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Solvents' influence on polyphenolic compound extractions from *Lippia multiflora* leaves (Mold, 1949), and their antioxidant activity

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ABSTRACT

The plants are the main source of bioactive compounds for new therapeutic strategy developments for numerous diseases' treatments. The study determined *Lippia multiflora* leaves' total polyphenol, and condensed tannin contents, and their antioxidant activities. So, aqueous, 70%Eth, and 70%Met solvents (alcohol/distilled water, v/v, 70/30) were prepared with ethanol and methanol, respectively. Then, these solvents were used to extract *Lippia multiflora* dried leaves. Thereafter, the phytochemical screening was performed to identify the groups of compounds. The extract total polyphenol contents were performed through Folin-Ciocalteu method, and condensed tannins were determined in the presence of sulfuric acid. Furthermore, the dry extract (DE) antioxidant activity was determined by using DPPH method. As a result, the total polyphenol and condensed tannin contents were 365±5; 635±5; 525±15 mg GAE/g.DE, and 105.14±3.36; 128.94±5.59; 143.36±9.35 mg Cat.E/g.DE, for the aqueous, 70%Eth and 70%Met extracts, respectively. Additionally, the inhibition rates ranged from 3.95% to 60.69% for the distilled water, from 12.94% to 82.94% for 70%Eth, and from 17.54% to 84.78% for 70%Met extracts. So, 70%Eth was more efficient for *Lippia multiflora* bioactive compounds extraction, compared to 70%Meth, and distilled water.

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INTRODUCTION

The plants have long been used by humans to meet various needs, such as food, ornamentation, and shelter. They are also used to treat numerous diseases, including malaria, diabetes, and abdominal pain (Masengo *et al.*, 2024). According to Lagnika *et al.* (2016), the plants' curative effects are primarily due to their secondary metabolites. Indeed, bioactive compounds such as flavonoids, tannins, saponins, alkaloids, glycosides, and anthocyanins found in plants play crucial roles in maintaining human health. Their importance stems from their diverse biological activities (Chandrasekara and Shahidi, 2018). Therefore, the plants have significant bioactive molecules, capable of treating various diseases (Falleh *et al.*, 2021). They represent both finished products for consumption and raw materials for obtaining active substances (Hamia *et al.*, 2014). These plant metabolites are the subject of much research for the development of new preventive and therapeutic strategies (Iloki-Assanga *et al.*, 2015). Various parameters and conditions, including the type of extract (aqueous or with alcohol), the polarity of the solvent, and geographical and climatic conditions, influence the chemical composition and biological activities of these metabolites (Hayat *et al.*, 2020). Studying the influence of different extraction solvents is essential (Iloki-Assanga *et al.*, 2015). Indeed, Falleh *et al.* (2021) work has highlighted the crucial role of extraction solvents in the quantification of phytochemical compounds. Several solvents are recommended for the extraction of phenolic compounds, among whose are methanol, ethanol, and water (Turkmen *et al.*, 2007).

Ivory Coast is rich in medicinal plants, including *Lippia multiflora*. Numerous studies reported diverse biological properties of *Lippia multiflora* different parts, notably their antihypotensive, anti-inflammatory, analgesic, antipyretic, scabicidal, antimalarial, antioxidant, antimicrobial, and muscle-relaxant properties (Masunda *et al.*, 2020). *Lippia multiflora* leaves are used to treat stomach aches, fever, malaria, toothaches, high blood pressure, itching, induced lactation after childbirth, and various seizures (Atanasso *et al.*, 2017). They are also an ingredient in improved traditional African medicines, such as Malarial® in Mali and Tetra® in Congo. According to Etou-Ossibi *et al.* (2005), softened leaves cooked over low heat improve sleep and relieve stress. The study focused on quantifying the phenolic compounds in various extracts, including aqueous, 70%Eth, and 70%Met extracts of *Lippia multiflora* leaves. These different extracts antioxidant activities were also determined.

MATERIALS AND METHODS

Lippia multiflora leaves were harvested at the flowering stage in the wild in Yamoussoukro. They were then dried in the shade at room temperature, around 25°C for 7 days. The dried leaves were ground into flour, which was used for various extractions and analyses.

Extracts by maceration

Distilled water was used as the aqueous solvent, while 70% ethanol (70%Eth) and 70% methanol (70%Met) solvents were prepared from diluted ethanol and methanol, respectively, to obtain hydro-alcohols, using Gay-Lussac alcohol wetting table. Then, 10 g leaf powders were added to 100 mL of each solvent. The mixtures were macerated for 40 minutes on magnetic stirrers (Shewale and Rathod, 2018). Thereafter, the mixtures were filtered using filter paper, and the solvents were evaporated at 40°C for 24 hours in an oven (Biobase, HAS-T105, China) to obtain the dry extracts.

Lippia multiflora leaves' extract phytochemical screening

The phytochemical screening was performed by identifying several major groups of secondary compounds in the leaf extract. Their detection was based on the chemical reactions induced by these compounds upon the contact with appropriate reagents (Wagner and Bladt, 2001). These tests were carried out by using Abo (2013), and Mea *et al.* (2017) analytical techniques. The detection tests and the different groups sought are listed in Table 1.

Table 1. Tests for the detection of phytochemical compounds

Phytochemical compounds	Highlighting test	Observations in case of positive results
Sterols and polyterpenes	Liebermann	Green coloring
Polyphenols	Ferric chloride	Bluish-black coloration
Flavonoids	Cyanidine	Pinkish-orange or purplish coloring
Saponosides	Vigorous agitation	Foam height greater than 1 cm
Quinonic compounds	Borntraeger	Red to purple coloring
Alkaloids	Dragendorff	Reddish-brown coloration
Alkaloids	Bouchardat	Reddish-brown coloration
Catechetical tannins	Stiasny	Precipitated in large flakes
Gallic tannins	Hydrochloric acid	Intense blue-black hair color

Phytochemical compounds

Total polyphenol content

Total polyphenols were determined using Folin-Ciocalteu method adapted by Wood *et al.* (2002). The determination is based on polyphenols ability to reduce Folin-Ciocalteu reagent to tungsten oxide. Molybdenum turns blue, indicating the amount of total polyphenols in the mixture (Dewanto *et al.*, 2002). A 2.5 mL solution of Folin-Ciocalteu reagent diluted 1:10 was added to 30 µL of extract. The mixture was kept at room temperature for 2 minutes in the dark, and then 2 mL of 75 $g.L^{-1}$ sodium carbonate solution was added. The mixture was then incubated for 15 minutes in 50°C water bath and subsequently cooled rapidly. The absorbance was measured at 760 nm using a UV-visible spectrophotometer against a blank prepared under the same conditions as the test samples. A standard curve was prepared with gallic acid. The phenolic compound contents are expressed in mg of gallic acid equivalent per gram of dry extract (mg GAE/g DE). The tests were carried out in triplicate .

Condensed tannin content

The condensed tannins were quantified in the presence of concentrated sulfuric acid (37% (v/v)). The tannins depolymerize, and through the reaction with vanillin, are transformed into anthocyanins with a specific red color. This red coloration is measurable by spectrophotometry at 500 nm. A 0.05 µL aliquot of the extract was mixed with 3 mL of 4% vanillin and 1.5 mL of 12 M hydrochloric acid. After homogenization, the mixture was incubated at room temperature (25°C) for 15 min. The absorbance was read at 500 nm against a blank containing 96% methanol. The condensed tannin content was determined using catechin as a standard. The result was expressed as mg catechin equivalent per gram of dry extract (mg Cat.E/g DE). The assays were performed in triplicate (Kouamé *et al.*, 2021).

Extracts' antioxidant activity

The leaves' antioxidant activity was determined by using 2,2-Diphenylpicrylhydrazine (DPPH) method described by Mansouri *et al.* (2005). The previously unstable DPPH becomes stable after the acceptance of a hydrogen free radical (Samarth *et al.*, 2008). The antioxidants present in the extract induce this reduction, which results in a color change from purple to yellow. To determine the antioxidant activity, the concentrations from 0 to 2500 µg / mL of aqueous, ethanolic, and methanolic leaf extracts were prepared. A 70 µM DPPH solution was prepared with 96% methanol. A 100 µL volume of each solution was mixed with 3.9 mL DPPH solution. The mixture was homogenized and then incubated at room temperature at 25°C for 15 min. After incubation, the absorbance of the solutions was read at 517 nm against a blank prepared from 96% methanol and containing no extract. The percentages of inhibition were calculated with the formula given below. The concentration that inhibits 50% DPPH was determined by graphical projection onto the percentage inhibition curve.

$$I(\%) = \frac{A0 - A\ Sample}{A0} \times 100$$

With I(%) = percentage DPPH inhibition; A0 = absorbance of diluted DPPH, A sample = absorbance of sample + diluted DPPH.

Statistical analysis

All the data were generated in triplicate. The total polyphenol, and condensed tannins contents' computation, and inhibition percentages was performed

by using Excel software. The data were analyzed with XLSTAT software version 2014.5.03. Means were classified into homogeneous groups using Newman-Keuls test with a 95% confidence interval.

RESULTS AND DISCUSSION

Lippia multiflora leaves screening

Phytochemical screening of *Lippia multiflora leaves* revealed the presence of numerous compound families, including catechins and gallic tannins, saponins, sterols and polyterpenes, total polyphenols, flavonoids, and the absence of quinone compounds (Table 2). Masengo *et al.* (2023) previous studies reported the alkaloids absence in phytochemical screening of *Lippia multiflora* leaves, which is not the case here, as Dragendorff 's reagent detected alkaloids. In addition to the compounds detected by Masengo *et al.* (2023), anthocyanins and leucoanthocyanins were also identified by Allo *et al.* (2020). Previous work reported the absence of saponins in *Lippia multiflora* leaves harvested in Côte d'Ivoire, which is not the case here. The differences in *Lippia multiflora* leaves' composition can be explained by the influence of various parameters, including climatic, soil conditions, and the plant development stage. Indeed, according to Hayat *et al.* (2020), and Naczk and Shahidi (2004), the chemical composition and biological activities of medicinal plants are dependent on the geographical and climatic conditions of the harvesting areas.

Table 2. Lippia multiflora leaves' phytochemical compounds

Sought-after compounds		Test or reagents	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		Ferric chloride	+
Flavonoids		Cyanidine	+
Saponosides		Vigorous agitation	+
Quinonic compounds		Borntraeger	-
Alkaloids		Dragendorff	+
		Bouchardat	-
Tannins	Catechisms	Stiasny	+
	Gallic	Hydrochloric acid	+

Total polyphenol content

The polyphenols are secondary metabolic compounds derived from plants and possess strong antioxidant activities. These antioxidant activities confer numerous beneficial effects on human and animal health, such as protective effects against chronic degenerative diseases (Hossen *et al.*, 2017), and cardiovascular diseases (Giglio *et al.*, 2018). The total polyphenol content of aqueous extracts, 70%Met and 70%Eth of *Lippia multiflora* leaves are shown in Fig. 1. The highest total polyphenol content was observed in 70%Eth extract for 635±5 mg GAE/g.DE, followed by 70%Met extract for 525±15 mg GAE/g.DE. The lowest total polyphenol content was obtained with distilled water extract for 365±5 mg GAE/g.DE.

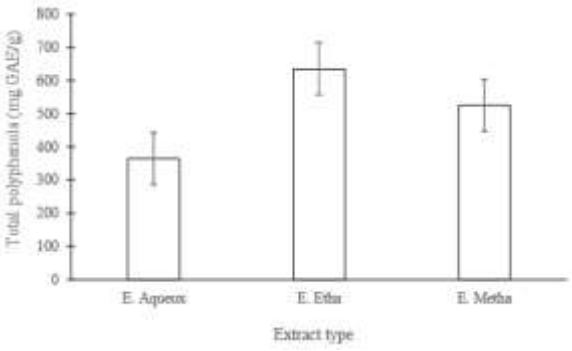

Fig. 1. Total polyphenol content of different extracts of *Lippia multiflora*

E. Metha: Methanol extract; E. Etha: Ethanol extract; E. Aqueux: Distilled water extract

According to Iloki-Assanga *et al.* (2015), the solubility of polyphenols in solvents depends on several parameters, including the hydrocarbon chains' length, the presence and the hydroxyl groups' position, and the molecular size.

The phenolic compounds are often extracted more readily with more polar solvents. This would explain the results, given the methanol and ethanol

high polarity compared to distilled water. Indeed, according to Asghar *et al.* (2016), methanol and ethanol possess a high extraction capacity for phenolic compounds due to their polarity. Furthermore, several authors have revealed that mixed solvents have proven very effective at extracting polyphenols compared to aqueous solvents. According to Farahmandfar *et al.* (2019), this could be explained by the fact that water molecules can only be involved in oxygen-hydrogen bonds, while alcohol molecules can be involved in both oxygen-hydrogen and carbon-oxygen bonds.

The highest concentrations of phenolic compounds were obtained with mixed solvents, specifically 70%Met, 70%Eth, and 70% acetone of *Euphorbia helioscopia* compared to aqueous extracts (Bourgou *et al.*, 2016). Here, the 70%Eth solvent exhibited the highest polyphenol content. This result is consistent with Farahmandfar *et al.* (2019) results, who obtained the highest levels of polyphenolic compounds in *Arum maculatum* with the ethanol/water (50/50, v/v) solvent using ultrasound. The polyphenol values obtained with 70%Met and aqueous extracts of *Carapa procera* were 414.79±0.69, and 295.17±0.07 mg GAE/g, respectively (Ipona *et al.*, 2023). Their result values were lower than ours obtained with *Lippia multiflora* leaves. Furthermore, the total polyphenol value of 336 mg GAE/g reported by Masengo *et al.* (2023) with the aqueous extract of *Lippia multiflora* is lower than that of 365±5 mg GAE/g.DE obtained in the present study with the aqueous extract.

Condensed tannin contents

The condensed tannins have numerous health benefits. Many studies have highlighted their strong antioxidant potential and diverse biological activities, including anticancer, anti-inflammatory, antidiabetic, anti-obesity, and antimicrobial properties (Amarowicz and Pegg, 2024). The condensed tannin content of aqueous, 70%Eth, and 70%Met extracts of *Lippia multiflora* leaves is in Fig. 2.

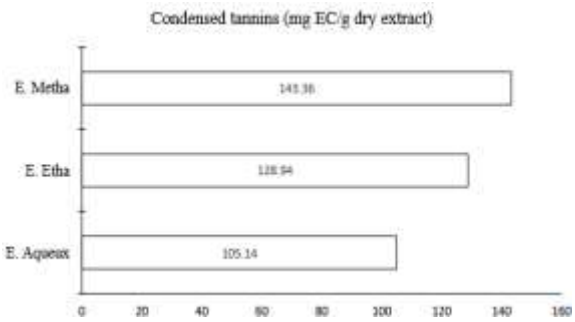

Fig. 2. *Lippia multiflora* leaves different extract' condensed tannin contents

E. Metha: Methanol extract; E. Etha: Ethanol extract; E. Aqueux: Distilled water extract

The lowest condensed tannin contents were recorded in the aqueous extract. Statistical analyses did not reveal any significant differences between the condensed tannin contents of 70%Eth and 70%Met extracts. Furthermore, the highest contents were recorded in these extracts. They were 128.94±5.59, and 143.36±9.35 mg Cat.E/g.DE for 70%Eth, and 70%Met solvents, respectively. This study indicates that the extraction solvents strongly influence the extraction of condensed tannins. Indeed, according to Iloki-Assanga *et al.* (2015), the extraction of a particular component depends on the solvent polarity, and the solute/solvent ratio. The tannin contents for 105.14±3.63; The concentrations of 128.94±5.59 and 143.36±9.35 mg Cat.E/g.DE, with the aqueous, 70%Eth and 70%Met extracts, respectively, were higher than those obtained by Masengo *et al.* (2024). For example, with aqueous, 70%Eth and 70%Met solvents with *Hibiscus acetosella,* Masengo *et al.* (2024) obtained 29.6±0.02; 26.4±0.08; 34.5±0.12 mg TAE/g, respectively.

***Lippia multiflora* leaves' extracts antioxidant activities**

The aqueous extracts, 70%Eth and 70%Met different concentration inhibition percentage was determined (Fig. 3). The 50% inhibitory concentrations of each extract were also determined to evaluate antioxidant efficiencies.

Extracts with concentrations ranging from 156 µg/mL to 2500 µg/mL exhibited inhibition rates that varied from 3.95% to 60.69% for the aqueous extract, from 12.94%

to 82.94% for the 70%Eth extract, and from 17.54% to 84.78% for the 70%Met extract (Fig. 3). The 70%Eth and 70%Met extracts showed higher inhibition rates compared to the aqueous extract. Furthermore, the lowest IC50 values were recorded for 70%Eth and 70%Met extracts, with respective values of 333.33 µg/mL and 375 µg/mL, compared to 1896.06 µg/mL for the aqueous extract. The 70%Eth. and 70%Met extracts exhibited better antioxidant activities compared to the aqueous extract of *Lippia multiflora* leaves, with the highest activity observed in 70%Eth extract. These results are similar to those obtained by Hayat *et al.* (2020) who reported IC50 values of 324.55 µg/mL, 333.58 µg/mL, and 752.43 µg/mL for ethanol, methanol, and water, respectively.

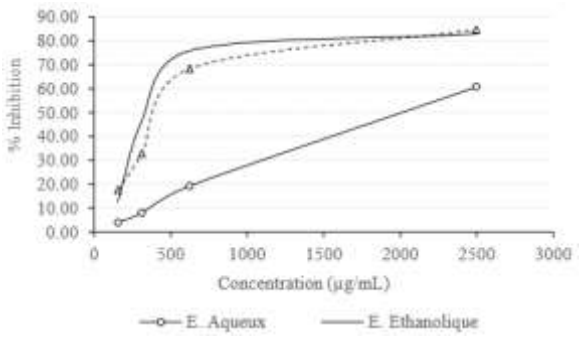

Fig. 3. Evolution of the inhibition rate as a function of concentration

E. Aqueux : Distilled water extract; E. Ethanolique: Ethanolic extract; E. Methanolique : Methanolic extract

Furthermore, Falleh *et al.* (2021) reported that, in general, ethanolic extracts exhibit stronger antioxidant activities than aqueous extracts. The antioxidant activity of plant extracts is primarily attributed to the polyphenols present in these extracts. Indeed, the ability of antioxidants such as ascorbic acid, tannins, and flavonoids to donate a proton, thereby reducing and decolorizing DPPH, has been demonstrated (Ipona *et al.*, 2023). Therefore, the higher antioxidant activity observed in the 70%Eth and 70%Met extracts can be explained by the high polyphenol extraction capacity of these solvents. Indeed, the highest levels of total polyphenols and condensed tannins were obtained with 70%Eth, followed by the 70%Met extract of *Lippia multiflora* leaves.

CONCLUSION

The study involved phytochemical screening of *L. multiflora* leaves, evaluating the extraction capacity for phenolic compounds and the antioxidant activity of various solvents, including aqueous solvents, 70% of ethanol (70%Eth), and 70% of methanol (70%Met). The phytochemical screening revealed the presence of tannins, flavonoids, and alkaloids in the aqueous extract. 70%Eth and 70%Met solvents were more effective in extracting total polyphenols and condensed tannins from *Lippia multiflora* leaves than distilled water. These solvents also displayed superior antioxidant activity compared to distilled water. Hydroethanol (70%Eth) was more suitable for extracting phenolic compounds from *Lippia multiflora* leaves. However, more studies should be conducted with other solvents and by varying preparing extract methods, in order to optimize *Lippia multiflora* leaves phenolic compounds extraction.

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