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Lippia chevalieri Moldenke: A brief review of traditional uses, phytochemistry and pharmacology

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Abstract

Lippia chevalieri Moldenke is an herbaceous, belonging to the Verbenaceae family largely used in folk medicine in Burkina Faso and in certain African countries. The aim of the present review was to give a detailed literature survey on its traditional uses, and phytochemistry and therapeutical properties in order to systematize achievements and direct further research. Composition and biological activities of its essential oil have been largely investigated. Literature shows that this species of plant is used mostly for the treatment of malaria, mental disorder, liver pathologies, hepatitis, anemia and respiratory disorders. We note that the most published studies on this plant are concerned to its content of essential oil.

Keywords: traditional uses, phytochemistry, essential oil, literature, *Lippia chevalieri*

Introduction

The genus of *Lippia* belongs to the family of Verbenaceae, and includes approximately 200 species native of Central and South America, but also tropical Africa [1, 2, 3, 4]. *Lippia chevalieri* Moldenke synonymous *Lippia adoensis* Hochs and *Lippia rugosa* A. Chev is a shrub spread in western Africa (Figure 1). The bloom is made in rainy season. Its natural environment in Africa is the soudano-Guinean and Guinean savanna. It grows spontaneous in the fallows and on banks, generally on armored grounds or gravel soils [5, 6, 4]. The differentiation of *Lippia chevalieri* from *Lippia multiflora* Moldenke (this last one commonly known as "tea of Gambia") is difficult, because both are morphologically very similar. Its bluish leaves (*Lippia multiflora*), exhale a deeper smell of camphor when rustle. This species (*Lippia multiflora*) is found often in the shade and in wet places [5, 6, 4].

Reports have been published concerning to the uses of *L. chevalieri* in folk medicine in many region of the world. Likewise many works on the phytochemistry and pharmacological activities of its essential oil have been already appeared [7, 8, 4].

The aim of this paper was to make a summary of the vast literature published about the traditional uses, the chemical composition and pharmacological properties of some of the chemical constituents of *Lippia chevalieri* in order to systematize achievements and direct further research.

Traditional uses

Lippia chevalieri is used as a painkiller in folk medicine to treat pathologies related to the malaria, but also in the treatment of respiratory diseases [9]. Ethno medicinal investigations indicated that its leaves are used in treatments of diarrheas and arterial high blood pressure [5, 7, 4]. In Africa, *L. chevalieri* is traditionally used as an antimalarial, as well as a sedative and also for the treatment of respiratory diseases [9, 10]. Pousset [11] indicated that *L. chevalieri* was used in association with other species of plants as traditional drugs, improving the effects against malaria; that association is named Malarial-5 [9], and is formed by *L. chevalieri* (32 %), *Cassia occidentalis* L. (62%) and *Spilanthes oleracea* L. (6%).

In Burkina Faso, Nacoulma [5], performed an ethnomedicinal investigation and showed the traditional medicinal uses of *L. chevalieri,* classifying them according to the internal and/or external uses. As external traditional uses, the employ against the gouty rheumatisms, the painful and infected wounds can be highlighted, and as internal uses, the application of the flowery leading heads against the pathologies of the liver, the bucco-anal and digestive candidiasis, anaemia, nervousness of the menopause, menorrhagia of the menopause, malaria, painful menses, insomnias, irritability, fevers, nervous hepatitis, gout and the mental disorders are relevant.

Current data on the phytochemistry and biological activities of *L. chevalieri* have been mostly focused on its essential oil content [5], since, like other members of Lamiales order, this species synthesize and accumulate great quantities of essential oil.

Phytochemistry investigation

The most frequently found components of the essential oils of the tissues of *Lippia chevalieri* are: limonene, ß-caryophyllene, *p*-cymene, camphor, linalool, ⍺-pinene, and the thymol [10]. A study, carried out at Burkina Faso, showed that the major component of the essential oils of *L. chevalieri* leaves is thymol (27.4 %), followed by *p*-cymene (21.1 %), and 2-phenylethylproprionate (12.6 %) [7], the flowers essentially accumulating ß-caryophyllene, 1,8-cineol, and germacrene [12]; those results revealed that the compounds accumulated in different organs of the plant are distinct. A quite different composition was reported by [13] for the essential oil of *L. chevalieri* leaves from Senegal, which, according to these authors, contain 1,8-cineole (23 %) and many sesquiterpenes, the most relevant being the germacrene D (12 %) and β-caryophyllene (11 %). Those reports suggest that an important difference in the essential oils composition of *L. chevalieri* leaves can be found related to the geographical origin of the plants, which in turn, can be related to the natural genetic variability of that species. According to the former investigations [4] on *L. chevalieri* leaves essential oils of Burkina Faso, the major components of leaves are ß-caryophyllene (27.3 %), elemol (22.0 %) and caryophyllene oxide (18.6 %), while that of the flowers are ß-caryophyllene (29.8 %), elemol (11.0 %) and germacrene D (15.0 %). The differences between the results of [7], [12], and [4] can be explained by the variability that can be found in the soil constitutions, which can affect the composition of the secondary metabolites of plants, like [14] reported. The variability of the secondary metabolites in plants also reflects adaptation processes to climatic and edaphic conditions to which they face, and in many cases that variability gives to plants (motionless organisms) defense mechanisms against herbivores [15].

Among the essential oils (90 %; w/w on dry weight basis) found by [4] in the leaves of *Lippia. chevalieri*, 88 % was represented by sesquiterpenes, against nearly 70 % detected by [7] in a different season of the year. Thirty-six constituents were identified by the first authors and twenty-five by the second ones, indicating the importance of the phenologic and development stages in the composition of leaves essential oils of *L. chevalieri*, importance reported for other plants species and other secondary metabolites by [16], who emphasized the capital importance of the nature of soil, the microclimate, and also of the harvest time on the biosynthesis of those compounds.

Initially, isotopic studies indicated that the carbon skeleton of terpenes came from acetate [17]. Later, it was demonstrated that the mevalonic acid was a universal precursor of these compounds [17]. The initial stage of the process involves the condensation of the thioethers of the acetic acid: formation of the aceto-acetate and the condensation of this one with a molecule of acetyl-coenzyme A synthase. At the end of the reaction, a compound of five carbons, the dimethylallylpyrophosphate, is obtained. Five types of enzymes participate in this series of reaction [17]. Once that C5 compound is obtained, C10 and others are formed by condensation [17]. Figure 2 shows the structure of major essential oils found in *Lippia chevalieri* by the former studies.

Recently Bangou [18] reported rutin and caffeic acid in the methanolic extract (Figure 2) of *Lippia chevalieri*. Those same authors reported 20 phenolic compounds using HPLC-DAD method, providing for most compounds only information about the component type but not the complete chemical identification. Others authors showed that certain positions of the hydroxyls on the flavonoids increases the antibacterial activity [19, 20]. Particularly the position 7, 3' and 4' increase the activity of these flavonoids on the streptococcus ones [19]. Methoxyl and hydroxyl groups are important to define retention times under a particular HPLC method and to determine values in a TLC analysis, using a specific solvent system [21, 22]. Greenham [21] Greenham and Tsimogiannis [23] reported that flavones such as scutellarein (5,6,7,4'-tetrahydroxyflavone) 7 and 4'-methyl ester, which are similar of the apigenin and their counterparts (Table 1), can be distinguished by their frontal references and by their UV spectrum, but were not separable by HPLC [21, 24, 23]. According to [21], flavones, like luteolin derivatives, when contain methoxyl groups, display higher retention time. Indeed the number of substitution of hydroxyls increases not only the polarity of the compound but also decreases its time of retention. On the other hand a methoxyl substitution makes the compound lipophilic and lengthens its time of retention [21], suggesting, according to the structural information they obtained, those multimethoxylate and multihydroxylate flavones were present in the extracts. According to [21], the flavones generally difficult to separate in HPLC were: luteolin (5,7,3',4'-tetrahydroxyflavone), tricetin (5,7,3',4',5'-pentahydroxyflavone), 6-hydroxyluteolin (5,6,7,3',4'-pentahydroxyflavone), 6,8-dihydroxyapigenin (5,6,7,8,4'-pentahydroxyflavone) and hypolaetin (5,7,8,3',4'-pentahydroxyflavone) (Table 1). By comparing the results found by [20] and [21], it arises that each of compounds above-mentioned could be incriminated in the investigation of the first author.

Table 1 Possible compounds include in the methanolic extract of *Lippia chevalieri*

Compounds	Derivative	Possible substitutions								
		3	4	5	6	7	8	3'	4'	5'
Rutine		O-rutinoside	H	OH	H	OH	H	OH	OH	H
Flavone		H	H	OH	H	OH	H	OH	OH	H
	Saponarin	H	H	OH	O-glc	H	H	H	OH	H
	5,8-dihydroxyflavone	H	H	OH	H	H	OH	H	H	H
	6,7-dimethylether (Cirsimaritin)	H	H	H	CH_3O	CH_3O	H	H	H	H
	5,7,8-trimethoxyflavone	H	H	CH_3O	H	CH_3O	CH_3O	H	H	H
	5,3',4'-trimethoxyflavone	H	H	CH_3O	H	H	H	CH_3O	CH_3O	H
	5,7,4'-trihydroxyflavone	H	H	OH	H	OH	H	H	OH	H
	5,7,3'4'-tetrahydroxyflavone	H	H	OH	H	OH	H	OH	OH	H
	5,7,3'4'5'-pentahydroxyflavone	H	H	OH	H	OH	H	OH	OH	OH
	3,5,7,8,4'- pentahydroxyflavone	OH	H	OH	H	OH	OH	H	OH	H
	3,5,6,7,8,4'- hexahydroxyflavone	OH	H	OH	OH	OH	OH	H	OH	H
	3,5,7,8,3'4'- hexahydroxyflavone	OH	H	OH	H	OH	OH	OH	OH	H

Figure 1: *Lippia chevalieri* Moldenke (Photo catch in the forest of Gonsè, 25 km of Ouagadougou by Bangou M. Jean; 15h35mn/04 July 2011)

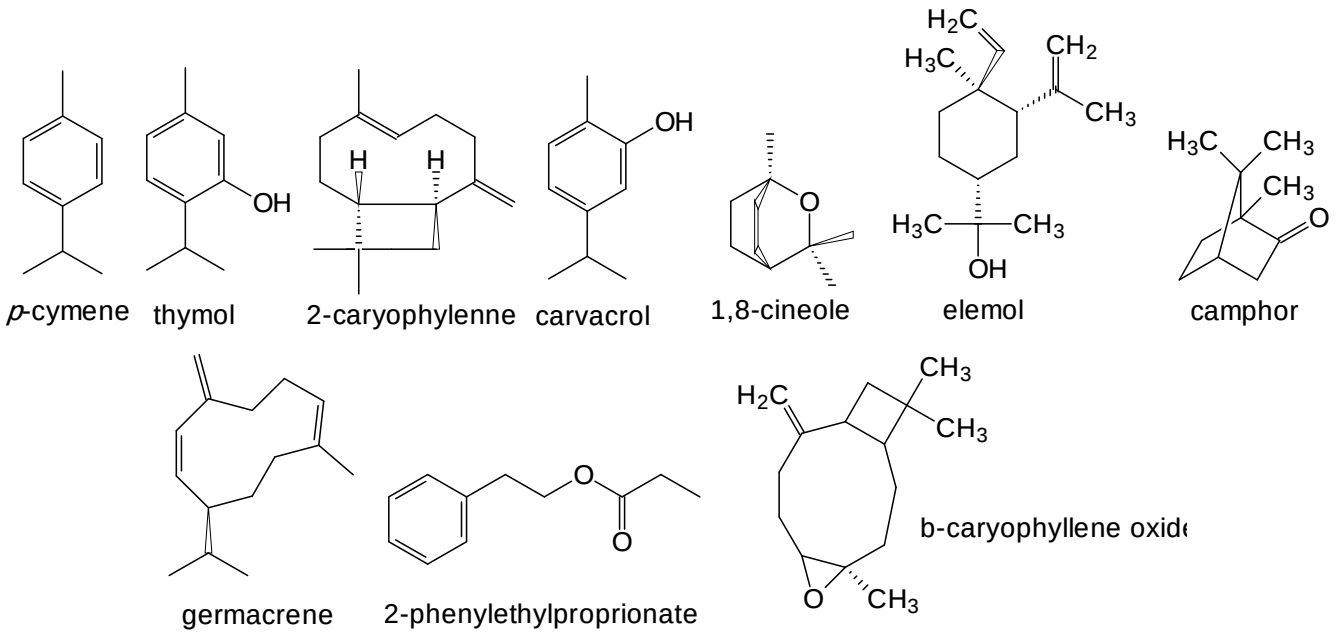

Figure 2: Major essential oil isolated from *Lippia chevalieri* Moldenke.

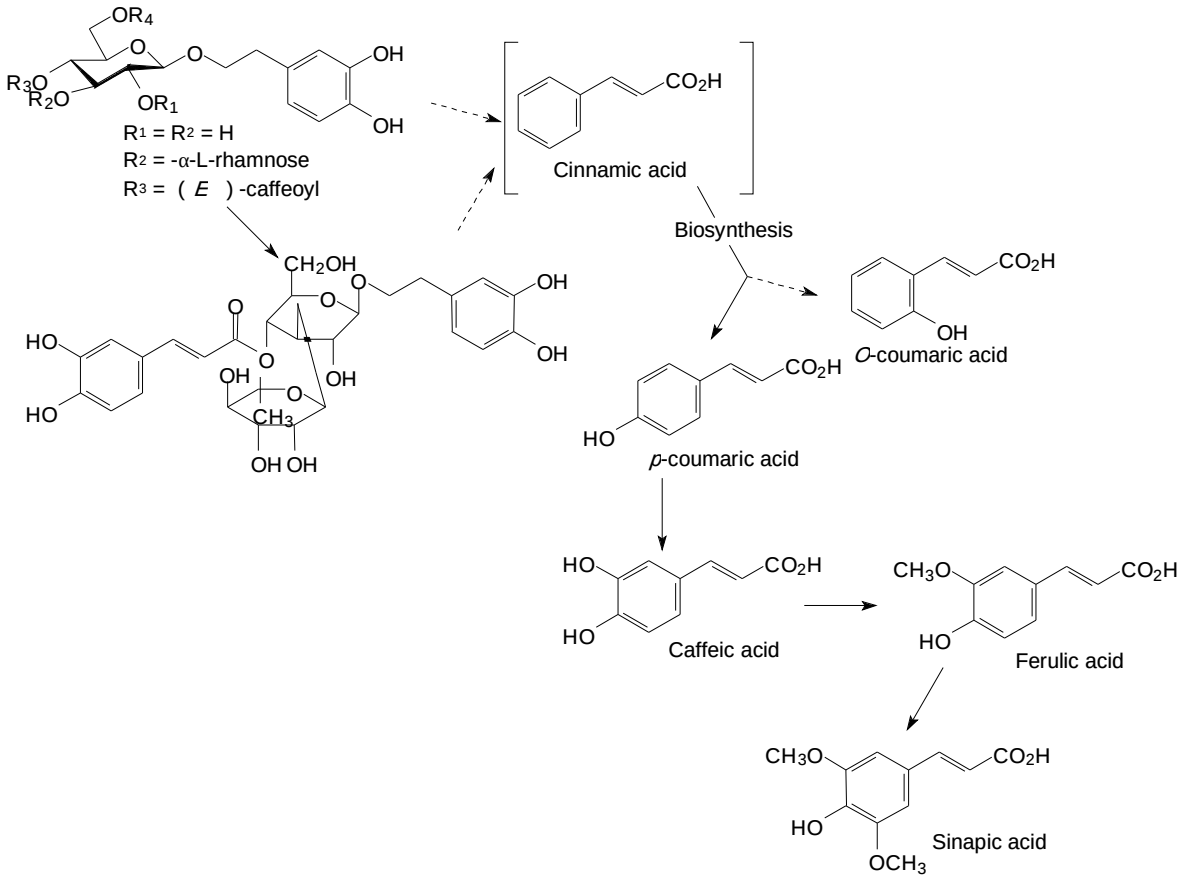

Figure 3: Possible pathway of caffeic acid biosynthesis

In a general way, flavones and flavonols represent approximately 80 % of known flavonoids [17]. The ring A is, in more than 90 % of the cases, substituted by two hydroxyl groups in C5 and in C7. These hydroxyls can be free or etherifies, one of them can be hired in a heterosidic connection [17]. Other substitutions can intervene, with variable frequencies: free hydroxyls or etherifies in C6 and/or in C8, isoprenylation or methylation in C6 or in C8, implication of C6 and/or it in a connection carbon-carbon with a sugar [17]. The ring B is substituted in 80 % of the cases in 4', can be 3', 4'-disubstituted or, less frequently, 3', 4', 5'-trisubstituted; the major substitution are groups -OH or -OCH3. Other positions (2' and 6') are only exceptionally substituted [17]. [10] Pascual (2001) showed that the genus Lippia was rich in verbascoside (Figure 3), which is a heteroside. According to [17], this compound is a phenylpropanoic heteroside ester, constituted by 1, 2 or 3 oside, and a molecule of dihydroxyphenylethanol, and the caffeic acid was the major phenylpropanoic acid reported by that author; but [18] also isolated p-coumaric acid derivatives and ferulic acid derivatives. In the same way, the dihydroxyphenylethanol can be etherified or, more rarely, hydroxyled on the carbon 2 [2-('-3 4 '-dihydroxyphenyl-dihydroxyphenyl)-dihydroxy-ethane]. These compounds seem to be the most abundant in Lamiales [17]. Four ions were also found in the methanolic extracts of Lippia chevalieri at different concentrations by [25]; those ions were magnesium (1.765 mmol L-1), sodium (0.461 mmol L-1), potassium (4.81 mmol L-1), and calcium (0.43 mmol L-1) [25]. These ions are important for the human cellular and nervous balance regulation (Mg2+, K+; Ca2+), increase human body defense reaction (Mg2+), and control renal insufficiencies (K^+), as was reported by [5].

Pharmacological investigation

Antimicrobial activity

Essential oil of *Lippia chevalieri* has bactericidal effect on *Proteus mirabilis* CIP588104, *Salmonella enterica* CIP105150 and *Staphylococcus camorum* LMG13567, belonging to the gram-negative bacteria [7]. The studies related to the measurement of MIC and MBC, with a range concentration of essential oil varying from 0.03 to 0.8 % [7]. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibits any visible organism growth and the minimal bactericidal concentration (MBC) were defined as the lowest extract concentration at which 99.9% of the bacteria were killed [25]. The carvacrol, a component of the essential oil of *Lippia chevalieri*, exhibit a prominent antimicrobial activity. However an antagonistic effect between *p*-cymene, thymol and carvacrol in the oil of *L. chevalieri* may explain its low antimicrobial activity [7]. Other studies concerning to *L. chevalieri* reported that some compounds such as the elemol, 1,8-cineol, the camphor and the *p*-cymene found in its essential oil have important antimicrobial effects [26, 27, 28, 29]. That could be due to the presence of the camphor which its toxicity is recognized starting from a certain dose [30]. Thus, the strongest antimicrobial activity could be justified by its content of toxic compounds. The mechanism of toxicity against the micro-organisms could be explained by inhibition of the hydrolytic enzymes (proteases) and carbohydrates or with other interactions for inactivating microbial adhesion, the proteins of transport of the cellular envelope and the non specific interaction with the carbohydrates [18]. Recently methanolic extract of *Lippia chevalieri* was showed to have antibacterial activity on *Staphylococcus epidermidis, Klebsiella pneumoniae, Pantoea sp,* and *Staphylococcus aureus* ATCC25923. However *Bacillus cereus* ATCC9144, *Citrobacter freundii, Escherichia coli*, *Shigella flexneri, Staphylococcus aureus* (wild strain), *Streptococcus agalactiae* and the two genus of *Vibrio* were insensitive to those extracts [18]. Comparing previous studies [7, 26, 27, 28, 29, 25], we could conclude that essential oils are more active than methanolic extract. But the last author used very weak concentration (25 µg mL^{-1}).

Antioxidant activity

The evaluation of the antioxidant activities of the methanolic extract of *Lippia chevalieri*, by estimating the DPPH* and the ferric reducing capacities have been evaluated by [18]. The following results were found: the reducing capacity of the methanolic extract of the Fe(III) in Fe(II) gave 15.16 mmol $AAEg^{-1}$, which was higher than that of the ascorbic acid (5.86 mmol $AAEg^{-1}$) and comparable at those of quercetin (13.19 mmol $AAEg^{-1}$) and the gallic acid (18.46 mmol $AAEg^{-1}$). With regard to the DPPH* scavenging activity, an IC_{50} of 6.23 µg mL^{-1} was found, which was weaker than the values registered for the references (1.8 µg mL^{-1}, 0.93 µg mL^{-1} and 0.60 µg mL^{-1} for ascorbic acid, quercetin and gallic acid,-respectively).

By using thin layer chromatography rutin, has been identified in the methanolic extracts of *Lippia chevalieri* [18], confirming the results reported by [31] and [32]. The presence of rutin in the extracts of *L. chevalieri* may explain the antioxidant capacity found, since rutin has been proven to possess a significant antioxidant activity, even at low levels such as 500 ng [33]. However, some other derivative compounds like luteolin [24, 34], derivatives of rosmarinic acid [35], or caffeic acid could explain that antioxidant capability [36, 37, 38, 39, 40, 41]. Caffeic acid is thought to prevent lipidic peroxidation of food and diseases triggered by free radicals such as cancer atherosclerosis; those compounds also can prevent the ageing of fabrics. The magnesium is recognized to be implicated in certain antioxidant activities through in the inhibition of the oxidation of the fatty acids and in the reduction of the levels of cholesterol [42]. From a chemiluminescence analysis it was showed that neither the leaf oil nor that of the flowers of *L. chevalieri* possesses an antioxidant property compared to ⍺-tocopherol, both oils having low values of IC_{50} (2%) [4].

Enzymes activities

The evaluation of a certain number of enzymatic activities of the extracts of *Lippia chevalieri* was carried out by [43], including the anti-acetylcholinesterase, anti-glutathione-S-transferase, anti-carboxylesterase and anti-xanthine oxidase activities. With the exception of the inhibiting activity of the xanthine oxidase (14.00 %), the methanolic extract inhibited the three last enzymes with a level higher than 35 % at a concentration of extract of 100 µg mL^{-1}. To give an idea of the potential of the *L. chevalieri* activity as enzymatic inhibitor, the following results from the report of [43] are mentioned. The galantamine, used as reference for the inhibition of the acetylcholinesterase, displayed 50.76% inhibition, against the 39.26 % showed by the extract of *L. chevalieri*. The ascorbic acid, (56.72 %), used as reference for the carboxylesterase inhibition, showed 56.72 % inhibition, while the extract of *L. chevalieri* showed 37.68 %; and the allopurinol activity was 98.38 % as inhibitor of xanthine, while the extract of *L. chevalieri* showed 14.00 %.With regard to the glutathion-S-transferase, the reference (ethacrynic acid) did not displayed any inhibition, but the extract of that species gave an inhibiting activity of 42.99 %.

Others activities

Studies tending to establish the toxicity of the essential oil such as of *Lippia chevalieri* and in particular those of the terpenes, were made by [30], most of evaluations concerning to the mode of absorption. According to [30] the passage of the essential oil by the cutanous layers would be dangerous. Höld [44] showed that the thuyone is incriminated in the neurotoxicity of mice. In every case it is assumed that the liver is the place of a first hepatic passage [30], because of the importance of the enzymes of that organ and their activity. The reactions in which the most frequent terpenes are involved *in vivo* are the oxidations by the CYP450, the glucuronoconjugations, and the conjugations with glutathion [30], the last one being important for the deletion of the toxic potential of electrophiles compounds in tissues [30]. If renal or hepatic insufficient have a particular sensibility to these compounds is unknown, but that it influences the elimination could be involved.

Among toxic essential oil met in the literature such as rosemary anethole, pinocamphone, pulegone, fenchone, camphor, cineole [45, 30], the two last were found in *Lippia chevalieri* [7, 4]. The ingestion of the camphor in a dose superior to 50 mg/kg is toxic for the human health [30]. In spite of the toxicity know for camphor, it is described as "quickly absorbed by the gastronomic skin and the tract-intestinal [30]. The thymol, administered by oral route, can provoke abdominal pains even a light collapse in dose from 0.3 to 0.6 grams [30].

Conclusion

Few phytochemical studies have been made on *Lippia chevalieri*, out of concerning its essential oil. Pharmacological studies have been oriented on its antimicrobial, antifungal, repellant and larvicidal effects. The present paper highlighted *Lippia chevalieri* as a medicinal plant. Further analysis of *Lippia chevalieri* should be focused to (1) the identification and isolation of the 20 polyphenolic compounds found in its methanolic extract, (2) to take back this activity with other type of extracts to isolate more to polyphenolic compounds, (3) the evaluation of the capacity of the extracts and/or compounds isolated from its extracts to increase the rate of the superoxide dismutase, the catalase and of the glutathion in the human organism.

Author's contribution

Norma Almaraz-Abarca and Nâg-Tiero Roland Meda have supervised article writing. Yougbaré-Ziébrou Mouhibatou was solicited to read the manuscript. The plant species identification and the validity of the research works were carried out by Jeanne Millogo-Rasolodimby and Odile Germaine Nacoulma.

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