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Phytochemical screening, DPPH scavenging and antimicrobial activities of leaves of *Eucalyptus camaldulensis*, *Cassia mimosoides* and *Vepris heterophylla* from Northern Cameroon

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Abstract

The aim of this study was to evaluate phytochemical constituents, antioxidant and antimicrobial activities of hexane, acetone and methanol extracts of three medicinal plants (*Eucalyptus camaldulensis* (Myrtaceae), *Cassia mimosoides* (Fabaceae) and *Vepris heterophylla* (Rutaceae)) traditionally used in the treatment of microbial diseases and food preservations. Qualitative phytochemical screening of the large family of compounds followed by quantitative evaluation of phenolic compounds and flavonoids was realised. The antioxidant activity was evaluated by the scavenging of DPPH radicals and the antimicrobial activity by agar well diffusion method. The results showed presence of phenolics, flavonoids, coumarins, alkaloids, triterpenes and steroids in the various extracts. The total amount of phenolic compounds ranges from 6098.76±30, 04 to 626.34±16, 13 mg of GAE and the amount of flavonoids from 57.95±1, 23 and 27.50±8,11 mg QE. The largest amount of phenolics and flavonoids was found in the acetone extract of *C. mimosoides* and the lowest in the methanol extract of *V. heterophylla*. The highest inhibition percentage of DPPH was obtained with the acetone extract of *C. mimosoides* which was 80% and the lowest was the methanol extract of *V. heterophylla*. The hexan extract of selected plants showed the higher inhibition diameter range from 12 to 15 mm with the maximum obtained with extract of *E. camaldulensis*.

Keywords: Phytochemical screening, total phenolic compounds, total flavonoids, antioxidant activity, antimicrobial activity

1. Introduction

Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Shariff, 2001) [23]. Whereas, the usage of herbs and medicinal plants to cure various diseases was in practice from the time immemorial (Samatha *et al.*, 2012) [19]. The need to search for plants of medicinal value is primordial. In Cameroon, septentrional regions possesses a vast array of medicinal plants which are traditionally used for various preventive and curative abilities (Egwaikhide and Gimba, 2007) [7]. The beneficial effects on human health is mainly due to the presence of some chemical compounds produce by the plants for their on defences against diseases and predators. The phytochemical compounds give them an advantage, such as antimicrobial property, antioxidant property and food storage (Cravotto *et al.*, 2010; Mahama *et al.*, 2017 and Mahama *et al.*, 2018) [6].

Eucalyptus camaldulensis (Myrtaceae), *Cassia mimosoides* (Fabaceae) and *Vepris heterophylla* (Rutaceae) are traditionally used for food preservation and against infectious diseases. Previous work had been more focused on the biological activities of the essential oils of these plants (Kim *et al.*, 2005; Negahban and Moharrampour, 2007; Ngamo *et al.*, 2007; Ghalem and Mohamed, 2008) [12, 16, 17, 9]. Then, it is important to evaluate these activities on organic solvent extracts.

The main objective of this study is to evaluate the phytochemical content, antioxidant activity and antimicrobial activity of the different extracts of *E. camaldulensis*; *C. mimosoides* and *V. heterophylla*.

2. Materials and Methods

2.1 Plant material

The young leaves of *Cassia mimosoides* and *Vepris heterophylla* were collected respectively at Koza and Godola two localities located in the Far North region of Cameroon.

The young leaves of *Eucalyptus camaldulensis* were collected at Ngaoundere Dang in the Adamawa region (Cameroon). The collections were carried out during the period of August to September 2014. The leaves were air-dried for 15 days, milled into powder and sieved with 1 mm mesh. The various powders obtained were stored in glass bottles and then kept in a refrigerator at 4 °C. until the extraction period.

2.2 Extraction procedure

The extraction procedure was cold maceration method as used by Perry *et al.* (2007) [18]. One kilogramme of each leaves powder plant were macerated in 3 L of hexane for 3 days in the glass jar (5 L) and the maceration was turned twice a day. Then the maceration were filtrated through Whatman No.1 filter paper to obtain hexane filtrate and residue for each plant. Then, the same procedure was reproduce respectively with acetone and methanol to give acetone and methanol extracts. The dry plant extracts were stored at -4°C until use for phytochemical screenings and bioassays.

2.3 Phytochemical screening

The phytochemical composition of different extracts was evaluated according to the methods described by Harborne (1973); Samatha *et al.*, 2012 [19] and Anjali and Sosa, 2013) [25] with slight modifications.

2.3.1 Test for identification of phenolic compounds

2.3.1.1 Ferric chloride test: To 0.2 mL of extract solution was added a drop of the 3% ferric chloride solution. The appearance of a darkish blue or green coloration more or less dark characterizes the presence of phenolic derivatives

2.3.2 Test for identification of Flavonoids

2.3.2.1 Shinoda test: Magnesium chips and concentrated hydrochloric acid were added drop wise to the test solution. Reddish-pink colour with effervescence indicates the presence of flavonoid

2.3.3 Test for identification of Tannins

Five grams of the ground powder was extracted with 10 mL ammonical chloroform and 5 mL chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

2.3.4 Test for identification of coumarins

In test tube containing 2 mL of extract, 3 ml of NaOH (10%) were added. After shaking the solution, the appearance of a yellow colour indicates the presence of coumarins.

2.3.5 Test for identification of alkaloids

2.3.5.1 Dragendorff's test: To 1 mL of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.

2.3.5.2 Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was added. A creamish white precipitate was formed indicating the presence of alkaloids.

2.3.6 Test for identification of Terpenoids

Five milliliter of the methanol extract was mixed with 2 mL of chloroform and 2mL of concentrated sulphuric acid. The

layer interface was observed for reddish brown coloration which indicates the presence of Terpenoids.

2.3.7 Test for identification of Steroids

In a test tube containing 0.5 mL of extract solution, 2 mL of acetic anhydride was added and along the sides of the tube 2 mL of sulfuric acid were also added. The appearance of the purple or green-blue colour indicates the presence of steroids.

2.3.8 Test for identification of saponins

Persistent foam, resulting from shaking the extract in distilled water for 10-15 min indicated the presence of saponins.

2.4 Quantitative analysis

Quantitative analysis was carried out to evaluate total phenolics compounds and total flavonoids.

2.4.1 Determination of total Phenolic Compounds

The Folin–Ciocalteu assay was used for the determination of total phenolic content as described by Lôpez *et al.* (2014) with slight modifications. Briefly, an aliquot of 0.1 mL of extract (0.2 mg/mL) was allowed to react with 0.2 mL of Folin–Ciocalteu reagent, 2 mL of H₂O for 3–5 min, then 1 mL of 20% Na₂CO₃ and the mixture was incubated at room temperature for 60 min. Absorbances were then taken at 765 nm. Gallic acid was used as standard and results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant material.

2.4.2 Total flavonoid content

The total flavonoid content of each extract was investigated using the aluminum chloride colorimetry method described by Chang *et al.* (2002) [5] with slight modifications. In brief, the extract sample was diluted with ethanol until 100 µg/mL. The calibration curve was prepared by diluting quercetin in methanol (0–500 µg/mL). The diluted extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM sodium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 415 nm using a UV–VIS spectrophotometer. TFC was expressed as milligram quercetin equivalent per gram dry weight (mg QE/g DW).

2.5 Antioxidant Activity: DPPH radical Scavenging

The antioxidant activity of the extract was measured with the DPPH method (Shimada *et al.*, 1992) with slight modifications. A solution of DPPH was freshly prepared by dissolving 6 mg of DPPH in 50 mL methanol (about 0.3 mM). The extract (2.5 mL) with varying concentrations (0.2 – 1 mg/mL) and DPPH solution (2.5 mL) was mixed together in a test tube. The test tube was then incubated in the dark for 30 minutes at room temperature. The decrease in absorbance was measured at 517 nm using a UV–VIS spectrophotometer. The percentage inhibition of radicals was calculated using the following formula:

$$\% \text{ Inhibition percentage} = 1 - \frac{A_{\text{test}}}{A_{\text{control}}}$$

where A_{control} is the absorbance of DPPH solution without extract and A_{test} is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%. The concentration (IC_{50}) required to scavenge 50% of DPPH free

radical was calculated from logarithmic regression analysis of the different curve of DPPH inhibition.

2.6 Evaluation of antibacterial potential of plant extracts

2.6.1 Bacterial strains and Preparation of Inocula

The microbial stains used for evaluation of the antimicrobial potential of plant were Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Salmonella* sp., Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus cereus* ATCC 19615, *Listeria monocytogenes* ATCC 19115 provided from the Food Microbiology and Biotechnology Laboratory, University of Ngaoundere. The inocula were prepared with distilled water according to NCCLS (1997): According to plant extracts, the stock solutions of corresponding fractions were prepared in dimethyl sulfoxide (DMSO)

2.6.2 Antibacterial assay

The antimicrobial activity of plant extract with different solvent was determined by the well diffusion method on Mueller Hinton agar medium as described by Satish *et al.* (1999) [21]. Briefly, 15 ml of sterile medium (at 45°C) was uniformly mixed 100 µL of inoculum (corresponding to 10⁶ CFU/ml) of different strains and then poured in Petri dishes. After solidification of medium, wells (6 mm diameter) were made in each Petri-dish, to which 30 µL of the different extracts were added. At the center of Petri dish, antibiotics disc of Chloramphenicol (30 µg/mL) placed and used for the control. The plates were incubated at 36±2 °C for 24 h and the diameter of the resulting zone of inhibition, if any, was measured.

2.7 Statistic analysis

The results were recorded after repeating the experiments three times. The experimental results were expressed as mean ± standard error. For the statistical analysis, data were submitted to ANOVA analysis using statistical analysis software SAS, Tukey test ($P < 0, 05$) was applied for mean separation (Zar, 1999; SAS, 2003) [26, 20].

3. Results

3.1 Phytochemical evaluation of different plant extracts

Table 1 presents the results of the phytochemical screening carried out on the crude extracts with hexane, acetone and methanol of *V. heterophylla*, *C. mimosoides* and *E. camaldulensis*. This screening revealed the presence of several secondary metabolites. This suggests that extracts of *V. heterophylla*, *C. mimosoides* and *E. camaldulensis* contain a wide range of compounds including alkaloids, triterpenes, sterols, flavonoids, phenolics, sterols and coumarins. A remarkable presence of triterpenes, steroids and saponins was observed in the hexane extracts as well as in the acetone and methanol extracts for the three plants. Similarly, in the acetone and methanol extracts, a significant presence of the total phenol compounds, flavonoids, tannins, coumarins, alkaloids and saponins for the three plants was noted. Apart from the acetone extract of *E. camaldulensis*, the other two extracts with acetone and the three with methanol contain triterpenes. Similarly, the presence of steroids in the acetone extracts of *V. heterophylla* and *C. mimosoides* was noted.

Table 1: Phytochemical screening of different extracts

Metabolites	<i>V. heterophylla.</i>			<i>E. camaldulensis.</i>			<i>C. mimosoides.</i>		
	Hexane	Acetone	Methanol	Hexane	Acetone	Methanol	Hexane	Acetone	Methanol
TPC	-	++	++	-	++	++	-	++	++
Flavonoids	-	++	+	-	+	++	-	++	++
Tanins	-	+	+	-	++	+	-	+	+
Coumarine	-	+	-	-	+	-	-	+	+
Alcaloides	-	+	+	-	++	+	-	-	++
Triterpenes	++	+	+	++	-	+	+	+	+
Steroids	++	+	-	++	-	-	+	+	-
Saponins	+	+	+	-	+	+	+	+	+

+: positif; ++: abondant; -: absent; *V. h.*: *Vepris heterophylla*; *E. c.* *Eucalyptus camaldulensis*; *C. m.* *Cassia mimosoides*; TPC: Total Phenolic compounds

3.2 Determination of Total Phenolic Compounds and total Flavonoids

Table presents the results of the quantitative determination carried out on the crude extracts with acetone and methanol of *V. heterophylla*, *C. mimosoides* and *E. camaldulensis*. This table shows that the amount of total phenolic compounds

varies from 6098.76 to 626.34 mg GAE and the amount of flavonoids varies from 27.50 mg QE to 57.95 mg QE. The extract which obtained the highest value in total phenolic compounds and flavonoid was the acetone extract of *C. mimosoides* and the lowest values were obtained by the methanol extract of *V. heterophylla*.

Table 2: Total Phenolic Compounds (mg EAG) and Flavonoids (mg EQUER) for 100gMs

Extract	TPC (mg GAE/100DW)	Flavonoids (mg QE/100DW)
Acétone <i>Vepris heterophylla</i>	691.36±15,80 ^d	42.54±3,75 ^c
Acétone <i>Eucalyptus camaldulensis</i>	1991,77±10,03 ^b	52.18±4,71 ^{ab}
Acétone <i>Cassia mimosoides</i>	6098.76±30,04 ^a	57.95±1,23 ^a
Méthanol <i>Vepris heterophylla</i>	626.34±16,13 ^c	27.50±8,11 ^d
Méthanol <i>Eucalyptus camaldulensis</i>	1995.88±4,33 ^b	51.77±3,21 ^b
Méthanol <i>Cassia mimosoides</i>	914.40±8,54 ^c	37.11±2,11 ^{cd}
F (5; 6)	544,23***	123,24***

GAE: Gallic Acid Equivalent; TPC: Total Phenolic Compounds. QE: Quercetine Equivalent

3.3 Determination of antioxidant activity

The Figure 1 below shows the results of the antioxidant tests carried out on extracts of *V. heterophylla*, *C. mimosoides* and *E. camaldulensis*. This figure shows that the acetone extracts

of *C. mimosoides* and the methanol of *E. camaldulensis* had the highest antioxidant activity (inhibitory percentage greater than 80% at the lowest dose of 0.2 mg / ml), while the acetone and methanol extract of *Vepris heterophylla* were low.

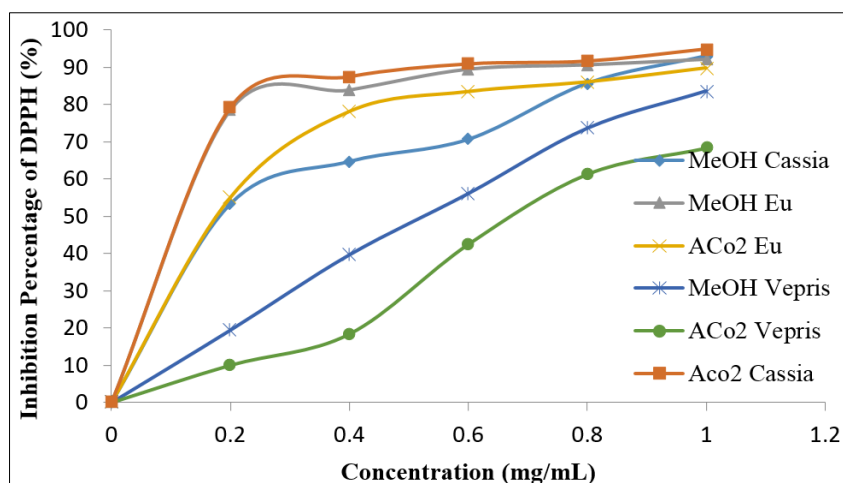


Fig 1: Inhibition percentage of DPPH of acetone and methanol extracts of: *V. heterophylla*; *E. camaldulensis* and *C. Mimosoides*

Table 3: Inhibitory concentration 50% of extracts of *V. heterophylla*, *C. mimosoides* and *E. camaldulensis*

Extracts	Plants	CI ₅₀ (mg/mL)
Methanol	<i>Eucalyptus camaldulensis</i> .	1.48
	<i>Cassia mimosoides</i>	2.30
	<i>Vepris heterophylla</i>	2.82
Acetone	<i>Eucalyptus camaldulensis</i> .	2.16
	<i>Cassia mimosoides</i>	1.36
	<i>Vepris heterophylla</i>	2.65

3.4 Antimicrobial activity

A total of 06 plants extracts was tested for their antimicrobial

activity against alteration pathogenic bacteria. The inhibition diameter values are very variable and reach 15 mm and three extracts showed best inhibition diameter as shown in Table 4. Our results revealed that hexan extract of *V. heterophylla*, *E. camaldulensis*, *C. mimosoides* showed significantly higher inhibitory activity. According to the methanolic extracts, the inhibition diameter were variables and depend from both extracts and bacterial strains. The inhibition diameter of chloramphenicol was significantly ($P < 0.05$) different with the extracts one exempt with the *B. cereus* strains were the difference was not significant.

Table 4: Diameter of inhibition (mm) of different plant extracts

Plants Extracts	Diameter of inhibition (mm)				
	<i>E. coli</i>	<i>Salmonella sp</i>	<i>L.monocytogenes</i>	<i>B.cereus</i>	<i>S.aureus</i>
Hex. <i>V. heterophylla</i>	10.4±1.3	11.7±0.6	10.7±1.6	13.3±1.5	14.0±1.5
Meth. <i>V. heterophylla</i>	6.0±0.0	7.0±0.0	8.0±1.0	8.3.0±1.3	7.0±1.0
Hex. <i>E. camaldulensis</i>	11.8±0.6	8.7±1.0	15.3±0.1	12.0±1.5	13.3±1.5
Meth. <i>E. camaldulensis</i>	9.3±1.5	8.7±1.6	12.3±0.6	12.0±1.0	11.3±0.6
Hexan <i>C. mimosoides</i>	8.7±1.3	7.7±0.6	9.7±1.1	10.3±1.5	7.7±0.6
Meth. <i>C. mimosoides</i>	6.3±0.7	6.0±0.0	7.0±1.0	7.0±1.0	7.5±1.3
Chloramphenicol	17.0±1.0*	16.0±1*	18.0±1.5*	14.0±1.3	23.0±2*
DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

V: *Vepris*; E: *Eucalyptus*; C: *Cassia*; * significant difference ($P < 0.05$); Meth: Methanol; Hex: Hexane

The most sensitive strains were the bacteria *Bacillus cereus* ATCC 19615, *Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* all Gram-positive bacteria. On the other hand, Gram-negative bacteria were more resistant, especially *Escherichia coli* ATCC 25922, *Salmonella sp.*, No activity was observed with the DMSO which were considered has control.

4. Discussion

The results obtained on the phytochemical screening of the extracts show that the crude extracts of *C. mimosoides*, *E. camaldulensis* and *V. heterophylla* contain a wide range of secondary metabolites including alkaloids, triterpenes, sterols, flavonoids and phenolic compounds, sterols and coumarins. Total phenolic compounds, flavonoids were abundant in polar solvent extracts (acetone and methanol) and absent in the

apolar solvent (hexane). Similarly, a remarkable presence of triterpenes, steroids and alkaloids was noted in the hexane extracts as in the acetone and methanol extracts. The presence of these compounds in these extracts is explained by the fact that during their growth, plants synthesize aromatic compounds or secondary metabolites that are involved in the many physiological processes such as cell growth, rhizogenesis, seed germination, fruit ripening, defense against external aggression. (François, 2010, Hartmann, 2008) [8, 10]. The absence of certain compounds in the hexane extracts is explained by the fact that hexane is an apolar solvent which cannot fix certain polar compounds. Similarly, the presence of triterpenes and steroids in the acetone and methanol extracts is explained by the fact that certain polar compounds are associated in their structures with apolar compounds such as triterpenes.

The high percentage of DPPH inhibition of the acetone extract (94.98%) compared to the methanol extract (92.98%) is justified by the presence of several secondary metabolites and the very high content of the phenolic compounds. Extracts with acetone and methanol show high percentages of inhibitions, which could be explained by their polyphenol richness. Our results are similar to those obtained by Lamien-Meda *et al.* (2008) [13] who found that the amount of total phenolic, the amount of total flavonoids and antioxidant activity of acetone extract is higher than methanol extract for more than 10 plants. Indeed several studies have shown the involvement of phenolic compounds, flavonoids in extracts of a plant that has antioxidant activity (Lamien-Meda *et al.*, 2008; Soni and Sheetal, 2013) [13, 25].

A considerable presence of triterpenes, steroids and saponins was observed in the hexane extracts. Several authors reported that the antimicrobial activity of plants was associated to the presences of those metabolite or compounds (Silva et Fernandes. 2010; Selvamohan *et al.*, 2012; Baloch *et al.*, 2013; Amenu *et al.*, 2014; Javid *et al.*, 2015) [24, 22, 3, 2, 11]. According to them, several plants contain numerous compounds with antimicrobial activities for protection against aggressor agents, especially microorganisms. The site of action of these compounds can be the cytoplasmic membrane, the cytoplasm, some proteins and in some cases the DNA. Similarly, these activities could be due to one/single molecule or to the synergistic action of two or more molecules. Burt. (2004) [4]; Silva et Fernandes. (2010) [24] explained that action mechanisms of those compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force, electron flow, active transport and coagulation of the cell content. Not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms. In the present work, the antimicrobial activity of extracts could be explaining by the presence and the action of triterpenes, steroids and saponins. The mechanism of action of each compound is not yet well elucidated, but according to some authors, triterpenes act by inducing the membrane disruption, alkaloids by intercalation into cell wall and/or DNA and the saponins act by forming pores in the cytoplasmic membrane which cause disruptions in cell function (Silva et Fernandes. 2010) [24].

Javid *et al.* (2015) [11] showed that extract of *A. indica* and *M. falcata* with apolar solvent (n-hexane, chloroform and ethyl acetate) have active phytochemical compounds such as saponins, tannins, alkaloids, steroids, which inhibit the growth of pathogenic bacteria and fungi. *A. indica* and *M. falcata* showed significantly higher inhibitory activity respectively against *E. coli*, *P. aeruginosa* and *S. aureus* (between 15-19 mm) and against strains of *P. aeruginosa*, *S. Typhi* and *S. aureus* (between 12-13 mm). Similarly, Baloch *et al.* (2013) [3] obtained good antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* with the chloroform, ethyl acetate and n-hexane extracts of *Medicago* spp. which are comparable with the standard drugs. These activities may be due to strong occurrence of compounds such as alkaloids, steroids, phenols and saponins. Our results is different to the one obtained by Adeniyi and Ayepola. (2008) [1] working on the antibacterial activity of methanol extracts of leaves of *Eucalyptus camaldulensis* and *Eucalyptus torrelliana* against *Klebsiella species* UCH 2101, *P. mirabilis* UCH 2102, *P. mirabilis* UCH 2204, *S. typhi* UCH 2201, *E. coli* CHO 3101, *E. coli* UCH 2103, *P. aeruginosa* CHO 3102 and *P. aeruginosa* UCH 2203. The diameter of zones of inhibition

exhibited by the extracts was between 10 to 22mm. They explained that this could be due to the detection in both extracts of tannins, saponins, cardiac glycosides. The difference observed with our results could be explain by many factors including the plant such as part of the plant, old of plant, the climate season and the analytical methods use for the evaluation (Selvamohan *et al.*, 2012) [22].

5. Conclusion

In conclusion, we can say that *Eucalyptus camaldulensis*, *Vepris heterophylla*, *Cassia mimosoides* contain several active compounds. The antioxidant activity of different extracts is due to the presence of phenolics compounds, flavonoids, tannins and the antimicrobial one is due to alkaloids, triterpenes, steroids, phenolic compounds. This investigation confirmed the use of selected plants as traditional medicine. However, it is important to submit these selected medicinal plants to more pharmacological and toxicity studies in order to bring out new drugs with antimicrobial and/or antioxidant activities.

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