

Research Article

Anti-Inflammatory and Anti-Pyretic Activity of the Leaf, Root and Saponin Fraction from *Vernonia amygdalina*

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Abstract: Studies have shown that *Vernonia amygdalina* possess saponin as one of the bitter phyto-constituents. This study was aimed at determining the anti-inflammatory and antipyretic activity of the aqueous extract of the leaf, root and saponin fraction from the herb. Standard procedures using ear thickness measurement in xylene induced inflammation and anal temperature readings in *Saccharomyces cerevisiae* induced pyrexia in rats were followed. Data indicated significant ($p \leq 0.05$) inhibitory activity for all the dose levels of the extracts in the anti-inflammatory and antipyretic evaluations. Saponin fraction at the dose of 100 and 200 mg/kg with 10.5 and 19.6% inhibition respectively, showed significant ($p \leq 0.05$) anti-inflammatory activity. The antipyretic evaluation of the saponin fraction showed no anal temperature reduction at 50 mg/kg dose level. Finding suggests the antipyretic and non-steroid like anti-inflammatory activity of the saponin fraction. This may partly explain the observed activity of the herbal extract which has found use traditionally as remedy for similar ailments.

Keywords: Anal temperature, ear thickness, inflammation, pyrexia, rat, *Vernonia amygdalina*

INTRODUCTION

Vernonia amygdalina is a widely used local vegetable in Uganda, Nigeria and other African countries. It grows in a range of ecological zones in Africa and the Arabian Peninsula (Bonsi *et al.*, 1995). The leaf is commonly referred to as bitter leaf and locally, “omubirizi” or “omululuza” (West and Central Uganda); “olusia” (Luo, Kenya); or “ewuro”, “etidot” and “olugbo” (Southern Nigeria). Apart from the nutritional use of the herb, the leaf and root are known for their therapeutic benefits due to the presence of numerous phytochemicals (Izevbogie, 2005). The bitter taste of the leaf has been attributed to the presence of anti-nutritive principles like saponins, alkaloids, tannins

and glycosides (Buttler and Bailey, 1973). Perhaps, the mode of preparing the leaf for human consumption which involves the soaking and washing in warm water is aimed at reducing these bitter tasting principles.

Saponins in foods have traditionally been considered bitter and unpleasant (Izevbogie, 2005). In some cases this has limited their use and therefore, most of the earlier research on processing has targeted their removal to enable human consumption (Güçlü-Ustündağ and Mazza, 2007). Food and non-food sources of saponins have, however, come into renewed focus in recent years due to increasing evidence of their health benefits (Shi *et al.*, 2004). Studies have shown that saponins are active components in many herbal medicines and also major contributor to the health

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benefit of herbs as food (Liu and Henkel, 2002). Similar emanating evidences are renewing interest in the commercial potential of saponins and also in the development of new processing strategies for saponin containing herbs (Muir *et al.*, 2002).

V. amygdalina can be put to better use if the potential therapeutic benefit of the saponin constituent can be substantiated through necessary evaluations. Despite the various pharmacological studies on the use of this herb (Iwalokun *et al.*, 2004; Iwalokun *et al.*, 2006; Ojiako and Nwanjo, 2006; Anoka *et al.*, 2008; Taiwo *et al.*, 2009; Asuquo *et al.*, 2010; Adiukwu *et al.*, 2012) there is the need for further investigations and data to support the rational use of the herb in a rapidly evolving world of health care (World Health Organization, 2005). Therefore, the objective of this study was aimed at investigating *Vernonia amygdalina* leaf and root aqueous extract and the crude saponin chromatographic fraction for anti-inflammatory and antipyretic properties.

MATERIALS AND METHOD

Chemicals, drugs and test agents: All the solvents (methanol, n-butanol, diethyl ether, chloroform, xylene and acetone), obtained from BDH sales representative in Kampala (Uganda) were of the analytical grade. Other agents used include lubricant (KY jelly[®], India) for the anal insertion of thermometer probe; acetic acid (Sigma-Aldrich, Germany); and active dry *Saccharomyces cerevisiae* (GRIFFCHEM[®], Kenya) which was used to induce pyrexia in the antipyretic study. 250 mg/kg Acetylsalicylic Acid (ASA) (Pinewood, Caprin[®]) was used as standard in all the evaluations in this study. Steroidal standard, dexamethasone (Agog Pharma., India) at a dose of one milligram per kilogram and a placebo of 10 mL/kg distilled water was used in the anti-inflammatory activity evaluations. Five milliliters per kilogram normal saline (Albert David, India) was used as placebo in the antipyretic evaluations. Based on previous reports three dose levels: 400, 600 and 800 mg/kg leaf extract; 200, 400 and 600 mg/kg root extract; and 50 mg/kg, 100 mg/kg and 200 mg/kg *Vernonia amygdalina* saponin fraction B (Va-SB) were used both in the anti-inflammatory and antipyretic evaluation of the leaf, root and Va-SB respectively (Okokon and Onah, 2004; Adiukwu *et al.*, 2011 and 2012).

Plant material and extraction: The fresh leaves and roots of *Vernonia amygdalina* identified by a botanist were collected in the morning, between June and July in the South-Western Uganda region. Specimens were retained with voucher number 16-20 and 21-20 in the

department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology. The plant's parts were separately shade air-dried and ground into coarse powder. The powders were sieved to 3500 g of leaf and 2000 g of root fine powders. The separate moistened powders were allowed to stand for 15 minutes before maceration for three hours in warm (<80°C) distilled water at a ratio of 131 g to 9 litres with intermittent shaking (Singh, 2008; Adiukwu *et al.*, 2011). The obtained infusions were filtered while warm using filter-papers. The filtrates were further filtered using buckner filter assemblage (aided by a suction pump) and subsequently evaporated to dryness using an oven (at ≤80°C) to obtain 630 g (18% yield) leaf and 350 g (17.5% yield) root residue. The obtained residues were stored in a desiccator for further use.

Phytochemical screening of extracts: Preliminary screening of the leaf and root aqueous extract for phytochemicals was carried out using standard procedures (Harborne, 1973; Trease and Evans, 1983).

Isolation of crude saponin: The liquid-liquid extraction technique as described by Obadoni and Ochuko (2001) was adopted for the isolation. A forty millimetre solution was prepared in distilled water using 20 g of the dried aqueous extract of *V. amygdalina* leaf. This was extracted thrice with 20 ml diethyl ether. The diethyl ether layer was discarded and the retained aqueous layer extracted further with 60 ml n-butan-1-ol (four times). The n-butan-1-ol extracts were bulked together and washed four times using 10 ml of five percent NaCl. The washed extract was concentrated at <80°C in an oven and air dried at room temperature to yield 1.81 g (9.1% w/w) of crude saponin residue. The residue was screened for saponin using the foaming test (Harborne, 1973).

Flash column chromatography fractionation: The crude saponin dissolved in methanol was adsorbed onto a TLC grade silica gel (CSI 010, Unilab) at a ratio of two to five and dried in an oven at <80°C to produce a 21 g free flowing powder. The powder was loaded and fractionated on a silica gel (May & Baker Dagenham, England: 0.2-0.5 mm, pore size 40 amstrom, 30-70 mesh) containing flash column (Stil *et al.*, 1978). The column was eluted with a gradient mobile phase solvent system of increasing polarity starting with xylene; combination of chloroform and methanol; and methanol, in multiples of 100 ml. An air pump (*Merck, Germany*) was used to facilitate the rate of elution. Each 100 mL effluent collected was profiled using TLC with a mobile phase system of acetone, chloroform and methanol (at a ratio of one to four to two) (Hostettmann

and Marston, 1986). Spots were located using saturated iodine chamber. Effluents with similar profile were combined together, concentrated over a water bath and allowed to evaporate to dryness at room temperature. This resulted in 2 fractions: *Vernonia amygdalina* saponin fraction A (Va-SA) 0.932 g, eluted with chloroform/methanol and *Vernonia amygdalina* saponin fraction B (Va-SB) 1.35 g, eluted with methanol. A preliminary screening of both fractions according to Harborne (1973) using foaming test was conducted. Va-SB was positive and was preserved in a desiccator for further use.

Animal handling: 120-200 g wistar rats of both sexes which have been acclimatized in the animal facility of Mbarara University of Science and Technology were placed in standard cages where they were maintained on standard animal pellets (obtained from Nuvita Feeds Ltd., Kampala) and water ad libitum. Using the CTO 12667 electronic probe thermometer (China), rats for antipyretic study were selected based on their measured basal anal temperature not exceeding 37°C and achieving anal temperature elevation of at least 0.1°C in response to intra peritoneal (i.p) administration of 15% w/v *Saccharomyces cerevisiae* at a dose of 10 ml/kg body weight. Animals were randomly divided into five groups in the antipyretic study and six groups in the anti-inflammatory study and fasted overnight.

The National Institute of Health Guide for the care and use of Laboratory Animals approved by the Institutional Ethical Committee was adopted for the animal protocol in this study (NIH, 1978).

Preparation and administration of doses: Distilled water was used to prepare the different dose solutions for anti-inflammatory study, while normal saline was used in the antipyretic study. In both instance a concentration of 100 mg/ml dexamethasone, 50 mg/ml ASA, 100 mg/ml leaf extract, 80 mg/mL root extract and 100 mg/mL Va-SB were prepared. Each animal was administered the appropriate dose orally using the prepared solutions via a syringe/canula assemblage.

Anti-inflammatory evaluation: Prior to inducing inflammation (edema) the thickness of the right ear of each rat was measured using a digital caliper micrometer (Neiko Tools, USA). Inflammation was induced thirty minutes after dose administration by applying 0.05 ml of xylene using a microliter pipette (Transferpette®, Germany) through the ear canal until the inner and outer surface of the ear is uniformly moistened (Junping *et al.*, 2005; Akindele and Adeyemi, 2007). Each animal was etherized 2 h later for anesthesia using diethyl ether and the measurement

of the inflamed ear thickness repeated. The thickness of the ear as the index of anti-inflammation was used to measure the extent of inhibition (Jaijoy *et al.*, 2010). The experimental procedure was the same in the evaluation of the leaf and root aqueous extract; and Va-SB. Obtained data were documented as group mean. Percentage anti-inflammatory inhibition was calculated as:

$$(\Delta T_p - \Delta T_s/t) / \Delta T_p \times 100$$

ΔT_p : Mean change in ear thickness of placebo group
 $\Delta T_s/t$: Mean change in ear thickness of standards or test group

Anti-pyretic evaluation: Procedure as described by Okokon and Onah (2004) was adopted for this study. 20 h after yeast administration (induction of pyrexia) the anal temperature of each animal was taken before dose administration. Four h after dose administration, the anal temperature reading of each animal was repeated.

The same experimental procedure was followed in the evaluation of the leaf and root aqueous extract; and Va-SB. Data were expressed as group mean.

Statistical analysis of data: Results and calculations were based on the numerical expression of data as mean±SEM (standard error of mean). Analysis of variance (ANOVA) was used to analyse values within groups and student t-test to analyse data between groups. $p \leq 0.05$ was taken as level of significance in all cases.

RESULT

Phytochemical screening: Standard test for phytochemical constituents revealed the presence of similar principles in both the leaf and root extracts: saponins, alkaloids, sophisticated lactones, triterpenoids, reducing sugars, amino acids, flavonoids, terpenoids, tannins and cardiotoxic glycosides. However, quinine was absent.

Anti-inflammatory evaluation: Significant ($p \leq 0.05$) anti-inflammatory activity was observed for all the dose levels used in the evaluation of the leaf and root extract. However, data suggest more inhibitory activity with the leaf (Table 1) than in the root extract (Table 2). In both case, activity was dose dependent. Va-SB indicated a relatively lower inflammation inhibitory activity compared to the extracts. However, observed activity at the dose levels of 100 and 200 mg/kg were significant ($p \leq 0.05$) and dose dependent (Table 3).

Table 1: *Vernonia amygdalina* leaf aqueous extract inhibitory activity on xylene induced inflammation (edema) in rats

Group	Dose (30 minutes before inflammation)	Ear Thickness (μm)			% Inflammation Inhibition
		Before dose & inflammation	2 hrs after inflammation	Change in ear Thickness (μm)	
Control	Water 10 ml/kg	132.2 \pm 4.3	202.1 \pm 1.2	69.9 \pm 3.9	0
Test	Extract 400 mg/kg	192.5 \pm 6.2	211.3 \pm 1.7	18.8 \pm 3.1*	73.1
Test	Extract 600 mg/kg	191.4 \pm 5.3	201.3 \pm 2.1	9.9 \pm 2.6*	85.8
Test	Extract 800 mg/kg	174.2 \pm 7.1	182.8 \pm 3.5	8.6 \pm 5.1*	87.7
NS Standard	ASA 250 mg/kg	201 \pm 4.1	166.8 \pm 5.2	34.2 \pm 4.3*	51.1
Steroidal Standard	Dexamethasone 1 mg/ml	145.3 \pm 4.7	151.1 \pm 6.4	5.8 \pm 2.1*	91.7

Data are mean \pm SEM (standard error of mean) value (n = 5). *Significantly ($p < 0.05$) different from placebo group. Non-Steroidal (NS)

Table 2: *Vernonia amygdalina* root aqueous extract inhibitory activity on xylene induced Inflammation (edema) in rats

Group	Dose (30 minutes before inflammation)	Ear Thickness (μm)			% Inflammation Inhibition
		Before dose & inflammation	2 hrs after inflammation	Change in ear Thickness (μm)	
Control	Water 10 ml/Kg	197.2 \pm 2.2	267.7 \pm 4.1	70.5 \pm 3.2	-
Test	Extract 200 mg/Kg	169.4 \pm 2.7	211.8 \pm 3.3	42.4 \pm 3.1*	39.8
Test	Extract 400 mg/Kg	154.6 \pm 6.1	191.8 \pm 2.7	37.2 \pm 3.7*	47.3
Test	Extract 600 mg/Kg	188.4 \pm 5.1	210.3 \pm 5.5	21.9 \pm 5.2*	68.9
NS Standard	ASA 250 mg/kg	178.1 \pm 4.5	211.7 \pm 3.9	33.6 \pm 4.2*	52.4
Steroidal Standard	Dexamethasone 1 mg/ml	171.2 \pm 2.7	176.1 \pm 5.2	4.9 \pm 3.7*	93.1

Data are mean \pm SEM (standard error of mean) value (n = 5). *Significantly ($p < 0.05$) different from placebo group. Non-Steroidal (NS)

Table 3: *Vernonia amygdalina* saponin fraction B (Va-SB) inhibitory activity on xylene induced Inflammation (edema) in rats

Groups	Dose (30 min before inflammation)	Ear Thickness (μm)			% inflammation inhibition
		Before dose & inflammation	2 hrs after inflammation	Change in ear Thickness (μm)	
Control	water 10 ml/kg	149.2 \pm 2.2	211.3 \pm 2.9	62.1 \pm 7.5	-
Test	Va-SB 50 mg/kg	158.7 \pm 3.8	221.2 \pm 7.1	62.5 \pm 5.4 [#]	-
Test	Va-SB 100 mg/kg	200.1 \pm 4.3	255.7 \pm 3.7	55.6 \pm 3.5*	10.5
Test	Va-SB 200 mg/kg	198.2 \pm 3.7	248.1 \pm 6.3	49.9 \pm 3.9*	19.6
NS Standard	ASA 250 mg/kg	162.3 \pm 4.5	193.9 \pm 4.1	31.6 \pm 2.7*	49.2
Steroidal Standard	Dexamethasone 1 mg/ml	190.4 \pm 8.1	205.0 \pm 3.1	8.6 \pm 5.1*	86.1

Data are mean \pm SEM (standard error of mean) value (n = 5). *: Significantly ($p < 0.05$) different from placebo group. Non-steroidal (NS);

[#]: Change in ear thickness \geq change in ear thickness of placebo group is considered zero inhibitory effect

Table 4: Effect of *Vernonia amygdalina* leaf aqueous extract on the anal temperature in rats

Group	Dose	Mean Anal Temperature $^{\circ}\text{C}$		Anal Temperature Change $^{\circ}\text{C}$ ($T_2 - T_1$)
		0 h after dose (T_1)	4 h after dose (T_2)	
Placebo	Normal saline 5 ml/kg	0.11	39.46 \pm 0.10	0.44 \pm 0.05
Test	Extract 400 mg/kg	39.02 \pm 0.18	39.02 \pm 0.17	0.00 \pm 0.03*
Test	Extract 600 mg/kg	38.50 \pm 0.20	38.43 \pm 0.10	-0.07 \pm 0.17*
Test	Extract 800 mg/kg	39.01 \pm 0.12	38.92 \pm 0.20	-0.09 \pm 0.15*
Standard	ASA 250 mg/kg	39.10 \pm 0.10	38.80 \pm 0.07	-0.30 \pm 0.13*

Data are mean \pm SEM (standard error of mean) value (n = 5). *Significantly ($p < 0.05$) different from placebo group. Acetylsalicylic acid (ASA)

Table 5: Effect of *Vernonia amygdalina* root aqueous extract on the anal temperature in rats

Group	Dose	Mean Anal Temperature $^{\circ}\text{C}$		Anal Temperature Change $^{\circ}\text{C}$ ($T_2 - T_1$)
		0 hrs after dose (T_1)	4 hrs after dose (T_2)	
Placebo	Normal saline 5 ml/kg	38.50 \pm 0.10	38.95 \pm 0.19	0.45 \pm 0.15
Test	Extract 200 mg/kg	38.70 \pm 0.25	39.00 \pm 0.05	0.03 \pm 0.10*
Test	Extract 400 mg/kg	38.08 \pm 0.19	38.09 \pm 0.10	0.01 \pm 0.13*
Test	Extract 600 mg/kg	39.06 \pm 0.11	39.02 \pm 0.05	-0.04 \pm 0.60*
Standard	ASA 250 mg/kg	38.90 \pm 0.11	38.52 \pm 0.12	-0.38 \pm 0.22*

Data are mean \pm SEM (standard error of mean) value (n = 5). *Significantly ($p < 0.05$) different from placebo group. Acetylsalicylic acid (ASA)

Antipyretic evaluation: As observed with ASA, data indicated significant ($p < 0.05$) anal temperature decrease for all the dose levels in the different test doses

(extracts and Va-SB) except at 50 mg/kg of Va-SB. Dose dependence was observed for all the test samples, though antipyretic activity was noted to be higher in the

Table 6: Effect of *Vernonia amygdalina* saponin fraction B (Va-SB) on the anal temperature in rats

Group	Dose	Mean Anal Temperature °C		Anal Temperature Change °C (T ₂ - T ₁)
		0 hrs after dose (T ₁)	4 hrs after dose (T ₂)	
Placebo	Normal saline 5 ml/kg	39.05±0.12	39.04±0.11	-0.01±0.02
Test	Va-SB 50 mg/kg	38.75±0.25	38.92±0.24	#0.17±0.02
Test	Va-SB 100 mg/kg	38.78±0.19	38.75±0.18	*-0.03±0.01
Test	Va-SB 200 mg/kg	38.66±0.11	38.29±0.11	*-0.09±0.01
Standard	ASA 250 mg/kg	39.10±0.11	38.95±0.08	*-0.15±0.04

Data are mean±SEM (standard error of mean) value (n = 5). *Significantly (p≤0.05) different from placebo group. # Anal temperature change ≥ anal temperature change in placebo group is considered zero inhibitory effect. Acetylsalicylic acid (ASA)

leaf (Table 4) than the root (Table 5) extract. Comparatively, Va-SB (Table 6) showed much lower activity than the root extract at a similar dose of 200 mg/kg.

DISCUSSION

Inflammation is a common tissue phenomenon when exposed to trauma or injury (Mohamed *et al.*, 2011; Ijeoma *et al.*, 2011; Wang *et al.*, 2010; Amazur *et al.*, 2010). In the anti-inflammatory evaluation, xylene was used to induce acute inflammation in the form of ear edema. Anti-inflammatory data for the leaf and root extract indicated reduction in the induced edema which was significantly (p≤0.05) different from the placebo group for all the three dose levels. Observed activity was comparable to dexamethasone dose at higher dose levels of 800 mg/kg leaf extract and 600 mg/kg root extract. Higher dose levels of Va-SB (100 and 200 mg/g) indicated mild activity which was significant (p≤0.05) and comparable to the inhibitory activity of ASA. In all the evaluations test doses indicated dose dependence.

Topical application of xylene is known to produce marked edema formation and increase in myeloperoxidase enzymatic activity (Ravelo-Calzado *et al.*, 2011). Such inflammation has been associated with increase in cystolic prostaglandin-E₂, a powerful vasodilator which synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation (Georgewill *et al.*, 2010a; Foyet *et al.*, 2011). Agents or substances with potential to inhibit activities associated with inflammation are considered to have anti-inflammatory property.

Xylene ear edema model has been shown to have bias for anti-inflammatory steroids and less sensitive to non-steroidal anti-inflammatory agents (Igbe *et al.*, 2010). Inflammation induction using xylene is considered to be partially associated with substance P (tachykinin neuropeptide), an undecapeptide which is widely distributed in the central and peripheral nervous system and functions as a neurotransmitter or neuromodulator. Prior study has shown the role of substance P activity in neurogenic inflammation, which can be inhibited by steroidal anti-inflammatory agents

(Junping *et al.*, 2005). As such, obtained data for Va-SB suggests a non-steroidal-like activity, similar to ASA which is associated with peripheral inhibition of prostaglandin-E₂ (David *et al.*, 2001). The anti-inflammatory activity of the extracts which was comparable in magnitude to the activity of dexamethasone may be explained in part, to be a consequence of the presence of flavonoids, tannins, glycosides (shown to be present in the aqueous extract by this study) and trace elements: zinc, copper and manganese as shown to be present by prior study (Ibrahim *et al.*, 2001). Earlier studies have reported the anti-inflammatory property of these substances (Ahmadiani *et al.*, 2000; Bittar *et al.*, 2000; Kim *et al.*, 2004; Agbaje *et al.*, 2008; Di-Silvestro and Marten, 1990; Michael and Murray, 1996).

Finding agrees with previous anti-inflammatory activity study of *V. amygdalina* (Koko *et al.*, 2008; Nwangwu *et al.*, 2011; Georgewill and Georgewill, 2010b). However, this study was able to show that Va-SB has a non-steroid like anti-inflammatory activity which may be similar to the mechanism of its antipyretic activity (Adiukwu *et al.*, 2012).

Brewer's yeast has been shown to induce pyrexia in laboratory animals, in a similar manner to lipopolysaccharides. Such induction activates the arachidonic acid pathway and has been associated with elevated prostaglandin E₂ (PGE₂) level in the hypothalamus (Walter, 2003). Antipyretic agents have been shown to antagonize the PGE₂ elevation by inhibiting the activity of cyclo-oxygenase there by suppressing pyrexia (David *et al.*, 2001). The aqueous extract of the leaf and root at the three dose levels in the antipyretic evaluation showed significant (p≤0.05) and dose dependent anal temperature decrease. Observed activity was similar, though less than data obtained for ASA. Va-SB showed significant (p≤0.05) anal temperature decrease at 100 and 200 mg/kg, which was much lower than that for the extracts and standard. In similar antipyretic studies, the leaf and root extract of *V. amygdalina* were shown to possess antipyretic property (Okokon and Onah, 2004; Adiukwu *et al.*, 2011). In this study, data shows that the antipyretic activity was strongly noticed with the leaf extract as compared to the root. Also, Va-SB was identified,

perhaps to be partially responsible for the observed activity in the herbal extracts.

Previous *in vivo* studies using rats, mice and rabbits showed that saponins are not absorbed in the alimentary channel but hydrolyzed to their corresponding sapogenins (aglycone) and sugar(s) by enzymatic action in the gastrointestinal tract (Güçlü-Ustündağ and Mazza, 2007; Gestetner *et al.*, 1968). The readily more absorbable aglycone is because of this reason, usually considered to be responsible for most of the associated activities with the orally administered saponins. This may explain the safety of the orally administered saponin rich *Vernonia amygdalina* extracts and Va-SB in this study (George, 1965; Adiukwu *et al.*, 2012).

CONCLUSION

This study has been able to show the anti-inflammatory and antipyretic activity of the aqueous extract of the leaf, root and saponin fraction, Va-SB from *Vernonia amygdalina*. However, further study on Va-SB may help to elucidate on the specific principle(s) associated with the observed activity of the fraction in this study.

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REFERENCES

- Adiukwu, C.P., A. Agaba, G. Nambatya, B. Adzu, L. Imanirampa, S. Twinomujuni, O. Twikirize, M. Amany, J.O. Ezeonwumelu, J. Oloro, G.A. Okoruwa and B. Katusiime, 2012. Acute toxicity, antipyretic and antinociceptive study of the crude saponin from an edible vegetable: *Vernonia amygdalina* leaf. *Int. J. Biol. Chem. Sci.*, 6(3): 1019-1028.
- Adiukwu, C.P., A. Agaba and G. Nambatya, 2011. Pharmacognostic, antiplasmodial and antipyretic evaluation of the aqueous extract of *Vernonia amygdalina* leaf. *Int. J. Biol. Chem. Sci.*, 5(2): 709-716.
- Agbaje, E.O., A.A. Adeneye and T.I. Adeleke, 2008. Anti-nociceptive and anti-inflammatory effects of a Nigerian Polyherbal Tonic (PHT) extract in rodents. *Afr. J. Compl. Alt. Med.*, 5(4): 399-408.
- Ahmadiani, A., J. Hosseiny, S. Semnanian, M. Javan, F. Saeedi, M. Kamalinejad and S. Saremi, 2000. Anti-nociceptive and anti-inflammatory effect of *Eleagnus angustifolia* fruit extract. *J. Ethnopharmacol.*, 72: 287-292.
- Akindele, A.J. and O.O. Adeyemi, 2007. Anti-inflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*. *Fitoterapia*, 78: 25-28.
- Amazu, L.U., C.C.A. Azikiwe, C.J. Njoku, F.N. Osuala, P.J.C. Nwosu and A.O. Ajugwo, 2010. Antiinflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals. *Asian Pac. J. Trop. Med.*, 3(11): 884-886.
- Anoka, A.N., B. Adzu, A. Amon, D. Byarugaba, S. Diaz-Llera and D. Bangsberg, 2008. The analgesic and antiplasmodial activities and toxicology of *Vernonia amygdalina*. *J. Med. Food*, 11(3): 574-580.
- Asuquo, O.R., A.O. Igiri, J.E. Akpan and M.I. Akpaso, 2010. Cardioprotective potential of *vernonia amygdalina* and *ocimum gratissimum* against streptozotocin (Stz)-induced diabetes in wistar rats. *Int. J. Trop. Med.*, 7(1), DOI: 10.5580/907.
- Bittar, M., M.M. de Souza, R.A. Yunes, R. Lento, F. Delle Monarche and V. Cechinel-Filho, 2000. Antinociceptive activity of 13, II8-binaringenin: A biflavonoid present in plants of the guttiferace. *Planta Med.*, 66: 84-86.
- Bonsi, M.L.K., P.O. Osuji, A.K. Tuah and N.N. Umunna, 1995. *Vernonia amygdalina* as a supplement to teff straw fed to ethiopian menz sheep. *Agroforestry Syst.*, 31(3): 229-241.
- Buttler, G.W. and R.W. Bailey, 1973. *Chemistry and Biochemistry of Herbage*. Academic Press, London and New York.
- David, M., M.D. Aronoff, G. Eric and M.D. Neilson, 2001. Antipyretics: Mechanisms of action and clinical use in fever suppression. *AJM*, 111(4): 304-315.
- Di-Silvestro, R.A. and J.T. Marten, 1990. Effects of inflammation and copper intake on rat liver and erythrocyte Cu-Zn superoxide dismutase activity levels. *J. Nutr.*, 120(10): 1223-1227.
- Foyet, H.S., B.A. Abdou, R. Ponka, A.E. Asongalem, P. Kamtchouing and V. Nastasa, 2011. Effects of *Hibiscus asper* leaves extracts on carrageenan induced oedema and complete Freund's adjuvant-induced arthritis in rats. *J. Cell Anim. Biol.*, 5: 69-75.

- Gestetner, B., Y. Birk and Y. Tencer, 1968. Soybean Saponins: Fate of Ingested Soybean Saponins and the Physiological aspect of their Hemolytic Activity. *J. Agric. Food Chem.*, 16: 1031-1035.
- George, A.J., 1965. Legal status and toxicity of saponins. *Food Chem. Toxicol.*, 3: 85-91.
- Georgewill, O.A., U.O. Georgewill and R.N.P. Nwankwoala, 2010a. Antiinflammatory effects of *Moringa oleifera* leaf extract in rats. *Asian Pac. J. Trop. Med.*, 3(2): 133-135.
- Georgewill, O.A. and U.O. Georgewill, 2010b. Antiarthritic activity of *Vernonia amygdalina* in albino rats. *Plant Prod. Res. J.*, 14: 36-38.
- Güçlü-Ustündağ, O. and G. Mazza, 2007. Saponins: Properties, applications and processing. *Crit. Rev. Food Sci. Nutr.*, 47(3): 231-258.
- Harborne, J.B., 1973. *Phytochemical Methods*. Chapman and Hall, London, pp: 113.
- Hostettmann, M. and A. Marston, 1986. *High Pressure Liquid Chromatography: Preparative Chromatography Techniques*. Springer-Verlag, New York Heidelberg, Berlin, pp: 56-61.
- Ibrahim, N.D.G., E.M. Abdurahman and G. Ibrahim, 2001. Elementary analysis of the leaves of *Vernonia amygdalina* and its biological evaluation in rats. *Nig. J. Nat. Prod. Med.*, 5: 13-16.
- Igbe, I., P.F. Ching and E. Aigbe, 2010. Anti-inflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* k. schum in acute and chronic inflammation. *Acta Polon. Pharmaceut. Drug Res.*, 67(1): 81-85.
- Ijeoma, U.F., S.O. Aderonke, O. Ogbonna, M.A. Augustina and I. Chijioke-Nwauche, 2011. Antinociceptive and anti inflammatory activities of crude extracts of *Ipomoea involucreta* leaves in mice and rats. *Asian Pac. J. Trop. Med.*, 4(2): 121-124.
- Iwalokun, B.A., B.U. Efedede, J.A. Alabi-Sofunde, T. Oduala, O.A. Magbagbeola and A.I. Akinwande, 2006. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *J. Med. Food*, 9(4): 524-530.
- Iwalokun, B.A., S.B. Bamiro and O.O. Durojaiye, 2004. An antimicrobial evaluation of *Vernonia amygdalina* (*Compositae*) against gram-positive and gram-negative bacteria from Lagos, Nigeria. *W. Afr. J. Pharmacol. Drug Res.*, 19: 9-15.
- Izevbogie, E.B., 2005. *Phytochemotherapy for cancer*. United States, Patent: 6, 849, 604; Issued: February 1.
- Jaijoy, K., N. Soonthornchareonnon, A. Panthong and S. Sireeratawong, 2010. Anti-inflammatory and analgesic activities of the water extract from the fruit of *Phyllanthus emblica* Linn. *Int. J. Appl. Res. Nat. Prod.*, 3(2): 28-35.
- Junping, K., N. Yun, N. Wang, L. Liang and H. Zhi-Hong, 2005. Analgesic and anti-inflammatory activities of total extract and individual fractions of Chinese medicinal ants *Polyrhachis lamellidens*. *Biol. Pharmaceut. Bull.*, 28: 176-180.
- Kim, H.P., K.H. Son and S.S. Kang, 2004. Anti-inflammation plant flavonoids and cellular action mechanism. *J. Pharmacol. Sci.*, 96: 229-245.
- Koko, W.S., M.A. Mesaik, S. Yousaf, M. Galal and M.I. Choudhary, 2008. In vitro immunomodulating properties of selected Sudanese medicinal plants. *J. Ethnopharmacol.*, 118: 26-34.
- Liu, J. and T. Henkel, 2002. Traditional Chinese Medicine (TCM): Are polyphenols and saponins the key ingredients triggering biological activities? *Curr. Med. Chem.*, 9: 1483-1485.
- Michael, T. and N.D. Murray, 1996. *The Encyclopaedia of Nutritional Supplements: The Essential Guide for Improving your Health Naturally*. In: Bowman, B. and R. Russel (Eds.), *Present Knowledge in Nutrition*, pp: 159-180, Retrieved from: www.organicfoodee.com/vms/magcit-vitaminB6.
- Mohamed, S.T.K., A.K. Azeem, C. Dilip, C. Sankar, N.V. Prasanth and R. Duraisami, 2011. Anti inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees. *Asian Pac. J. Trop. Biomed.*, 4(2): 147-149.
- Muir, A.D., D. Paton, K. Ballantyne and A.J. Aubin, 2002. Process for recovery and purification of saponins and saponinogens from quinoa (*Chenopodium quinoa*). U.S. Pat. No. 6, 355, 249.
- NIH (National Institute of Health), 1978. *Guide for the Care and Use of Laboratory Animals* (revised). 1978. NIH Publication No. 83-23. National Institute of Health, Bethesda, MD.
- Nwangwu, S., D.O. Adesanya, S. Josiah, O.F. Amegor, H. Njoya and A.A. Oladele, 2011. The effects of aqueous and ethanolic leaf extracts of *Vernonia amygdalina* on some vital organs in adult Wistar rats. *J. Cell Molecul. Biol.*, 9(1): 75-82.
- Obadoni, B.O. and P.O. Ochuko, 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in edo and delta states of Nigeria. *Glob. J. Pure Appl. Sci.*, 8: 203-208.
- Ojiako, O. and H. Nwanjo, 2006. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in Rats. *Afr. J. Biotechnol.*, 5(18): 1648-1651.
- Okokon, J.E. and M.I. Onah, 2004. Pharmacological studies on root extract of *Vernonia amygdalina*. *Nig. J. Prod. Med.*, 8: 59-61.
- Ravelo-Calzado, Y., V. Molina-Cuevas, S. Jiménez-Despaine, Y. Pérez-Guerra, A. Oyarzábal-Yera, D. Carbajal-Quintana and R. Mas-Ferreiro, 2011. Effects of D-002 on xylene-induced oedema in ear of mice. *Revista CENIC Sci.*, 42(1): 13-16.

- Shi, J., A. Konesh, D. Yeung, Y. Kakuda, G. Mittal and Y. Jiang, 2004. Saponins from edible legumes: Chemistry, processing and health benefits. *J. Med. Food*, 7(1): 67-78.
- Singh, J., 2008. Maceration, Percolation and Infusion Techniques for Extraction of Medicinal and Aromatic Plants. In: Handa, S.S., P.S. Khanuja, G. Longo, D.D. Rakesh (Eds.), *Extraction Technologies for Medicinal and Aromatic Plants*. ICS-UNIDO, Lucknow, pp: 67-82.
- Stil, W.C., M. Kahn and A. Mitra, 1978. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.*, 43: 2923-2925.
- Taiwo, I.A., P.G.C. Odeigah and L.A. Ogunkanmi, 2009. The glycaemic effects of *Vernonia amygdalina* and *V. tenoreana* with tolbutamide in rats and the implications for the treatment of diabetes mellitus. *J. Sci. Res. Dev.*, 11: 122-130.
- Trease, G. and M. Evans, 1983. *Textbook of Pharmacognosy*. 12th Edn., Bailliere Tindall, London, pp: 343-383.
- Walter, F., 2003. *Medical physiology: A cellular and molecular approach*. Elsevier/Saunders, 58: 1300.
- Wang, D., W. Tang, G.M. Yang and B.C. Cai, 2010. Anti-inflammatory, antioxidant and cytotoxic activities of flavonoids from *oxytropis falcata* Bunge. *Chin. J. Nat. Med.*, 8(6): 461-465.
- World Health Organization, 2005. *National policy on traditional medicines and regulation of herbal medicines*. Report of a WHO Global Survey, Geneva.