Chemical Composition Evaluation of Egyptian Lemongrass, *Cymbopogon citratus*, Essential Oil

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Abstract— Medicinal plants are widely used in traditional medicine for health care due to its contents of biological active substances. One of the most important natural substances is essential oil. This experiment was conducted during the successive season 2016/2017 to evaluate the quality of lemongrass, *Cymbopogon citratus*, and its essential oil. Lemongrass showed high contents of total phenolic (7.55 mg GAE/g) and total flavonoids (1.96 mg CE/g), as well as high free radical scavenging capacity with DPPH of 37.5 mg TE/g. The average essential oil percentage was 0.66% fresh weight mass. Using GC analysis, 22 compounds representing 97.83% of essential oil compositions were identified. These compounds were mainly monoterpenes (96.37%), and small content of sesquiterpenes and diterpenes (1.25 and 0.21%, respectively). The major compound was citral which was 79.69% of total essential oil composition. Citral was divided into two major compounds; 42.86% citral A (geranial) and 39.83% citral B (neral). Other major compounds were myrcene (8.05%), geraniol (3.22%), and cis-verbanol (1.84%). These percentages are consistent with high quality lemongrass essential oil under good agricultural and management practices. Therefore, these results suggest the efficiency of growing lemongrass under organic farming system to produce high quality essential oil.

Index Terms—Citral, *Cymbopogon citratus*, Essential oil, GC-Mass, Geranial, Lemongrass, Neral

1 Introduction

MEDICINAL plants constitute an independent and economic group in the plant systems [1]. The term "medicinal plant" refers to any plant that contains biologically active substances within its tissues with therapeutic and medicinal properties [2]. The history of using medicinal and aromatic plant returns to the beginning of mankind. Our ancestors have used the natural substances found in nature to relieve pains, treat illnesses, and heal wounds that called nowadays "traditional medicine" [3]. In Africa, traditional medicine is used for primary health care by up to 80% of the population. In the industrialized countries, the renaissance of using medicinal plants has brought a different type of traditional medicine in the form of herbal medicines, which are termed "Complementary" or "Alternative" and are used by up to 65% of the population [4, 5]. WHO has elaborated a traditional medicine strategy to promote the suitable use of traditional/alternative medicine focusing on policy, safety, quality/efficacy, access, and rational use [6-9]. In view of the greatly increasing demands on these remarkable natural resources, medicinal plant researches should pay more attention to explore the biologically active substances of plants that determine its healing and therapeutic properties.

Plenty of chemical reactions are taking place in plant cells at any single moment which is called metabolism. The sum of metabolism processes is the synthesis of primary and secondary metabolites. The primary metabolites, chemical essentials, are generally occurring in most plants. The primary metabolites include mainly carbohydrates, lipids, proteins, and nucleic acids. On the other hand, the secondary metabolites are the products of the special metabolism of valuable ingredients. The secondary metabolites include a wide range of compounds that are not found in all species [10]. The main groups of secondary metabolites are alkaloids, terpenoids, phenolic compounds, glycosides, essential oils, etc [11]. In the medicinal and aromatic plants, the secondary metabolites are mainly utilized and frequently are metabolic end products with no relevant role in the metabolism. For example, essential oils are excreted by glandular hairs. Some others such as alkaloids are actively transported and even translocated throughout the plant [12]. Plants containing essential oils are important in medical practice. The chemical composition of essential oil is complex and may contain hundreds of components. It is a mixture of fragrant volatile substances, monoterpenes, sesquiterpenes, aromatic compounds, and their derivatives. Essential oils have been used as antimicrobial, anti-inflammatory, sedative, expectorants, and diaphoretics. There are several medicinal plants contain essential oils in their tissues such as sage (*Salvia officinalis* L.), eucalyptus species, peppermint (*Mentha piperita* L.), Scotch pine, elecampane (*Inula helenium* L.), linden (*Tilia cordata* Mill), garden heliotrope (*Valeriana officinalis* L.), etc.

Lemongrass, *Cymbopogon citratus*, is a perennial medicinal plant belonging to family Geramineae, and it is distributed worldwide especially in tropical and subtropical areas of Africa, Asia, and America [13-15]. The main effective compounds of lemongrass are citral and essential oil [16, 17]. The chemical composition of lemongrass essential oil depends on many factors such as genetic diversity, temperature, light intensity, maturity stage, and agricultural practices [18, 19]. The essential oil percentage is about 1-2% of herb dry mass for lemongrass [16]. The main objectives of this study were to determine the active substances content, and to identify the chemical composition of lemongrass essential oil cultivated under southern Nile delta conditions (Bilbeis, Sharqia, Egypt).

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2 Materials and Methods

2.1 Field Planting and Agronomic Practices

In March 2016, stalks of lemongrass were planted in a private farm in Bilbeis city, Sharqia governorate, Egypt. The planting area was planned with 4 terraces. Each terrace was 80 cm width and 6 meter long. Stalks were cultivated on both sides of terraces, and distance between plants was 30 cm, with total of 160 plots. Normal agricultural practices were carried out. Drip irrigation system was used for irrigation purpose. Organic farming system was used to feed plants by adding and mixing 10m^3 compost per acre (Feddan) in soils before planting. Plants were harvested by cutting the shoots 10 cm above ground after 4, 8 and 12 months from planting.

Plant height, shoot fresh weight, and shoot dry weight were recorded. Leaf area was measured according to [20]. Total chlorophyll was measured using SPAD chlorophyll meter (SPAD-502plus, Konica Minolta, INC., Osaka, Japan). Shoots were air dried for further chemical analysis.

2.2 Total Phenolic, Total Flavonoids and DPPH Analysis

Dried shoot (5 g) were grinded and subjected to hydro alcoholic extraction using soxhlet apparatus. Rotary evaporator under pressure was used to concentrate and dryness the extracts, and then dissolved in 100 mL of 80% methanol. Total Phenolic content were assayed using the Folin-Ciocalteu method [21] with some modifications. Briefly, the extract (100 µL) was transferred into a test tube and the volume adjusted to 3.5 mL with distilled water and oxidized with the addition of 250 µL of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 mL of 20% aqueous sodium carbonate (Na_2CO_3) solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank using UV-Visible spectrophotometer. Gallic acid was used for standard and total phenolic content was expressed as mg Gallic Acid Equivalents (mg GAE).

Total flavonoid content was measured by aluminium chloride colorimetric assay [22]. Briefly, 300 µL of 5% sodium nitrite ($NaNO_2$) was mixed with 100 µL of extract. After 6 min, 300 µL of a 10% $AlCl_3$ solution was added and the volume was adjusted to 2.5 mL using distilled water. After 7 min, 1.5 mL of 1 M NaOH was added, and the mixture was centrifuged at 5000g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank using UV-Visible spectrophotometer. Catachine was used for standard and total flavonoid content was expressed as mg Catachine Equivalents (mg CE).

Free radical scavenging capacity of extracts was determined using the stable DPPH according to [23]. The final concentration was 200 µM for DPPH and the final reaction volume was 3 mL. The absorbance was measured at 517 nm against a blank of pure methanol after 60 min of incubation in a dark condition. Inhibition of the DPPH free radical was calculated by the following equation:

$$Inhibition\ (\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the blank reaction and A_{sample} is the absorbance with the test compound. Extract concentration of sample providing 50% inhibition (IC50) was calculated using linear regression analysis.

2.3 Hydro-Distillation and GC Mass Analysis

The fresh shoot (50 g) was hydro-distillated using Clevenger-type apparatus for three hours [24]. The extracted essential oils have been collected and dried using anhydrous sodium sulphate for chemical constituents' identification. GC-MS analysis has been done at Department of Medicinal and Aromatic Plants Research, National Research Center, using Gas Chromatography–Mass Spectrometry with the following specifications; a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 °C for 1 min; rising at 4.0 C/min to 160 °C and held for 6 min; rising at 6 °C/min to 210 °C and held for 1 min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Mostly, the constitutions were identified using two different analytical methods; (a) mass spectra (authentic chemicals, Wiley spectral library collection and NIST library), and (b) KI, Kovats indices in reference to n-alkanes (C9-C22) matching with the National Institute of Standards and Technology published data [25].

2.4 Statistical Analysis

Plant samples were randomly selected. The experiment incorporated four replicates, each replicate comprised 40 plots. Four individual samples have been taken from each cut, and the mean values were calculated.

3 Results and Discussion

Modern approaches in production and uses of medicinal and aromatic plants strongly focus on the importance of quality, safety and efficacy of medicinal plants and their products [6-9]. The importance of research in medicinal plants will continue to seek new and valuable sources of drugs. This study was conducted using organic farming system to evaluate the production and quality of lemongrass, *Cymbopogon citratus*, under southern Nile delta conditions. Table (1) showed the mean values of the morphological and chemical parameters of lemongrass. The average plant height was (55.70 cm), the average shoot fresh and dry weights were (205.25 and 69.10 g per plant, respectively), and leaf area was (19.25 cm2).

The quality characters of medicinal plants are affected by weather condition, especially temperature. Due to higher kinetic energy under hot conditions, molecules bonds are broken and the mobility and solubility are increasing. Godwin et al. [26] showed that total phenolic content is affected by temperatures in lemongrass. The total phenolic content ranged between 1.3-4.7 mg GAE/g under cold conditions, and this rang increased to 2.6-7.3 mg GAE/g under hot conditions. This result was comparable with [27] who observed average total phenolic content of 6.44 mg GAE/g from lemongrass leaves. Flavonoid is a simple group of phenols and is expected

to become more soluble than complex phenols. In this study, average total chlorophyll content was 33.42 mg/g. The total phenolic content was 7.55 mg GAE/g, while total flavonoid was 1.96 mg CE/g. On the other hand, free radical scavenging capacity represented by DPPH was 37.49 mg TE/g (Table 1). These results reflected the high content of total phenolic contents which related to higher flavonoid and scavenging capacity.

TABLE 1
MORPHOLOGICAL AND CHEMICAL ANALYSIS OF LEMONGRASS. DATA REPRESENT THE MEAN VALUE ± STANDARD DEVIATION OF THREE CUTS.

Plant height (cm)	55.70±4.64
Shoot fresh weight (g/plant)	205.25±84.94
Shoot dry weight (g/plant)	69.10±27.03
Leaf area (cm2)	19.25±2.73
Total chlorophyll(mg/g)	33.42±4.82
Total phenolic (mg GAE/g)	7.55±0.49
Total flavonoid (mg CE/g)	1.96±0.56
Essential oil content (%)	0.66±0.12
DPPH (mg TE/g)	37.49±4.28

One of the effective natural products obtained from plants is the essential oil. The current industry showed increased demand of essential oil due to its bioactive compound with various therapeutic effects [28]. Lemongrass, *Cymbopogon citratus*, is a perennial medicinal plant with long and thin leaves, and it is largely cultivated for its essential oils in tropical and subtropical regions of Asia, Africa and America [15]. Leaves of lemongrass contain pale yellow essential oils with average of 0.25-0.90% [29, 30]. In this study, the percentage of essential oil content was around 0.66% of a fresh leaves mass. This result is in consistent with previous works who reported that oil content could be yielded up to 0.66–0.90% with good management and selected strains [29].

Essential oil was detected to gather at parenchyma tissues, specifically in the adaxial surface of leaf mesophyll [31]. Essential oils consist of hundred components in the form of a complex mixture of fragrant volatiles, monoterpenes, sesquiterpenes, and aromatic derivatives [11, 32]. The chemical composition of lemongrass oil is varying widely upon genetic diversity, habitat, agronomic practices, and geo-climatic factors [19, 33, 34]. The quality of lemongrass oils determines by citral content [30, 35]. Citral is a combination of geranial (α-citral) and neral (β-citral) isomeric aldehydes, and it is used for several chemical syntheses [16, 17, 36, 37]. Other isolated components from lemongrass essential oil are β-myrcene, ocimene, β-ocimene, linalool, citronellal, citronellol, caryophyllene and β-pinene [17, 38]. Tajidin et al. [18] analyzed lemongrass oil compositions at different growth stage, and they detected 65 chemical compounds, but only 13 of the compounds were present in each stage. Figure 1 showed the GC-MS analysis of lemongrass essential oil which identified 22 components, representing 97.83% of total composition. The essential oil consisted 96.37% monoterpenes, 1.25% sesquiterpenes, and 0.21% diterpenes. The major essential oil component was citral (79.69%) consisted of 42.86% geranial and 36.83% neral, followed by myrcene which was 8.05%. Other components identified in oil were geraniol 3.22%, cis-verbenol 1.84%, etc (Table 2). This result was in consistent with other studies in lemongrass chemical composition which reported average citral content of 65 to 80% [39], consisting 20-50% of geranial and 30-40% of neral [28, 40, 41, 42]. Mohamed Hanaa et al. [30] analyzed essential oil components of lemongrass and she reported major component as geranial (31.5-39.9%), neral (30.1-35.5%), and myrcene (14.5-16.6%). In another study, Masamba et al. [35] showed that lemongrass oil consisted of 82% citral (41.67% geranial and 40.33% neral), and 10% myrcene. These results reflected the high quality of Egyptian lemongrass grown under organic farming system.

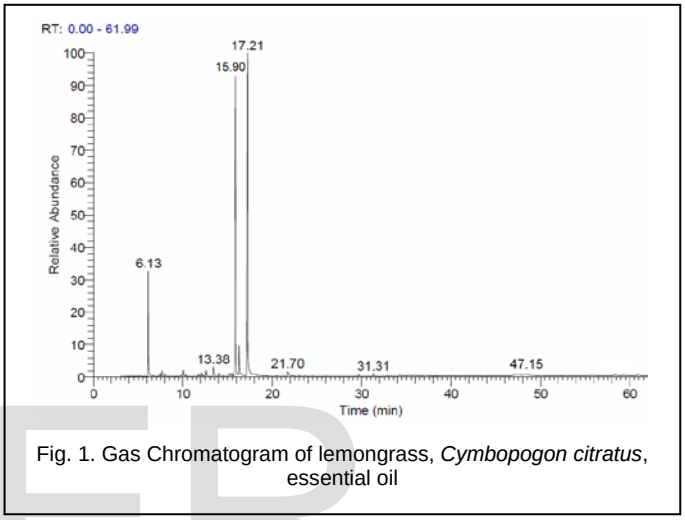

Fig. 1. Gas Chromatogram of lemongrass, *Cymbopogon citratus*, essential oil

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TABLE 1

CHEMICAL COMPOSITIONS (%) OF THE LEMONGRASS, CYMPOBOGON CITRATUS, ESSENTIAL OIL

	RT	Name	Area %	Molecular weight	Kovates index	Molecular formula	Classification
1	4.41	á-Pinene	0.04	136	893	$C_{10}H_{16}$	monoterpenes
2	6.13	á-Myrcene	8.05	136	957	$C_{10}H_{16}$	monoterpenes
3	7.35	o-Cymene	0.12	134	1001	$C_{10}H_{14}$	monoterpenes
4	7.43	D-Limonene	0.25	136	1003	$C_{10}H_{16}$	monoterpenes
5	8.00	cis-Ocemene	0.90	136	1018	$C_{10}H_{16}$	monoterpenes
6	9.51	trans-Linalool Oxide	0.11	170	1058	$C_{10}H_{18}O$	monoterpenes
7	10.04	3,7-Dimethyl-1-ocyanol	0.75	154	1072	$C_{10}H_{18}O$	monoterpenes
8	10.36	trans-3-Caren-2-ol	0.14	152	1081	$C_{10}H_{16}O$	monoterpenes
9	12.08	2,2-Dimethylocta-3,4-dienal	0.37	152	1123	$C_{10}H_{16}O$	monoterpenes
10	12.20	Citronella	0.12	154	1125	$C_{10}H_{18}O$	monoterpenes
11	13.38	cis-Verbenol	1.84	152	1153	$C_{10}H_{16}O$	monoterpenes
12	14.04	Bornel	0.35	168	1168	$C_{10}H_{16}O_2$	monoterpenes
13	15.17	Nerol	0.15	154	1194	$C_{10}H_{18}O$	monoterpenes
14	15.29	Citronellol	0.27	156	1196	$C_{10}H_{20}O$	monoterpenes
15	15.90	Citral B (Neral)	36.83	152	1210	$C_{10}H_{16}O$	monoterpenes
16	16.28	Geraniol	3.22	154	1218	$C_{10}H_{18}O$	monoterpenes
17	17.21	Cital A (Geranial)	42.86	152	1239	$C_{10}H_{16}O$	monoterpenes
18	21.70	Geranyl acetate	0.63	196	1338	$C_{12}H_{20}O_2$	sesquterpenes
19	23.00	trans-Caryophellene	0.11	204	1368	$C_{15}H_{24}$	sesquterpenes
20	29.62	Caryophyllene oxide	0.21	220	1521	$C_{15}H_{24}O$	sesquterpenes
21	31.31	Juniper Camphor	0.30	222	1560	$C_{15}H_{26}O$	sesquterpenes
22	47.15	Phytol	0.21	296	1989	$C_{20}H_{40}O$	diterpenes
Total identification			97.83				
Total monoterpenes			96.37				
Total sesquterpenes			1.25				
Total diterpenes			0.21				

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