

In Vitro Studies of Anticoagulation Activity of *Pentaclethra Macrophylla*

Anslem O. Ajugwo^{1*}, Teddy C. Adias², Felix N. Osuala³, Kevin Aghatise⁴, Diamreyan O. Onoriode¹,
Adaora U. Anosike¹

¹Department of Haematology and Blood Transfusion, Madonna University Elele, Nigeria

²Bayelsa State College of Health Technology, Ogbia, Nigeria

³Department of Pharmacognosy, Madonna University Elele, Nigeria

⁴Department of Medical Laboratory Science, Achievers University Owo, Nigeria

*Corresponding author: slemjugwo@yahoo.com

Received June 8, 2013; Revised July 26, 2013; Accepted July 30, 2013

Abstract There is an increasing need to source for pharmacological and medicinal materials from plant source. An exploratory effort towards identifying and characterizing new anticoagulants from plants is worthwhile. Extract from *Pentaclethra macrophylla* (African oil bean) was subjected to anticoagulation activity and compared with EDTA and sodium citrate anticoagulants using some haematological parameters. The seeds of *Pentaclethra macrophylla* were dried and ground into powdered form. 2ml of blood sample was introduced into each tube containing 0.1g, 0.2g and 0.5g of the powdered extract. Coagulation was achieved in 2520 sec and 105 sec in 0.2g and 0.5g tubes respectively while in 0.1g tube coagulation was unattained. The extract-anticoagulated blood was compared with EDTA/sodium citrate-anticoagulated blood. The results of PCV, Hb, WBC and platelet count showed no significant difference ($p>0.05$) when compared. However, PT and APTT were significantly different ($p<0.05$). In vitro anticoagulation activity of *Pentaclethra macrophylla* was established. It may also be of interest as an anticoagulant for laboratory use.

Keywords: *Pentaclethra macrophylla*, anticoagulation, pharmacological, In vitro, haematological, laboratory use

Cite This Article: Ajugwo, Anslem O., Teddy C. Adias, Felix N. Osuala, Kevin Aghatise, Diamreyan O. Onoriode¹, and Adaora U. Anosike. "In Vitro Studies of Anticoagulation Activity of *Pentaclethra Macrophylla*." *World Journal of Nutrition and Health* 1, no. 1 (2013): 10-12. doi: 10.12691/jnh-1-1-2.

1. Introduction

Pentaclethra macrophylla (African oil bean) produces its seeds used for ugba production from a perennial legume tree [1]. The trees are often planted along the sides of roads as shade trees and around communities as cash crops. African oil bean is a tropical tree crop found mostly in the Southern rain forest zone of West Africa and has been cultivated since 1937. It belongs to the leguminosae family and sub-family Mimosoideae. African oil bean tree grows to about 21 meters in height and to about six meters in girth and it is well branched, forming crown-like canopy. The leaves have stout angular petiole. The compound leaves are usually about 20 centimeters large and covered with rusty hairs. The flowers are creamy, yellowish or pinkish-white and sweet smelling. The main flowering season is between March-April after which the pods (brown and woody when matured) open by explosive mechanism, dispersing the seeds and curls up, releasing about eight flat, glossy brown seeds measuring about 5-7 cm in diameter and weighing between 15-20g [2].

In Nigeria it thrives in the eastern and western parts. Ugba is the Igbo name for the fermented African oil bean seeds. It is called *Apara* by the Yoruba's and *Ukana* by the Efiks [2]. The African oil bean seed is fermented and

consumed especially in eastern states of Nigeria. The fermented product Ugba (old Imo State) or Ukpaka (Old Anambra State) is eaten alone or with other ingredients like stockfish, garden egg, sliced tapioca or can be mixed with vegetable popularly known as Africa salad in the Eastern part of Nigeria [3,4].

The oil bean seeds contain 8-10% carbohydrate and 47-48% of fatty acid [5]. About 75% of the total oil in the seed is made up of unsaturated fatty acids, predominantly oleic and linoleic acid. The pharmacological importance derivable from its seeds, roots, bark and leaves are of benefit in the treatment and management of obesity, itching, heart problems, high blood pressure [6]. The seed is a source of oil used for candle making and soaps. The seed shells are decorative and often used as craft beads, which are worn as necklaces and sometimes as local dance apparels.

Anticoagulants are substances or agents which prevent or retard clotting of blood [7], by removing calcium [8]. It could be used in vivo (inside the body) or in vitro (outside the body). This work therefore is an exploratory research effort towards identifying and characterizing new anticoagulants. Extract of *Pentaclethra macrophylla* was subjected to anticoagulation activity and compared to EDTA and sodium citrate.

2. Materials and Methods

2.1. Plant Extract

The seeds of *Pentaclethra macrophylla* were procured from a local market in Elele, Rivers State. Then washed clean with tap water before they were manually cracked to extract the edible cotyledons, which were sliced and dried to constant weight at 75°C in an air draught oven (Astell-Hearson, London, UK). The dried samples were pulverized, mixed thoroughly and stored in polythene bags in a refrigerator. The dried sample was weighed out (0.1g, 0.2g and 0.5g) and introduced into plain containers, 2ml of blood was equally added to each container and observed for clotting.

2.2. Sample Collection and Analysis

Verbal consent was obtained from ten (10) human volunteers. 4ml of blood sample was collected from ante cubital part of the arm and dispensed 2ml each into 0.1g of the extract and EDTA/sodium citrate container. The blood samples were analyzed within six hours of collection. Packed cell volume (PCV), Platelet count, total white blood cell count, activated partial thromboplastin time (APTT), prothrombin time (PT) and haemoglobin concentration were analyzed using standard methods [8,9].

3. Results

Table 1. Clotting time for different concentrations of the extract

Concentration (g/2ml of blood)	Time (sec)
0.1	Not achieved
0.2	2520 (42mins)
0.5	105

When 2ml of blood was introduced into plain bottles containing 0.2g and 0.5g of the extract, clotting was achieved at 2520 sec and 105 sec respectively while at 0.1g/2ml of blood clotting was unattained.

Table 2. Mean \pm SD of extract compared to EDTA/sodium citrate

Parameters	Extract (Test)	EDTA/sodium citrate (Control)	pvalue
PCV (%)	39.2 \pm 3.83	40.0 \pm 4.00	p>0.05
Hb (g/dl)	13.0 \pm 1.69	13.3 \pm 2.19	p>0.05
WBC ($\times 10^9/l$)	9.4 \pm 1.55	8.7 \pm 2.26	p>0.05
Platelet count ($\times 10^9/l$)	307.4 \pm 86.30	305.2 \pm 84.54	p>0.05
APTT (sec)	9.0 \pm 1.41	44.2 \pm 5.63	p<0.05
PT (sec)	20.8 \pm 1.30	14.8 \pm 2.16	p<0.05

4. Discussion

Some plants have been attributed to possess anticoagulation property [10,11,12] but have not been properly utilized. Anticoagulant from plant source should definitely have better safety margin and eliminate monitoring of therapy. Such anticoagulant may present with little or no side effects both for laboratory and clinical use [13]. In this present study, the extract of *Pentaclethra macrophylla* clotted 2ml of human blood

sample at concentration of 0.2g and 0.5g in 2520sec and 105sec respectively while at 0.1g/2ml of blood, coagulation was not achieved. This indicates that the extract of *Pentaclethra macrophylla* exhibits anticoagulation activity. Medicinal plants show good therapeutic effect comparable to orthodox drugs and yet exhibit minimal unwanted side effects [14]. *Salvia multiorrhiza* and *D. chrysanthum* have also been shown to possess anticoagulation activity [12,15].

The extract-anticoagulated blood was compared with other anticoagulants, EDTA and sodium citrate using some routine haematological parameters. The PCV of the extract was 39.2 \pm 3.83% while that of EDTA was 40.0 \pm 4.00% which is statistically not significant (p>0.05). Also, at haemoglobin concentration of 13.0 \pm 1.69g/dl and 13.3 \pm 2.19g/dl for the extract and EDTA respectively, the difference was not significant (p>0.05). The extract is rich in potassium/calcium content [16], vitamins and iron [17]. This could possibly be the reason for its anticoagulation activity.

White blood cell count (WBC) of the extract-anticoagulated blood gave 9.4 \pm 1.55 $\times 10^9/l$ while EDTA gave 8.7 \pm 2.26 $\times 10^9/l$. The difference was insignificant (p>0.05) when compared. Platelet count which is used to assess coagulation was also analyzed. The platelet count was 307.4 \pm 86.30 $\times 10^9/l$ and 305.2 \pm 84.54 $\times 10^9/l$ for extract-anticoagulated blood and EDTA respectively. Though much literature is unavailable to compare with previous works, the difference between the extract of *Pentaclethra macrophylla* and EDTA showed little or no difference. This shows a promising prospect for its use for in vitro studies.

The extract-anticoagulated blood recorded a reduced APTT value of 9.0 \pm 1.41sec compared to that of sodium citrate which was 44.2 \pm 5.63sec. The prothrombin time was 20.8 \pm 1.30sec and 14.8 \pm 2.16sec for the extract and sodium citrate respectively. The APTT and PT were both statistically significant (p<0.05). Based on this significant difference, the extract might not be of importance in coagulation studies. In laboratory practice, some anticoagulants are reported to cause deleterious effects on blood cellular elements, while others confer unwanted bizarre colouration on cells [18]. This makes the choice of anticoagulant to be selective depending on the type of investigation so desired.

In this work, in vitro anticoagulation activity of the extract of *Pentaclethra macrophylla* have been established. The extract has potential which could be harnessed and produced into anticoagulant for in vitro/laboratory use.

Statement of Conflicting Interest

We declare that we have no conflict of interest.

Abbreviations

PCV	Packed Cell Volume;
Hb	Haemoglobin concentration;
WBC	White Blood Cell count;
APTT	Activated Partial Thromboplastin time;
PT	Prothrombin time.
EDTA	Ethylene diamine tetra acetic acid.
Sec	seconds

References

- [1] Ogueke, C.C., Nwosu, J.N., Owuamanam, C.I. and Iwouno, J.N. "The Fermented African Oilbean; Its Production, Chemical Composition, Preservation and Health Benefits". *Pakistan Journal of Biological Sciences*. 13. 489-496. 2010.
- [2] Ikuoria, E.U., Aiwonegbe, A.E., Okoli, P. and Idu, M. "Characteristics and Composition of African Oil bean seed (*Pentaclethra macr (Benth)*". *Journal of Applied Sciences*. 8 (7). 1337- 1339. 2008.
- [3] Achinewhu, S. C. "The effect of fermentation on carbohydrate and fatty acid composition of African oil bean seed" *Food Chem*. 19. 105-116. 1986.
- [4] Mbajunwa, O. K., Akungbala, J. O., Mulongoy, K. and Oguntimein, G. "Starter culture evaluation for the production of ugba from African oil bean seed". *Journal of Science Food Agriculture*. 77. 127-132. 1998.
- [5] Odoemelam, S.A. "Proximate Composition and Selected phytochemical Properties of African Oil Bean (*Pentaclethra Macrophylla*)" *Pakistan Journal of Nutrition*. 4 (6). 382-383. 2005.
- [6] Ugbogu, O. C. and Akukwe, A. R. "The antimicrobial effect of oils from *Pentaclethra macrophylla*, *Chrysophyllum albidum* G. Don on some local clinical bacteria isolates" *African Journal of Biotechnology*. 8. 285-287. 2009.
- [7] Roger, N. *Pocket Medical Dictionary*. 13th Edition, ELBS/Churchill Livingstone, Edinburgh. 1978. 21.
- [8] Cheesbrough, M. Haematological tests. In *District laboratory practice in tropical countries*. Part 2 Cambridge University Press, London. 2000. 297.
- [9] Ingram, G.C. "A suggested schedule for rapid investigation of acute haemostatic failure". *Journal of Clinical Pathology*. 14. 156 -160. 1961.
- [10] Son, D. J., Cho, M. R., Jin, Y. R., Kim, S. Y., Park, Y. H., Lee, S. H., Akiba, S., Sato, T. and Yun, Y. P. "Antiplatelet effect of Green Tea Catechins: A possible mechanism through arachidonic acid pathway" *Prostaglandins, Leukokienes and Essential Fatty Acids*. 71. 25-31. 2004.
- [11] Lee, S. H., Alhukorala, Y., Lee, J. S. and Jeon, Y. J. "Simple separation of anticoagulant sulfated galactan from marine red algae" *Journal of Applied Physiology*. 10. 1007. 2008.
- [12] Adams, J. D., Wang, R., Yang, J. and Eric, J. "Preclinical and clinical examinations of *Salvia multiorrhiza* and its transinones in ischemic conditions" *China Medicine*. 1. 3-5. 2006.
- [13] Ajugwo, A. O. and Ezimah, A. C. " In vivo studies of anticoagulation activity of *Triclisia dictyophylla* using albino Wistar rats". *Int. Journal of Pharmaceutical Science Inventions*. 2 (5). 37-40. May 2013.
- [14] Bielory, L. "Complementary and alterative interventions in asthma, allergy and immunology". *Ann Allergy Asthma immunology*. 93. 45-54. 2004.
- [15] Ping, L., Yiqun, T.L. and Zyang, G. "Preliminary studies on anticoagulant function of *Dendrobium chrysanthum*". *Chinese Journal of Natural Medicine*. 3. 44-47. 2005.
- [16] Onwuliri, V.A., Attah, I. and Nwankwo, J.O. "Anti-Nutritional factor Essential and Non-Essential Fatty Composition of Ugba (*Pentaclethra macrophylla*) Seeds At Different Stages of Processing and Fermentation". *Journal Of Biological Sciences*. 4. 671-675. 2004.
- [17] Umelo, R. "Potentials for utilization of African star apple (*Chrysophyllum albidum*) for jam making in Nigeria". in *National workshop on the potentials of star apple in Nigeria*. 1997. 103.
- [18] Bain, B. J. *Blood cells: A practical guide*. Gower Publishers, London. 1989.