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PHYTOCHEMICAL ANALYSIS AND DPPH FREE RADICAL SCAVENGING ACTIVITY OF METHANOL LEAF EXTRACT OF *LAGGERA PTERODONTA* SCH. BIP. EX OLIV.

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ABSTRACT

Laggera pterodonta is a typical Nigerian medicinal plant. There are roughly 20 species in the genus *Laggera*, which is a member of the Asteraceae family. The aim of this study was to assess the phytochemical compounds present in the Methanol leaf extract of *Laggera pterodonta* and to evaluate its antioxidant activity. The total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and total saponin content (TSC) were evaluated by employing the Folin-Ciocalteu's reagent, aluminum chloride (AlCl3) technique, Folin-Ciocalteu, and Makkar methods; respectively. Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Phytochemical contents estimated were 854.5 ± 0.03, 1,277 ± 0.01, 5.794 ± 0.01 and 4.586 ± 0.01 for flavonoids, phenolics, tannins and saponins; respectively. Antioxidant activity study revealed that *Laggera pterodonta* leaf extract had IC50 of 3.06 µg/ml while ascorbic acid used as the standard had an IC50 of 0.58 µg/ml. Phytochemical study of the extract of *Laggera pterodonta* leaf shows the presence of medicinally important bioactive compounds and this justifies the use of the plant in traditional medicine for treatment of various diseases. The radical scavenging activity of both *Laggera pterodonta* and ascorbic acid at different concentrations was observed. Scavenging of DPPH was found to rise with increasing concentration of the extract, the highest scavenging activity was observed at the concentration of 100 µg with value 59.71% while that of ascorbic acid was 86.38%. The outcome of this study has shown that *L. pterodonta* extract has strong antioxidant property and is rich in flavonoid and phenolic compounds.

Keywords: Antioxidant, DPPH free radical, *Laggera pterodonta*, phytochemical, reactive oxygen species.

INTRODUCTION

Laggera pterodonta (DC) Sch. Bip. (Compositae) is a robust herb that grows up to 1.7 m in height. It is viscid and strongly aromatic. It has white flowers; and its basal leaves are bigger than the upper ones. It is a weed found in open waste land and partially shaded galleried forest (Burkil, 2000). It is a native of Africa and Asia. In Africa, it can be found in Senegal, Sierra Leone (Burkil, 2000), Nigeria and west Cameroon. In Nigeria, it's called *tabaagbe* by the Yorubas and *taba-taba* by the Hausas. Studies on chemically induced hepatic injury in neonatal rat hepatocytes showed that the phenolics from *Laggera pterodonta* have hepatoprotective and antioxidant effects (Wu *et al.*, 2007).

Plants such as *Laggera pterodonta* have limitless abilities to synthesize phytochemicals that have enormous therapeutic potentials. Large number of

medicinal plants have been investigated for their antioxidant properties (Tariq; *et al.,* 2021). It has also been reported to be very effective in preventing the destructive processes caused by oxidative stress (Zengin *et al*., 2011). The human body is naturally antioxidative, and many biological processes, including those that prevent cancer, aging, and mutagenesis, stem from this characteristic (Gocer and Gulcin, 2011; Gulcin, 2012). Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Nunes *et al*., 2012). Because naturally occurring antioxidants are complex in their multiplicity and quantity of action and provide vast potential for rectifying imbalances, interest in their usage in food, cosmetic, and pharmaceutical products has surged (Wannes *et al*., 2010). It is generally recognized that free radical reactions have a role in the pathophysiology of many acute and chronic diseases in humans, including diabetes, atherosclerosis, aging, immunosuppression, and neurodegeneration. (Saeed *et. al*., 2012).

Reactive oxygen species (ROS) and the body's natural antioxidant capacity were out of balance, necessitating the need of dietary and/or medicinal supplements, especially during the illness attack (Filomeni; *et al*., 2015). Research on fruits, vegetables, and herbal plants has revealed the existence of antioxidants such as proanthocyanidins, flavonoids, phenolics, and tannins (Patro; *et al*., 2016). The antioxidant contents of medicinal plants may contribute to the protection they offer from various diseases. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin, 2012). Liver diseases remain a serious health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury (Saeed, *et. al*., 2012). Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Saeed *et. al*., 2012). The use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages. Therefore, this study is embarked upon in order to study the phytochemical compounds present in methanol leaf extract of *Laggera pterodonta* and to evaluate its antioxidant activity.

MATERIALS AND METHODS

Plant Collection and Identification

The fresh leaves of *Laggera pterodonta* were collected from Kufena Zaria in November 2021 and authenticated at the Herbarium section of the Department of Biology, Kaduna State University, Kaduna State. A voucher specimen number was assigned as KASU/BSH/642 and the voucher sample of the leaves was deposited on the plant for future references.

Preparation of the Leaf Extract

The leaves were separated from the rest of the plant material and allowed to air dry at room temperature. It was crushed into a coarse powder after drying using a pestle and mortar. Two hundred gram (200g) of the sample was weighed out for the extraction, and 200 ml of aqueous methanol (80:20 v/v) was then added. It was allowed to macerate for 24 hours at room temperature in a conical flask. Following that, grade 1 Whatman paper was used to filter the sample. According to Kumar *et al* (2010), the methanol was removed by evaporation in an evaporating dish over a water bath at 50 degrees Celsius. Extract from the process was stored for subsequent analyses. The percentage yield of the extract obtained from the solvent of extraction was calculated using the formula:

$$\text{Yield of Extract (\%)} = \frac{\text{weight of Crude Methanol Extract}}{\text{Weight of Plant}} x\ 100\%$$

Estimation of Total Phenolic Content

The spectrophotometer method was used to determine the concentration of phenolic compound in the extract. The Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 ml of plant extract and 9 ml of distilled water. One (1) ml of Folin-Ciocalteu phenol reagent was added to the mixture and well shaken. After 5 minutes, 10 ml of 7% Na_2CO_3 solution was used to neutralize the mixture. A set of Gallic acid standard solutions (20, 40, 40, 60, 80, and 100 µg/ml) were prepared. These were incubated for 90 min at 30°C, the absorbance was analyzed for test and standard solutions with a reagent blank at 550 nm using a UV-visible spectrophotometer. The content of the total phenolic compound was denoted as mg of GAE/gm of extract (Alhakmani *et al*., 2013).

Estimation of Total Flavonoid Content

Colorimetric assay was used to determine the total content of flavonoid using aluminium chloride for the reaction. The plant extract of 1 m (1mg/ml) and distilled water of 4 ml was taken in a 10 ml of flask. After five minutes, 0.3 ml of 10% aluminum chloride was added to the flask along with 0.30 ml of 5% sodium nitrite. After five minutes, 10 ml of distilled water was used to treat and dilute 2 ml of 1M NaOH. A set of standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance was measured for test and standard solutions using reagent blank at 510 nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/g of extract (Alhakmani *et al*., 2013).

Estimation of Total Tannin Content

Folin-Ciocalteu method was used to quantify the tannin total content. About 20ul of plant extract was added in 10 ml of volumetric flask containing the distilled water of 1.5 ml and Folin-Ciocalteu phenol reagent of 0.1 ml (100 µl), 0.2 ml (200 µl) of 35% Na_2CO_3 solution and diluted to 2ml using distilled water. The reagent mixture was well shaken and kept at 30°C temperature for 30 min. A set of Gallic acid solutions (20, 40, 60, 80 and 100 µg/ml) were prepared. Absorbance of standard and test solutions was analyzed with blank at 725nm wavelength using UV-Visible spectrophotometer. The total content of tannin was expressed as mg of GAE/g of extract (Alhakmani *et al*., 2013).

Estimation of Total Saponin Content

This was done using the method described by Makkar. To 250 µL of distilled water, about 40 µL of plant extract was added. This was combined with about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol). Next, 2.5 ml of 72% sulfuric acid were added, it was mixed thoroughly and heated on a water bath at 60^0c for 10 min. After which the mixture was cooled on ice and the absorbance was measured at 544 nm against reagent blank. Diosgenin was used as a standard material and compared the assay with Diosgenin equivalents.

Free Radical Scavenging Activity (DPPH Assay)

The free radical scavenging activity of the leaves of *Laggera pterodonta* was determined using (2,2-diphenyl-1-picrylhydrazyl) (Villano *et al*., 2007). The 0.1 mM DPPH solution was prepared in 95% methanol. The stock solution of the extracts was also prepared in 95% methanol. After that from the stock solution, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were taken in five test tubes and diluted with the same solvent

to get a final concentration of 20 µl/mg, 40 µl/mg, 60 µl/mg, 80 µl/mg, and 100 µl/mg respectively. The sample extracts were taken in 1 ml of each test tube and added 2 ml fresh DPPH solution in each of these test tubes. As a control, 95% methanol was used. The mixture was shaken vigorously and was left to stand in dark for 30 mins. The absorbance of the resulting solutions was measured using UV spectrophotometer at 520 nm. The percentage scavenging activity of the extract on DPPH radical was calculated as follows.

$$\text{Scavenging activity (\%)} = (Ab - Ae\backslash Ae) \times 100$$

Where; Ab = absorbance of blank sample

Ae = absorbance of the solution containing the extract

The IC_{50} (concentrations of the extracts that inhibit 50% of the free radicals) were calculated from the graph of scavenging activity plotted against sample concentrations using Microsoft excel software (Viturro *et al*., 1999).

Statistical Analysis

Analysis of the result was carried out using SPSS version 21.0. Student-test was used for the statistical analysis, at 95% confidence level. A value of $p \leq 0.05$ was considered statistically significant. Results were presented in Tables and line graphs.

RESULTS

Determination of Phytochemical Contents

The following results were obtained from the determination of phytochemical contents: TPC of 1,277 mg GAE/g, TFC of the extract was 854.5 mg QE /g, TTC 5.794 mg GAE /g and TSC 4.586 mg DE/g (Table I). Result showed that extract is highly rich in phytochemical constituents evaluated having high quantity of phenolic compounds, which is followed by flavonoid.

Table I: Phytochemical contents of aqueous methanol leaf extract of *Laggera pterodonta*

S/N	Characteristics	Values
1	Total phenolic content	1,277 mg GAE /g
2	Total flavonoid content	854.5 mg QE /g
3	Total tannin content	5.794 mg GAE /g
4	Total saponin content	4.586 mg DE/g

DPPH radical scavenging activity of aqueous methanol leaf extract of *Laggera pterodonta*

Ascorbic acid was chosen as the reference antioxidant for this test. Below are the comparative % scavenging activity of the plant extract and the standard (Figure I), the IC_{50} value for the plant extract (Table II), Calculation of IC_{50} value of leaf extract by regression analysis (Figure II), IC_{50} value of ascorbic acid by regression analysis (Table III), and calculation of IC_{50} value of ascorbic acid by regression analysis (Figure III).

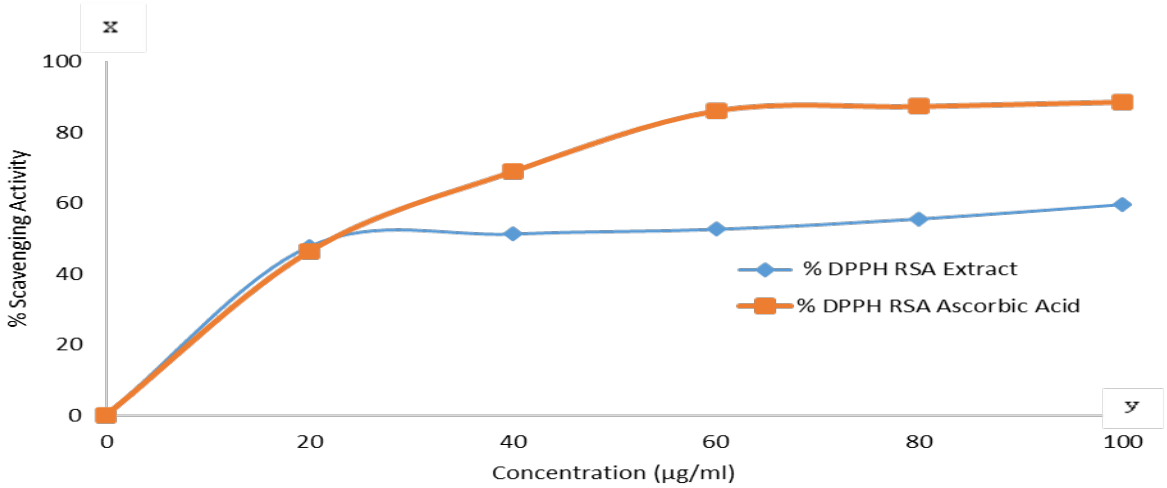

Figure 1: Comparative percentage methanol leaf extract of *Laggera pterodonta*

Table II: IC_{50} values for the methanol leaf extract of *Laggera pterodonta*

Concentration (µg/ml)	log concentration	Absorbance	% DPPH RSA	IC_{50} (µg/ml)
20	1.861	0.844	47.81	
40	2.292	0.776	51.36	
60	2.544	0.754	52.67	3.06
80	2.723	0.707	55.62	
100	2.861	0.652	59.71	

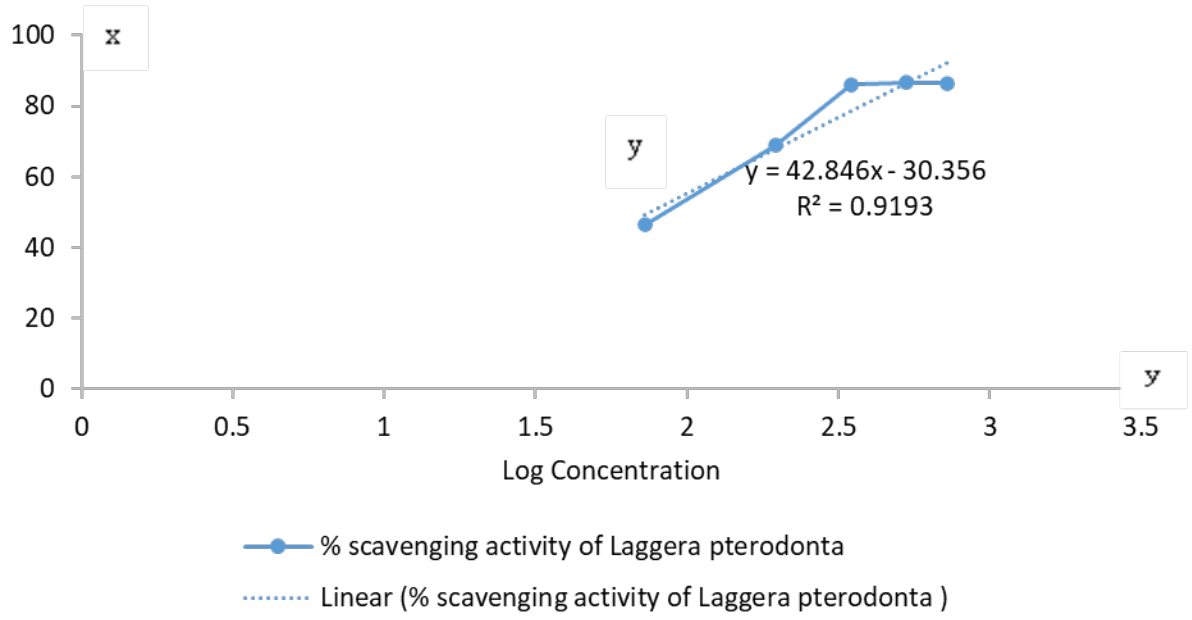

Figure 2: Calculation of IC_{50} value of methanol leaf extract of *Laggera pterodonta* by regression analysis

Table III: IC_{50} value of ascorbic acid by regression analysis

Concentration (µg/ml)	log concentration	Absorbance	% DPPH RSA	IC_{50} (µg/ml)
20	1.861	0.858	46.35	
40	2.292	0.495	68.93	
60	2.544	0.221	86.12	0.58
80	2.723	0.217	86.63	
100	2.861	0.213	86.38	

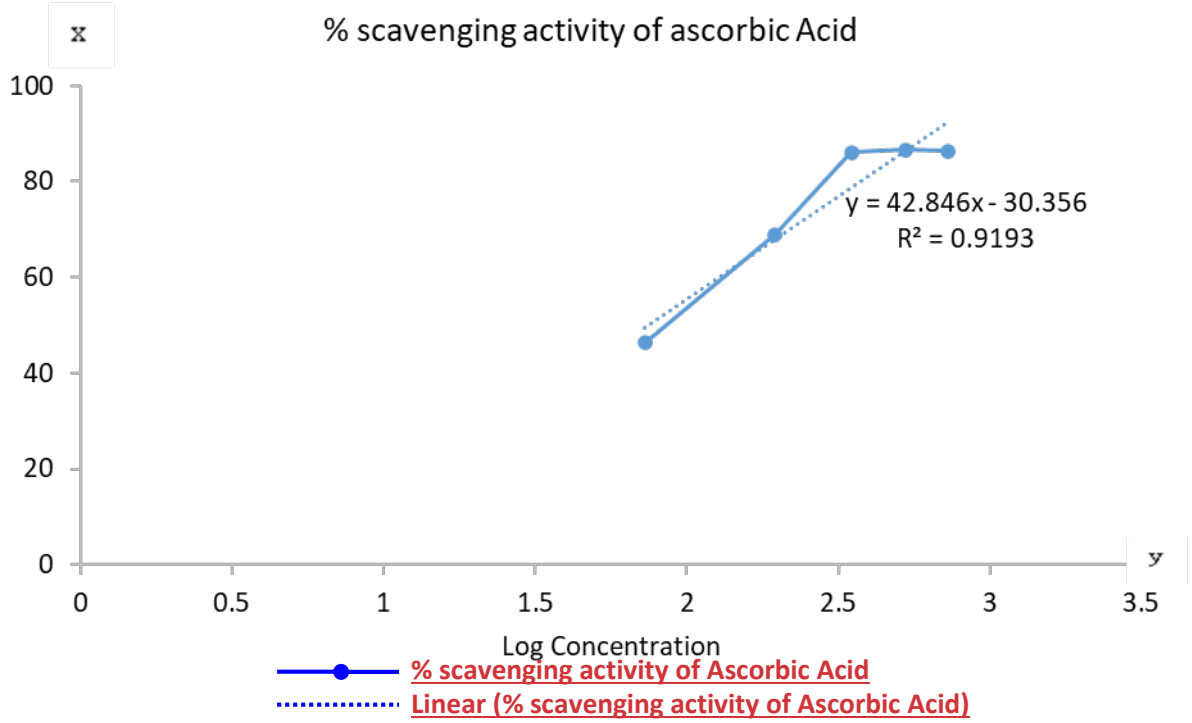

Figure 3: Calculation of IC_{50} value of ascorbic acid by regression analysis

DISCUSSION

It has been noted that plant secondary metabolites like flavonoids and phenolics are effective at scavenging free radicals (Crépin *et. al*; 2014). The results of this research are consistent with a prior study by Crépin *et al.* (2014), which shown that members of the genus, including *Laggera aurita* L., are rich in flavonoids and phenolic compounds, which accounts for the plant's good anti-oxidant activity. Furthermore, the results of Indradi *et al*. (2017) demonstrated that the Asteraceae family is a rich source of phenolic and flavonoid chemicals, which makes it a highly promising exogenous antioxidant source.

This study's phytochemical quantification result agreed with the findings of Wu *et al*. (2007) in that the percentage of the phenolic compound was higher than that of the other phytoconstituents. Contrary to our findings, the leaf extract in a related study by

Ikiyenge *et al*. (2012) did not test positive for saponins. This may be due to environmental factors, which also play a vital role in the presence, absence, and composition of phytochemical constituents in plants. The phytochemical makeup of flora, especially aromatic and medicinal plants, is being altered by climate change. Plant phytochemical composition is influenced by environmental factors such as soil type, sun exposure, grazing stress, location, climate, and seasonal variations (*Hussain et al.,* 2009; Aryal, 2015). One of the most practical ways to assess the antioxidant capacity of plants is the DPPH evaluation, among many other assays. The DPPH solution was decreased in the presence of antioxidant compounds with hydrogen-donating groups, such as flavonoids and phenols, as a result of the creation of non-radical (Mensour *et al.*, 2011). The radical scavenging activity of *L. pterodonta* observed in this study may be due to the presence of polyphenols, flavonoids, and phenolic compounds. It was earlier reported that most of the antioxidant activity of plants may be due to the presence of the phenols (Mansouri *et al*., 2005). It was discovered that when extract concentration increased, so did DPPH radical scavenging. It has been established that phenolic compounds and the like (phenolic terpenes, tannins, flavonoids, and polyphenols) are primarily responsible for the antioxidant action of plant products due to their ability to scavenge free radicals (Rahman and Moon, 2007).

The fundamental source of phenolic compounds' antioxidant activity is their redox characteristics, which can be useful for adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or dissolving peroxides (Hasan *et al*., 2008). Phenolic chemicals also have antioxidant, anti-inflammatory, anticancer, and antibacterial properties (Balakrishnan *et al*., 2020). It is well-established that the phenolic compounds found in plants play a vital role in offering defense against the free radicals produced in the biological system (Kumar and Goel, 2019). Free radicals are thought to be the root cause of a number of major diseases in human beings, including lung, breast, and oral cancers (Phaniendra; *et al*., 2015). In this study, a significant amount of flavonoid content was found in *Laggera pterodonta.*

Flavonoids contain antioxidant characteristics that inhibit the formation and progression of tumors and lower the risk of coronary heart disease. Haslam (1998) draws attention to the growing interest in the possibility of treating illnesses with no more complicated measures than increasing dietary intake of minerals with antioxidant qualities, such as vitamin E, vitamin C, -carotene, and carotenoids, as well as plant phenolics like tannins and flavonoids.

CONCLUSION

The outcome of this study has shown that *L. pterodonta* extract has strong antioxidant property and is rich in flavonoid and phenolic compounds. Consequently, the plant may be a source of bioactive compounds with the potential to mitigate oxidative stress.

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