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Chemical Composition, Antioxidant and Antibacterial Activity of *Curcuma longa* L. Essential Oils

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Abstract

The objective of this study was to characterize the chemical composition of the essential oil of *Curcuma longa L* and its anti-oxidant, antimicrobial and physicochemical properties.The chemical composition of the hydrodistilled essential oils of *Curcuma longa L* was analysed by gas chromatography- mass spectrometry. Sixty five compounds representing 93 % of the total oil were identified; the major components were α-tumerone (28.02 %), β-sesquiphellandrene(9,98%),Zingiberene(10,37),β-tumerone (9.85 %), ar-tumerone (2.61 %), and ar-curcumene (8.57 %), β-curcumene (5.70 %).The antioxidant activities of the oil and various extracts of *Curcuma longa L* were evaluated by using 2,2- diphenyl-1-picrylhydrazyl and superoxide radical-scavenging assays in 100 g of *Curcuma langa L* powder The half-maximal inhibitory concentration of oil and methanol extract showed value (17.976 and 21.678 µg/ml) respectively, ethanol extract (36.446 µg/ml) showed moderate radical scavenging activity toward ascorbic acid (14.105 µg/ml). The antimicrobial activity was carried using diffusion agar method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*.The highest and broadest activity was shown on *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Curcuma longa L.*, Essential oil, Chemical composition, physicochemical properties, Antibacterial activities, potential antioxidant, gas chromatography- mass spectrometry

1. Introduction

Curcuma longa L. (Zingiberaceae) has been widely studied by the scientific community due to its diverse properties usually attributed to substances present in its rhizomes. It's essential oil generally contains turmerone, dihydro turmerone, and aromatic ketone, in addition to a variety of other volatile components such as aliphatic or oxygenated mono- and sesquiterpenes (Ferreira FD et al., 2013; Liu CH and Chang FY 2011; Peret-Almeida L et al., 2008; Prakash B et al., 2012; Silva FilhoCRM,et al.,2009) .Currently been characterized by gas chromatography and mass spectrometry systems, as well as through research of their antimicrobial and antioxidant activities (Lang G and Buchbauer G, 2012). Variations in climatic conditions may interfere with the composition of the essential oils (Zhang L. et al., 2017).

Aromatic plants are a potential natural source of biomolecules; they are the subject of rigorous scientific studies for their possible use as alternative to drugs. Curcuma longa L., popularly known as turmeric, is a perennial rhizomatous plant belonging to the Zingiberaceae family (Gupta, A.K. et al., 2015). Presumably from India, Curcuma longa is also present in most tropical regions, in Latin America, in the islands of the Pacific Ocean, and even in Africa. Previous research reports suggested that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly

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cardiovascular diseases and cancer (Sathisha AD et al., 2011). In turmeric rhizome two major classes of secondary metabolites i.e. phenolic curcuminoids and essential oil (Funk et al., 2010). The rhizomes of several Curcuma species are widely used in indigenous medicine owing to their pharmacological activity, mainly the antimicrobial (Wilson et al, 2005), anticancerous (Azuine and Bhide, 1992; Upadhyayand et al., 2013), antidiarrheal (Owolabi et al., 2012) and anti-inflammatory (Jang et al., 2001).

Turmeric powder and crude extracts have been evaluated for some pharmacological activities such as hepatoprotective(Hossen M et al.,2017) antifungal, (Neelofar K et al., 2011; Sharma M. 2010) neuroprotective (Kadri Y., 2018 ;Yuliani, S, 2018) and memory improvement(Serafini, M.,2017).

The members of the family Zingiberaceae were also reported as natural antimicrobial agents, especially their essential oils (Chen IN et al., 2008; Akarchariya N., 2017) and their phenolic extracts.

In this context, the chemical profile (EH polyphenolic) and antioxydant effect from Curcuma longa L were determined then the antibacterial activity were evaluated against some pathogenic bacteria such as Escherichia coli, Bacillus subtilis, and Staphylococcus aureus.

2. Materials and methods

2.1. Plant material

The *Curcuma longa L* plant in the form of dry rhizome (figure 1) which is the subject of our chemical and biological study has been identified and recorded in the raw state by a botanist in the department of Biology (University of SBA). A referenced specimen (RH00034) was introduced in the WAMAP-base of the Laboratory of Bioconversion, Microbiological Engineering and Sanitary Safety (LBMESS) of our university.

Plant rhizome

Figure 1: *Curcuma longa L.*

2.2. Preparation of rhizome extract

A mass 100 g of air-dried *Curcuma longa L* rhizome powder was extracted with ethanol and methanol separately at room temperature for 72 hours. The filtrates subjected to rotary evaporation under reduced pressure. Then the residue was dried, scraped and stored at 5°C in test tubes protected from air and light until use. The extraction process gave extracts of ethanol (5.4 g) and methanol (6.8 g). Solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, France).

2.3. Essential oil extraction

100 g of dried turmeric powder are introduced into a 500 ml flask, containing 300 ml of distilled water and some pumice stones. The mixture was heated to the boil for about 5 hours in Clevenger-type apparatus. Repeat the operation several times to obtain a sufficient quantity of the EOs. The sodium chloride (NaCl) was added to the distillate and filtered. The EOs which separated on the water column was collected, and dried over anhydrous sodium sulfate. The oils were centrifuged and stored in hermetically sealed colored vials at 4°C before analysis and test. EOs extractions were done in three replications and the extraction yield was expressed as the weight of EOs volume on the weight of turmeric powder used (W/W).

2.3. Physicochemical analysis

Our analyses were done in the physico-chemical analysis laboratory (CRAPC) dependent on the delegated ministry in charge of scientific research. Determination of physical and chemical indices is crucial to characterize the EOs of the studied plant. All chemicals used were of analytical grade.

2. 2.3.1. Physical index

The specific density (NFT-75 111) of the essential oil is the ratio of the mass of a certain volume of the essential oil and the mass of same volume of water taken at the same temperature. The measure the density of the essential oil was conducted by using a pycnometerm at 20°C, refraction index **(**NFT-75 112) is the ratio between the sine of the angle of incidence of the light beam in the air and the sine of the refraction angle of the refracted ray in the considered environment. We have conducted the measurement of refractive index of the essential

oil using a conventional refractometer of Bellingham type. The refractive index is given by a direct reading on the refractometer; temperature set at 20 ° C.The optical rotation (NFT-75 113) by polarimetry at 589 nm and 25°C as a solution in dichloromethane.

Miscibility with ethanol (NFT-75 101) in an Erlenmeyer flask containing 1 ml of essential oil, through fraction of 0.2 ml and using a burette of 20 ml we pour ethanol (70%) stirring after each addition. When a limpid solution is achieved we register directly the volume of alcohol added.and freezing point (NFT-75 102) of an essential oil is the constant or maximum temperature observed during the latent heat release phase of solidification, when this essential oil in the liquid state is cooled according to the method described. The essential oils are placed in test tubes, inside a freezer, accompanied by a thermometer all analyses are determined by the methods in accordance with A.F.N.O.R (1992) codex.

2.3.2. Chemical index

Methods used for the determination of the acid value (NFT-75 103)) is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the free acids contained in (01) gram of the essential oil and ester index (NFT-75 104) is the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the liberated acid by a hydrolysis operation of the esters in basic environment are also in conformity with the standards A.F.N.O.R (1992) codex.

2.4. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The composition and characterization of EOs were analyzed by the chromatographic and mass spectroscopy analysis (GC/MS). The identification of volatile compounds was performed on a gas chromatograph HP –MS Hewlett Packard Agilent 6890 interfaced with a HP-MS Packard Agilent 5973 mass spectrometer with electron impact ionization (70 eV). Using HP-5 MS capillary column (30 m × 0.25 mm, coated with 5% phenyl methyl silicone and 95% dimethylpolysiloxane, 0.25 mm film thickness) with helium as the carrier gas (0.5mL/min). The column temperature was programmed from 60 to 250ºC at a rate of 4 ºC/min, and finally held isothermal at 250 °C for 10 min. The volume injected was 0.1 µL. The split flow ratio was 80:1. The injection temperature was 250°C and the gas chromatography/mass spectroscopy interface temperature was set at 270°C.The compounds of the EOs were identified based on the comparison of mass spectra and Kovats retention indices with a series of n-alkanes with those of data available in the NIST library, Wiley GCMS library and literature (Adams, R.P., 2007).

3. Antibacterial activity

Antibacterial susceptibility and resistance tests were carried out using the distribution of records (Rota and al., 2008). The following bacterial species were used for their pathogencity, Gram-positive bacteria: Staphylococcus aureus (S. aureus) (ATCC 25923) and Bacillus cereus (B. cereus) (ATCC 14931), Gram-negative bacteria: Escherichia coli (E. coli) (ATCC 25922). Antibacterial activity was analyzed by the disc diffusion method (Oonmetta-aree J et al., 2006).

The Mueller-Hinton ar and dimethyl sulfoxide (DMSO) solutions (in ratio 1:25, v/v) were vortexed for 2 min and immediately 20 ml were poured into sterile Petri dishes (90 mm diameter) and left to set for 30 min. A sterile paper disc (6 mm diameter) impregnated with 5µl of EOs or phenolic extracts at final concentrations of 20µg/ml, was placed on the surface of each plate, and incubated for 24 h at 37°. The control test by aqueous DMSO alone showed no toxicity in the concentrations used for these bacteria. The results of agar diffusion assays were evaluated by measuring the inhibition zone diameters (in mm), after incubation. All the experiments were calculated for the inhibition zone diameters.

A microplate method was used to determine minimal inhibitory concentration (MIC) values of plant extracts and EOs. The product extracts were serially diluted, ranging from 1/2 up to a 1/100 dilution. The MIC readings were performed by a spectrophotometer with a microplate reader at 595 nm. MICs values were calculated by comparing growth in control wells and the extracted blank, which consisted of inoculated plates. The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition of more than 90% at 48 h, as compared to the control.

4. Antioxidant Activities

4.1. Free radical scavenging assay (DPPH)

The free radical scavenging assay of the EOs was determined by using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) (Alam et al., 2008; Kumaraswamy MV and Satish S, 2008). Briefly, 3mL of EOs were added to 1mL of DPPH solution (0.2mM in methanol) as the free radical source.

The mixture was vigorously shaken and left for 30 min in the dark. The range of reduction of the DPPH radical was measured at 517 nm using UV-VIS spectrophotometer (Shimadzu).

As a reference standard, ascorbic acid was prepared under the same conditions to serve as positive control. The negative control consists of DPPH and methanol.

The DPPH assay is based on the measurement of the free radical scavenging activity of a test compound (Kedare and Singh, 2011). The solution was kept in a shaker and incubated in dark for 30 min at 37 °C. After an incubation period of 30 min at room temperature, the absorbance was read against a blank at 517 nm. The inhibition percentage of free radical DPPH was calculated as follows:

DPPH - scavenging activity (%) = [(Absc-Abss)/Absc] x100

Absc: Absorbance of the negative control without Eos; Abss: Absorbance in the presence of EOs.

5. Statistical analysis

All the experiments were carried out in triplicate. The results are presented as mean±SEM. Statistical analysis was performed one way analysis of variance (ANOVA) and $p \leq 0.05$ were considered as significant.

Results and discussion

Yield and Physico-chemical analysis of EOs

The essential oil content, obtained *from Curcuma longaL* is (1.97 ± 0.03 %) (table1), compared to witch obtained (2.2%) (Raina,V.K. et al., 2002).

Table1
Extraction yield

Plants	Mass of plant(g)	Mass of Extract (g)	Y (**%**)
Curcuma Longa L	100	1.989	1 .97

The extraction yield of EOs is influenced by the nature of the soil, the apparatus used, the temperature, pression and the time of distillation method. Physical and chemical characteristics of *C. langaL* EOs are exposed in (table 2). These physicochemical properties are used as means of verification and quality control of essential oil (Afssaps, 2008).

The measured refraction index corresponds to the standards where their value (1.406) is higher than the refraction index of water. Boukhatem et al., (2010) affirm that the refraction index varied with the monoterpenes content and derived oxygen's. A high content of monoterpenes gave a high index. While the density (0.825) is lower than that of water (1.0).

Our EOs have +19.5 of optical rotation and -16.8 °C of freezing temperature, concerning the miscibility with ethanol, the EOs is less miscible in this solvent (1v : 3v).

The measured acid value is 0.56 mg KOH/g. Indeed, fresh oil contains very little free acid. This is related to the storage period that the oil can be degraded as the hydrolysis of esters. Also, it is interesting to note that the ester index was found to have a value of 15.78 mg KOH/g .These results put-into work with steep Lazouni et al., (2007) who affirmed that the oil of high quality should have a higher index of ester and acid value lower than low oil quality. We note also variations of the ester and acid values during time and temperature. Besides, an essential oil of high quality will have a relative density, optical rotation and an index of ester higher than low oil quality, but has a lower index of refraction (Dumortier, 2006).

Table2.

Physicochemical parameters of *C. langa*essential oils

Physical indices				
Density D	Refractive index N_{20}	Optical rotation	Freezing point (°C)	Solubility in ethanol 90%
0.825	1.406	+19.5°	-16.8°C	1v: 3v
Chemical indices				
Acidity (mg KOH/g)			Ester index (mg KOH/g)	
0.56			15.78	

GC-MS

The complex nature of the essential oil from common wormwood is demonstrated in the chromatogram **(Fig. 1).**The chromatographic analysis of EOs C. longa essential oil by GC-MS allowed the identification of 65 compounds which represented 93.0232% (**table 2**). The proportions of the components present in EOs vary greatly. The dominated constituents were oxygenated monoterpenes: α-turmerone (28,02%), β-turmerone (9,85%) ar-turmerone (2,61%), ar-curcumene (8,57%), 1,8−ceneole (6,31%), ar-curcumene (8,57%) and the sesquiterpenes: β-sesquiphelladrene (9,98%), α-phellandrene (4,84%), (E)-β-caryophyllene (4,61%), caryophyllene (3,54%) and a significant percentage for Zingiberene (10,37%). In contrary for Sabinene(0,009%) which has a very low value.Sesquiterpenes in plants are related to a protective function of these compounds against fungi, bacteria, insects and other pests, i.e., for plant preservation (Bakkali F., Averbeck, S., Averbeck D. &Idaomar M. M. (2008).

Hassan et al 2016 determined that the major components of a sample of C. longa essential oil that showed high antimicrobial and antioxidant actions were β-sesquiphellandrene, α-curcumene, and p-mentha-1,4(8)-diene. Another study (Avanço GB. et al ., 2017) pointed to the presence of α-turmerone, β-turmerone, and ar-turmerone, mainly, and to antifungal and antimycotoxigenic activities.

The results reported by another researcher are relatively in agreement with the results presented here except for the amounts of the compounds. However, it is not worthy that the composition of the EOs from a particular species of C. longa plant can differ between harvesting seasons, extraction methods, and geographical sources, and that those from the different parts of the same plant can also differ widely (Burt, 2004, Bordolol et al., 1999).
This chemical composition is relatively similar to those reported by (Avanço,G.B.,and al., 2017; Singh et al., 2010 and Gounder and Lingamallu 2012).

Curcuma longa L.(Zingiberaceae) has been widely studied by the scientific community due to its various properties usually attributed to substances present in its rhizomes.It's essential oil usually contains turmerone, dehydroturmerone and aromatic ketone, in addition to a variety of other volatile components such as aliphatic or oxygenated mono- and sesquiterpenes. Studies on the characterization of turmeric essential oil show that among the most abundant Components are ar-tumerone, α-tumerone, β-tumerone, zingiberene, ar-curcumene and β-sesquiphellandrene.Following the work of (Zhang et al. 2017) evaluated samples collected from 20 different habitats in China and found that the composition and bioactivity of essential oils were varied.Among the most abundant components, they found ar-turmerone, β-turmerone, α-zingiberene, ar-curcumene and β-sesquiphellandrene. Another study found the presence of ar-turmerone (36.04%), curlone (8.78%), β-turmerone (7.05%), 8,9-dehydro-9-formyl-cycloisolongifolene (5.69%), β-sesquiphellandrene (5.39%) (Zheng, et al., 2020).

The same for Tefiani.C. 2015 where aromatic Tumerone is the major compound with a rate of around 35.0% .On the other hand, (Singh et al. 2011) have confirmed that ar-turmerone is the major constituent of turmeric rhizome oil from different origins. This majority of tumerones and also observed in the work of (Naz S. et al. 2010) , (Essien EE et al.,2015) and (Avanço et al., 2017) also the major components identified in the oils by gas chromatography were ar -turmerone (40.00% ± 13.20%), α-turmerone (10.05% ± 2.90%) and curlone (22.73% ± 12.72%) (Guimarães , et al., 2020).

In contrast to that observed in rhizomes, where the sesquiterpenesar-turmerone is frequently reported as the major compound in EO from C. longa (Ferreira, F.D. et al., 2013; Tavares et al., 2013; Sandeep et al., 2016).

On the other hand, Priya *et al*. (2012) obtained β-sesquiphellandrene as the majority compound with a rate of 22.8% followed by terpinolene (9.5%) and aromatic curcumene (7.8%).

But in other studies, zingiberene has been observed with a rate that is slightly higher than that of Tumerone, as in the case of the volatile components of C. longa contained zingiberene (13.18%), α-tumerone (10.94%), ar-tumerone (10.57%), α-santalene (8.77%), β-sesquiphellandrene (7.45%). (Suphrom et al.,2017) and for (Gonçalves et al., 2019) the major component was zingiberene (11%), followed by sesquiphellandrene (10%), -turmerone (10%) and α-curcumene (5%).

(Sindhu *et al*. (2011) analyzed the essential oils of leaves extracted by hydro distillation. The resultshowed α-phellandrene (24.35%), terpinolene (13.10%), p-cymene (11.07%) and 1,8-cineole (7.04%) as the main components of the oil of turmeric leaf.

A variety of component results are observed. Variations in climatic conditions can interfere with the composition of essential oils (Zhang L *et al*., 2017).

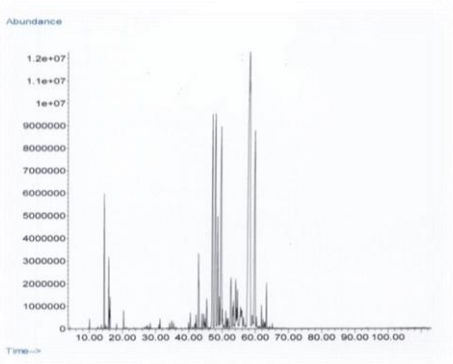

Fig. 1. Chromatogram of the essential oil from the *Curcuma longa L*

Table 3:

Chemical composition (%) of essential oil from *C. longa L* by GC/MStechnique with retention indices on HP-5MS capillary Column

N°	KI	Composé	%
1	931	α-Pinene	0.1333
2	976	Sabinene	0.0094
3	978	β-Pinene	0.0299
4	992	Myrcene	0.0601
5	1002	δ-2-Carene	0.0131
6	1007	**α-Phellandrene**	2.8433
7	1011	δ-3-Carene	0.0611
8	1017	α-Terpinene	0.0376
9	1025	**p-Cymene**	1.2468
10	1029	**D-Limonene**	0.3074
11	1031	Eucalyptol	0.469
12	1059	γ-Terpinene	0.0766
13	1088	α-Terpinolene	0.3314
14	1092	2-Nonanone	0.0183
15	1100	Linalool	0.0116
16	1101	Nonalool 2	0.0271
17	1129	Limona ketone	0.0158
18	1175	Terpinen-4-ol	0.0274
19	1182	Paramethylacetopnenone	0.0309
20	1184	p-Cymen-8-ol	0.044
21	1189	α-Terpineol	0.0571
22	1193	Decanone 2	0.0158
23	1197	Estragole	0.0256
24	1238	cumin aldehyde	0.0793
25	1242	Carvone	0.1739
26	1284	E-anethole	0.1016
27	1293	Thymol	0.197
28	1302	Carvacrol	0.1052
29	1312	p-vinyl-Gualacol	0.0361
30	1390	β-Elemene	0.0408
31	1400	β-Longipinene	0.0722
32	1418	**(E)-Caryophyllene**	1.6074
33	1420	α-Santalene	0.5804
34	1435	Trans-α-Bergamotene	0.2714
35	1444	(Z)-β-Farnesene	0.3111
36	1447	β-epi-Santalene	0.13
37	1452	β-Humulene	0.203
38	1459	(E)-β-Farnesene	0.8774
39	1472	β-acoradiene	0.0265
40	1476	γ-Muurolene	0.0106
41	1480	γ-Curcumene	0.054
42	1488	**ar-Curcumene**	8.5682
43	1503	**Zingiberene**	10.3734
44	1513	β-bisabolene	2.9134
45	1516	**β-curcumene**	0.1027
46	1521	6-methyl-α-lonone	0.6311
47	1532	**β-Sesquiphellandrene**	9.9806
48	1536	E-γ-Bisabolene	0.2975
49	1544	Cis-Sesquisabinene Hydrate épi-Longipinanol	0.1171
50	1561	Longipinanol	0.2253
51	1566	**Ar-Turmerol**	0.2514
52	1582	Viridiflorol	2.1433
53	1592	ar-dihydro-Tomerone	0.5425
54	1594	1,3,5-Bisabolatrien-7-ol	0.5116
55	1606	Platyphyllo	1.5573
56	1611	1,10-Cubenol	0.4582
57	1616	**α-Turmerone**	0.9933
58	1685	**ar-Turmerone**	28.0228
59	1686	E-1,10-Dihydroatlantone	2.6102
60	1695	Germacrone	0.553
61	1699	**β-Turmerone**	0.1345
62	1713	Chamazulene	9.8515
63	1733	(6S,7R)-Bisabolone	0.0615
64	1749	E-α-Atlantone	0.4468
65	1778		0.9055
		Total	93,0232

Total phenolic content

The of total phenolics content in the extracts were determined spectrometrically according to the Folin–Ciocalteu method and calculated as gallic acid equivalents. The standard curve equationis, ($y = 0.01145 x + 0.1095, R^2=0,995$) The total phenolic content found in the rhizome was highest in the polar methanol extract (92.3 GAE mg/g of) than the low polar ethanol extracts(65.4 GAE mg/g). It seems clear that presence of polar phenolics is fundamental in the evaluation of free radical-scavenging. Therefore, it could be concluded that the phenolic compounds were highly involved in the antioxidant activity found in organic extracts of *C. longa* rhizome and also able to complement their activity.

Antioxidant activity

The DPPH free radical has been widely used as tool to estimate free radical-scavenging activity of antioxidants. The antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character (Archana and al., 2005). The color of the reaction mixture changes from purple to yellow, and a decrease in absorbance. The DPPH radical-scavenging activity of the EOs and the phenolic extracts are shown in **Fig. 2**. Lower IC50 value indicates higher antioxidant activity. Methanolic extracts (Polar) exhibited stronger activity than ethanolic extracts (non-polar). Of all samples studied, the EOs and methanol extract had the strongest free radical-scavenging activity with an IC_{50} value of 17.976±0.789 and 21.678±1.254 µg/ml, respectively, while ethanol extract (IC_{50} = 36.446±1.955 µg/ml) showed moderate DPPH radical scavenging activity toward ascorbic acid (IC_{50} = 14.105±0.532 µg/ml). Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (Decker, 1997) (Al-Reza S M, and al.,2010) .Regarding turmeric, it has been shown that curcumin is ten times more antioxidant than vitamin E (Aggarwal *et al*., 2006).

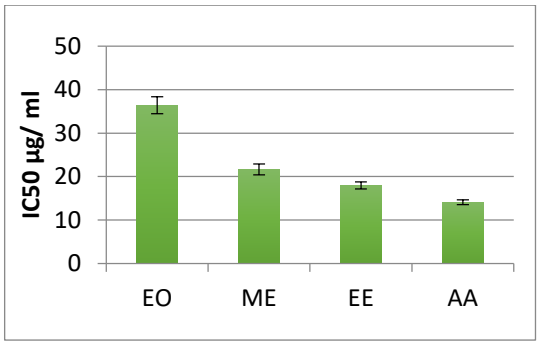

Fig.2.Antioxidantactivities of essential oil and phenolic extracts of *C. Longa*.By using DPPH radical-scavenging activity. Data are given as mean ± S.D. (n = 3). EO, essential oil; MEE, methanol extract; EE, ethanol extract; AA, ascorbic acid (control).

The ethanolic extract gave an IC50 of around 36.44 mg/ml is higher than the methanolic and ascorbic acid extracts. The lower the IC50, the greater the antioxidant activity of the compound tested (Villano, Det al., 2007).In comparison with the study of Boukri N.,2014**.**The IC50 of the ethanolic extract of *C. longa* rhizomes was found to be 17.974 µg/ml. It seems that the anti-free radical activity is stronglydependent on the concentrations of turmeric extracts. The phenolic extracts showed different values of IC50 and generallysolubilization of polyphenols which has a high number of hydroxyl groups exhibit the highest antioxidant activity and of compounds insoluble in water such as curcuminoids the main polyphenol compound of *C. longa L.*

The IC_{50} values of Denre (2014) are identical for turmeric from West Bengal, in India with value (5.99 mg / mL), this value is very close with the results of Tanvir et al. (2017), while the Turmeric from Indonesia from Widowati et al. (2009).

The IC50 values were higher (8.33 µg / ml) than the values for curcumin (7.85 mg / ml) in the same study. The results of the studies by Ghasemzadeh et al. (2012) and Panpatil et al. (2013) of the same species of turmeric which are superior compared to the other studies IC50 =. 600.7 µg / ml and 183, 383 µg / ml respectively.

Indeed, this has been confirmed by studies carried out by Bitemou et al. (2020) and by Indis and Kurniawan. (2016) who revealed that Curcumin exhibits good antioxidant activity which reaches 415.178µg / ml and 212.70µg / ml respectively.Kim et al. (2011) also obtained a percentage inhibition of 74.2% for the extract of the rhizome of the same species (Curcuma). This value is higher than the result of Chen et al. (2008) (72.1%) and Maizura et al. (2011) (64.6%) and Qader et al. (2011) (62.3%) and Trinidad et al. (2012) (54%).

Antibacterial activity

The antibacterial activity phenolic extracts or EOs from *Curcuma longa L.* has been tested against three bacterial (*E.coli, Staphylococcus aureus, Baccilus subtilis*), by the method of diffusion on discs. The activity of the plant studied was evaluated by the presence or absence of inhibition bacterial growth. The presence of antibacterial activity is reflected by the appearance of clear areas around disc soaked with phenolic extracts or EOs and the absence of inhibition results in the absence of areas clear around the discs. The diameter of the clear zone or inhibition zone varies depending on of the strain tested, the values are listed in the

Table 4
Diameters of microbial inhibition zones Methanolic and ethanolic extract of *Curcuma longa L.*

Extract	Diameters of microbial inhibition zones (mm)		
	E. coli ATCC 25922	*B.subtilis* ATCC 14931	*S.aureus* ATCC 25923
Methanolic	16±0.3	18±0.4	16±0.7
	14±0.3	16±0.4	14±0.7
	12±0.3	16±0.4	10±0.7
	12±0.3	14±0.4	10±0.7
ethanolic	26±0.2	17±0.3	20±0.5
	16±0.2	14±0.3	13±0.5
	10±0.2	12±0.3	9±0.5
	7±0.2	0±0.3	2±0.5

The results of the antibacterial activity of ethanolic extract (ME) and methanolic extract (EE) of *Curcuma longa L.* (**Fig 3; Fig 4**).

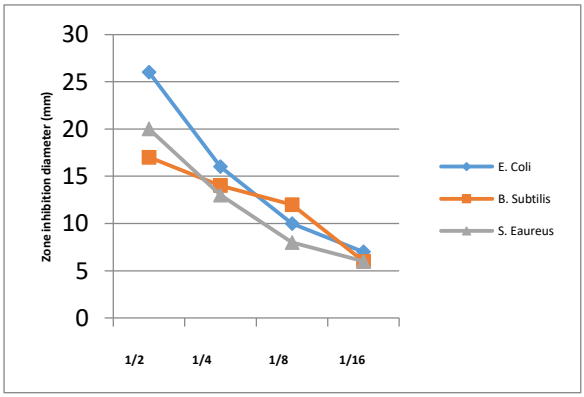

Fig. 3. Antibacterial activity of ethanolic extract of *Curcuma longa L*

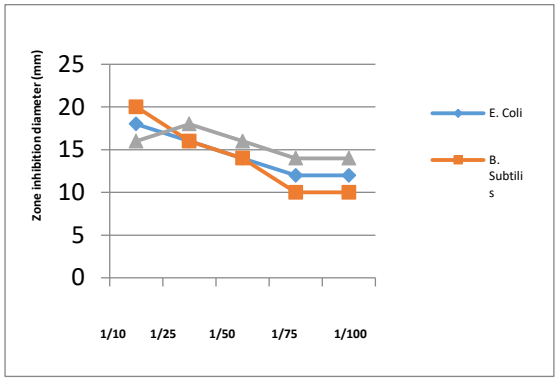

Fig. 4. Antibacterial activity of methanolic of *Curcuma longa L*

We observed variable antimicrobial activity against all strains tested. The results of the diameters of the inhibition zones of the methanolic extracts reveal a moderate activity for *Staphylococcus aureus* ATCC 25923 with an inhibition diameter varying from (8 to 16mm ± 0.7), greater with an inhibition zone ranging from (8 to 18mm ± 0.4) for *Escherichia coli* ATCC 25922 and (10 to 20 ± 0.3) for *Baccilussubtilis* ATCC 14931. This demonstrates the sensitivity of these strains to our extract.

According to (Zeghad, 2008), the zone of inhibition of *E-Coli* ATCC 25922 varies from (8 to 18) mm, which is consistent with the result of our study. The same study reveals that for *Staphylococcus aureus* ATCC 25923, the interval of inhibition zones is between (10 to15) mm; this also converges with our results. On the other hand, Negi and al. (1999) speak of a weak inhibition against Escherichia coli, and even against *Staphylococcus aureus*. It has been found that various ethanobotanical studies of*Curcuma* longahave revealed effective antibacterial properties (RahmanMdet al., 2014).

In our work the antibacterial activity of the ethanolic extract of *Curcuma L* has shown that*S. aureus*exhibit a highsensitivity at all dilutions, the diameter of the inhibitions varies from (9 to 18 mm) followed by *E. coli*of (6 to 15 mm)and*B. subtilis*(6 to 17 mm).**(**Naz, S., et al. 2010) showed a notable sensitivity towards Staphylococcus aureus which demonstrated the antibacterial effectiveness of curcumin and essential oil extracted from Curcuma longa with areas of inhibition of 12.2 and 10 mm respectively vis-à-vis *Bacillus subtilis* with a zone of inhibition of 10 mm.

This converges with our results but on the other hand by Simay et al. (2008) who had no antimicrobial activity against this strain *Escherichia coli*. Harit et al., (2013) their results show that the ethanolic extract of *Curcuma longa* which inhibits *B. subtilis* and *S. aureus*.So we notice that there is generally some antibacterial activityfor our extracts on Gram-positive and Gram-negative bacteria, on the other hand Stanojevic et al. (2015) indicated that, essential oils obtained from *Curcuma longa* exhibit a more potent antimicrobial activity on Gram-positive bacteria (compared to Gram-negative bacteria).

Similarly,(Gupta, A., et al. 2015)reported extracts obtained from *Curcuma longa* rhizome exhibited antimicrobial activity against *S. aureus.*As for (Thakur et al., 2013) found that ethanolic turmeric extract inhibited the growth of all bacterial isolates (*E.coli, P. aeruginosa, K. pneumonia*).

In this context, Apisarlyakul et al. (1995) attributed the antimicrobial activity of the essential oils of *Curcuma longa* to the importance of its aromatic compound turmerone. This deduction was also supported by Singh et al. (2011). A critical review by Shahidi and Hossain (2018) revealed that the reduced potential toxicity of methanol extract against *S. aureus* was due to poor solubility and low bioavailability of curcumin in the extract.According to Ikpeama et al., (2014) all bacteria are sensitive to the methanolic extract of Curcuma longa, other than that in Niamsa and Sittiwet (2009) they found that the bacteria*(K. pneumonia, S. epidermidis, E. coli and S. aureus)*are sensitive to the aqueous extract of Curcuma longa, andaccording to AŞKAR and Deveboynu (2018) the bacterium *S. aureus* is only sensitive to the commercial extract of Curcuma longa with a lower inhibition at 6.24 mg / disc.Indeed, the differences in results could be attributed to the very nature of the chemical composition of the oils, because according to Oussalah et al., (2006), the biological activity of essential oils is to be related to its chemical composition. In the literature, it has been stated that the antimicrobial activities of plant extracts depend on the nature and structure of the phenolic compounds. By their hydroxyl group; phenolic compounds have the ability to bind to proteins in bacterial membranes to form complexes (Zongo et al., 2011).In addition, like Kwiatkowski et al., (2020) mentioned, Gram-positive bacteria can facilitate the infiltration of hydrophobic EO compounds due to the lipophilic ends of lipoteichoic acid present in the cell membrane.

Antimicrobial compounds in plants can inhibit bacterial growth by different mechanisms. Indeed, Caillet and Lacroix (2007) have shown that the antimicrobial action of essential oils and their extracts takes place in three phases:(1) attack of the bacterial wall by the extracts, causing an increase in permeability followed by the loss of cellular constituents; (2) acidification of the interior of the cell, blocking the production of cellular energy and the synthesis of structural components;(3) destruction of genetic material, leading to the death of the bacteria. Antimicrobials may therefore have important clinical value in the treatment of resistant microbial strains. The varying sensitivity degree of turmeric extracts against bacterial strains might be due to the difference in the cell wall structure of Gram-positive and Gram-negative bacteria.

The mechanism of antimicrobial action varies depending on the type of EO or the strain of the microorganism used. It is well known that compared to Gram-negative bacteria, Gram-positive bacteria are more sensitive to EO (Huang, D.F.2014; Azhdarzadeh, F 2016.The varying degree of sensitivity of turmeric extracts against bacterial strains could be due to the difference in the cell wall structure of Grampositive and Gram-negative bacteria.It has been described by Chouhan et al. (2017) that Gram-negative bacteria such as E. coli and S. typhimurium present a rigid lipopolysaccharide on an outer membrane, thus limiting the diffusion of hydrophobic compounds through it.

The minimum inhibitory concentrations (MIC) of the EOs were determined using the broth dilution assay. It was possible to observe that EOs present high antimicrobial activity, with MIC of 4.76×10^{-3}µg/mL for S. aureus, 4.88 µg/mL for *B. subtilus*, and 39.06 µg/mL for *E. coli*. Considering the bioactive potential of EOs could be of great interest for development of antimicrobial agents for therapeutic use in treatment of bacterial infections in humans.

Conclusion

In this study, we carried out the extraction of the essential oil of *Curcuma longa L* with the gas chromatography-mass spectrometry method for the detection of the natural compounds of these essential oils. The result revealed the presence of α-tumerone (28.02%), β-sesquiphellandrene (9.98%), Zingiberene (10.37), β-tumerone (9.85%), ar -curcumene (8.57%) as major compounds.The antibacterial activity of Curcuma longa L was found to be pronounced with *B. subtilis* and *E. coli*, and moderate with *S. aureus*. The antioxidant activity is satisfactory with variable values for the methanolic and ethanolic extract which is confirmed by the IC_{50} values (21,678; 17,976).

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