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ORIGINAL RESEARCH PAPER

PHYTOCHEMISTRY, ANTIMICROBIAL AND ANTIRADICAL ACTIVITIES EVALUATION OF ESSENTIAL OILS, ETHANOLIC AND HYDROETHANOLIC EXTRACTS OF THE LEAVES OF *EUCALYPTUS CITRIODORA* HOOK FROM BENIN

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Abstract: The precipitation and coloration reactions implemented in this study revealed in the leaves of *E. citriodora* (Myrtaceae) of Benin the presence of polyphenols, anthocyanins, flavonoids, saponins, tannins, mucilages, anthraquinones, leucoanthocyanins, cardiac glycosides, coumarins, proteins, sterols and triterpenes. The essential oil from the leaves of this plant was extracted with a yield of 3% and then analyzed by GC/MS. Nine compounds, representing 94.46% of the chemical composition of the oil, were identified, three among them being majority: citronellal (65.45%), citronellol (13.5%) and isopulegol (10.33%). The contents of polyphenolic compounds of ethanolic and hydroethanolic extracts were respectively 4.52 mg EAG.g^{-1} and 4.38 mg EAG·g^{-1} for total polyphenols, 78.76 mg EC·g^{-1} and 81.56 mg EC·g^{-1} for total flavonoids and 62.62 mg EC·g^{-1} and 67.09 mg EC·g^{-1} for condensed tannins. The radical scavenging activity of hydroethanolic extract (IC_{50}=0.23 mg·mL^{-1}) was more pronounced than that of ethanolic extract (IC_{50}=0.42 mg·mL^{-1}) and essential oil (IC_{50}=0.9 mg·mL^{-1}). Regarding antimicrobial activity, *Staphylococcus aureus* and *Candida albicans* were sensitive to hydroethanolic extract of *E. citriodora* leaves while *Escherichia coli* developed resistance against this extract. As for the essential oil extracted from the leaves of *Eucalyptus citriodora* it showed fungicidal activity against *Candida albicans* and bacteriostatic with *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Candida albicans, Escherichia coli, essential oil, polyphenolic compounds, radical scavenging*

INTRODUCTION

The medicinal plant use at therapeutic ends was a multisecular practice. But in spite of this use all mysteries of this over the plant world were not yet clarified. The survey of the plants chemistry was therefore always of a burning actuality in spite of its seniority and the exponential developments of the fields as the biotechnology and the synthesis chemistry. It results mainly from the fact that the plant reign was an inexhaustible source of biologic active principles.

The African flora and in particular the one from Benin interest overflows of several thousands of medicinal plants which got a little interest from scientific studies.

Eucalyptus was a large genus of Myrtaceae family comprised of about 900 species and subspecies [1]. *Eucalyptus citriodora* is a tree which height varying between 20 and 40 m. The leaves are alternate, hanging, to limb in the shape of forgery, of green color and propertied gray a strong characteristic odor. The flowers are in clusters or in umbels, propertied, each, a chalice accrescent tubes some slightly flared, of numerous stamens half notes, very decorative and an ovary infers to short style. The fruit is present under shape of capsule. Flowering spreads from May to September [2, 3].

Many species of the genus *Eucalyptus* were used in Africa folk medicine for a variety of medical conditions. The leaves of *E. citriodora* were used by the tradipraticiens to treat several pathologies of which among others: the obesity, the ageing, the cardiovascular illnesses and the diabetes [4]. For instance, hot water extracts of dried leaves of *Eucalyptus citriodora* Hook were traditionally used as analgesic, anti-inflammatory, and antipyretic remedies for the symptoms of respiratory infections, such as cold, flue and sinus congestion. Essential oils from *Eucalyptus* species were also widely used in modern cosmetics, food, and pharmaceutical industries [5]. In this regard, monoterpenoid components of the aromatic constituents of the oils were commercially available for the treatment of the common cold and other symptoms of respiratory infections [6-8].

A lot of studies dedicated to the essential oil of the leaves of *Eucalyptus citriodora* existed in the literature, but to our knowledge, very few works were about the ethanolic and hydroethanolic leaves extracts of this plant. For our contribution to the scientific knowledge, it seemed necessary to us to orient the reflection toward the valorization of leaves extracts of *Eucalyptus citriodora.*

The aim of the present study was to determine the chemical composition of the essential oil of the leaves, to identify the present secondary metabolites in the leaves which induced the antimicrobial, antifungal and antiradical activities of *Eucalyptus citriodora* leaves extracts.

MATERIAL AND METHODS

Plant material

The plant material used in the present survey was constituted of leaves of *Eucalyptus citriodora* harvested in Abomey-Calavi (Benin) in January 2013 and identified in the National Herbarium of Benin.

Biological material

Biological material was constituted of the reference strains of *Staphylococcus aureus (*ATCC 25923), *Escherichia coli* (ATCC 25922*)* and *Candida albicans* (*ATCC* 10231). These strains have been provided by the National Health Laboratory of Benin.

Methods

After plant material collection, the samples have been appropriated, then dried over the temperature of the laboratory during two weeks and then reduced into powder for a part and another was devoted to hydrodistillation extraction.

Phytochemical screening

The research of the sterols and terpenes has been made according to the reaction of Liebermann. The characterization of the compounds belonging to the group of the polyphenols has been performed by the reaction to the ferric chloride. Flavonoids have been put in evidence by the reaction of the cyanidine. The compounds belonging to the group of the tannins have been determined following the reaction of Stiasny. The free compounds or combined quinonics has been put in evidence according to the reaction of Borntraeger. The saponosides has been identified by the calculation of the moss indication and the alkaloids with the help of the reagents of Mayer and Dragendorff [9-12].

Extraction of the essential oil

The essential oil has been gotten on average by hydrodistillation with the help of an extractor of Clevenger type during two hours [13, 14]. After recuperation, it has been dried over the anhydrous sodium sulphate, and then preserved in dark small bottles, no permeable to the solar rays, until the analysis. The output (R) of the extraction was calculated by the formula:

$$R = \frac{Mass\ of\ essential\ oil\ extracted}{Mass\ of\ plant\ material\ used}\ X100$$

Determination of essential oil chemical composition

The chromatography in sparkling phase coupled to the mass spectrometry (CPG/SM) has been used for the determination of essential oil chemical composition.
The CPG/SMS coupling have been achieved on a device of type Hewlett Packard-Quadrupôle (Model 5970). The chromatograph was equipped with a capillary column of melted silica of 30 m of length and 0.25 mm of interior diameter with a phase transplanted stationary of type dimethyl polysiloxane (HP5) of 0.25 µm of thickness [14 - 16]**.**

Dosage of the flavonic aglycones and anthocyanidins

For flavonic aglycones and anthocyanidins research, 2 g of plant material were hydrolysis in presence of 160 mL of HCl 2 N. The solutions were placed then in to the double boiler at 100°C during 40 min. After cooling, flavonic aglycones and anthocyanidins were extracted as followed:

Flavonic aglycones

The differential proportioning of flavones and flavonols was carried out based on their chelating properties with $AlCl_3$ 1% in ethanol 95°. The absorbance was measured by using a UV–vis spectrophotometer ((ENWAY 50/60Hz) at the wavelength of 380 nm to 460 nm. The differential height of the peaks against a sample consisting of a extract solution with ethanol 95° without $AlCl_3$ is proportional to concentration of flavonic aglycones in the sample. The flavones have a maximum of absorption between 390 and 415 nm, whereas that of flavonols lies between 420 and 440 nm [41].

$$T_{aglycones} = \frac{A.M.V.f}{\varepsilon.P}$$

A: Absorbance of differential peaks
ε: Molar absorption coefficient of the quercetol (= 23000 $L \cdot mol^{-1} \cdot cm^{-1}$)
M: molar mass of quercetol (= 302 $g \cdot mol^{-1}$)
V: Volume of ethanolic solution (mL)
f: factor of dilution
p: mass of dry vegetable material (g)

Anthocyanidins

The aqueous phase was extracted three times with n-butanol which extracted anthocyanidins. The absorbance of butanolic phase was measured at the maximum wavelength (λmax) using the Spectrophotometer (JENWAY 50/60 Hz) and anthocyanidin content was calculated by following formula described in literature [17 - 18]:

$$T_{anthocyanidins} = \frac{\gamma.A.M.V.f}{\varepsilon.P} \text{ (mg of anthocyanidin} \cdot g^{-1} \text{ of dry matter)}$$

γ: Factor of correction ($\gamma = 6$), of proanthocyanidins transformation output (= 17%)
A: Absorbance at the maximum wavelength
ε: Molar absorption coefficient of the cyanidol (= 34700 $L \cdot mol^{-1} \cdot cm^{-1}$)
M: molar mass of leucocyanidol (=306 $g \cdot mol^{-1}$)
V: Volume of butanolic solution (mL)
F: factor of dilution
P: mass of dry vegetable material (g)

Preparation of hydroethanolic and ethanolic extracts

50 g of powder plant of every organ of *Eucalyptus citriodora* have been introduced in a small bottle of 500 mL containing 250 mL of extraction solvent. The small bottle was

plugged and was let under continuous agitation during 72 h. After decanting and filtrating on Wattman paper, the filtrate was concentrated to the rotary evaporator and the dry extract was preserved in the refrigerator.

Quantitative evaluation of polyphenolic compounds

Total Phenolic Content (TPC) was determined by a Folin-Ciocalteu assay using gallic acid as standard [19, 20]. The last one was constituted by a mixture of phosphotungstic and phosphomolybdic acids that were reduced, at the time of the oxidization of the phenols, in mixture of blue oxides of tungsten and molybdenum. The produced blue coloration possessed a maximal absorption around 765 nm, by reference to a range stallion gotten with phenolic acids (gallic acid), and permitted to determine the quantity of total polyphenols present in an extract. It was expressed in mg of equivalent gallic acid per gramme dry matter. The method with the aluminum trichlorure ($AlCl_3$) was used to quantify the total flavonoids [18, 21]. Technique was based on the formation of flavonoids-aluminum complex that possessed a maximal absorption at 500 nm.
The dosage of the tannins condensed was achieved by the method using the sulfuric vanillin. The principle of this dosage was based on the fixing of the aldehydic group of the vanillin on the carbon in position 6 of the cycle to the catechin to form a complex red color chromophore that absorbed at 510 nm and the absorbance was measured to the spectrophotometer (JENWAY 50/60 Hz) after 10 min rest of the mixture in the darkness [22].

Biological activity

Antiradical activity
The antiradical activity has been valued by the method in the DPPH [23 - 25]. The principle of this method was based on the measure of the free radicals trapping of DPPH solution. This trapping was visualized by the disappearance of the crimson color of the DPPH and the absorbance of the fading was measured to the spectrophotometer at 517 nm. The percentage of trapping has been determined by the formula:

$$P = \frac{(Ab - Ae)}{Ab} X\ 100$$

P: percentage of trapping; Ab: absorbance of the white; Ae: Absorbance of the sample.

Antimicrobial activity of hydroethanolic extract and essential oil of *Eucalyptus citriodora* leaves

Preparation of suspensions
This preparation was carried out from three stocks of tested bacteria. A pure colony of each stock was suspended in 5 mL of Mueller Hinton broth. After incubation at 37 °C for 2 h, we obtained 10^6 cfu·mL^{-1} corresponding to the scale 2 of Mc Farland standard [26].

Antimicrobial activity of the hydroethanolic extract

The methods reported by NCCLS [27]; Aladesanmi *et al*. [28]; Alshawsh *et al*.[29] have been used for this work.

A solution mother of hydroethanolic extract was prepared at the concentration of 400 $mg \cdot mL^{-1}$ in the mixture of ethanol/water (40/60). 100 µL of the medium Mueller-Hinton Broth were set down in every well of the microplaque. 100 µL of the extract solution - mother were set down in the first well. After homogenization well by aspiration - repression with the help of a micropipette one got 200 µL of an extract solution to 200 $mg \cdot mL^{-1}$. 100 µL of this new solution were appropriated and mixed in the MHB Muller Hinton Broth solution contained in the 2nd well and one pursued this set of dilution of reason two well by well until the 9th well, the remaining aliquot was thrown away. Finally 100 µL of a soup of 18h of microbial culture (1.510^6 $CFU \cdot mL^{-1}$at scales 2 of McFarland) were added in every well. The 10th and 12th wells corresponded respectively to the positive witness and to the negative one and contained 100 µL of MHB + 100 µL of microbial culture medium for the positive witness and 150 µL of MHB+50 µL of extract solution - mother to test for negative witness. The microplaque was covered with the aluminum paper and was placed during 24 hours in the incubator at 37°C. The MIC was estimated visually compared to the witnesses and every well was spread on the MHA gelose and placed to 37°C during 24 hours. The MBC corresponded to the smallest concentration of extract for which any microbial colonies development was not observed.

Antimicrobial activity of the essential oil

The method used was reported by Yehouenou *et al*. [26]. 2 mL of Mueller Hinton broth, 0.02 $g \cdot L^{-1}$ of phenol red in which were added 40 µL of essential oil and 1 to 2 drops of Tween 80. The mixture introduced in a hemolyse test tube was homogenized.

100 µL of bubble Mueller Hinton broth containing phenol red were distributed in all the 96 wells of microplate. 100 µL of essential oil emulsion (initial solution) were added well in the first column of the second line and successive dilutions of reason 2 were carried out well by well till the 12th one and the remaining aliquot (100 µL) were rejected. 100 µL of Mueller Hinton which not containing phenol red were introduced on the first well of the first column and successive dilutions of reason 2 were carried out as above. All the wells of the second column received 100 µL of bacteria suspension, the first line which represents the negative control and the second line, the positive control. The microplate was finally covered with parafilm paper and was incubated at 37°C during approximately 18 hours.

RESULTS AND DISCUSSIONS

Secondary metabolites

Secondary metabolites identified in the leaves of *Eucalyptus citriodora* were showed in Table 1.

Various secondary metabolites have been more or less materialized in the leaves of *Eucalyptus citriodora* by a set of the coloration or precipitation reactions specific to every active principle class.

Table 1. *Chemical groups of the leaves of Eucalyptus citriodora*

Secondary metabolites		**Leaves of *Eucalyptus citriodora***
Anthocyanins		+/-
Alkaloids		+
Free anthraquinoneses		+/-
Combined anthraquinones	O-heterosides	-
	O-heteroside with reduced genine	+
	C-heterosides	+
Flavonoids		+
Gallictannins		+
Catechic tannins		+
Mucilages		+/-
Saponosides		+
Polyphenols		+
reducingComposed		-
Leuco-anthocyanins		+
Proteins		+/-
Quinones		+/-
Coumarins		+

+: presence; +/-:Trace; -: Absence

Among the number of these secondary metabolites, we can mention the alkaloids, coumarins, quinones, saponosides, polyphenols, leuco-anthocyanins, mucilages, gallic tannins and catechic tannins, flavonoids, free anthraquinones, combined anthraquinones (O-heteroside with reduced genine, C-heterosides) and traces of anthocyanins. The wealth of the leaves of *Eucalyptus citriodora* in secondary metabolites could explain its use in threshold of the traditional medicine.

Chemical composition of essential oil extracted from the leaves of *Eucalyptus citriodora*

The Table 2 indicated the chemical composition of the essential oil extracted from the leaves of *E. citriodora.*

Table 2.*Chemical composition of the essential oil extracted from the leaves of E. citriodora*

Kovat's index	**Compound**	**Percent Composition**
940	α-Pinene	0.10
977	β-Pinene	0.42
1033	1,8-Cineole	0.52
1152	**Isopulegol**	**10.33**
1161	**Citronellal**	**65.45**
1231	**Citronellol**	**13.05**
1358	Citronellylacetate	2.05
1419	β-Caryophyllene	0.51
1726	(E, E) Farnesol	0.90
	Total	94.46

Nine compounds representing 94.46% of the total chemical composition of oil have been identified among which three majority one: the citronellal (65.45%); the citronellol (13.05%) and the isopulegol (10.33%). according to Djossou, the essential oil extracted from the leaves of *Eucalyptus citriodora* of Sèmè-Kpodji (Benin) contained 71.79% of citronellal; 11.29% of citronellol and 5.21% of isopulegol [30]**.** Gbenou [31] had determined in the essential oil extracted from the leaves of *E. citriodora* of Abomey-Calavi (Benin) citronellal (78.61%); isopulegol (5.81%) and citronellol (7.86%) whereas *Eucalyptus citriodora* of Ketou (Benin) was rich in citronellal (74.45%), isopulegol (6.46%) and citronellol (7.18%). The essential oil from the leaves of *E. citriodora* harvested in the Bucaramanga (Colombia) was rich in citronellal (40.0%), isopulegol (14.6%) and citronellol (13%) [32] where as the one of the leaves harvested in Congo has for main compounds, the citronellal (72.7%); the citronellol (6.3%); the eugenol (3.5%); the citronellyl acetate (2.3%) and theβ-caryophyllene (2.6%) [33]. Essential oil of the leaves of *Eucalyptus citriodora* of Cuba contained 90% of oxygenated compounds of which 70% of citronellal [34]. The difference observed at the level of the chemical composition of the essential oil of *Eucalyptus citriodora* by comparison of our results to the previous works highlighted some factors like the harvesting, the period, the pedological structure of the soil and some the climatic factors [35].

Yields of extraction and phenolic compounds content

Yields of extraction

The yields of the extracts expressed in percentage are consigned below in the Table 3.

Table 3.*Yields from the leaves of Eucalyptus citriodora extract*

Extracts	Essential oil	Non volatile	
		Ethanolic	Hydroethanolic
Yields (%)	3	20.8	23.4

The output in essential oil of the leaves of *E. citriodora* was equal to 3%. This content was slightly superior to the one gotten by Djossou for *E. citriodora* harvested in Semè - Kpodji that was 2.7% [30] while Cimanga *et al*. were found an output of 1.63% for the *E. citriodora* harvested in Congo [33]. This difference noted, could be assigned either to age, at the period of harvesting of the species and to the nature of soil [35]**.** The outputs of the ethanolic and hydroethanolic extracts were respectively 20.8% and 23.4%. The output of the hydroethanolic extract was more important than the one of the ethanolic extract. Other researchers demonstrated in their works that the addition of water to the system of extraction improve the output in polyphenolic compounds [36].

Phenolic compounds content

The Figure 1 translated the content in total polyphenols (PT) expressed in mg equivalent of gallic acid; the content in total flavonoids (FT) and the content in tannins digests (TC) expressed in mg per g of dry matter ethanolic and hydroethanolic extracts equivalent in catechin.

The contents in total polyphenols, in total flavonoids and in tannins condensed of the ethanolic extracts of the leaves of *Eucalyptus citriodora* were respectively: 4.515 mg EAG·g^{-1}; 78.76 mg EC·g^{-1} and 62.62 mg EC·g^{-1} of dry matter, whereas the one of the hydroethanolic extracts was of 4.38 mg EAG·g^{-1}of dry matter; 81.56 mg EC·g^{-1}and 67.09 mg EC·g^{-1}. Exceptionally the content in total polyphenols was practically insensible to the nature of the solvent, those in flavonoids and in tannins digests are raised more for the hydroethanolic extract than the one of the ethanolic extract.

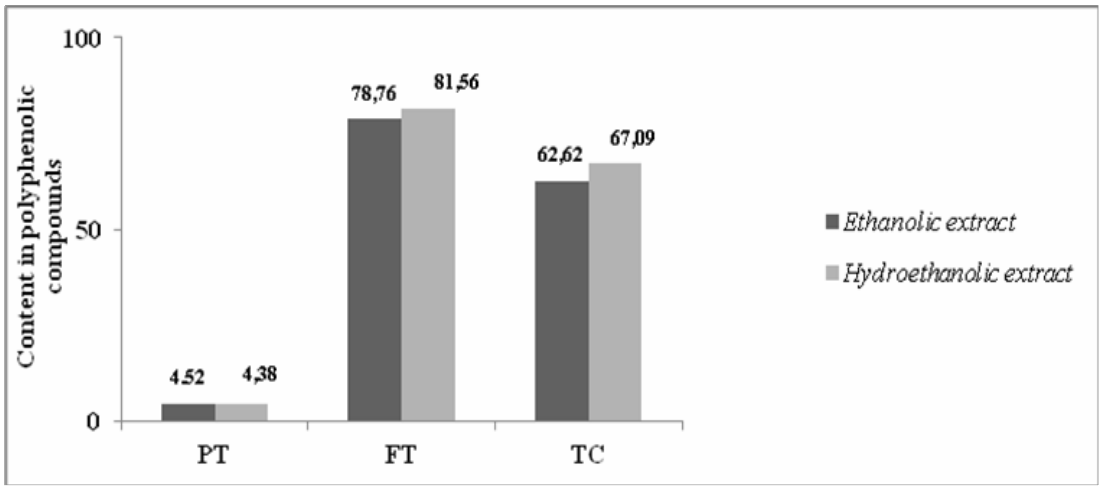

Figure 1. *Phenolic compounds Content of ethanolic and hydroethanolic extracts from Eucalyptus citriodora leaves*

Flavonics aglycones and anthocyanidinins Contents

The Figure 2 indicated the contents in flavonics aglycones (Tagly) and in anthocyanidinins (Tantho) expressed respectively in mg equivalent of quercetol and in mg equivalent of Cyanidols per g of dry matter. These contents were respectively 1.21 mg·g^{-1} and 2.3 mg·g^{-1}.

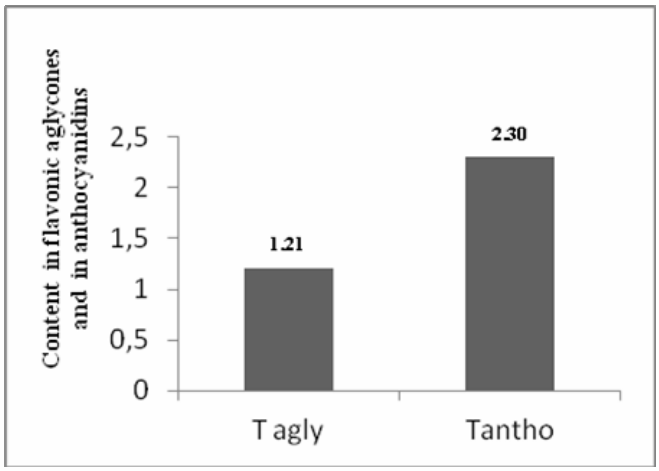

Figure 2. *Flavonics aglycones and anthocyanidinins Contents (mg.g^{-1}) of Eucalyptus citrodora leaves*

Antiradical activity of the extracts from leaves of *Eucalyptus citriodora*

Essential oil

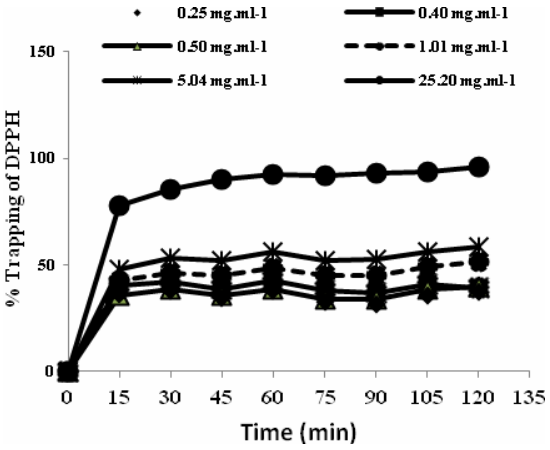

Figure 3. *Kinetic of radical trapping (DPPH) at different concentrations of the essential oil*

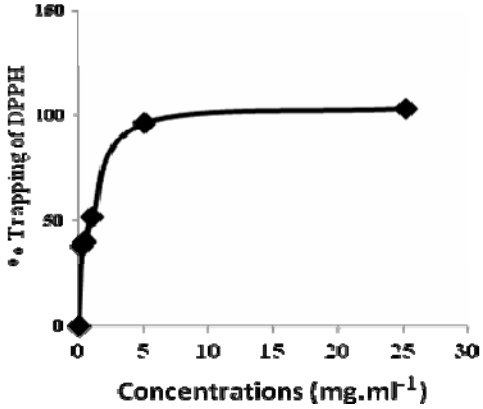

Figure 4. *Percentage of trapping according to the concentrations of the essential oil extracted from the leaves of Eucalyptus citriodora*

The Figure 3 translated the percentage of trapping of DPPH by the essential oil extracted from the leaves of *Eucalyptus citriodora* at different concentration according to the time.

For concentrations of essential oil composed between 8.4 mg·mL^{-1}and 168 mg·mL^{-1}, we noted a progressive increase of the rate of trapping of the free radicals (DPPH) in the two hours of reaction whereas from an equal concentration at 840 mg·mL^{-1} the percentage of trapping becomes constant and practically equal to 100% at the end of 15 min; within sight of these results, we noticed that the percentage of trapping was bound strongly to the concentration of the essential oil and that the adequate concentration range to the determination of the antiradical activity was from 8.4 mg·mL^{-1} to 840 mg·mL^{-1} with one time of balance of two hours. While taking into account the concentrations of the extract inferior to 840 mg·mL^{-1}, it was necessary to use the percentages of trapping after two hours of reaction to value the concentration of the essential oil that permits to trap 50% of radicals.

The tracing of the graph expressing the rate of trapping of the radical DPPH according to the concentration of extract (figure 4) permitted to determine by graphic extrapolation the concentration of the essential oil permitting to trap 50% of radicals of DPPH (IC_{50} = 0.9 mg·mL^{-1}).

The Figure 5 translated the percentage of trapping of the ethanolic extract of *Eucalyptus citriodora* to different concentration in function of time.

With the concentrations of the ethanolic extract situated between 0.78 mg·mL^{-1} and 3.64 mg·mL^{-1}, it was noted a progressive increase of the rate of trapping of the free radicals on a length of two hours while at superior concentrations or equal to 3.64 mg·mL^{-1} the percentage of trapping reaches its maximal value (92.4%). The gotten kinetic profile revealed an antiradical activity greatly dependent of the extracts concentration. The time

of balance is therefore dependent of the extract concentration. This time passed from two hours to thirty minutes when the concentration of extract passed from 0.78 mg·mL^{-1} to superior values.

Ethanolic extract

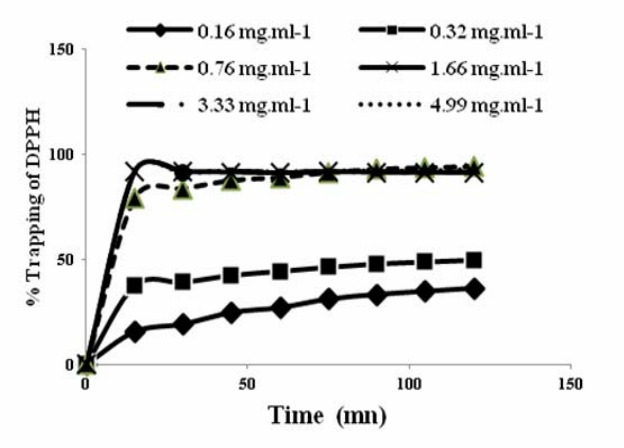

Figure 5*. Kinetic of radical trapping (DPPH) at different concentrations of the ethanolic extract from the leaves of Eucalyptus citriodora*

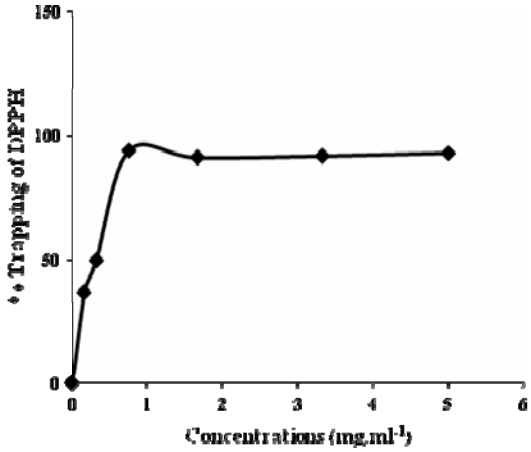

Figure 6. *Percentage of trapping according to the concentrations of the ethanolic extract from leaves of Eucalyptus citriodora*

Hydroethanolic Extract

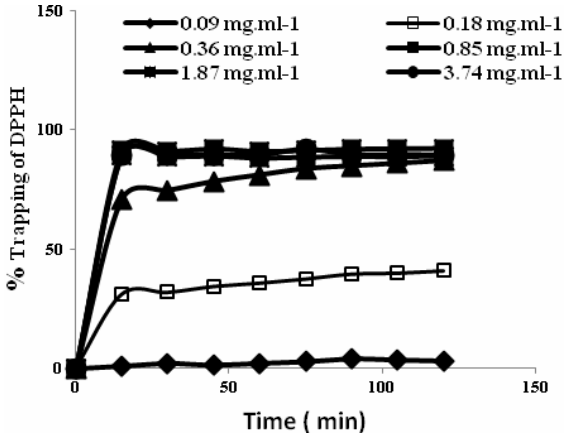

Figure 7*. Kinetic of radical trapping (DPPH) at different concentrations of the hydroethanolic extract from leaves of Eucalyptus citriodora*

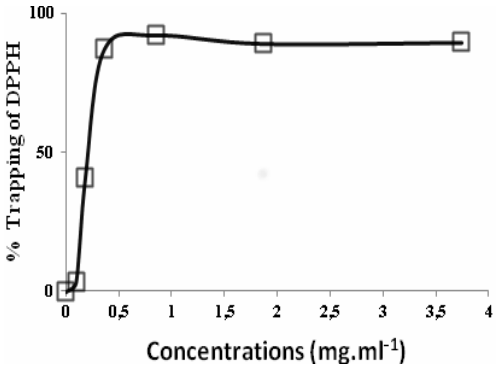

Figure 8. *Percentage of trapping according to the concentrations of the hydroethanolic extract from leaves of Eucalyptus citriodora*

The Figure 7 translated the percentage of trapping of the hydroethanolic extract of *Eucalyptus citriodora* at different concentrations according to the time.

For the concentrations of the hydroethanolic extract leaves of *Eucalyptus citriodora* at the lower or equal to 0.4 mg·mL^{-1}, it was noted a practically equal trapping percentage

to zero after two hours of reaction. When the concentration of the extract was situated between 0.78 mg·mL^{-1} and 1.54 mg·mL^{-1}, it was observed a progressive growth of the trapping rate during two hours while for concentrations of extract superior or equal to 3.64 mg·mL^{-1}, the percentage of trapping became maximal (90%) after 30 min of reaction. Within sight of these results, it was necessary to signal that the antiradical activity increased with the concentration of this extract.
The tracing of the graph expresses the rate of trapping of the radical DPPH according to the concentration of the extract (Figures 6 and 8); after two hours reaction permitted to determine the concentrations of the ethanolic and hydroethanolic extracts favoring to trap 50% of free radicals (IC_{50}).
The hydroethanolic extract that was revealed the richest in phenolic compounds presented an antiradical activity more pronounced (IC_{50} = 0.23 mg·mL^{-1}) than the ethanolic extract (IC_{50} = 0.42 mg·mL^{-1}) and the essential oil (IC_{50} = 0.9 mg·mL^{-1}). The antiradical activity of these extracts would be bound therefore to their content in polyphenolic compounds.

Antimicrobial activity

Minimum inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) of the investigated extracts and Minimal fungicide concentration (MFC) of extracts from *Eucalyptus citriodora* leaves were consigned in Table 4.

Table 4. *Minimum inhibitory concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicide Concentration (MFC) of extracts from leaves of Eucalyptus citriodora*

Stumps	**MIC, MBC and MFC of the investigated extracts of *Eucalyptus citriodora***		
		Hydroethanolic extract [mg·mL^{-1}]	**Essential oïl [µl·mL^{-1}]**
C. albicans (ATCC 10231)	MIC	25	0.63
	MFC	100	1.25
E. Coli (ATCC 25922)	MIC	-	1.25
	MBC	-	-
S.aureus (ATCC 25923)	MIC	6.25	1.25
	MBC	50	-

The hydroethanolic extract of the leaves of *E. citriodora* has a fongistatic activity to a concentration of 25 mg·mL^{-1} and fungicide to 100 mg·mL^{-1}on *C. albicans* whereas the essential oil was fongistatic at 0.625µl·mL^{-1}and fungicide at 1.25 µl·mL^{-1} on the same strain. The essential oil extracted therefore from the leaves of *E. citriodora* displayed an antifungal power more pronounced on *C. albicans* than the hydroethanolic extract. *E. coli* developed a resistance against the hydroethanolic extract from the leaves of *Eucalyptus citriodora* whereas the essential oil was bacteriostatic at equal concentration of 1.25 ·mL^{-1}. *S. aureus* was inhibited for a concentration of the hydroethanolic extract at 6.25 mg·mL^{-1} whereas the extract became bactericidal to an equal concentration at 50 mg·mL^{-1} as for the essential oil of the leaves of *E. citriodora*, it do not inhibit *S. aureus* to an equal concentration at 1.25 µl mL^{-1}, thus presented no bactericidal activity. The

antifungal activity noted to the level of the essential oil could be due to its main compound or to a synergy between the majority compounds and its minorityone. These results were in concordance with the previous works that showed the essential oil extracted from the leaves of *E. citriodora* proved to be more efficient, opposite to the bacteria than, of the mushrooms [37]**.**

The antibacterial and antifungal activities of the hydroethanolic extract of the leaves of *E. citriodora* respectively on *S. aureus* and *C. albicans* could explained themselves by the presence in this extract of the secondary metabolites like the flavonoids, the tannins and the coumarins [38-40].

According to the obtained results, it was noticed that *E. coli* developed a resistance against the hydroethanolic extract from the leaves of *E. citriodora*. This resistance could be due to the nature of the membrane of this strain that was probably impervious to the active principles contained in the extract. This same remark had been made by other researchers who showed that the bacterial partition of the Gram- would developed a resistance to the active principle penetration contained in the extracts of plants [41], essentially in relation of with its wall made by lipopolysaccharides and phospholipids, which block the penetration of the extract inside the cell cytoplasma [26].

CONCLUSION

The present work has been dedicated to the survey of the essential oil, ethanolic and hydroethanolic extracts from the leaves of *Eucalyptus citriodora* of Benin. After determination essential oil chemical composition, the identification of the secondary metabolites and the dosage of the different composed polyphenolic of the ethanolic and hydroethanolic extracts, the antimicrobial and antiradical activities of these extracts have been valued.

The phytochemical screening revealed the presence of several secondary metabolites (polyphenols, anthocyanins, flavonoids, saponosides, tannins, mucilages, anthraquinones, leuco-anthocyanins, cardiotonic glycosides, sterols, triterpenes, coumarinins and proteins) in the leaves of *Eucalyptus citriodora*.

Nine compounds have been identified in the essential oil of the leaves of *Eucalyptus citriodora* representing 94.46% of its chemical composition. Three of these majority compounds were the citronellal (65.45%), the citronellol (13.5%) and the isopulegol (10.33%). This essential oil possessed the antifungal activity and interesting antibacterial activity.

The contents in total polyphenols and in total flavonoids were raised more in the hydroethanolic extract than in the ethanolic extract, what could explained the antiradical activity more pronounced of the hydroethanolic extract.

With regard to the antimicrobial activity, *C. albicans* and *S. aureus* were sensitive to the hydroethanolic extract of the leaves of *Eucalyptus citriodora* whereas *E. coli* developed a resistance against this extract.

These interesting exploratory results will be pursued for the determination of the chemical structure of the molecules that underlies the different activities noted to the level of the hydroethanolic and ethanolic extracts in order to propose a structure - activity relation.

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