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Evaluation of In Vitro Antioxidant Activity of Stem Bark Extract of *Lophira Lanceolata* (Ochanaceae) Van Tiegh Ex Keay

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Research Article

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Abstract

Plants have been the mainstay of many traditional cultures for their low cost and wide accessibility for preventive and therapeutic uses. *Lophira lanceolata* van tiegh ex keay. is a plant belonging to the family Ochnaceae. It is a medicinal plant commonly used in west and central African regions. In Nigeria it is found in the North western region. This plant is well known and has been employed in the treatment of dermatosis, toothache, arthritis, headache, Cardiovascular diseases and many others. In this study, the in vitro antioxidant activity of the methanolic stem bark extract of *L. Lanceolata* methanolic leaf extract was investigated by determining the total phenolic content and DPPH radical scavenging activity using varied concentrations (31.25-500µg/ml) of the plant extract at absorbance of 515nm using ELISA plate reader. Ascorbic acid was used as the standard. From the results of the study, preliminary phytochemical screening indicated the presence of major Phyto-compounds like Flavonoids, alkaloids, steroids, terpenoids and saponins. The highest absorbance value that presents with the highest concentration revealed that the plant possesses radical scavenging activities. The present study indicates that, *Lophira lanceolata* has significant antioxidant activity and thus, support the claimed ethnomedical uses of the plant in the management of conditions associated with oxidative stress.

Keywords: *Lophira Lanceolata*; Phyto Compounds; Antioxidant; Radical Scavenging

Introduction

Plants and their products have been used since time immemorial as principal ingredients of various traditional medicines and have played a key role in global health improvement. The use of herbal medicines in Africa represents a long history of human interactions with the environment therefore, plants have also been the mainstay of many traditional cultures for their low cost and wide accessibility in addition to their preventive and therapeutic value. Traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities [1-3].

In the last 2 decades, antioxidants have attracted considerable attention in cancer prophylaxis and therapy, and longevity and in prevention and treatment Oxidative stress is pathological state that arises when free radicals (collectively known as reactive oxygen species or ROS) chemically interact with and damage biological molecules [4]. Medicinal plants have strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals [5] of many ailments. They have also received attention in formulation of supplements and nutraceuticals. Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes [6]. Much evidence shows that consumption of natural source substances confers chemopreventive and cytoprotectant activities [7]. There are numerous antioxidants in dietary plants: carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids,

proanthocyanins, stilbenes, coumarins, lignans, and lignins [8]. Other antioxidants include; vitamin A, E and C, glutathione transferase, catalase, superoxide dismutase, omega -3-fatty acid and some other polyunsaturated fatty acids. Free radicals have the capability to damage cellular components such as DNA and the cell membranes when they react. To reduce their energy load, free radicals react with certain chemicals in the body, and in the process, interfere with the cells' ability to function normally [9]. Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases [10]. The plant *Lophira lanceolata* is a multipurpose tree, valued especially for its oil, timber and many medicinal applications. The oil and other products of the tree are traded on a local scale. In Mali pounded roots, mixed with flour are used to treat constipation, while its concoction is used to cure chronic wounds [11]. Other medicinal uses have also been reported while other research findings are still ongoing to explore the medicinal value of this important plant. The present study aimed to explore the antioxidant property of this plant through In vitro assessment.

Materials and Method

Materials, Chemicals and reagents

Beakers, Conical flask, Separating funnel, Aluminum foil, Cotton wool, Retort stand , Plant powder, Test tube, Test tube holder, Glass rod, Filter papers, Measuring cylinder , Glass funnel, u.v. spectrophotometer, Measuring cylinder, Weighing balance, Capillary tube, Mortar and pestle, Drying oven, Incubator, Solvent and reagent used, Methanol, Distilled water, DPPH, Folin – ciocalteu, Alluminium chloride, Sodium nitrate (5%), Hydrochloric acid, Sodium bicarbonate, 1% ferric chloride, 1% potassium ferric cyanide.

Collection and Identification of Plant Sample.

The leaves and stem bark of *Lophira lanceolata* was collected from Niger state of Nigeria in June 2016. The plant was identified and authenticated by Dr. Mshelia of the department of Pharmacognosy and Ethnopharmacy faculty of pharmaceutical science Usmanu Danfodiyo University Sokoto. A voucher specimen with number was preserved at the herbarium for future reference.

Preparation of Sample

The stem bark of the plant was washed, air dried and powdered using pestle and mortar. It was labelled and kept in air tight container prior to extraction.

The plant sample was weighed and macerated with 1000ml of 80% methanol for 72 hours and later 500 ml was added each for 72 and 48 hours (total of 8 days). The extract obtained was dried using a vacuum with rotary evaporator at 45oCanda brownish black extract was obtained. The extract was stored in an oven until when required for the experiment. Percentage yield was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Mass of extract}}{\text{Initial mass of powder drug}} \times 100$$

Qualitative Phytochemical Analysis

Phytochemical analysis of methanolic extract of *Lophira lanceolata* van Tieag.ex keay was carried out using standard method of analysis Silva, Trease and Evans [12] in Pharmacognosy lab, faculty of pharmaceutical science Usmanu Danfodiyo University Sokoto. Using the following procedure.

Test for Saponins

Frothing Test: Small amount of extract was put in a test tube and about 10 mL of water was added to it. It was thoroughly shaken for 30 seconds. Honeycomb froth was formed and persisted in the test tube for 10-15 minutes.

Test for Tannins

Lead Sub acetate Test: a little amount of extract of *Lophira lanceolata* was put in a test tubes and dissolved in chloroform. Few drops of lead sub acetate were added. The formation of heavy precipitate indicates the presences of tannins.

Ferric Chloride Test: a little amount of extract was put in a test tube. A small amount of water was added. Few drops of ferric chloride were added. Presence of blue-black colour indicates the presence of tannins.

Tests for Flavonoids

Sodium Hydroxide Test: a little amount of sodium hydroxide was added to the extract of *Lophira lanceolata* formation of yellow coloration which turns colourless on addition of dilute hydrochloric acid indicates the presences of flavonoids.

Shinoda's Test: methanol was added to a small amount of extract. A few drops of dilute hydrochloric acid were added. A few metallic magnesium turnings were put in each sample. The presence of effervescence indicates the presence of flavonoids in the sample.

Test for Anthraquinones

Bontrager's Test: a small amount of extract was dissolved in chloroform and an equal volume of 10% ammonium

hydroxide was added to the extract and shaken. Appearance of a bright pink colour in the upper layer indicates the presence of Anthraquinones.

Test for Cardiac Glycosides

Keller-Killiani's Test: a small amount of extract was dissolved in glacial acetic acid. Few drops of ferric chloride were added, shaken and few drops of concentrated sulphuric acid was added by the wall of the test tube. Appearance of a purple-brown ring at the interface indicates the presence of cardiac glycosides.

Salkowski's Test: A small amount of extract was taken and dissolved in some chloroform. Equal amount of conc. Sulphuric acid was added. Appearance of a brown ring indicates the presence of cardiac glycosides.

Tests for Steroids

Liebermann-Burchard's Test: 0.15g extract was dissolved in 1mL chloroform and equal volume of acetic anhydride and then a few drops of concentrated sulphuric acid were added. The test is positive for steroids if the upper layer is blue-green in color while the lower layer is red.

Tests for Alkaloids

Mayer's Test: 0.5g of extract was put in a test tube each. Water was added to each extract and then filtered. Then a few drops of Mayer's reagent were added. The test is positive for alkaloids if there is appearance of buff colored precipitate.

Wagner's Test: 0.5g of extract was put in a test tube each. Water was added to each extract and then filtered. To each sample a few drops of Wagner's reagent was added. Appearance of brown-reddish precipitate indicates the presence of alkaloids.

Dragendoff's Test: 0.5g of extract was put in a test tube each. Water was added to each extract and then filtered. To the sample a few drops of Dragendoff's reagent was added. Appearance of red color precipitate indicates the presence of alkaloids.

Chromatographic Study

Thin layer chromatography:

n – Hexane extract: a precoated TLC plates was taken and spotted with the n- Hexane extract of *Lophira lanceolata* using a capillary tube. Then it was immersed in the solvent system containing Hexane and Ethyl acetate in the ratio of 8:2 respectively. The TLC plate was dried and viewed under UV- light at wavelength of 254 nm. It was then sprayed with 10% sulphuric acid (which is the detecting reagent) and allowed to dry. Then the TLC plate was place in the oven at 105oC for 3minsand observe.

Methanol extract: the same procedure as described above was repeated using methanol extract of *Lophira lanceolata* and a solvent system of chloroform and methanol in the ratio of 4:1 respectively Evans and Tease.

In Vitro Antioxidant Assay

Determination of Total Phenolic Content in the extract.

The total phenolic content was determine using folin-ciocalteu reagent method as adapted by McDonald, and was slightly modified. 1mL of the extract was added to 2 mL of distilled water and 1 mL of folin-ciocalteu phenol reagent, the mixture was allowed to stand at room temperature for 5 minutes and 0.6 ml of Na_2CO_3 (200 mg/mL) were added to the mixture and further allowed to stand at room temperature for 10min. The resulting blue complex was then measured at an absorbance of 760 nm. The content of phenolic compounds was expressed as mg/mL. Gallic acid was used as positive control. Two-fold serial dilutions were used for the gallic acid and the extract. Total phenolic content was expressed as gallic acid equivalent based on calibration curve and was calculated using the formula:

$$T = \frac{C \times V}{M}$$

Where T = total phenolic contents in milligram per gram of plant extract; C = the concentration of gallic acid establishes from the calibration curve in milligram per milliliter; V = the volume of extract in milliliter and M = weight of the plant extract McDonald.

In Vitro Free Radical Scavenging Activity

The radical scavenging activities of the plant extracts was determined using 2.2-diphenyl-1-picryl-hydrazyl or 1.1-diphenyl-2-picryl-hydrazyl (DPPH) assay and UV spectrophotometer at 517nm. Radical scavenging activity was measured by a slightly modified method adapted by Ayoola. The following concentrations of the extracts were prepared, 1.25, 2.5, 5, 10 and 20 mg/cm^3 in methanol. Vitamin C was used as the antioxidant standard at concentration of 0.625, 1.25, 2.5, 5, and 10 mg/cm^3. 1cm3 of the extract was placed in a test tube, and 2 cm^3 of methanol was added and followed by 0.5 cm^3 of the DPPH solution. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\{Ab - Aa\}}{Ab} \times 100$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the extract. The IC50 of the various extracts were also calculated by plotting a graph of percentage inhibition against logarithm of concentration and then extrapolating from the 50 % inhibition to obtain the IC50 values Ayoola.

Results

Percentage yield of the plant extract

The total weight of dried extract obtained was 44g and the Percentage yield of methanolic extract was 17.60% (Tables 1-5).

Preliminary phytochemical screening

The results of the preliminary phytochemical screening are shown below in Table 1 below.

S/N	TESTS	RESULT
1	SAPONINS	+
	Frothing Test	+
2	TANNINS	+
	Lead sub acetate	+
	Ferric chloride	+
3	FLAVONOIDS	+
	Sodium hydroxide	+
	Shinoda's	+
	Ferric chloride	+
4	ANTHRAQUINONES	+
	Borntrager's	+
5	CARDIAC GLYCOSIDE	+
	Keller – Kelliani	+
	Salkowski's	+
6	STEROIDS	+
	Liebermann-Burchard's	+
	ALKALOIDS	+
	Mayer's	+
	Wagner's	+
	Dragendoff's	+

Key: + = Present

Table 1: Showing phytochemicals present in methanolic stem bark extract of *L. lanceolata*.

Thin Layer Chromatography()

S/No	Spot	Methanolic Extract	Hexane Extract
1	A	+	+
2	B	+	+
3	C	+	-
4	D	+	-
5	E	+	-
6	F	+	-
7	G	+	-
8	H	+	-

Key: + Indicate activity, - Indicate no activity.

Table 2: Qualitative Antioxidant Screening with DPPH Using Pre-coated TLC Plates.

S/No	Gallic acid		Methanolic Extract	
	Concentration (g/mL)	Mean absorbance (nm)	Concentration (g/mL)	Mean absorbance (nm)
1	0.02	0.995±0.001	0.05	0.966±0.001
2	0.01	1.022±0.001	0.025	0.926±0.008
3	0. 05	0.764±0.001	0.0125	0.752±0.001
4	0.0025	0.725±0.002	0.00625	0.572±0.001

Key: The Mean Absorbance is express as Mean ± Standard deviation.

Table 3: Showing the Total Phenolic Content of Gallic Acid and Extract.

S/No	Concentration (mg/mL)	Vitamin C		Methanolic Extract	
		Mean absorbance (nm)	% Inhibition (%)	Mean absorbance (nm)	% Inhibition (%)
1	10	0.135 ± 0.001	83.97	0.419±0.001	52
2	5	0.301± 0.000	65.55	0.491±0.001	43.8
3	2.5	0.383 ± 0.003	56.16	0.512±0.002	41.36
4	1.25	0.121 ± 0.001	86.11	0.523±0.000	40.18
5	0.625	0.452 ± 0.002	48.27	0.167±0.001	80.85

Key: The mean absorbance is express as Mean ± Standard deviation.

Table 4: Showing Free Radical Scavenging Activities of Vitamin C and Extract.

S/No	Extract/ Compounds	IC50 (mg/mL) ± S.D
1.	Methanolic Extract	0.419±0.001
2.	Vitamin C	0.452±0.002

Key: IC50 = Concentration sufficient to obtain 50% of a maximum effect estimate in 100%.
S.D= Standard Deviation.

Table 5: Showing IC50 Values of Vitamin C and Methanolic Extract.

Discussion

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [13]. Different solvents are being employed for extraction of bioactive compounds from plants. The solvents used vary in their polarity. One of the most commonly used solvent employed for extraction of plant material is methanol Biglari and was used as extraction solvent for this study. Generally, any part of plant can be used for antioxidant studies but the most commonly used part is leaves followed by fruit and stem bark Brighente hence the use of stem bark extract of *L. lanceolata* to evaluate antioxidant activity.

Phytochemicals are bioactive, non-nutrient naturally occurring compounds in plants Okarter. The various phytochemical compounds detected in this study are believed to have pharmacological and overall health benefits when used similar to what was reported by Jaberian, et al. [14] following investigation of three plants for their antioxidant and antibacterial activity. The result of the phytochemical analysis is also in accordance with the study of Sani, et al. and Ghogomu, et al. who both reported the presence of similar compounds in *L. lanceolata*. The antioxidant properties shown by the present extract could be due to presence of tannins and other phenolic and flavonoid compounds since these compounds are known to exhibit high antioxidant activity Chung. The result of this study is also in accordance with another study which reported that, the consumption of *Lophira lanceolata* will aid in production of polyphenolic flavonoid which is effective in lowering the risk of coronary heart diseases Rice-Evans and Miller. The high amount of the total phenolic content in the methanolic stem bark extract of *L. lanceolata* may explain why the extract has been found to be rich in flavonoids compounds based on their chemical reducing capacity relative to standard gallic acid. Therefore, this indicates that, there is a positive relationship between antioxidant activity potential and the number of phenolic compounds of the methanolic extract Elliot. This also indicates a direct correlation between total phenolic content and antioxidant activity as reported by Saravana, et al. The antioxidant activity of phenolic compounds found in this extract could also be due to the redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet triplet oxygen and decomposing peroxides Osawa.

The stable DPPH free radical assay used in this study is a reliable method because the results obtained could be reproducible under a given condition [15-22]. The results of the DPPH experiment showed that, methanolic extract of *Lophira lanceolata* has free radical scavenging activity and this could be due to the presence of the polar phenolic compounds (Flavonoids and Tannins) which are good scavengers of free radicals Zheng and Wang. The IC_{50} of the methanolic extract was also evaluated using standard (vitamin C). It can be deduced that, the extract has high free radical scavenging activity, having notably lower IC_{50} value, thus showing a relatively good free radical scavenging ability, because the lower the IC_{50} the better the cytotoxicity as reported by Danielle and Lall.

Conclusion

The free radical scavenging activity and the total phenolic content of the methanolic stem bark extract of *Lophira lanceolata* indicates a significant antioxidant potential. The antioxidant potential of this plant could provide a chemical basis in food industries, Medicine and overall health benefits in the production of antioxidants for the treatment and prevention of various diseases. Further studies on isolating and purifying active compounds from the extract should be carried out to ascertain the compounds and their mechanism could be essential to evaluate detailed antioxidant activity of the stem bark extract of *L. lanceolata*.

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