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Phytochemical composition and antioxidative potential of Purple Canary (*Canarium schweinfurthii*) fruit

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Abstract
The extracts of matured seed of purple canary (*Canarium schweinfurthii bursaraseae*) 'atili' in different solvents were evaluated for phytochemicals and antioxidative potential using standard methods. Preliminary phytochemical screening of the plant extracts showed the presence of phytochemical constituents such as tannin, resin, saponins, flavonoids, terpenoids, steroids, alkaloids, protein, glycosides, carbohydrates and fixed fat and oils in methanol extract with highest concentrations followed by water extracts. Antioxidant potential of various extracts of n-hexane, acetone, methanol and water respectively were; total phenol (416.14, 412.03, 620.31 and 598.47) g GAE/100 g, total flavonoids (1.18, 1.83, 3.56 and 2.71) mg RE/g, Ferric reducing property (106.28, 232.03, 258.96 and 284.11), DPPH radical scavenging (13.61, 17.28, 48.15 and 32.27), vitamin C (32.85, 31.32, 34.21 and 45.21) mg/100g and vitamin E (487.20, 451.40, 338.0 and 338.0) ppm. The results indicate that *Canarium* seed possessed appreciable amount of phytonutrients and antioxidant especially in methanol and water extracts which could serve as alternative medicine for people suffering from cancer, diabetic, hypertension and other cardiovascular diseases.

Keywords: Phytochemical, Antioxidant, Purple canary, Plant extracts

1. Introduction
Indigenous fruits play a vital role in the livelihoods of many rural communities in Nigeria, especially those living in the dry-lands. Ethnobotanical records available on useful wild plants in Nigeria highlight the importance of *Canarium* sp. medicinal roles and human consumption of their edible fruits. The combined action of two or more components in fruits or vegetables often potentiates a specific therapeutic action [1] and with no observed secondary or collateral effects as is the case with chemically synthesized compounds [2]. Antioxidants enzymes (made in the body) and antioxidant a nutrient (found in foods) can scavenge or deactivates the secretive free radicals turning them into harmless particles [3]. Foods from plant origin usually contain natural antioxidants that can scavenge free radicals [3]. Phytonutrients are naturally occurring plant chemicals which do not have any nutritional value, but which have the ability to act as antioxidants. Minerals also act as antioxidants. Selenium is the main mineral antioxidants. It involves in the production of powerful enzymes which 'mop- up' free radicals and deactivates them [4]. Antioxidants, including phenolic compounds have diverse biological effects such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic effects. Phenolic antioxidants commonly used in foods with one exception have two hydroxyl groups or one hydroxyl and one substituted hydroxyl group in ortho or para positions. These compounds are effective at extremely low concentrations, some lose effectiveness as their concentration is increased. At high concentration some may accelerate the rate of autoxidation [5, 6, 7]. In general the most effective antioxidants are highly reactive and are readily destroyed at heat. In this study, the phytonutrient components of *Canarium* seed was determined, also investigating the antioxidative potential and bioactive substances of *Canarium* seed in deferent solvents to establish the scientific evidence on nutritive and bioactive substances of plant seed.

2. Materials and methods
2.1 Sample collection and preparation
The matured disease-free atili (*Canarium schweinfurthii burseraceae)* seeds were purchased directly from a farmer in Pankshin a local Government in Jos, Plateau State, Nigeria. The seeds were brought to Akure and identification/authentication was done at the Forestry and Wood Technology Department of the Federal University of Technology, Akure Ondo State, Nigeria. The seeds were screened in the Laboratory by hand-picking to remove the bad ones

and soaked in warm water at 65 ºC for about 20 mins to soften the seed coat for easy removal.

2.2 Preparation of the extract

40 fresh fruits were sorted, soaked in warm water and the pulp was separated from the endocarp using a sterile knife. The edible portion of the fruit was homogenized using blender and the homogenate was then stored at 4 ºC in a refrigerator. Hexane-methanol-acetone-distilled water was used for extraction of phytonutrients using soxhlet extraction method. The extraction was carried out for 6hrs. The extracts were concentrated at 55 ºC using rotary evaporator and resultant residues were then made-up to 50 ml and stored under refrigerated conditions prior to analysis.

2.3 Phytochemical analyses

Phytochemical screening and estimation of the plant extracts were carried out using standard procedure of [8, 9].

2.4 Evaluation of antioxidant activity

The phenolic contents of the extracts were determined using Follin-Ciocalteu reagent and expressed as Gallic Acid Equivalents (GAE) [10], total flavonoids content (TFC) was determined spectrophotometrically using the method described by [11] based on the formation of flavonoid-aluminium complex and DPPH radical scavenging activities of the extracts were determined using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) [12], Iron reducing power assay of the sample was determined according to the method described by [13]. The vitamin C and E content of the extracts were determined spectrophotometrically using the method of [14].

2.5 Statistical analysis

The experimental results were expressed as mean ± Standard Deviation (SD) of three replicates. Data obtained were statistically analysed using one way Analysis of Variance (ANOVA), a tool in Statistical Packages for Social Sciences (SPSS 14.0). The level of significance was set at $P < 0.05$. Means were separated with Duncan Multiple Range Test (DMRT).

3 Results and discussion

3.1 Phytochemical Screening and Determination

Various solvent extracts of *Canarium* fruit was prepared and the percentage yield was calculated. Among the extracts, maximum yield was obtained using methanol followed by water. Prepared extracts were analysed for the presence of various phytochemical constituents and the results were presented in table 1 while table 2 revealed the concentrations of phytochemicals in different extracts.

Table 1: Phytochemical Screening of Canerium Fruit in Various Extracts

Phytochemical constituents	Solvents			
	n-hexane	Acetone	Methanol	Water
Yield (%)	5.2	3.4	11.6	10.8
Tannin	-	-	+	+
Resin	+	+	+	-
Saponins	+	-	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Steroids	-	+	+	+
Glycosides	+	-	+	-
Carbohydrates	+	+	+	+
Protein	+	+	+	+
Terpenoids	+	+	+	+
Fixed oils & fat	+	+	+	-

Key: (+) = Present (-) = Absent

Table 2: Phytochemical Components (mg/g) of Canerium Fruit in Various Extracts

Phytochemicals	n-hexane	Acetone	Methanol	Water
Tannin	0.34 ± 0.00^{c}	0.34 ± 0.00^{c}	2.06 ± 0.17^{a}	1.83 ± 0.05^{b}
Saponins	0.42 ± 0.03^{c}	0.16 ± 0.01^{d}	1.87 ± 0.02^{a}	1.24 ± 0.03^{b}
Flavonoids	1.18 ± 0.11^{d}	2.61 ± 0.13^{c}	4.84 ± 0.32^{a}	3.01 ± 0.01^{b}
Alkaloids	0.62 ± 0.03^{c}	0.33 ± 0.00^{d}	3.26 ± 0.00^{a}	2.53 ± 0.02^{b}
Glycosides	0.51 ± 0.01^{c}	0.11 ± 0.00^{d}	2.91 ± 0.15^{a}	2.14 ± 0.00^{b}
Terpenoids	0.87 ± 0.05^{c}	0.28 ± 0.01^{d}	6.62 ± 0.81^{a}	4.21 ± 0.63^{b}
Total	**3.94**	**3.83**	**21.50**	**14.96**

Mean values followed by the same superscript within the rows are not significantly different at $p<0.05$

Phytochemical analysis: Preliminary phytochemical screening of the plant extracts showed the presence of various phytochemical constituents such as tannin, resin, saponins, flavonoids, terpenoids, steroids, alkaloids, protein, glycosides, carbohydrates and fixed fat & oils. The result showed that all tested phytochemicals were (+ve) present in methanol extract, while tannin, saponins, and glycosides were absent (-ve) in acetone extract but contained resin, flavonoids, steroids, glycosides, carbohydrate, protein, terpenoids and oils. Also resin, glycosides and oils were not detected in water extract but tested positive (+ve) to tannin, saponins, flavonoids, alkaloids steroids, carbohydrates, protein and terpenoids. Only tannin and steroids are absent in hexane extract but showed positive (+ve) to the rest of phytochemicals. The presence of all phytochemicals in ethanol extract was an indication that methanol was the best solvent for extraction of phytoconstituents in Canarium fruit; this result conformed to what was obtained for methanol extracts of *A. soonei*, *M.*

lucida and T. orientalis [15]. The results of phytochemical analysis of the extracts as presented in table 2 revealed that the sum of concentrations of phytochemicals are more abundant in methanol and water (21.50 and 14.96) extracts than acetone and hexane (3.83 and 3.94) extracts. This implies that there was a correlation between the result obtained for phytochemical screening and its quantitative determinations.

3.2 Antioxidant Activities

All the fruits and vegetables materials used as sources of nutrition and medicine contain some degree of antioxidants. Free radicals are critically involved in various pathological condition such as cancer, cardiovascular disorder, arthritis, inflammation and liver diseases [16]. The antioxidant activity of various extracts of *Canarium pulp* was investigated against various *in vitro* models. Since, free radicals are of different chemical entities, it is essential to test the extract against many free radicals to prove their antioxidant activity. Hence, a large number of *in vitro* methods were used for the screening. IC_{50} values obtained were compared with the standards used, that is, garlic acid, ascorbic acid and rutin. The results of antioxidant activity of *Canerium* pulp in various extracts are shown in Table 3.

Table 3: Antioxidant Properties of *Canerium* Fruit Extracts

Solvents	Total phenol	Ferric reducing power	DPPH	Total flavonoids	Vitamin C	Vitamin E
n-Hexane	416.14±2.71^{c}	106.28±8.13^{d}	48.15±4.13^{a}	1.83 ± 0.00^{c}	32.85±1.03^{c}	487.2±17.21^{a}
Acetone	412.03±6.63^{d}	232.03±15.07^{c}	32.27±1.05^{b}	1.68 ± 0.02^{d}	31.32±0.03^{d}	451.4±15.62^{b}
Methanol	620.31±15.82^{a}	358.96±21.72^{a}	13.61±1.26^{d}	3.06 ± 0.15^{a}	34.21±1.01^{b}	338.0±13.15^{c}
Water	598.47 ± 13.9^{b}	284.11±13.21^{b}	17.28±0.17^{c}	2.71 ± 0.34^{b}	45.31±2.14^{a}	338.0±13.15^{c}

Mean values followed by the same superscript within the rows are not significantly different at $p<0.05$

Total phenolic and flavonoids content: Polyphenol compounds are commonly found in both edible and medicinal plants and they have been reported to have various biological effects including antioxidant activity. The antioxidant activities of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [16]. The level of phenolic compound in various extracts showed strong antioxidant activities in all prepared extracts. The mean total amount of phenolic concentration is statistically significant ($p<0.05$) for the extracts acetone, n-hexane, water, methanol; 412.03, 416.14, 598.47 and 620.31 g GAE/100 g respectively. The highest concentration was obtained in methanol extract followed by sample extracted with water. This showed that the appropriate solvent for extraction of phenolic compound is ethanol which means that the polarity of category of *Canarium* main substance is as high as methanol's polarity. Moreover the plant extracts also contain terpenoids, alkaloids, glycosides, saponins and tannins. The antioxidant effect of various extracts of *Canarium* could be due to the presence of various phytochemical compounds present. Flavonoids are very effective antioxidants [17]. The total flavonoids content of *Canarium* pulp was also statistically significant ($p<0.05$) and followed almost the same trend as phenolic content from acetone, n-hexane, water, ethanol as 1.68, 1.83, 2.71 and 3.56mgRE/g respectively. The highest flavonoids content was obtained in methanolic extract of fruit sample; this was higher than the values of 1.01-2.35 ug RE/g reported for three *vitex* species [18].

Ferric reducing antioxidant property (FRAP): In the measurement of the reducing ability, it has been investigated from the Fe^{3+} - Fe^{2+} transformation. Fe^{3+} reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with other antioxidant properties [19]. The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The data obtained in the present study suggest that it is likely to contribute significantly towards the observed antioxidant effects. However, the antioxidant activity has been attributed by various mechanisms, like prevention of chain initiation, binding of transition metal ion catalysts, prevention of continued hydrogen abstraction, reductive capacity, radical scavenging activity and decomposition of peroxides. The value obtained in various extract varies from hexane, acetone, water and methanol as 106.28, 232.03, 284.11 and 358.96 respectively. In the antioxidant activity, the reducing power of the extracts increases with increasing concentration. In this method, methanol and water extracts showed good activity and the order of activity is as follows: methanol > water > acetone > Hexane.

Scavenging assay effect of 2, 2-diphenyl-1-picrylhyrazyl: The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [20]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [21]. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. The experimental data (13.61, 17.28, 32.27 and 48.15) of the extracts methanol, water, acetone and hexane as shown in table 4.5 revealed that the extracts were likely to have the effects of scavenging free radicals especially the methanolic extract. It has been found that cysteine, glutathione, ascorbic acid, tocopherols, flavonoids, tannins and aromatic amines reduce and decolorize the DPPH by their hydrogen donating ability. In this study methanol and water extracts were found to be active against DPPH radical scavenging method. The order of activity was as follows: methanol>water>acetone>hexane. Presence of flavonoids and phenolic compounds in the extracts are possibly involved in their antiradical activity.

Vitamins: The vitamin E and vitamin C content of *Canarium* pulp in various extract varied significantly as shown in Table 3. The result of vitamin C in hexane, acetone, methanol and water extracts are; 32.85, 31.32, 34.21 and 45.21 mg/100 g respectively. This revealed that the highest concentration was obtained in sample extracted with water which could be as a result of solubility of vitamin C in water. Ascorbic acid is

generally used for protein metabolism and collagen synthesis. The vitamin C content of *Canarium* fruit extracts of 31.32 – 45.21 mg/100 g is within the RDA value (60 mg/100 g) for adult [22]. Likewise, the vitamin E content of hexane extract gave the highest concentration (487.20 ppm), followed by acetone (451.4 ppm), methanol and water gave the same concentration of (338.0 ppm) respectively. This showed that vitamin E (tocopherols) belongs to non-polar group. Therefore it dissolved well in no polar solvent such as hexane. Vitamin E act as antioxidants, but tocopherol is the principal fat-soluble antioxidant in the body and is found in lipoproteins, especially Low Density Lipoproteins (LDL). It is found within membranes both inside and outside cells, enhancing the cell's protection against free radical attack. Vitamin E enhances immune function and can play a vital role in the repair of damaged membranes [23, 24].

4. Conclusion

Phytochemical screening and determination of the seed in various extracts of hexane, acetone, methanol and water revealed that ethanol was the best solvent for extracting phytochemicals in *Canarium* seed pulp followed by water as both of them gave high percentage yield and tested positive to show the presence of all tested phytoconstituents. Likewise, the concentrations of phytochemicals are more abundant in methanol and water extracts than acetone and hexane extracts. The results of antioxidant activity of *Canarium* seed in total phenolic and flavonoids contents, ferric reducing power, scavenging ability of DPPH radical, Vitamin C and E showed strong but varied antioxidant activities in various extracts and the highest concentration was obtained in methanol extract. Hexane extract gave the best antioxidant activity of vitamin E and the best antioxidant activity of vitamin C was obtained in water extract. The study established the presence of some phytochemicals and appreciable amount of antioxidants in *Canarium* seed, it is recommended to be eaten by people suffering from cancer, diabetic, hypertension and other cardiovascular diseases.

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