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Comparative Phytochemistry and Antioxidant Activities of Water and Ethanol Extract of *Annona muricata* Linn Leaf, Seed and Fruit

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Abstract: All parts of *Annona muricata* **(**soursop) are widely applied in alternative medicine and they are traditionally used in the treatment of infectious and chronic non-communicable diseases. In this study, we assessed the phytochemical and antioxidant profile of leaf, seed and fruit of *Annona muricata* (soursop). Using established conventional analytical methods qualitative/quantitative phytochemistry was carried out on aqueous/ethanol extract of air-dried samples of plant parts. Antioxidant potential of the parts was assessed using DPPH radical scavenging activity**.** Results showed that fruit-endocarp extract of *A. muricata* had the highest percentage yield (5.8%); closely followed by the leaf (4.9%). The qualitative phytochemical conducted on *A. muricata* revealed the presence of some bioactive compounds in all the parts. Aqueous extracts of the plant parts showed higher *in vitro* antioxidant potential compared to ethanol extracts, except the fruit-endocarp. The radical scavenging potential of the plants compared highly with that of standards, gallic (92%) and ascorbic acid (92%). Notable are those of leaf (80%) and fruit-epicarp (76%). In conclusion, *A. muricata* (leaf, fruit-bark, fruit-epicarp, fruit-endocarp and seed) has been shown to possess significant DPPH scavenging activities. This may give credence to the folklore use of the plants for myriad of disease conditions.

Key words: *Annona muricata* · Phytochemistry · Antioxidant · Cancer · DPPH

INTRODUCTION

The use of medicinal plants as major source of formulated drugs in the industrialized society can be traced back to the traditional use in folk medicine. Scientific utilization of medicinal plants is important as they can be toxic [1], also, can offer a wide range of therapeutic application [2]. *Annona muricata* (soursop) belongs to the genus Annona of the custard apple tree family and has age-long traditional use as medicinal plant. Soursop is a tropical plant that is also cultivated in Saharan Africa. They are perennial crops with edible fruits and they are commercially underutilized [3]. The plant has been reported to contain nutrients like vitamins B and C; phosphorus, iron and calcium [4]. These nutrients are thought to be complimentary in the traditional use of the plant. For instance, the iron ameliorates effects of massive haemolysis.

All parts of soursop are widely applied in alternative medicine. The bark, leaves and seeds possess diverse biological activities and they are traditionally used in the treatment of infectious and chronic non-communicable diseases such as diabetes, hypertension and inflammation. The leave has been demonstrated to be hepatoprotective, antiplasmodic [5] and anti-diabetic [6]. The fruit has been reported to possess antimicrobial, antitumor and antiviral effects. The juice of ripe fruit is applied as diuretic and the powdered immature fruits can be used as remedy for dysentery. Like other medicinal plants, *Annona muricata* contains phytochemicals that are responsible for their healing properties. The phytochemical screening of this plant revealed the presence of flavonoids (group of polyphenolic antioxidants), saponins, tannins, glycoside and alkaloids [7]. The leaves and seed of *Annona muricata* contain 50 mono THF acetogenins

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which is a key intermediate in acetogenins synthesis. Family Annonaceae family, to which soursop belongs, also contain neurotoxic alkaloids named annonacin. This neurotoxin is suspected to cause atypical Parkinsonism and other neurological effects on large or frequent consumption [8, 9].

Reactive oxygen species are formed in minute quantities during physiological cell metabolism. However, at high concentration, excessive free radical production or decreased capacity of endogenous antioxidant leads to state named oxidative stress [10]. This condition have potential of causing damage to cellular macromolecules including as carbohydrate, protein, lipids and nucleic acids thereby disrupting the functional and structural integrity of biological cells [11, 12]. Oxidative stress plays important roles in the pathogenesis of diseases such as cancer, neurological disorders, atherosclerosis, hypertension, ischaemic disease, diabetes, acute respiratory syndrome, fibrosis, pulmonary disease and asthma [13-15].

Under pathological condition, endogenous antioxidant system can be overwhelmed [16]; hence, there is necessity for recruitment of antioxidants derivable from medicinal plants. Exogenous antioxidants obtainable from plant sources can augment cellular defences and help to prevent cellular damage. Medicinal plants contain substantial amounts of phenolic compounds and flavonoids which possess multiple biological effects such as antioxidant, anti-inflammatory and antitumor [17,18]. Phenolic compounds as antioxidants operate as reducing agent, metal chelators or singlet oxygen quencher [19]. Studies have shown that plant constituents with antioxidant activities are capable of protecting biological system against oxidative stress [20, 21]. Crude aqueous or alcoholic extracts of plant materials have been confirmed to be rich in phenols and flavonoids which are known to have positive linear correlation with their antioxidant activities [22]. In this study, we assessed the phytochemical and antioxidant profile of leaf, seed and fruit extracts of *Annona muricata* (soursop).

MATERIALS AND METHODS

Plant Processing and Samples Preparation: *Annona muricata* was obtained from Ibadan, south-west Nigeria. Authentication was done by Fred Yakubu, Forestry Research Institute of Nigeria (FRIN). *Annona muricata* was separated into leaf, fruit-bark, seed, fruit-epicarp and fruit-endocarp. The different parts were air-dried on laboratory bench, to constant weight. Dried plant parts were pulverised and cold extraction was carried out by soaking 500g of each part in ethanol for 72 hrs. Thereafter, the filtrates were concentrated using Rotary evaporator to obtain the samples. Percentage yield was calculated from ratio of extraction to pulverised plant part. The extracts were stored refrigerated in amber coloured sample bottles for further investigations.

Phytochemical Screening: Chemical tests were carried out on the samples using standard procedures below:

Test for Alkaloids: About 200 milligrams of the grounded samples were boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 min. Filtrate of cool mixture was divided equally into 3 test tubes. On adding of 2 drops of Draggendorff's reagent to 1 ml portion of the mixture, a red precipitate indicated presence of alkaloids while a creamy white coloured precipitate of another portion treated with 2 drops of Mayer's reagent indicated the presence of alkaloid [23, 24].

Test for Flavonoids: A mixture of 500 mg pulverised samples and 10 ml of ethyl acetate was heated for a minute. Development of yellow colour on addition of 1ml dilute ammonium solution to a mixture 4 ml of the filtrate and 1ml of 1% ammonium chloride indicated flavonoid presence [25].

Test for Saponins: Drops of olive oil were added to a portion of filtrate from boiled the samples and shaken. Presence of stable froth on vortex of a mixture of the filtrate from olive oil-boiled sample and 4 ml distilled water indicated presence of saponins [25].

Test for the Presence of Tannins: Gelatinous precipitate on addition of drops of solution of lead acetate to filtrate of boiled mixture of sample-ethanol indicated presence of tannin [24].

Test for Glycosides: Development of brick red precipitate on adding Fehling solutions A and B to and boiled filtrate of the sample indicated presence of glycosides [26].

Test for Terpenoids and Steroids: Presence of reddish brown and greenish blue colours on gently adding concentrated sulphuric acid to a mixture of vortexed sample-chloroform indicated presence of terpenoids and steroids, respectively [25].

Quantitative Phytochemical Analysis

Determination of Flavonoids: A mixture of 5ml ethyl-acetate and 1% ammonium was added to filtered portion of boiled HCL solution of test sample. The quantitative presence of flavonoids was done by spectrophotometry [23].

Determination of Saponins: Ferrous sulphate reagent and concentrated H_2SO_4 were, in turn, added to acetone-ethanol solution of residue obtained from boiling HCL solution of test sample and adding petroleum ether. Quantitative saponins presence was determined through spectrophotometry [27].

Determination of Tannins: Solutions of $FeCl_3$- NH_4Cl and $K_2Fe(CN)_6$ were, in turn, added to filtered aqueous solution of test sample plus methanol. Quantitative determination of tannin was done by spectrophometry [28].

DPPH Radical Scavenging Activity: DPPH scavenging profile of the extracts was assessed using method described by Blios, (1958) with modification [29]. Briefly, 0.5ml of extracts (200 to 1000ig/ml) was mixed with methanol solution of 0.1mM DPPH. The resulting mixture was vortex and absorbance read after 30 minutes at 517 nm.

RESULTS AND DISCUSSION

Endocarp extract of *A. muricata* had the highest percentage yield (5.8%; Figure 1) which implies that with little quantity of the raw material; more of the yield will be produced thereby making it a good candidate in drug discovery from medicinal plant. Plants are a good source of synthetic and semi-synthetic drugs [30]. Sometime insight into research for development of candidate synthetic drugs is from plants [31]. Thus, percentage yield is a vital issue in the process of drug development. Next in percentage yield was the leaves (4.9%) followed by fruit-bark and epicarp (2.6 and 2.3% respectively).

Table 1: Qualitative phytochemical components of *A. muricata* plant parts

Phytochemical constituents	Fruit (Endocarp)	Fruit (Epicarp)	Seed	Leaf	Bark
Alkaloids	+	+	+	+	+
Anthraquinones	+	+	-	+	-
Flavonoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Saponins	+	+	+	+	-
Steroids	+	+	+	+	+

Key: (+) = present, (-) = absent

The phytochemistry (Table 1) conducted on *A. muricata* plant parts showed the existence of some biologically active compounds known to exhibit medicinal activities [32]. These include tannins, saponins, steroids, flavonoids, alkaloid, anthraquinones, terpenoids and glycoside. Tannins have been shown to have exhibit antimicrobial properties. This activity has been related to interference with vital endogenous microbial protein synthesis and binding to preformed endogenous microbial protein; depriving microbes of such proteins [33, 34]. Furthermore, tannins chelate iron which is necessary for enzymatic microbial protein production. Thus reducing microbial growth and proliferation.

Flavonoids are the most prevalent phenol from plants [35]. They exhibit effects against allergy, oxidative stress and inflammation. Sources of flavonoids have found

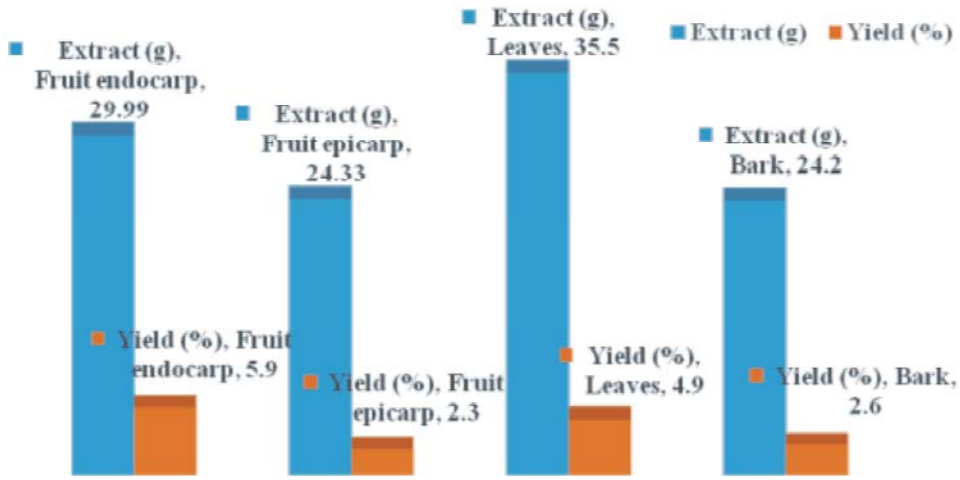

Fig. 1: Percentage yield of *A. muricata* (leaves, bark, seed, epicarp and endocarp)

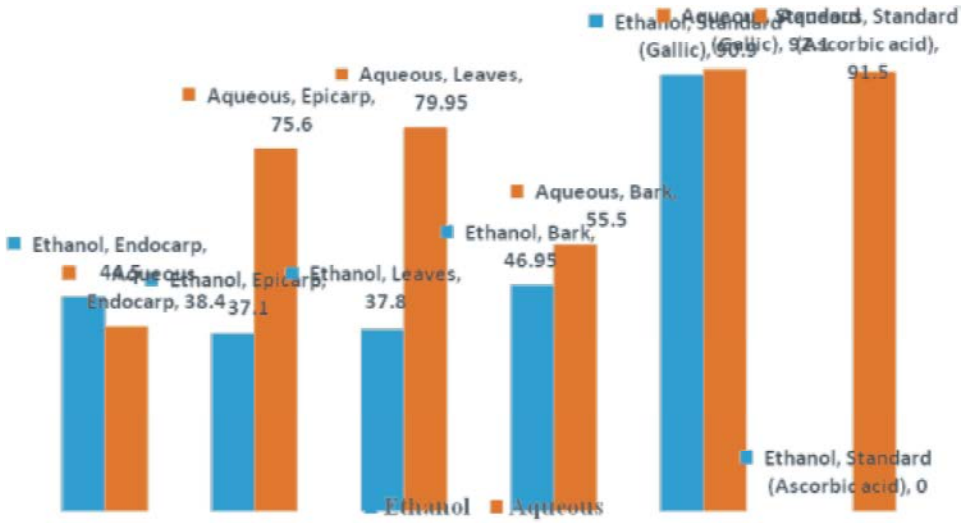

Fig. 2: Evaluation of *in vitro* antioxidants using DPPH radical scavenging assay

Table 2: Quantitative (%) phytochemical components of *Annona muricata* plant parts

Plant Parts	Flavonoids	Saponins	Tannins
Bark	2.20±0.28	6.35±0.80	2.08±1.06
Fruit (Endocarp)	24.00±0.28	1.00±0.57	1.63±1.45
Fruit (Epicarp)	9.70±0.42	1.40±0.57	1.42±1.14
Leaf	3.10±0.71	7.40±0.28	1.60±0.28
Seed	5.90±0.42	4.80±0.47	1.07±0.08

Results were expressed as mean±SD of triplicate determinations.

usefulness as antimicrobials, antitumor and antitoxins [36]. They stand as potent prevention of damages from oxidative stress by mopping up reactive oxygen species.

Steroids, alkaloids, saponins are noted for their anti-inflammatory, analgesic and anti-cholesterol properties, respectively.

The quantitative phytochemical estimation of percent crude chemical constituent in the *A. muricata* (leaves, bark, epicarp, endocarp, seed) is presented in Table 2. Results revealed that endocarp has the highest percentage of flavonoids (24%), bark has the lowest (2.2%) but the highest yield of saponin (6%) was found in leaf. Highest percentage of tannin was obtained from the fruit-bark (2%).

Aqueous extracts of the plant parts showed higher *in vitro* antioxidant potential compared to ethanol extracts, except the endocarp (Fig. 2). This may explain the habitual use of water in preparation of plants parts for local medicinal benefits. The radical scavenging ability of the plants compared highly with that of standards, gallic (92%) and ascorbic acid (92%). Notable are those of leaves (80%) and fruit-epicarp (76%).

Progression, morbidity and ultimate mortality as a result of diseases like microbial infection, metabolic disorders (like diabetes, atherosclerosis), cardiovascular disorders and neoplasm are related to inflammation and reactive oxygen species [15]. Expectedly, radical scavengers will not only ameliorate morbidity but halt disease progression, reducing mortality.

The plant under study showed high scavenging ability which may be ascribed to the flavonoid (phenolic compound) constituent. The anti-nutritive/anti-cholesterol activity of saponins present in the plant parts may further mitigate atherosclerosis in cardiovascular disease. Astringent characteristic of tannins make them valuable in inflammation and ulceration which are hallmarks in diabetes and neoplasm [37].

The plant parts under study contain bioactive medicinal components at various percentages. This may explain the folklore medicinal uses of combined therapy of aqueous preparations of *A. muricata* fruit and leaves.

CONCLUSIONS

Annona muricata (leaves, bark, epicarp, endocarp and seed) has been shown to possess significant DPPH scavenging activities. This may give credence to the folklore use of the plants for myriad of disease conditions.

REFERENCES

1. Keen, R.W., A.C. Deacon and H.T. Delves, 2004. Indian herbal remedies for diabetes as a cause of lead poisoning. Postgrad Med. J. Afri., 70: 113-114.

2. Bailey, C.J. and C. Day, 2009. Traditional plant as treatment for diabetes. Diabetes Care, 12: 553-564.
3. ICUC, 2002. Fruits of the future Annona: International Centre for Underutilized Crops.
4. Enweani, I.B., J. Obroku, T. Enahoro and C. Omoifo, 2004. The Biochemical Analysis of Soursop (*Annona Muricata L.)* and Sweetsop (*A. Squamosa L.)* and their potential use as oral rehydration therapy. Journal of food agriculture and environment, 2: 39-43.
5. Gleye, C., P. Duret, R. Laurens, R. Hocquenniller and O. Laprevote, 1998. Cis Monotetrahydrofuran Acetogenins from the roots of Annona Muricata. J. Nat. Prod., 61: 576-579.
6. Adewole, S.O. and J.A Ojewole, 2008. Protective effects of *Annona muricata Linn*. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin- treated diabetic rats. Afr. J. Trad. Complement Altern Med., 6: 30-41.
7. Arthur, F.K., E. Woode, E.O. Terlabi and C. Larbie, 2011. Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals. Eur. J. Exp. Biol., 1: 115-124.
8. Caparros-Lefebvre, D., A. Elbaz and C.P.S. Group, 1999. Possible relation of atypical parkinsonism in the French west indies with consumption of tropical plants: A Case-Control Study. The Lancet, 354: 281-286.
9. Champy, P., A. Melot, V. Guérineau Eng, C. Gleye, D. Fall, G.U. Höglinger, M. Ruberg, A. Lannuzel, O. Laprévote and A. Laurens, 2005. Quantification of Acetogenins in Annona Muricata linked to atypical parkinsonism in Guadeloupe. Movement Disorders, 20: 1629-1633.
10. Anosike, C.A., N.E. Ogbodo, A.L. Ezugwu, R.I. Uroko, C.C. Ani and O. Abonyi, 2015. DPPH radicaal scavemging activity of some thnomedicinal plants in Nigeria. American-Euraasian Journnal of Toxicologocal Sciences, 7(2): 104-109.
11. Marnett, L., 1999. Lipid Peroxidation--DNA damage by Malondialdehyde. Mutat Res., 424: 83-95.
12. Normaizatul Aftzah, I., W. Ahmad, F. Jamshed and S. Al-Jasabi, 2016. Quantitative biochemical analysis of antioxidant properties of *Carissa carandas* fruit ethanol and N-hexane extracts. Middle East Journal of Scientific Research, 24(8): 2418-2423.
13. Toshniwal, P.E. and Zarling, 1992. Evidence for increased lipid peroxidation in multiple sclerosis. Neurochem Res., 17: 205-207.
14. Lyras, L., N. Cairns, A. Jenner, P. Jenner and B. Halliwell, 1997. An assessment of oxidative damage to proteins, lipids and DNA in brain from patients with Alzheimer's disease. J. Neurochem., 68: 2061-2069.
15. Jenner, P., 2003. Oxidative stress in Parkinson's disease. Ann Neurol., 53: S26-S36.
16. Birben, E., U. Sahiner, C. Sackesen, S. Erzurum and O. Kalayci, 2012. Oxidative stress and antioxidant defense. World Allergy Organization Journal, 5: 9-19.
17. Kirti Jain, M., M. Abhishek, A. Ramesh, B.V. Somashekaraiah and D.N. Saraswathi, 2016. *In-vitro* antioxidant activity of lleaf extract of *Justicia wynaadensis*. World Engineering & Appplied Sciences Journal, 17(2): 99-106.
18. Andersen, Ø.M. and K.R. Markham, (Eds.), 2006. Flavonoids: chemistry, biochemistry and applications.: broken sound parkway Nw: Crc Press, Taylor & Francis Group.
19. Rice-Evans, C.A., N.J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids dnd phenolic Acids. Free Radical Biology And Medicine, 20: 933-956.
20. Cao, G., E.R. Sofic and R.L. Prior, 1996. Antioxidant capacity of tea and common vegetables. Journal Of Agricultural and Food Chemistry, 44: 3426-3431.
21. Ness, A.R. and J.W. Powles, 1997. Fruit and vegetables and cardiovascular disease: A Review. International journal of epidemiology, 26: 1-13.
22. Alali, F. and T. El-Elimate, 2007. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: An ICBG project. Natural Product Research, 21: 1121-1131.
23. Harbone, J.B., 1998. Phytochemical Methods*:* A guide to modern techniques of plant analysis, Chapman and Hall London.
24. Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 2nd ed, Braille tiridel and Macmillan publishers.
25. Siddiqui, AA. and M. Ali, 1997. Practical pharmaceutical chemistry. 1st ed., Cbs Publishers and distributors, New Delhi, pp: 126-131.
26. Sofowora, A., 1993. Recent trends in research into African medicinal plants. Journal of Ethno Pharmacology, 38(2): 197-208.
27. Oloyede, O.I., 2005. Chemical profile of unripe pulp of *Carica Papaya.* Pak. J. Nutr., 4: 379-381.
28. Onwuka, G.I., 2005. Food analysis and instrumentation theory and paractice, 1st ed, Naphthali Prints, Lagos, pp: 114-169.

29. Blois, M.S., 1958. Antioxidant determination by the use of stable free radicals. Nature, 181: 1199-1200.
30. Harvey, A.L., 2008. Natural Products in drug discovery. Drug Discovery Today, 13(19): 894-901.
31. Fabricant, D.S. and N.R. Farnsworth, 2001. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives, 109(1): 69.
32. Gajalakshmi, S., S. Vijayalakshmi and V. Devi Rajeswari, 2012. Phytochemical and pharmacological properties of *Annona muricata*: A review. International Journal of Pharmacy and Pharmaceutical Sciences, 4(2): 3-6.
33. Adeosun, A.M., S.O. Oni, O.M. Ighodaro, O.H. Durosinlorun and O.M. Oyedele, 2016. Phytochemical, Minerals and free radical scavenging profiles of Phoenix Dactilyfera L. Seed extract. Journal of Taibah University Medical Sciences, 11(1): 1-6.
34. Ncube, N.S., A.J. Afolayan and A.I. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African Journal of Biotechnology, 7(12): 1797-1806.
35. Balasundram, N., K. Sundram and S. Samman, 2006. Phenolic compounds in plants and Agri-industrial By-Products: Antioxidant activity, occurrence and potential uses. Food Chemistry, 99(1): 191-203.
36. Benavente-Garcia, O. and J. Castillo, 2008. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular and anti-Inflammatory activity. Journal of Agricultural and Food Chemistry, 56(15): 6185-6205.
37. Aggarwal, B.B., S. Prasad, S. Reuter, R. Kannappan, V.R. Yadev, B. Park, J.H. Kim, S.C. Gupta, K. Phromnoi, C. Sundaram and S. Prasad, 2011. Identification of Novel anti-inflammatory agents from ayurvedic medicine for prevention of chronic Diseases: "Reverse Pharmacology" and "bedside to bench" Approach. Current Drug Targets, 12(11): 1595-1653.