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Study on hemostatic activities of *Aspilia africana* (Pers.) C. D. Adams and *Conyza canadensis* L.

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Abstract

The process of hemostasis involves the body turning blood into a thick, gel-like mass at the site of injury or damage in order to stop bleeding. Africa and Latin America are the native habitats of *Aspilia africana* (Family Asteraceae). Hemorrhage plant and wild Sunflower are frequent names for this plant, which grows in savanna wastelands and forested areas. *Conyza canadensis* (Family Asteraceae) is commonly known as Canadian horseweed or Canadian fleabane. It is present in all warm nations, but because of its global distributions from America, it is thought to have originated there and spread to the rest of the world. This study investigated hemostatic activities of the leaves extract of *Aspilia africana* and *Conyza canadensis* on mice. Fresh leaves of the plant samples were extracted with methanol and thirty-two (32) mice grouped into eight of four mice were used for the study. Incision was made on the tail of each mouse. Warfarin, a standard drug (3 mg/kg), was used as positive control while each plant extract was made into concentrations of 50, 100 and 200 mg/kg. The bleeding time (seconds) of breaking tail and volume of bleeding were measured to evaluate the hemostatic activity after administration of treatments for 5 days (once per day). The results of this study showed reduction in the volume of bleeding in all the treatment groups when compared to untreated (negative control) group but highest activity was recorded in warfarin (Positive control). *Aspilia africana* (200 mg/kg) methanol extract (1.12±0.01) was not statistically different from the mean values of warfarin (1.11±0.01). The results of bleeding time (Seconds) in tails of the mice of warfarin, *Aspilia africana* methanol extract and *Conyza canadensis* methanol extract groups reduced when compared to the negative control group, but *Aspilia africana* extract gave highest activity at 200 mg/kg. The significant ability of *Aspilia africana* leaves extract to effectively arrest bleeding from fresh wounds and accelerate wound healing process might be attributed to the metabolites present. However, further studies should be carried out on its toxicity and mechanism of actions.

Keywords: Hemostasis; Asteraceae; *Aspilia africana*; *Conyza canadensis*; Incision

1 Introduction

Wounds are a significant global health issue that place a lot of strain on healthcare organizations, caregivers, patients, and their families on acommercial, psychological, and social level (Benbow, 2011). The term "wound" refers to any physical, chemical, or thermal injury or insult that causes the skin to open or break, as well as any disorder of the anatomical and functional integrity of living tissues (Meenakshi *et al*., 2006). Untreated wounds may result in discomfort, inflammation, infection, and occasionally organ failure (Kumar *et al*., 2007). The fundamental response in wound healing involves a process of connective tissue repair and is characterized by four overlapping phases such as hemostasis, inflammation, proliferation, and remodeling in which the repair process requires the coordination of different cells, growth factors and cytokines(Cianfarani *et al.,* 2013).

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Herbal medicine is a part of human health care for thousands of years. Myriad of chemical constituents obtained from herbs are active against a number of diseased condition. *Aspilia africana* (Figure 1) is a plant that falls under the group of Neglected and Underutilized Species (NUS). It is a perennial herb belonging to the family Asteraceae which is native to Africa and Latin America (Ndam *et al.,* 2019). In English, it is referred to as hemorrhage plant or wild Sunflower, Kissinyana in Sierra Leone. In Nigeria, the Efik calls it "Edemeedon", "Yunyun" in Yoruba, "Orangila" in Igbo and "Jamajina" in Hausa (Ajeigbe *et al.,* 2013). In herbal medicine, the bruised leaves and flowers of *Aspilia africana* are used to clean the surfaces of sores to promote healing.Decoctions and herbal extracts of the powdered leaves and roots of this plant are used to treat fever frequently due to malaria (Chono *et al.,* 2009), corneal opacities, stomach disorders, cough, gonorrhea, rheumatic pains, tuberculosis and the leaf infusion is taken as a tonic for women immediately after delivery (Christina *et al.,* 2017; Oyesola and Okoye, 2010).Several scientific studies have attributed the numerous medicinal properties of *Aspilia. africana* to the abundant bioactive secondary metabolites in it such as alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoids (Christina *et al.,* 2017; Etiosa *et al.,* 2017).*Conyza canadensis* (Family Asteraceae;figure 2) is commonly known as Canadian horseweed, Canadian fleabane or Pleat by the locals (Mahmood *et al.,* 2011). It is found in all warm countries, but is presumed to be of American origin and spread on the rest of the globe because of its distributions from that continent. *Conyza canadensis* is used locally as traditional vegetable and sweetening agent in Northern areas of Pakistan (Yan *et al.,* 2010) and for the treatment of various pathological conditions including its use in acute pain, inflammation, fever and especially the microbial infections including urinary infections, respiratory tract infections, diarrhea and dysentery (Shakirullah *et al.,* 2011).In Africa, *Conyza canadensis* is used for the treatment of ringworm and eczema (Shakirullah *et al.,* 2011).It is reported to possess anti-inflammatory, hemostatic, antidiuretic, antioxidant, antibacterial, antiplatelet, cytotoxic, antiviral and antimelanoma activities (Edzir *et al.,* 2011; Yan *et al.,* 2010; Hayet *et al.,* 2009). However, the phytochemicals present in plants are responsible for their pharmacological activities (Aslam *et al.,* 2018). Phytochemical studies revealed that *Conyza canadensis* contained saponins, diterpenoids, terpenoids, glycosides, tannin, anthraquinone, steroids and flavonoids (Al-Snafi, 2017). This study investigated hemostatic abilities of the methanol leaves extract of *Aspilia africana* and *Conyza canadensis* on mice.

Figure 1 *Aspilia africana*(Pers.) C. D. Adams

Figure 2 *Conyza canadensis* L

2 Material and methods

2.1 Preparation of Plant Materials

The matured leaves of *Conyza canadensis* and *Aspilia africana* were collected at Akungba-akoko and identified by Dr. O. A. Obembe, a taxonomist at the Department of Plant Science and Biotechnology, AAUA. A portion (500 g)of fresh leaves of each plant wascrushed with mortar and pestle and extracted with 500 ml of methanol. The mixtures were filtered separately through muslin cloth and each methanol extract was lyophilized. The dried extract was weighed and maintained in a tightly closed container until being used for the analysis.

2.2 Experimental Design and Treatment Administrations

Thirty-two (32) mice were purchased and fed with standard pellets and water *ad libitum*. This animal study was approved by the Adekunle Ajasin University, Akungba-Akoko animal care and use committee thereby following the guidelines for animal welfare and experimental conduct. The animals were divided into 8 groups of 4 animals each as follows:the first and second groups were regarded as negative and positive controls and the animals received normal saline (2 ml) and warfarin (3 mg/kg) respectively. Groups 3, 4 and 5 received 50,100 and 200 mg/kg methanol extract of *Aspilia africana* respectively while groups 6, 7 and 8 received 50,100 and 200 mg/kg methanol extract of *Conyza canadensis* respectively. All the animals were pretreated with varied extract concentrations, standard drug and normal saline for 5 days. Each tail of the animals was cut at 2 cm from the tip and tail lesion were blotted with filter paper and weighed to determine the volume of bleeding. The interval from the time of the tail incision to the time that blood no longer dropped on the filter paper was recorded as the bleeding time (seconds) with the aid of stopwatch (Ozgur *et al*., 2013; Hatalchanok *et al*., 2012).

2.3 Statistical Analysis

The data obtained were subjected to Analysis of variance (ANOVA) using statistical package for Social Science (SPSS) version 22 to generate the mean and standard error. Mean generated were separated and compared by Duncan's New Multiple Range Test (DNMRT).

3 Results

The results of volume of bleeding and bleeding time of the hemostatic activities of *Aspilia africana* and *Conyza canadensis* methanol extract are presented in Tables 1 and 2 respectively.

Table 1 showed reduction in the volume of bleeding of all the treatment groups when compared to untreated (negative control) group but highest activity was recorded in warfarin (positive control). *Aspilia africana* (200 mg/kg) methanol extract (1.12±0.01) was not statistically different from the mean values of warfarin (1.11±0.01). *Aspilia africana* methanol extract exhibited higher activity than *Conyza canadensis* methanol extract. The activity of *Aspilia africana* extract occurred in concentrations dependent increased pattern. The volume of bleeding was at the lowest in 50 mg/kg of *Conyza canadensis* extract group when compared with 100 and 200 mg/kg of *Conyza canadensis* extract groups. The activity of the extract was decreasing as the concentration increases.

In Table 2, the results showed that the bleeding time (Seconds) in tails of the mice of warfarin, *Aspilia africana* methanol extract and *Conyza canadensis* methanol extract groups reduced when compared to the negative control group, but *Aspilia africana* extract gave highest activity at 200 mg/kg.

Table 1 Volume of bleeding of *Aspilia africana* methanol extract and *Conyza canadensis* methanol extract on mice

Parameters	Negative control(2 ml/kg)	Warfarin (3 mg/kg)	50 mg /kg	100 mg/kg	200 mg/kg
Aspilia africana methanol extract	1.56±0.03f	1.11±0.01a	1.20±0.01d	1.14±0.01b	1.12±0.01a
Conyza canadensis methanol extract	1.56±0.03f	1.11±0.01a	1.17±0.04c	1.18±0.01c	1.35±0.26e

Note: Mean values with the same superscript alphabets are not significantly different from each other (P>0.05)

Table 2 Bleeding time (Sec.) of *Aspilia africana* methanol extract and *Conyza canadensis* methanol extract on mice

Parameters	Negative control (2 ml/kg)	Warfarin (3 mg/kg)	50 mg /kg	100 mg/kg	200 mg/kg
Aspilia africana methanol extract	76.00±1.00g	33.00±1.02c	38.00±1.20d	30.10±0.50b	25.50±0.55a
Conyza canadensis methanol extract	76.00±1.00g	33.00±0.02c	58.00±0.03e	58.50±1.10e	62.50±1.05f

Note: Mean values with the same superscript alphabets are not significantly different from each other (P>0.05)

4 Discussions

The increasing demand and availability of medicinal products have prompted the need to understand the principles responsible for their therapeutic activities and effectiveness. For instance, stimulation of fibroblasts by plant extracts has been observed as one of the mechanisms by which medicinal plants enhance the wound healing process (Suguna *et al.,* 2002). In this study, an attempt was made in evaluating the hemostatic activities of mice administered with leaves of *Aspilia africana* methanol extract and *C. canadensis* methanol extract on mice. The plants' extracts and warfarin could significantly shorten the volume of bleeding and bleeding time in mice. The result of this study also revealed that the groups treated with 50, 100 and 200 mg/kg *Aspilia africana* leaves extract showed reduction in the volume of bleeding and bleeding time in a dose dependent increased manner. Contrarily to *Aspilia africana* leaves extract, *C. canadensis* methanol extract (50, 100 and 200 mg/kg) showed its activity in a concentration dependent decreased manner when compared to the normal control.

This result revealed that the methanol extract of *Aspilia africana* leaves at 200 mg/kg exhibited higher hemostatic activity than *Conyza canadensis* extract. The *Aspilia africana* extract arrested bleeding from fresh wounds by reducing the volume of bleeding and the bleeding time more thanwarfarin (in the case of bleeding time) which are important indices of hemostatic activity.

Dandjesso *et al.* (2012) demonstrated that astringent activity of medicinal plants can be the presence of tannins. Astringent activity promotes vasoconstriction, which is an important parameter in hemostasis. The reduction in volume of bleeding and bleeding time observed in the plants' extracts might be attributed to the metabolites present in the extract. Phytochemical analysis of *Aspilia africana* indicated the presence of typical plant constituents such as alkaloids, saponins, sterols, terpenoids, carbohydrates, glycosides and tannins (Christiana *et al*., 2017). Al-Snafi (2017) reported that *C. canadensis* contained saponins, diterpenoids, terpenoids, glycosides, flavonoids, anthraquinone, steroids andtannin. Ebrahimi *et al.* (2020) propounded that the bioactive compounds which are often involved in the bleeding control are categorized as tannins, saponins, glycosides and other phenolics. Extracts of medicinal plants have been documented to arrest bleeding from fresh wounds, inhibits microbial growth and promotes wound healing (Lau *et al.,* 2008). In addition, the effects of *Aspilia africana* and *C. canadensis* observed in volume of bleeding and bleeding time may be linked to the free radical scavenging action of compounds in the extracts acting either singly or synergistically. These active compounds enhance the process of wound healing by increasing the viability and strength of collagen fibrils thereby increasing the circulation or prevention of cell damage (Okoli *et al.,* 2007; Esimone *et al.,* 2008).

5 Conclusion

The *in vivo* study confirmed the significant ability of *Aspilia africana* leaves extract to effectively arrest bleeding from fresh wounds and accelerate wound healing process. However, further studies should be carried out on its toxicity and mechanism of actions.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declared no conflicts of interest.

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