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PROXIMATE AND PHYTOCHEMICAL ANALYSES OF MATURE STEM BARK OF *Terminalia macroptera* Guill. & Perr.

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Abstract

The proximate and phytochemical constituents of matured stem bark of *Terminalia macroptera* (TM) were investigated. Aqueous and ethanolic extracts of the plant's stem bark were obtained by conventional solvent extraction (CSE) and analyzed using spectrophotometry and gravimetry methods. The results showed moisture (5.26 ± 0.11 %), ash (2.68 ± 0.06 %), fibre (6.67 ± 0.16 %), lipid (0.53 ± 0.01 %), protein (11.84 ± 0.45 %) and carbohydrate (73.01 ± 0.60 %) contents. The stem bark of TM also revealed the presence of alkaloids, cyanogenic glycosides, anthraquinones, terpenoids, flavonoids, tannins and saponins with extractive values of 1.54 ± 0.18 % and 5.20 ± 0.60 % for the aqueous and ethanol solvents respectively. The therapeutic claims arising from the use of the stem bark extracts may be attributed to the presence of these phytochemicals.

Keywords: Phytochemicals, Conventional solvent extraction, *Terminalia macroptera*.

Introduction

There is a growing global interest in phytomedicine arising from the therapeutic claims on a number of medicinal plants used monoherbally or polyherbally [1]. *Terminalia macroptera* is one of such plants acclaimed to be effective against a number of diseases in Northern Nigeria. *Terminalia macroptera* Guill. & Perr. belongs to the family Combretaceae. It grows in the savannah to a height of about 1,200 m and 30 m in width where it is easily recognized by the prominent tufts of nearly stalkless, light green leaves that are oppositely decussated and by its large fruits that are pale green to purple bloom with a seed chamber bordered by a papery wing [2-3]. Its growth is favoured by the tropical climate of Ghana, Nigeria, Uganda, Mali, Senegal and Sudan. In Nigeria, it is commonly found in Kwara and other states along the savannah belt, where it is referred to as '*Kwandari*' or '*bayankada*' among the Hausas; and '*Orin idi odan*' among the Yorubas [2]. The root, stem barks and leaves are used for the treatment of stomach aches [4], cough [5] malaria and infections [6]. Earlier reports have shown that the leaves contain steroids, chlorogenic acid, quercetin, cutin, mucilage, phenolic compounds, fixed oils, starch and calcium oxalate crystals [3]. The root, stem bark and leaves showed the presence of resins, hydrolyzable tannins and triterpenes [7-9]. Terminolic, ellagic and trimethylellagic acids have also been isolated from the ether extract of the plant's stem wood while the flowers were found to contain flavonoids [10]. Zou *et al* [11] discovered bioactive polysaccharides from water extracts (using accelerated solvent extractor, 50 ^{o}C and 100 ^{o}C) of plant stem bark. The methanol crude extract of the plant's leaves have shown high radical scavenging activity and moderate xanthine oxidase inhibition [12].

No doubt, a lot of studies have been carried out on the non-polar extracts of *Terminalia macroptera* [12-14] and those done on polar solvents have utilized extreme temperature to extract the crude drugs for analysis giving rise to possible denaturation of countless medicinally useful phytochemicals; most times the plant components are successfully extracted but the alien solvents/condition may alter the functional group or structure responsible for the pharmacological activities of the compounds [15]. Interestingly, traditional healers in practice have applied aqueous and alcohol decoctions of the plant parts to their patients using conventional extraction methods. The aim of this study therefore was to determine the proximate and phytochemical components of aqueous and ethanolic extracts of the stem bark of this medicinal plant at ambient temperature.

Materials and Methods

All chemicals and reagents were of analytical grade and were purchased from JHD, China; BDH and M & B, England. Equipments used include: thermostatically-controlled muffle furnace (600 °C), air oven of temperature range 100 – 250 ^{o}C (Saisho, Model: S-916, China), water-bath (NL-420S, New Life Medical Instrument, England), spectrophotometer (KJ23A No. 23A10044), mettler H80 weighing balance, freeze dryer (FD-10M, PEC MEDICALS, USA).

Collection of Plant Material

Matured stem barks of *Terminalia macroptera* were obtained from open forest in Ilorin, Kwara State in February, 2014. A taxonomist in the Department of Plant Biotechnology, University of Benin, Benin City identified and documented it with voucher number UBH_T 0232.

Preparation of Powdery Plant Sample

The bark was washed, cut into bits, sun dried and pulverized. The powdery sample was filtered with a 2 mm screen. Then, it was stored in a large plastic drum, sealed and kept in a cool dry room after which samples were immediately taken for extraction and analyses.

Proximate Analysis of Powdery Plant Sample

Moisture content was determined by drying 2 g of sample in an oven at 105 °C for 24 h without lid [16] and the dry matter calculated by subtracting the % moisture from 100 %. Total ash was determined by incinerating 2 g of sample for 3 hours in a muffle furnace at 600 °C until grayish white residue was obtained [16].

Crude fibre was determined after the defatted sample (2 g) was digested sequentially for 30 minutes in 1.25 % sulphuric acid and 1.25 % sodium hydroxide solution. The sample was ashed at 550 °C in a muffle furnace for 2 hours, cooled in a desiccator and reweighed. Extracted fibre was expressed as percentage of the original undefatted sample [16].

Crude protein was determined by digesting 0.2 g of the sample in Kjeldahl flask containing 25 ml of H_2SO_4 mixed with ground selenium dioxide, potassium sulphate, copper (II) sulphate as catalyst (in the ratio 20:1:1) for 3 hours till the solution became clear. Ten (10) ml aliquot from the digest was added to 2.5 ml of alkaline phenol (88.125 g, liquefied phenol + 0.30 g of sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H2O$) + 100 g of NaOH in 200 ml H_2O allowed to cool and made up to 1 L mark), 1 ml sodium potassium tartrate (17.8 g of $Na_2HPO_4.2H_2O$ + 50 g of $C_4H_4KNaO_6.4H_2O$ + 108 g of 50 % NaOH and made up to 1 L mark with distilled water) and 2.5 ml of sodium hypochlorite in a test tube. Serially diluted standard solutions containing 0.2 mg/L - 1.2 mg/L of ammonium chloride-N was treated similarly. The resultant solution was read spectrophotometrically at 636 nm against blank. Dilution was done following high colour intensity. Nitrogen amounts (ppm) obtained from the standard curve were converted to percentage protein using the formula below.

Conc. (N) ppm = (SR x ABS x DF x EV x FCV) / (Sample weight x aliquot). Where EV = Extraction volume, DF = Dilution factor, FCV = Final colour volume, SR = Slope Reciprocal of a standard curve for nitrogen determination using ammonium

chloride, ABS = Absorbance, %N = Conc.(N) ppm * 10^{-4} and %Protein = %N * 6.25 (Protein Conversion Factor) [17].

Crude fat and carbohydrate were determined by the methods of FAO [18] and AOAC [16] respectively.

Preparation of Aqueous Plant Extract

Conventional Solvent Extraction Method: CSE-H_2O (R: 1/4, 8-15 ^{O}C)

The powdery bark sample (200 g) was weighed and soaked in 800 ml cold distilled water (single phase extractant) in an open amber glass jar and placed in a refrigerator. The plant sample having been submerged in the water, was allowed to percolate for 72 hours at 8-15 ^{o}C accompanied by periodic stirring. This was followed by filtration through several folded clean white muslin piece to remove the shaft. The filtration process was repeated using whatman filter paper 1(Qualitative/cat No.1001240) and the filtrate pooled and allowed to stand in a refrigerator adjusted to 8-10^{o}C after which it was concentrated with a rotary evaporator at reduced pressure. The concentrated solution was then dried to powdery form using a freeze dryer giving an extractive value of 1.54 %. It was thereafter kept in an air-tight bottle and stored at 4 ^{o}C.

Preparation of Ethanolic Plant Extract

Conventional Solvent Extraction Method: CSE-C_2H_5OH (R: 1/4, 25^{O}C).

The test sample (200 g) was weighed and submerged in a glass jar containing 800ml absolute ethanol. The plant sample having been submerged in the alcohol, was allowed to percolate for 72 hours at 25^{o}C, accompanied by vigorous agitation of the sealed jar at intervals. This was followed by filtration through severally folded clean white muslin piece to remove debris. The filtration process was repeated using whatman filter paper 1. Thereafter, the filtrate was pooled and concentrated with a rotary evaporator, freeze dried into powdery form (with an extractive value of 5.2 %), after which it was stored at 4^{o}C.

Determination of Thermochromic Properties of Extract

A measured sample weight of 1 g of extract was dissolved in distilled water, alkaline and acidic solution and the volume made up to 10 ml in a boiling tube. A thermometer was inserted into the tube, clamped to a retort stand. The test tube was then placed in ice.

The colour of the solution was noted at 0 ^{o}C under ultra violet rays. After which heat was applied to the system and colour changes noted at 5 ^{o}C, 25 ^{o}C, 40 ^{o}C, 80 ^{o}C and 100 ^{o}C under ultra violet light.

Phytochemical Screening

The powdery experimental plant bark was evaluated for phytochemicals according to standard methods. Alkaloid was analyzed in accordance with the alkaline precipitation gravimetric method [19]. Crude saponins, anthraquinone glycosides and terpenoids were determined gravimetrically after respective extraction with butanol [20], chloroform [21] and petroleum ether [22], while tannin was evaluated by spectrophotometric method after treatment with Folin Dennis reagent [15]. Flavonoids was precipitated with ethyl acetate and determined gravimetrically [23]. Cyanogenic glycosides was determined by the alkaline pictrate method [24].

Statistical Analysis

All investigations were performed in triplicates. Difference in variables between two studies (aqueous and ethanolic groups) were analyzed by student's paired *t*-test. Results were reported as means ± standard error using SPSS Version 19.0 (2010). $P < 0.05$, $P < 0.01$ or $P < 0.001$ was taken to indicate a significant difference between groups as the case permitted.

Results and Discussion

The use of herbal extracts in pharmaceutical industries has suffered setbacks due largely to the dearth in scientific information upholding their folkloric claims, chemical composition and mode of action. The result of this study showed that the stem bark of *Terminalia macroptera* contains ash (2.68 ± 0.06%), fibre (6.67 ± 0.16%), lipid (0.53 ± 0.01%), protein (11.84 ± 0.45%) and carbohydrate (73.01 ± 0.60). The dry matter content was 94.74 ± 0.11% (Fig. 1). The bark sample showed very small content of lipid. This accounts for its dry and non-sticky feel.

Ash contents of the stem bark of guava (*Psidium guajava)* and barley (*Hordeum vulgare*) compared well with the result obtained in this study. The result of the proximate analysis also showed similarity in carbohydrate content with the stem bark of guava (*Psidium guajava*) [25]. However, the ash content of the stem bark of *Terminalia macroptera* in this study was less compared to that in the leaves (11.30 %) as reported by [3] (this possibly means that the mineral deposits are more in the leaves than in the stem bark). This was also low when compared to the ash content (18.5 % - 21.30 %) of the stem bark of *Terminalia arjuna* [26]. Similarly, a lower fibre content was revealed in comparism to *T. arjuna* stem bark (16.2 % - 18.9 %). This showed that the insoluble cellulose and lignin are quite small in quantity. It has been known that fibre has the capacity to unite with intestinal bile salts and dietary cholesterol, preventing their absorption from the gut and hastening their eliminaton via the intestinal tract. It has been advocated as a check against gallstone formation and the production of atherosclerotic plaques in blood vessels (27). The alcohol and water extractive values of 12.7 % and 17.0 % respectively reported in the leaves of this plant [3] were higher than what was obtained in the stem bark of the same plant as revealed in this study. This is a possible indication that there are more phytochemicals in the leaves than in the stem of the plant and the ethanol solvent is a better extractant, however, the method of extraction could also be a limiting factor.

The proximate analysis also showed that the stem bark of *Terminalia macroptera* is a rich source of protein when compared with cereals like rice (*Oryza sativa*) 1.80%, maize (*Zea mays*) 4.50 % and oat (*Avera sativa*) 4.90% [28-29]. The protein content compares well with that of *Terminalia arjuna* apical bark in summer [26]. The stem bark of the plant could become a possible supplement for animal feed if further nutritional investigations are carried out on it.

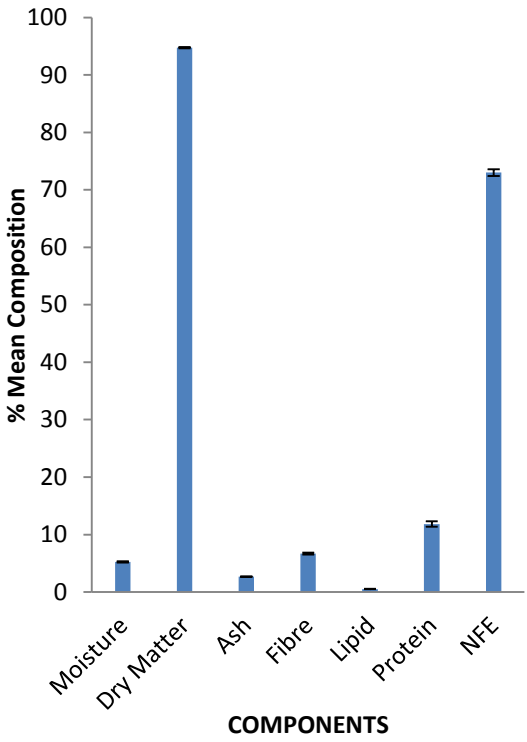

Fig. 1**:** Proximate analysis of the powdery stem bark sample of *Terminalia macroptera*. Height of each bar is mean value ± SE for triplicate determinations.

Results from the thermochromic analysis showed a slight deepening in colours of extracts' solutions as temperature increases and dissimilar colours in acid medium (Table 1). The characteristic colour change of the bark extract could be used as

colorant in food and confectionaries against synthetic additives. It would be important to note that a deviation in these physicochemical properties may point to a possible adulteration of the drug. An initial examination of this sort informs the herbal practitioner and scientist that the right plant is being used [27].

Most tree barks extracts possessing pharmacological activities are uniquely coloured indicating the presence of bioactive molecules. Dark red coloration most likely predict the presence of phlobaphene (hydrolysed tannin) and flavonoids [15]. This suspicion was strongly confirmed by the phytochemical analysis shown in figure 2, revealing high concentrations of tannins (a basic support for isolated tannins and triterpenes [7-9]) and moderate amount of alkaloids, flavonoids and saponins in the stem bark. The powdery stem bark of some previously examined trees have shown similar colouration backed by definite phytochemical tests [30]**.**

Table 1: Thermochromic properties of Aqueous and Ethanolic Extracts of *Terminalia macroptera* Stem Bark.

Form	Temperature	Colour Under Daylight	
		Aqueous extract	Ethanolic extract
Freeze dried	25ºC	Chocolate / black-brown	Purplish black
Aqueous solution	0ºC - 5ºC	Brown	Peach red
Aqueous solution	25 ºC - 40ºC	Amber	Reddish brown
Aqueous solution	80ºC-100ºC	Black - Brown	Deep red
Acidic solution	25ºC	Deep orange	Slight pink
Alkaline solution	25ºC	Brown	Brown

Preliminary phytochemical investigation of the plant stem bark showed the absence of cardiac glycoside and steroids in both extracts. A similar screening of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark has shown absence of steroids and very low amount of glycosides [31].

Previously, high tannin and total polyphenol contents have been shown in the stem bark of *Terminalia arjuna* extracted in water at 100 ºC for 30 minutes and acetone respectively [26]. Although, tannin contents were high for both extracts in the current study, these still remain relatively low when compared to soybean seed that is popularly consumed as food [32], indicating an insignificant antinutritional property. These phytochemicals may act independently, synergistically or antagonistically among themselves on different target organs associated with physiological processes [33].

The variation in the composition of the phytocomponets can be dependent in part on the abiotic factors of the biome, nature of extractant and the method used for extraction [34]. It is therefore noteworthy to point out that the phytochemical screening done on plant crude extract is only dependent on the selectivity of the extractant based on the solubility of the phytochemicals therein. The similarities in the phytochemical contents of the different extracts may be attributed to the presence of a similar functional group (-OH) in both extractants. However, these phytochemicals vary in quantity probably due to the differences in polarity and strength [35], except for alkaloid and tannins that did not show significant differences in quantity between aqueous and ethanolic extracts. These findings are consistent with earlier preliminary investigations [36].

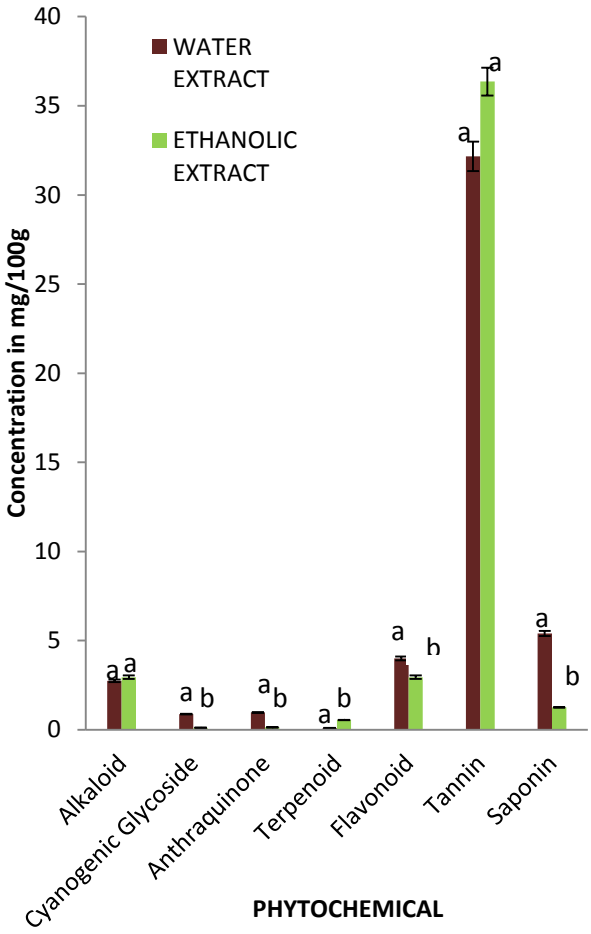

Fig. 2 Phytochemical analysis of aqueous and ethanolic stem bark extracts of the *Terminalia macroptera*. Height of each bar is mean value ± SE for triplicate determinations. Statistical difference between extracts at $P < 0.05$ is indicated by different lower case alphabets.

These phytocomponents incapacitate oxidants by mopping up free radicals [37] or by preventing the decomposition of hydroperoxides into free radicals [38]. Flavonoids have been known to directly scavenge molecular species of active oxygen due to the presence of phenolic hydroxyl groups [39]. Tannins which have reportedly been extracted from a similar *Terminalia* species have shown hepatoprotective properties [40]. Previous investigation has shown similar phytochemicals in the stem bark of *Terminalia superba* whose aqueous extracts reflected gastric and ant-ulcer activity [41].

Conclusion

The proximate analysis in this study was necessary to serve as a comparative tool to judge between the investigated plant sample and other herbal drugs. The results of the study also provides useful information to predict the possible intake and digestability of the stem bark of *Terminalia macroptera* as well as its nutritional and energy value needed to guide against adulteration. The phytochemical composition of the extracts showed the presence of major bioactive molecules that may be linked to the acclaimed curative properties of the stem bark. There is no doubt that these findings provide some basis for the development of African pharmacopeia.

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