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Phytochemical Evaluation of Dry, Wet and Oil of Leaf of Annona muricata for Medicinal Activities

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Abstract
Qualitative and quantitative phytochemical analyses were carried out on the dry, wet and oil of the leaves of *Annona muricata* using standard methods. The phytochemicals detected in the aqueous extracts of dry, wet and oil of the leaf were flavonoids, cardiac glycoside, tannins, saponin, terpenoids and steroids. The result of the quantitative analyses showed that oil extract had the highest total phenolics content (TPC: 3.09 mg GEA/g), total flavonoids content (TFC: 16.67 mg QE/g), total saponins content (TSC: 16.86 mg DE/100g) and Total tannins content (TTC: 1.13 mg GAE/g) followed by dry leaf extract (TPC: 1.66 mg GEA/g; TFC: 7.067 mg QE/g; TSC: 14.55 mg DE/100g; and TTC: 0.78 mg GAE/g) and the least was recorded for the wet leaf extract (TPC: 0.83 mg GEA/g; TFC: 3.92 mg QE/g; TSC: 12.07 mg DE/100g; and TTC: 0.44 mg GAE/g). The findings indicate that an *Annona muricata* leaf is a potential source of phytomedicine with the oil rich in phytochemicals, than dry and wet leaf extracts and any other biochemical analyses should be done using the oil.
Keywords: Phytochemicals, leaf, *Annona muricata,* dry, wet, oil

Introduction
The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). *Annona muricata* commonly called Sour-sop is commonly found in southern part of Nigeria. It is mostly eaten as fresh fruits. Sour-sop has found its uses in many areas. It is consumed as a desert fruit. It is made into a fruit jelly with the addition of some gelatin or used in the preparation of beverages, ice creams and syrups. A number of medicinal properties are attributed to the leaves and juice of the soursop. This study is therefore conducted to determine phytochemical properties of the dry, wet and oil of the leaves of *Annona muricata* plant.

Material and Methods
Chemicals
Ethanol, distill water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid, Ammonia solution, picric acid, Hexane.

Sample Collection
The leaves of *Annona muricata* (sour-sop) were collected from the local suppliers in Akure, Ondo state (Nigeria). The leaves were used for the purpose of phytochemical analysis. The plants collected were identified botanically in Department of Plant science, School of Agriculture, Federal University of Technology, akure. Fresh and tender leaves of selected plants were used for phytochemical analysis.

Preparation of plant extract
The leaves of of *Annona muricata* (sour-sop) were removed from the plant and then washed under running tap water to remove dust.

Fresh leaves extract
The leaves were crushed, 5 % HCl was added and the concentrated aliquot extracts obtained were used for phytochemical analysis.

Dry leaves extract
The leaves were air dried for few days and crushed into powder and stored in air-tight stopped glassware for use. The plant powder was taken in a test tube and distilled water was added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis.

Extration of oil from *Annona muricata* (sour-sop) leaf
10 g portion of the milled sample was soaked in 100ml of n hexane and reflux at 60°C 3 times. The evaporation of the n hexane was performed using a rotary evaporator on a water bath for 1 h leaving the oil. The oil were concentrated and stored in air tight bottles until used.

Qualitative phytochemical analysis of *Annona muricata* (sour-sop) leaves and Oil
Chemical tests were done on the wet and dry leaves and oil obtained from *Annona muricata* (sour-sop) using standard procedures to identify the constituents as described by Sofowora (1993).

Test for alkaloids
For the purpose of phytochemical analysis of wet and dry leaves and oil obtained from *Annona muricata* (sour-sop), 5 ml of the extract was added to 5mL of aqueous HCl (1%) in a steam bath. The solution was filtered and the filtrate was treated with a few drops of Dragendorff's reagent. Turbidity or precipitate showed the presence of alkaloids (Trease and Evans, 1978).

Test for saponins
1 ml of the extract was shaken with 5mL of water in a test tube and warmed. Frothing indicated the presence of saponins (Trease and Evans, 1978).

Test for steroids
2ml of acetic anhydride was added to 0.5ml of each plant extract with 2ml H_2SO_4. The colour change from violet to blue or green indicates the presence of steroids (Edeoga, 2005).

Test for tannins
5 ml of the extract was stirred with 10mL of distilled water. The mixture was filtered and the filtrate treated with ferric chloride. A blue-green – black-green precipitate indicated the presence of tannins (Trease and Evans, 1978).

Test for cardiac glycosides
Keller – Killiani test
5 mL of the extracts was treated with 2mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for flavonoids
For the confirmation of flavonoid in the wet and dry and oil obtained from the leaves of sour-sop, 0.5 g of each extract were added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract (Sofowora, 1993).

Test for terpenoids
An amount of 0.8 g of the dry, wet and oil extracts were taken in test tubes, then poured 10 ml of methanol, shake well and filtered to obtain 5 ml extracts of each sample. Then 2 ml of chloroform were mixed with each extract of the sample and 3 ml of sulphuric acid were added to the samples extract. Formation of reddish brown color indicates the presence of terpenoids in the samples (Edeoga, 2005).

Test for phlobatannins
Plant wet, dry and oil extracts were separately mixed with distill water in test tubes, then shake well, and filtered to take plant extract. Then to each extract, 1% aqueous hydrochloric acid was added and each sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.

Quantitative phytochemical analysis
The phytochemicals which are present in the wet crushed leaves and aqeous extracts of dry powdered leaves and the oil extracts of sour-sop were determined and quantified by standard procedures.

Determination of total phenolics
100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of distilled water. 1.5 ml of this solution was transferred to a test tube, then 1 ml 2N of the Folin-Ciocalteu reagent and 2 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Hageman *et al.,* 2000).

Determination of total flavonoids

The total flavonoid content of the extract was determined using a slightly modified method reported by Meda *et al.* (2005). 0.5mL of the extract samples were mixed with 0.5mL methanol, 50µl of 10% $AlCl_3$, 50µl of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard by making use of a seven point standard curve (0-100 µg/mL). The total flavonoids content of samples was determined in triplicates and the result was expressed as mg quercetin equivalent per gram of the sample.

Determination of total tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

Determination of total saponins

Estimated of total saponins content was determined by the method described by Makkar *et al.* based on vanillin-sulphuric acid colorimetric reaction with some medications (Makkar *et al.,* 2007). About 50 µL of the extract was added with 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 ^{o}C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

Results

Qualitative phytochemical analysis

The present study revealed that the aqueous extracts of dry and wet leaves and oil of the leaves of *Annona Muricata* contained saponins, steroids, flavonoids, tannins, terpenoids and cardiac glycosides. However, steroids were detected only in the oil extract and the tannins were found in the aqueous extract from dry leaf and oil extract. Flavonoids, saponins, terpenoids and glycosides were present in all the samples. Alkaloids and phylobatanins were absent in all the tested extracts (Table 1).

Quantitative phytochemical analysis

Total phenolics content

Total phenolics content (TPC) of oil, dry and wet leaves extracts of sour-sop plant showed that the oil extract had the highest TPC (3.09 mg GEA/g) followed by dry leaf extract (1.66 mg GEA/g) and the wet leaf had the least TPC value (0.83 mg GEA/g) (Table 2).

Total flavonoid content

The total flavonoids content was highest in oil extract (16.67 mg QE/g) followed by dry leaf extract (7.067 mg QE/g) and the wet leaf had the least content (3.92 mg QE/g) (Table 2).

Estimation of tannins content

The tannins content of the extracts was determined and found to be 1.13 mg GAE/g in oil extract, 0.78 mg GAE/g in dry leaf extract and 0.44 mg GAE/g in wet extract (Table 2).

Estimation of saponins content

The total saponins content of the samples were found to be highest in oil extract (16.86 mg DE/100g) followed by dry leaf extract (14.55 mg DE/100g) and the least value was seen in wet extract (12.07 mg DE/100g).

Table 1: Phytochemical analysis of Oil, Dry and Wet leaves of *Annona muricata* (sour-sop)

Compounds	*Annona muricata* Oil	*Annona muricata* Dry leaf	*Annona muricata* Wet leaf
Alkaloids	--	--	--
Saponins	+	+	+
Steroids	++	--	--
Flavonoids	++	+ +	+
Tannins	+	+	--
Terpenoids	++	+	+
Cardiac glycosides	++	+	+
Phylobatanins	--	--	--

++ = highly present, + = moderately present, -- = absent

Table 2: Quantitative analysis of phytochemicals (mg/g) of Oil, dry and wet leaves of *Annona muricata* (sour-sop)

Compounds	*Annona muricata* Oil	*Annona muricata* Dry leaf	*Annona muricata* Wet leaf
[1]Flavonoids	16.67 ± 7.16^{a}	7.067 ± 0.87^{b}	3.92 ± 1.06^{b}
[2]Phenolics	3.09 ± 1.11^{b}	1.66 ± 0.53^{a}	0.83 ± 0.19^{b}
[3]Tannins	1.13 ± 0.15^{a}	0.78 ± 0.13^{a}	0.44 ± 0.075^{b}
[4]Saponins	16.86 ± 1.05^{c}	14.55 ± 1.05^{b}	12.07 ± 1.06^{a}

Values were performed in triplicates and represented as mean±SD. [1]; mg QE/g extract, [2]; mg GAE/g extract, [3]; mg GAE/g, [4]; mg DE/g extract. Mean values followed by different superscript in a column are significantly different ($P<0.05$)

Discussion

Phytochemical analysis conducted on the dry, wet and oil leaves extracts of *Annona muricata* plant revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993). Analysis of the plant extracts revealed the presence of phytochemicals such as tannins, flavonoids, saponins, cardiac glycosides, steroids and terpenoids. These secondary metabolites reported to have many biological and therapeutic properties when ingested by animals (Charalampos *et al.,* 2013).Plants used in the treatment of diseases are said to contain bioactive principles with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Evans, 2002). The qualitative phytochemical screening of *Annona muricata* was in agreement with the works of Foong and Hamid, (2012), Falodun *et al*, (2011), and Vijayameena *et al* (2013).

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al*., 2013; Pourmorad *et al*., 2006; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995).

Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001), thus supporting the reasons why *Annona muricata* has position among medicinal plants used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues. (Okwu and Emineke, 2006; Li *et al*., 2003; Adegboye *et al.*, 2008).

Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.*, 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011).

Steroids have been reported to have antibacterial properties (Raquel, 2007) and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001).

Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995). *Annona muricata* is used for the treatment of cardiac infections along with other ailments such as cough, and chest pain in Jamaica, Haiti, and the West Indies (Technical Data Report for Graviola, 2005; Taylor, 2002).

This study shows that oil extract from *Annona muricata* are rich in phytochemicals than dry and wet leaves extracts and that the utilization of these extracts should be strongly recommended for good health. *Annona muricata* leaves are reservoirs for free radical scavenging molecules such as tannins, terpenoids, phenolic acids, flavonoids and other metabolites, which are basically rich in antioxidant activities.

Conclusion

The oil extracts from the leaf of *Annona muricata* plant has varieties of phytochemicals such as flavonoids, phenolics, tannins, saponins, steroids and cardiac glycosides at higher concentration than those obtained from dry and wet extracts qualitatively and quantitatively.

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