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Phenolic and Flavonoid contents, and Scavenging Activity of *Terminalia catappa* Leaves Extracts

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ABSTRACT

This study determined phenolic, flavonoid contents and scavenging potentials of *Terminalia catappa leaves* in various solvents: hexane, ethyl acetate, acetone and methanol. The Total phenol, flavonoids and extracts scavenging activity on DPPH were determined by spectrophotometry at different waveengths. The percentage extract yield for hexane, ethyl acetate, acetone and methanol extracts of *Terminalia catappa* were 4.24, 4.32, 5.65 and 4.56%w/w respectively. Acetone extract showed the highest percentage yield followed by methanol. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins, anthraquinones and phenols. Total phenolic content ranged from 27.38±0.36 mg GAE/g of extract in hexane extract to 76.87±87 mg GAE/g in acetone extract. Least flavonoid value of 2.82±0.19 mgQE/g of extract in ethyl acetate was observed and acetone was the highest with 10.93±0.93mg QE/g. Scavenging activity values on DPPH were in the range of 36.37±0.33% to74.65±2.76% in hexane and acetone respectively. The observed scavenging or antioxidant activity could be attributed to the high level of phenolic content and presence of flavonoids and other secondary metabolites with known antioxidant capacities.

Keywords: Flavonoid, phenolic, scavenging and *Terminalia catappa.*

INTRODUCTION

Plant tissues contain different antioxidants such as flavonoids, tannins, and lignin precursors, which act as reactive oxygen species (ROS) scavenging compounds [1]. ROS scavenging potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins [2]. These compounds are of

great value in preventing the onset and/or progression of many human diseases. The health-promoting effects of antioxidants from plants are thought to arise from their protective effects by counteracting reactive oxygen species [3].

Phenolic compounds are widely distributed in plants and in recent years they have gained much attention, due to their antioxidant activity and free radical-scavenging ability with potential beneficial implications in human health [4]. Phenols are a member of a group of aromatic chemical compounds with weakly acidic properties and are characterized by one or more hydroxyl (OH) groups attached directly to an aromatic ring. The simplest of phenols derived from benzene is also known as phenol and has the chemical formula C_6H_5OH [5]. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds, which are in the highest oxidation state [6].

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent antioxidant activity against the superoxide radical [7]. Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. The antioxidant property of flavonoids was the first mechanism of action studied, in particular with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumor promotion. A number of flavonoids have been shown to suppress carcinogenesis in various animal models [8]. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as “biological response modifiers.” Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity [9]. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages [9].

Africa and Nigeria's focus on the institutionalization of traditional medicine (phytomedicine) parallel with orthodox medicine into the national health care scheme in order to move the new health agenda forward. Effective health care cannot be achieved in Africa by orthodox medicine alone unless it has been complemented with traditional medicine as recorded by [10].

Terminalia catappa is a large tropical tree in the Leadwood tree family, *Combretaceae*. It grows to 35 metres (115 ft) tall, with an upright, symmetrical crown and horizontal branches. It has corky, light fruit that is dispersed by water. The nut within the fruit is edible when fully ripe, tasting almost like almond. The leaves are large, 15–25 cm long and 10–14 cm broad, ovoid, glossy dark green and leathery. *Terminalia catappa* contains several chemicals that are distributed on every parts of this plant. The seed consist of fatty acids and tannins like punicalin, punicalagin, terflavins A,B, and tercatein. The dried fallen leaves are known to contain flavone glycosides like apigenin 6-*C*-(2²-*O*-galloyl)-b-*D*-glucopyranoside, apigenin-8-*C*-(2²-*O*-*g*alloyl)-b-*D*-glucopyranoside, isovitexin, vitexin, isoorientin, and rutin [12]. Leaves and bark are used in different traditional medicines for various purposes. In Taiwan, fallen leaves are used as herb to treat liver diseases. In Suriname, a tea made from the leaves is prescribed against dysentery and diarrhea. The leaves are thought to contain agents for prevention of cancers, although they have not demonstrated anticarcinogenic properties and antioxidant as well as anticlastogenic characteristics. The leaves kept in an aquarium is said to lower the pH and heavy metal content of the water. It is also believed that it helps prevent fungus forming on the eggs of the fish [13].

Natural scavengers are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additives due toconsumers' preference, because of reported adverse effects of synthetic antioxidants such as toxicity and carcinogenicity [14]. The antioxidant activities of extracts of several plants, including their leaves, bark, roots, fruits, and seeds have been extensively studied. However, little or no information has been documented on *Terminalia catappa.* The present study was carried out to evaluate total phenolic and flavonoid contents, and scavenging activity of leaves extracts of various solvents (hexane, ethyl acetate, acetone and methanol).

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Terminalia catappa were* collected within Kashere, Gombe State. The plant was identified and authenticated by Dr. Kolawole Opeyemi Saheed in Plant Science Department of Biological Sciences, Federal University, Kashere and voucher specimen deposited in herbarium for reference purposes.

Extraction of Plant Materials

The sample was carefully washed under running tap water followed by sterile distilled water. It was shade dried at room temperature of 28 ±2.0ºC for two weeks and pulverized to a fine powder using a sterilized mixer grinder. One hundred (100g) of pulverized leaves were mixed with 1000 ml hexane and placed on a magnetic stirrer for 24 hours. The extract was filtered using a sterilized Whatman filter paper Number 1. The filtrate was concentrated by evaporation in water bath at 55 ºC [15]. Same procedure was repeated for acetone, ethyl acetate and methanol, below their respective boiling points. Percentage extract yield w/w was calculated accordingly using equation below

EY% =EDW(g) / PDW(g) × 100……Eqn. (1)

EY=Extract yield; EDW=Extract dried weight in grams; PDW=Powdered dried weight in grams

Estimation of total phenolic content

The total phenolic content of extracts in different solvents were determined using the Folin-Ciocalteu assay [16]. Six milligrams samples of each of the different extracts was dissolved into methanol (1 ml), deionized water (1 ml), and 95% ethanol (1 ml) respectively, and then 11.4µl aliquots of each of these solutions was mixed with Na_2CO_3 (2%, 227.3 µl). The mixtures were allowed to stand at room temperature for 2 min before the addition of Folin-Ciocalteu reagent (50%, 11.4 µl) to each sample mixture. After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured at 750 nm, spectrophotometrically. Gallic acid (0.2–1.0 mg/ml in methanol was used as a standard, and the total phenolic contents of extracts were expressed in milligram gallic acid equivalents (mg GAE/g extract dry weight).

Estimation of total flavonoid content

According to Zhishen *et al.* [17], Methanol was mixed with the same volume of extracts (0.4mg/ml) to 5 ml of 2% aluminium chloride ($AlCl_3$). An absorption reading at 415 nm was taken after 1 h against a blank (methanol). The total flavonoid content was determined using a standard curve with catechin. Total flavonoid content was expressed as mg of catechin equivalents (CE/g of extract).

Free radical scavenging activity on DPPH

The radical scavenging assay was conducted as described by Blois [18]. Various solvents of the extracts adjusted in a final volume of 2.5 ml were mixed with 5 ml of 0.1 mM 2,2 Diphenyl-1-picrylhydrazyl (DPPH) solution. The tubes were shaken properly and incubated for 20 min in the dark. The changes in the absorbance of the samples were measured at 517nm using a spectrophotometer. The radical scavenging activity of the extracts of different solvents were determined and compared with that of the standard antioxidant (quercetin). DPPH solution without the extract and standard formed the control. The percentage of DPPH scavenging activity was calculated using the equation below.

% Scavenging Activity on DPPH= Abc – Abs. / Abc × 100 …….Eqn. (2)

Abc = Absorbance of control; Abs=Absorbance of sample

Phytochemical Screening

The extracts were subjected to the qualitative phytochemical screening for the presence of some phytoconstituents like steroids, tannin, saponin, alkaloids, anthraquinones, phenols and flavoniods. They were identified by characteristics colour change using standard procedures [19].

Statistical analysis

The experimental results were expressed as mean of three replicates ± SD. The statistical analysis of data was done using one way ANOVA (Analysis of variance) to compare variance in test samples. Test of significant differences between means were determined using the post hoc test with level of statistical significance taken at $p<0.05$.

RESULTS AND DISCUSSION

The higher yield of extract obtained in acetone as an extractive solvent, compared to slightly lower values in other solvents could be due to presence of dissolved phytochemicals at low concentrations in the other extracts, hence the phytochemicals were more dissolved in acetone 5.65% as shown in Table 1 below, followed by methanol 4.56%, then ethyl acetat 4.32% and the least was hexane 4.24%. This suggests that, *Terminalia catappa* was poorly dissolved in these solvents. Hexane with zero polarity index recorded closer value to methanol with highest polarity index [20]. Acetone has two methyl groups, which are non-polar and one polar carbonyl group.

This implies that, the extract contains more non polar than polar phytoconstituents. The extraction yield and, consequently, the biological activity of vegetal extracts can be strongly affected by the solvent applied [21]. The critical extraction parameters include solvent, time, solid-to-solvent ratio, number of extractions, temperature, and partial size of the sample material [22]. Some of these parameters could be responsible for the observed variation. However there is no significance difference at $p<0.05$ in their respective percentage yields

Table 1: Yield Value of *Terminalia catapppa* in various solvents

Plant	Solvent	Yield (g)	Yield %
Terminalia catappa	Hexane	8.47	4.24
	Ethyl Acetate	8.63	4.32
	Acetone	11.29	5.65
	Methanol	9.12	4.56

Table 2 , showed acetone to contain highest phenolic and flavonoid values of 7.87±2.56 mg GAE/g of extract and 10.93±0.93 mg QE/g respectively. Least values of same were seen in ethyl acetate 27.38±0.36 and 2.82±0.19. Phytochemical compounds, such as flavonoids, phenols, saponins, and tannins, are considered major secondary metabolites in plants. Phenolic compounds,which have a broad spectrum of biochemical activities, represent the largest group [23]. As obtained in this study, phenolic compounds generally displayed higher values compared with flavonoid. This is in agreement with the findings of Paramita and Camelia [24]. They reported phenolic content of Jack fruit as 411.5±11.2 mg GAE/g with flavonoid total of 0.24±0.02 mg QE/g .This present study demonstrated that, phenolic compound interacts favourably in these solvents more than flavonoids. It may likely be due to differences that exist in their structures as phenols have aromatic rings with one or several hydroxyl groups attached which may have been responsible for maximum reaction, hence elevated phenolic levels. Crude extracts of fruits, vegetables, and other plant materials are rich in phenols [25].

Table 2: Total Phenolic and Flavonoid Contents in Various Solvents

Plant	Solvent	Total Phenolic (mg GAE/g of extract)	Flavonoid (mg QE/g of extract)
Terminalia catappa	Hexane	33.55 ± 0.36^{c}	4.56 ± 0.32^{c}
	Ethyl Acetate	27.38 ± 0.36^{cd}	2.82 ± 0.19^{cd}
	Acetone	76.87 ± 2.56^{a}	10.93 ± 0.93^{a}
	Methanol	55.63 ± 3.35^{b}	8.04 ± 0.13^{ab}

The results are given as means± SD of three determinations Values with different superscript down the column are significantly different from each other at $p<0.05$. mg GAE/g = milligram garlic acid equivalent per gram; mg QE/g= milligram quecetin equivalent per gram

Percentage DPPH scavenging as depicted on Table 3, ranged from 74.65±2.76% to 36.37±0.33% in acetone and hexane respectively. This study revealed that, the quantity of phenolic content in extract is proportional to the observed scavenging effect on DPPH. As seen in Tables 2 and 3, the scavenging activity is in the order acetone>methanol>ethyl acetate>hexane. The ability to scavenge DPPH may be principally due to the presence of phenolic compounds. Obviously, total phenolic content could be regarded as an important indication of scavenging properties of plant extracts [26]. Significance difference exists between acetone and others so also between methanol and others, but there is no significant difference between ethyl acetate and hexane at $p<0.05$ (Table 3). Some authors have reported excellent linear correlations between antioxidant activity tests and total phenolic content [27].

Table 3: Free radical Scavenging Activity on DPPH

Plant	Extract	% Scavenging Activity on DPPH
Terminalia catappa	Hexane	36.37 ± 0.33^{cd}
	Ethyl Acetate	40.40 ± 0.41^{c}
	Acetone	74.65 ± 2.76^{a}
	Methanol	66.14 ± 1.55^{b}

The results are given as means± SD of three determinations. Values with different superscript down the column are significantly different from each other at $p<0.05$

Phytochemical analysis in Table 4, revealed alkaloid, phenols, flavonoids, saponins and steroid presence in all the solvents except that anthraquinones was only found in hexane and absent in others. Equally, tannins was absent in only in acetone. The presence of most of these phytocomposition in most extracts could be responsible for the observed scavenging capabilities. Phenolic compounds are secondary plant metabolites which contribute to overall antioxidant activities of plants mainly due to their redox properties [28]. Flavonoids could be extremely helpful as they possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities [29]. Tannins can also be effective in curbing haemorrhages as well as restrict bare swellings. While tannins are proved homeostatic, they are also beneficial when applied on mucosal coating in mouth. Conventionally, tannins have also been used to cure diarrhea [30]. So, the identification of tannins in *Terminalia catappa* could be of great advantage. Several workers have reported the analgesic, antioxidant, antispasmodic and antibacterial properties of alkaloids [31]. The observed scavenging activity could be achieved due to their synergistic action.

Table 4: Phytoconstituents of *Terminalia catappa*

Phytochemicals	Hexane	Ethyl Acetate	Acetone	Methanol
Alkaloid	+	+	+	+
Anthraquinones	+	–	–	–
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	–	+
Steroids	+	+	+	+

Key: += Present; - =Absent

CONCLUSION

In the present study, we investigated the total phenolic, flavonoid contents, and scavenging activities of four extracts of *Terminalia catappa.* It has shown to be a potent source of natural scavenger, with phenolic-rich and poor flavonoid contents. Elevated scavenging activity was observed in the acetone extract when compared with other tested extracts. These findings now provide a basis for developing a valuable food additive to enhance human nutrition via phenolic composition and consequent scavenging activity.

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