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Phytochemical profiling, acute toxicity and haemostatic effects of methanol leaf extract of *Acanthus montanus* (Nees) T. Anderson (Acanthaceae)

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Abstract

Acanthus montanus leaf is used in African trado-medicine as a haemostatic agent, which could be ascribed to its bioactive principles. This study evaluated phytoconstituents and haemostatic potential of *Acanthus montanus* methanol leaf extract in albino Wistar rats, as well as acute toxicity in mice. Methanol extract of *Acanthus montanus* was analysed for phytochemicals by standard protocol and High Pressure Liquid Chromatography (HPLC). Acute toxicity was determined using established method, while the haemostatic parameters were determined using tail bleeding time and blood clotting time in albino Wistar rats at 200, 400 and 800 mg /kg b.w.; administered by gavage once daily for nine days. Alkaloids, tannins, saponins, flavonoids, glycosides and steroids were inferred from the phytochemical screening. Nineteen phytoconstituents were identified and quantified via HPLC, with catechin and rutin showing documented evidence of haemostatic effect. *Acanthus montanus* was considered safe since no death or change in behaviour was observed at maximum dose of 5000 mg/kg b w. A significantly dose dependent decrease ($P<0.001$) in clotting and bleeding time was noted across the groups. The 800 mg/kg treated group was comparable to vitamin K (10mg/kg b w) treated group. This reaffirms the traditional use of *Acanthus montanus* leaf as haemostatic agent.

Keywords: Bleeding time; Clotting time; *Acanthus montanus*; Haemostasis; Acute toxicity; Phytochemicals

INTRODUCTION

Adequate perfusion of the organs with oxygen and nutrition are enabled by the blood in the cardiovascular system [1]. Cuts and wounds are basic unavoidable incidences in human life, which could amount to bleeding thus leading to life threatening consequences. The result of severe blood loss includes but not limited to multiple organ failure, shortness in oxygen and nutrition, infections and ultimately death [2]. Haemorrhage can be described as the loss of blood and its components from the vascular system, which could be as a result of trauma or abnormal increase in the pressure within the blood vessels. Its aetiology varies with the socioeconomic prospect and lifestyle

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of the individual [3]. Haemorrhage can be grouped based on their different anatomical locations; skull, chest, abdomen and retro peritoneum [4]. Haemorrhage from external wounds has been reported to be accountable for about 35% of before hospital admission mortality and more than 40% of death after 24 h of hospital admission [5]. Thus, prevention of haemorrhages and its attendant complications have become necessary to reduce mortality.

Haemostasis is the natural process of the body in preventing and stopping bleeding, which may involve several enzymatic and cascade steps, with vascular spasms and formation of platelet plug which culminates into coagulation [6]. In the face of severe injury and blood loss, the body's natural process of haemostasis may become overwhelmed and the use of haemostatic agents becomes mandatory. However, some of the available haemostatic agents; aprotinin, desmopressin, epsilon-aminocaproic acid and tranexamic acid have been shown to produce untoward effects such as hyponatremia, hypertension and tachycardia [7]. This has led to the continuous search for newer drugs with better efficacy, cost effective, safe and readily accessible.

Bleeding disorders are now being treated globally using medicinal plants. Herbs with substantiated evidence of haemostatic effects have been documented [8]. Plants with medicinal properties are veritable sources of new drug candidates. The presence of biologically active principles (phytochemicals) may be responsible for their acclaimed effectiveness in treatment and management of diseases. *Acanthus montanus* (Nees) T. Anderson (Acanthaceae) is one of such medicinal plants. It originated from tropical Africa, though have become widely apportioned in Eastern Europe and Mediterranean. *Acanthus montanus* is a prickly little shrub with thinly branches and tender stem, commonly called mountain thistle [9]. In southern eastern (Igbo speaking) Nigeria, it is known as Agamebu, Agamsoso, Agamefu and Ogwu-aga (name varies according to dialects) [10]. It is reported to be one of the species that is underutilized and has almost gone into extinction [11]. It grows to a height of 90 cm tall with spikes of pink flowers and the leaves occurs as basal clusters of oblong lance-shaped, shiny with deep green colour, having silvery marks with wavy margins [12]. Roots, stems and leaves are used locally in traditional medical practice to treat various ailments [9].

Aqueous extract of *A. montanus* is traditionally utilized for the relief of pain, treatment of female infertility and as a remedy for threatened abortion [13]. In Cameroon, *A. montanus* is popular in reliving of cough, convulsion and cramp during menstruation, and for the prevention of miscarriages and pre-term labour. Some countries in West Africa, the leaves are used as vegetable in soups, to treat abdominal discomfort and indigestion [14]. In south-eastern Nigeria, the root is effective as a treatment for furuncles [9], while the leaves are used to bath to relieve aches and pains [15]. The poultice of the leaves is used to cover fresh bleeding wound or knife cut to arrest bleeding. It is one of the key ingredients in fever remedies especially febrile convulsions. *A montanus* is also popular in the treatment of pain in the urethra, endometritis and infections affecting urinary tract and the genitals [16]. Some of these traditional uses of this plant have been validated pharmacologically and documented, they include spasmolytic, anti-inflammatory, antirheumatic, antiulcer, digestive and vasoprotective properties [17,18].

Phytochemical screening of the leaf of *A. montanus* yielded alkaloids, flavonoids, steroids, saponins, terpenoids, glycosides and tannins [19]. Putative phytochemicals that have been identified from the leaves by GC-MS analysis include esters, fatty acids and alkaloids such as Benzoxazolone; 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and

2-(1-pyrrolidinyl)-bicyclo (3.3. 1) nonan-9-one [10]; N, N-dimethyl valeramide [20]. The compounds from the aerial parts of *A. montanus* have been characterized and include; acanmontanoside, decaffeoyl verbascoside, isoverbascoside, verbascoside, leucosceptoside A, (2R)-2-O-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one, (2R)-2-O-β-D-glucopyranoside and ebracteatoside B [21]. However, search in the literature has shown paucity of information regarding the determination of the phytochemical contents using HPLC analysis and the in-vivo haemostatic potential of the leaf extract of *A. montanus*. Thus, this study aims to profile the methanol leaf extract of *A. montanus* for phytochemicals using HPLC analyses and evaluate its haemostatic effect in experimental animals.

EXPERIMENTAL METHODS

***Acanthus montanus* collection, preparation and extraction.** Matured leaves (fresh) of *Acanthus montanus* were harvested by Ide Emeka Desmond Ezesuokwu in July, 2022 from Igbo-Ukwu (6^o 1’ 0’’N, 7^o 1’0’’E), Aguata Local Government Area of Anambra state, Southeast Nigeria. Identification was done by Dr. Felix I. Nwafor, (Plant Taxonomist, University of Nigeria Herbarium). Specimen number of UNN/11780 was issued and specimen was kept in the herbarium. The leaves were rinsed with clean running water to remove sand particles and other contaminants, air-dried under a shade at ambient temperature for two weeks and pulverized into powder using a locally fabricated hammer mill. This was then stored in an air-tight container prior to use.

The pulverized leaf (800g) was extracted by cold maceration in 96 % methanol (1L) for 72 h with intermittent shaking. The supernatant extraction mixture was filtered using Whatman filter paper size one. The resultant filtrate was concentrated in vacuo using a Rotary evaporator at 45^{o}C, to obtain the crude methanol leaf extract. The weight was noted and subsequently stored at 4^{o}C in a refrigerator until required.

Phytochemical Screening. The crude methanol leaf extract was screened for phytochemicals using standard documented methods. Test for alkaloids, tannins, carbohydrate, protein, steroids, saponins, flavonoids and glycosides were evaluated [22, 23].

High Pressure Liquid Chromatography Analysis. The crude extract of *A. montanus* was analysed using a Shimadzu HPLC with binary pump of two-fold (LC-10AD), a column (CTO-10AS) in an oven and an Ultra violet/Visible detector (SPD-20A). The column utilized was a C-12 phase column with thickness (5 µ), length (200 mm) and internal diameter (4.8 mm). The mobile phase A is composed of a mixture with 2.8 pH, made from deionized water in acetic acid, while acetonitrile was used as the second mobile phase (B) at 0.8 mL/min flow rate. Twenty minutes was used to balance the column by passing 5% of solvent B it, before each sample injection. Column temperature was kept constant (38^{o}C), while injecting 20 µL of the extract and detection wavelength was set at 280 nm. Qualitative and quantitative analyses was based on peak areas and retention times extrapolated from plot calibrated from external standard.

The mobile phase (A and B) were altered sequentially; within 0-5 min, solvent A (95-91 %): solvent B (5-9 %), 5-15 min, solvent A (91 %): solvent B (9 %), 15-22 min, solvent A (91-89 %), solvent B (9-11 %), 22-38 min, solvent A (89-82 %): solvent B (11-18%), 38-43 min, solvent A (82-77 %): solvent B (18-23 %), 43-44 min, solvent A (77-10 %): solvent B (23-90 %), 44-45 min, solvent A (10-2 %): solvent B (90-98 %), 45-55 min, solvent A (0 %): solvent B (100 %). Standards include proanthocyanin, lunamarin, kaempferol, anthocyanin, steroids, epicatechin, catechin, cyanogenic glycoside, naringenin, cardiac

glycoside, spartein, flavonones, flavan-3-ol, ribalinide, rutin, resveratrol, oxalate, phytate and sapogenin [24, 25].

Experimental animals. Both sexes of adult Wistar rats (110-150 g) and mice (15-25 g) were used for the study. They were bred in the Animal House facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele. Ethical approval (Pharm120241011) dated 2nd June 2022, was issued from the Office of the Research Ethnics Committee, Madonna University, Nigeria, following the request from our research group. The animals were fed with standard livestock pellets (Topfeed) and allowed free access to drinking water *ad libitum*. The animals were placed in steel cages and allowed fourteen (14) days acclimatization period on transfer to the study space at ambient temperature. The study was in compliance with the National Institute of Health (NIH) Guideline for tending and utilizing of laboratory animals (Pub No. 85-23 revised, 1985) [26].

Oral acute toxicity test in mice. Lorke's method [27] was used in evaluating the acute toxicity of the methanol leaf extract of *A. montanus* in mice. This was done in two stages. In the first stage, nine (9) mice were divided randomly into three (3) groups of three (3) mice per group. These were given the methanol leaf extract of *A. montanus* reconstituted in Tween 80 at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg b.w. respectively. The mice were observed for 24 h for any change in behaviour and mortality. Since no death or change in behaviour was observed, the second stage was commenced with separate set of mice in three (3) groups of one mouse per group and treated with 1600 mg/kg, 2900 mg/kg and 5000 mg/kg bw respectively. Behavioural changes and mortality were also monitored for 24 hours. All administrations were done by gavage.

Experimental protocol. A total of 25 healthy strains of Wistar rats of both sexes were used for this study. These were randomly divided into five groups of five rats each. Group A, B and C served as the treatment groups and received 200mg/kg, 400mg/kg and 800mg/kg b.w. respectively of the methanol leaf extract of *A. montanus* reconstituted with Tween 80. Group D served as the positive control and were treated with vitamin K 10mg/kg b.w. Group E was the negative control and received 1ml of distilled water across board. All administrations were by gavage once daily for a period of 9 days.

Bleeding time determination. The Duke's method [28] as described by Okoroiwu and co-workers [29] was used in this determination. Briefly, the tail of each rat from the respective group was disinfected with methylated spirit and subsequently infiltrated with plain lidocaine (1%). The animal was firmly held on a disinfected table and quickly a disposable lancet was used to cut off the tip of the tail (0.5 cm length) and immediately the stop clock was started as bleeding commenced. The cut was dabbed with filter paper every 15 seconds until there were no more blood stains on the filter paper. Bleeding time was then taken as the time when the bleeding started and when it stopped bleeding from the cut. Cut-off time was set at 120s. This procedure was done on day 3, 6 and 9 on every animal in each group [30].

Clotting time determination. The method by Ivy was utilized to evaluate the clotting time [29, 31]. A drop of blood from the animal was placed on a clean grease free glass slide with a stop watch started at the same time. A pin was passed every 15 seconds to check for the formation of a thread of fibrin and the stopwatch was stopped once thread of fibrin was noticed. This measurement was also achieved on day 3, 6 and 9 [30].

Statistical analysis. Results are presented as mean ± standard error of mean (SEM). One-

way analysis of variance (ANOVA) was used to analyze the data, followed by Dunnett's post hoc test. All analyses were carried out using GraphPad Prism software (version 5.01). $P \leq 0.05$ was considered statistically significant.

RESULTS

Phytochemical analysis. Qualitative phytochemical analysis of the methanol leaf extract of *A. montanus* revealed the presence of carbohydrates, reducing sugar, alkaloids, glycosides, saponins, tannins, proteins and flavonoids (Table 1).

Phytochemical profiling. The results revealed nineteen (19) compounds with retention time varying from 0.1900 min to 40.7060 min (Table 2). The compounds identified and quantified include proanthocyanin (7.2917 ppm), lunamarin (8.6196 µg/mL), cardiac glycoside (11.3284 µg/mL), anthocyanin (5.0215 µg/mL), ribalinidine (13.1597µg/mL), flavan-3-ol (7.5576 ppm), rutin (9.0117µg/mL), naringenin (2.2881µg/mL), cyanogenic glycosides (5.0080µg/mL), spartein (9.5901µg/mL), flavonones (10.9228ppm), steroids (14.6479 ppm), kaempferol (3.3761 µg/mL), epicatechin (20.5907µg/mL), phytate (12.3475µg/mL), resveratrol, (20.6849 ppm), oxalate (27.5258 µg/mL), catechin (1.1328 µg/mL) and sapogenins (21.7710 µg/mL)

Acute toxicity. The acute toxicity test revealed that the methanol leaf extract of *A. montanus* is safe at the maximal administered dose (LD_{50} >5000 mg/kg b. w.) There was no notable change in behaviour, breathing, cutaneous changes and mortality was recorded post administration of the methanol leaf extract of *A. montanus*.

Pharmacological activities. On the 3rd day of treatment (Figure 1), there was a significant reduction in the bleeding time and clotting time on comparing varying doses of the extract with the negative control (distilled water). The 800 mg/kg b. w showed significant reduction ($P \leq 0.05$) in bleeding time and clotting time better than the vitamin K treated group (positive control). The 400 mg/kg b. w treated group also produced a non-significant lower clotting time than vitamin K.

On the 6th day of treatment, there was a significant reduction in the mean bleeding time and clotting time at all dose levels (Figure 2). There was an improvement on the reduction in bleeding time and clotting by the 200 mg/kg, 400 mg/kg and 800 mg/kg b w of the extract. The 800 mg/kg was still comparable to the positive control (10 mg/kg of vitamin K). There was also a reduction in the clotting time and bleeding time of the control group (I ml of distilled water). This is essentially because of recruitment of the body's normal defence. The reductions in bleeding time and clotting time across the treatment groups however, remained dose-dependent ($P \leq 0.05$).

On the 9th day, at all dose levels, the significant ($P \leq 0.05$) reduction in bleeding time and clotting time appears to be the same (Figure 3). This shows the effect of the extract as well as the internal defence mechanism which may have been signalled.

DISCUSSION

Phytochemical screening of the methanol leaf extract revealed the presence of phytochemicals as presented in Table 1. Nwachukwu and co-workers [19] and Orakwue and co-worker [32] have independently documented the presence of flavonoids, alkaloids, glycosides, phenols, tannins and saponins in the leaves of *A. montanus*. This result of the phytochemical screening therefore, agrees with earlier reports. The presence of phytochemicals in an extract shows that such plant is endowed with different biological activities. Activities such as anti-inflammatory, immunological [9], antioxidant [33], antimicrobial [19] and Hypolipidemia [10] have been reported in different parts of *A. montanus*.

Table 1: Results of phytochemical screening of the leaf extract of *A. montanus*

Phytochemicals	Inference
Carbohydrates	+
Reducing sugar	+
Proteins	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Saponins	+
Tannins	+

Key + = present

Table 2: HPLC analysis of the phytochemicals in the methanol leaf extract of *A. montanus*

Phytochemicals	Retention Time (min)	Area (m^2)	Concentration
Proanthocyanin	0.1900	5184.3944	7.2917ppm
Lunamarin	1.5830	4709.7496	8.6196µg/mL
Cardiac glycoside	2.6330	12170.5138	11.3284µg/mL
Anthocyanin	3.5500	3903.4112	5.0215µg/mL
Ribalinidine	4.4000	10229.5051	13.159µg/mL
Flavan-3-ol	12.6200	6505.2012	7.5576ppm
Rutin	12.9900	7261.1404	9.0117g/mL
Naringenin	13.2730	5414.6802	2.2881µg/mL
Cyanogenic glycosides	13.9730	3725.9862	5.0080µg/mL
Spartein	15.6200	5351.2845	9.5901µg/mL
Flavonones	18.9500	6368.0202	10.9228ppm
Steroids	22.4560	8539.7226	14.6479ppm
Kaempferol	25.5630	4875.0349	3.3761µg/mL
Epicatechin	27.9100	13725.1531	20.5907µg/mL
Phytate	28.2760	9186.5206	12.3475µg/mL
Resveratrol	33.8100	18147.5364	20.6849ppm
Oxalate	35.6500	17427.5578	27.5258µg/mL
Catechin	36.5260	5159.9954	1.1328µg/mL
Sapogenins	42.7060	13247.6644	21.7710µg/mL

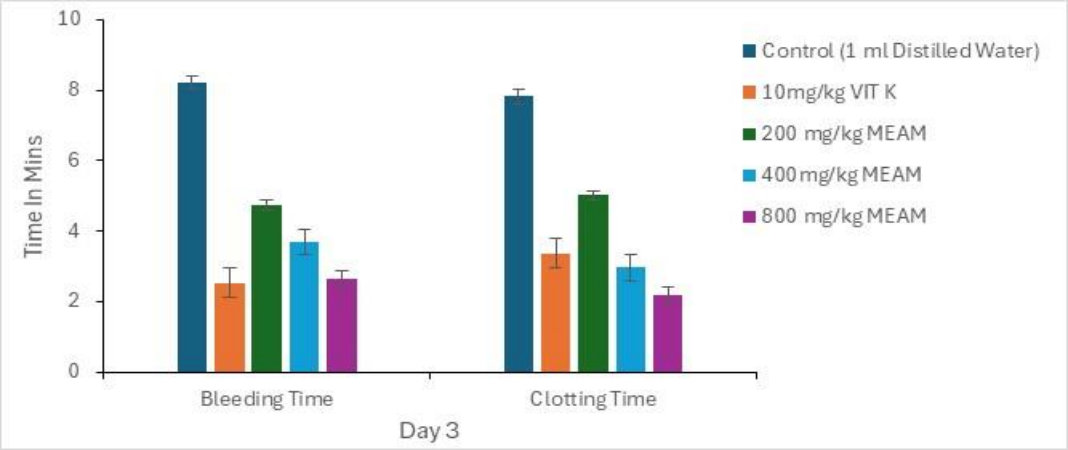

Figure 1: Bleeding time and clotting time after 3 days of treatment

Effect of methanol extract of *Acanthus montanus* leaf extract on bleeding and clotting times of rats after 3 days of treatment. P≤ 0.05 represents the level of significance when compared with the control groups. Values represent mean ± standard error of the mean (SEM) n = 5.

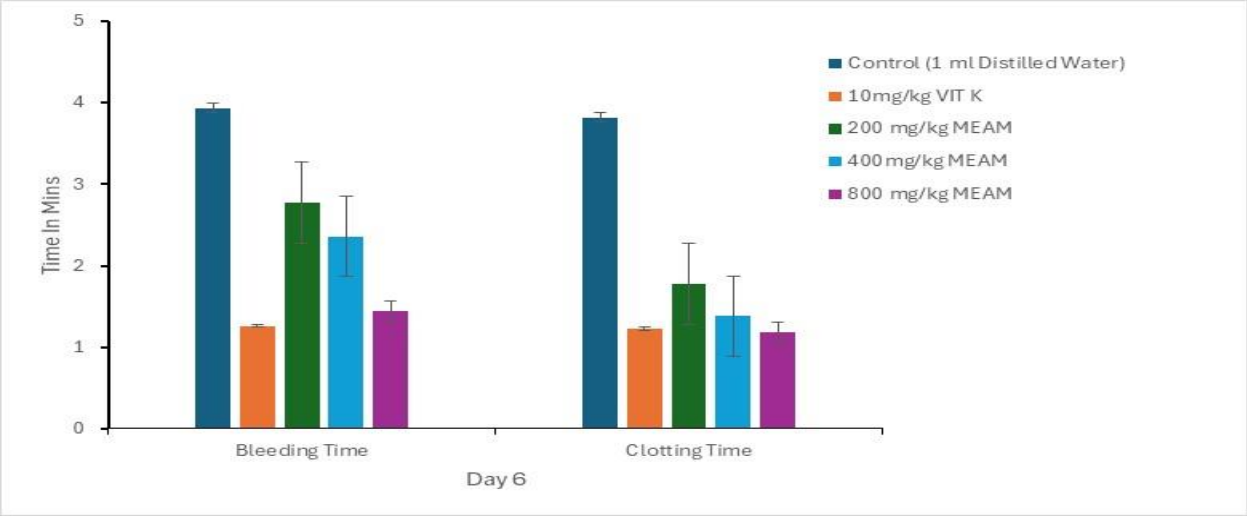

Figure 2: Bleeding time and clotting time after 6 days of treatment
Effect of Methanol extract of *Acanthus montanus* (MEAM) leaf extracts on bleeding and clotting time in Wistar rats after 6 days of treatment. P≤ 0.05 compared with the control group. Values represent mean ± standard error of the mean (SEM) n = 5

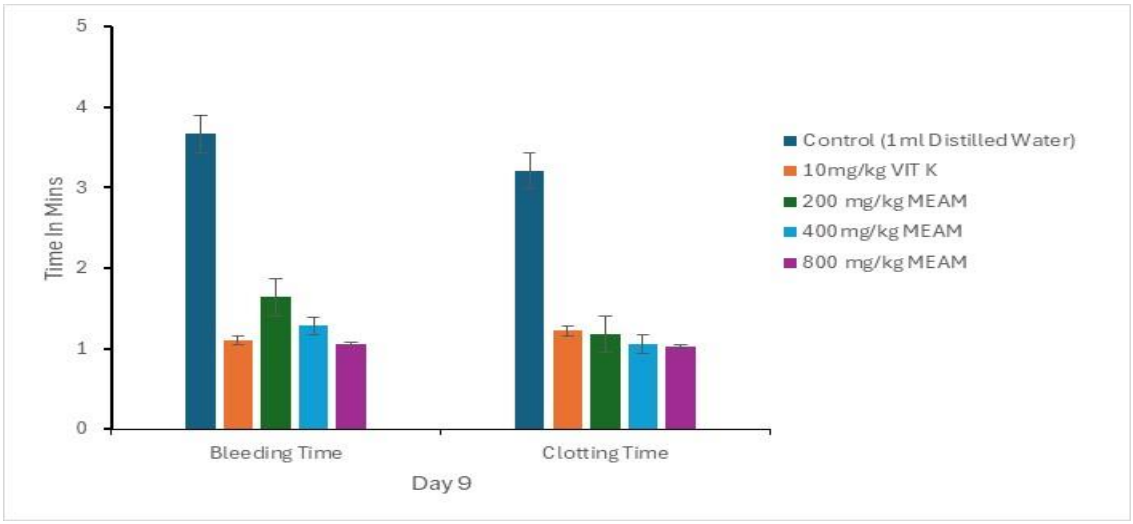

Figure 3: Bleeding time and clotting time after 9 days of treatment
Effect of Methanol extract of *Acanthus montanus* (MEAM) leaf extracts on bleeding and clotting time of rats after 9 days of treatment. P≤ 0.05 compared with the control group. Values represent mean ± standard error of the mean (SEM) n = 5

Phytochemicals that have been reported with haemostatic activity include tannins, glycosides, saponins and phenolic compounds. Their likely mechanisms of action are activation of coagulation by increasing factor XII action and plasma fibrinogen levels, fibrinolysis inhibition, vascular or smooth muscle constriction and aggregation of the platelet [34].

Compounds identified and quantified from the HPLC analysis are broadly placed under the following headings: anti-nutrients and phytochemicals. The anti-nutrients include oxalate and phytate which are known to interfere with proteolytic digestion due to the fact that phosphorus in its molecular form is not available to monogastric animals that ingest the leaves [35]. They also bind essential divalent cationic minerals preventing their

absorption into the gastrointestinal system [36]. Though the leaves have been reported to be in use as vegetable, this implies that the percentage of anti-nutrition compounds are low and may not significantly affect the absorption of other important compounds. However, the presence of these anti-nutrients in plant materials has been used pharmacologically to lower plasma cholesterol, as anticancer agent and reduce the risk of developing chronic diseases [37]. These phytochemicals present are alkaloids, steroids, glycosides, flavonoids, phenolics and saponins. Flavonoids include flavan-3-ol, flavonones, kaempferol, rutin, epicatechin, catechin, anthocyanin, proanthocyanin and naringenin. Lunamarine, spartein and ribalinidine are alkaloids. Resveratrol are phenolic compounds. Cardiac and cyanogenic glycosides were appropriately grouped, while sapogenins are group under saponins. Catechin which are considered to be polyphenolic compound with antioxidant potential have been reported to have haemostatic properties [38, 39]. Rutin have been shown to increase the number of platelet and encourage its aggregation [40].

Aqueous leaf extract of *A. montanus* administered at varying doses of 500-8000 mg/kg was observed to be safe [41]. It was reported that leaves with stalks of *A. mntanus*, extracted with 95 % ethanol administered to Wistar rat at doses of 10-10000 mg/kg b w showed no significant change in behaviour or death after 21 days [42]. Sub-acute toxicity evaluation with aqueous extract of *A. montanus* for 30 days produced neither mortality nor change in behaviour of the experimental animal [13]. The result of the acute toxicity in this study is consistent with documented reports.

Platelet function is assessed clinically by bleeding time measurement; this can be achieved by making standard incision and recording bleeding cessation [43]. The method used in this study is considered to be accurate but have the risk of scarring, infection and bleeding. The result in this study showed decrease in the bleeding time throughout the period of measurements (3,6 and 9 days). It could therefore, be inferred that one or more of the phytochemicals present in the methanol leaf extract of *A. montanus* may have been responsible in reducing the bleeding time and clotting time significantly ($P \leq 0.05$). The time it takes for a blood sample to form a fibrin clot is considered as the clotting time and this is in the range of 4-10 minutes for humans. The administered extract showed a gradual decrease in clotting time from day 3-9. In day three, 5.01±0.63 min was noted as the clotting time for 200 mg/kg b w dose as against 2.17±0.07 min for 800 mg/kg b w of extract. The clotting time decreased to 1.18±0.06 min for 200 mg/kg and 1.02±0.02 min for 800 mg/kg b w of extract. This implies that the extract encouraged the formation of fibrin thread, thus resulting in the reduction of the clotting time. The day nine result also showed that the varying doses of the extract (200-800 mg/kg) produced no significant difference in their effect on the values, although significant difference ($P \leq 0.05$) was observed with the controls. This may be indicating that site of action of the active principles in the extract may have been saturated from day 3-9.

Conclusion. *A. montanus* leaf is safe and contains phytochemicals that may act in synergy to demonstrate an array of biological activities including haemostatic activity. There are documented reports on the haemostatic activity of catechin and rutin which are also present in *A. montanus* leaf extract. This calls for further work to isolate and characterize the active principle responsible for the observed haemostatic activity. This could be achievable through bioactivity guided assays.

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REFERENCES

1. Donley ER, Munakomi S, Loyd JW. Hemorrhage Control. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535393/ [Accessed on 12 August, 2024].

2. Heckbert SR, Vedder NB, Hoffman W, Winn RK, Hudson LD, Jurkovich GJ, Copass MK, Harlan JM, Rice CL, Maier RV. Outcome after hemorrhagic shock in trauma patients. Journal of Trauma. 1998; 45:545-549.

3. Johnson AB, Burns B. Hemorrhage In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542273/

4. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. Journal of Trauma. 2006; 60: S3-11.

5. Curry N, Hopewell S, Dorée C, Hyde C, Brohi K, Stanworth S. The acute management of trauma hemorrhage: a systematic review of randomized controlled trials. Critical Care. 2011;15(2).R92. doi: 10.1186/cc10096.

6. McRae S. Physiological Haemostasis. In:R Fitridge, M Thompson, editors. Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists. Adelaide (AU): University of Adelaide Press; 2011. 9. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534253/.

7. Das SK, Reddy MM, Ray S. Hemostatic agents in critically ill patients. Indian Journal of Critical Care Medicine. 2019 (Suppl 3):S226-S229. doi: 10.5005/jp-journals-10071-23258.

8. Baunthiyal M, Semwal P, Dwivedi S. Haemostatic potential of medicinal plants and their phytochemicals. Journal of Mountain Research. 2021;16(1),93-102 DOI: https://doi.org/10.51220/jmr.v16i1.8

9. Okoli CO, Akah PA, Onuoha NJ, Okoye CT, Nwoye AC, Nworu CS. Acanthus montanus: an experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. BMC Complementary and Alternative Medicine. 2008;8:27. doi: 10.1186/1472-6882-8-27.

10. Onuoha OU, Osuocha KU, Chukwu EC. Phytochemical, hypolipidemic, haematological and body weight of Acanthus montanus leaf extracts in male and female albino rats. European Journal of Experimental Biology. 2018;8:5-30.

11. Osuala FN, Fagbenja KS, Mounmbegna PP. Pharmacognostic and antidysentery screening of mixed ethanol leaf extract of Parkia biglobosa and Acanthus montanus (50:50). Magna Scientia Advanced Research and Review. 2021. https://doi.org/10.30574/msarr.2021.3.2.0073.

12. Huxley AJ. The New Royal Horticultural Society dictionary of gardening. London: Macmillan; 1992. *Acanthus montanus* plant description and geographical distribution.http://www.doacs.state.fl.us/pi/enpp/98-mar-apr.htm..

13. Djami TA, Asongalem EA, Nana P, Choumessi A, Kamtchouing P, Asonganyi T. Subacute toxicity study of the aqueous extract from Acanthus montanus. Electronic Journal of Biology. 2011;7(1):11-15.

14. Noumi E, Fozi FL. Ethnomedical botany of epilepsy treatment in Fongo-Tongo village, Western Province, Cameroon. Pharmaceutical Biology. 2023;41(5):330-339.

15. Ibe AE, Nwufo MI. Identification, collection and domestication of medicinal plants in Southeastern Nigeria. Africa Development 2005;30(3) DOI:10.4314/ad.v30i3.22230.

16. Teklehaymanot T, Gidey M. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula North Western Ethiopia. Journal of Ethnobotany and Ethnomedicine. 2007;3:12 https://doi.org/10.1186/1746-4269-3-12

17. Adeyemi OO, Okpo SO, Young-Nwafor CC. The relaxant activity of the methanolic extract of Acanthus montanus on intestinal smooth muscles. Journal of Ethnopharmacology. 1999; 68:169-173.

18. Foyet HS, Nana P, Chounfack E, Asongalem EA, Dimo T, Kamtchouing P. Protective effect of Acanthus montanus in carrageenan-induced models of local inflammation: inhibitory effect on nitric oxide (NO) production. Pharmacologyonline. 2008;2:161-169

19. Orakwue FC, Ojiako EN, Okoye EI. Phytochemical and antimicrobial analysis of Acanthus montanus leaves. International Journal of Natural and Applied Sciences. 2012;5(4):1-5

20. Igwe OU, Nnaji JC. Chemical characterization and investigation of the bioeffects of the leaves of Acanthus montanus (Acanthaceae) on some selected microorganisms. International Journal of ChemTech Research. 2014;6(14):5554-5561.

21. Noiarsa P, Ruchirawat S, Kanchanapoom T. Acanmontanoside, a new phenylethanoid diglycoside

from Acanthus montanus. Molecules. 2010;5(12): 8967-72. doi: 10.3390/molecules15128967

22. Sofowora A. Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition. Spectrum Books Ltd., Nigeria, 2009;150-156.

23. Evans WC. Trease and Evans Pharmacognosy. 16th Edition. Elsiver Ltd. 2009;135-147.

24. Odion EE, Nwigwe GN, Ambe DA, Nnamani MN, Osigwe CC, Odiete EC, Iyanyi LU. Phytochemical Profiling of Passiflora edulis Vines. Sciences of Phytochemistry. 2024;3(1):11-19.https://doi.org/10.58920/sciphy0301219]

25. Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of Functional Foods. 2011;(3):88–99.

26. National Institutes of Health Guide for the Care and Use of Laboratory Animals. NIH Publication 1985 Number 85-23, US Department of Health, Education and Welfare, Bethesda, MD.

27. Locke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;53:275-289. doi:10.1007/BF01234480.

28. Duke WW. The relation of blood platelets to hemorrhagic disease. Journal of American Medical Association. 1983;250(9):1201–9.

29. Okoroiwu HU, Atangwho IJ, Uko EK and Okafor IM. Haemostatic property of Chromolaena odorata leaf extracts: in vitro and in vivo evaluation in Wistar rats. Journal of Biological Research 2016; 89(6211):57-60

30. Tanko Y, Eze ED, Jimoh A, Yusuf K, Mohamed KA, Balarabe F and Mohamed A. Haemostatic effect of aqueous extract of mushroom (Gandonerma lucidium). Pelagia Research Library. European Journal of Experimental Biology 2012;2 (6):2015-2018. Available online www.pelagiaresearchlibrary.com

31. Ivy AC, Shapiro PF, Melnick P. The bleeding tendency in jaundice. Surgery Gynecology Obstetrics. 1953;60:781–4.

32. Nwachukwu AA, Ogbulie TE, Nwachukwu CU, Evans-Kemka CI, Onyekachi VC. Assessment of proximate, phytochemical and selected mineral content of Acanthus montanus Leaf. Asian Journal of Biotechnology and Bioresources Technology. 2020;6(2):45-54.

33. Igwe A, Eleazu C. Effect of processing on the biochemical contents of Acanthus montanus (Nees) T. Anderson (Acanthaceae) leaves. Food Science and Nutrition. 2017;6(2):388-394.

34. Ebrahimi F, Torbati M, Mahmoudi J, Valizadeh H. Medicinal plants as potential haemostatic Agents. Journal of Pharmacy and Pharmaceutical Sciences. 2020;23(1):10-23. https://doi.org/10.18433/jpps.30446.

35. Oboh G, Ekperigin MM, Kazeem MI. Nutritional and haemolytic properties of egg plant. (Solanum macrocarpon) leaves. Journal of Food Composition and Analysis. 2005;18:153-160.

36. Ojiako OA, Ogbuji CA, Agha NC, Onwuliri VA. The proximate, mineral, and toxicant compositions of four possible food security crops from southeastern Nigeria. Journal of Medicine and Food. 2010;13:1203-1209.

37. Salim R, Nehvi IB, Mir RA, Tyagi A, Ali S, Bhai OM. A Review on anti-nutritional factors: Unraveling The Natural Gateways to Human Health. Frontera in Nutrition. 2023;10:1215873. doi:10.3389/fnut,2023.1215873.

38. Taylor L. Plant based drugs and medicine. Rain Tree Nutrition. 2000.

39. Bae J, Kim N, Shin Y, Kim SY, Kim YJ. Activity of catechins and their applications. Biomedical Dermatology. 2020;4(1):8. doi: 10.1186/s41702-020-0057-8.

40. Mu K, Liu Y, Liu G, Ran F, Zhou L, Wu Y, Peng L, Shao M, Li C, Zhang Y. A review of hemostatic chemical components and their mechanisms in traditional Chinese medicine and ethnicmedicine. Journal of Ehnopharmacology 2023; 307:116200 https://doi.org/10.1016/j.jep.2023.116200

41. Paulina N, Achab AE, Simplicea FH, Théophilea D, Pierrea K. Acute Toxicolgical Studies of Acanthus montanus (Nees) T. Anderson (Acanthaceae) In Wistar Rats. Pharmacologyonline. 2007;1:339-348.

42. Oshadu DO, Ajanusi JO, Chiezey PN, Abubakar MS, Tnako JT, Sambo SJ, Mohammed B. Toxicological evaluation and therapeutic index of ethanolic leaf extract of Acanthus montanus (Acanthaceae) in Mice. Journal of Pharmacology and Toxicology. 2022;17(1):28-35.

43. Russeau AP, Vall H, Manna B. Bleeding Time. In: StatPearls. Treasure Island (FL): Stat Pearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537233/