

Chemical Composition, Antioxidant, Antimicrobial And Acetylcholinesterase Inhibitory Properties of *Lannea Barteri* (Anacardiaceae)

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Abstract: *Lannea barteri* (Oliv.) Engl (Anacardiaceae) is a medicinal plant used in west African countries such as Côte d'Ivoire for the treatment of various diseases (wound, rheumatic, diarrhoea). Dichloromethane and methanol extracts from the roots and stem bark of *L. barteri* were screened for their antibacterial, antifungal, radical scavenging and acetylcholinesterase inhibitory activities. TLC bioautography and agar overlay assay for antifungal activity were run with *Cladosporium cucumerinum*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *lycopersici* and *Candida albicans* respectively. Also extracts were tested on bacteria (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*), some of which were multidrug resistant bacteria. DPPH and Acetylcholinesterase solutions sprayed on TLC plates were used for radical scavengers and acetylcholinesterase inhibitors. *L. barteri* gave high positive responses in all four tests, exhibiting activity against bacteria, fungi, free radicals and acetylcholinesterase. The phytochemical screening showed that all the extracts contained at least trace amount of steroids, terpenoids, saponins, quinones, tannins and flavonoids. This study which is the first report on the biological activities and phytochemicals of *Lannea barteri*, supports its traditional uses in the treatment of infectious and non infectious diseases.

Key words: Acetylcholinesterase inhibitors, Antibacterial, Antifungal, Côte d'Ivoire, Free radical scavengers, *Lannea barteri*.

INTRODUCTION

The increasing incidence of microbial diseases and non infectious diseases in man and associated therapeutic difficulties required the search for new drugs. Bacterial and fungal infections are widespread worldwide. Antimicrobial resistance is a global problem that affects all countries (Chatterjee and Fleck, 2011). It is not a new problem but one that is becoming more dangerous, particularly in West African countries where high prevalence of multidrug resistant bacteria (MRSA, bacteria producing beta-lactamases) have been reported (Aka, *et al.*, 1987; Keasah, *et al.*, 1998; Okesola, *et al.*, 1999; Benbachir, *et al.*, 2001; Kacou-N'Douba, *et al.*, 2001; Akoua, *et al.*, 2004; Akinyemi, *et al.*, 2005). This emergence of resistance to numerous antibiotics curbs the control of microbial infections.

In addition, morbidity and mortality of non transmitted diseases are on the increase within the populations including the young. One of the main etiologic factors of these ailments is free radicals which are implicated in number of pathological conditions (Thomas and Kalyanaraman, 1997; Ferry, 2004; Harman, 2009). High levels of free radicals tend to cause increased cellular damage and are responsible for oxidative stress that may contribute to cardiovascular and inflammatory diseases, AIDS, neurodegenerative diseases (Alzheimer, Parkinson), diabetes, cancer and ageing. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (Halliwell, 1994; Harman, 2009).

The pathogenesis of Alzheimer's disease has been linked to a deficiency in the brain neurotransmitter acetylcholine (Tabet, 2006). Subsequently inhibitors of acetylcholinesterase currently form the basis of the newest drugs available for the management of this disease (Marston, *et al.*, 2002).

Against these public health problems, urgent and consolidated efforts are required at local and national level in West African countries. Such as an effort should continue the search for new drugs. Bioactive plant constituents play an important role in various fields, ranging from medicine, over agriculture to veterinary medicine (Cepleanu, 1993). These chemical compounds found in some medicinal plants may decrease the accumulated effects of a disease, thus helping to cure or prevent infectious and non infectious diseases. As such medicinal plants are still widely used by populations who have strong beliefs in the use and efficacy of ethnomedicines (Chinsebu and Hedimbi, 2010).

Such a medicinal plant is *Lannea barteri* that stem bark and roots are consumed by natives from Northern Côte d'Ivoire, as traditional remedies for the treatment of diarrhoea, gastritis, rheumatic, sterility, intestinal

helminthiasis (Koné, *et al.*, 2005). Also, in different West African areas, this plant species is prescribed against oedema, rickets, wounds, scurvy, scorbout, epilepsy (Arbonnier, 2002). *L. barteri* is a tree with a height of 12 m, usually highly branched and distributed throughout the Sudanese and Guinean Savannah (Arbonnier, 2002).

The present study investigates effects of *Lannea barteri* against bacteria, human and plant pathogenic fungi, free radicals and acetylcholinesterase. The plant also was analyzed with phytochemical screening methods.

MATERIALS AND METHODS

Plant Materials:

The stem bark and roots of *Lannea barteri* were collected in September 2009 from the savannah of the Ferkessedougou region (Northern Côte d'Ivoire). Plant species was identified by the ivorian botanist Téré Henri and was deposited at the herbarium of the "Centre Suisse de Recherches Scientifiques en Cote d'Ivoire" (CSRS). The authentication of botanical name was made using the Flora of West Africa (Hutchinson and Dalziel, 1954).

Preparation of Extracts and TLC:

The air-dried and grounded stem bark and roots were successively extracted at room temperature, with dichloromethane (DCM), 3 x 24 h, using a 5-fold excess of solvent, followed by methanol (MeOH). The DCM and MeOH were concentrated in a rotavapor at 40 °C to dryness and then lyophilized. For the study of antifungal, radical scavenging activities and acetylcholinesterase inhibitory activities, each extract (10 mg/ml in methanol) was applied on aluminium or glass-backed silicagel 60 F₂₅₄ plates (Merck). The plates were eluted in mobile phases: hexane-ethyl acetate (1:1) for dichloromethane extracts and chloroform-methanol-water (65:35:5) for polar extracts (methanol). Two plates were developed for each assay: one for the biological test and the other as a reference, detected with vanillin/sulphuric acid reagent (Godin, 1954).

Antifungal Assays:

The extracts were tested for antifungal activity by direct bioautography on aluminium-backed TLC sheets (Homans and Fuchs, 1970; Goustein, *et al.*, 1984) against plant phytopathogenic filamentous namely *Fusarium oxysporum* f. sp. *vasinfectum* and *Fusarium oxysporum* f. sp. *lycopersici*. Activity against the yeast *Candida albicans* was assessed on glass-backed TLC plates in agar overlay assay (Rahalison, *et al.*, 1991). After elution, the chromatograms were thoroughly dried to remove any solvent residues before being sprayed with suspension of each fungus. Nystatine (Sigma) and Amphotericin B (Sigma) were used as controls.

Test against *F. oxysporum* strains:

Inoculated Sabouraud maltose liquid medium with *F. oxysporum* f. sp. *vasinfectum* was sprayed on TLC. Clear inhibition zones were observed against a blue-reddish background after 48 h incubation at room temperature in humid atmosphere. Conidial suspension of *F. oxysporum* f. sp. *lycopersici* was supplemented with a solution of thiazolium (0.25 % MTT) before being sprayed on the TLC. The activity of the extract appears as clear inhibition zones against reddish background 48 h post-incubation.

Test against *Candida albicans*:

Inoculated malt agar medium was overlaid on the TLC plate, which was then incubated overnight at 30°C. Then, plates were sprayed with solution of thiazolium (MTT). The active compounds appeared as clear zones against purple background 2 h after incubation.

Radical Scavenging Assays with DPPH:

The TLC-DPPH assay was performed as described by (Takao, *et al.*, 1994; Cuendet, *et al.*, 1997). Briefly, after developing and drying, aluminium-backed TLC sheets were sprayed with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution (2 mg/ml in MeOH). Active compounds appeared as yellow spots against purple background.

The extracts showing qualitative positive reaction were selected for quantitative estimation of radical scavenging activity (RAS) according to the method described by (Blois, 1958). 2.5 ml of 0.04% DPPH radical solution (MeOH) was added to each extract solutions (100 µl) ranging from 192.5 to 6 µg/ml. The mixtures were vortex-mixed and kept under darkroom conditions for 30 min. The optical density (OD) was measured at 517 nm. Methanol was used as baseline control. Ascorbic acid and Gallic acid were used as positive controls. The antioxidant activity was carried out in triplicate and the reading was averaged. The scavenging activity was measured as a decrease in absorbance of the samples versus DPPH standard solution. The DPPH radical concentration was calculated using the following equation: Scavenging effect (%): $(A_0 - A_1) \times 100\%/A_0$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of tested extracts.

The IC₅₀ (concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs the corresponding scavenging effect.

Detection of Acetylcholinesterase Inhibitors:

The extracts were screened for acetylcholinesterase inhibitory activity by bioautography on thin-layer chromatograms (Marston, *et al.*, 2002). After migration and drying, the chromatograms were sprayed with the enzyme stock solution and thoroughly dried again. After incubation at 37°C during 20 min, 10 ml of the naphthyl acetate solution and 40 ml of the Fast Blue B salt solution were mixed and sprayed onto the plate to give a purple coloration after 1-2 min.

Antibacterial Assays:

The antibacterial screening was carried out on 15 reference cultures and clinical isolates, provided by the Institut Pasteur de Côte d'Ivoire. The bacteria included Gram positive strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis* and *Enterococcus faecalis*.) and Gram negative ones (*Escherichia coli* and *Pseudomonas aeruginosa*). Some strains were sensitive and some were multidrug resistant (MRSA). The lyophilized plant extracts were solubilized in DMSO (30 mg/ml) and serially diluted from 1500 to 3 g/ml. Two methods were used to assess the antibacterial activity: a diffusion method on agar plates and microdilution in liquid medium. First, susceptibility of all the bacteria to crude extracts was determined using the diffusion method. The extracts showing an inhibitory diameter of at least 8 mm were selected for quantitative assessment of inhibitory (MIC) and bactericidal (MBC) concentrations in Mueller Hinton liquid medium (Koné, *et al.*, 2004) in triplicate. Tetracycline (Sigma) and gentamicin (Sigma) were used as controls.

Phytochemical Screening:

For phytochemical investigations, 10 g of air-dried material were successively extracted with ten-fold excess of petroleum ether, ethyl acetate and methanol. The extraction was carried out at room temperature under constant agitation during 3 h. Extracts were filtered on Whatmann paper. Separately, an aqueous extract was prepared from 10 g mixed with 100 ml of distilled water and boiled during 15 min. Then the extract was filtered on Whatmann paper. Phytochemical tests for bioactive constituents were carried out on portions of these extracts using standard phytochemical procedures (Kapoor, *et al.*, 1969; Harborne, 1973; Pelissier, 1981). By this analysis, the presence of terpenoids, steroids, saponins, alkaloids, phenolic compounds, tannins, flavonoids and anthraquinones.

Estimation of Total Phenolic Content:

Total phenolic content of *L. barteri* roots and stem bark was determined using the Folin-Ciocalteu reagent method (Singleton, *et al.*, 1965; Lister and Wilson, 2001). To 500 µl of each extract dilutions (6, 12, 24, 48, 96 and 192 µg/ml), 2.5 ml of Folin-Ciocalteu reagent (1/10 dilution) were added. After 2 min incubation at ambient temperature, 2 ml of 7.5% Na₂CO₃ (w/v) were added and mixed well. The blend was incubated at 50°C for 15 min. The absorbances of all samples were measured at 765 nm with Na₂CO₃ solution (2 ml of 7.5% Na₂CO₃ in 2 ml of distilled water) as blank in triplicate determination. The results were expressed as GAE (Gallic acid equivalence) in µg/g.

Statistical Analysis:

The data were presented as mean ± standard deviation (SD) for the three determinations. The data of DPPH assays were analyzed for statistical significance using analysis of variance (one-way ANOVA) with STATISCA 8.0.

RESULTS AND DISCUSSION

The stem bark and root extracts of *Lannea barteri* showed activity at least against bacteria, fungi, free radicals and acetylcholinesterase. The results of antibacterial screening revealed that *L. barteri* inhibited the growth of all tested bacteria, except *Escherichia coli* (Table 1). The most active extracts come from the methanol extracts. Quantitative assessment showed bactericidal and bacteriostatic activities, with MIC values ranging between 47 and 375 µg/ml (Table 2).

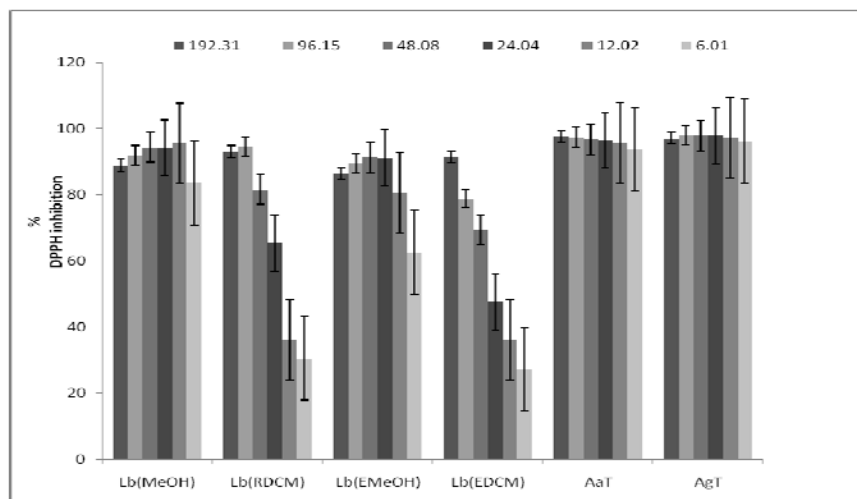


Fig. 1: Percentage of DPPH inhibition of extracts prepared from *Lannea barteri*.

Against *Fusarium oxysporum* f. sp. *vasinfectum* and *Fusarium oxysporum* f. sp. *lycopersici*, DCM and MeOH extracts of both stem bark and roots exhibited activity at 100 µg (Table 3). Only DCM extracts of both roots and stem bark showed activity against *Candida albicans* (Table 3).

Acetylcholinesterase inhibitors were detected from methanol and dichloromethane extracts of the stem bark and roots at 10 µg (Table 3). The extract of roots showed distinct inhibition zones.

Table 1: Inhibitory diameters of *Lannea barteri* roots and stem bark against tested bacteria.

Bacteria	Roots		Stem bark	
	DCM	MeOH	DCM	MeOH
<i>Enterococcus faecalis</i> ATCC 29212	0	7	0	6
<i>Proteus mirabilis</i> (clinical strain)	0	12	0	11
<i>Pseudomonas aeruginosa</i> ATCC 27853	0	10	0	10
<i>Staphylococcus aureus</i> ATCC 29213	0	11	0	10
MRSA (clinical strain)	0	9	0	10
MLS _B /R (clinical strain)	0	8	0	9
<i>Staphylococcus epidermidis</i> (clinical strain)	0	8	0	6

DCM = dichloromethane ; MeOH = methanol ; MRSA = methicillin resistant *Staphylococcus aureus*, MLS_B/R = resistance to macrolides, lincosamides, streptogramin B

Table 2: Antibacterial activity of the MeOH extracts from stem bark and roots of *Lannea barteri* and controls (µg/ml).

Bacteria	Stem bark (MeOH)	Roots (MeOH)	Gentamycin	Tetracycline
<i>Staphylococcus aureus</i> ATCC 29213	94 (bs)	47 (bs)	1,6	> 50
Sensitive	188 (bc)	47 (bs)	> 50	> 50
MRSA	94 (bc)	47 (bc)	25	> 50
MLS _B /R	47 (bc)	94 (bs)	> 50	> 50
<i>Staphylococcus epidermidis</i>	47 (bc)	94 (bs)	25	> 50
<i>Enterococcus faecalis</i> ATCC 29212	47 (bs)	94 (bc)	25	25
Sensitive	188 (bc)	188 (bc)	> 50	> 50
Resistant	94 (bs)	47 (bs)	> 50	> 50
<i>Pseudomonas aeruginosa</i> ATCC 29853	94 (bs)	188 (bc)	25	25
Sensitive	94 (bs)	94 (bc)	> 50	50
Resistant	188 (bc)	94 (bc)	25	25
<i>Proteus mirabilis</i>	375 (bc)	188 (bs)	50	50

Bold type = value of MIC ≤ 100 µg/ml, bc = bactericidal, bs = bacteriostatic, MeOH = methanol, MRSA = methicillin resistant *Staphylococcus aureus*, MLS_B/R = resistance to macrolides, lincosamides, streptogramin B

Table 3: Antifungal activity and Acetyl cholinesterase inhibitors detected at 100 µg and 10 µg respectively for *Lannea barteri* stem bark and roots.

Plant parts	Extracts	<i>Candida albicans</i>	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Acetyl cholinesterase
Stem bark	Methanol	i	a	a	a
	Dichloromethane	a	a	a	a
Roots	Methanol	i	a	a	a
	Dichloromethane	a	a	a	a

i = inactive ; a = active, bold type = high activity

In the DPPH test, DCM and MeOH extracts of stem bark, MeOH and DCM extracts of roots gave positive reaction (Table 4). The activity seems to be similar for both roots and stem bark. However, roots contained more radical scavengers than stem bark, considering the number and intensity of the spots. Quantitative assessment gave radical scavenging activity (RAS) ranging between 58.5-91.4% for both roots and stem bark (Figure 1). The highest RAS were for MeOH extracts. These extracts that IC₅₀ values were 4.71 and 3.54 µg/ml for stem bark and roots respectively were statistically active as controls namely gallic acid (IC₅₀ = 3.09 µg/ml) and ascorbic acid (IC₅₀ = 3.14 µg/ml).

Table 4: Radical scavenging activity and IC₅₀ values of *Lannea barteri* stem bark and roots.

Plant parts and controls	Extracts	TLC-DPPH (100 µg)	% DPPH inhibition ± SD	IC ₅₀ (µg/ml)
Stem bark	Methanol	a	83.64 ± 4.51	4.71a
	Dichloromethane	a	58.47 ± 10.36	25.76b
Roots	Methanol	a	91.43 ± 1.85	3.54a
	Dichloromethane	a	66.89 ± 11.47	17.38b
Gallic acid		a	97.35 ± 0.27	3.09a
Ascorbic acid		a	96.32 ± 0.57	3.14a
F			5.9964	
P			< 0.001	
dl			30	

a = no significant difference ; b = significant difference

Table 5: Results of preliminary qualitative analysis of phytochemicals present in *Lannea barteri* roots and stem bark .

Extracts	Sterols/ Polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids (Dragendorff)
				catechic	gallic		
Stem bark							
Petrol ether	+	-	-	-	-	-	-
Ethyl acetate	+	++	+++	+	-	+	-
Methanol	++	+++	-	++	-	-	-
Water	++	++	+++	+++	+++	++	+
Roots							
Petrol ether	+	-	-	-	-	-	-
Ethyl acetate	+	+	+++	+	-	-	-
Methanol	+	++	-	++	-	-	-
Water	++	++	+++	+++	+++	-	++

- = absence; + = presence; ++ = abundant; +++ = abundant in appreciable quantity

The phytochemical analysis of the roots and stem bark extracts by qualitative study showed the presence of steroids, triterpenoids, saponins, polyphenols, flavonoids, tannins, alkaloids and quinones (Table 5). TLC derivation with Godin reagent showed that radical scavenging compounds of the DCM extract of roots are terpenoids (purple colour).

Quantitative estimation proved that both extracts of roots and stem bark have considerably high constitutions of phenolic compounds. Similar total phenolic contents were obtained for the stem bark and roots with respectively 254.46 and 254.96 $\mu\text{g/g}$ GAE (Figure 2).

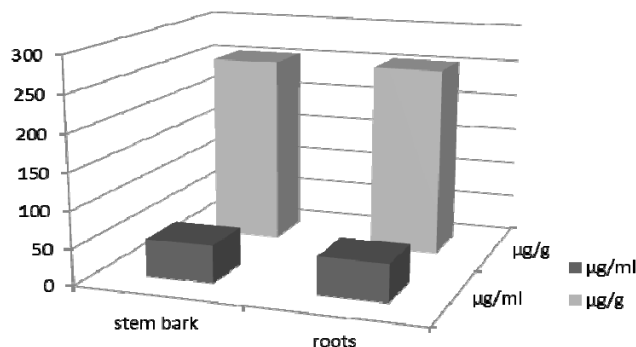


Fig. 2: Total phenolic contents of *Lannea barteri* stem bark and roots.

Discussion:

In this study, 4 crude extracts from the roots and stem bark of *Lannea barteri* showed antifungal, antibacterial, radical scavenging activities and acetylcholinesterase inhibitors. These findings which reveal that *L. barteri* is a promising medicinal plant, are in full agreement with the traditional uses of this plant for treating microbial infections, wounds healing, rheumatic (Arbonnier, 2002; Koné, 2005; Koné, *et al.*, 2005). To the best of our knowledge, this study is the first report on the antimicrobial, radical scavenging and acetylcholinesterase inhibitory properties of *L. barteri*. Previous study showed anthelmintic activity of *Lannea barteri* against the nematode *Haemonchus contortus* (Koné, *et al.*, 2005).

Other species of the genus *Lannea* also exhibited antifungal, antibacterial and radical scavenging activities. This is the case of *Lannea velutina* which was shown to display antifungal activity against *Cladosporium cucumerinum* and free radical scavenging activity (Diallo, *et al.*, 2001; Maïga, *et al.*, 2006). *Lannea acida* possessed antibacterial activity against a wide range of bacteria including methicillin resistant *Staphylococcus aureus* (Koné, *et al.*, 2004). *Lannea coromandelica* inhibited the motility of zoospores of *Aphanomyces cochlioides*, a phytopathogenic fungus (Islam, *et al.*, 2002). *Lannea welwitschii* possesses cytotoxicity activity against NCI-60 cells (Groweiss, *et al.*, 1997). While *Lannea edulis* showed a low mutagenic activity against the strain TA97a of *Salmonella typhimurium* and antioxidant activity (Queiroz, *et al.*, 2003).

The preliminary phytochemical screening showed that *Lannea barteri* contained sterols, polyterpenes, tannins, flavonoids, quinones, saponins, and traces of alkaloids. Roots and stem bark showed a similar phytochemical composition and total phenolic content. This is the first report on the chemical content of this plant species. Previous phytochemical studies on other species of the genus *Lannea* allowed isolation of flavonoids from *Lannea coromandelica* (Islam and Tahara, 2000), tannins polyflavonoids (Islam, *et al.*, 2002), hydroquinones from *Lannea welwitschii* (Groweiss, *et al.*, 1997), alkylphenols, dihydroalkylhexenones from *Lannea edulis* (Queiroz, *et al.*, 2003). The latter alkylphenols and dihydroalkylhexenones were found to be radical scavenging compounds.

The rational use of *L. barteri* in traditional medicine can be explained by the biological activities observed and phytochemical contents. The use in wound healing and treatment of rheumatic, diarrhoea is supported by the antioxidant and antibacterial properties as well as the presence of some phytochemicals such tannins, flavonoids. According to (Gupta and Jain, 2010), agents that demonstrate a significant antioxidant activity may preserve viable tissue and facilitate wound healing. Also a reducing of bacterial load of a wound may be necessary to facilitate wound healing as well as to reduce local inflammation and tissue destruction. Phenolic compounds such as tannins and flavonoids are known to be antibacterial and antioxidant compounds (Bruneton, 1999). The presence of alkaloids might explain in part the anti-acetylcholinesterase activity. Acetylcholinesterase inhibitors proposed at the moment against Alzheimer's disease are from alkaloid origin (Marston, *et al.*, 2002).

Interestingly, in this study *L. barteri* inhibited the growth of phytopathogenic fungi such as *Fusarium oxysporum* f. sp. *vasinfectum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Fusarium* diseases are among the most devastator diseases of crops, especially tomato (Hibar, *et al.*, 2007), cotton (Assigbetsè, *et al.*, 1990) and palm

tree (Allou, *et al.*, 2003). These findings give some evidence that *L. barteri* could have interest in crop protection.

The present results of the study indicated that *Lannea barteri* commonly used by West African traditional medical practitioners to cure diarrhoea, gastritis, wounds were active against bacteria, fungi, free radicals and acetylcholinesterase. For medicinal purposes, the safety and toxicity of this plant species need to be addressed. Deep phytochemical study was launched to isolate the active molecules of *L. barteri*.

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