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Full Length Research Paper

PROXIMATE, MINERALS AND PHYTOCHEMICAL EVALUATION OF PERICARP AND SEED OF *TERMINALIA CATAPPA* FRUIT

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ABSTRACT

Terminalia catappa commonly known as Tropical almond is a plant of the lead wood family, Combretaceae. *T. catappa* is deciduous and grows well in the tropics. Tropical almond fruit can exist in different colors but the yellow and red-pink varieties are most common. This variation however depends on how the mature fruit ripen, with the pink red ones considered very mature and ripe. Phytochemicals evaluation was carried out on the aqueous extract of the pericarp, in pulp form and seed. Standard procedures were followed to test ten different phytochemicals quantitatively in the seed and pericarp. The result showed the presence of flavonoids, anthraquinones, tannins, steroids, saponins, terpenoids and phenol in both the pericarp and seed. The seed, however showed no presence of alkaloids, glycosides and phlobatannins though they were present in the pericarp. The pericarp contain the highest concentration of phytochemical for the both plant parts in the form of phenol (149.33±0.00 GAE/100g), while the highest concentration of phytochemical for the seed is saponin (84.15±0.25mg/100g). The proximate analysis revealed that the seed has higher moisture, crude protein and crude lipid (23.14±1.06, 22.85±1.27 and 22.20±1.19 % respectively), than in the pericarp(14.31±1.29, 6.40±0.60 and 1.28±0.28 respectively),while the ash, carbohydrate and crude content were found to be higher in pericarp (6.40±0.60, 59.98±4.76 and 13.00±0.98 % respectively), than the seed (3.97±0.16, 24.39±3.98 and 3.19±0.27 % respectively). The mineral contents showed that both the seed and pericarp have relatively higher concentration of magnesium, calcium, iron,

zinc, potassium and sodium.From this study, it has been established that the pericarp and seed of *T. catappa*contain phytochemicals, which are active agents that can be employed as basic elements for the synthesis of drugs in pharmaceutical industries and also contain adequate dietary nutrients that can be used as food supplements.

Keywords: Mineral analysis,*Terminalia catappa*, Phytochemicals, Proximate composition

INTRODUCTION

Plants have since being used for medicinal purposes through it was initially thought to be an archaic method with local application mainly beneficial for the poor and people in the rural areas. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powers and other herbal formulations (Balick *et al*., 1997; Sammuelson, 2004). This idea however has greatly changed. Drug discovery from medicinal plants led to the isolation of early drugs like cocaine, codeine, digitoxin and quinine in addition to morphine of which some are still in use (Newman et al., 2000). Plants that have healing properties are called medicinal plants (Krishnaveni *et al*., 2015).

Terminalia catappa belongs to the fruit-bearing family called Combretaceae that contains lots of nutritional benefits (Chinemezu *et al*., 2018). It is requires rainfall in the range of 1000-3500mm per annum, distributed rather uniformly throughout the year and sandy soil for its fast growth (Krishnaveni *et al*., 2015). It is invariably found near the coast, at elevations of less than 300-400m, where there is little seasonal variation in temperatures these areas are typically flat, but they may have dunes or rocky bluffs (Orwa *et al.*, 2009).

It also serves as food for animals like barts and lizards who feed on the fruit. The foliage is used as feed for silkworm and other animal feeds, the bark contains gums and resin and the kernel contains an extractable oil that is used for cooking. Tropical almond also offers a range of environmental benefits which include ornamental uses, use as fence, hedge or visual/noise barrier. The vast soil binding ability of the root of tropical almond makes it an excellent control of erosion. It serves as shade for shelter because of its large canopy, a promising species for reforestation as it is tolerant to drought and salt spray, a good improver of mulch for protection of soil and young crops, eradication of unwanted weeds and grasses (Orwa*et al*., 2009).

The root, bark, fruit and leaf extracts of *Terminalia catappa* using petroleum either, methanol and aqueous solutions has been found to possess antidiabetic (Nagappa *et al*., 2003; Ahmed *et al*., 2005: Lakshminarasimhaiah and Goyal, 2015), antimicrobial (Pawar and Pal, 2002; Nair and Chanda, 2008), antibacterial (Tagana *et al*., 2011) anti-inflammatory (Fan *et al*., 2004), antixodant (Lin *et al*., 2001) and anticancer (Yeh *et al*., 2012; Pandya *et al*., 2013) activities.

Terminalia catappa is chosen for this study to examine the phytochemicals present in its pericarp and seed both qualitatively and quantitatively, so the claims on its medicinal values and health benefits can be ascertained and proved scientifically.

MATERIALS AND METHOD

Collection of plant sample and preparation

Fresh, mature and whole fruits of *Terminalia catappa* were collected by hand picking in March, 2021 from the trees at the University of Ilorin, , Kwara State, which lies in the southern Guinea savanna zone of Nigeria (a tropical country) at Latitude 8.500^{o}N and Longitude 4.5500^{o}S. The fruits were collected in the early hours of the day between 7am-9am during the dry season and identified and authenticated at the Department of Plant biology, University of Ilorin.

Each of the fruit mesocarp was separated from seed and air dried for 7days after which they were separately ground into fine powder with the laboratory mill. After sieving with the aid of 2.00mm wire mesh, the fine powder with the laboratory mill. After sieving with the aid of 2.00mm wire mesh, the fine powder was then used for extraction and the phytochemical analysis of the extracts was carried out in laboratory.

Extraction procedure

Aqueous extracts were prepared by soaking 20g each of the fine powder of the pericarp and seed in 200ml of water for 24 hrs. The extracts were filtered after 24hrs, first through a Whatmann filter paper (125mm) and then through cotton wool. The extracts were concentrated by gentle evaporation on heating mantle (John-Dewole and Oni, 2013). This concentrated solution was subjected to both qualitative and quantitative phytochemical screenings.

Preliminary phytochemical screening

Phytochemical test was carried out on the aqueous extract of the pericarp and seed using standard procedures to test for the presence of secondary metabolites following the standard procedures of Sofowora (1993) as adopted by Ayoola *et al*. (2008).

Test for alkaloids

Firstly, 0.5g of the extract was diluted to 10ml with acid alcohol, boiled and filtered. Then 5ml of the filtrate was transferred into a test tube followed by addition of 2ml of dilute ammonia and 5ml of chloroform. The mixture was shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into three portions and the test for alkaloids was conducted using Mayer's, Wagner's Hager's and Drangendorff's reagents was added to the last portion.

Test for Tannins

About 0.5g of the extract was boiled in 10ml of water in atest tube and then filtered. A few drops of 0.1% chloride were added. A brownish green, dark green or blue-black coloration indicates the presence of tannins.

Test for Steroids

To the extract 2ml of chloroform was added followed by 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. Formation of bluish red to cherry red color ring at chloroform layer indicates the presence of steroids.

Test for Phlobatannins

The extract of 10ml was boiled with 1% hydrochloric acid in a test tube. A deposition of red precipitate will occur if phlobatannins are present.

Test for Flavonoids

5ml of dilute ammonia was added to a portion of aqueous filtrate of the extract

(0.5g of ethanolic extract in 5ml of water). 1ml of concentrated sulphuric acid was added. A yellow coloration that appears on standing indicates the presence of flavonoids. A few drops of 1% aluminium solution were added to a portion of aqueous filtrate of the extract (0.5 g of ethanolic extract in 5ml of water). A yellow coloration indicates the presence of flavonoids.

A portion of the extract was heated with 10ml of ethyl acetate over a bath for 3 minutes. The mixture was filtered and 4ml of filtrate of the extract was shaken with 1ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for Saponins

5ml of distilled water was added to 0.5g of the extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20minutes. The frothing was mixed with 3 drops of olive oil and shaken vigorously. The formation of emulsion indicates the presence of saponins.

Test for Terpenoids

Salkowski's test was carried out to determine the presence of terpenoids. 0.5g of extract was mixed with 2ml of chloroform and 3ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.

Test for Phenols

Three drops of 1% (w/v) solution of ferric chloride was added to 0.5g of the extract followed by 1% (w/v) gelatin in sodium chloride of the same concentration. The formation of a precipitate indicates the presence of phenols.

Test for Anthraquinones

Sulphuric acid of 10ml was boiled with 0.5g of the extract and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for color changes which indicate the presence of anthraquinones.

Test for Glycosides

0.5g of the sample diluted to 5ml of glacial acid containing one drop of ferric chloride solution was added to the extract. This was under laid with 1ml of concentrated sulphuric acid. A brown ring at the interface indicate the presence of 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 above the brown ring and gradually spread throughout the layer.

Quantitative phytochemical analysis

The concentration of alkaloid, saponins, tannins, glycosides, steroids, flavonoids, terpenoids, phenol, and anthraquinone was determined on the grinded sample of *Terminaliacatappa*using standard procedures as described by Sofowora, 1993; Onwuka, 2005; and, Oyedemi *et al*., 2010.

Proximate Analysis

The fresh samples of *Terminalcattapa*pericarp and seed were analysed for moisture, ash, fibre, protein, fatand carbohydrate by the methods of Association ofOfficial Analytical Chemist (AOAC, 2019).

Mineral Analysis

The dried sample (0.5g) of the pericarp and seed was transferred intodigestion glass tube. Ten milliliters (10 ml) of $HNO_3$was added to the sample. The resulting mixture waskept overnight at room temperature. Thereafter, 4.0ml perchloric acid ($HClO_3$) was added to the mixtureand kept in fume cupboard. The temperature wasincreased gradually stating from 50 ℃and increasingup to 300℃ in the digestion unit. The digestionwas achieved in about 70-80 minutes as indicatedby the appearance of the white fumes. The mixturewas allowed to cooled and transferred into 100mlvolumetric flasks and the volume of the contentswere made up to 100 ml with distilled water. Theresulting solution was filtered. The filtrate was thenused for the determination of mineral composition. Calcium(Ca), iron (Fe), magnesium (Mg), and zinc (Zn)were determined with the use of Atomic AbsorptionSpectrophotometer (AAS) (Shimazzu, Japan AA-6200). Other mineral elements such as sodium (Na) and potassium (K) were determined by Flame Photometer (Olayinka and Etejere, 2018).

Statistical data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS). Each experiment was run in triplicate. The results of phytochemical, proximate, mineral compositions were analyzed for statistical significance using paired t-test ($p \leq 0.05$). The data were expressed as mean ± SE of three replicates

RESULTS

The results of the qualitative analysis of the aqueous extracts of the pericarp and seed of *Terminalia catappa* fruit are shown in Table 1. In both pericarp and seed, saponin, tannin, anthraquinone, flavonoids, terpenoids, steroids and phenols were found present. Phlobatannin, glycoside and alkaloid were found absent in the seed of *Terminalia catappa*

Table 1: Quantitative phytochemical screening of the percarp and seed of *Terminalia catappa* fruit

Phytochemicals	Pericarp	Seed
Saponins	+	+
Alkaloids	+	_
Tannins	+	+
Steroids	+	+
Flavonoids	+	+
Terpenoids	+	+
Glycosides	+	_
Phenols	+	+
Anthraquinones	+	+
Phlotobannins	+	_

N. B.: + = present; – = absent

The result of quantitative analysis of the aqueous extract of the seed and pericarp of *Terminalia catappa* fruit is presented in Table 2. Phytochemical constituent, phenol was found to be the highest occurring phytochemical in both plant parts under study with its concentration in the pericarp (149.33GAE/g) highest for both pericarp

and seed. Saponin was found to be the highest occurring phytochemical in the seed (84.17 mg/100g). Terpenoids were found to be higher in the seed (11.41 mg/100g) than in the pericarp (4.27 mg/100g) while tannin (8.71 mg/100g), steroid (2.94 mg/100g), phenol (149.26 mg/100g) and anthraquinone (9.63 mg/100g) were higher in the pericarpthan in the seed. Since alkaloid, glycoside and phlobatannin were found to be absent in the seed, their concentration in the pericarp can't be related to that of the seed as they were found absent in the pericarp.

Table 2: Quantitative phytochemical screening of the pericarp and seed of *Terminalia catappa* fruit.

Phytochemicals	**Pericarp**	**Seed**	**Mean**	**t-test value**
Terpenoid (mg/100g)	4.27±0.04	11.41±0.21	7.14	<0.001
Alkaloids (mg/100g)	34.42±0.47	0.00±0.00	34.42	<0.001
Tannins (mg/100g)	8.72±0.18	1.37±0.08	7.35	<0.001
Steroids (mg/100g)	2.94±0.10	1.39±0.18	1.55	<0.002
Flavonoids (mg/100g)	5.97±0.15	7.05±0.07	1.08	0.1
Saponin (mg/100g)	41.98±0.31	84.16±0.17	42.19	<0.001
Glycosides (mg/100g)	76.36±0.50	0.00±0.00	76.36	<0.001
Phenols (GAE/g)	149.26±0.40	27.39±0.21	121.87	<0.001
Anthraquinones (mg/100g)	9.63±0.17	1.82±0.10	7.82	<0.001
Phlotobannins (mg/100g)	1.30±0.05	0.00±0.00	1.30	<0.001

Values are presented as mean ± SE

Proximate Analysis

Table 3 showed the proximate composition of the pericarp and seed of *Terminaliacatappa*. The proximate composition parameters observed in seed is higher than in the pericarp except in carbohydrate and crude fibre. The moisture, ash, carbohydrate, crude fibre, protein and lipid contents differ significantly. The carbohydrate content was found to be highest in the pericarp with 59.98%, while ash content was found to be least with 6.40%,similar trend was observed in the seed.

Table 3: Proximate composition of the pericarp and seed of *Terminalia catappa*

Proximate composition	**Pericarp(%)**	**Seed(%)**	**Mean(%)**	**t-test value**
Moisture	14.31±1.29	23.14±1.06	9.10±0.23	<0.001
Ash Content	6.40±0.60	3.97±0.16	16.45±0.67	<0.001
Carbohydrate	59.98±4.76	24.39±3.98	35.59±1.09	<0.001
Crude Fibre	13.00±0.98	3.19±0.27	9.81±0.70	0.002
Crude Protein	6.40±0.60	22.85±1.27	16.45±0.67	<0.001
Crude Lipid	1.28±0.28	22.20±1.19	20.93±0.92	<0.001

Values are presented as mean ± SE

Mineral Analysis

The mineral composition recorded in pericarp is higher than that of seed (Table 4). The calcium, magnesium, iron, potassium, and sodium differ significantly, while that of zinc were statistically similar. In all the mineral elements analyzed, magnesium was found to be the highest in the pericarp, while calcium was the highest in the seed. The mineral composition was found to be higher in pericarp than seed. No significant different was observed in the Iron, Zinc, potassium and sodium contents of the pericarp and the seed, while the calcium, and magnesium contents differ significantly.

Table 4: Mineral composition the pericarp and seed of *Terminalia catappa*

Mineralcomposition	Pericarp(ppm)	Seed(ppm)	Mean(ppm)	t-test value
Calcium	40.41±1.41	32.20±0.37	36.31±1.04	0.005
Magnesium	46.46±1.45	23.80±1.58	35.13±0.13	<0.001
Iron	7.05±0.07	5.55±1.33	6.3±1.27	0.177
Zinc	1.30±1.20	1.04±0.08	1.17±1.12	0.724
Potassium	2.77±0.46	2.04±0.04	2.405±0.42	0.93
Sodium	5.27±0.46	3.46±0.47	4.37±0.27	0.007

Values are presented as mean ± SE

DISCUSSION

The result presented in this study correlates with the findings of Oduro *et al*., (2009), who reported the presence of photochemical compounds like saponin, glycoside, flavonoids, alkaloids, anthraquinone in the pericarp pulp that was gotten from the fruit of the red variety. The presence of alkaloid, steroid, flavonoids, anthraquinone, tannin, phenol, glycosides and saponin in the fruit flesh (perciarp) as reported by Krishnaveni M. and Dhanalaskshmi (2015) corresponds with the findings of this present investigation pertaining to the pericarp extract.

The presence of glycoside and alkaloid in the nut as reported by Krishnaveni and Dhanalakshmi (2015) contradicts the findings of this current study as these two phytochemicals were found absent in the nut (seed). This study also disagrees with the report of Oduro *et al*., (2009) who reported the presence of alkaloid in the nut of the red variety which is the variety used for this study.

Acetonic extract of the pericarp of *T.catappa* indicates the presence of alkaloids, tannins, saponnins and steroids (Arumugnam*et al*., 2015) which agrees with the findings of this study though aqueous extract was used.Rajaram*et al*., (2013) also reported that the acetonic extract of *T. catappa* fruit showed the presence of saponin, steroid and tannin which is in line with the phytochemicals found present by this study using aqueous extract. However, glycoside and phlobatannins were absent in the result of their study and this did not align with the findings of this study though the varying extracts might be responsible for the variation in the results.

The highest concentration of phytochemicals in this study is phenol in the pericarp. Phenols are chemicals components that occur ubiquitously as natural colour pigments responsible for the color of fruits of plants. Their most important role may be in plant defense against pathogens and herbivore predators and thus are applied in the control of human pathogenic infections. Phenols essentially represent a host of natural antioxidants, used as nutraceuticals for their enormous ability to combat cancer, they are taught to prevent heart ailment to an appreciable degree and are sometimes used as anti-inflammatory agents (Fan *et al*., 2004). Therefore, the pericarp of *T.catappa* fruit with such a concentration of phenol should be investigated for use against cancer.

Saponin is the highest occurring phytochemical in the seed. Saponins are regarded as high molecular weight hydrocarbons. They are soluble in water and gives aglycones called sapogenin upon its hydrolysis. Saponins are reported to be extremely poisonous (Kar, 2007), which is greater pointer for humans to be cautious about the amount of the seed of *T.catappa* one takes. Saponins are important therapeutically as they are shown to have hypolipidemic and anticancer activity. They are important sources of steroid hormones (Sarker and Nahar, 2007). Since the seed contains a high concentration of saponin it corroborates the report by Ratnasooriya and Dharmasiri(2000), who established that the seeds of *T.catappa*showed aphrodisiac activities in male rats.The high concentration of glycoside in the pericarp as opposed to its absence in the seed according to this present study will make the pericarp more likely to be effective as an astringent than the seed as the bitter properties of glycosides in the presence of tannic acids serve as astringents, as antiprotoxoan or to reduce throxine and metabolism. Amygdalin, a form of glycoside has been used as cough suppressants in various preparations although excessive ingestion of cyanogenic glycoside can be fatal. Some foodstuff containing cyanogenic,glycosides can cause food poisoning (severe gastric irritation and damage) if not properly handled (Sarker and Nahar, 2007).

Alkaloids though absent in the seed is present in the pericarp according to the phytochemicals analysis in this study. Alkaloids like phenols function in the defence of the plant against pathogens and herbivore predators. Therefore they are widely exploited as pharmaceuticals, stimulants, narcotics and poisons due to their potential biological activities. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al*., 2010). These functions are therefore expected of the pericarp of *T. Catappa* not the seed according to the result of this study.The pericarp and seed both contain flavonoids. Flavonoids are made up of more than one benzene ring in their structure and numerous reports support their use of antioxidants or free radical scavengers (Kar, 2007). Dietary intake of flavonoids exceeds that of antioxidative vitamins and provitamins (Sies *et al*., 2005).Terpenoids are classified based on the number of isoprene units involved in its formation and can be mono-, di-, tri- and sesqui depending on the number of carbon

atoms. Terpenoids are present in both the pericarp and seed under study. Terpenoids have anti-inflammatory, sedative, insecticidal and cytotoxic activity (Martinez *et al*., 2008).

Plant steroids are known to be important for their cardiotonic activities; they possess insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicine and cosmetics. They are routinely used in medicine because of their profound biological activities (Janporn *et al*., 2015).Anthraquinones are derivatives of phenolic and glycoside compounds. It is present in the pericarp of *T. catappa* but not the seed, therefore its laxative action will only be expressed in the pericarp. The concentration of phlobatannin in the fruit is quite low, very little in the pericarp and absent in the seed, therefore any phlobatannin character will be barely expressed.

The moisture content of the pericarp was found to be very low compared to the one reported by Oduro *et al*., 2009 who reported moisture of 31.05±0.04 %, while the moisture content of the seed was in agreement with that of Akpakpan and Akpabio, (2012) who reported moisture content of 2523%. The moisture content in both pericarp and seed are higher than that reported by Anuforo *et al*., (2017) who reported 5 %. It was concluded that higher moisture content enhances the growth of microorganism and accelerates food spoilage (Anuforo *et al.*, 2018). The ash content which signifies the level of mineral present was found to be in agreement with Akpakpan and Akpabio (2012), who reported ash content of 5 %, and Hassan *et al*., (2011), who reported ash content ranging from 7.83 – 8.33 %, but lower than that reported by Anuforo *et al*.,(2017) who reported 11.03 %. The carbohydrate content in both seed and pericarp disagreed with what was reported by Hassan *et al*., (2011), who reported 78.31 – 64.90 %, but the carbohydrate content of the pericarp agreed with what was reported by Akpakpan and Akpabio (2012), who reported 25.47 %. The carbohydrate content of the seed is higher than the value (30.85%) reported by Anuforo *et al*., (2017).

The lipid content of the pericarp is low and is in agreement with the value(1.33 %) reported by Hassan *et al.*, (2011) while the lipid content observed in the seed is high, which agreed with what was reported by Akpakpan and Akpabio, (2012). This is an indication that they are not sources of fat and oil (Hassan *et al*., 2004). The protein content of the pericarp and seed was in agreement with the amount (8.28 %) reported by Anuforo *et al*., 2017), but the content of the seed is lower than that reported by Akpakpan and Akpabio (2012), while it was found to be higher than the value (3.92 %) reported by Hassan *et al*., (2011). The fiber content agreed with what was reported by Hassan *et al*., (2011) in yellow fruit sample of *Terminalia catappa*, and also in agreement with the report of Akpakpan and Akpabio, (2012) who reported 3.10%, but was found to be lower than the value (36.33 %) reported by Anuforo *et al*., (2017).

The mineral analysis revealed that the calcium concentration is in agreement with

the value (36.1 ppm) reported by Akpakpan and Akpabio, (2012), while it was found to be lower than the value (22.02 ppm) reported by Anuforo *et al*., (207). The magnesium concentration agreed with the value (43.66 ppm, 36.1 ppm) reported by Anuforo *et al*., (2017) and Akpakpan and Akpabio, (2012) respectively. The potassium concentration reported in this study agreed with what wasreported by Anuforo*et al*., (2017); Akpakpan and Akpabio, (2012), who reported 37.5 ppm, and 35.0 ppm respectively. Iron concentration reported was found to be lower than that reported by Akpakpan and Akpabio, (2012). The phosphorous concentration found to be lower than the value reported by Akpakpan and Akpabio, (2012) who reported phosphorous concentration of 4.19 ppm, but lower than the value (1.00 ppm) reported by Akpabio, (2012). The mineral contents reported in this study showed that *Terminalia catappa* could be recommended a dietary supplement.

CONCLUSION

The present investigation has identified the various phytochemicals, mineral and proximate composition of the pericarp and seed of *Terminalia catappa* fruit. This study has also established the variation in the distribution of each of analysis in the two fruit parts. Furthermore, we were able to ascertain that the pericarp of *T. catappa* contain all of the phytochemicals, mineral and proximate composition tested for and often in great concentrations.

It may also be accurately inferred from this study that based on valid prove of the phytochemical, mineral and proximate analyses of the fruit's pericarp and seed under study, they can serve as good basic ingredients in drug making for use in treatment and prevention of diseases.

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