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Chemical Analyses of *Annona muricata* Leaf Extracts

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ABSTRACT

The chemical analyses of *Annona muricata* leaves were determined using standard methods. The proximate analysis was done on the dry sample while the phytochemicals, minerals and vitamins were performed on the leaf extracts. The proximate compositions of the leaves were moisture content (5.79%), ash content (8%), crude fats (2.33%), crude fibre (46.95%), crude protein (22.58%) and carbohydrate content (14.20%). The phytochemicals composition showed that phenols, flavonoids, tannins, glycosides, alkaloids and saponins were present in both extracts in significantly ($P<0.05$) different concentrations. The minerals (calcium, zinc, copper, manganese, iron, sodium, magnesium and potassium) and vitamins (A, B_1, B_2, B_3, C, D, E and K) differed significantly between the extracts. The result showed that *A. muricata* leaves contain chemical properties which have pharmacological effect and are vital in the treatment and prevention of diseases and illness in man.

Keywords: *Annona muricata,* chemical analyses, proximate, vitamins and diseases.

INTRODUCTION

Plants are grown on the surface of the earth and are sources of food or medicine for human beings [1].Its usage as medicine has been an immemorial practice [2].The use of herbs (medicinal plants) is widely embraced in different countries, both developed and developing countries such as United Kingdom, North America, China, Australia, Ghana, India, Nigeria and Switzerland [3].

Annona muricata belongs to the family, *Annonaceae*. It is known mainly for its comestible fruit. *A. muricata* is usually called "Soursop" in English, its local names vary from country to country including *Graviola* (Portugual), *Corossol* (French) and *Brazilian pawpaw* (Brazil) [4]. In Nigeria, the plant is called "*Abo"* in Yoruba and "*Shawshop"* in Igbo [5]. *A. muricata* is found in the Caribbean, Central America, South America and also in West African Countries [6]. *A. muricata* leaves extracts have been reported to have many properties which include anti-inflammatory [7], gastroprotective activity [8], antioxidant activity [9], antidiabetic activity [10]; [11] and anticancerous property [12]; [13]; [14].

In view of the above listed pharmaceutical activities of *A. muricata*, there is need for further research into the chemical components of *A. muricata* leaves considering the fact that the constituents may vary depending on the solvent used for extraction. This study was aimed at investigating the chemical properties of

Annona muricata aqueous and ethylacetate leaf extracts.

MATERIALS AND METHODS

Materials

The fresh leaves of *A. muricata* were collected from No 2 New heaven Akama, Ngwo Village, Enugu State, Nigeria. They were washed with distilled water, dried at an ambient temperature and ground into powdered form. Deionized water and ethylacetate were used for the extraction, according to a method by [15]. The ground leaves (750g) were macerated in 1500ml of deionized water and ethylacetate respectively for 48hours. The mixture was filtered with muslin cloth, then with filter paper. The filtrate was concentrated by evaporation to dryness using a rotary evaporator.

Methods

Proximate Composition of *A. muricata* Leaves: Crude fiber, crude protein, carbohydrate, ash, moisture and fat contents were determined using method described by the Association of Official Analytical Chemists (2000).

Phytochemicals Analysis of the Extracts: Alkaloids, tannins and phenols were determined by the method described by [16]. Saponins and cyanogenic glycosides determined using methods as described by [17] and [18] respectively, while flavonoids was determined using a method described by [17].

Mineral Analysis of the Extracts: Sodium, calcium, magnesium, manganese, iron, zinc, potassium and copper were determined using atomic absorption spectrophotometer [18] as described by [19].

Vitamin Analysis of the Extracts: The vitamin content (Vit. A, B_1, B_2, B_3, C, D, E and K) of the extracts were determined by the methods described by [20] and [21].

Statistical Analysis

Data obtained were expressed as mean ± standard deviation. The data were analyzed using analysis of variance (ANOVA).

RESULTS

The proximate composition of *Annona muricata* leaves is presented in figure 1, the phytochemicals and minerals constituents of the leaf extracts are presented in figures 2 and 3 respectively, while vitamins constituents of the leaf extracts are presented in table 3.

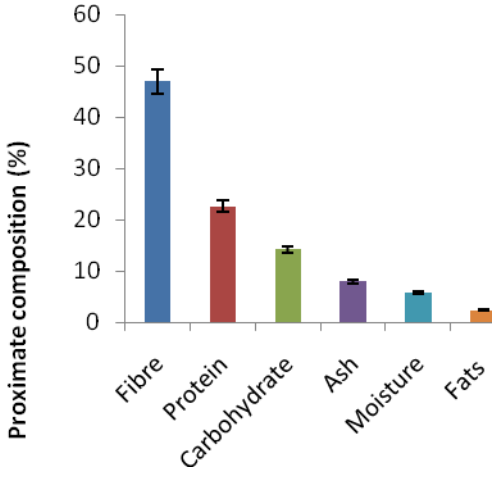

Figure 1: Proximate composition of *A. muricata* leaves. Values are mean±S.D; n=3

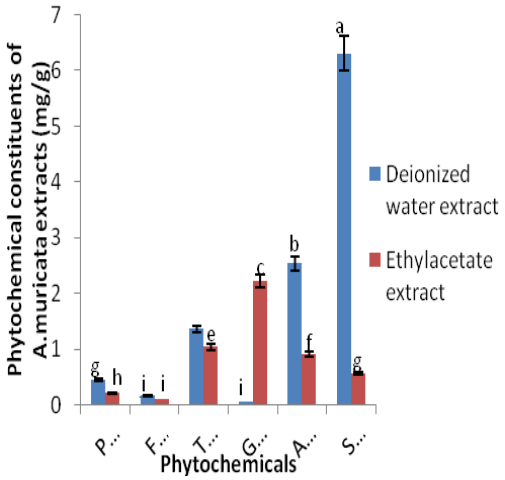

Figure 2: phytochemical constituents of deionized water and ethylacetate extracts of *A.muricata* leaves. Values are mean±S.D; n=3. Bars bearing different alphabet differs significantly (P<0.05)

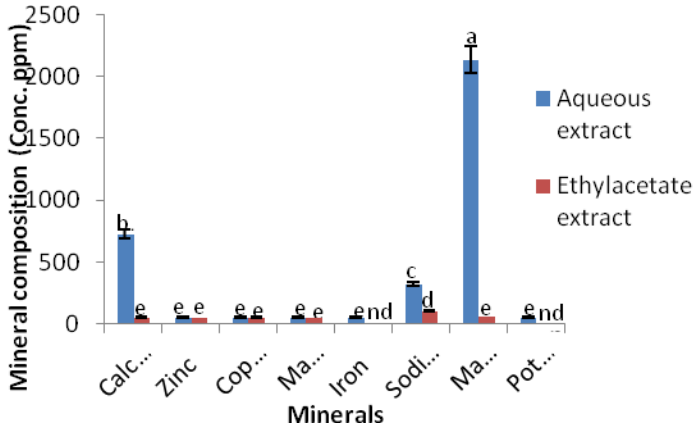

Figure 3: Mineral content of *A. muricata* leaf extracts. Values are mean±S.D; n=3. Bars bearing different alphabet differs significantly (P<0.05). nd=not detected.

Table 1: Vitamin Constituents of the extracts

Vitamins (mg/g)	Aqueous leaf extract (mean±SD)	Ethylacetate leaf extract (mean±SD)
Vit. A	0.004 ± 0.01^{g}	0.032 ± 0.04^{e}
Vit. B_1	0.002 ± 0.03^{g}	0.010 ± 0.05^{f}
Vit. B_2	0.050 ± 0.01^{e}	0.011 ± 0.05^{f}
Vit. B_3	1.390 ± 0.04^{b}	0.100 ± 0.01^{d}
Vit. C	1.550 ± 0.09^{a}	0.850 ± 0.05^{c}
Vit. D	0.900 ± 0.05^{c}	5.370 ± 0.09^{a}
Vit. E	0.007 ± 0.02^{f}	0.044 ± 0.01^{e}
Vit. K	0.400 ± 0.03^{d}	2.590 ± 0.08^{b}

DISCUSSION

The proximate composition of *A. muricata* leaves showed that it contain moisture, ash, crude fats, carbohydrate, protein and higher value of crude fibre (46.95%) as shown in Figure 1. The high content of crude fibre may be partly responsible for the hepato-protective effect of the leaves and its usefulness in the management of diabetes and hypercholesterolemia [22]; [23]; [24].

The value of crude protein in *Annona muricata* leaves (22.58%) is also high. Thus it is considered as a good source of protein and can meet the requirement of protein in the body. Proteins are one of the macromolecules which are essential in the structure and function of all living things. It functions as digestive enzymes, transporters, antibodies and acts as alternative source of energy in the body [25]. The protein value of *Moringa olefera* leaves (28.08%) is higher than that of *Annona muricata*, while *Psidium guajava* leaves (18.64%) is lower [26]; [27]. The carbohydrate content of *Annona muricata* leaves (14.2%) is lower than the carbohydrate content of *Moringa olefera* leaves (47.09%) (Bamishaiye *et al.,* 2011)

and *Carica papaya* leaves (78.22%) [28]. Breakdown of carbohydrate is one of the major sources of energy in the body. Energy is needed to drive the basic functions of the body and to carry out physical activities [29].

The ash content is a reflection of the mineral contents present in the plant materials. The *Annona muricata* leaves ash value (8.00%) is higher than the ash value of *Psidium guajava* leaves (4.35%) as observed by [30]. This was consistent with the observations of [31], stating the high mineral content of *A. muricata* leaves. The moisture content of *A. muricata* leaves (5.79%) is comparatively low (*Allium satium* bulb-82.00% and *Newbouldia laevis* leaves-55.00%) [32]. The low moisture content would prevent microbial growth and spoilage [33]. Although dietary fats increase the palatability of the diet or food, it contribute to overconsumption of energy because of the high energy content per unit weight which can lead to obesity or weight gain. *Annona muricata* leaves exhibited low level of crude fat (2.34%) just like *Moringa olefera* leaves (2.5%) [34] and *Carica papaya* leaves (1.37%) [35].
Phytochemicals (alkaloids, tannins, phenols, saponins, cyanogenic glycosides and flavonoids) were present in leaf extracts *A. muricata*. The presence of these observed phytochemicals maybe partly responsible for the medicinal properties of the leaf extracts. Flavonoids and phenols have been reported to possess antioxidant, anticancer and hepato-protective activities. This is because of their ability to scavenge free radicals which are implicated in cancer, renal and hepatic damages [36]; [37]. Phenols can interfere at all stages of cancer formation [38] and [39]. Saponins are also reported to have antioxidant property, inhibiting lipid peroxidation in the body [40]. Alkaloids (catharanthine, vindolinine and vindoline) and glycosides (allen) have been reported to lower blood glucose levels [41]. Alkaloids also act as an antimicrobial by interposing with the DNA of microbes [26] Tannins have also been disclosed to inhibit superoxide radical generation [42].

The difference in the concentrations of the phytochemicals in the leaf extracts maybe because of their different solubility in the solvents. The concentration of phytochemicals observed in this work is relatively high compared to the values reported by [3] on the phytochemicals of *Zingiber officinale* ethanolic stem extract. The phytochemicals of *Moringa olefera* leaf extracts recorded by [37],showed similar concentrations in the leaf extracts of *A. muricata* except that they recorded high content of tannins in *Moringa olefera* aqueous (9.36mg/g) and ethanolic (9.19mg/g) leaf extracts when compared to *Annona muricata* aqueous (1.36mg/g) and ethylacetate (1.04mg/g) leaf extracts.

The mineral analysis of leaf extracts of *A. muricata* revealed that aqueous extract contain high content of magnesium, calcium and minute amount of zinc, copper, manganese, iron, sodium and potassium. The ethylacetate leaf extract contained trace amount of zinc, copper, manganese, sodium and magnesium. The high concentration of magnesium and calcium could mean that *Annona muricata* helps in wound-healing, meditating some hormonal responses, bone formation and neuro-response. The presence of zinc, copper and iron in the leaf extracts could mean that *Annona muricata* aids in immunity. Minerals play important roles in the metabolic activities of mammals [38]. For instance, sodium and calcium are implicated in nerve and muscle contraction. They serve as co-factors for

many metabolic reactions, for example, magnesium is needed mostly in ATP depended-reactions and DNA/RNA synthesis [39]. Iron is an important component of cytochromes which are required for oxidative phosphorylation, it is needed for the transportation of oxygen in the blood and the enhancement of white blood cells in immunity fight [40]. Zinc has been reported to play a role in antioxidant defense. [42], examined the mineral contents of *Annona muricata* leaves. The result showed the presence of magnesium (9619mg/kg), calcium (11183.50mg/kg), zinc (8.34mg/kg), copper (14.25mg/kg), manganese (8.25mg/kg), iron (139.50mg/kg), sodium (694.86mg/kg) and potassium (14.25mg/kg) in the *A. muricata* leaves. This result also confirmed the high content of magnesium and calcium in *A. muricata* leaves. *A. muricata* leaves can be source of these minerals in the body as they are present in dry leaves and in their extracts.

Vitamin C value was significantly higher ($P< 0.05$) in aqueous leaf extract than the other vitamins. The high content of vitamin C may be responsible for the antioxidant property of the extracts. Vitamin C has been reported as an antioxidant which helps in fighting the free radicals that manifest as a result of chemical, drugs and disease (Ibrahim and Abdullahi, 2015). Vitamin B_3 is essential in formation of coenzymes NAD^+ and $NADP^+$ which are vital in redox reactions. The vitamin D and K values were higher in ethylacetate leaf extract. This can also explain the anti-inflammatory and wound-healing properties of *Annona muricata* leaves because vitamin K acts as blood coagulant and also helps in bone formation (as well as vitamin D). Vitamin E is also known for its antioxidant property [39], Vitamin B_1 and B_2 aid in blood circulation, maintenance of heart muscles and prevents nerve damages [34]. [42], reported higher value of vitamins in *Annona muricata* fruit drink than in the leaf extracts.

CONCLUSION

The results showed that the leaves of *Annona muricata* contained chemical compounds which may be responsible for the effect of *Annona muricata* leaf extracts. The result also justifies the use of *A. muricata* as a multipurpose medicinal plant.

REFERENCES

1. Mbagwu, F. N and Ajero, C. M. (2005). Advances in biotechnology, biological weapons and phytomedicine. Oweeri mega soft publishers, medical publishers ltd, New Delhi. 130-234.
2. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Review,* **6**(11): 1-5.
3. World Health Organization. (1998). Guidelines for the appropriate use of herbal medicine. Manila, Philippines.
4. Dayeef, A. M., Karyono, S and Sujuti, H. (2013). The influence of *Annona muricata* leaves extract in damaging kidney cell and inducing caspase-9 activity. *Journal of Pharmacy and Biological Studies,* **8**(5): 48-52.
5. Oreagba, A. I., Usman, S. O., Aina, O. O., Ogunkorede, T. O., Olawale, T and Olayemi, S. O. (2013). Evalution of the antimalarial

effects of the leaf extract and fruit juice of *Annona muricata* against *Plasmodium berghei* infection in mice. *University of Lagos Journal of Basic Medical Sciences,* **1**(2): 1-6.

6. International Organization for Plant Information (2008). Plant name detail: *Annonaceae Annona muricata* L. International plant name index.
7. Fong, C. P and Hamid, R. A. (2012). Evalution of anti-inflammatory activities of ethanolic extract of *Annona muricata* leaves. *Brazilian Journal of Pharmacognosy*, **22**(6):1301-1307.
8. Moghadamtous, S. Z., Rouhllah, E., Karimian, H., Fadaeinasab, M., Abdulla, M. A and Kadir, H. A (2014). Gastroprotective activity of *Annona muricata* leaves against ethanol-induced
9. Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F and Aei-Shemy, H. (2014). Phytochemical screening, antioxidant activity and in-vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). *Asian Pacific Journal of Tropical Medicine,* **7**(1): S355-S363.
10. Adewole, S. O and Ojewole, J. A. (2009). Protective effects of *Annona muricata* leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines,* **6**(1):30-41.
11. Adeyemi, D. O., Komolafe, O. A., Adewale, O. S., Obuotor, E. M and Adenowo, T. K. (2009). Antihyperglycemic activities of *Annona muricata. African Journal of Tradiational, Complementary and Alternative Medicines*, **6**(1): 62-69.
12. Asare, G. A., Afriye, D., Ngala, R. A., Abutiate, H., Doku, D., Mahmood, S. A and Rahman, H. (2015). Antiproliferative activity of aqueous leaf extract of *Annona muricata* L. on the prostate, BPH-1 cells and some target genes. *Integrative Cancer Therapies,* **14**: 65-74.
13. Eggadi, V., Gundamedi, S., Sheshagiri, S. B., Revoori, S. K., Jupally, V. R and Kulandivelu, U. (2014). Evalution of anticancer activity of *Annona muricata* in 1,2-dimethyl hydrazine induced colon cancer. *World Applied Sciences Journal,***32**: 444-450.
14. Harborne, T. B. (1998). Phytochemical Methods: A guide to modern technique of plant analysis. 5th edition. Chapman and Hill limited, London. 49-188.
15. Obadoni, B. O and Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta states of Nigeria. *Global Journal of Pure and Applied Science,***8**:203-208.
16. Siddiqui, A. A and Ali, M. (1997). Practical pharmaceutical chemistry. 1st edition. CBS Publishers and Distributors, New Delhi. 126-131.
17. Trease, G. E and Evans, W. C. (2002). Pharmacognosy. 15th edition. Saunders publisher, London. 380-393.
18. James, C. S. (1995). Analytical chemistry of food. Chapman Hall, New York. 145-320.
19. Ijeh, I. I., Ejike, C. E., Nkwonta, O. M and Njoku, B. C. (2010). Effect of traditional processing techniques on the nutritional and phytochemical composition of African bread fruit (*Treculia africana*) seeds. *Journal of Applied*

Sciences and Environmental management, **14**(4): 169-173.
20. Pearson, D. (1976). The chemical analysis of foods. 7th edition. Churchhill Livingstone, Edingburg.
21. Baraket, M. Z., Shehab, S. K., Darwish, N and Zahermy, E. I. (1973). Determination of ascorbic acid from plants. *Analytical Biochemistry*, **53**: 225-245.
22. Devinder, D., Mona, M., Hradesh, R and Patil, R. T. (2011). Dietary fibre in foods: a review. *Journal of Food Science and Technology,* **49**(3): 255-266
23. Okaka, J. C., Akobundu, E. N and Okaka, A. N. (2011). Food and human nutrition: an integrated approach. 2nd edition. OJC Academic Publishers Enugu, Nigeria. 104-174.
24. Usunobun, U., Okolie, N. P., Anyanwu, O. G., Adegbeji, A. J and Egharevba, M. E. (2015). Phytochemical screening and proximate composition of *Annona muricata* leaves. *European Journal of Botany Plant Science and Phytology,* **2**(1): 18-28.
25. Nwokocha, L. M and Williams, P. A. (2009). New starches: physicochemical properties of sweet sop (*Annona squamosa*) and soursop (*Annona muricata*) starches. *Carbohydrate Polymers,* **78**(2009): 462-468.
26. Bamishaiye, E. I., Olayemi, F. F., Awagu, E. F and Bamshaiye, O. M. (2011). Proximate and phytochemical composition of *Moringa oleifera* leaves at the three stages of maturation. *Advance Journal of Food Science and Technology,* **3**(4): 233-237.
27. Offor, C. E. (2015). Phytochemical and proximate analyses of *Psidium guajava* leaves. *Journal of Research in Pharmaceutical Science.* 2(6): 05-07.
28. Nwofia, G. E., Ojimelukwe, P and Eji, C. (2012). Chemical composition of leaves, fruit pulp and seeds in some *Carica papaya* (L) morphotypes. *International Journal of Medicinal and Aromatic Plants,***2**(1): 200-206.
29. Barasi, M. E. (2003). Human nutrition: a health perspective. 2nd edition. Hodder Arnold group, London. 57-228.v
30. Ayoola, A. A., Yusuf, A. O and Oki, D. G. (2016). Phytochemical screening and proximate analysis of *Newbouldia laevis* and *Allium satium. Nigerian Journal of Animal Sciences,* **1**: 242-256.
31. Tapas, A. R., Sakarkar, D. M and Kakde, R. B. (2008). Flavonoids as nutraceuticals:a review. *Tropical Journal of Pharmaceutial Research,* **7**(3): 1089-1099.
32. Siqueira, A. D., Moreira, A. C., Melo, E. D., Stamford, T. C and Stamford, T. L. (2015). Dietary fibre content, phenolic compounds and antioxidant activity in soursops (*Annona muricata*). *Revista Brasileira de Fruticulyura,* **37**(4): 1020-1026.
33. Ibrahim, N. S and Abdullahi, M. (2015). Cytotoxicity, total phenol in contents and antioxidant activity of the leaves extracts of *Annona muricata. Chemsearch Journal,* **6**(1): 46-51.
34. Gemede, H. F and Ratta, N. (2014). Antinutritional factors in plant foods: potential health benefits and adverse effects. *International Journal of Nutritional and Food Sciences,* **3**(4): 284-289.
35. Gaikwad, S. B., Mohan, K. G and Rani, M. S. (2014). Phytochemicals for diabetes management. *Pharmaceutical Crops,***5**(1): 11-28.

36. Rarassari, M. A and Maftuch, H. N. (2016). Phtochemicals and antibacterial activities of soursop leaf (*Annona muricata*) against *Edwardsiella tarda* (in vitro). *Journal of Life Science and Biomedicine,* **6**(1): 6-9.
37. Nweze, N. O and Nwafor, F. I (2014). Phytochemical, proximate and mineral composition of leaf extracts of *Moringa oleifera* Lim from Nsukka, South-Eastern Nigeria. *Journal of Pharmacy and Biological Sciences,***9**(1): 99-103.
38. Ritter, J. M., Lewis, L. D., Mant, T. G and Ferro, A. (2008). A textbook of clinical pharmacology and therapeutics. 5th edition. Hodder Arnold publishers, UK. 23-50.
39. Vasudevan, D. M., Sreekumari, S and Vaidyanathan, N. K. (2011). Textbook of biochemistry for medical students. 6th edition Jaypee Brothers Medical Publishers LTD, New Delhi. 379-431.
40. Mbadianya, J. I., Echezona, B. C., Ugwuoke, K. I and Wokocha, R. C. (2013). Phytochemical constituents of some medicinal plants. *International Journal of Science and Research,* **2**(4): 18-22.
41. Onyechi, A. U., Ibeanu, V. N., Eme, P. E and Madubike, K. (2015). Nutrient, phytochemical composition and consumption pattern of soursop (*Annona muricata*) pulp and drink among workers in University of Nigeria Nsukka community. *Pakistan Journal of Nutrition,* **14**(12): 866-870.
42. Ayoola, A. A., Yusuf, A. O and Oki, D. G. (2016). Phytochemical screening and proximate analysis of *Newbouldia laevis* and *Allium satium. Nigerian Journal of Animal Sciences,*3(1): 1-14.