International Journal of Phytopharmacy
ISSN: 2277-2928 (Online)
Journal DOI: https://doi.org/10.7439/ijpp

Research Article

Phenolic compounds' dosage and antioxidant activities of aqueous and ethanolic extracts of *Lophira lanceolata*'s Van Tiegh. ex Keay (Ochnaceae) leaves

Djidénou Ahoton, Mahoudo Fidèle Assogba, Eléonore Chikani Yayi Ladekan, Arnaud Davo, Mansourou Moudachirou and Joachim D. Gbenou*

Laboratory of Pharmacognosy and Essential Oils, Faculty of Sciences and Technology and Faculty of Health Sciences, University of Abomey-Calavi, 01 BP: 918, ISBA Fairground, Cotonou Benin
Research Laboratory of Fragrant, Aromatic, Food and Medicinal Plants, FAST-ENS, Natitingou, UNSTIM Abomey

Abstract

Hepatic disease is a public health problem because of the devastation it causes to people. The purpose of this work is to evaluate the antioxidant activity of a plant, such as *Lophira lanceolata*, commonly used in the treatment of liver diseases in Benin. Phytochemical screening of aqueous and ethanolic extracts according to traditional use revealed the presence of alkaloids, polyphenols, flavonoids, tannins, saponosids, anthocyanins, free anthracene derivated, leuco-anthocyanins, quinone derivated and reducing compounds. Chemical analysis dosage of phenolic compounds has shown that the ethanolic extract of *Lophira lanceolata*'s leaves was rich in total polyphenols, flavonoids, tannins and anthocyanins than the aqueous extract. The evaluation of the antioxidant activities by the DPPH and the ferric Reduction antioxydant power (FRAP) tests showed that the ethanolic extract of *Lophira lanceolata*'s leaves is more active than the aqueous extract. The larval toxicity test on *Artemia salina* indicate that the aqueous and ethanolic extracts of *Lophira lanceolata* are practically non-toxic. That can justify its use to the treatment of liver diseases by populations in Benin.

Keywords: *Lophira lanceolata*, extracts, antioxidant, larval toxicity.

***Correspondence Info:**
Joachim D. Gbenou
Laboratory of Pharmacognosy and Essential Oils, Faculty of Sciences and Technology and Faculty of Health Sciences, University of Abomey-Calavi, 01 BP: 918, ISBA Fairground, Cotonou Benin

***Article History:**
Received: 03/09/2019
Revised: 22/12/2019
Accepted: 24/12/2019
DOI: https://doi.org/10.7439/ijpp.v9i6.5262

QR Code

How to cite: Ahoton D, Assogba M. F, Ladekan E. C. Y, Davo A, Moudachirou M and Gbenou J. D. Phenolic compounds' dosage and antioxidant activities of aqueous and ethanolic extracts of *Lophira lanceolata's* Van Tiegh. ex Keay (Ochnaceae) leaves. *International Journal of Phytopharmacy* 2019; 9(6): e5262. Doi: 10.7439/ijpp.v9i6.5262
Available from: https://ssjournals.com/index.php/ijpp/article/view/5262

1. Introduction

Many diseases such as atherosclerosis, cancer, arthrosis, diabetes, asthma, heart disease, liver disease come from free radicals produced in the body [1]. The diet of natural products (fruits, vegetables) which are rich in antioxidants, could play an important role in the prevention of these diseases [2]. In fact, polyphenols are natural compounds that are widely used in the plant kingdom and that are of growing importance, in particular because of their beneficial effects on health [3]. Their role as natural antioxidants is attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4]. Traditional medicine remains the main recourse of a large majority of populations to solve their health problems, not only because it constitutes an important element of the cultural heritage, but also for the limited financial means compared to conventional products [5]. According to the World Health Organization, nearly 80% of people depend on traditional medicine for primary health care [6]. This is explained by the fact that populations are attached to the ancestral culture and civilization for the treatment of diseases by herbal medicine because of the effectiveness, accessibility and availability of medicinal plants [7].

That is why we were interested in studying the antioxidant activity of aqueous and ethanolic extracts of leaves of *Lophira lanceolata* (Ochnaceae), a plant commonly used in the treatment of liver diseases in Benin. *Lophira lanceolata* is widely distributed in the Sudano-Guinean savanna zone from Senegal, through the Central African Republic and the extreme North of the DR of Congo, to Uganda. *Lophira lanceolata* is also distributed in Benin.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

The leaves of *Lophira lanceoalata* were collected in the forest of Pahou on the road to Ouidah. Voucher specimen was deposited at the National Herbarium of Abomey-Calavi's University and identified under the number AAC 231/HNB. They are then dried, crushed and stored in glass bottles away from light and moisture for extracts' preparation.

2.1.2. Chemical reagents

The reagents used are: DPPH (2,2'-diphenyl-1-picryl-hydrazyl), Folin-Ciocalteu reagent, ascorbic acid ($C_6H_8O_6$), gallic acid ($C_7H_6O_5$), aluminum trichloride ($AlCl_3$), quercetin ($C_{15}H_{10}O_7$) . All these products come from Sigma and the solvent used is ethanol 96°.

2.2. Methods

2.2.1. Preparation of the ethanolic extract

A test portion of 50 g of *Lophira lanceolata*'s leaves powder was macerated in 500 mL of ethanol 96° for 72 h with magnetic stirring. The supernatant was then filtered on Whatman paper and the solvent evaporated under reduced pressure at 50 °C using a rotary evaporator (Büchi Rotavapor 200).

2.2.2. Preparation of the aqueous extract

A test portion of 50 g of leaves powder of *Lophira lanceolata* was decocted for 30 minutes. The decoction was filtered on Whatman paper and the filtrate deposited in an oven and scraped. The extract obtained is kept in the freezer.

2.2.3. Phytochemical Screening

Phytochemical analysis based on differential reactions (staining and precipitation) of the main groups of chemical compounds contained in the aqueous and ethanolic extracts were carried out according to the method of Houghton and Raman [8].

2.2.4. Determination of total polyphenols

125 µL (10 mg/mL) of the extract are removed and dissolved in 625 µL of Folin-Ciocalteu reagent. After incubation for 5 min at 28 - 30 °C, 500 µL of sodium carbonate (Na_2CO_3) at 75 mg/mL and 4.75 mL of distilled water were added. The vortex mixture is incubated for 2 hours at 28 - 30 °C. Absorbance reading was performed on the Biomate spectrophotometer Thermo Spectonic Genesys 6 (Rochester NY USA) at 760 nm.

2.2.5. Determination of flavonoids

The total flavonoids content of plant extracts can be estimated by the aluminum trichloride ($AlCl_3$) method. Quercetin is used as the reference compound to obtain the calibration curve [9, 10]. 500 µL of a ethanol solution of $AlCl_3$ (2%) are taken and 500 µL of sample are added. To this mixture, 3 mL of ethanol is added. The blank consists of 500 µL of $AlCl_3$ and 3.5 mL of ethanol. The absorbances are read spectrophotometrically at 415 nm after 10 min of incubation at 28 - 30 °C.

2.2.6. Determination of condensed tannins

To 500 µL of the sample (10 mg/mL) or standard, 3 mL of the Sulfuric vanillin (4%), 1.5 mL of concentrated hydrochloric acid and 2 mL of ethanol are added. The mixture is incubated for 15 minutes and the absorbance is read at 500 nm. Concentrations of condensed tannins are deduced from calibration curves established with pyrogallol (0-300 µg/mL) and are expressed in µg of pyrogallol equivalent per milligram (µg eq pyrogallol/mg).

2.2.7. Determination of anthocyanins

Anthocyanins are red pigments in acid medium, turning in blue in alkaline medium; they are very common in flowers and fruits. The extraction of anthocyanins and flavonic aglycones is carried out according to the Lebreton technique [11]. The anthocyanin content was determined by the following formula:

$$T(\text{anthocyanins}) = (OD.MVd) / (\varepsilon.p)$$

With T(anthocyanins): anthocyanin content (in % or mg/g);
OD: Optical density at the maximum absorption wavelength;
ε: molar absorption coefficient of cyanidine (34,700) ;
M: molar mass of procyanidin (306);
V: volume of the butanolic solution;
D: dilution factor;
p: dry weight of hydrolyzed plant material

The formula for determining the aglycone content is as follows:

$$T(\text{aglycones}) = (OD.MVd) / (\varepsilon.p)$$

With T(aglycones): anthocyanin content (in % or mg / g);
OD: Optical density at the maximum absorption wavelength;
ε: Absorbance coefficient

2.2.8 Antioxidant activity

2.2.8.1. FRAP method (ferric reducing antioxidant power)

The Iron Reducing Antioxidant Reduction (FRAP) test is performed according to the Penpun method [12] with some modifications. This method measures the reducing power of antioxidants present in an extract by their ability to reduce ferric tripyridyltriazine (Fe^{3+} -TPTZ) to ferrous tripyridyltriazine (Fe^{2+} -TPTZ) at acidic pH.

2.2.8.2. Determination of antioxidant activity with DPPH (2,2'-diphenyl-1-picrylhydrazyl)

The ability of the extracts to trap DPPH (2,2'-diphenyl-1-picrylhydrazyl) was determined using the procedure described by Lamien-Meda [13]. A stock solution is prepared at a concentration of 1 mg/mL of the ethanol extract which is diluted 1/10. Then, the 1.5 mL mixture of each extract (dilute solution) is prepared with 3 mL of the ethanolic solution of DPPH (Cm = 0.04 mg/mL). The mixture is incubated for 15 minutes at room temperature and the absorbance is read at 517 nm. The antioxidant capacity of the extracts was determined using a calibration curve of ascorbic acid (0-10 mg/mL). Each test is performed in triplicate. The antioxidant capacity is expressed in mmol of ascorbic acid equivalent per gram of extract or fraction (mmol of EAA/g).

2.2.8.3. Determination of antioxidant activity (IC_{50}) extracts

For this test, the samples were prepared by dissolving in ethanol [14]. For each extract, a stock solution in ethanol is prepared at 200 µg/mL. This solution is then diluted in a geometric series of the reason 2 to have different concentrations: 0.048875; 0.09775; 0.1955; 0.391; 0.781; 1,562; 3.125; 6.25; 12.5; 25 µg/mL. In dry sterile tubes, 1 mL of the test extract solution is added, 1 mL of DPPH solution (0.04 mg/mL) is added. After vortexing, the tubes are protected from light at laboratory temperature for 30 minutes. The absorbance is measured at 517 nm using a spectrophotometer (Biomate UV/VIS). For each dilution, a blank consisting of 1 mL of the test solution and 1 mL of ethanol is prepared. The positive control is represented by ascorbic acid (200 µg/mL) and is treated under the same conditions as the test sample.

2.2.9 Larval toxicity

The test was carried out on shrimp larvae *Artemia salina* according to the technique proposed by Michael [15].

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening has allowed us to highlight the presence of chemical groups in leaves of *Lophira lanceolata.* The results obtained are grouped together in **Table 1.**

Table 1: Chemical compounds' groups from leaves of *Lophira lanceolata*

Groupes chimiques	aqueous extract	ethanolic extract
Alkaloides	+	+
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Mucilage compounds	+	-
Triterpenoids	-	-
Steroides	-	-
Cardenolides	-	-
Anthocyanins	+	+
Leucoanthocyanins	+	+
Saponosides	+	+
Reducing compounds	+	+
Quinones derivates	+	+
Anthracene derivates	-	-
Cyanogenic derivates	-	-
Essential Oil	+	+

NB: - Absent, + Present

Phytochemical screening revealed in both extracts the presence of chemical groups such as alkaloids, polyphenols, flavonoids, tannins, saponosides, anthocyanins, leuco-anthocyanins, quinone derivatives, reducing compounds and essential oil. The difference is in the mucilages observed in the aqueous extract and not in the ethanol extract. *Lophira lanceolata* is therefore endowed with antioxidant properties due to the presence of polyphenols and flavonoids in this plant. Flavonoids are recognized for their hepatoprotective activities [16,17]. The presence of phenolic compounds (polyphenols, flavonoids, tannins, etc.) could be the basis of the oxidizing and hepatoprotective activities of *Lophira lanceolata*'s leaves extracts.

3.2. Total contents of phenols, flavonoids, tannins and anthocyanins

The contents of polyphenols, flavonoids and tannins are given in Table 2 and those of aglycones are given in Table 3.

Table 2: Content of polyphenols, flavonoids and tannins

Extracts	T Polyphenols	T flavonoids	T Tannins
L.l. aqext	100 321 ± 0 000	11 261 ± 0,014	282 956 ± 0,750
L.l. ethext	119 995 ± 0,235	25 929 ± 0,152	501 511 ± 3 707

Table 3: Aglycone content

Extracts	T Anthocyannins	T Aglycones-flavonoids	T Flavones	T C-glycosyl-Flavones	T Flavonols
L.l. aqext	0,171 ± 0,000	0,106 ± 0,000	0,164 ± 0,000	0,823 ± 0,006	0,003 ± 0,000
L.l.ethext	0,174 ± 0,000	0,338 ± 0,000	0,584 ± 0,000	0,826 ± 0,003	0,007 ± 0,000

Determination of the total phenol and flavonoid content in both extracts of *Lophira lanceolata* was performed using separately the colorimetric methods (Folin-Ciocalteu and aluminum trichloride ($AlCl_3$).) The total phenol content estimated by the method of Folin-Ciocalteu for each extract was reported in mg of gallic acid/g of dry vegetable material The results show that the ethanolic extract has a high content of total phenols (11 9.99 ± 0.25 mg/g) relative to that of the aqueous extract (100, 321 ± 0.000 mg/g).

The flavonoid content determined by the aluminum trichloride method for each extract was reported in mg equivalent of catechin/g of dry vegetable matter. The results reveal that the flavonoid contents of the two aqueous and ethanolic extracts are respectively 11,261 ± 0,014 and 25,929 ± 0,152. Based on these data, it can be deduced that the flavonoids represent 21.60 % of the total phenols in the ethanolic extract. This level does not exceed 11.22 % in the aqueous extract.

The tannin content determined by the sulfuric vanillin method (4%) was reported in mg equivalent of pyrogallol/g of dry plant material. The results reveal that the two extracts have higher levels: 282.956 ± 0.750 and 501.511 ± 3.707 respectively for the aqueous extract and the ethanolic extract.

The anthocyanin content determined by the method of Lebreton (1967) for each extract was calculated by the formula [11] expressed in mg equivalent of procyanidin/g of dry plant material. The results reveal that both extracts have very low levels: 0.171 ± 0.000 and 0.174 ± 0.000 respectively for the aqueous extract and the ethanolic extract.

3.3. Antioxidant Activity with DPPH (2,2'-diphenyl-1-picrylhydrazyl) and Iron Reduction Test (FRAP)

Table 4 summarizes the antioxidant activity at DPPH and the iron reduction test (FRAP).

Table 4: antioxidant activity at DPPH and (FRAP)

Extracts	IC_{50} µg/mL	EC_{50}	APR	I% at	100 µg/mL	CA (mmol EAA/g extract)	Fe^{2+} (mmol/g extract)
L.l. aqext	4,71	117,708	0,008		92,762	44,016 ± 9 334	5,801 ± 0,016
L.l. ethext	1,24	31,034	0,032		97,126	56,540 ± 5,470	10,949 ± 0,033

CA: Antioxidant capacity

The curves of inhibition of the free DPPH radical as a function of the different concentrations of *Lophira lanceolata* leaves extracts are shown in **figures 1** and **2**.

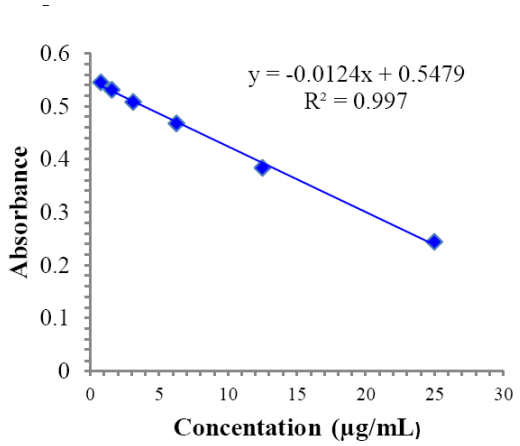

Figure 1: *Lophira lanceolata* leaves aqueous extract's inhibition of the free DPPH radical

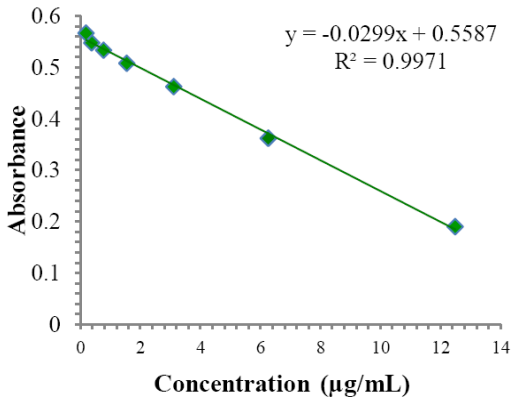

Figure 2: *Lophira lanceolata* leaves ethanol extract's inhibition of the free DPPH radical

The antioxidant activity of both extracts was evaluated by two methods FRAP and DPPH for confirmation purposes.

The iron reduction test (FRAP) is a simple, fast and reproducible test [18]. It is universal and can be applied in plants as well as in plasmas and in organic and aqueous extracts [19]. The presence of reducing agents in the plant extracts leads to the reduction of Fe^{3+}/ferricyanide complex in ferrous form. Fe^{2+} can therefore be evaluated by measuring and monitoring the increase in the density of the blue color in the reaction medium at 700 nm [20]. The reducing power of the dose - dependent plant extracts (depending on the concentration). At the concentration of 0.5 mg / mL, the reducing power of the ethanolic extract of *Lophira lanceolata* is twice as high (10.949 ± 0.033 mmol Fe^{2+}/g extracted) compared to the aqueous extract (5.801 ± 0.016 mmol Fe^{2+}/g extracted). The reducing power of the species *Lophira lanceolata* is probably due to the presence of hydroxyl groups in the phenol compounds that can serve as electron donors. As a result, antioxidants are considered to be reducing and inactivating oxidants [21]. Some previous studies have also shown that the reducing power of a compound may be a significant indicator of its potential antioxidant activity [22,23]. The ethanolic extract of *Lophira lanceolata* would have greater antioxidant activity than that of the aqueous extract.

According to the results recorded (Table 4), the aqueous extracts (44,016 ± 9.334 mmol of EAA/g of extract) and ethanolic (56.540 ± 5.470 mmol of EAA/g of extract) have a proven antioxidant capacity, their EC_{50} values of 31.034 µg/mL and 117.708 µg/mL respectively. Antioxidant chemical groups such as flavonoids and tannins have been shown to reduce and discolor DPPH due to their ability to release hydrogen [24]. The polyphenols contained in the extracts of *Lophira lanceolata* are probably responsible for the antioxidant activity of these extracts. The DPPH free radical scavenging test of the ethanolic and aqueous extracts of *Lophira lanceolata* with respect to the DPPH radical was evaluated using a spectrophotometer following the reduction of this radical accompanied by its passage from the purple color ($DPPH^{\bullet}$) with yellow color (DPPH-H) measurable at 515 nm. This reduction capacity is reflected in a decrease in the absorbance induced by antiradical substances [25]. The results of the antioxidant power of the extracts tested show that the percentage inhibition of the aqueous and ethanolic extracts of *Lophira lanceolata* is greater than 90% at the concentration of the order of 100 µg/mL for each of the aqueous and ethanolic extracts. The ethanolic extract of *Lophira lanceolata* would be more active than the aqueous extract. This could be explained by the high content of phenolic compounds, especially flavonoids.

3.4. Larval toxicity

The test was carried out on shrimp larvae *Artemia salina* according to the technique proposed by Michael [15]. The lethal concentration (LC_{50}) was determined.

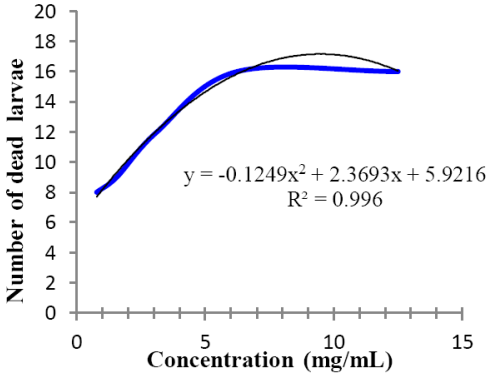

Figure 3 : Larval toxicity of the aqueous extract of *Lophira lanceolata*'s leaves

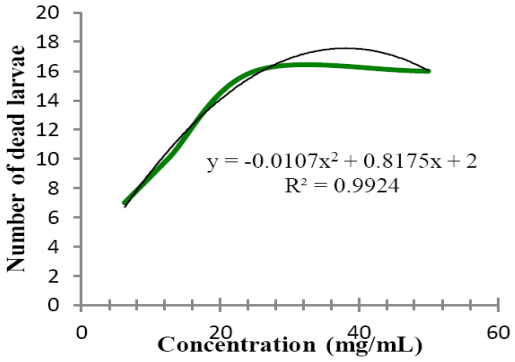

Figure 4 : Larval toxicity of the ethanolic extract of *Lophira lanceolata's* leaves

The consumption of a plant requires scientific evidence. The safety of a substance requires toxicity testing and the larval toxicity test is the preliminary test. This is a test that uses *Artemia salina* shrimp larvae. Indeed, there is a correlation between cytotoxicity on larvae of *Artemia salina* and on 9PS and 9KB cells (human nasopharyngeal carcinoma on the one hand [26], pulmonary carcinoma A-549 cells and colonic carcinoma HT-29 cells on the other hand [27]). The larval toxicity test consists of observing the behavior of these larvae with the two aqueous and ethanolic of *Lophira lanceolata.* Knowing that any substance capable of eliminating cancer cells is toxic, the determination of LC_{50} of aqueous (LC_{50} = 0,922 mg/mL) and ethanolic (LC_{50} = 8,159 mg/mL) extracts of *Lophira lanceolata* and their comparison with the ladder of Mousseux [28] showed that all extracts had an LC_{50} greater than 100 µg/mL, both extracts being non-toxic to *Artemia salina* larvae.

4. Conclusion

The study of the antioxidant activity of *Lophira lanceoalta* leaves' extracts according to the FRAP reduction method and that of the DPPH free radical scavenging showed that the ethanolic and aqueous extracts have a moderate antioxidant activity. This activity is due to the presence of total polyphenols, flavonoids, tannins and anthocyanins. But *Lophira lanceolata* ethanolic leaves' extract would be more active than aqueous'. The larval toxicity test showed that the aqueous and ethanolic extracts of *Lophira lanceoalta'*s leaves would not be toxic to the larvae.

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