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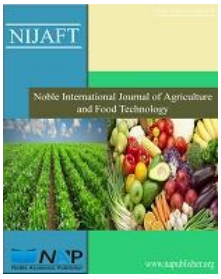

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EFFECT OF DRYING METHODS AND EXTRACTANTS ON SECONDARY METABOLITE COMPOSITIONS OF AZANZA GARCKEANA PULP AND SHAFT

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ABSTRACT: *Azanza garckeana* are used in Nigeria by herbal practitioners for the treatment of diseases. In the present study, effect of drying methods and extraction solvents on secondary metabolite compositions of *Azanza garckeana* pulp and shaft were evaluated. Results revealed that the shaft had higher moisture loss of 6.35% and 12.34 % while the pulp recorded the least moisture loss of 4.67 % and 7.46 % during air and sun-drying respectively. Methanol produced higher pulp extract yield than ethyl-acetate. *Azanza garckeana* pulp contains phenols, flavonoids, tannins, alkaloids, saponins, glycosides, terpenoids, steroids and phlobatanins but absence of anthraquinone. Similarly, phlobatannins and anthrquinone were absent in the shaft. Tannins are the most abundant phytochemicals in both air dried (5.43±0.68 mg/100g) and sun dried (4.19±0.21 mg/100g) shaft followed by phenol (2.34±0.25 and 2.35±0.35mg/100g), flavonoids (2.04±0.21 and 2.78±0.54 mg/100g) while alkaloids (1.04±0.02 and 1.35±0.25 mg/100g) was the least. Methanol extract had higher amounts of phenols, flavonoids and alkaloids but lower tannin than ethylacetate extract. Drying significantly decreases the amounts of all these phytochemicals for both methanol and ethylacetate extracts. In conclusion the shaft and pulp of *Azanza garckeana* contains significant amounts of phytochemicals with medicinal reputation. However, the pulp contains higher mounts of these phytochemicals than the shaft. Also, sun drying significantly decreases the amounts of these phytochemicals.

Keywords: Azanza Garckeana, Pulp and Shaft, Phytochemicals, Solvents, Drying Methods.

1. INTRODUCTION

Humans have depended directly or indirectly on plants for use in the treatment of several ailments either due to high cost of orthodox drugs or due to persistent adverse effects of synthetic drugs [1, 2]. According to the World Health Organization (WHO), 80 % of the world's population uses medicinal plants in the treatment of diseases. In African countries this rate is much higher [3]. The secondary metabolites in plants have been the drive for pharmaceutical discovery and the active components of these plants have stimulated significant interest [4]. Several pharmacologically active compounds that may act individually, additively or synergistically to improve health are contained in medicinal plants and these herbal drugs are widely preferred owing to their availability, affordability (cost effectiveness) and assumed safety [4]. Plants have maintained a backbone in everyday existence providing food, oxygen and serving as raw materials for so many industrial products. In developing countries, about 60 % of the populations use traditional medicine [5]. Preparation of traditional system of medicine is usually from a single or combination of more than one plant, however the growth of plants, their efficacy as well as the quality of herbal ingredients present in particular plant species from same country may differ due to some environmental factors like rainfall, climate and the presence of primary and secondary metabolites [6]. In

addition it is well documented that the processing methods as well as the dissolution medium of these plants effects the quality of the active ingreadients.

Azanza garckeana (F. Hoffm.) Exell and Hillc. have been found to play a vital role in traditional medicine. In tropical Africa, *Azanza garckeana*. is among the popular multipurpose fruit trees, characterized by edible fruits with different plant parts used as herbal medicines and plant products sold to local markets generating substantial incomes for the household [7]. The World Agroforestry Centre identified *A. garckeana* as one of the fruit trees that should be integrated in the domestication process in farming systems to support nutritional, health and income security of local communities in tropical Africa [8]. Bearing in mind that plants contains bioactive agents that could offer protection against several diseases [9] coupled with the fact that *Azanza garckeana* are used in Nigeria by herbal practitioners in the treatment of diseases together with other medicinal plants. There is a need to study the secondary metabolite compositions in order to validate its uses in traditional medicine. In addition, there is also a need to evaluate the most efficient processing method and extraction solvents for better yield of the secondary metabolites of this plant.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

The *Azanza garckeana* were collected from Gombe village, in Gombe State, Nigeria in the month of July, 2019. The plant was identified by A Botanist at the Department of Plant Biology, Federal University of Technology, Minna

2.2. Reagents and Chemicals

Methanol and ethylacetate used for extraction of the plant material were of analytical grade obtained from Sigma Chemical Co St. Louis M.O (USA) All other chemicals used were also of analytical grade and were prepared in didtilled water

2.3. Experimental Design

The whole nuts and the shaft were rinsed under clean running water and were separated into the pulp and the shaft. Each of the pulp and the shaft were divided into 2 portions. First portion was air dried for two weeks while the second portion was sundried for 2 weeks. The change in weight after drying was recorded and the percentage weight loss was computed. The dried materials were pulverized into coarse powder with mortar and pestle, milled into fine powder with an electric miller and stored in a clean container till ready for use. The percentage moisture loss was computed.

2.4. Extraction from Plant Material

Two hundred gram (200 g) of powdered nuts was weighed into a reflux flask (100 g per turn), 2.5 liters of methanol was used in succession and extraction step was exhaustively carried out for two hours with reflux extractor. The mixture was sequentially filtered with chess cloth and Whatman's paper (No.1). The final filtrate was first concentrated in a rotary evaporator and then later in a water bath at 65^{o}C. The dried extract was stored in sample bottle in the refrigerator at 4˚C. The Same procedures were repeated for aqueous and ethyl acetate extracts. The weight of the extract was recorded and the percentage extract yield was computed using the formular below

$$\% \text{ Yield} = \frac{\text{Weight of the Crude Extract (g)}}{\text{Weight of Dried powdered sample (g)}} \times 100$$

Tsado, et al. [10]

2.5. Qualitative Phytochemical Analysis

Flavonoids tannins, saponins, phenols, alkaloids, phlobatannins, steroids, terpenoids and cardiac glycosides were assayed using standard procedures [11, 12].

2.6. Quantitative Phytochemical Analysis

2.6.1. Total Flavonoids

This method is based on the formation of flavanoid-aluminium complex with its maximum spectrophotometric absorption at 415 nm. Fifty micolitre (50 µl) of extract was mixed with 50 µl of 10 % aluminium trichloride in methanol and drop of acetic acid was added. This was then diluted with distilled

water to 5 ml. Absorbance was read at 412 nm after 30 minutes. Blank was prepared from 50 µl of distilled water in place of extract and same procedures for the sample was repeated. Quercetin was used as standard [13].

2.6.2. Total Phenol

The 100 mg extract was dissolved in 100 ml of distilled water. One milliliter (1ml) of the mixture was pipetted into sample test tube, 0.5 ml of 2 M Folin-Ciocalteu reagent and 1.5 ml 20 % of Na_2CO_3 solution were added to the sample test tube. Distilled water was added to make up the volume to 8 ml. This was then mixed thoroughly and incubated for two hours at room temperature. Thereafter, tube content was mixed again and absorbance was read at 765 nm. The total phenol content was then determined using the standard calibration curve gotten from various dilution concentration of garlic acid as standard [14].

2.6.3. Alkaloids

The extract (0.5 g) was dissolved in 1ml of 96 % ethanol: 20 % H_2SO_4 (1:1) and filtered. One millitre (1 ml) of filtrate was added to 5 ml of 60 % H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 ml of 0.5 % formalaldehyde was added and allowed to stand again for further 3 hours. Absorbance was read at 565 nm and vincristine was used as standard at 0.1 M concentration [11].

2.6.4. Tannins

The extract (100 mg) was weighed into a beaker. Fifty millilitres (50 ml) of distilled water was added and shaken in a mechanical shaker for one hour. One millilitre (1 ml) of the filtrate was pipetted into sample test tube. Two millilitres (2 ml) of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide were added to the filtrate and mixed thoroughly. Absorbance was read at 120 nm within 10 minutes. Tannic acid (1 M) was used as standard.

2.6.5. Saponin

The extract (0.5 g) was added to 20 ml of 1 M hydrochloric acid and boiled for 4 hours. This was filtered after cooling and 50 ml of petroleum ether was added to the filtrate and evaporated to dryness. Five millilitres (5 ml) of acetone/ethanol was added to the residue. 0.4 ml was taken into 3 different test tubes. 6 ml of ferrous sulphate reagent was added and 2 ml of concentrated H_2SO_4 was added after. It was thoroughly mixed after 10 min and the absorbance was read at 490 nm [14].

2.6.6. Statistical analysis

Data collected were subjected to statistical analysis using the statistical package for social science version 21.0 and express as mean ± standard error of mean (SEM). Statistical significance of the results between groups was determined using One-way analysis of variance (ANOVA) followed by Duncans multiple range test (DMRT) to check differences between the individual groups. Differences in mean were considered to be significant at $p<0.05$.

3. RESULTS

3.1. Moisture Loss

Effect of drying methods on moisture loss of *Azanza garckeana* pulp and shaft are shown in table 1: The shaft of the *Azanza garckeana* had higher moisture loss of 6.35% and 12.34 % for both air dried and sundried samples respectively. The pulp materials on the other hand recorded the least moisture loss of 4.67 % and 7.46 % for both air dried and sundried samples respectively**.**

Table 1. Effect of drying methods on moisture loss of *Azanza garckeana* pulp and shaft

Plant part	**Drying-Methods**	**Moisture loss (%)**
Pulp	Air dry	4.67 %
	Sundry	7.46 %
Shaft	Airdry	6.35 %
	Sundry	12.34 %

3.3. Percentage Yield

The percentage extract yield of air dried and sundried *Azanza garckeana* pulp and shaft are shown in table 2. for the Shaft materials air drying yielded high extract quantity of 17.5% while the sun drying yielded 15.56% extract. Methanol extract of the *Azanza garckeana* pulp produced higher extract yield of 7.84% and 7.09% when compared with the ethyl-acetate extract that recorded 5.67% and 5.09% extract yield for both air dried and sun-dried samples respectively.

Table 2. Percentage extract yield of air dried and sundried *Azanza garckeana* pulp and shaft

	Shaft		**Pulp**	
Phytochemicals Sundry	**Air drying**	**Sun-drying**	**Air drying**	**Sun-drying**
Aqueous Extract	17.5	15.56		
Methanol Extract	-	-	7.87	7.09
Ethyl-acetate Extract	-	-	5.67	5.09

3.4. Phytochemical Compositions

The qualitative phytochemical composition of air dried and sun-dried *Azanza garckeana* shaft and pulp are shown in table 3. All the phytochemicals analyzed including phenols. Flavonoids, tannins, alkaloids, saponins, glycosides, terpenoids, steroids and phlobatanins were present in both air dried and sun-dried samples of *Azanza garckeana* pulp. However, anthraquinone was absent. The shaft materials also contain the phytochemicals listed above except the terpenoids, phlobatannins and anthrquinone which were completely absent in air dried and sundried *Azanza garckeana* shaft

Table 3. Comparative qualitative phytochemical composition of air dried and sun-dried *Azanza garckeana* shaft and pulp

	Pulp		**Shaft**	
Phytochemicals	**Air dried**	**Sun dried**	**Air dried**	**Sun dried**
Total phenol	+	+	+	+
Total flavonoids	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Glycoside	+	+	+	+
Terpenoids	+	+	-	-
Steroids	+	+	+	+
Phlobatannins	+	+	-	-
Anthraquinonne	-	-	-	-

Key: + represents a positive result and - represents a negative result

3.5. Quantitative Phytochemical Composition of Air Dried and Sun-Dried *Azanza Garckeana* Shaft

The quantitative phytochemical composition of air dried and sun-dried aqueous extracts of *Azanza garckeana* shaft are shown in table 4. Tannin is the most abundant phytochemicals in both air dried (5.43±0.68 mg/100g) and sun dried (4.19±0.21 mg/100g)*Azanza garckeana* shaft followed by phenol (2.34±0.25 and 2.35±0.35mg/100g), flavonoids (2.04±0.21 and 2.78±0.54 mg/100g), and saponin (2.54±0.21 and 2.03±0.35 mg/100g) while alkaloids (1.04±0.02 and 1.35±0.25 mg/100g) was the least abundant phytochemicals in both air dried and sun dried sample respectively. On the basis of drying methods, no significant differences were found in the phytochemicals composition except for tannin which was slightly higher in air died sample when compared with the sundried samples (Table 4)

Table 4. Quantitative phytochemical composition of air dried and sun-dried aqueous extracts of *Azanza garckeana* shaft

Phytochemicals	**Air dried (mg/100g)**	**Sun-dried (mg/100g)**
Total phenol	2.34±0.25^{b}	2.35±0.35^{b}
Total flavonoids	2.04±0.21^{b}	2.78±0.54^{b}
Tannins	5.43±0.68^{c}	4.19±0.21^{c}
Alkaloids	1.04±0.02^{a}	1.35±0.25^{a}

Saponins	2.54±0.21 [b]	2.03±0.35[b]

Data are Mean ± SEM of triplicate determination. Data followed by different superscript alphabet along the same column are significantly different ($p<0.05$).

3.6. Effect of Solvent and Drying Condition on Phytochemical Composition *Azanza Garckeana* Pulp

The effect of Solvent and drying condition on quantitative phytochemical composition *Azanza garckeana* pulp are presented in table 5. Methanol extract had higher amounts of phenols, flavonoids and alkaloids when compared with the ethylacetate extract. Higher amounts of tannin s were however recorded for ethyl acetate extract. Drying significantly decreases the amounts of all these phytochemicals for both methanol and ethylacetate extracts

Table 5. Effect of Solvent and drying condition on quantitative phytochemical composition *Azanza garckeana* pulp

	Air drying		Sun drying	
Phytochemicals	**Methanol**	**Ethylacetate**	**Methanol**	**Ethylacetate**
Total phenol	34.32±2.34[c]	25.34±0.32 [a]	30.45±1.78 [b]	22.34±0.55[a]
Total flavonoids	13.45±0.89 [c]	7.65±0.24[a]	10.45±0.78 [b]	7.46±0.19[a]
Tannins	8.65±0.94 [a]	15.23±0.67 [c]	7.35±0.56[a]	10.34±0.56 [b]
Alkaloids	43.24±2.95 [c]	32.34±3.24 [ab]	39.45±0.34 [b]	27.57±0.78 [a]
Saponins	5.43±0.67[b]	5.35±0.67[b]	3.46±0.32[a]	3.05±0.11 [a]

Data are Mean ± SEM of triplicate determination. Data followed by different superscript alphabet along the same row are significantly different ($p<0.05$)

4. DISCUSSION

In recent times, secondary plant metabolites that occur in various parts of plants have been exploited for the prevention, management and treatment of many health conditions as well as their physiological activity as facilitating natural healing with little or no side effects [15]. Preliminary phytochemical screening of the *Azanza garckeana* showed the presence of secondary metabolites such as Flavonoids, tannins, alkaloids, saponins, glycosides, terpenoids, steroids and phlobatanins. Several authors have established that compounds, such as phenols, flavonoids, alkaloids and tannins, have antimicrobial antioxidants, anti-inflammatory, antimalarial and analgesic activity [16-21]. The antimicrobial activities of these phytochemicals arise from polyphenol and tannin capacity to inhibit enzymes and alter membrane characteristics.

The presence of flavonoids suggests the ability of this plant to play an important role in preventing disorders associated with oxidative stress. Alkaloid is the most efficient therapeutically significant plant substance [22]. Alkaloid rich plants are recommended for patients as alkaloids possess a significant pharmacological property. The presence of tannin suggests the ability of this plant to play major roles as antifungal, antidiarrheal, antioxidant and antihemorrhoidal agents [23]. Saponin & Steroid also have relationships with sex hormones like oxytocin which regulate the onset of labour in pregnant women and subsequent release of milk [24]. The presence of this phytochemicals is an indication that this plant can be given to expectant ruminant animals and those that deliver without the expulsion of their placenta.

Glycoside showed positive result in the shaft and pulp. This perhaps suggests the ability of this plant in the treatment and management of hypertension [25]. The presence of important phytochemicals in the shaft and pulp of *Azanza garckeana* is an indication that this plant if properly screened could yield a drug of pharmaceutical significance. However, the absence of phlobatannins and anthrquinone in *Azanza garckeana* shaft but present in pulp agrees with early studies which also found that not all phytochemicals are present in all plants or plant parts [26].

It was observed that the methanol extract had higher amounts of phenols, flavonoids and alkaloids when compared with the ethylacetate extract this is could be attributed to the high polarity of methanol which causes high solubility of the bioactive metabolite when compared with the ethylacetate which is a known mid polar extractant and thus less compounds are soluble in it. This finding is in concordance with the results of the previous studies who reported that secondary metabolites in medicinal plants vary with the solubility of the solvents used in the extraction process [26-28]. On the other hand, quantitative assays revealed that drying significantly decreases the amounts of all these phytochemicals for both methanol and ethylacetate extracts, this decrease in bioactive metabolites will correlate to low biological activity of the sundry sample when compared with the air dried samples.

5. CONCLUSION

In conclusion the shaft and pulp of *Azanza garckeana* contains significants amounts of phytochemicals with medicinal reputation. However, the pulp contains higher mounts of these phytochemicals than the shaft. Methanol extract of the *Azanza garckeana* pulp had high amounts of these phytochemicals than the ethylacetate extract. Also, sun drying significantly decreases the amounts of all these phytochemicals for both methanol and ethylacetate extracts

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