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Extraction and characterization of Essential Oil and Hydrolate obtained from an Algerian Lemongrass (*Cymbopogon citratus*)

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ABSTRACT/RESUME

Abstract: *Lemongrass (Cymbopogon citratus) is a medicinal plant which largely used in popular medicine. It has cultivated for the commercial production and used in pharmaceutical, cosmetics and food industries. The aim of this work was to characterize lemongrass essential oil and hydrolate. The lemongrass was cultivated and grown under Algerian ecological conditions. The essential oil and hydrolate were obtained from leaves of lemongrass by hydrodistillation on an industrial scale (yield (v/w): 0.8±0.1%). The physicochemical properties of the essential oil were investigated to determine its quality. The lemongrass essential oil and hydrolate were then analysed by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). The lemongrass essential oil was also analysed by gas chromatographic coupled with mass spectrometry (GC/MS) for the determination of its chemical composition. The essential oil was light yellow aromatic liquid which was non soluble in ethanol. Lemongrass essential oil and hydrolate exhibited approximate pH, relative density and refractive index values of 4±1 and 6±1, 0.891±0.001 and 0.998±0.001, 1.488±0.001 and 1.333±0.001, respectively. The lemongrass essential, oil acid value, iodine value, and peroxide value were 1.402±0.036 mg KOH/g, 69.795±1.521 g/100 g and 3±0.082 meq O2/kg, respectively. FTIR spectra of both essential oil and hydrolate showed the presence of alkanes, alkenes, aldehydes, alcohols, carboxylic acids. Ethers and aromatics appeared in the essential oil only. The GC-MS analysis revealed nineteen components in lemongrass. The oxygenated monoterpenes are the most abundant chemical class (92.33%). Lemongrass essential oil has Isogeranial (41.77%), Neral (43.75%), β-Pinene (5.77), Geranial (3.78%) and Isoneral (1.90%) as major compounds. The established constituents of lemongrass essential oil suggested that it has an insecticidal value which should be investigated deeply.*

I. Introduction

Plant extracts constitute an indispensable part of the traditional medicine to treat several diseases because of their active substances. The use of plant extracts in continuously increased and become a part of a competitive market in pharmaceutical, food, cosmetic, and perfumery industries [1,2] because of consumers' demand for natural products with less chemicals. Nowadays interest of consumers with natural products is continuously raising. Essential oils (EOs) and hydrolates (HDs)

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obtained from plant extraction are studied by many researchers [3-7]. In fact, EOs and HDs have relevant chemical constituents which can possess several biological activities. Therefore, their incorporation in formulations can reduce the use of chemicals.

Lemongrass (*Cymbopogon citratus*) which belongs to Poaceae family is commonly used in traditional popular medicine. It is an originated herb from India and is one of the widely cultivated medicinal plants for its leaves in other tropical and subtropical countries [8].

Lemongrass contains 1-2% of EO on dry basis [1,9]. Previous investigations reported anti-oxidant [10,11], insecticidal [12], antibacterial [14], anti-yeast [13], anti-fungal [15,16], anti-inflammatory [15], and anti-herpetic [8] activities of lemongrass EO. Anti-oxidant, anti-yeast, antibacterial and anti-fungal activities of lemograss EO give them a promoted properties as natural food preservatives replacing to the chemical ones which are less healthier and safer.

Lemongrass EO is characterized by a strong lemony odour due to its main content of citral [2] which is used as a raw material for the production of ionone, vitamin A and betacarotene [1,9]. Citral also has which has an promising insecticidal activity [12] and lemongrass EO might be replaced the insecticides which the accumulation of their residues contaminate the aquatic system and repeatedly compromised aquatic food resources, fisheries, and aquaculture.

The chemical composition of lemongrass EO is varying widely upon genetic diversity, habitat and agronomic treatment of the culture [1] which suggested the specificity in properties and composition of each plant extracts. The present study is focused on extraction and characterization of lemongrass EO and HD. Lemongrass was cultivated in the North of Algeria. The characterisation of lemongrass extracts allows to evaluate their qualities and their chemical compositions as well as functional groups for a possible valorisation as a natural product instead of chemical ones in order to preserve human health and aquatic fauna and flora.

II. Material and methods

II.1. Plant material, EO extraction and obtention of HD

Aerial parts (leaves) of lemongrass at the vegetative stage were collected in August 2018 in the morning of the day from a field of culture of Medicinal and Aromatic Plants of "Extral-bio" Company located in the plain of Mitidja (Chiffa, Wilaya of Blida, Algeria). The collected vegetative components were air-dried at room temperature in the shade.

The leaves of lemongrass (200 kg) were subjected to hydrodistillation for 3 h in an alembic, on an industrial scale, in the distillatory of "Extral-bio" which also located in Chiffa Wilaya of Blida, Algeria. The obtained EO and HD were separated by decantation. The lemongrass EO and HD were stored in closed amber colored bottles and stored at room temperature.

II.2. Yield of lemongrass EO

Yield of lemongrass EO that obtained was calculated as given in equation (1).

$$\%\ Yield\ (v/w) = \frac{Volume\ in\ litre\ of\ extracted\ EO}{Weight\ in\ kg\ of\ plant\ used} * 100 \quad \textbf{(1)}$$

II.3. Organoleptic and physicochemical characterization of lemongrass EO and HD

The different organoleptic characteristics (appearance, color and odor) of both EO and HD of lemongrass were evaluated. Each EO and HD was separately placed in a transparent test tube over a white background and the appearance and color were observed. The characteristic odors were determined by smelling.

The pH of lemongrass EO and HD were determined using pH paper and approximate values were obtained.

Specific gravity is an important criterion of the quality and purity of volatile oils [17]. The relative density of EO and HD of lemongrass at 20 ° C were measured using an AP PAAR DMA48 densimeter according to the ASTM D4052 – 95 standards [18].

The refractive index is frequently used in determination of the identity, the quality and the purity of EOs [17]. The refractive index value of lemongrass EO and HD were obtained by using the digital refractometer Hanna HI 96801.

The solubility of the lemongrass EO was determined by mixing in test tube one volume of the EO in specified volumes of ethanol 96%.

The acid value expresses the number of milligrams of potassium hydroxide (KOH) required for the neutralization of the free acids present in 1 g of EO. The acid value of lemongrass EO was performed according to ISO 1242 standard [19].

The iodine value is expressed as the number of grams of iodine per 100 g of oil. It is the amount of iodine that can be fixed per 100g of substance by breaking the double bond to the two neighboring carbons. Iodine value measures can indicate the potential of a fat to be oxidized. The iodine value of lemongrass EO was performed according to the Algerian legislation [20].

The peroxide value indicates the initial step of oxidation (i.e. incipient rancidity) and the conservation state of fatty matter. It is concerned with the number of active oxygen in the organic

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chains of a fatty substance. The peroxide value of lemongrass EO was expressed as milliequivalents of oxygen per kg of oil (meq O2/1kg) and was accomplished according the Algerian legislation [21].

II.4. Spectral analysis: Fourier transform infrared spectroscopy (FTIR)

Lemongrass EO and HD Fourier transform infrared spectroscopy characterizations were performed on KBr pellet at various wavenumber infrared rays with a range of frequencies from 4000 to 400 cm-1 with an SP 2000 Saias Monaco FTIR spectrophotometer.

II.5. Chromatographic analysis: Gas chromatography- mass spectroscopy analysis (GC-MS)

The chemical composition of the lemongrass EO was determined using Agilent GC 7890B, equipped with Agilent MS 5977A detector. The chromatographic separations were performed on a capillary column HP-5MS (5% Phenyl Methyl Siloxane) (30 m length × 0.32 mm inner diameter × 0.25 µm film thickness). High purity helium was used as the carrier gas at a constant flow rate of 1.3 ml/min. An 1 µl sample was injected in the split mode. The injector temperature was 280 °C. The oven temperature was started at 50 °C. It was raised to 150 °C at a rate of 5 °C/min, to 250 °C at a rate of 14 °C/min, and the to 300 °C at a rate of 10 °C/min. The detector was operating in the electron impact mode (70 eV). The temperatures of the ion source were 250 °C. The amount of each compound was calculated by computing its area against the area of the internal standard in the gas chromatogram. The chemical compositions of the product were directely identified by MassHunter Software. The relative percentages of the constituent compounds were percentages from the GC peak areas based on the total ion chromatogram.

III. Results and discussion

III.1. Yield

Quality and quantity of EO depend on environmental factors, maturity stage [22], extraction procedures [23] and drying methods [24,25]. The yield of 0.8±0.1 % (v/w) was obtained by the extraction of lemongrass EO. The obtained result was fairly higher than that obtained by the same extraction method in the distillatory of "Extral-bio" and the same location of lemongrass which collected in May 2013 (i.e. Yield: 0.6%) [15]. The obtained yield was closed to lemongrass EO yield (0.7%) obtained by hydrodistillation of air-dried lemongrass from India [1] and Cost-Ivory [26], and higher than that by hydrodistillation of fresh lemongrass from Brazil which yield of 0.37% was obtained [8]. However, the obtained yield is lower than the hydrodistillation of Egyptian (2.12%) [25] and Indian (2.65%) [24] shade-dried lemongrass, Nigerian (1.03%) air-dried lemongrass [27] and fresh lemongrass (1.10%) from Benin [28].

III.2. Organoleptic and physicochemical properties of lemongrass EO and HD

The organoleptic and physical characteristics of lemongrass EO and HD, and chemical properties of lemongrass EO are listed in Table 1. The obtained lemongrass EO was a pale yellow mobile liquid and the HD was transparent mobile liquid. Similar colour of lemongrass EO was observed by Suryawanshi et al. [1] and Boukhatem et al. [15]. The transparency of HD is probably due to its richness with water. Both of EO and HD had a same fresh and strong lemon odour which was accentuated in EO. This lemony scent which also noticed by Boukhatem et al. [15] was probably due to the presence of citral (neral and/or geranial). The organoleptic properties of lemongrass were in the agreement with standard [29].

Lemongrass EO had an acidic pH of 4. This value was lower than that obtained by hydrodistillation of air-dried lemongrass of Nigeria (pH=6) [27]. pH of lemongrass EO was lower than that of HD which was probably due to the richness of HD (pH=6) by water. The obtained results also supposed that EO was richer than HD with acidic character components.

Relative densities of lemongrass EO and HD were 0.891±0.001 and 0.998±0.001, respectively. The EO was less dense than water as generally expected. Refractive index is a measure of how fast light travels through a substance. It is used to confirm purity and detect rancidity of the oil. It increases with chain length and unsaturation in oil. Refractive index of lemongrass EO (1.488±0.001) was higher than lemongrass HD (1.333±0.001). It was close to that Nigerian (1.484), and Ivorian-cost (1.481) lemongrass EO studied by Olayemi et al. [27] and Kanko et al. [26], respectively. Relative density and refractive index of lemongrass EO met the international [29]. HD relative density and refractive index are close to that of water because of the HD water richness.

No data of organoleptic and physical characteristics are found in the literature about lemongrass HD to make comparisons.

As can be seen from the Table 1 lemongrass EO is immiscible in ethanol which is due to the storage

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duration which speed up polymerization and thus decrease the solubility of EO in ethanol [29].

The acid value is an index which helps to identify any rancidity, to estimate the oxidation, and to specify the quality of oil. The lower of the acid value of oil makes it less exposed to rancidity. The studied lemongrass EO showed acid value of 1.402±0.036 mg KOH/g which is higher than Nigerian lemongrass EO (0.55 mg KOH/g) [27]. The result is in accordance with international regulations which threshold is 4 mg KOH/g [30].

The iodine value gives an idea of the average degree of unsaturation in oil and its stability to oxidation. Higher the iodine value, greater the number double bonds in the sample and therefore greater care will be needed to slow down oxidation [27,31]. In contrast, low iodine value may be indicative of low susceptibility to oxidative rancidity [32]. The iodine value of the tested EO was 69.795±1.521 mg/100g which is higher than Indian lemongrass EO (0.115 mg/100g).

The peroxide value is an index of oxidation progress, quality and shelf-life of oil because of peroxides are the main initial products of oil oxidation. Peroxide value could be used as an indication of the quality and stability of oil. The peroxide value increases with the storage time, temperature and contact with air of the oil [33] and implies its lower oxidative stability [31]. Lemongrass EO presents low peroxide value of 3±0.082 meq O2/kg. The obtained result is in accordance with the standard which threshold is 10 meq O2/kg [30].

The low acid and peroxide values of lemongrass EO are indicative of its resistance toward oxidative deterioration. Also, chemical properties of EO confirm its good quality and stability.

III.3. Spectral analysis: Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of lemongrass EO and HD are shown in Figure 1. The functional groups associated with respective wave numbers of lemongrass EO and HD are listed in Tables 2 and 3, respectively. The comparison between absorption peaks of lemongrass EO and HD indicated the similarities in their FTIR spectra. In facts EO FTIR spectrum had more pronounced peaks than HD. The obtained results revealed the presence in lemongrass EO and HD of alkanes, alkenes, alcohols, aldehydes, and carboxylic acids. Ethers and aromatics were present in the EO only. FTIR spectra of lemon grass EO had characteristic peaks at 3483, 2968, 2921, 2859, 2761, 1675, and 1445 cm-1. Similar peaks were observed in lemongrass EO analysed by Jamuna et al. [10]. The intense peaks at 1675 and 1635 cm-1 in EO and at 1637 cm-1 in HD were due to vibration of C=C (cis and trans), confirming the presence of conjugated double bonds (C=C-CHO) in citral [10]. The large and pronounced band at 3436 cm-1 of HD in comparison with EO due to the vibration of O-H indicated HD water richness.

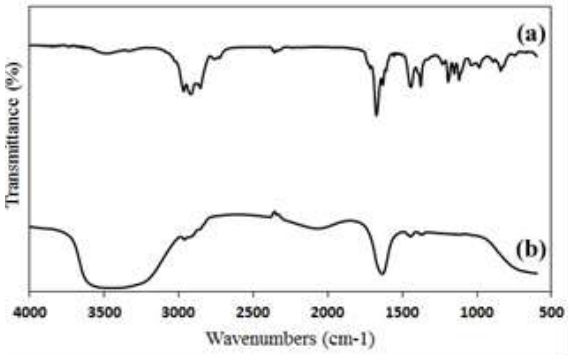

Figure 1. *FTIR spectra of the lemongrass EO (a) and HD (b).*

III.4. Gas chromatography-mass spectroscopy analysis

Chemical composition of the lemongrass EO determined using GC/MS are presented in Table 4. Nineteen (19) compounds were identified which are in agreement with functional groups identified by FTIR analysis. Boukhatem et al. [15] identified 23 volatile compounds in the lemongrass EO extracted by the same process and which lemongrass was collected the same location. 18, 14, 35, 16, 15-50, and 8 compounds were identified in Brazilian [8,13], South African [34], Benin [28], Malaysian [22] and Togolese [16] lemongrass EO, respectively.

Lemongrass EO was mainly composed by oxygenated monoterpenes (92.33%). It had Isogeranial (41.77%), Neral (43.75%), β-Pinene (5.77), Geranial (3.78%) and Isoneral (1.90%) as major compounds. Lemongrass EO studied by Boukhatem et al. [15] was also principally composed by oxygenated monoterpenes at the less extent (73.97%). Their major compounds were Geranial (42.16%), Neral (31.52%), β-Myrcene (7.45%), Geranyl acetate (4.3%), Isopulegol (1.39%) and Terpinolene (1.3%).

The essential oil chemical analysis (Table 4) revealed a Citral richness (47.66%) profile which confirm an intense pic of citral observed in its FTIR spectrum. Presence of significant amount of Citral compounds indicated high antimicrobial potential and antioxidant activity of lemongrass EO [31].

Previous works [8,13,15,22,28,34] reported that lemongrass EOs were mainly composed with Geranial and Neral. However, in the present work the studied lemongrass EO were mainly composed with Isogeranial and Neral. No authors found Selin-6-en-4α-ol and m-Camphorene in the composition of their studied lemongrass EO.

Differences in the chemical composition and quantities of components in EOs, which modulate their qualities, can be explained by many factors

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such as ecological region and field conditions [35,36], genotype [37], maturity stage of plant [22], drying methods [25], extraction methods [32], and duration of extraction process [38]. According to Tajidin et al. [22], the lemongrass EO composition differed significantly at different harvesting stage. Because of the aforementioned, a complete description of the ecological and geographic conditions, pre-treatment and harvesting stage of the plant material should be presented in order to understand the origin of the chemical composition and quantities components variation in the essential oils.

IV. Conclusion

The present study focused on characterization of extracts (i.e. EO and HD) from lemongrass cultivated in in the North of Algeria to evaluate their qualities and chemical compositions. The results indicated that organoleptic and physicochemical characteristics of lemongrass EO met the international standards which proved its good quality. Organoleptic and physicochemical properties of lemongrass HD indicated its water richness which was confirmed by FTIR analysis. FTIR spectra of lemongrass EO and HD exhibited similar functional groups except for ethers and aromatics which were appeared in EO only. FTIR spectra also showed the presence of Citral as one of the major component which was confirmed by GC-MS analysis for lemongrass EO. Nineteen compounds were identified on lemongrass EO with oxygenated monoterpenes as the most abundant chemical class (92.33%). Lemongrass essential oil has Isogeranial (41.77%), Neral (43.75%), β-Pinene (5.77), Geranial (3.78%) and Isoneral (1.90%) as major compounds. Selin-6-en-4α-ol and m-Camphorene in the composition of the present lemongrass EO not found by any previous work. It would be interesting to determinate the chemical composition of lemongrass HD in order to confirm the presence of Citral as major compound and to compare HD chemical composition with that of the EO which suggested that it has an insecticidal value which should be investigated deeply. Also further research is needed to evaluate antimicrobial potential and antioxidant activity of both EO and HD which supposed to be due to the Citral.

Table 1. *Organoleptic and physicochemical properties of lemongrass EO and HD.*

Characteristics	Essential oil	Hydrolat
Appearance	Mobile, clear liquid	Mobile, clear liquid
Color	Pale yellow	Transparent liquid
Odor	Fresh with strong lemon odour	Fresh with strong lemon odour
pH	4±1	6±1
Relative density at 20°C	0.891±0.001	0.998±0.001
Refractive index at 20 °C	1.488±0.001	1.333±0.001
Solubility in ethanol 96%	Immiscible	/
Acid value (mg KOH/g)	1.402±0.036	/
Iodine value (mg/100 g)	69.795±1.521	/
Peroxide value (meq O2/kg)	3±0.082	/

Table 2. *Results of FTIR analysis of lemongrass EO.*

Wavenumbers (cm^{-1})	Bonds	Functional groups
3483	O-H stretch	Alcohols
2968	C-H stretch	Alkanes
2921	C-H stretch	Alkanes
2859	C-H stretch	Aldehydes
2761	C-H stretch	Aldehydes
2731	C-H stretch	Aldehydes
1675	C=C stretch	Alkenes
1635	C=C stretch	Alkenes
1559	C=C stretch	Aromatics
1540	C=C stretch	Aromatics
1445	C-H bend	Alkanes
1380	-C-H bend	Alkanes

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1230	C-O stretch	Carboxylic acids
1192	C-O stretch	Alcohols
1155	C-O stretch	Alcohols
1120	C-O stretch	Alcohols
1037	C-O stretch	Ethers
987	=C-H bend	Alkenes
893	=C-H bend	Alkenes
842	=C-H bend	Aromatics
749	=C-H bend	Aromatics
669	=C-H bend	Aromatics

Table 3. *Results of FTIR analysis of lemongrass HD.*

Wavenumbers (cm^{-1})	Bonds	Functional groups
3436	O-H stretch	Alcohols
2963	C-H stretch	Alkanes
2730	C-H stretch	Aldehydes
1637	C=C stretch	Alkenes
1447	C-H bend	Alkanes
1375	C-H bend	Alkanes
1330	C-O bend	Alcohols
1230	C-O stretch	Carboxylic acids
1190	C-O stretch	Alcohols
1153	C-O stretch	Alcohols
1117	C-O stretch	Alcohols

Table 4. *Lemongrass EO chemical composition identified by GC–MS analysis.*

N°	RI*	Components	References**	Retention time (min)	Relative amounts (%)
1	986	6-methyl-5-Hepten-2-one	[8,13,28,34]	9.84	0.28
2	979	β-Pinene	[34]	10.01	5.77
3	1049	E-β-Ocimene	[12,15]	11.46	0.43
4	1038	Z-β-Ocimene	[12,15,28]	11.79	0.35
5	1099	Linalool	[8,28]	14.76	0.10
6	1153	Trans-chrysanthemal	[22]	15.10	0.12
7	1153	Citronellal	[12,15,16,28]	15.19	0.78
8	1170	Isoneral	[12]	15.58	1.90
9	1185	Isogeranial	[12]	16.17	41.77
10	1240	Neral	[8,12,15,16,28]	18.44	43.75
11	1270	Geranial	[8,12,15,16,28]	19.55	3.78
12	1276	Citral	[8,12,28]	22.17	0.13
13	1382	Geranyl acetate	[8,12,15,16,28]	23.27	0.09
14	1419	β-Caryophyllene	[8,12,15,16]	23.57	0.12
15	1435	α-Bergamotene	[15,22]	24.74	0.06
16	1491	α-Franesene	[15,16]	26.38	0.05
17	1581	Caryophyllene oxide	[8,15,16]	26.88	0.12
18	1636	Selin-6-en-4α-ol	/	30.26	0.21
19	1960	m-Camphorene	/	30.57	0.19
Oxygenated monoterpenes					92.33
Monoterpenes hydrocarbons					6.55
Oxygenated sesquiterpenes					0.33
Sesquiterpenes hydrocarbons					0.23
Diterpenes					0.19
Others					0.37
Total identified					100.00

** : Retention index.*

***: Molecules detected in previous works at similar RI.*

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