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ANTIPYRETIC AND ANTINOCICEPTIVE PROPERTIES OF THE AQUEOUS EXTRACT AND SAPONIN FROM AN EDIBLE VEGETABLE: *VERNONIA AMYGDALINA* LEAF

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ABSTRACT

Vernonia amygdalina is commonly used for food and health purposes. The processing of the leaf for food is usually aimed at removing bitter tasting principles like saponins. This study was designed to determine the antipyretic and antinociceptive properties of the aqueous extract, crude saponin and the chromatographic fraction of the crude saponin from the leaf. In the antipyretic evaluation, anal temperature change in fasted rats with prior induced pyrexia using intra-peritoneal administered 15% $^{w}/_{v}$ *Saccharomyces cerevisiae*, was measured four hours after dose administration. In the antinociceptive evaluation, 0.6% acetic acid solution administered by intra peritoneal route at a dose of 15 ml/kg was used to induce writhing in the writhing test; hot plate at 55 °C ± 1 to induce thermal pain in the hot plate test; and cold mixture of water and ethylene glycol (1:1) maintained at -10 °C to initiate pain sensation in the cold tail flick tests. Antipyretic data showed significant ($P \leq 0.05$) anal temperature decrease for all the test doses compared to the placebo. This was markedly observed with the aqueous extract at 0.55 ^{o}C ± 0.03 anal temperature decrease, compared to the 0.29 ^{0}C ± 0.01 of the crude saponin, at a similar dose level of 400 mg/kg. At 200 mg/kg dose, the crude saponin induced an anal temperature decrease of 0.14 ^{0}C ± 0.02. This was higher than the response obtained with the chromatographic fraction which produced 0.06 ^{0}C ± 0.01 anal temperature decrease. Antinociceptive data was significant ($P \leq 0.05$) for the crude saponin in the writhing (52.58 % antinociceptic effect) and hot plate tests (14.24 maximum percent analgesia), contrary to observation in the cold tail flick test. Findings showed the antipyretic and non-steroid like antinociceptive property of the crude saponin, which may support the rationale for the use of *Vernonia amygdalina* leaf to reduce fever and/or pain.

Key words: Anal temperature, writhing, pain, pyrexia

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INTRODUCTION

Some vegetables commonly eaten as food have been shown to be beneficial in herbal therapy [1]. *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 [2]. It 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). The leaf is used for both its nutritional and therapeutic benefits [3]. In Southern Ethiopia, dairy farmers feed the leaf to their stock during the dry period, after boiling to decrease the bitter taste. Prior study showed that feed intake, body weight gain and feed efficiency were not affected when the leaf was used to replace 300 g/ kg of maize-based diet fed to broilers [2].

Numerous phytochemicals have been shown to be present in the leaf extract of this plant [3]. The bitter taste of the leaf has been attributed to the presence of anti-nutritive principles like saponins, alkaloids, tannins and glycosides [4]. Leaf processing for human consumption, which involves the soaking and washing in warm water tends to reduce these bitter tasting principles.

Saponins are glycosides widely distributed in plants. They are characterized by the number of sugar chains attached to the triterpene or steroid aglycone backbone, also called sapogenin. While evidence has shown that the main dietary sources of saponins are the dicotyledon like legumes, the main non-food sources used in health and industrial applications include the soap bark tree, *Medicago sativa*, *Aesculus hippocastanum* and *Glycyrrhiza glabra* [5].

Saponins in foods have traditionally been considered bitter and unpleasant thereby limiting their use [3]. Most of the earlier studies on processing techniques targeted their removal to facilitate human consumption of the resulting product [6]. However, the food and non-food sources of saponins have come into renewed focus in recent years due to increasing evidence of their health benefits [7]. Recent evidence highlighted saponin contribution to the health benefit of some foodstuffs, and also as one of the active components in some herbal medicines [8, 9]. The major objective of this study was to investigate the antinociceptive and antipyretic properties of the aqueous extract, crude saponin and the chromatographic fraction of crude saponin (Va-SB: *Vernonia amygdalina* saponin fraction B) from *Vernonia amygdalina* leaf.

MATERIALS AND METHODS

Chemicals, Drugs and Test Agents:

Analytical grades of methanol, n-butanol, diethyl ether, chloroform, acetone, and xylene from BDH, Uganda and acetic acid (Sigma-Aldrich, Germany) were used. Active dry *Saccharomyces cerevisiae*: brewer's yeast (Griffchem®) was used to induce pyrexia in the antipyretic study. A dose of 300 mg/kg acetylsalicylic acid (ASA) (Pinewood, Caprin®) as reference standard, 5 ml/kg normal saline solution (Albert David, India) as placebo, *V. amygdalina* leaf aqueous extract and crude

saponin as test samples were used in all the experiments. Morphine sulphate (Ministry of Health Molago, Kampala) as opioid reference standard at a dose of 5 mg/kg was used in the antinociceptive study while Va-SB (*V. amygdalina* saponin fraction B), was used as one of the test samples in the antipyretic study.

Plant Material and Extraction

The fresh leaves of *Vernonia amygdalina* identified by a botanist were collected in the morning, between June and July (raining season) in south-western Uganda region. Specimens were retained with voucher number 16-20 in the Pharmacy Department, Faculty of Medicine, Mbarara University of Science and Technology. The leaves were shade air-dried and ground into a coarse powder that was sieved to 2000 g fine powder. The moistened powder was allowed to stand for 15 minutes before maceration for three hours in warm (<80 ^{o}C) distilled water at a modified ratio of 131 g to 9.0 litres with intermittent shaking [10]. The obtained infusion was filtered through filter-paper while warm. The filtrate was further filtered using buckner filter assemblage (aided by a suction pump) and subsequently evaporated to dryness in an oven (at $\leq$ 80^{o}C). The total amount of yield (leaf residue) obtained was 18 %. This was stored in a desiccator at room temperature (25 ^{o}C) until required for use.

Phytochemical Screening

Preliminary screening of the aqueous extract for phytochemicals was carried out using standard procedures [11]. Test procedures carried out were, foaming assay for saponins; dragendorff's test for Alkaloids; baljet test for sophisticated lactones; liebermann-burchard test for triterpenes and steroids; fehling test for reducing sugars; ninhydrine test for amino acids; shinoda assay for flavonoids; borntragers test for quinines; salkowski test for terpenoids; ferric chloride test for tannins; and kedde's assay for cardiotonic glycosides.

Isolation and Fractionation of crude Saponin

ISOLATION: The liquid-liquid extraction technique as described by Obadoni and Ochuko in 2001 [12] 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 ^{0}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 [11].

FLASH COLUMN CHROMATOGRAPHIC 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^{0}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 amgstrom, 30-70 mesh) containing flash column [13]. The column was eluted with a gradient mobile phase solvent system of increasing polarity starting with xylene; combination of chloroform and

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methanol (one to one); and methanol, in multiples of 100 ml. An air pump (Merck, Germany) was used to facilitate the rate of elution. Each 100ml 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) [14]. 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 using foaming test was conducted [11]. Va-SB was positive and was preserved in a desiccator for further use.

Animals

Twenty five Wistar rats weighing between 120-200 g (antipyretic study) and 25 albino mice weighing between 20-25 g (antinociceptive study) of both sexes were placed in standard cages where they were maintained on standard animal pellets (Nuvita Feeds Ltd., Kampala) and water *ad libitum*. The rats for antipyretic evaluation were selected based on their measured basal anal temperature ($\leq$ 37 ^{0}C) using a thermistor probe (with a resolution of 0.0005 and accurate to 0.001 ^{o}C), and also achieving a minimum of 0.1 ^{0}C increase in anal temperature in response to 10 ml/kg intra-peritoneal (*i.p*) administered 15% $^{w}/_{v}$ *Saccharomyces cerevisiae*. The animals for the two evaluations (antipyretic and antinociceptive) were randomly divided into five groups of five animals each and fasted over night.

National Institute of Health (NIH) guide for the care and use of laboratory animals approved by the Institutional Ethical Committee was adopted for the animal protocol in this study [15].

Preparation and Administration of Drug

All the standards and tests samples were prepared using sterile normal saline solution which also, served as placebo in the different experiments. Oral administration was carried out using a canula-syringe assemblage.

Antipyretic Study

Standard procedure as described by previous study was adopted for this study [16]. Wistar rats earlier selected and induced pyrexia as described above, were randomly divided into five groups of five animals each. The animals were allowed to starve overnight in their respective cages. Twenty hours after the administration of *Saccharomyces cerevisae* solution, the anal temperature of each animal in their respective group was measured. Oral administration of normal saline to group one, ASA to group two and aqueous extracts (400 mg/kg, 600 mg/kg or 800 mg/kg) to groups three to five was immediately carried out after the anal temperature measurement. Four hours after the administration of the dose, the anal temperature of each animal was measured again.

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Similar antipyretic evaluation procedure was adopted for the crude saponin using three dose levels: 200, 400 and 600 mg/kg of crude saponin; and for the Va-SB also, using 200, 300 and 400 mg/kg of the chromatographic fraction, Va-SB as test doses. Doses were based on prior study of crude saponin content in *V. amygdalina* leaf [17].

Antinociceptive Study

WRITHING TEST: Following literature techniques, 0.6% acetic acid solution administered by intra peritoneal (i.p) route at a dose of 15 ml/kg was used to induce writhing (typical abdominal constriction response consisting of abdominal wall contraction, pelvic rotation and hind limb extension) 30 minutes after oral administration of acetylsalicylic acid (non-opiate reference standard), morphine sulphate (opiate reference standard), normal saline (placebo), *V. amygdalina* aqueous extract (800 mg/kg) or crude saponin (600 mg/kg) to the mice. Each mouse was returned to the cage [18]. Five minutes after the administration of acetic acid, writhing counts were taken for five minutes under a double blind observation. Antinociceptive effects of the standard and test samples were measured by comparing their mean abdominal writhes count to the mean writhe count of the placebo group

$$[(c - t \text{ or } s)/c] \times 100$$

where c is mean writhes count for placebo; t or s is mean writhes count for any of the tests or standards.

HOT PLATE TEST: Prior to dose administration, the mice were selected on the basis of their basal reaction time (fore paw licking or jumping off) when placed on the hot plate which was maintained at 55 °C ± 1. Animals with ≤ 8 seconds reaction time were regarded as appropriate for inclusion [19]. Sixty minutes after oral administration of acetylsalicylic acid, morphine sulphate, normal saline, *V. amygdalina* aqueous extract (800 mg/kg) or crude saponin (600 mg/kg), to the mice in the respective groups, the mice were exposed to the hot plate. The Time taken for either fore paw licking or jumping off the hot plate was documented as reaction time. The group reaction time was taken as mean of the reaction times. Fifteen seconds was taken as cut-off period to avoid damage to paws. Maximum possible analgesia (MPA) was calculated as follows [20].

$$[(T_1-T_0) / (15- T_0)] \times 100$$

T_1 is mean reaction time for standard or test; T_0 is mean reaction time for placebo.

COLD TAIL FLICK TEST: The mice were selected for this study based on their latent period of tail-flick response. A modified literature procedure was followed [21]. Prior to dose administration, two cm length of the animal's tail was immersed into a cold one to one mixture of water and ethylene glycol maintained at -10 °C with an ultra low temperature freezer (UF 650 Napcoil, Thermo Electric Corporation). Animals with response ≤ 15 seconds were selected. Sixty minutes after oral administration of acetylsalicylic acid, morphine sulphate, normal saline, *V.*

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amygdalina aqueous extract (800 mg/kg) or crude saponin (600 mg/kg), animals were similarly exposed to the mixture. The response for each animal was taken as the elapsed time for tail deflection from the mixture. Three different reaction time readings were taken for each mouse. The first reading was discarded and the mean of the last two readings taken as the reaction time. The response for each group was as the mean of the reaction times of the five mice. Using 30 seconds as the reaction cut-off period to avoid damage to the tail, the MPA was calculated as

$$[(T_1-T_0) / (30- T_0)] \times 100$$

T_1 is mean reaction time for standard or test; T_0 is mean reaction time for placebo.

Statistical Analysis of Data

Results and calculations were based on the numerical expression of data as mean ± SEM. 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.

RESULTS

Phytochemical Screening

Standard test for phytochemical constituents revealed the presence of saponins, alkaloids, sophisticated lactones, triterpenoids, reducing sugars, amino acids, flavonoids, terpenoids, tannins and cardiotonic glycosides but absence of quinine.

Antipyretic Study

The antipyretic study showed a dose dependent response with the extract (table 1), crude saponin (table 2) and Va-SB fraction (table 3). Statistical analysis using ANOVA indicated no significant ($P \leq 0.05$) difference in the yeast induced anal temperature within and among groups. T-test analysis indicated significant ($P \leq 0.05$) dose induced anal temperature decrease for all the test samples in the different evaluations. However, at similar dose levels of 400 (figure 1) and 200 mg/kg (figure 2) crude saponin produced higher anal temperature decrease than the Va-SB fraction.

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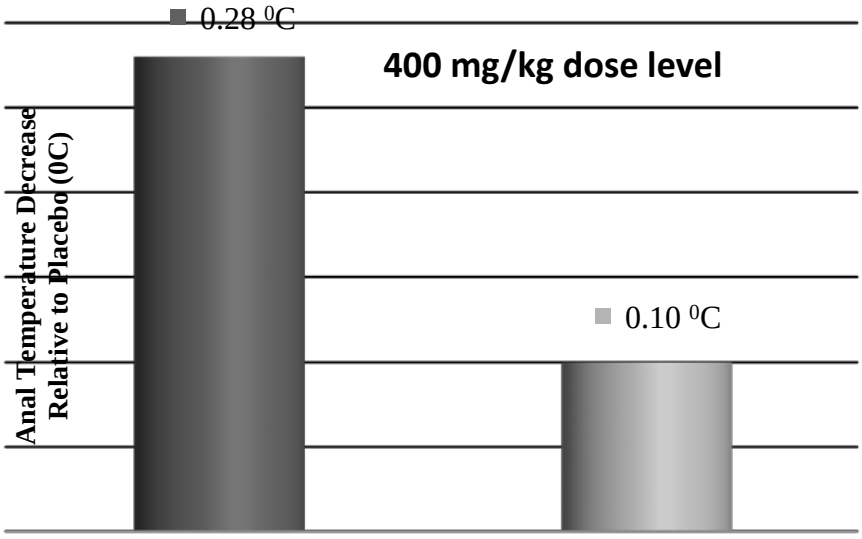

Figure 1: Comparing the relative induced anal temperature decrease of the crude saponin and *Vernonia amygdalina* Saponin fraction B (Va-SB) in rats at 400 mg/kg dose level

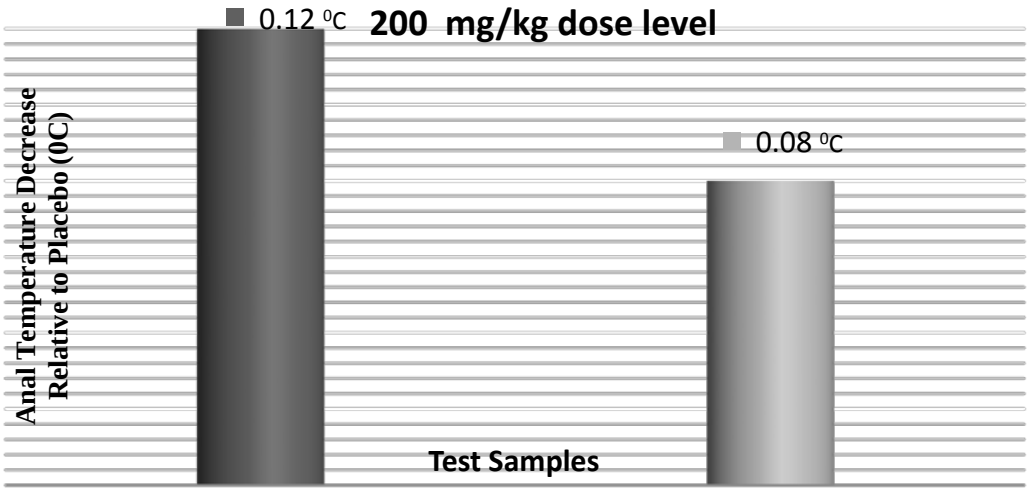

Figure 2: Comparing the relative induced anal temperature decrease of the crude saponin and *Vernonia amygdalina* saponin fraction B (Va-SB) in rats at 200 mg/kg dose level

Antinociceptive Study

Writhing Test: The acetic acid induced writhe count data for standards, aqueous extract and crude saponin doses were significantly ($P \leq 0.05$) different from that of normal saline (placebo). The antinociception effect was markedly observed with morphine sulphate at 89.69% and ASA at 86.60% (table 4).

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Hot Plate Test: Relative to the placebo (normal saline) group, result obtained with this test showed significant ($P \leq 0.05$) reaction time for all the standards and test samples. Lower data were obtained with the crude saponin at 14.24% MPA (table 5).

Cold Tail Flick Test: Reaction time obtained with the cold tail flick test was significant ($P \leq 0.05$) for morphine sulphate and the aqueous extract. Acetylsalicylic acid at 8.17% MPA showed a marginal antinociceptive property (table 6).

DISCUSSION

Vernonia amygdalina leaf is used by local communities in Africa and Arabian Peninsula. The use of the leaf as food has always required a prior processing leading to the loss of possible medicinal principle [22]. In the antipyretic study, brewer's yeast was used as an exogenous stimulus to induce pyrexia; the mode of action may be similar to that reported for lipopolyssacharides in laboratory animals [23]. Such induction commonly activates the arachidonic acid pathway and has been associated with increase in prostaglandin E_2 (PGE_2) [24]. According to David *et al*. [25], inhibiting the arachidonic acid pathway, and specifically the activity of cyclo-oxygenase, may result in the reduction of PgE_2 level within the hypothalamus [25]. Such inhibitory activity ultimately decreases elevated body temperature [25]. The antipyretic activity data obtained in the present study indicated that the aqueous extract, crude saponin and Va-SB at the doses used significantly ($P \leq 0.05$) decreased the yeast induced anal temperature in the rats. This suggests that the test samples just like the ASA standard possess inhibitory activity against the arachidonic acid pathway.

At the same dose levels of 400 and 600 mg/kg, the data indicated higher anal temperature decrease (0.55 ^{o}C and 0.91 ^{o}C) for the leaf extract used in this study, compared to the observed 0.32 ^{o}C and 0.85 ^{o}C obtained with the root extract of the plant by Okokon and Onah [16].

Data obtained for crude saponin and its chromatographic fraction, Va-SB at similar dose levels (figure 1 and figure 2) suggest the presence of other principle(s) in the crude saponin. Such principle(s) like flavonoid compounds which could have been lost during the chromatographic process, may have antipyretic or potentiating effect as reported by a prior study [26]. However, no test was carried out to determine the extent of difference in the phyto-constitution between the crude saponin and Va-SB samples.

The antinociceptive study was carried out using the writhing, hot plate and cold tail flick test method. This approach was focused on determining and identifying the antinociceptive mechanism of the test samples. In the writhing test, intra peritoneal administration of 0.6% acetic acid induced pain in form of writhes in the mice. The acid produces this effect by stimulating the release of various endogenous pain mediators peripherally which excite the nociceptors [27, 28]. Obtained data showed that the aqueous extract and crude saponin, like the opioid and non-opioid control

standards, were able to significantly ($P \leq 0.05$) decrease the number of writhes obtained. This observation suggests the inhibition of the effects of the endogenous pain mediators by these test samples. However, writhing test is limited by its lack of bias for either central or peripheral activity [28].

In hot plate test, thermal pain has been demonstrated to be induced in smaller animals like mice at 55 °C ± 1. Such pains are known to be initiated via the Aδ fibers through the neospinothalamic pathway [27]. In this evaluation, obtained antinociceptive data for the aqueous extract were significantly ($P \leq 0.05$) different from the placebo. At a reaction time of 3.02 seconds relative to the placebo, the extract produced lesser activity compared to the opioid standard, morphine sulphate (6.52 seconds). Marginal increase in reaction time which was also significantly ($P \leq 0.05$) different from the placebo was observed with the crude saponin. However, while findings may suggest the inhibition of the neospinothalamic pathway (central activity) by the extract, data obtained in this study cannot strongly support the central niociceptive inhibitory activity of the crude saponin. The hot plate technique have bias for centrally acting antinociceptive agents, but it is also known to lack sufficient sensitivity in distinguishing between opiates and non-opiates with central niociceptive inhibition [20].

The cold tail flick test has been demonstrated to sufficiently identify opiates from non-opiates with central activity [20]. Data in the cold tail flick test showed that the aqueous extract produced increased reaction time which was significantly ($P \leq 0.05$) different from the placebo (normal saline). This observation was similar, although lower than data obtained with opioid standard, morphine sulphate.

The findings of the antinociceptive activity test supports previous data on the peripheral and central activity of *Vernonia amygdalina* leaf aqueous extract [28]. However, data obtained in this study gave a strong indication that the crude saponin constituent could be a major participant in the peripheral antinociceptive activity of the aqueous extract. This agrees with an earlier *in vivo* study with *Vernonia cinerea* that suggests the presence of phytochemicals like saponins to be responsible for the observed antinociceptive property [29].

Generally, saponins are considered to be toxic with majority being powerful haemolytics *in vitro*. However, large doses may be needed to produce toxic effect when administered parenterally and larger doses due to poor absorption, when administered orally. Several toxicity tests has shown that the lethal oral dose of saponins is 3–1000 times as large as the lethal intravenous dose
[30]. As such, toxicity of saponins to warm blooded animals is dependent on the method of administration, source, composition, and concentration of the saponin mixture [8]. Data obtained from 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, by enzymatic action in the gastrointestinal tract [5]. The readily more absorbable aglycone is because of this reason, usually considered responsible for most of the associated activities with the

orally administered saponins. This may explain the safety of orally administered *Vernonia amygdalina* extract or products [30].

In conclusion, the result obtained in this study indicates that the aqueous extract of *Vernonia amygdalina* leaf possesses antipyretic and antinociceptive activity. The crude saponin and Va-SB fraction indicate that saponins are major participants in these activities. The Va-SB fraction should be studied further for identifiable pure component(s) that may be associated with the observed antipyretic and antinociceptive activities.

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AUTHOR(S) DISCLOSURE STATEMENT
No competing financial interests exist.

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Table 1: Effect of the aqueous extract on the anal temperature in rats 4 hrs after Administration

Group	Dose	20 hrs after yeast administration the anal Temp.^{0}C readings are		Dose Induced Anal Temp.^{0}C Decrease
		0 hrs (after Dose admin.)	4 hrs (after Dose admin.)	
1. Placebo	Normal saline 5 ml/kg	39.02 ± 0.11	39.01 ± 0.11	0.02 ± 0.02
2. Standard	ASA 300 mg/kg	39.12 ± 0.10	38.97 ± 0.07	*0.15 ± 0.04 (P = 1.65x10^{-6})
3. Test 1	Extract 400 mg/kg	39.21 ± 0.18	38.66 ± 0.17	*0.55 ± 0.03 (P = 5.3x10^{-10})
4. Test 2	Extract 600 mg/kg	39.09 ± 0.20	38.18 ± 0.15	*0.91 ± 0.09 (P = 6.8x10^{-}6)
5. Test 3	Extract 800 mg/kg	39.08 ± 0.13	37.58 ± 0.19	*1.50 ± 0.20 (P = 6.7x10^{-7})

Data are mean ± SEM (standard error of mean) value (n = 5). *Significantly ($P \leq$ 0.05) different from placebo

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Table 2: Effect of the crude saponin on the anal temperature in rats 4 hrs after Administration

Group	Dose	20 hrs after yeast administration, the anal Temp.^{0}C readings are		Dose Induced Anal Temp.^{0}C Decrease
		0 hrs (after Dose admin.)	4 hrs (after Dose admin.)	
1. Placebo	Normal saline 5 ml/kg	39.054 ± 0.12	39.04 ± 0.11	0.014 ± 0.02
2. Standard	ASA 300 mg/kg	39.10 ± 0.11	38.95 ± 0.08	*0.15 ± 0.04 ($P = 3.95x10^{-3}$)
3. Test	Crude saponin 200 mg/kg	38.75 ± 0.25	38. 62 ± 0.24	*0.14 ± 0.02 ($P = 2.4x10^{-4}$)
4. Test	Crude saponin 400 mg/kg	38.78 ± 0.19	38.49 ± 0.18	*0.29 ± 0.01 ($P = 1.2x10^{-4}$)
5. Test	Crude saponin 600 mg/kg	38.66 ± 0.11	38.29 ± 0.11	*0.39 ± 0.01 ($P = 1.06x10^{-5}$)

Data are mean ± SEM (standard error of mean) value (n = 5). *Significantly ($P \leq 0.05$) different from placebo

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Table 3: Effect of the *Vernonia amygdalina* chromatographic fraction, *Va*-SB on the anal temperature in rats 4 hrs after Administration

Group	Dose	20 hrs after yeast administration, the anal Temp.^{0}C readings are		Dose Induced Anal Temp.^{0}C Decrease
		0 hrs (after Dose admin.)	4 hrs (after Dose admin.)	
1. placebo	Normal saline 5 ml/kg	38.01 ± 0.29	37.96 ± 0.28	0.05 ± 0.04
2. Standard	ASA 300 mg/kg	37.84 ± 0.22	37.64 ± 0.23	*0.19 ± 0.01 ($P = 4.7x10^{-4}$)
3. Test	Va-SB 200 mg/kg	37.87 ± 0.27	37.81 ± 0.27	*0.06 ± 0.01 ($P = 3.1x10^{-3}$)
4. Test	Va-SB 300 mg/kg	37.83 ± 0.28	37.69 ± 0.28	*0.14 ± 0.01 ($P = 2.2x10^{-3}$)
5. Test	Va-SB 400 mg/kg	37.82 ± 0.25	37.67 ± 0.25	*0.15 ± 0.01 ($P = 1.45x10^{-3}$)

Data are mean ± SEM (standard error of mean) value (n = 5). *Significantly ($P \leq 0.05$) different from placebo

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Table 4: **Effect of *V. amygdalina* leaf aqueous extract and crude saponin in the acetic acid induced writhing test in mice**

Groups	Dose	Mean Writhes Count	% Antinocicepti c Effect
1. Placebo	Normal Saline 5 ml/kg	19.4 ± 2.51	0
2. Opiate Standard	morphine sulphate 5 mg/kg	*2 ± 0.71 ($P = 8.7x10^{-5}$)	89.69
3. Non-opiate Standard	300 mg/kg acetylsalicylic acid	*2.6 ± 0.55 ($P = 6.6x10^{-5}$)	86.6
4. Aqueous Extract	800 mg/kg	*5.6 ± 0.89 ($P = 7.1x10^{-5}$)	71.13
5. Crude Saponin	600 mg/kg	*9.2 ± 0.84 ($P = 1.1x10^{-3}$)	52.58

Data are mean ± SEM value (n = 5). *Significantly ($P \leq 0.05$) different from placebo

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Table 5: **Effect of *V. amygdalina* leaf aqueous extract and crude saponin in the thermal stimulus-induced hot plate test in mice**

Groups	Dose	Mean Reaction Time (sec)	MPA
1. Placebo	Normal Saline 5 ml/kg	5.49 ± 0.07	0
2. Opiate Standard	morphine sulphate 5 mg/kg	*9.62 ± 0.12 ($P = 6.2x10^{-8}$)	43.39
3. Non-opiate Standard	300 mg/kg acetylsalicylic acid	5.51 ± 0.49 ($P = 0.1.9x10^{-1}$)	0.21
4. Aqueous Extract	800 mg/kg	*7.90 ± 0.74 ($P = 8.4x10^{-4}$)	25.3
5. Crude Saponin	600 mg/kg	*6.84 ± 0.30 ($P = 2.7x10^{-4}$)	14.24

Data are mean ± SEM value (n = 5). *Significantly ($P \leq 0.05$) different from placebo.

MPA: maximum percent analgesia

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Table 6: **Effect of *V. amygdalina* leaves' aqueous extract and crude saponin in the thermal stimulus-induced cold tail flick test in mice**

Groups	Dose	Mean Reaction Time (sec)	MPA
1. Placebo	Normal Saline 5 ml/kg	19.72 ± 1.11	0
2. Opiate Standard	morphine sulphate 5 mg/kg	*26.24 ± 1.29 ($P = 1.6x10^{-4}$)	63.42
3. Non-opiate Standard	300 mg/kg acetylsalicylic acid	*20.56 ± 0.77 ($P = 0.008.2x10^{-3}$)	8.17
4. Aqueous Extract	800 mg/kg	*22.74 ± 0.90 ($P = 0.003.5x10^{-3}$)	29.38
5. Crude Saponin	600 mg/kg	19.04 ± 0.73 ($P = 1.4x10^{-1}$)	#-6.62

Data are mean ± SEM value (n = 5). *Significantly ($P \leq 0.05$) different from placebo.

MPA: maximum percent analgesia. # considered to be zero MPA

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