Chemical investigation and antioxidant activity of fractions of *Lannea humilis* (Oliv.) Engl.

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Abstract: The aim of this experiment was to establish the phytochemical constitution and antioxidant activity of the fractions of *Lannea humilis.* Chemical investigation and antioxidant activity of the fractions were carried out using standard methods. Steroids and terpenes were available in the hexane, ethyl acetate, and methanol fractions, while tannins, flavonoids and alkaloids were available in the ethyl acetate and methanol extracts. Carbohydrate and saponins were available in the methanol fraction. The antioxidant activity of this plant extracts demonstrated a dose-dependent increment. The ethyl acetate extract displayed most noteworthy antioxidant activity of 98% at 240 $\mu g.mL^{-1}$, followed by the hexane extract which had a percentage antioxidant activity of 92 % at 240 $\mu g.mL^{-1}$. The methanol extract demonstrated percentage antioxidant activity of 71 % at 240 $\mu g.mL^{-1}$. This result shows that this plant can be used as a good antioxidant. These observations demonstrated that this plant has antioxidant activity and consequently can be used as an antioxidant agent.

Keywords: Antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl; phenolic compounds; antioxidant activity; *Lannea humilis*.

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INTRODUCTION

Antioxidants have various applications because of their different parts in reducing destructive impacts of oxidative stress (1). Antioxidant agents respond by free radical or molecular oxygen extinguishing, being able to either postpone or restrain oxidation which happens under the influence of molecular oxygen species. Antioxidants are in charge of the shielding mechanism of the living being against the pathologies connected with the assault of free radicals, in this manner the consumption of plant related antioxidants is responsible for the avoidance of degenerative ailments brought about by oxidative anxiety, for example, Cancer, Parkinson, Alzheimer, or Atherosclerosis [2]. Free radicals are broadly accepted to facilitate the development of a few ailments by bringing about oxidative anxiety and eventually oxidative harm which are reasons for some pathological diseases (3-6). Researchers recommend that if oxidative harm could be observed to be in charge of the continually increasing occurrence of different neurotic conditions, then quest for normal cancer prevention agents that could prevent the oxidation of free radicals would be scientifically valuable (7, 8). The phytochemicals in plants, green tea for example, have antioxidants properties used to enhance and give security against numerous ailments connected with reactive oxygen species (ROS, for instance, tumor and neurodegenerative diseases) (9, 10). It belongs to the family *Anacardiaceae*, it a deciduous bush growing up to 3 meters tall, at times turning into a tree with a level or spreading crown. A decoction of the stem bark of this plant is utilized in the treatment of sickness, hack, bodily torments intense looseness of the bowels, cholera, and asthma (11).

MATERIALS AND METHODS

Collection and identification of plant

The fresh stem bark of *L. humilis* was collected from Otukpo, Benue state, Nigeria in January 2015. The plant was identified with a voucher specimen number 3231 by Mal. S. Namadi at the Herbarium section, Department of Botany, Faculty of Life Science, Ahmadu Bello University, Samaru, Kaduna State, Nigeria.

Extraction of plant materials

The stem bark of *L. humilis* were air-dried at room temperature (27 °C) for 2 weeks, after which it was grinded to a uniform powder. The crude methanol extract was prepared by soaking 100 g of the dry powdered plant materials in 1 L of methanol at ambient temperature for 48 h. The extract was filtered after 48 hrs and was concentrated using a rotary evaporator with the water bath set at 40 °C. A portion of this extract was reconstituted in water to yield a water-soluble fraction and water-insoluble fraction. The two fractions were subsequently partitioned successively and exhaustively using hexane and ethyl acetate, which were then concentrated using a rotary evaporator.

Phytochemical screening

The extract and the fractions were qualitatively examined for phytochemicals following standard procedures (12, 13).

Determination of antioxidant activity

The radical scavenging activities of the extract against 2,2-diphenyl-1-picrylhydrazyl (DDPH) radical were determined using UV spectrophotometer at 515 nm. Radical scavenging activity was measured by a slightly modified method previously described (14). The following concentrations of the extracts were prepared, 15, 30, 60, 120, 240 $\mu g.mL^{-1}$ in methanol.

Vitamin C was used as the antioxidant standard at concentrations of 15, 30, 60, 120, 240 $\mu g.mL^{-1}$. 1 mL of the extract was placed in a test tube, and 1 mL of 0.3 mM methanolic solution of DPPH added. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\%radical\ scavenging\ activity = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

RESULTS AND DISCUSSION

Table 1: Phytochemical Screening of the Extracts of the Stem Bark of *L. humilis*

Metabolites	LHH	LHE	EHM
Terpenes	+	+	+
Sterols	+	+	+
Carbohydrates	_	_	+
Glycosides	-	_	+
Tannins	-	+	+
Flavonoids	-	+	+
Anthraquinones	_	_	+
Alkaloids	_	+	+

Key: + =present, - = absent, LHH: hexane fraction, LHE: ethyl acetate fraction, LHM: methanol fraction.

The result of the phytochemical screening of the fractions of the stem bark of *L. humilis* is reported in Table 1. The phytochemical examination of the hexane, ethyl acetate and methanol extracts of the stem bark extract of *L. humilis* has uncovered the availability of some bioactive principles. Steroids and terpenes were available in the hexane, ethyl acetate, and methanol fractions, while tannins, flavonoids, and alkaloids were available in the ethyl acetate and methanol fractions. Carbohydrate and saponins were available in the methanol

fraction. Anthraquinones were absent in all the fractions. The availability of these metabolites has proven earlier report that various plants in same family with *L. humilis* likewise have a large portion of the phytochemicals as observed in this plant (11). The presence of flavonoids, carbohydrate and cardiac glycosides in the ethyl acetate and methanol extract is not unexpected as the majority of these constituents are basically polar in nature. Flavonoids which are normal cancer prevention agent are acquired chiefly from plants, and are utilized for the treatment of degenerative infections (15). The inconceivable number of these chemical constituents in the stembark of *L. humilis*, some of which are be bioactive legitimizes the ethnomedicinal uses of this plant in the treatment of diseases.

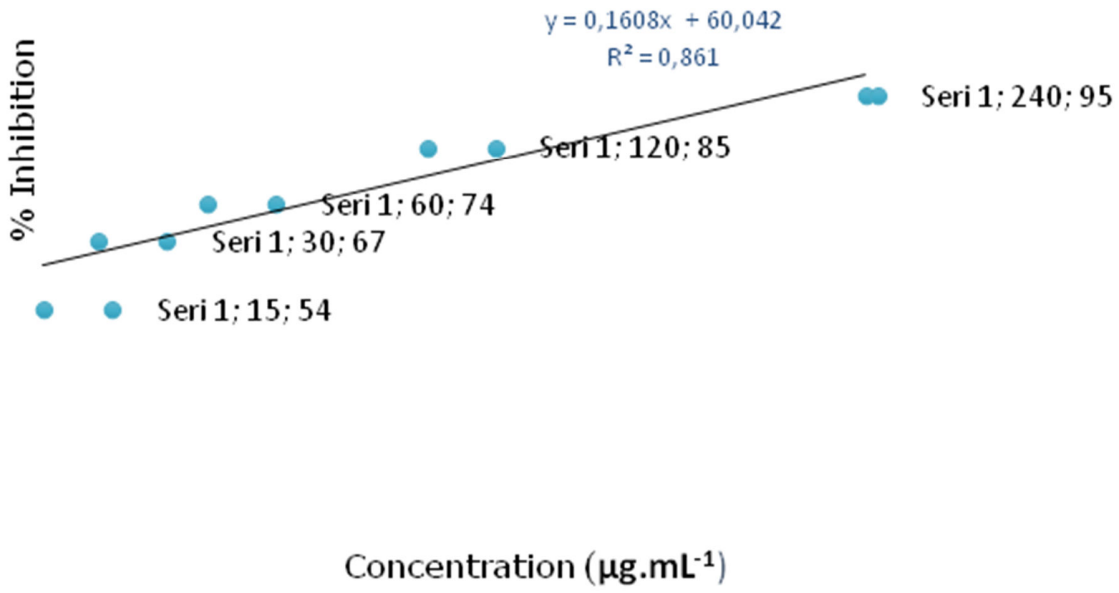

Figure 1: DDPH Scavenging Activity of the Hexane Extract of *Lannea humilis.*

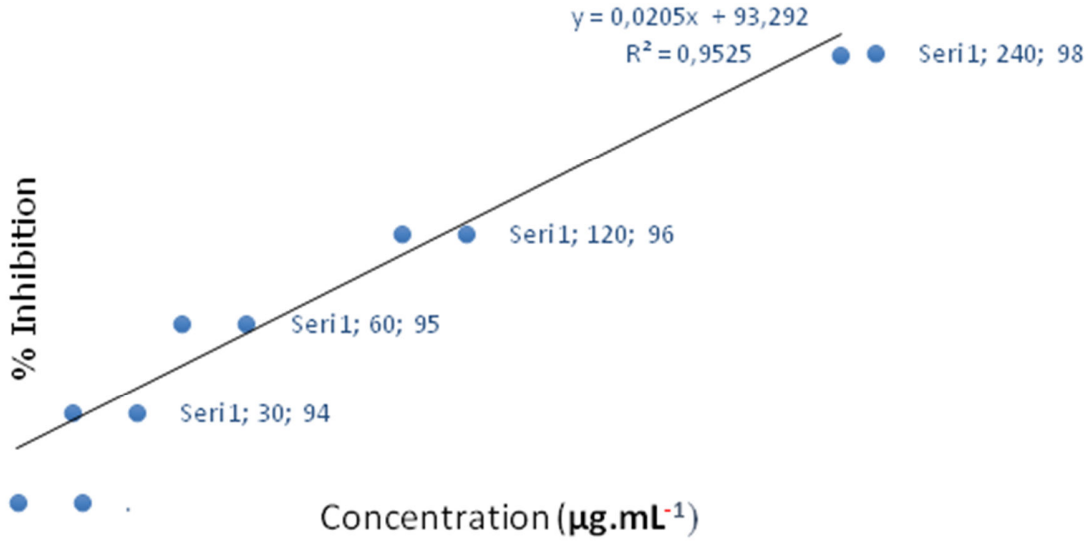

Figure 2: DDPH Scavenging Activity of the Ethyl acetate Extract of *Lannea humilis.*

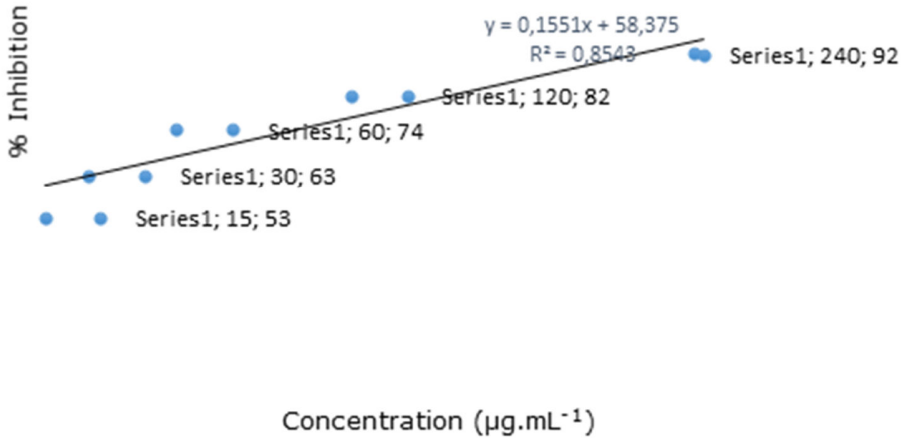

Figure 3: DDPH Scavenging Activity of the Methanol Extract of *Lannea humilis.*

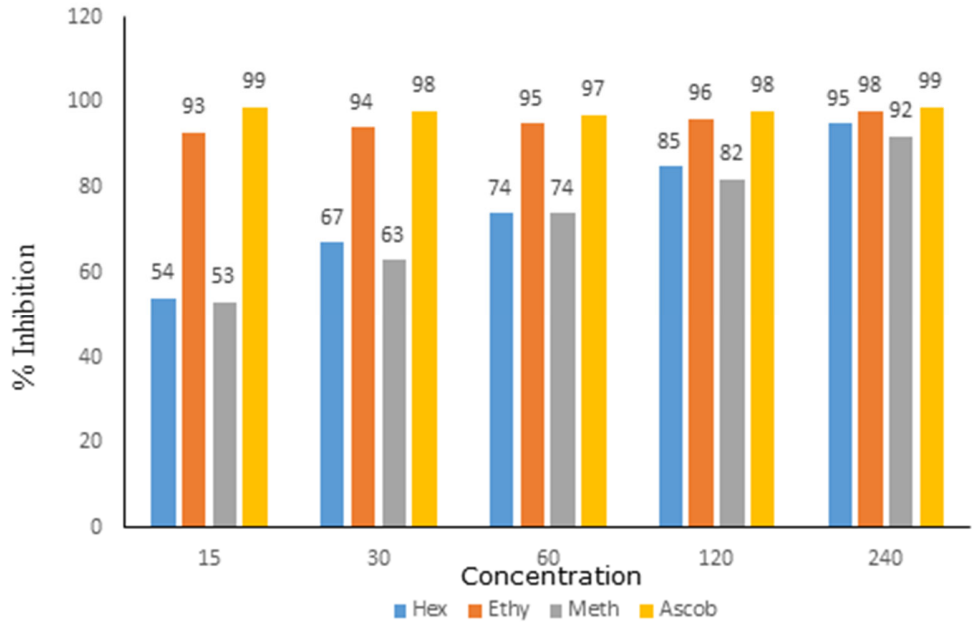

Figure 4: Inhibition of DPPH by the Hexane, Ethyl acetate and Methanol Extracts of *Lannea humilis* in Comparison with Ascorbic acid (µg.mL^{-1}).

The antioxidant capacity of the hexane, ethyl acetate and methanol fraction of *L. humilis* to repress and extinguish free radicals and responsive oxygen species was analyzed in this study. DPPH radical has been utilized widely as a free radical to test the reductive capacity of fractions or chemicals as free radical scavengers or hydrogen contributors and to assess the antioxidant activity of plant fractions (16, 17). Antioxidants respond to DPPH• by giving electron or hydrogen particle, in this way reducing it to 1,1-diphenyl-2-hydrazine (DPPH-H) or a substitute practically equivalent to hydrazine. The profound violet shade of DPPH at most extreme absorption of 515 nm is changed to light yellow, colorless or bleached product, resulting in decrease in absorption (18, 19). The hexane extract of *L. humilis* displayed noteworthy antioxidant activity of 95% at 240 µg.mL^{-1} (Figure. 1) and compared well with the standard ascorbic acid. In like manner, the test technique for DPPH scavenging action connects amazingly with the adjustments in the different concentration of the hexane utilized,

with relationship coefficients (r^2) of 0.861. This infers that the DPPH scavenging activity of the extracts of this plant is concentration dependent as highest percentage inhibitions were observed at a corresponding highest concentration for all the extracts. The percentage inhibition created by the ethyl acetate extract was 98 % at a concentration of 240 $\mu g.mL^{-1}$, while that of the methanol fraction of *L. humilis* was 92 % at the same concentration as shown in Figure 2 and 3. The three fractions of this plant showed comparable antioxidant activity with the positive standard antioxidant agent, ascorbic acid (Figure 4). These results show that the fractions of this plant can scavenge free radicals. The scavenging activity of the plant fractions on DPPH has been shown to be related to the phenolic concentration of the fractions (20, 21, 22), which is accepted to add to their electron exchange/hydrogen giving capacity. It could hence be inferred that flavonoids contents of the fractions of this plant as revealed in the phytochemical screening results is responsible for stabilizing radicals or scavenge their activities.

In conclusion, this result suggests that the phenolic compounds might be major contributors to the antioxidative activities of the stem bark *L. humilis*. Further efforts are underway to isolate and identify the active phenolic compounds from the plant.

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