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# Content of Polyphenolics Constituents and the Antioxidant and Antimicrobial Activities of Extracts from Leaves and Fruits of *Lannea microcarpa* Engl. & K. Kraus (Anacardiaceae)

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**Abstract:** Nowadays, a number of studies on health benefits associated with natural compounds have been demonstrated. Polyphenolics in fruits, leaves, herbs and vegetables possess potent antibacterial and antioxidant activities. In the present study, a portion of *Lannea microcarpa's* lyophilised fruits and leaves aqueous acetone extract, was fractionated by successive liquid-liquid partitioning to obtain ethyl acetate and n-butanol fraction. The total polyphenolic constituents and total flavonoid constituents from the extract and the two fractions were determined and were found to be the highest respectively in leaves butanol fraction (22.05±0.78 mg GAE/100 mg) for polyphenolic constituents, and in leaves and fruits ethyl acetate fraction (1.61±0.02 mg QE/100 mg) for the flavonoids constituents. Antioxidant activity of the extract and the two fractions were determined by in vitro model using DPPH; Antibacterial activity of these extracts and fractions was also assayed on two Gram+ bacteria and six Gram-bacteria by using disc diffusion and broth micro dilution method. Data indicated highest antioxidant activity obtained from leaves extracts and fractions IC50 (1.55±0.19 µg/mL), and inhibitory effect both on Gram- and Gram+ bacteria with highest antibacterial activity MIC (0.15625 mg/mL), obtained with leaves ethyl acetate and n butanol fraction on *Bacillus cereus* (ATCC 9144). All the results obtained from this study, showed that *Lannea microcarpa*'s fruit and leaves extract could be candidate for news antibacterial and antioxidant compounds.

**Key words:** Antibacterial, antioxidant, *Lannea microcarpa,* polyphenol

## INTRODUCTION

Since the beginning of history, mankind has used a variety of plants found in his environment to treat pain and disease. Certain plants were used as food before their prophylactic or curative effects were discovered. In some developing countries, particularly Burkina Faso, this fact is still true both because of the weakness and inefficiency of the modern healthcare system and also the widespread ineffectiveness of certain synthetic molecules against resistant microorganisms.

Because of opportunistic infections associated with AIDS and the increase in functional diseases (e.g., cardiovascular diseases, diabetes, obesity) in Africa, scientific interest in antimicrobials and antioxidants that originate from plants has increased. Africa is rich in flora, and its traditional medicinal knowledge can contribute to the discovery of new compounds of pharmacological interest. While investigating ethnobotany in the central region of Burkina Faso, it was discovered that the leaves and fruits of *Lannea microcarpa* are commonly used to treat bacterial and oxidative stress diseases.

*Lannea microcarpa* Engl & K. Krauss (Anacardiaceae) is a wild fruit tree found in the Sudano-Sahelian regions of Africa. This tree's fruits are commonly consumed (and used in the production of local drinks) and are also utilised in traditional medicine to treat scurvy, rickets and cough.

*Lannea microcarpa* leaves are used for livestock feed and also for the treatment of diarrhoea, gastroenteritis, malaria, bacterial infections, toothaches and wound care (Irvine, 1961; Nacoulma, 1996; Arbonnier, 2002; Tapsoba and Deschamps 2006). Several studies have shown the presence of anthocyanins and tannins in extracts of the fruit epicarp of *Lannea microcarpa,* fractionation and analysis of *Lannea microcarpa*'s polar extract allowed the identification of 4'-methoxy-myricetin 3-O-α-$_l$-rhamnopyranoside, myricetin 3-O-α-$_l$-rhamnopyranoside,

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myricetin 3-O-β-$_d$-glucopyranoside, vitexin, isovitexin, gallic acid and epi-catechin (Pale, 1998; Picerno *et al*., 2006). The antiradical activity of the hydro-methanolic extract's of the tree's bark (Lamien-Meda *et al*., 2008; Ouattara *et al*., 2011) and even the extract's antihypertensive activity (Ouedraogo *et al*., 2010) have been studied.

Despite the widespread use of extracts of leaves and fruits of this species, the scientific literature provides little data on the extract's antibacterial and anti-oxidative properties, especially in the case of the ecotype of the Sahel. As the results of the ethnobotanical survey showed that traditional use of the species involved the polar compounds of the extracts, the purpose of this work is to characterise the polyphenol extracts and assess their antioxidant and antibacterial activities.

## MATERIALS AND METHODS

This research was conducted at the University of Ouagadougou (Burkina Faso), UFR/SVT, Departement of Biochemistry-Microbiology, in the Laboratory of Biochemistry and the Laboratory of Microbiology during the Months from May 2010 to February 2011.

**Plant material:** The leaves and fruits of *Lannea microcarpa* were collected in the botanical garden of Gonsé (Lat/Long N 12º26, 624′ W 001º18,817′ Kadiogo Province, Burkina Faso) and authenticated by Professor Jeanne Millogo botanist. A herbarium specimen (No. 003/B) was deposited in the herbarium of the University of Ouagadougou. Fresh fruits were stored in a freezer at 4ºC before use, while the leaves were dried at room temperature and subsequently pulverised and stored in a bag before utilisation.

**Reagents and solvents:** The following reagents and solvents were used in the laboratory analysis: 1,1-diphenyl-2-picrylhyhydrazyle (DPPH), sodium chloride (NaCl), iodonitrotetrazolium violet (INT) [Sigma-Aldrich St Louis USA]; N-butanol, hexane, dichloromethane, acetone [Fluka,Seelze. Germany]; Aluminum trichloride ($AlCl_3$), dimethyl sulphoxide (DMSO) [Prolabo, rue Carnot, France]; Mueller Hinton agar and Mueller Hinton broth [Liofilchem s.r.l Bacteriology products, Italy] were used.

**Extraction:** Fifty grammes (50 g) of fruits were ground with the grain using a grinder (Microton 550 MB). Next, the paste was extracted with acetone (70%) for 24 h using an electric mixer. Fifty grammes (50 g) of *Lannea microcarpa* leaf powder were extracted with acetone (80%) following the same procedures as that used with the fruit. The various macerates obtained were filtered (through Whatman No. 1 paper), concentrated using a rotary evaporator and lyophilised. A portion of the lyophilised material that was obtained was fractionated by successive liquid-liquid partitioning with n-hexane, dichloromethane, ethyl acetate and n-butanol. The ethyl acetate and n-butanol fractions, as well as crude extracts, were used in the tests.

**Determination of total flavonoids content:** Levels of flavonoids were determined by the method of Dowd, as adapted by Arvouet-Grand *et al*. (1994). A volume of 2 mL of 2% $AlCl_3$ (in methanol) was mixed with a volume of 1.25 mL of extract (0.1 mg /mL in methanol). The absorbances were read after 10 min at 415 nm using a spectrophotometer, and the levels of flavonoids in extracts were determined from the regression equation ($Y = 0.0886 X + 25.1$, $R^2 = 0.999$) that were obtained from a dilution series of quercetin in methanol. The concentrations were calculated from the formula below. Three readings were performed per sample, and the results are expressed in mg of quercetin equivalents per 100 mg of extract (mg QE/100 mg):

$$C = \frac{c \times D \times 10}{m}$$

C = Mg QE per 100 mg of extract
C = Concentration readed
D = Dilution factor
m = Extract weight

**Determination of total polyphenols:** The total polyphenols were estimated by the method of Singleton *et al*. (1999). The method assesses all of the phenolic compounds using phosphomolybdotungstic reducing reagent or Folin-Ciocalteu (FCR). A volume of 50 µL of FCR (0.2 N in distilled water) was mixed with 10 µL of extract from leaves or fruits (0.1 mg/mL in distilled water) in a 96-well plate. After 5 min, 40 µL of $Na_2CO_3$ (75 g/L) was added to the preceding mixture. The mixtures that were obtained were left to stand in the dark for 2 h. The absorbance was subsequently read at 760 nm using a BioteckEpoch spectrophotometer. The content of polyphenol extracts were determined from the regression equation ($Y = 0.014X + 0.145$; $R^2 = 0.997$) obtained from a dilution series of gallic acid in water, and the concentrations were calculated using the formula below. Three tests were conducted, and the result was expressed as milligrammes of gallic acid equivalent to 100 mg of extract (mgGAE/100 mg).

$$C = \frac{c \times D \times 10}{m}$$

C = MgGAE per 100 mg of extract
C = Concentration readed

D = Dilution factor
m = Extract weight

**Evaluation of antiradical activity:** The antiradical capacity of leaves and fruits extracts of *Lannea microcarpa* was determined using a method measuring inhibition of DPPH ions according to the model described by Velazquez *et al*. (2003). A methanolic extract of leaves or fruits (0.75 mL) was mixed with 1.5 mL of a methanol solution of DPPH (200 mg/L). After stirring, the mixture was incubated for 15 min, and the absorbance was read at 517 nm against a control consisting of methanol (0.75 mL) and DPPH (1.5 mL). The result was expressed as microgrammes of extract inhibiting 50% of control using the following formula.

$$\text{Inhibiotion (\%)} = [(\text{Abs c} - \text{Abs e}) / \text{Abs c}] \times 100$$

**Abs c:** Absorbance of the control, Abs e: absorbance of the tested sample.

Each test was repeated three times, and the value of the IC 50 (the minimum concentration that inhibits 50% of control) was determined graphically using the plot obtained from different concentrations of each extract. A low IC 50 value indicates strong antioxidant activity.

**Antibacterial activity:**

**Bacterial strains and inoculum preparation:** The antibacterial activity of *Lannea microcarpa* (leaves and fruits) was tested on gram negative bacteria, including *Shigella dysenteria* (CIP 5451), *Escherichia coli* (CIP 105182), *Escherichia coli* (ATCC 25922), *Escherichia coli* (isolate Hospital) *Proteus mirabilis* (ATCC 6538) and *Samonella typhimirium* (Salad isolates). Gram-positive bacteria were also tested: *Bacillus cereus* (ATCC 9144) and *Staphylococcus aureus* (ATCC 6538). A bacterial culture of 18 h was diluted in a solution of 0.9% sodium chloride and standardised at $1{,}5.10^6$ cfu/mL by adjusting the optical density of 0.1 at a wavelength of 600 nm (Tereschuk *et al*., 1997).

**Evaluation of the effect on bacterial growth inhibition by the disc method:** The diameter of the inhibition zone from discs soaked in extracts of leaves and fruits of *Lannea microcarpa* was determined by the disc-diffusion method (Rabe and Van Staden, 1997). After dissolving the extracts from leaves or fruits in 10% DMSO (20 mg/mL), Sterile paper Whatman No. 1 (6 mm) discs were soaked in 15 µL of the resulting solution. The discs were then placed on agar inoculated with100 µL of a bacterial suspension ($15.10^6$ cfu/mL). Next, 10% DMSO was used as the negative control; ampicillin and ciprofloxacin as positive controls. All Petri dishes were incubated for 24 h, and the eventual zone of inhibition was measured around the discs. Diameters of inhibition (including that of the disk) ≥9 mm were considered to be antibacterial. All tests were repeated three times.

**Evaluation of the effect of inhibition on bacterial growth in the broth microdilution method:** The broth microdilution method (Ellof, 1998) was adapted for determining the minimum inhibitory concentration (MIC) with a microplate (96 wells). Fifty microliters (50 µL) of extracts of fruits or leaves of *Lannea microcarpa* were diluted in 50 µL of broth to obtain a concentration ranging from 0.1 to 10 mg/mL. The final concentration of DMSO in each well was less than 5%. Next, 5 µL of inoculum ($1.5\times10^6$ CFU/mL) was added to the test medium. The negative control consisted of 100 µL of Mueller Hinton Broth (MHB) and 5 µL of inoculum (Zgoda and Porter, 2001). The microplates were covered with sterile lids and agitated to mix the contents of the wells and were incubated at 37ºC for 24 h. Each test was repeated three times. The minimum inhibitory concentration of extracts was determined by the addition of 40 µL (0.2 mg/mL) of a solution of iodonitrotetrazolium salt (INT) after 30 min of incubation in the dark. Live microorganisms reduce INT (colourless) to a pink colour. The MIC is defined as the lowest concentration of extract which allows no visible growth (Kuete *et al*., 2008).

**Statistical analysis:** The values all tests obtained were analysed using the statistical software XLSTAT 7.5.2-ANOVA. The results were expressed as the mean value±standard deviation.

## RESULTS AND DISCUSSION

**Total polyphenol and total flavonoids contents:** The results for flavonoids and polyphenol contents and those for the antioxidant activity of *Lannea microcarpa* fruits and leaves extracts are shown in Table 1. The results presented in Fig. 1 show levels of total flavonoids ranging from 0.52±0.07 to 1.27±0.05 mg QE/100 mg for the leaf extract and its fractions and from 0.25±0.02 to 1.61±0.02 for the fruits extract and its fractions. The results presented in Fig. 3 show that the levels of total polyphenols are ranged from 1.21±0.08 to 2.36±0.02 for the fruit extracts and its fractions ($p<0.05$) and from 3.45±0.06 to 22.05±0.78 for the leaf extract and its fractions ($p<0.05$). The highest levels of flavonoids were obtained from the ethyl acetate fractions of leaves and fruits. There was an increase in the total flavonoid content with butanol and ethyl acetate fractions from the fruit extracts. For the extracts from leaves, the total flavonoid content is increased in the ethyl acetate fractions. These values (Fig. 1)show that the flavonoids in fruits and leaves of *Lannea microcarpa* are more soluble in ethyl acetate.

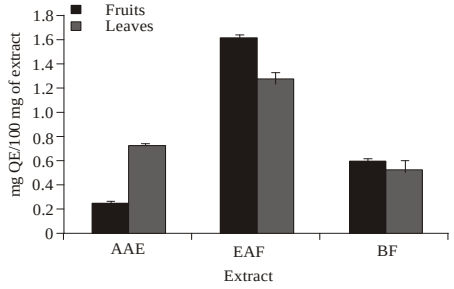


Fig. 1: Total flavonoid content of *Lannea microcarpa* fruits and leaves extract and fraction

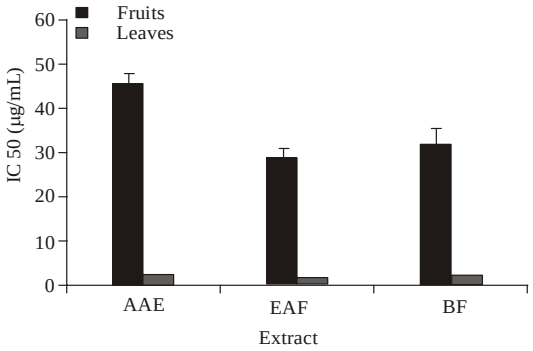


Fig. 2: Antioxidant activity of *Lannea microcarpa* fruits and leaves extract and fraction

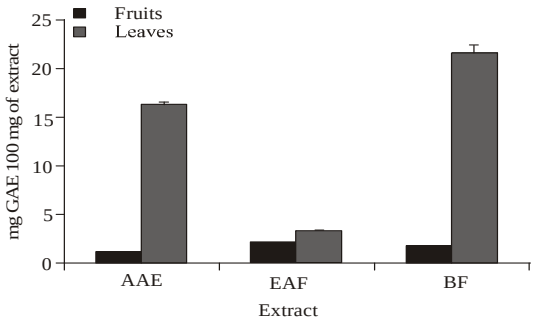


Fig. 3: Total polyphenol content of *Lannea microcarpa* fruits and leaves extract and fractions

However, the highest content of total polyphenols was obtained with the n-butanol fraction from leaves, which was followed by the macerated aqueous-acetone and ethyl acetate fractions of leaves. There is an increase of total polyphenol content of fruits in both the butanol and ethyl acetate fractions but only for the butanol fraction from leaves. Kim and Kim (2010) showed that the fractions of ethyl acetate and n-butanol have both a high total polyphenol content and high antioxidant activity. Compared to fruits, leaves have a high content of total polyphenols, but these values are lower than those obtained with extracts from bark (Ouattara *et al*., 2011). This unequal distribution of polyphenols in varying parts of the plants was observed by (Falleh *et al*., 2006).

Table 1: Total flavonoid content, total phenolic content, and antioxidant activity of extracts and fractions from fruits and leaves

| | AAE | EAF | BF |
|---|---|---|---|
| **Total phenolic content (mg GAE/100 mg )** | | | |
| Fruits | 1.21±0.08$^{e}$ | 2.36±0.02$^{d}$ | 1.96±0.02$^{de}$ |
| Leaves | 16.61±0.34$^{b}$ | 3.45±0.06$^{c}$ | 22.05±0.78$^{a}$ |
| **Total flavonoid content (mg QE/100 mg)** | | | |
| Fruits | 0.25±0.02$^{c}$ | 1.61±0.02$^{a}$ | 0.60±0.02$^{b}$ |
| Leaves | 0.72±0.03$^{b}$ | 1.27±0.05$^{a}$ | 0.53±0.07$^{bc}$ |
| **IC50 (µg/mL)** | | | |
| Fruits | 46.67±2.05$^{c}$ | 29.55±1.80$^{b}$ | 32.67±3.77$^{b}$ |
| Leave | 2.57±0.12$^{a}$ | 1.55±0.19$^{a}$ | 2.27±0.11$^{a}$ |

Data are means±SEM (n = 3); Values showing the same letter are not significantly different (p>0.05) from another in the same line

**Antioxidant activity:** Antioxidant activity was determined by calculating the IC50, as expressed in microg/mL. As showed in Fig. 2, the IC50 values are ranged from 46.67±2.05 to 29.55±1.8 for the fruit extracts of *Lannea microcarpa* and from 2.57±0.12 to 1.55±0.19 for leaves extracts. These results show that the aqueous-acetone maceration extract of leaves and their fractions are generally more active than the fruit extract and its fractions. However, we noted a low activity of the leaf extracts of *Lannea microcarpa* compared to the activity of quercetin (0.60±0.01 microg/mL). No significant difference was observed between the aqueous-acetone maceration extract of leaves and its fractions (p>0.05). Conversely, the ethyl acetate fraction and n-butanol extract of fruits show better activity compared with their aqueous-acetone maceration extract. The results show a correlation between the polyphenol contents of the extracts of leaves and fruits and their antioxidant activities (R = 0.2463, p<0.0362). Many studies have shown a correlation between the total polyphenol contents of plants and their abilities to inhibit DPPH radicals (Karou *et al*., 2005; Lamien-Meda *et al*., 2008).It is well-known that the antioxidant activities of plant extracts are primarily due to the phenolic compounds found in most plant organs, such as fruits, nuts, seeds, roots and bark (Hajaji *et al*., 2010).

**Antibacterial activity:** The result of inhibition diameters (expressed in mm) and that of the broth microdilution (expressed in mg/mL) are shown in Table 2 and 3. The diameters of inhibition ranged from 7±00 to 13.5±0.71 for the fruit extract and its fractions and from 8.5±0.71 to 17±00 for the leaf extract and its fractions. The Minimum Inhibitory Concentrations (MIC) that were obtained ranged from 0.625 to more than 10 for the fruit extract and its fractions and from 0.15 to 2.5 for the leaf extract and its fractions. These values show that the extract from leaves and its fractions are more active than that from the fruit and its fractions. All strains tested were somewhat sensitive to the extracts and fractions tested. Ciprofloxacin has a high activity on all the bacterial

Table 2: Determination of strain sensitivity

| | Inhibitory diameter (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fruits (300 µg/disc) | | | Leaves (300 µg/disc) | | | | |
| | AAE | EAF | BF | AAE | EAE | BF | Ampi (10 µg) | Cip (5 µg) |
| E c | 11±00[b] | 12.5±0.58[a] | 12±0.00[a] | 10.5±0.8[b] | 12±00[a] | 12.5±0.8[a] | 19±0 | 023.5±0.58 |
| *E c H* | 11±00[d] | 12±00[c] | 11±00[d] | 12.5±0.8[c] | 16.5±0.58[a] | 13.5±0.8[b] | 28.5±0.071 | 33.5±0.58 |
| *E c a* | 9±00[d] | ND | 12±00[c] | 13±00[b] | 16±00[a] | 15.5±0.1[a] | 6±00 | 28.5±0.58 |
| *St*(sal) | 10.5±0.8[d] | 10.5±0.58 | 10±00 | 14±00[b] | 15±00[a] | 12±00[c] | 6±00 | 30±00 |
| *P m* | 11±00 | ND | ND | 8.5±0.58 | 10±00 | 9±00 | 6±00 | 27±00 |
| *S d* | 7±00[d] | 7±00[d] | 9±00[c] | 14±00[b] | 17.5±0.58[a] | 6±00 | 6±00 | 25.5±0.58 |
| *B c* | 10.5±0.8 | 12.5±0.58[b] | ND | 11±00 | 14±00[a] | 13.2535[ab] | 6±00 | 25±00 |
| *S a* | 13.5±0.8[b] | 11.5±0.58 | 12±00[c] | 10±00 | 16±00[a] | 11.5±0.8[c] | 6±00 | 24.5±0.71 |

Data are means±SEM (n = 3); ND: Not determined; Ec: *Escherichia coli* (ATCC 25922); Ec a: *Escherichia coli* CIP 105 182; Ec H: *Escherichia coli* (Hospital isolate); S t: *Samonella typhimirium* (Salad isolate); Sd: *Shigella dysenteria* (CIP 5451); S a: *Staphylococcus aureus* (ATCC 6538); Ampi: Ampicilline; Cip: Ciprofloxacine; Values showing the same letter are not significantly different (p>0.05) from another in the same line

Table 3: Antibacterial activity of extracts and fractions from the fruits and leaves of *lanneamicrocarpa*

| | MIC (mg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Fruits | | | Leaves | | |
| | AAE | EAF | BF | AAE | EAF | BF |
| *E. coli* | 05.00 | 02.50 | 02.50 | 01.25 | 01.25 | 01.25 |
| *E. coli* (hospital) | 10.00 | 10.00 | 05.00 | 01.25 | 00.63 | 00.63 |
| *E. coli* a | 01.25 | ND | ND | 01.25 | 01.25 | 01.25 |
| *S. typhi* (salad) | 05.00 | 05.00 | 01.25 | 00.63 | 00.32 | 00.32 |
| *P. mirabilis* | 05.00 | ND | ND | ND | 05.00 | ND |
| *S. dysenteriae* | ND | ND | >10 | 00.32 | 00.63 | ND |
| *B. cereus* | 10.00 | 05.00 | >10 | 00.32 | 00.16 | 00.16 |
| *S. aureus* | 00.63 | 05.00 | 10.00 | 02.50 | 00.32 | 01.25 |

ND: Not determined

strains tested compared to the activity from the plant extracts. However, ampicillin is less active than the extracts, except with *Escherichia coli* (hospital isolate) and *Escherichia coli* (ATCC 25922). The most sensitive bacterial strain to the extracts tested was Bacillus cereus (ATCC 9144) while the least sensitive was *Proteus mirabilis* (ATCC 6538). The antibacterial activity obtained with extracts of *Lannea microcarpa* leaves could justify their use in traditional medicine for treating ailments, such as diarrhoea, gastroenteritis, bacterial infections, toothaches and wound (Arbonnier, 2002; Tapsoba and Deschamps, 2006).

These results also show that the ethyl acetate fraction was the most active of all extracts and fractions tested. The antibacterial activity observed with this fraction extract could be due to the presence of polyphenol compounds, such as tannins, phenolic acids and flavonoids (Bruneton, 1999; Oleivera *et al*., 2008). Numerous studies on polyphenol compounds indicate that they have antibacterial activity (Zhentian *et al*., 1999; Meng *et al*., 2001; Berahou *et al*., 2007).

The fruit extract and its fractions showed moderate antibacterial activity against most of the bacterial strains tested. However, we noted a significant inhibition of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 6538) by the extract and fractions from the fruit of *Lannea microcarpa*. *Escherichia coli* and *Staphylococcus aureus* are recognised as food contaminants (Al-Zoreky and Nakahara, 2003). Thus, extracts from *Lannea microcarpa* fruits could be used as food additives or preservatives.

## CONCLUSION

This study demonstrates the antibacterial and antioxidant power of extracts and fractions obtained from the leaves and fruits of *Lannea microcarpa*. The leaf extracts have greater antibacterial and antioxidant activity than do the fruit extracts. The observed antibacterial activity may justify the use of leaves in traditional medicine to treat bacterial infections, diarrhoea, gastroenteritis, and toothaches. The extracts from the fruit of *Lannea microcarpa* showed moderate antibacterial and antioxidant activity compared to leaves extracts. However, the fruit of *Lannea microcarpa* could play an important role in the food industry as an additive, specifically as a colorant or antioxidant. This fruit could also be used as preservative because of it antibacterial activity. Further studies should be performed to isolate the bioactive compounds that are responsible for the biological activities of fruits and leaves of *Lannea microcarpa*.

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