab]	7.2%[ab]	10.3%[ab]	12.1%[a]	17.6%[ab]	17.6%[a]
7) Ethyl acetate	500	15.2%[ab]	0%	4.2%[a]	9.2%[a]	12.7%[a]	13.2%[a]
8) Ethyl acetate	1000	12.1%[ab]	2.0%[ab]	8.8%[a]	14.2%[ab]	12.3%[a]	14.2%[a]
9) Methanol	250	0%	4.3%[ab]	11.1%[ab]	15.4%[ab]	20.0%[ab]	29.7%[ab]
10) Methanol	500	0%	11.4%[ab]	17.4%[ab]	19.8%[ab]	21.8%[ab]	31.3%[ab]
11) Methanol	1000	0%	6.6%[ab]	11.9%[ab]	15.2%[ab]	17.7%[ab]	33.8%[ab]
12) Water	250	0%	6.3%[ab]	12.0%[ab]	16.6%[ab]	18.5%[ab]	22.2%[ab]
13) Water	500	0%	5.3%[ab]	12.7%[ab]	18.3%[ab]	19.8%[ab]	19.8%[ab]
14) Water	1000	0%	4.5%[ab]	18.7%[ab]	21.8%[ab]	24.0%[ab]	24.8%[ab]

n=5; [a]P<0.05 compared to Normal saline; [b]P<0.05 compared to Diclofenac min= minutes

Table 5: Effects of the extracts of *A. viridis* on reaction time in the hot plate test in mice

Group/Treatment	Dose (mg/kg)	30 min	60 Min	90 Min
1)Normal Saline	5 ml/kg	4.16 ± 0.56	5.58 ± 1.22	4.82 ± 1.52
2)Pentazocine	10	9.66 ± 0.86[a]	7.42 ± 1.29[a]	8.24 ± 0.76[a]
3)Hexane	250	4.98 ± 0.54[a]	4.96 ± 0.88	7.16 ± 2.60[a]
4)Hexane	500	6.26 ± 0.73[a]	7.44 ± 0.68[ab]	7.48 ± 0.48[a]
5)Hexane	1000	9.70 ± 1.22[ab]	8.46 ± 1.67[ab]	9.18 ± 0.78[ab]
6)Ethyl acetate	250	4.54 ± 0.86	4.62 ± 0.84	3.14 ± 0.85
7)Ethyl acetate	500	5.70 ± 0.40[a]	5.20 ± 0.78	5.52 ± 0.45[a]
8)Ethyl acetate	1000	7.18 ± 1.77[a]	5.36 ± 1.31	6.58 ± 1.03[a]
9)Methanol	250	4.64 ± 0.92	4.88 ± 0.80	4.56 ± 0.90
10)Methanol	500	5.10 ± 0.27[a]	5.28 ± 0.36	4.00 ± 0.64
11)Methanol	1000	6.24 ± 0.48[a]	5.46 ± 1.33	6.40 ± 0.60[a]
12)Water	250	5.46 ± 0.66[a]	5.60 ± 0.44[a]	3.34 ± 0.81
13)Water	500	6.34 ± 0.33[a]	6.26 ± 0.30[a]	5.72 ± 0.46[a]
14)Water	1000	5.78 ± 0.19[a]	7.32 ± 1.02[ab]	5.98 ± 1.27[a]

n=5; [a]P<0.05 compared to Normal saline; [b]P<0.05 compared to Pentazocine; Data presented as mean ± SEM min= minutes

Table 6: Effects of *A. viridis* leaf extracts on rectal temperature (°C) in Brewer's Yeast-Induced pyrexia in mice

Group/Treatment	Dose (mg/kg)	Basal Temp	Post Inducement Temp	Post Treatment Temp
1) Normal Saline	5 ml/kg	37.8 ± 0.17	38.1 ± 0.16	37.3 ± 0.18
2) Paracetamol	15	37.4 ± 0.13	37.3 ± 0.44	37.1 ± 0.08[a]
3) Hexane	250	37.8 ± 0.07	37.9 ± 0.03	37.0 ± 0.28[ab]
4) Hexane	500	37.9 ± 0.14	37.4 ± 0.23	36.6 ± 0.46[ab]
5) Hexane	1000	37.7 ± 0.22	37.5 ± 0.36	37.0 ± 0.42[ab]
6) Ethyl acetate	250	36.7 ± 0.12	36.9 ± 0.30	36.8 ± 0.22[ab]
7) Ethyl acetate	500	36.7 ± 0.18	37.5 ± 0.26	36.2 ± 0.25[ab]
8) Ethyl acetate	1000	37.3 ± 0.11	38.1 ± 0.04	36.7 ± 0.18[ab]
9) Methanol	250	37.3 ± 0.13	37.7 ± 0.24	36.9 ± 0.18[ab]
10) Methanol	500	37.8 ± 0.07	37.9 ± 0.16	36.9 ± 0.27[ab]
11) Methanol	1000	37.7 ± 0.09	38.1 ± 0.27	36.6 ± 0.15[ab]
12) Water	250	37.0 ± 0.12	37.3 ± 0.13	37.4 ± 0.19
13) Water	500	37.4 ± 0.05	37.3 ± 0.29	37.2 ± 0.35[a]
14) Water	1000	37.6 ± 0.21	37.5 ± 0.31	37.2 ± 0.26[a]

n=5; [a]P<0.05 compared to Normal saline; [b]P<0.05 compared to Paracetamol; Data presented as mean ± SEM

Analgesic effects of the extracts. The effects of the various extracts in relation to the controls (positive and negative) on the reaction time of mice to the pain caused by the hot plate at 30, 60 and 90 minutes post treatment were as shown in Table 5. The results show general increase in reaction time or pain threshold exhibited by all extracts, especially compared to the negative control (normal saline) in a dose dependent manner. At all observation

periods, hexane and water extracts showed much more significant analgesic activities compared to the controls, better than ethyl acetate and methanol extracts. The crude extracts also indicated significant analgesic activities as reported by Ashok Kumar et al. [37], Macharla et al. [13] and Ezekiel [14]. This is indicative of the pain killing property of this plant, including centrally mediated pain that can be generated using the hot plate as explained by Wannang et al. [24] and Amagon et al. [27]. This could justify the traditional use of this plant for pain management [10], which could also be attributable to the presence of steroidal triterpenes, alkaloids and saponins known for their analgesic properties [36].

Antipyretic effects of the extracts. The effects of hexane, ethyl acetate, methanol and water extracts in relation to the controls (positive and negative) on rectal temperature in Brewer's yeast-induced pyrexia in mice at 60 minutes post treatment were as shown in Table 6. Antipyretic property is equivalent to decrease in mice rectal temperature following induction and treatment as reflected by Subedi, Abdur Rahman & Akbar [26] and Amagon et al. [27]. So, from the results all the extracts showed significant ($p \leq 0.05$) antipyretic properties in a dose dependent manner in the following order: methanol > ethyl acetate > hexane > water. These agree with an earlier finding where crude methanolic extract of *A. viridis* had shown significant ($p<0.01$) antipyretic activity [15, 37].

This property could therefore explain the traditional application of the leaf preparations of *A. viridis* for the treatment of fevers in parts of West Africa and India [9,12], which could also be attributed to the presence of flavonoids and saponins, known for their antipyretic activities [36,38].

Conclusion. This work has been able to establish that the various extracts of *A. viridis* leaf contain constituents that are both safe and effective, as anti-inflammatory, analgesic, and antipyretic agents, thereby further providing scientific evidence for the traditional uses in the treatment of inflammations, pain, and fever.

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