Asian Journal of Chemical Sciences

6(3): 1-9, 2019; Article no.AJOCS.51333
ISSN: 2456-7795

Phytochemical Study and Biological Activities of Two Medicinal Plants used in Burkina Faso: *Lannea velutina* A. Rich (*Anacardiaceae*) and *Ximenia americana* L. (*Olacaceae*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DP and JYPN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SG, MN and AH managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2019/v6i318997
Editor(s):
(1) Prof. Pradip K. Bhowmik, Department of Chemistry, University of Nevada Las Vegas, Maryland Parkway, USA.
Reviewers:
(1) Maria Bintang, IPB University (Bogor Agricultural University), Indonesia.
(2) Debarshi Kar Mahapatra, Dadasaheb Balpande College of Pharmacy, India.
Complete Peer review History: https://sdiarticle4.com/review-history/51333

Original Research Article

Received 28 June 2019
Accepted 04 September 2019
Published 28 September 2019

ABSTRACT

Background: Bacterial infections are at the origin of a number of serious pathologies, including meningitis, bronchopneumopathies, typhoid fever and especially diarrhea, which are still a real public health problem for the child population in developing countries. In most of these countries, people use medicinal plants for primary health care. The objective of this study was to determine the content of phenolic compounds, the antioxidant and antimicrobial activity of extracts from two plants used in traditional medicine in Burkina Faso, *Lannea velutina and Ximenia americana.*
Place and Duration of Study: Laboratory of Biochemistry and Applied Chemistry (LABIOCA).
Methods: The total phenolics and flavonoid content of the ethanolic extract extracts were determined by spectrometric assay. The DPPH and FRAP method were used to measure the

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antioxidant capacity of the extracts. The antibacterial potential was determined on five bacterial strains.
Results: The ethanolic extracts of *Lannea velutina* showed the best polyphenol content with 969.67±8.23 mgGAE/g extract against 753.145917±66.31 mgGAE/g extract for *Ximenia americana.* On the DPPH radical *Ximenia americana* gave the best activity with a percentage inhibition of 62.32±0.17% compared to *Lannea velutina*. This species also gave the best reducing activity with a reducing capacity of 3.45±0.97 mmol EAA/10 g of extract. *Shigella dysenteria* and *Staphylococcus aureus* were susceptible to *Lannea velutina* and *Ximenia americana* with inhibition diameters greater than 8 mm.
Conclusion: These results showed that the extract has good antioxidant potential as well as antimicrobial activity. These extracts could be used to prevent damage from oxidative stress and infections.

Keywords: Antibacterial; antioxidant; medicinal plants; phenolic compounds.

1. INTRODUCTION

In West Africa, as in most African countries, people use medicinal plants for primary health care. Currently the World Health Organization (WHO) estimates that about 80% of populations use traditional herbal medicines for their primary health care [1]. The craze shown for the use of these plants is necessarily linked to their effectiveness against diseases. Also we can mention the resistance of certain germs to the treatments of conventional medicine called modern medicine; adverse effects of synthetic pharmaceutical drugs that drive these populations from underdeveloped countries to traditional medicine.

Bacterial infections are at the origin of a certain number of serious pathologies including meningitis, bronchopneumopathies, septicemia, peritonitis, typhoid fever and especially diarrhea which still remain a real public health problem for the infant population in developing countries [2].

Infection in an organ promotes the onset of oxidative stress that further destroys the body tissue. In bacterial infections, oxidative stress comes, at least in part, from altered metabolic pathways and is also involved in organic lesions and the development of malignant tumors [3]. With these public health problems, medicinal plants could be a therapeutic response that can be adapted and accessed by the population.

In Burkina Faso *L. velutina* and *X. americana* are two herbs used in traditional medicine against various ailments such as diarrhea, arterial hypertension, fever, jaundice, constipation, female sterility ect .

L. velutina is used in Mali for the treatment of pain, gastric ulcer, wounds, respiratory diseases and fever [4]. The species has shown positive results for its antifungal, molluscicidal, antioxidant and larvicidal activity on larvae of Aedes aegypti, Anopheles gambiae and Culex quinquefasciatus mosquitoes [5]. It is rich in rich in anthocyanins, hydroxyanthracene derivatives, coumarins, flavonoids, leucoanthocyanins, tannins, sterols and triterpenes [6].

X. americana is used to treat cancer, its leaves and stem have antimicrobial, antitrypanosomal, mollucicidal and analgesic activities [7]. Kabran, (2012) [8] showed that fruits of *X. americana* contain flavonoid aglycones, coumarins. The leaves contain cyanogenic glycosides of saponosides and the bark is rich in tannins [9]. Phytochemical screening of the aqueous extract of stem bark of *X. americana* revealed the presence of flavonoids and saponins which could be at the origin of the anti-inflammatory properties [10].

The objective of this study was to determine the antioxidant and antimicrobial capacity of ethanolic extracts of *L. velutina* and *X. americana* two medicinal plants from Burkina Faso, with the aim of contributing to the search for the beneficial use of these plants on the health.

2. MATERIALS AND METHODS

2.1 Plant Material

The barks *Lannea velutina* and the leaves of *Ximenia Americana* (Photo 1) were harvested in Lounbila (locality located 15 Km from Ouagadougou, Burkina Faso) in March 2019. The species were authenticated and herbaria were deposited at the herbarium of the UFR/ SVT (University of Ouagadougou) under the respective identification codes ID 16748 and ID

16730. The samples were dried under laboratory conditions, sheltered from the sun, then pulverized and kept in freezer bags for different extractions heading. The fine and homogeneous powder was obtained using a sieve of mesh 0.5 mm.

2.2 Bacterial Strains

Five bacterial strains were used, 3 Gram-negative bacteria (*Escherichia coli ATCC8739, Salmonella typhi, Shigella dysenteria*) and 2 Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, *Bacillus cereus*). These strains come from the Microbiology Laboratory of the University of Ouagadougou.

2.3 Extraction

The powder (50 g) of each plant material (*Lannea velutina* and *Ximenia americana*) was placed in bottles containing 500 ml of absolute ethanol. The bottles were subjected to mechanical stirring for 24 h at room temperature. The macerates were filtered and then concentrated in an evaporator equipped with a vacuum pump and then evaporated to dryness. These extracts are stored and used for different tests.

2.4 Phytochemistry

2.4.1 Determination of polyphenols

The total polyphenols of the extracts were determined by the method described by Singleton (1999) [11]. The extracts are dissolved in pure methanol to have a concentration of 0.1 mg/ml. A volume of 25 µl of the diluted solution (0.1 mg / ml) was then mixed with 125 .µl of the Folin reagent ciocalteu at 0.2 N and incubated for 5 min. 100 µl of a solution of sodium carbonate at 75 g/l in distilled water is added and the mixture incubated for 2 hours. At the end of the incubation, the optical densities are read at 760 nm using a spectrophotometer. Total phenolic contents are determined using a standard curve with gallic acid (y=0.0249x, R^2=0.99) as standard. The results are expressed in milligrams of gallic acid equivalent per 1 g of dry extract (mg GAE/1 g).

2.4.2 Determination of flavonoids

The total flavonoid contents of the extracts were determined by the colorimetric method described by Arvouet-Grand et al. (1994) [12]. A volume of 75 µl of 2% $AlCl_3$ in pure methanol is mixed with an equal volume of 1 mg / ml extract in methanol. The optical densities are read after 10 min at 415 nm using the spectrophotometer. Quercetin served as a standard for the development of the calibration curve. A mixture of 75 µL of extract and 75 µL of methanol without $AlCl_3$ served as a blank. In total, three (3) analyzes are performed for each extract and the result gave is an average of the three readings. The results are expressed in milligrams equivalent quercetin per 10 g of dry extract (mg EQ / 10 g)

2.5 Antioxidant Activities

2.5.1 Inhibition of the radical DPPH (2,2diphenyl-1-picrylhydrazyl)

The anti-radical activity of the ethanolic extracts (1 mg/ml) was evaluated by the DPPH method.

1 **2**

Photo 1. *Ximenia Americana (1)* and *Lannea velutina (2)*

(2,2-diphenyl-1-picrylhydrazyl) described by Velazquez et al., (2003) [13]. This method is based on the reduction of the absorbance at 517 nm of the stable free radical DPPH., In the presence of a radical donor H. Three (03) tests were carried out by mixing 100 µl of sample and 200 µl of DPPH (20 mg/L in methanol). The absorbance is read at 517 nm against a blank (100 µL of methanol and 200 µL of DPPH) using a spectrophotometer after 15 minutes of incubation. Gallic acid was used as a reference substance. The antiradical activity was expressed as a percentage inhibition

2.5.2 Ferric reducing antioxidant power (FRAP)

The FRAP method is based on the reduction of ferric ion (Fe^{3+}) in ferrous ion (Fe^{2+}) by the reducing compounds following electron-monoelectron transfer. The determination of the reducing power of the plant extract has been evaluated as described by Hinneburg et al., (2006) [14]. In a test tube containing 0.5 ml of test extract (1 mg / ml), 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium hexacyanoferrate. (1% aqueous) were added. The mixture was heated at 50°C in a water bath for 30 minutes. After cooling, trichloroacetic acid (1.25 mL, 10%) was added and the mixture was then centrifuged (2000 rpm for 10 minutes). Three aliquots (125 µl) of the supernatant were transferred into a 96-well microplate to which 125 µl of distilled water and then 25 µl of $FeCl_3$ (0.1% aqueous) were added. The evaluation of the reducing power was carried out at 700 nm against a standard curve of ascorbic acid) using a spectrophotometer. The reducing activity of the extract was expressed in mmol Equivalent Ascorbic acid per gram of extract (mmol EAA / g extract)

2.6. Antimicrobial Activity

The method described by Lennette et al. (1987) [15] reported by Mihin et al. (2019) [16] was used. A suspension of each bacterial strain was prepared in 10 mL of Mueller-Hinton Broth for 18-24 hours at 37°C. Using the sterile diluent (physiological saline), the concentration was adjusted in each tube to about 1.0 10^8 CFU/mL comparable to that of the McFarland 0.5 standard.

Evaluation of the bacterial growth inhibition effect by the well method:

The diameter of the inhibition zone due to the extracts was determined. A volume of 10 µl of extract (20 µg/ml) solubilized in 10% DMSO was placed in the wells previously made using a Pasteur pipette on a Mueller-Hinton agar inoculated by flooding with the bacterial suspension equivalent to Mac Farlan. All Petri dishes were incubated for 24 hours. All tests were repeated in duplicate. The results were read by measuring the diameters of the zones of inhibition corresponding to the clear zone around the wells [17]. The sensitivity of the strains was classified according to the diameters of inhibition [18]. Indeed, the microbial strain is insensitive to a diameter of less than 8 mm, sensitive for a diameter of between 9 mm to 14 mm, very sensitive for a diameter of between 15 mm to 19 mm and extremely sensitive for a diameter of inhibition Greater than 20 mm.

2.7 Statistical Analysis

All results were expressed as the mean value of several independent experiments (n=3) ± standard deviation. For statistical analysis, Graph Pad Prism software (version 5.0) and MS Excel software were used to obtain standard curves and graphs, percentages of inhibition, averages and standard deviations. Anova one way followed by the Tukey test was used to measure the degree of statistical significance of the results. A significant difference was considered for $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemistry

Total flavonoids and total polyphenols were quantified from pre-established quercetin and gallic acid standard curves. the ethanolic extracts of *Lannea velutina* and *Ximenia americana* showed high levels of polyphenol with respectively 969.67±8.23 mg GAE/g of extract and 753.145917±66.31 mg GAE/g of extract (Fig. 1). For flavonoid contents, the crude extract of *L. velutina* yielded 1.77±0.005 mg eq Q/10 g of extract against 1.97±0.08 for *X. americana*. (Fig. 1)

3.1.2 Antioxidant activities

The antioxidant capacity of extracts of both plants was evaluated in vitro by the use of different tests of antioxidant activities involving

different mechanisms of action. The extracts of *Lannea velutina*, and those of *Ximenia americana* showed good antioxidant activity.

For the test on the free radical potential on the radical DPPH *X. americana* gave the best activity with a percentage inhibition of 62.32±0.17% compared to *L. velutina* which showed a percentage inhibition of 52.8125±2.16% (Fig. 2.). But the antiradical activity of the extracts is lower than that of the gallic acid used as reference substance.

The reducing power of the extracts measures their ability to reduce ferric Fe^{3+} ion to ferrous Fe^{2+} ion. The best reductive activity is obtained with the extract of *X. americana* which gave a reducing capacity of 3.45±0.97 mmol EAA/10 g of the extract against 1.74±0.45 mmol EAA/10 g of extract for *L. velutina* (Fig 3). Quercetin used as a reference substance gave a better activity than our plant extracts.

3.1.3 Antimicrobial activity

The antimicrobial activity allowed to determine the ability of the extracts to inhibit bacterial growth.

Fig. 4. shows the inhibition diameters of the extracts according to the microbial strains. The inhibition diameters vary between 6 mm and 10 mm for the extracts.

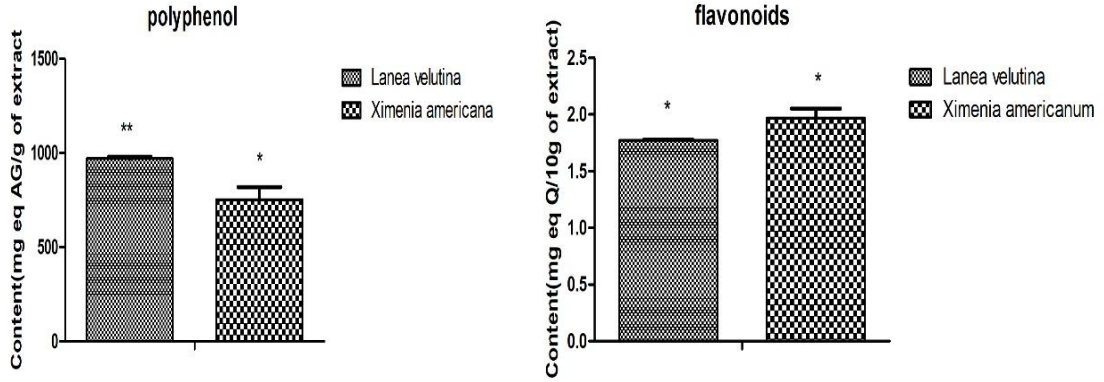

Fig. 1. Content of polyphenol and flavonoid extracts

***P-value is significant at p < 0.05; mean ± S.E.M. = mean values ± standard error of means of three experiments*

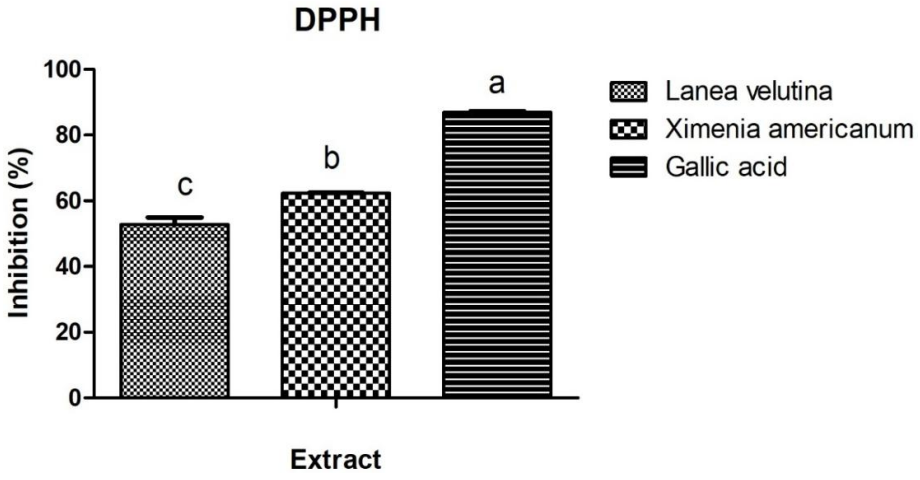

Fig. 2. The radical DPPH inhibition of extract

Results indicated by different letters are statistically distinct (p <0.05; Mean ± S.E.M = Mean values ± Standard error of means of three experiments)

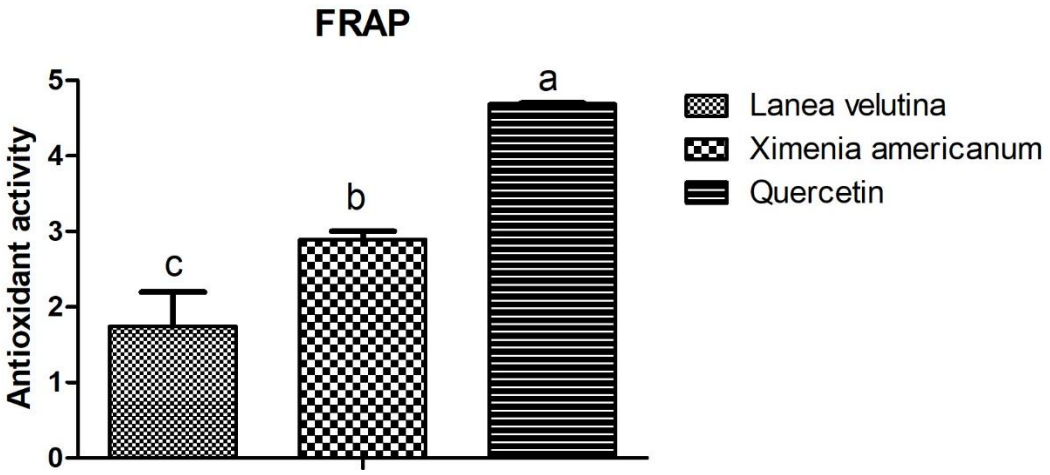

Fig. 3. Ferric reducing antioxidant power of extracts

Results indicated by different letters are statistically distinct (p <0.05; Mean ± S.E.M = Mean values ± Standard error of means of three experiments)

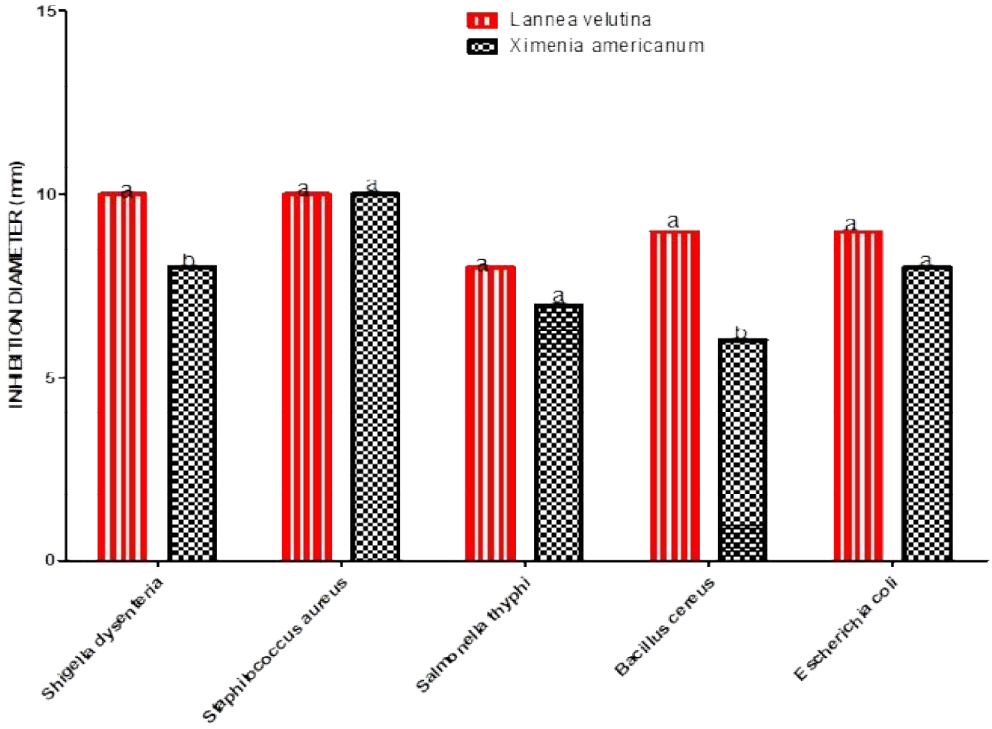

Fig. 4. Antimicrobial activity of extracts

Results indicated by different letters are statistically distinct (p <0.05; Mean ± S.E.M = Mean values ± Standard error of means of three experiments)

According to the recommendations of Negreiros et al. (2016) [18], *Shigella dysenteria* was sensitive to extracts of *Lannea velutina* and *Ximenia americana* with inhibition diameters of 10 and 8 millimeters respectively. *Staphylococcus aureus* was also sensitive to both extracts with 10 millimeters each as the inhibition diameter. *Salmonella thyphi, Bacillus cereus and Escherichia coli* were insensitive to *X. americana* extract with inhibition diameters of 7, 6 and 8 respectively. *Bacillus cereus, Escherichia coli* are sensitive to the extract of *L. velutina*.

4. DISCUSSION

Lannea velutina and *Ximenia americana* gave good polyphenol and flavonoid content. Our results corroborate with those of Ouattarra et al., 2011, [19] and Soro, 2015 [10] who respectively found that *L. velutina* and *X. americana* are rich in flavonoids.

Phenolic compounds generally have several biological properties such as anti-artherogenic, anti-inflammatory, hepatoprotective, antimicrobial, antiviral, antibacterial, anticarcinogenic, antithrombotic, cardioprotective and vasodilatory activities [20,21]. Some flavonoids such as quercetin also inhibit βcatenin, an intracellular protein involved in the regulation of cell proliferation of gastric and colorectal epithelia [22]. The presence of flavonoids in these two extracts could explain the use of these species against certain pathologies such as cancer, diarrhea, malaria, respiratory disorders, as analgesic. ect. These pathologies cause an increase in oxidative stress in patients. organizations. The extracts of *L. velutina* and *X. americana* have good antioxidant activity. They showed a good ability to neutralize the radical DPPH, and to reduce the iron ion. The polyphenols present in these species are able to trap free radicals permanently generated by an organism or formed in response to aggressions of our environment (bacterial infection, pollution ... ect). Some flavonoids have a potential ability to chelate metal ions such as Fe^{2+} and Cu^{+} that play a critical role in oxygen metabolism and free radicals. This potential allows the extracts to neutralize free radicals produced during infections.

Gram-positive bacteria such as *Staphilococcus aureus* and *Bacillus cereus* and gram-negative bacteria (*Escherichia coli, Shigella dysenteria*) are susceptible to *L. velutina* extract. Ouattarra et al., 2011 [19] also obtained a sensitivity of *Staphilococcus aureus* to *L. velutina. Staphylococcus aureus* is responsible for infections such as boils, paronychia, arthritis, pneumonitis, meningitis and urinary tract infections [23]. *B. cereus* is responsible for intoxications resulting in watery diarrhea, abdominal pain and occasional nausea [24].

Shigella dysenteria most often causes diarrhea, bloody and dysentery [25], *Escherichia coli* may be responsible for intestinal or urinary infection.

The antibacterial compounds act by different mechanisms: by preventing the synthesis of the bacterial wall, by inhibiting the synthesis of proteins and nucleic acids of the bacterium, by disrupting the structure and function of the bacterial membrane or by blocking the metabolic pathways by inhibition of major enzymes [26].

The inhibitory activity of the growth of bacteria obtained with the extracts could be due to the presence of flavonoids detected in this plant. Polyphenols, especially flavonoids and tannins, are recognized for their toxicity to microorganisms. The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins [27]. Some flavonoids would affect intracellular replication and thus reduce the infectious properties of bacteria [28]. Walid et al., 2015 [29] isolated quercetin (flavonoids) in the methanolic extract of *Shinus molle L*. which showed a very good antibacterial activated on *Bacillus subtilits, Staphylococcus aureus and Escherichia coli.* Noura et al., 2016 [30], have also shown that flavonoids such as quercetin and genistein were active on *Escherichia coli.* Valdiléia et al., 2016 [31] Isolated quercetin in *X. americana* extract, the presence of this flavonoid in this species could explain the good antibacterial activity obtained with our extracts and the traditional use of *X. americana* against microbial infections.

5. CONCLUSION

This study showed that the ethanolic extract of *Lannea velutina* and *Ximenia americana* have a good content of polyphenol and flavonoids. They have good antioxidant potential as well as antimicrobial activity. These extracts could be used to prevent damage from oxidative stress such as arterial hypertension, rheumatism and cancer. Our results confirm the traditional use of these plant species against bacterial infections. The determination of the bioactive molecules of these extracts as well as their mode of action is necessary for the production of a phytomedicine which will be accessible by the populations.

ACKNOWLEDGEMENTS

This work was supported by Laboratory of Biochemistry and Applied Chemistry in Burkina Faso. Special thanks to Center for Research in Nutritional Food Biological Sciences for their microbiological analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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