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PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF CRUDE AND PARTITION PORTIONS OF *Leptadenia hastata* ROOT EXTRACTS

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ABSTRACT

Leptadenia hastata is an edible, non-domesticated vegetable. It is cultivated and collected in the wild, and arid tropical regions characterised by sandy soil composition throughout Africa. The ethnobotanical data collected from Traditional Medical Practitioners in northern Nigeria indicates that L. hastata is utilized for the management of diabetes mellitus. Previous studies have documented the antibacterial and antimicrobial properties of L. hastata. This study was carried out to determine the chemical content and in-vitro antioxidant activity of the root of L. hastata. After exhaustive soxhlet extraction of the root, the extract was then fractionated. In-vitro antioxidant activity and qualitative phytochemical analysis of the fractionated and the residual portions were determined using a DPPH free radical assay and standard procedure. The extractive values of the ethanol extract, chloroform, ethyl acetate, n-butanol, and residual aqueous portions were 18.3% w/w, 0.89% w/w, 21.6% w/w, 13.2% w/w, and 45.3% w/w, respectively. The result of qualitative phytochemical analysis of these extracts revealed the presence of alkaloids, carbohydrates, cardenolides, cardiac glycosides, flavonoids, saponins, and terpenoids in all but alkaloids and saponins were not detected in the chloroform portion. The crude extract was found to be safe, as indicated by an oral mean lethal dose (LD_{50}) ≤ 3600 mg/kg body weight in rats. In-vitro antioxidant activity using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay revealed the presence of antioxidant principles in the crude extract, chloroform, ethyl acetate, and n-butanol portions similar to the standard ascorbic acid. However, only chloroform, ethyl acetate, and n-butanol showed concentration-dependent scavenging activity at concentrations of the solutions (10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, and 50 mg/ml). Conclusively, L. hastata has proven to be a good source of antioxidant compounds using chloroform, ethyl acetate, and n-butanol as solvents.

Keywords: Phytochemical Screening; *In-vitro*; Antioxidant; *Leptadenia hastata*

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are chemical entities that possess high reactivity. The phenomenon in question is a result of the existence of unpaired electrons within the system and their molecular structure [1]. Chemically reactive compounds that arise as inherent byproducts of cellular metabolism are referred to as endogenous metabolites which represent molecules that are formed as natural byproducts of cellular metabolism [2]. They are generated as incidental byproducts under physiological circumstances and function as valuable molecules for redox signaling. A sophisticated collection of endogenous antioxidants effectively manages these reactive species, enabling their utilization by our cells and tissues safely [3]. In many diseases, this fine balance is shifted so that ROS and RNS outnumber our antioxidants. The occurrence of oxidative stress can give rise to toxicity and cellular demise, hence posing grave and potentially fatal repercussions for the affected individual [4]. The impact of oxidative stress is of utmost importance in the context of pathophysiological cascade responsible for several diseases. Oxidative stress (O S) diminishes the functional capabilities of immune cell populations which are compromised, leading to an increased vulnerability to illnesses in animals [5]. For instance, adult dairy cows usually experience oxidative stress (O.S) around the time of calving [6,7]. While neonatal calves experience oxidative stress during the first few weeks of age [8,9]. The periods of heightened vulnerability to diseases in these animals are ascribed to impaired immunological responses. Evidence suggests that oxidative stress is implicated in the pathophysiology of a diverse array of cardiovascular disorders [10].

The role of oxidative stress in cardiac diseases of dogs has been reported by Cristina *et al*. [11]. oxidative stress. has been associated with chronic renal failure [12], diabetes mellitus [13], diabetic nephropathy [14], coronary artery diseases [15], and obesity [16]. Chronic accumulation of advanced oxidation protein byproducts and the activation of the angiotensin II type 1 receptor (AT1R) has been shown to facilitate inflammatory mechanisms inside the renal tissue of individuals with diabetes [14] and in chronic renal failure [12]. During inflammatory diseases like mastitis, there is an increase in lipid peroxidation, which causes a decrease in levels of some antioxidant molecules, leading consequently to oxidative stress [17,18]. An increase in ROS following experimentally induced acute mastitis with lipopolysaccharide injection has been reported [19]. Correlation between plasmodium infection and elevated levels of oxidative stress was reported by Vasquez *et al.* [20]. Therefore, oxidative stress is central to the pathogenesis/pathophysiology of many human and animal diseases.

The most optimal approach to eradicate and reduce the activity of free radicals, which instigate oxidative stress is through anti-oxidative defense mechanisms. However, the natural antioxidant system is usually overwhelmed by ROS and RNS, thus the need for antioxidant supplements. Antioxidants are those substances that inhibit the synthesis and propagation of free radicals. Recently, the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury is being pursued rigorously and is of interest to both pharmaceutical and food industries [21].

Leptadenia hastata is a plant species classified within the family Asclepiadaceae, which is commonly utilized as a vegetable in Tropical Africa [22]. The medicinal significance of the plant has been documented in many studies: hormonal therapy [23], antidiabetic [24], antifungal [25] and antibacterial effects [23]. *Leptadenia hastata* is an edible, non-domesticated vegetable, and it is collected in the wild throughout Africa. *Leptadenia hastata* is a voluble herb with creeping latex stems, glabrescent leaves, glomerulus, and racemes flowers, as well as follicle fruits [22]. It typically grows in regions characterized by tropical dry climates and sandy soil. The use of wild foods, such as *L. hastata*, plays a

crucial role in ensuring food security throughout periods of seasonal transitions. Additionally, these wild foods are widely utilized for their therapeutic properties in many regions.
The ethnobotanical data collected from Traditional Medical Practitioners in northern Nigeria indicates that *L. hastata* is utilized for the management of diabetes mellitus. Previous studies have documented the antibacterial and antimicrobial properties of *L. hastata* [26]. Furthermore, investigations into its toxicity have indicated that the plant exhibits a comparatively low level of harm [27]. Nevertheless, there is a need for more information confirming the claimed antioxidant potential of *L. hastata*. The objective of this study is to assess the antioxidant capacity of *L. hastata* root with the view to validating its antioxidant potential.

MATERIALS AND METHODS

Plant Collection

Leptadenia hastata root was collected from the outskirts of the University of Maiduguri and identified by a botanist affiliated with the Department of Biological Sciences at the University of Maiduguri, Nigeria. A voucher specimen (VET/LAB/LH362[a]) was kept in the research laboratory, Department of Chemistry, University of Maiduguri, Nigeria

Chemicals and Reagents

2, 2-diphenyl-1-picryhydrazyl (DPPH), ascorbic acid, ferric chloride ($FeCl_3$), ethanol, chloroform, ethyl-acetate, n-butanol and methanol were procured from Merck and Sigma Aldrich. The chemicals and solvents utilized in the experiment were of analytical grade.

Plant Extraction

After collecting, the root of L. *hastata* was separated from the other parts and dried under shed. The dried grounded root of *L. hastata* (3.0 kg) was extracted in a Soxhlet apparatus using 85% ethanol. The extraction process was conducted at 78^{o}C for six (6) hours. The extract obtained was evaporated under pressure at 50^{o}C to a constant weight. The crude ethanol extract was further partitioned using solvents of different polarity in the following order: chloroform, ethyl-acetate, n-butanol, and residual aqueous, and the partition yield was calculated. The yield of the evaporated dried root extract of *L. hastata* and that of the partition portions based on dry weight were calculated from the following equation: yield (g/100g of plant material) = (W1 × 100)/W2 Where W1 and W2 were the weight of the extract after the solvent evaporation and the weight of the dry plant material, respectively.

Phytochemical Analysis

A small quantity each of the *Leptadenia hastata* root extract and fractions (LHERE), EECF, EEEA, EENB, and EERA were subjected to qualitative phytochemical screening to test for the presence of the following secondary plant metabolites: alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides (cardiac, steroidal), terpenes/terpenoids as described by Harborne [28], Brain and Turner [29], Vishnoi [30], Silver *et al.* [31], Trease and Evans [32].

In-vitro Antioxidant Assay

DPPH Radical Scavenging Activity

The extract's capacity to scavenge free radicals was determined using 2,2-diphenyl-1-picrylhydrazyl (*DPPH)*. The *DPPH* solution (0.3mM) was prepared in 95% methanol. The sample extract of *L. hastata* was combined with a solution of 95% methanol in order to create the stock solution. (1 mg/ml). Freshly prepared DPPH solution was taken in test tubes, and extracts were added, followed by serial dilution (100-1000 µg) to every test tube such that the final volume was 2ml. The discoloration was measured at

517nm after incubation for 30 minutes in the dark (SP001 Matemeter, UV spectrophotometer, Thermo Electron Corporation, England, UK). Measurements were performed at least five times. The standard substance utilized in this experiment was ascorbic acid, which was dissolved in distilled water to create a stock solution of equal concentration with the same concentration (1mg/ml). The control sample, which contains the same volume without any extract and 1 ml of 95% methanol, was used as a blank. Percent scavenging of the DPPH free radical was determined using the following equation.

DPPH Scavenging effect (%) = $[Abs_{control} - Abs_{sample}] / Abs_{control} \times 100$

The variable "$Abs_{control}$" represents the absorbance value of the DPPH radical.

Methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/standard, i.e. crude, ethanol, chloroform, Ethyl-acetate, *n*-butanol, and residual aqueous of *L. hastata*, respectively). A comparison was made between the test compound's real absorption decrease and the positive controls.

Statistical Analysis

The statistical analysis of the data involved the application of one-way analysis of variance (ANOVA) and control Dennett's test. The software used for this analysis was SPSS version 8. The statistical analysis was deemed to be statistically significant at a significance level of $P \leq 0.05$.

RESULTS

Extraction and Partitioned Portions Profile *L. hastata* Root Extract

The extractive value for 85% ethanol extract, chloroform, Ethyl-acetate, n-butanol, residual aqueous, and insoluble substances of *L. hastata* from 500 g was found to be 21.8% w/w. The relative extractive percentage values for the organic solvent partitioned portions from 500 g of the crude extract were found to be EECH; 0.89% (4.45g dried mass), EEEA; 21.6% (108.8g Dried mass), EENB; 13.2% (65,9g gummy mass), EERA; 45.3% (226.5g sticky gummy mass), insoluble substance 14.4% (72.6g light brown) for chloroform, ethyl-acetate, n-butanol, residual aqueous and insoluble substance respectively as presented in Table 1.

Table 1: Percentage yield and characteristics of *L. hastata* root extracts

S/No.	*Extract/Portion#	Code	Weight (g)	Color	Texture	% Yield
1.	*85% ethanol	LHERE	11,200	Dark-brown	Gummy Mass	18.3
2.	Marc	-	10,428	Light brown	-	-
3.	Chloroform#	EECH	4.45	Dark-brown	Dried mass	0.89
4.	Ethyl-acetate#	EEEA	108.8	Dark-brown	Dried mass	21.6
5.	n-Butano #	EENB	65.9	Dark-brown	Gummy mass	13.2
6.	Residual aqueous#	EERA	226.5	Dark-brown	Sticky-Gummy mass	45.3
7.	Insoluble substance	-	72.6	Brown	Light brown	14.5

Key: LHERE= *Leptadenia hastata* Ethanol Root Extract, EECH= Chloroform portion, EEEA= Ethyl-acetate portion, EENB= n-Butanol portion, EERA= residual aqueous portion. *- extract, # - fractionated portion.

Phytochemical Constituents of the Crude Ethanol Root Extract and Partitioned Portions of *L. hastata*

Preliminary phytochemical screening of the extract revealed the presence of several bioactive constituents, including alkaloids and carbohydrates. Cardenolides, cardiac glycosides, flavonoids, saponins, and terpenoids are prominent in the crude ethanol extract, ethyl-acetate, n-butanol, and the residual aqueous. However, chloroform is only positive for cardenolides, cardiac glycosides, flavonoids, and Terpenoids, as presented in Table 2.

Table 2: Phytochemical constituents of crude ethanol and fractionated portion of the root extract of *L. hastata*

S/No.	Group constituents	Test	RESULTS				
			EE	**CF**	**EA**	**NB**	**RA**
1.	**Alkaloids**	Dragendorff's	+	-	+	+	+
		Mayer's	+	-	+	+	+
2.	**Anthraquinones**						
	Combined Anthraquinones	Bontrager	-	-	-	-	-
3.	**Carbohydrates**						
	General test	Molisch's	+	-	+	+	-
	Monosaccharide	Barfoed's	-	-	-	-	-
	Free reducing sugar	Fehling's	+	-	-	+	+
	Combined reducing sugar	Fehling's	+	-	-	+	+
	Ketoses	Salivanoff's	+	-	-	-	+
	Pentoses		-	-	-	-	-
4.	**Cardenolides**	Keller-Killiani's	+	+	+	+	+
5.	**Cardiac glycosides**						
	Salkowski's	L-Buchard's	+	+	+	+	-
	Lieberman-Buchard's	L-Buchard's	+	+	+	+	+
6.	**Flavonoids**	Shinoda's	+	+	+	+	-
		Ferric chloride	-	-	-	-	-
		Lead acetate	-	-	-	-	-
		NaOH	+	-	+	+	-
7.	**Phlobatannins**		-	-	-	-	-
8.	**Saponins**	Frothing's	+	-	+	+	+
9.	**Soluble starch**		-	-	-	-	-
10.	**Tannins**	Ferric chloride	-	-	-	-	-
		Lead acetate	-	-	-	-	-
11.	**Terpenoids**		+	+	+	+	+

Key: + = Present, - = Absent; EE = Ethanol extract, CF= Chloroform portion, EA= Ethyl-acetate portion, NB= n-Butanol portion, RA= Residual aqueous portion

Antioxidant Effect of the Crude and Partition Portions of *L. hastata* Root

The result of the antioxidant assay of the crude and the partition portion (chloroform, Ethyl-acetate, *n*-butanol, and residual aqueous) tested on free radicals generated by DPPH, as presented in Fig 1. showed that the chloroform, ethyl-acetate, and *n*-butanol extract demonstrated better tendency to scavenge free radicals (95.0 ± 2.91%, 93.2 ± 2.91% and 91.6 ± 2.91%) respectively. In comparison, the crude and residual aqueous extract had the lower scavenging effect (84.9 ± 2.91%) and (77.3 ± 2.91%) at 10mg/ml extract administration when compared to the standard ascorbic acid (96.9 ± 2.91). At 20 mg/ml extract administration, the chloroform and ethyl-acetate partition portion of the extract showed better scavenging properties (94.7 ± 2.71% and (93.5 ± 2.71%) when compared to ascorbic acid (97.2 ± 2.71%) which inhibited the free radicals readily as presented in Fig 2. At 30 mg/kg extract administration, the chloroform portion of the extract maintained higher scavenging (94.0 ± 2.33%) than the ethyl-acetate portion (91.3 ± 2.33%) on comparison with the standard ascorbic acid (97.3 ± 2.33%) as presented in Fig. 3. At 40 mg/kg extract administration similar scavenging effect was observed with chloroform portion of the extract scavenging better (93.6 ± 1.98%) than ethyl-acetate, n-butanol crude and the residual aqueous extract (89.8 ± 1.98%, 82.3 ± 1.98%, 85.3 ± 1.98% and 83.9 ± 1.98%) respectively when compared with the standard ascorbic acid (97.3 ± 1.98%). At the highest concentration of 50mg/ml extract administration, chloroform, ethyl-acetate, and residual aqueous portion slightly scavenge better (93.2 ± 3.35%), (91.0 ± 3.35%) and (85.6 ± 3.35%) respectively on comparison with the standard ascorbic acid (96.9 ± 3.35%). However, multiple comparisons of the data obtained using ANOVA showed a statistically significant scavenging effect of the extract and standard at $p \leq 0.05$.

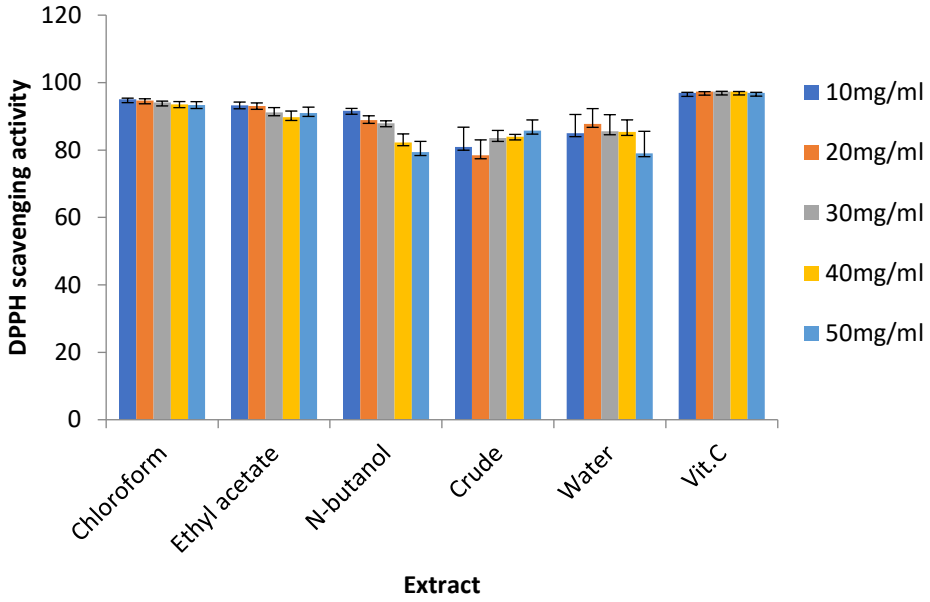

Fig.1: Percentage scavenging effect of DPPH free radicals by crude and partition portions of *L. hastata* root extract

DISCUSSION

The extractive value of 85% ethanol crude root extract of *L. hastata* from 300g was found to be 18.3% w/w (940g dark brown mass). The choice of ethanol as a solvent was because previous studies have reported that ethanol was a better solvent for more consistent extraction of antioxidant compounds from

medicinal plants than other solvents such as water, methanol, and hexane [33,34]. The preliminary phytochemical screening revealed alkaloids, carbohydrates, cardenolides, cardiac glycosides, flavonoids, saponins, and terpenoids. The partitioning process of the solvents demonstrated diverse extraction efficiencies. The observed variability can be ascribed to fluctuations in the polarity of the solvents used for extraction, which in turn can result in significant variances in the concentration of bioactive chemicals present in the extract. A higher extraction yield was observed in water, ethyl acetate, and n-butanol compared to chloroform, indicating that the extraction efficiency favors the highly polar solvents; this could be because the plant material contains high levels of polar compounds that are soluble in solvents with high polarities such as water, ethyl-acetate, and n-butanol is attributable to the higher solubility of these compounds in ethyl-acetate and n-butanol than the other solvents tested. In order to better understand the solvent's effect on extraction yield, further analysis was performed to measure the content of bioactive compounds in the extract. In accordance with the extraction yields, the content of bioactive compounds (cardiac glycoside, saponin, cardenolides, alkaloids, flavonoids, and terpenoids) varied amongst the extracts. The presence of cardiac glycosides, saponins, cardenolides, alkaloids, flavonoids, and terpenoids were observed in ethyl-acetate, n-butanol, and water extracts, thus resulting in a higher extraction yield. Alkaloids, saponins, and carbohydrates were absent in the chloroform extract, but the remaining compounds are present; these findings suggest that ethyl-acetate, n-butanol, and water are the best solvents for extracting bioactive compounds from *L. hastata*. This result is consistent with the extraction yield of Limophila aromatic [35]. Extraction solvents affect the extraction yield and the content of bioactive compounds and hence affect the biological activity of the extract (though not always) [36]. In this study, the extracts obtained from different solvents were studied for their antioxidant activity using DPPH scavenging activity assays. Although having the lowest extraction yield, chloroform produces the highest activity in terms of IC_{50} values of DPPH scavenging activity; this could be because of chloroform, this suggests that the DPPH scavenging activity in *L hastata* is not affected by the extraction yield. Perez-Bonilla *et al* [37] reported higher DPPH scavenging activity by solvent with lower extraction yield. In another study, negative Pearson's linear correlations between extraction yield and total phenolics and between extraction yield and total flavonoids were reported, suggesting that higher yield may not correspond with the amount of phenols and flavonoids, which are strong antioxidant compounds. Also, Babbar *et al*. [38] reported higher free radical scavenging activity for fruit extract with a lower content of phenolics compared to other fruit extracts. It may also suggest that the other non-phenolic antioxidant compounds may be present in *L. hastata.* In general, significant antioxidant activity was exhibited by all the solvents, but chloroform performed better than ethyl acetate and *n*-butanol in comparison to Vit. C. this could be due to the presence of potent phytochemicals, as reported in a previous study by Yesufu *et al*. [39]. The mechanism of action could be associated with the high Vit. C-like property of the plant material judging from the performance of the extract to ascorbic acid, which is a known antioxidant. It could also be attributed to the terpene moiety as the major pharmacological activity of plant materials has been attributed to the presence of phytochemicals such as flavonoids, terpenoids, and their glycosides. The result of this study indicated that chloroform, ethyl acetate, and *n*-butanol are the best solvents in the extraction of antioxidant components in *L hastata* The therapeutic attributes of traditional botanical species are mostly ascribed to the existence of flavonoids. Still, they may also be influenced by other organic and inorganic compounds such as terpenoids, coumarins, phenolic acids, and antioxidant micronutrients like Cu, Mn, and Zn [40].

The current understanding suggests that free radicals or oxidative damage play a central role in the development of several diseases in both animals and humans. In the context of diabetes, it has been

hypothesized that there is a concurrent elevation. A reduction in levels of antioxidants often accompanies the occurrence of oxidative stress. This imbalance can lead to the initiation of lipid peroxidation by oxygen free radicals. Consequently, this process can stimulate protein glycation, enzyme inactivation, and structural and functional changes in collagen basement and other membranes. Ultimately, these mechanisms are believed to contribute to the progression of chronic problems linked to diabetes [41,42]. In the context of carcinogenesis, the initiation of the multistage process is attributed to reactive oxygen species. These species induce DNA damage and the subsequent accumulation of genetic events in a limited number of cell lines. This accumulation results in the gradual development of dysplastic cellular appearance, deregulated cell growth, and, ultimately, the formation of carcinoma [43]. Therefore, the utilization of free-radical scavengers (known as antioxidants) in therapy has promise in the prevention, postponement, or improvement of numerous illness [44]. Spices and herbs are acknowledged for their capacity to function as a naturally occurring reservoir of antioxidants, which can effectively shield against oxidative stress. Consequently, they assume a significant role in the prevention of diseases that originate from and are influenced by reactive oxygen species, contributing to chemoprevention.

CONCLUSION

The result from this study demonstrated the antioxidant activity of *L. hastata* root extract and its partition portions by DPPH scavenging ability. Based on the results obtained in this study, the extract has significant *In-vitro* antioxidant activity against the DPPH oxidant system, with the highest activity in chloroform and ethylacetate partition portions. Therefore, the extracts may be a good source of antioxidant-related functional food for the prevention/amelioration of diseases and industrial purposes.

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