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Phytochemical screening, total polyphenols and flavonoids content and antiradical activity of methanolic extract of *Lannea welwitschii* (Hiern) Engl. (Anacardiaceae) from Gabon

Joanna Grace OMBOUMA[1*], Aimé-Jhustelin ABOGO MEBALE[2], Daniel Datch MEFOUET ABESSOLO[2], Ornella ABESSOLO MVE[2] and Raymonde MBOMA[3]

[1]*Département de Pharmacologie. Université des Sciences de la Santé (USS), BP : 4009 Libreville, Gabon.*
[2]*Laboratoire Pluridisciplinaire des Sciences (LAPLUS), Ecole Normale Supérieure, BP : 17009, Libreville, Gabon.*
[3]*Département de Biologie et d'Ecologie Végétale. Institut de Recherche en Ecologie Tropicale (IRET), BP : 13354, Libreville, Gabon.*
[*]*Corresponding author; E-mail: omboumajoanna@yahoo.fr; Tel: 00241 6627 247*

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ABSTRACT

The present study was carried out to identify chemical groups, measure polyphenols and total flavonoids, and evaluate the anti-free radical activity of the methanolic extract of the trunk bark of *Lannea welwitschii* who is a plant currently used to treat various diseases in the gabonese pharmacopoeia so as respiratory tract infections. The phytochemical screening was carried out on the methanolic (maceration) extract using standard laboratory methods. Total polyphenols and total flavonoids were measured by spectrophotometric assay while anti-free radical activity was assessed by ABTS method. The Phytochemical screening revealed the presence of polyphenols, tannin, flavonoids, alkaloids, reducing compounds, terpenes and coumarins. The determination of the total polyphenols of the methanolic extract from gallic acid showed a content of 598.18 ± 6.69 mg EAG/100 g of dry matter and the total flavonoids from quercetin showed a content of 91.71 ± 2.43 mg EQ/100 g of dry matter. Finally, the evaluation of the anti-free radical activity showed that the methanolic extract of the trunk bark of *Lannea welwitschii* has a weak free radical activity compared to that of gallic acid: IC_{50} are respectively 76.57 µg/ml and 0.47 µg/ml.

Keywords: *Lannea welwitschii*, methanolic extract, polyphenols, flavonoids, antiradical activity.

INTRODUCTION

Lannea welwitschii is a woody medicinal plant of the Anacardiaceae family (Osafo et al., 2016). Geographically, more particularly in Africa, this plant is found from Côte d'Ivoire to Cameroon while extending to Uganda and Angola. It grows in forests where the majority of trees have deciduous leaves (Agyare et al., 2013). *Lannea welwitschii*, commonly called «Ekika» or «Ewinwan» in Nigeria (Olatokunboh et al., 2010), «Trongba» or «Loloti» in Ivory Coast (Osafo et al., 2016),

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«Alum», «Mundungu» or «Ghébimbi» in Gabon (Breteler et al., 2017) has many pharmacological properties. Indeed, different studies have revealed that *L. welwitschii* has antimicrobial (Deji-Agboola et al., 2010), antioxidant (Agyare et al., 2013), anti-inflammatory (Obiri et al., 2013), analgesic (Obiri et al., 2013), antiallergic (Obiri et al., 2013), antidiabetic (Okine et al., 2005) and antidiarrheal activities (Olatokunboh et al., 2010). However, all these pharmacological properties have been determined on the plant in media other than the gabonese environment, while the gabonese populations use it to treat fever or even for the treatment of opportunistic HIV / AIDS diseases such as infections respiratory tract (Feuya Tchouya et al., 2015). To this end, the aim of this work was to determine the medicinal properties of the methanolic extract of the trunk bark of *Lannea welwitschii* from Gabon by evaluating its phytochemical constituents as well as its antioxidant activity.

MATERIAL ET METHODS

Plant material

The trunk bark of *Lannea welwitschii* was collected in August 2018 between Oyane 4 and FourPlace in Estuaire Provincial during an ethnobotanical survey of Gabonese antidiarrheal plants. The fresh trunk bark was dried for about fifteen days protected from light in an air-conditioned room. It was powdered using an electric grinder (Flour Mills Nigeria, El MOTOR N°1827). The resulting powder was stored at room temperature in a clear labeled packaging bag. The plant has been identified at the National Herbarium of Gabon.

Preparation of methanolic extract

200 ml of methanol were added to 30 g of plant powder contained in an Erlenmeyer flask with a magnetic bar. After hermetically closing the Erlenmeyer flask, the mixture is left under magnetic stirring for 24 hours and then filtered using Whatman N°1 filter paper. The filtrate was stored at 4°C until analysis.

Phytochemical screening

Methanolic extract of the trunk bark from *Lannea welwitschii* were screened for their qualitative chemical composition, using standard methods (Ciulei, 1964; Harborne, 1998). The identification of the following groups has been considered: alkaloids, reducing compounds, coumarins, flavonoids, polyphenols, sterols and triterpenes, saponins, and tannins.

Total polyphenols content

The evaluation of the total polyphenols was carried out by the Folin-Ciocalteu method described by Singleton et al. (1999). 1ml of Folin-Ciocalteu reagent (0.2 N diluted in MeOH) was mixed with 200 µl of extract dissolved in distilled water (1 mg/ml). After 5 min incubation in dark at room temperature, 800 µl of 20% sodium bicarbonate solution (w/v) was added. Sample was incubated at room temperature during 1 h. Absorbances were determined at 765 nm using a GENESYS 10 UV spectrophotometer. All tests were achieved in triplicate. Gallic acid was used as a standard to establish the standard range (0-150 mg/l). The results are expressed in mg of Gallic Acid Equivalent (GAE) per 100 g of dry extract.

Total flavonoids content

The total flavonoids are determined according to the method of Arvouet-Grant (1994) with some modifications. 1 ml of AlCl3 (2% in methanol) is mixed with 1 ml of methanolic extract (1 mg/ml) followed by 10 minutes of incubation. Absorbances were determined at 415 nm using a GENESYS 10 UV spectrophotometer. Quercetin was used as a standard to establish the standard range (0-50 mg/l). The results are expressed in mg of quercetin equivalent (QE) per 100 g of dry extract.

Evaluation of radical activity

Antiradical activity was determined by the ABTS method (Re et al., 1999). The antiradical activity of methanolic extract is deduced from its ability to inhibit ABTS•+ compared to a reference antioxidant: gallic acid. The ABTS•+ radical ion is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM) in distilled water for 16 hours at room temperature and in sunlight. The ABTS•+ solution obtained is diluted with sodium phosphate buffer (5 mM, pH = 7.4), in order to obtain a stock solution having an initial absorbance value of between 0.65 and 0.70 at 734 nm (UV spectrophotometer).

The inhibitory effect of ABTS was calculated according to the following formula:

Anti-free radical activity (%) = [1-Ar/Ai]×100

Ar = Absorbance remaining of ABTS•+

Ai= Initial Absorbance of ABTS•+

Three tests were performed for each concentration of extract which was prepared as follows:

In 100 ml of methanol, 25 g of Lannea welwitschii trunk bark were added. The whole was macerated for 30 min and gave the first solution. 25 ml of this solution was taken and diluted in 75 ml of methanol. This second solution represented the stock solution. From the latter, we performed a succession of dilutions (1/400ths; 1/800ths, 1/1600ths, 1/3200ths, 1/6400ths, 1/12800ths, 1/25600ths) in order to obtain 7 daughter solutions.

RESULTATS

Phytochemical screening

The results of the phytochemical screening of the methanolic extract of the trunk bark of *Lannea welwitschii* are presented in Table 1. The qualitative analysis showed the presence of alkaloids, tannins (gallic), reducing compounds, flavonoids (flavones, leucoanthocyanins), terpenes, coumarins and polyphenols. This extract doesn't contain catechic tannins, saponins and even less catechol-type flavonoids.

Total Phenolic and Flavonoid contents

The total phenol content was determined in comparison with a standard which is gallic acid. The Results (Table 2) were expressed in milligrams of gallic acid per 100 grams of dry matter (mg GAE/100 g DM), using the linear equation extract: $Y = 0.0047X + 0.0158$ (with $R^2 = 0.9988$) Thus, total phenolic content is 598.18 ± 6.69 mg EAG/100 g DM.

The total flavonoid content was determined in comparison with quercetin. Results (Table 2) were expressed in milligram equivalent of quercetin per 100 grams of dry matter (mg EQ/100g DM. using the linear equation $Y = 0.0258X + 0.043$ (with $R^2 = 0.9973$). Thus, total flavonoids content is 91.71 ± 2.43 mg EQ/100g DM.

Antiradical activity

The antiradical activities of gallic acid and methanolic extract from the trunk bark of *L. welwitschii* were reported in Figures 1 and 2.

For the gallic acid (Figures 1), the percentage of the antiradical activity increases with the concentration after 6 minutes of incubation. The antiradical activity reaches 100% after 6 minutes of incubation for concentrations greater than or equal to 0.84 µg/ml.

The antiradical activity reaches 100% for concentrations of the methanolic extract greater than or equal to 313 µg/ml (Figure 2).

The different regression equations (Figures 1 and 2) allow us to deduce that the IC_{50} of gallic acid (0.47 µg/ml) is much higher than that of methanolic extract (76.57 µg/ml) as shown in Table 3.

Table 1: Phytochemical composition of methanolic extract the trunk bark of *Lannea welwitschii*.

phytochemical compounds		Extract
Alkaloids		++
Tannins	Gallic	++
	Catechic	-
Saponosides		-
Reducing compounds		++
Flavonoids	Flavones	++
	Flavanones	-
	Flavonols	-
	Leucoanthocyanes	++
	Catechols	-
Sterols et triterpenoides	Sterols	-
	Terpenes	++
Coumarins		++
Polyphenols		++

(++) : Abundance ; (+) : presence ; (-) : absence

Table 2: Total phenolic and flavonoids contents in methanolic extract of trunk bark of *Lannea welwitschii*.

Extract	Total phenolic content (mg GAE/100 g DM)	Flavonoids content (mg QE/100 g DM)
Methanolic	309.18 ± 38,06	155.16 ± 21,06

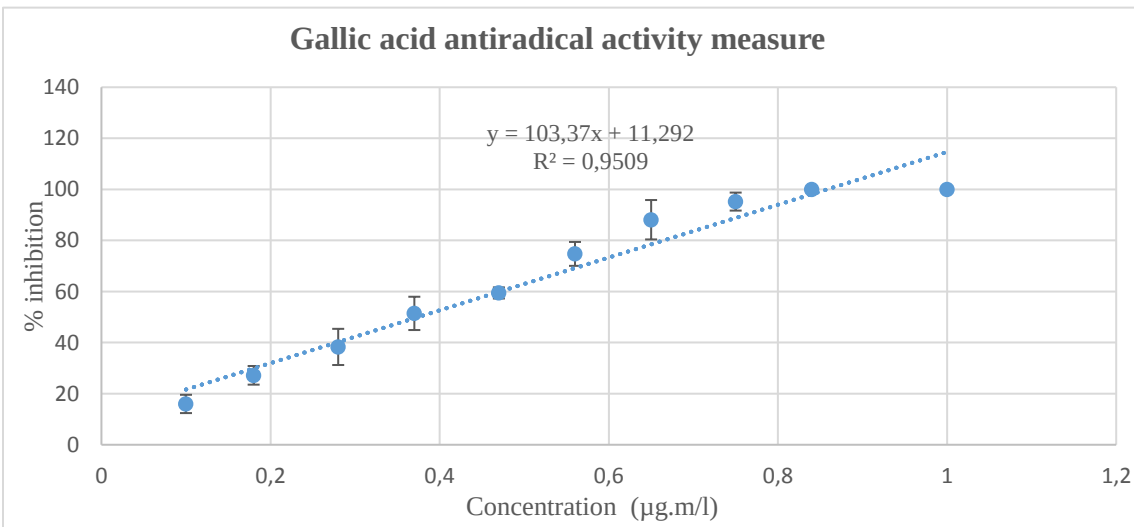

Figure 1: Gallic acid inhibition curve at different concentrations.

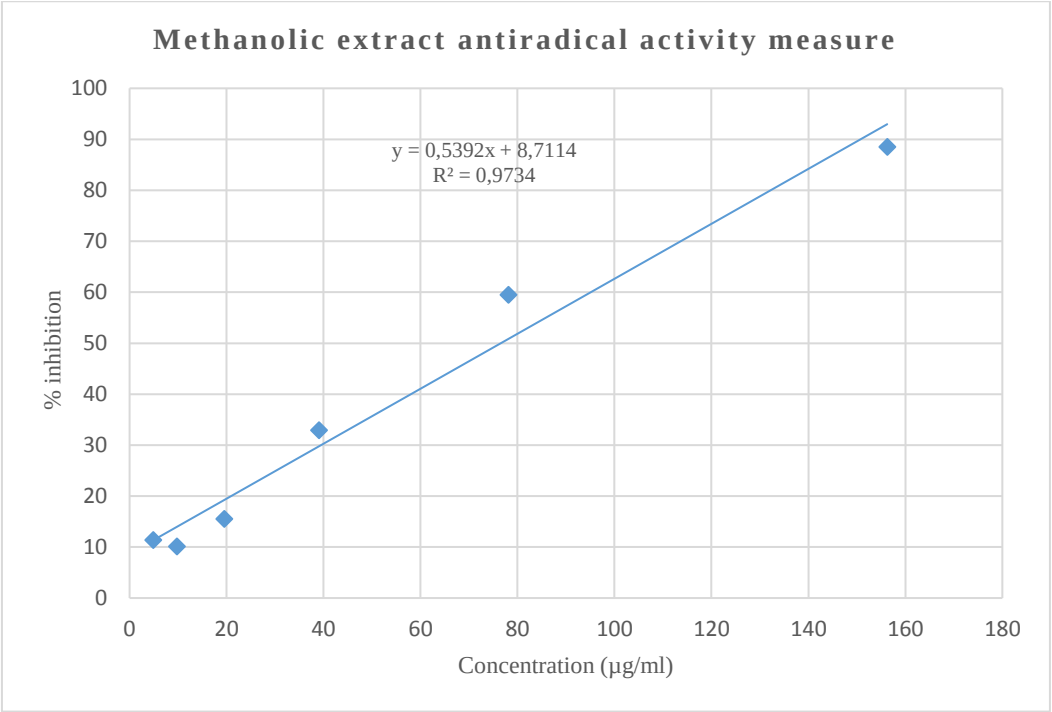

Figure 2: Methanolic extract inhibition curve at different concentrations.

Table 3: IC_{50} values of *gallic acid and* methanolic extract of *Lannea welwitschii.*

ABTS : IC_{50} of Gallic acid (µg/ml)	ABTS: IC_{50} of methanolic extract (µg/ml)
0, 47	76.57

DISCUSSION

Phytochemicals have been shown to be responsible for various biological activities such as antimutagenic, antioxidant, antimicrobial and anti-inflammatory properties (Bruneton, 2009). Indeed, Bribi (2018) revealed that plants that contain alkaloids are used as analgesics, antiseptics, sedatives or in the treatment of coagulation disorders and eye diseases. Olatokunboh et al. (2010) claim that tannins are known to decrease irritability in the gut. They are therefore very suitable for combating diarrheal diseases. In response to microbial infection, flavonoids are synthesized by plants. To this end, several of them have been shown to possess not only antibacterial activity against several microorganisms but also antioxidant, hepatoprotective, anti-inflammatory, anticancer and antiviral activities (Kumar et al., 2013). As for terpenes, they are described to have the same activities previously mentioned for flavonoids but also an antimalarial and hypoglycemic activity (Yang et al., 2020). The richness of our methanolic extract in active chemical compounds could explain the traditional use of *Lannea welwitschii* in various country for the treatment of edema, coagulation disorders, hemor-oids, gout, recovery, dysentery, gingivitis, topical infections, wounds, food

poisoning, naso-pharyngeal infections and vomiting…

The results on the antiradical activity of our reference (gallic acid) correspond to those obtained by N'negue et al. (2020) in their study to the antioxidant activity of an aqueous extract of dried calyxes of *Hibiscus Sabdariffa*. This guarantees us certainty of our results.

The IC_{50} of gallic acid (IC_{50} = 0.47µg/ml) is 160 times lower than that of our extract (IC_{50} = 76.57µg/ml). The methanolic extract from the bark of *L. welwitschii* appears to have low antioxidant activity compared to that of gallic acid. However, taking into account the high content of total phenolic compounds (598.18 ± 6.69 mg EAG/100 g DM), we can deduce that our methanolic extract has good antiradical activity. Indeed, our result is very close to those obtained by Obonga et al. (2017) on the antiradical activity of trunk bark of *L. welwitschii* and other plants using several methods (DPPH, NO, H_2O_2). In this work, the IC_{50} of methanolic extract from the trunk bark of *L. welwitschii* was 72.55 ± 0.07 by the DPPH radical scavenging method and 95.56 ± 0.12 by the H_2O_2 radical scavenging method.

Conclusion

This study involved the analysis of methanolic extract of *Lannea welwitschii* trunk bark for phytochemicals, total polyphenols and total flavonoids content, and antioxidant activity. The chemicals present in this plant demonstrate an antioxidant capacity against free radicals *in vitro*. Thus, this plant could be considered as a source of antioxidant. A correlation could be demonstrated between the antioxidant activity and the content of total polyphenols.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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