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Effect of the Leaf Extract of *Bidens pilosa* on Haemostasis

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Authors' contributions

This work was carried out in collaboration between both authors. Author PB proposed the project, run the experiment and wrote the initial draft of the manuscript. Author BB moderated the proposal and experimental design, data analysis and prepared the final manuscript. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: To determine the effect of the leaf extract of *Bidens pilosa* on the rate of haemostasis and validate its traditional use application to fresh wounds.
Study Design: Experimental
Place and Duration of Study: The study was conducted at the Biology Department, Faculty of Science and Education, Busitema University and Nagongera Health Center IV laboratory between April and May 2019.
Methodology: Different concentrations of the extract were applied to blood samples. Whole venous blood was collected by vein puncture in heparin tubes. The rate of **c**lotting in presence and absence of the extract was determined. The experiment was replicated.
Results: Increase in the concentration of the extract decreased the rate of haemostasis. Statistical analysis with a two-way ANOVA was significant, ***P*** = 0.02 at a 95% CI.
Conclusion: High concentration *Bidens pilosa* leaf extract decreases the rate of haemostasis but may have other healing activities attributed to its historical and traditional use and application to fresh wounds.

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Keywords: Haemostasis; leaf extract; clotting; Bidens pilosa; ANOVA.

ABBREVIATIONS

ANOVA : Analysis of variance

1. INTRODUCTION

Bidens pilosa commonly known as black jack is a cosmopolitan weed freely growing almost in every part of the tropics. It is a member of the Asteraceae family, one of the dominant families contributing to medicinal species worldwide. A plant reffered to as a medicinal herb, is one in which effective materials are constructed and stored in its configuration [1]. According the World Health Organization (2008) approximately 80% of Asia and Africa's population use traditional medicine as a form of healthcare for treatment of diseases including blood disorders. Plant extracts can be an alternative to currently used antiplatelet agents, as they constitute a rich source of bioactive chemicals. Compounds such as alkaloids, xanthones, coumarins, anthraquinones, flavonoids, stilbenes, and naphthalenes have been reported to have an effect on platelet aggregation [2]. Extensive research over the past decades has shown that *B. pilosa* has activities including; antiviral, antifungal, and antibacterial [3]. Hassan and colleagues investigated the wound healing potential of *B. pilosa* in Wistar rats. In their study histological examination revealed better collagenation, angiogenesis, and organization of wound tissue seven days after application of the extract. Epithelialization and total healing time in *B. pilosa*-treated rats were comparable to those of neomycin sulfate. Their report, shows that that *B. pilosa* may be a viable alternative to neomycin lotion for the treatment of wounds [4].

On the other hand, *Bidens pilosa* extracts have also been reported to contain two major classes of secondary metabolites - namely polyacetylenes and flavonoids. These phytochemical components have been found to be responsible for the various medicinal activities of *B. pilosa* including anticancer properties, antimalarial, treatment of headache [5,6,7,8] and anti–inflammatory properties [9,10,11].

Bidens pilosa, either as a whole plant or different parts, has been reported to be useful in the treatment of more than 40 disorders such as inflammation, immunological disorders, digestive disorders, infectious diseases, cancers and metabolic syndromes [12].

The use of plants in addressing medical challenges have been witnessed since antiquity and is regaining shape in the modern era due to their safety, effectiveness, cultural preferences, inexpensiveness, abundance, and availability. A recent research conducted in Uganda reveals the efficacy and use of *Bidens pilosa* in management of snake bites [13].

2. EXPERIMENTAL DETAILS

2.1 Preparation of the Extract

Fresh leaves of *Bidens pilosa* were washed with distilled water, dried on the table in the laboratory and finally ground into fine powder using a grinder. Methyl alcohol extract of the sample was obtained by dissolving 50 g of the powder with 500cc methanol. The methyl extracts were then concentrated by rotary evaporator. The aqueous and hydro alcoholic extracts were then stored in tightly closed dark vials at 4°C as previously described [14].

2.1.1 Blood sample collection

Blood samples were obtained from three - amongst our colleagues. Whole venous blood was collected by vein puncture in heparin tubes. The samples were collected at the Nagongera Health Centre IV laboratory. Clotting time in presence and absence of the extracts was determined and compared with the control.

2.1.1.1 Experimental procedure

A half gram of concentrated solvent free extract was obtained in test tubes and suspended in distilled water and the final volume made up to 15 mLl, the concentrations were varied by obtaining another 1.0 g and 1.5 g as above [15]. To 0.5 mL of various extracts of different concentrations, 1 mL of whole blood obtained using the above method was added. The control tubes contained 0.5 mL of normal saline instead of the extract preparations. The clotting time was recorded in minutes as previously describe [15].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Statistical data analysis

The above results were analyzed using a two-way ANOVA statistical data analysis. The results

are significant, *P* value (0.02), at a 95% CI and reference level (*P*=0.05).

3.2 Discussion

The mechanism of effective control of bleeding occurs through a complex of processes known as haemostasis. This occurs in four basic steps namely: Vasoconstriction, platelet activation, thrombus formation, clot dissolution. The mechanism is, however, initiated in one of the two ways which include; the intrinsic pathway/internal pathway and the extrinsic/external pathway. The intrinsic pathway occurs when the clot forms inside the blood vessel itself due to an abnormality or injury due to rapture of the blood vessel. The treatment of internal bleeding is beyond the scope of simple first aid, and should be considered by any first aider to be potentially life threatening [1]. The definitive treatment for internal bleeding is always surgical treatment, and medical advice must be sought urgently for any victim of internal bleeding. The extrinsic pathway occurs due to injury such as a cut when blood is exposed to the outside environment. In order to manage bleeding effectively, it is important to be able to readily identify both types of chemical and plants drugs [1]. No matter the mechanism of initiation, the clotting process follows a similar pathway; this is called the common pathway. Blood cells such as platelets along with other factors and molecules such as proteins, enzymes, vitamin K and calcium found in the blood plasma are involved in the clotting process [16].

Table 1. Showing the average clotting time (minutes) of each sample before and after application of a given plant extracts concentration

		Concentration of the extract / Time (minutes).		
Sample	**Control**	**0.5**	**1.0**	**1.5**
A	6.06	9.72	10.66	11.26
B	4.93	8.83	12.97	13.39
C	6.75	10.24	14.44	14.87
Mean.	5.95±3.33	9.60±3.33	12.69±3.33	13.17±3.33

Standard Deviation, s: 3.3341903065062

Count, N:	*4*
Sum, Σx:	*41.41*
Mean, x̄:	*10.3525*
Variance, s^2:	*11.116825*

Overall, increase in the concentration of the extract is proportional to the time taken for the blood sample to clot

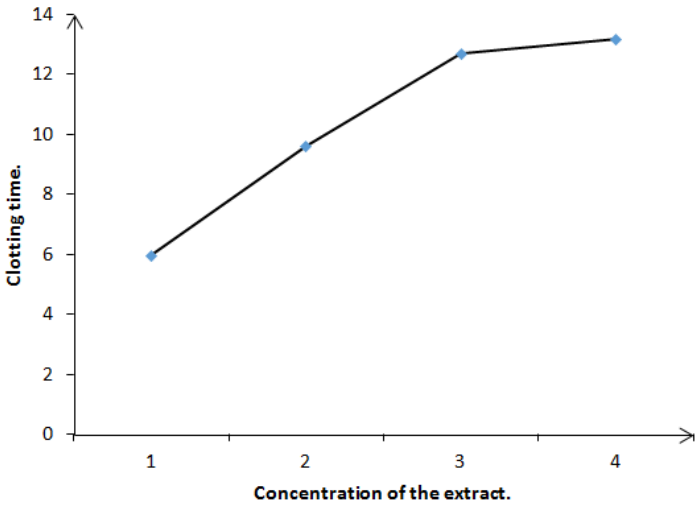

Fig. 1. A Graph showing the relationship between concentrations of the extract and clotting time (minutes)

Sample A clotted on average after 6.06 minutes, sample B blood clotted at an average of 4.93 minutes, while sample C blood clotted after 6.75 minutes without any extract (Table 1). At the lowest concentration, blood sample A clotted after 9.72 minutes, a delay by 3.67 minutes in reference to the control. At the same concentration, sample B took 8.83 minutes to clot, expressing a delay of 3.92 minutes while sample C, the clotted after 10.24 minutes expressing a delay of 3.48 minutes. Overall, increase in the concentration of the extract was proportional to the time taken for the blood sample to clot. Statistically the difference in the means was significant as indicated above.

Our results show that the extract of *Biden pilosa* may have anticoagulant activities in high concentration, contrary to the traditional perception of blood clotting. Interestingly what is not perceived are the anti-inflammatory and antimicrobial activities of *B. pilosa* attributed to the overall wound healing as previously reported in rats [4]. This is actually the main plausible benefit of the extract to man, though, unfortunately not perceived as such.

4. CONCLUSION

Based on the findings of our experiment high concentration of the *Biden's pilosa* leaf extract decreases the rate of haemostasis. The historical and traditional use and application of *Bidens pilosa* extracts to fresh wounds may be due to its anti-inflammatory and antimicrobial activities. This result provides a window for further experiments to validate the said activities of *B. pilosa*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hossein N, Zahra Z, Abolfazl M, Mahdi S, Ali K. Effect of Cinnamon zeylanicum e ssence and distillate on the clotting time. 2013;7(19):1339–43.
2. Cordier W, Steenkamp V. Herbal remedies affecting coagulation : A review; 2011.
3. da Silva J, Cerdeira CD, Chavasco JM, Cintra ABP, da Silva CBP, de Mendonça AN, et al. Triagem in vitro da atividade antibacteriana de Bidens pilosa Linné e Annona crassiflora mart. contra Staphylococcus aureus resistente à oxacilina (ORSA) provenientes do ambiente aéreo na clínica odontológica. Rev Inst Med Trop Sao Paulo. 2014;56(4): 333–40.
4. Hassan AK, Deogratius O, Nyafuono JF, Francis O, Engeu OP. Wound healing potential of the ethanolic extracts of Bidens pilosa and Ocimum suave. African J Pharm Pharmacol. 2011;5(2): 132–6.
5. Sundararajan P, Dey A, Smith A, Doss AG, Rajappan M, Natarajan S. Studies of anticancer and antipyretic activity of Bidens pilosa whole plant. African Heal Sci. 2006;6(1):27-30.
6. Brandão MGL, Krettli AU, Soares LSR, Nery CGC, Marinuzzi HC. Antimalarial activity of extracts and fractions from Bidens pilosa and other Bidens species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. J Ethnopharmacol. 1997; 57(2):131–8.
7. Jäger AK, Hutchings A, van Staden J. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. J Ethnopharmacol. 1996;52(2):95-100.
8. Hou D, Guo Q, Li Y, Li L, Wang Q, Yi Q, Tang M. Effects of anticancer No. 8 drugs on the experimental tumours in mice. J China Pharm Univ. 1989;20:348–50.
9. Geissberger P, Séquin U. Constituents of Bidens pilosa L. do the components found so far explain the use of this plant in traditional medicine?. Acta Trop. 1991; 48(4):251–61.
10. Chih HW, Lin CC, Tang KS. Anti-inflammatory activity of Taiwan folk medicine" ham-hong-chho" in rats. Am J Chin Med. 1995;23(03n04):273–8.
11. Chih HW, Lin CC, Tanga KS. The hepatoprotective effects of Taiwan folk medicine ham-hong-chho in rats. Am J Chin Med. 1996;24(03n04):231-240.
12. Vuguziga L, Livadariu O, Matei F. Screening among some eastern Africa ' S Indigenous Plants For Their Biotechnological Potential. 2019;LXIII(1): 555–64.

13. Omara T, Kagoya S, Openy A, Omute T, Ssebulime S. Antivenin plants used for treatment of snakebites in Uganda : ethnobotanical reports and pharmacological evidences. 2020;0:1–16.
14. Ezeonwumelu JOC, Julius AK, Muhoho CN, Ajayi AM, Oyewale AA, Tanayen JK. Oloro J. Biochemical and histological studies of aqueous extract of Bidens pilosa leaves from Ugandan Rift Valley in rats. Br J Pharmacol Toxicol. 2011;2(6):302–9.
15. Mehta JH, Kularni CG, Jadhav ST, Deshpande AM, Bhise SB. Evaluation of Indian herbs for haemostatic activity. Int J Res Pharm Chem. 2013;3(4):743–7.
16. Norris LA. Blood coagulation. Best Pract Res Clin Obstet Gynaecol. 2003;17(3): 369–83.

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