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In vitro Antioxidant Status and Polyphenol Content of *Vitex doniana* Leaves Extracts

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ABSTRACT

This study was designed to evaluate the antioxidant and phytochemical activities of *Vitex doniana leaves*. Hexane, ethyl acetate, acetone and methanol were used as extractive solvents. Total flavonoids, phenolic and extracts scavenging actitiviy on DPPH were determined spectrophotometrically at different wavelengths. The solvents yielded 5.24, 6.85, 7.48 and 7.99 % w/w respectively. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins, anthraquinones and phenols. Flavonoids with highest value of 59.99±2.23 were seen in methanol while least value of 29.88±2.15 was in hexane. Also phenolic value in methanol was 12.22±0.20 and the least value of 5.19±0.61 was recorded in hexane. Methanol scavenging activity on DPPH was 83.8±0.85% and the least value of 43.51±3.42% was observed in hexane. This study revealed that *Vitex doniana* possessed antioxidant status, quantitatively. Flavonoids demonstrated higher solubility than phenols. The presence of phytoconstituents could be responsible for the observed effects.

Keywords: Antioxidant, polyphenols, scavenging, v*itex doniana*

INTRODUCTION

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy. When our body cells use oxygen, a highly reactive atom, they naturally produce free radicals. Free radicals are electrically charged molecules. They have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves [1]. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And

until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Antioxidants delay the oxidation processes inhibiting free radical initiated chain polymerisation, and other subsequent oxidizing reactions [2]. These components include: Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoicaci [2].

It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating. Free radicals, reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage [1]. They are also capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction [3]. These antioxidants act as "free radical scavengers" that prevent and repair damage done by these free radicals. Antioxidants are capable of stabilizing, or deactivating free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being [4]. Many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Numerous other antioxidant phytonutrients are present in a wide variety of plant foods [5]. Antioxidants status of medicinal plants are today, recognised as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents[6] .There has been an increasing interest in the use of natural antioxidants, such as tocopherols, flavonoids and plant extracts for the preservation of food materials in recent years, because these natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants, such as butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT) and propyl gallate (PG) [7].

Vitex doniana is a small to medium- sized tree growing up to 25 m tall. It belongs to the order Lamiales. It is of the family Lamiaceae, genus *Vitex* and Specie *doniana.* It is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states [8]. It is variously called *dinya* (Hausa), *dinchi* (Gbagyi), *oriri* (Yoruba), *ejiji* (Igala) and *olih* [8,9]. It has quite a number of available ethnopharmacological significance among different African communities, including use of water decoctions of different parts for treatment of stomach and rheumatic pains as well as inflammatory disorders [9]. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery [8]. The roots and leaves are used for nausea, colic and epilepsy [9]. In eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meal.

A number of studies have been carried out on*Vitex doniana*. However, little or no report has been documented on its antioxidant status in different solvents, phenolic and flavonoid content analysis and otherphytoconstituents.It is therefore necessary to carry out scientific investigations in order to provide baseline information for drug discovery and development.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Vitex doniana* were collected within Yelmatu Deba, Gombe State. The plant was taxonomically 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 30±2.0 ºC (thermohygrometer) for two weeks and pulverized to a fine powder using a sterilized mixer grinder (model: BL400C, China). Two hundred (200 g) of pulverized leaves were mixed with the first solvent in the series (hexane, 1000 ml) and placed on a magnetic stirrer for 24 hours. The extract was filtered using a sterilized Whatman filter paper No.1.The filtrate was concentrated by evaporation in water bath at 55 ºC

[10] . Same procedure was repeated for acetone, ethylacetate and methanol. Percentage extract yield w/w was calculated accordingly using the equation below.

$$Extract\ yield\ \% = Extract\ Dried\ Weight\ (g)/Powdered\ Dried\ Weight\ (g)\ \times 100 \ldots\ldots\ldots\ldots Eq\ 1$$

Equipment, Chemicals/Reagents

Spectrophotometer, Thermohygrometer, Folin - Ciocalteau reagent, 1, 1- diphenyl - 2-picrylhydrazyl (DPPH), gallic acid, and quercetin were purchased from Zayo Sigma Pharmaceutical. Methanol, ascorbic acid, hydroxyl toluene, aluminium trichloride and sodium carbonate were purchased from Merck Scientific. All chemicals used were of analytical grade.

(DPPH) free radical scavenging assay

The radical scavenging assay was conducted as described by Blois [11]. Various solvents of the extracts adjusted in a final volume of 2.5 ml were mixed with 5 ml of 0.1 mM2,2 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.

$$\%DPPH\ scavenging\ activity = \left(Abs.control - Abs.\frac{Sample}{Abs}.control\right) \times 100 \ldots\ldots..Eq.2$$

Abs= Absorbance at 517nm

Estimation of total phenolic content

The total phenolic content of extracts in different solvents weredetermined using the Folin-Ciocalteu assay [12] . 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-Ciocalteau 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. 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.* [13] 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).

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 [14].

Statistical analysis

The experimental results were expressed as means 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 student t-test with level of statistical significance taken as $p<0.05$

RESULTS AND DISCUSSION

Selecting extracting solvent depends on the specific nature of the bioactive compound being targeted. The extraction yield and consequently, the biological activity of vegetal extracts can be strongly affected by the solvent applied [15]. Polar substances dissolved in polar solvents because it is thermodynamically favorable for the solvent-solute forces. The solubility of a drug in a given solvent is mainly a function of the polarity of the solvent [16]. As obtained in this study, methanol showed maximum extract yield (7.99 %), followed by acetone (7.48%), and the least was observed in n-hexane (5.24%) as shown in Table 1. This suggested that extraction yield increase with increase in polarity of solvents. It also revealed that the major phytoconstituents are high in polarity and hence soluble in methanol than hexane. The ranking order for the

percentage extraction was MeOH>DMK>EtOAc>Hex (Methanol>Acetone>Ethyl acetate>Hexane).

Table 1: Yield Values of *Vitexdoniana* leaves extracts in Different Solvents

Plant	Solvents	Yield (g)	Yield (%)
Vitex doniana	Hexane	10.48	5.24
	Ethyl acetate	13.70	6.85
	Acetone	14.96	7.48
	Methanol	15.98	7.99

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant [17]. It has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the anti-oxidative activity of plant extracts and foods [18]. As presented in Table 2, there is significant difference between methanol, acetone and hexane but there is no significant difference between acetone and ethyl acetate.

Table 2: DPPH scavenging activity of *Vitexdoniana* indifferent extracts

Plant	Extract	Percentage DPPH Scavenging Activity
Vitex doniana	Hexane	43.51 ± 3.42^{c}
	Ethyl A	52.50 ± 0.92^{bc}
	Acetone	65.46 ± 0.43^{b}
	Methanol	83.8 ± 0.85^{a}

The results are given as means± SD of three determinations.

Values with different superscript down the column are significantly different from each other at $p<0.05$.

Phenolic compounds are richly distributed in plants and in recent times, they have gained attention, due to their antioxidant activity and free radical-scavenging ability with potential beneficial implications in human health [19]. These antioxidants may help to relieve oxidative stress, i.e. preventing free radicals from damaging biomolecules such as proteins, DNA, and lipids [20]. .As shown in Table 3, total phenolic contents ranged from 59.99±2.23 to 29.88±2.15

mg GAE/g of extract. This study revealed significance difference in phenolic contentof methanol extract and ethyl acetate and no significance difference between hexane and ethyl acetate. Flavonoids and phenolic acid possess many biological activities including anti-inflammatory, anti-carcinogenic, and antiatherosclerotic activities. These activities might be related to their antioxidant activity [21]. The flavonoid content of the extracts (ranged from 12.22±0.20 to 12.22±0.20 mgQE/g of extract) obtained from *vitex doniana* leaves are shown below. Generally, the values of all the said parameters tended to increase with the increasing polarity of the solvents used as extraction medium.

Table 3: Total phenolic and flavonoidcontents in different extracts of *Vitexdonina* leaves

Plant	Solvent	Total Phenolics (mg GAE/g of extract)	Flavonoids (mgQE/gof extract)
Vitex doniana	Hexane	29.88±2.15^{b}	5.19±0.61^{e}
	Ethyl A	39.63±0.97^{b}	6.65±1.05^{de}
	Acetone	44.13±0.99^{ab}	9.32±0.34^{dc}
	Methanol	59.99±2.23^{a}	12.22±0.20^{c}

The results are given as means± SD of three determinations

Values with different superscript down the column are significantly different from each other at $p<0.05$.

Key: GAC=garlic acid equivalent, QE=Quercetin equivalent

Qualitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research [22]. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals [23].Saponins was found absent in methanol,ethyl acetate and hexane and steroids were only absent in acetone extract.

Table 4: Result of Phytochemical screening of *Vitex doniana* leaves extracts

Phytochemicals	Hexane	Ethyl A.	Acetone	Methanol
Alkaliods	+	+	+	+
Anthraquinones	+	+	+	+
Phenols	+	+	+	+
Flavoniods	+	+	+	+
Saponins	-	-	+	-
Tannins	+	+	+	+
Steriods	+	+	-	+

Key +=Present -= Absent

CONCLUSION

Higher values of antioxidant activity were observed in the methanol extract of *vitex doniana*l eaves when compared with other tested extracts. Thus, these extracts can be considered as new sources of natural antioxidants. Also, total phenolic content values in all extracts were higher than flavonoid contents.The extracts showed to contain other phytoconstituents of healthbenetifits. Our findings now provide baseline information for developing new drugs and a valuable food additive to enhance human nutrition via their phenolic composition and antioxidant activity.

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