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Essential Oils for Pest Control

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Lemongrass Oil: As a Green Pesticide

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Lemongrass Oil: As a Green Pesticide

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6.1 Introduction

Lemongrass (*Cymbopogon* sp.) is an herb that is widely distributed in the tropical and subtropical regions of Asia, Africa, Australia, and America. The genus *Cymbopogon* comprises 144 species and is famous for its high content of essential oil; it contains essential oil with a fine lemon flavor. On account of their diverse use in the pharmaceutical, cosmetics, food, flavor, and agriculture industries, the commercial value of some *Cymbopogon* species is further enhanced by their ability to grow in moderate and extremely harsh climatic conditions (Padalia et al. 2011). *Cymbopogon citratus* is ranked as one of the most widely distributed of the genus, being used in every part of the world. The traditional application in different countries shows high applicability as a common tea, medicinal supplement, insect repellant, insecticide, anti-inflammatory, and analgesic, and in flu control.

6.2 Botany of the Plant

6.2.1 Taxonomic Classification (Shah et al. 2011)

Kingdom: Plantae
Division: Nagmoleophyta
Class: Liliopsida
Order: Poales
Family: Poaceae or Gramineae
Genus: *Cymbopogon*
Species: *citratus*, *flexiuosus*
Botanical name: *Cymbopogon citratus*

6.2.2 Habitat and Distribution

Most lemongrass is native to South Asia and Australia. The crop grows well in both tropical and subtropical climates at an elevation up to 900 m (above sea level). However, the ideal conditions for lemongrass are a warm and humid climate with sufficient sunshine and 250–330 cm of rainfall per annum, evenly distributed over most of the year. A temperature ranging from 20°C to 30°C and sunshine throughout the year is conducive to a high crop yield. Lemongrass can also be grown in semiarid regions receiving low to moderate rainfall. Well-drained sandy loam is most suitable for the growth of the plant. It can be grown on a variety of soils, ranging from loam to poor laterite. Java citronella is mainly produced by Taiwan, Guatemala, Honduras, Brazil, Ceylon, India, Argentina, Equador,

Madagascar, Mexico, and the West Indies. In India, lemongrass is widely cultivated in the states of Kerala, Karnataka, and Tamil Nadu in the southern region; parts of Uttar Pradesh and Uttaranchal in the northern region; and Assam in the northeast region.

6.2.3 Botanical Description of the Plant

Lemongrass is a tall, perennial grass, about 1–1.8 m in height, and it throws up dense fascicles of leaves from a short rhizome. The culm is stout and erect.

6.2.4 Leaves

The leaves of *Cymbopogon* sp. are long, glaucous, green, and linear, tapering upward. Along the margins, the ligule is very short, the sheaths are terete, and those of the barren shoots are wide and tightly elapsing at the base, and others are narrow and separating.

6.2.5 Flower

It is a short-day plant, and it produces profuse flowering. The inflorescence is a long spike about 1 m in length.

6.3 Methods of Extraction of Oil

The common methods to extract essential oil from lemongrass are hydrodistillation (HD), steam distillation, and water distillation. Although the process is very simple, it can induce thermal degradation, hydrolysis, and water solubility of some fragrance constituents. In order to overcome these drawbacks, a number of novel methods have been studied from time to time, for example, microwave-assisted hydrodistillation (MAHD), subcritical water extraction (SWE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and ultrasound-assisted extraction (UAE).

6.3.1 Conventional Methods

6.3.1.1 Distillation

Distillation is an extracting oil process that converts volatile liquid (essential oils) into a vapor state and then condenses the vapor into a liquid state. There are different categories of distillation processes, such as water distillation, steam distillation, and hydrodiffusion (Ranjitha and Vijiyalakshmi 2014).

6.3.1.1.1 Water Distillation

In this process, the botanic material is completely immersed in water and the still is brought to boil. It is used to protect the oils to a certain degree since the surrounding water acts as a barrier to prevent overheating when condensed material cools down. The water and essential oil are separated, and the oil is decanted to use as an essential oil. Water distillation can also be done at reduced pressure (under vacuum) to reduce the temperature to less than 100°C, which is useful in protecting the botanical material for obtaining the essential oil.

It is a simple and easy-to-operate extraction method of oil from plant species. Due to the use of heat in this method, it may not be used on very fragile plant material. Oil components like esters are sensitive to hydrolysis, while others like cyclic monoterpene hydrocarbons, and aldehydes are susceptible to polymerization. Oxygenated compounds such as phenols have a tendency to dissolve in distilled water, so their complete removal is not possible. As water distillation tends to be a small operation, it takes a long time to accumulate much oil (Ranjitha and Vijiyalakshmi 2014).

6.3.1.1.2 Steam Distillation

In the steam distillation method, the botanical material is placed in a still and steam is forced over the material. The hot steam is used to release the aromatic molecules from the plant material. The steam forces the pockets to open, and then the molecules of these volatile oils escape from the plant material and evaporate in the steam. The steam containing the essential oil is passed through a cooling system to condense the steam, which forms a liquid form of essential oil. Finally, the water is separated (Masango 2005; Ranjitha and Vijiyalakshmi 2014).

The major advantage of steam distillation is that the temperature never goes above 100°C, so temperature-sensitive compounds can distilled. A disadvantage is that not many compounds can be steam distilled—only the aromatic ones.

6.3.1.1.3 Hydrodiffusion

The hydrodiffusion method is similar to the steam distillation process. The main difference between these two methods is how the steam is introduced into the still. In the case of hydrodiffusion, the steam is fed into the top, onto the botanical material, instead of from bottom, as in normal steam distillation. The steam containing the essential oil is passed through a cooling system to condense steam, which forms a liquid of essential oil, and then water is separated (Ranjitha and Vijiyalakshmi 2014).

The main advantages of this method are that less steam is used, the processing time is shorter, and there is a higher yield.

6.3.1.2 Soxhlet Extraction

Soxhlet extraction is a general and well-established technique that surpasses other conventional extraction techniques in performance, except for limited fields of application, for example, the extraction of thermolabile compounds. Most of the solvent extraction units worldwide are based on Soxhlet principles with recycling of solvents. Basically, the equipment consists of a drug holder extractor, a solvent storage vessel, a reboiler kettle, a condenser, a breather system, and supporting structures like a boiler, a refrigerated chilling unit, and a vacuum unit (William 2007).

This technique is based on the choice of solvent coupled with heat or agitation. In this process, the circulation of solvents causes the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix. This method maintains a relatively high extraction temperature and no filtration of extract is required (Shams et al. 2015).

However, the limitation of this technique is that there is a possibility of thermal decomposition of thermolabile targeted compounds because the extraction usually occurs at the boiling point of the solvent for a long time.

Nidia et al. (2013) compared supercritical fluid extraction using carbon dioxide with the Soxhlet and hydrodistillation processes for extraction of basil oil and reported a higher

yield of oil by Soxhlet extraction. The higher yield may be due to extraction of polar and nonpolar compounds.

6.3.2 Novel Extraction Methods

6.3.2.1 Microwave Extraction Method

Solvent-free microwave extraction is used to separate the essential oil from plant material. The method involves placing the sample in a microwave reactor without any addition of organic solvent or water. The internal heating of the water within the sample distends its cells and leads to rupture of the glands and oleiferous receptacles. This process frees essential oil, which is evaporated by the *in situ* water of plant material.

A cooling system outside the microwave oven continuously condenses the vapors, which are collected in specific glassware. The excess of water is refluxed back to the extraction vessel in order to restore the *in situ* water to the sample.

The microwave isolation offers a net advantage in terms of yield and better oil composition. Furthermore, it is environmentally friendly. In this method, low-boiling-point hydrocarbon compounds undergo decomposition (Marie et al. 2004; Ranjitha and Vijiyalakshmi 2014).

6.3.2.2 Subcritical Water Extraction

Subcritical water extraction is the extraction using hot water under pressure. It has recently emerged as a useful tool to replace traditional extraction methods. Subcritical water extraction is an environmentally clean technique that, in addition, provides higher extraction yields to extract solid samples. Subcritical water extraction is carried out using hot water (from 100°C to 374°C, the latter being the water critical temperature under high pressure [usually up to 10 bars]), enough to maintain water in the liquid state. The most important factor to take into account in this type of extraction procedure is the dielectric constant. This parameter can be modulated easily within a wide range of values by only tuning the extraction temperature.

Water at room temperature is very polar solvent, with a dielectric constant close to 80. However, this level can be significantly decreased to values close to 27 when water is heated up to 250°C while maintaining its liquid state applying pressure. The dielectric constant value is similar to that of ethanol, and therefore is appropriate for solubilizing less polar compounds.

Basically, the experimental setup needed to use this technique is simple. The instrumentation consists of a water reservoir coupled to a high-pressure pump to introduce the solvent into the system, an oven where the extraction cell is placed and where the extraction takes place, and a restrictor to maintain the pressure along the extraction line. Extracts are collected in the collector vial placed at the end of the extraction system (Shams et al. 2015).

The use of subcritical water extraction provides a number of advantages over traditional extraction techniques. These are low extraction time, higher-quality extracts, lower cost of the extractant agent, and being an environmentally cleaner technique (Shams et al. 2015).

6.3.2.3 Supercritical Fluid Extraction

Supercritical fluid extraction is used for the extraction of flavors and fragrances. SFE is a separation technology that uses supercritical fluid as solvent. Every fluid is characterized

by a critical point, which is defined in terms of the critical temperature and critical pressure. Fluids cannot be liquefied above the critical temperature regardless of the pressure applied, but may reach the density close to the liquid state. A substance is considered to be a supercritical fluid when it is above its critical temperature and critical pressure. Several compounds have been examined as SFE solvents (e.g., hexane, pentane, butane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons).

The main supercritical solvent used is carbon dioxide. Carbon dioxide (critical condition 30.9°C and 73.8 bar) is cheap, environmentally friendly, and generally recognized as safe. Supercritical carbon dioxide is also attractive because of its high diffusivity and easily tunable solvent strength. Another advantage is that carbon dioxide is gaseous at room temperature and ordinary pressure, which makes analyte recovery very simple and results in a solvent-free analyte (Taylor 1996; Ranjitha and Vijiyalakshmi 2014).

6.3.2.4 Accelerated Solvent Extraction

Accelerated solvent extraction is sometimes called pressurized solvent extraction (PSE). It uses organic solvents at elevated pressure and temperature in order to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, and elevated pressure keeps the solvent in a liquid state, thus enabling safe and rapid extraction. Furthermore, high pressure forces the solvent into the matrix pores, and hence facilitates the extraction of analyte. High temperature decreases the viscosity of the liquid solvent, allowing a better penetration of the matrix and a weakened solute matrix interaction. Elevated temperature enhances diffusivity of the solvent, resulting in increased extraction speed. The solvent is selected based on the polarity of the analyte and compatibility with postextraction processing equipment. In ASE applications, generally organic solvents are used in conventional techniques, such as methanol. The use of hot water as the extraction solvent under atmospheric or higher pressure is very efficient for extracting phytochemicals.

ASE has been reported to be more efficient than other extraction methods by consuming less solvent and allowing faster extraction. However, since the extraction is performed at elevated temperature, the thermal degradation is a cause of concern, especially for thermolabile compounds in extracts.

The efficiency of ASE is influenced by factors such as pressure, temperature, static extraction time, flush volume, and vessel void volume (Shams et al. 2015).

6.3.2.5 Ultrasound-Assisted Extraction

The mechanical effect of ultrasound accelerates the release of organic compounds within the plant body due to cell wall disruption, mass transfer intensification, and easier access of the solvent to the cell content. Ultrasound-assisted extraction is reported to be one of the important techniques for extracting valuable compounds from the vegetable material (Vilkhu et al. 2008). General ultrasonic devices are the ultrasonic cleaning bath and ultrasonic probe system.

The efficiency of UAE depends on various factors, such as the nature of the tissue being extracted, the location of the component to be extracted, and pretreatment of the tissue prior to extraction. UAE can extract analytes under a concentrated form, free from any contaminants or artifacts. It has also advantages in terms of yield, selectivity, operating time, energy input, and preservation of thermolabile compounds (Shams et al. 2015).

6.4 Composition of Oil

The genus *Cymbopogon* is known to include about 140 species, of which more than 52 have been reported to occur in Africa, 45 in India, 6 each in Australia and South America, 4 in Europe, 2 in North America, and the remaining are distributed in South Asia. There is a considerable variation in the qualitative and quantitative composition of essential oils from different cultivars of *Cymbopogon*. On the basis of chemical similarity, the cultivars of the genus *Cymbopogon* are divided into five chemical variants or groups within two series, that is, Citrati and Rusae.

As explained earlier, lemongrass oil (*Cymbopogon winterianus*) contains a number of fragrant fractions, of which citronellal, geraniol, and citronellol are the major components and are responsible for the real chemistry of this essential oil (Leung 1980; Evans 1989). Citronella oil has two chemotypes: Ceylon type and Java type (Jowitt 1908; Guenther 1950).

- *Ceylon type*: The oil obtained from *Cymbopogon nardus* consists of camphene, dispentene, citronellal, geraniol, granylacetate, nerol, citronellol, thuzylalcohol, borneol, farnesol, linalool, and methyl eugenol. In this type, the content is 18%–20% for geraniol, 9%–11% for limonene, 7%–11% for methyl isoeugenol, 6%–8% for citronellol, and 5%–15% for citronellal.
- *Java type*: This type of oil is obtained from *C. winterianus* Jowitt and consists of limonene, citronellal, citral, geraniol, citronellol, citronellate, eugenol, methyl eugenol, chavicol, sesquicitronellene, elemol, citronellyl oxide, γ- and ∂-cadinene, vanillin, isovaleraldehyde, hexane-2-al, and 3-methyl pentanal. In this oil, the content is 32%–45% for citronellal, 11%–13% for geraniol, 3%–8% for geranyl acetate, and 1%–4% for limonene. The differences in two varieties and the chemical composition of the essential oil have been recorded since early times (Jowitt 1908; Guenther 1950). It was believed that the Java type variety contained around 85% of geraniol. On the other hand, the Ceylon type variety was reported to contain only 55%–65% of geraniol. A geraniol-rich mutant containing as high as 60% of geraniol content has been developed (Ranaweera and Dayananda 1996).

Gas chromatography–mass spectrometry (GC-MS) analysis of citronella oil revealed the presence of many monoterpene hydrocarbons, amounting to more than 20% of the oil in the Ceylon type, but only 3%–4% in the Java type. There is a high proportion of hydrocarbons in the Ceylon type; the most abundant was found to be camphene. The other hydrocarbons present were α- and β-pinene, sabinene, myrcene, car-3-ene, α- and β-phellandrene, α- and β-terpenes, cis/*trans*-ocemene, terpinolene, and p-cymene. The Java-type oil contains more oxy-terpenes than the Ceylon type. There is a great difference in the amount of geraniol in the two oils. However, the Java type contains much more citronellal and citronellol. Another distinguishing feature of the Ceylon type is the presence of methyl eugenol and methyl isoeugenol. Different *Cymbopogon* species contain varying major compounds, such as citral, geraniol, citronellol, piperitone, and elemin. The major components of the *Cymbopogon* species observed are mentioned in Table 6.1.

TABLE 6.1

Major Components of *Cymbopogon* Species

Serial Number	Compound	Molecular Formula	Species	Country/ Region	Content %	Reference
1	Citronellal	$C_{10}H_{18}O$	*C. winterianus*	India	32.7	Wany et al. (2013)
			C. nardus	Malaysia	29.6	Wei et al. (2013)
			C. winterianus	Brazil	36.19	Leite et al. (2011)
			C. winterianus	Southeast Brazil	27.44	Quintans-Júnior et al. (2008)
2	Citronellol	$C_{10}H_{20}O$	*C. winterianus*	India	15.9	Wany et al. (2013)
			C. winterianus	Brazil	11.34	Leite et al. (2011)
			C. winterianus	Southeast Brazil	10.45	Quintans-Júnior et al. (2008)
3	Geraniol	$C_{10}H_{18}O$	*C. winterianus*	India	23.9	Wany et al. (2013)
			C. martini	India	84.16	Dubey et al. (1999)
			C. winterianus	Brazil	32.82	Leite et al. (2011)
			C. winterianus	S.E. Brazil	40.06	Quintans-Júnior et al. (2008)
4	Myrcene	$C_{10}H_{16}$	*C. citratus*	Egypt	15.69	Mohamed et al. (2012)
			C. citratus	Zambia	18.0	Chisowa et al. (1998)
			C. citratus	Nigeria	25.3	Kasali et al. (2001)
			C. citratus	Mali	9.1	Sidibé et al. (2001)
5	Neral	$C_{10}H_{16}O$	*C. flexuosus*	India	30.0	Chowdhury et al. (2010)
			C. flexuosus	Burkina Faso	34.6	Bassolé et al. 2011
			C. flexuosus	Brazil (North)	30.1	Andrade et al. (2009)
			C. flexuosus	Egypt	34.98	Mohamed et al. (2012)
			C. flexuosus	Zambia	29.4	Chisowa et al. (1998)
			C. flexuosus	Kenya	33.31	Matasyoh et al. (2011)
			C. giganteus	Benin Republic	19.93	Gbenou et al. (2013)
			C.giganteus	Nigeria	26.5	Kasali et al. (2001)
			C.citratus	Angola	28.26	Soares et al. (2013)
			C.citratus	Malaysia	50.81	Ranitha et al. (2014)
			C.citratus	Brazil	4.53	Leite et al. (2011)
6	Geranial	$C_{10}H_{16}O$	*C. flexuosus*	India (Kumaon)	33.1	Chowdhury et al. (2010)
				India (Bihar)	42.4	Kumar (2013)
				Brazil	50.0	Andrade et al. (2009)
				Egypt	40.72	Mohamed et al. (2012)
				Zambia	39.0	Chisowa et al. (1998)
				Kenya	39.53	Chisowa et al. (1998)

(*Continued*)

TABLE 6.1 (CONTINUED)

Major Components of *Cymbopogon* Species

Serial Number	Compound	Molecular Formula	Species	Country/ Region	Content %	Reference
			C. citratus	Nigeria	33.7	Kasali et al. (2001
				Angola	40.55	Soares et al. (2013)
				Ivory Coast	34.0	Sidibé et al. (2001)
				Mali	45.3	Sidibé et al. (2001)
				Iran	39.16	Farhang et al. (2012)
7	Camphene	$C_{10}H_{16}$	*C. pendulus*	India	9.1	Wei et al. (2013)
			C. winterianus	India	8.0	Wany et al. (2013)
8	Lemonene	$C_{10}H_{16}$	*C. giganteus*	Cameroon	7.4	Jirovetz et al. (2007)
			C. giganteus	Burkina Faso	42.0	Bassolé et al. (2011)
			C. proximus	Burkina Faso	3.9	Menut et al. (2011)
9	Elemecin	$C_{12}H_{16}O_3$	*C. pendulus*	India	53.7	Shahi et al. (1997)
10	Linalool	$C_{10}H_{18}O$	*C. flexuosus*	India	2.6	Chowdhury et al. (2010)
			C. winterianus	India	1.5	Wany et al. (2013)
			C. martin	India	2.0	Dubey et al. (1999)
			C. nardus	Malaysia	11.0	Wei et al. (2013)
11	Pipertone	$C_{10}H_{18}O$	*C. oliovieri*	Iran	72.8	Mahboubi et al. (2012)
			C. parkeri	Iran	80.8	Bagheri et al. (2007)

6.4.1 Effect of Method of Extraction on Composition of Lemongrass Oil

It has been proven through a number of studies that the quality of essential oil mainly depends on the procedure used to extract it. In contrast, these common methods can induce thermal degradation, hydrolysis, and water solubilization of some fragrance constituents. Ranitha et al. (2014) evaluated the effect of microwaves on the extraction of essential oil. The concentrations of key compounds found in lemongrass oil were similar for both methods, but the oil composition revealed that a higher amount of oxygenated monoterpenes, such as linalool, geranic acid, and citronellol, are present in essential oil isolated by MAHD. The quality of lemongrass oil obtained by pressurized liquid extract (PLE) was compared with that of conventional extraction methods, hydrodistillation, and the Soxhlet extraction method. PLE gave the significantly highest amount of neral and geraniol, followed by Soxhlet and hydrodistillation. This was due to the ability of the solvent n-hexane to extract almost all nonvolatile and volatile compounds, compared with hydrodistillation, which can only extract the volatile compounds (Nur et al. 2013). Obtained from dried lemongrass stems found to be better quality containing 90% citral in comparision to steam distillation method (Ha et al. 2008).

6.5 Methods of Analysis

The classical methods of analysis of the essential oil of *Citronella* were primarily based, on the one hand, on the estimation of total acetylizable material and, on the other hand, on

various rough solubility checks, such as Schimmel's test, raised Schimmel's test, and the London solubility test. In addition, refractive index and optical rotation were specified. As the new instrumental methods emerged, the new techniques of the characterization of chemical compounds based on spectroscopic methods resulted in a major surge in natural products research in the early 1960s, followed by the development of chromatographic techniques such as gas–liquid chromatography (GLC), GC-MS, and GC–ion mobility spectrometry (IMS).

The quality of lemongrass oil is determined by its citral content. Various methods have been reported in literature for the estimation of citral in lemongrass oil, and also for the separation of citral from lemongrass oil. The common methods for the estimation and separation of citral are the bisulfite method, neutral sulfite method, hydroxylamine method, and colorimetric methods.

6.5.1 Bisulfite Method

The bisulfite method is based on adduct formation. Upon shaking of a measured quantity of oil with a hot aqueous solution of sodium bisulfite, an adduct is formed, which dissolves on heating the solution. The noncitral portion of the oil separates as an oily layer, which can be measured conveniently in the neck of a Cassia flask, and thereby the citral content of the oil can be determined.

6.5.2 Neutral Sulfite Method

This is also an adduct formation reaction. In this method, the liberated sodium hydroxide has to be neutralized periodically with acid to permit the reaction to go to completion. However, the solution must not be permitted to turn acidic, as this would result in the formation of the stable dihydrosulfonic compound, from which citral cannot be regenerated. This method has all the disadvantages of the bisulfite method as a method of estimation and also of separation. However, it offers certain advantages over the bisulfite method. By using the indicator (phenolphthalein), the exact end point of the reaction can be determined.

6.5.3 Hydroxylamine Method

This method is also used for the estimation of citral in lemongrass oil. It makes use of both hydroxylamine and hydroxylamine hydrochloride. After the reaction of this with the carbonyl group, the mixture is titrated with standard alkali. The hydroxyl amine method also has some defects. All the carbonyl groups present in lemongrass oil will react with hydroxyl amine, and the value obtained will be much higher. However, this method offers some advantages over the adduct formation process. Relatively small amounts of the oil are required for estimation. The reaction of hydroxylamine with aldehyde is rapid, thereby shortening the time required for the estimation. This method proves to be exceptionally applicable to oils that contain large amounts of aldehydes. The solution used for the standard procedure is stable and can be kept for longer periods.

6.5.4 Colorimetric Methods

The citral content of lemongrass oil has also been estimated by the coloring agent of Ehrlich Miller. This coloring agent has been found to give better results, and the development of

color takes place rapidly and remains quite stable for a long time. The coloring agent is prepared according to Ehrlich Miller and consists of the following solutions:

- 5% p-dimethylaminobenzaldehyde solution in acetic acid
- 10% phosphoric acid solution in acetic acid

One milliliter of each of the above solutions is added to different amounts of citral in acetic acid, whereby a marked color change from blue to pink can be observed. The percentage absorbance and extinction of the colored citral is then measured using a colorimeter, and calibration graphs are plotted. The amount of citral in solutions can be compared with that of known strength, and thus the percentage of citral can be determined. Here, we also need solutions of citral with known strength.

6.5.5 Gas–Liquid Chromatography and Gas Chromatography–Mass Spectrometry Methods

The GLC technique depends on the effectiveness of the volatility of the compounds and constituents of the essential oils. However, in most cases a large number of chemical compounds in essential oils were dependent on the extent up to which they could be effectively separated. Thus, GLC offered a method of separation that could be achieved with a very minute quantity of sample. The separated constituents are subjected to the new technique, such as mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, to determine their chemical structure. The separation of constituents is affected by the type of stationery phase (column) and the temperature condition of separation. Various researchers used various methods for the separation, identification, and quantification of constituents (Table 6.2).

6.6 Physicochemical Properties of Lemongrass Oil

The physicochemical properties, such as appearance, color, solubility, specific gravity, refractive index, and angular rotation, have been studied by various researchers. These properties may vary from type to type and by method of extraction. There are several standards framed at the national and international levels to maintain the quality of the oil. The specifications for physicochemical properties per the Food Chemical Codex are shown in Table 6.3.

Hazwan et al. (2014) studied the physical properties of citronella oil from different sources obtained by three different extraction methods, such as ohmic heated hydrodistillation, hydrodistillation, and steam distillation. The refraction index and specific gravity at 20°C for all three different sources of oil were about 1.47 and 0.89, respectively. In addition, the color, which is an important feature to determine the consumer's acceptability of citronella oil products, was also measured. Normally, commercial citronella oil is yellow. In this study, the intensities of yellow and red were measured. The citronella oil extracted by ohmic heated hydrodistillation was in the range of 0.7 R 2.0 Y, whereas extracted citronella oil by steam distillation exhibited color in the range of 0.5 R 3 Y. The characteristics of the color red were possibly due to *trans*-β-caryophyllene and γ-cadinene constituents in the citronella oil (Abena et al. 2007; Harjeet et al. 2011). However, no red color was found

TABLE 6.2

Methods Used for Analysis of Lemongrass Oil

Serial Number	Source of Oil	Method of Extraction	Number of Compounds	Method Used	Reference
1	Aerial parts of *C. citratus*	Hydrodistillation	18	GC-FID Column: HP-5 capillary 30 m × 0.32 mm i.d., 0.25 µ thickness Detector: FID Temp program: 50°C for 2 min @ 8°C/min to 240°C Detector temp: 280°C Injector temp: 240°C	Mohamed et al. (2012)
2	Aerial parts of *C. citratus*	Hydrodistillation	18	GC-MS Column: VF-5MS capillary 30 m × 0.25 mm i.d., 0.25 µ thickness Detector: MSD Temp program: 50°C–180°C @ 5°C/min to 250°C Carrier gas: Helium Flow rate: 1 ml/min Split ratio: 1:20 Ionization energy: 70 eV	Mohamed et al. (2012)
3	Aerial parts of *C. nardus*	Distillation	13	GC-MS Column: HP-5MS capillary 30 m × 0.25 mm i.d., 0.25 µ thickness Detector: MSD. Temp program: 70°C for 5 min @ 3°C/min to 325°C Carrier gas: Helium Flow rate: 1 ml/min Split ratio: 100:1 Ionization EI mode energy: 70 eV Detector temp: 280°C Injector temp: 240°C	Hazwan et al. (2014)
4	Aerial parts of *C. citratus*	Pressurized liquid extraction	2	GC-MSD Column: DB-5 capillary 20 m × 0.188 mm i.d., 0.4 µ thickness Detector: FID Temp program: 100°C for 1 min @ 1°C/min to 120°C Detector temp: 250°C Injector temp: 300°C	Nur Ain et al. (2013)

(*Continued*)

TABLE 6.2 (CONTINUED)

Methods Used for Analysis of Lemongrass Oil

Serial Number	Source of Oil	Method of Extraction	Number of Compounds	Method Used	Reference
5	Leaves of *C. citratus*	Microwave-assisted hydrodistillation	7	GC-MSD Column: HP-5MS capillary 30 m × 0.25 mm i.d., 0.25 μ thickness Detector: MSD Temp program: 50°C for 5 min, then rise @ 3°C/min to 240°C, then @ 5°C/min to 300°C Carrier gas: Helium Flow rate: 1 ml/min Split ratio: 1:10 Ionization EI energy: 70 eV	Ranitha et al. (2014)
6	Citronella oil, Ceylon and Java type	Distillation	60	GC-FID Column: 10% Carbowax Chromosorb W (2.7 × 3.2 mm) Detector: FID Temp program: 60°C @ 2°C/min to 220°C Base attenuation: ×16	Wijesekara (1973)

Note: EI, electron ionization; FID, flame ionization detector; Temp, temperature.

TABLE 6.3

Physicochemical Properties of Lemongrass Oil as per Food Chemical Codex

Serial Number	Parameter	Lemongrass Oil, East Indian Type	Lemongrass Oil, West Indian Type	Reference
1	Description	Dark yellow to light brown–red liquid with lemon odor	Light yellow to light brown liquid with light lemon odor	Food Chemical Codex (2014)
2	Specific gravity at 20°C	0.894–0.904	0.869–0.894	Food Chemical Codex (2014)
3	Angular rotation at 20°C	–10° to +0°	–10° to +0°	Food Chemical Codex (2014)
4	Refractive index at 20°C	1.483–1.489	1.483–1.489	Food Chemical Codex (2014)
7	Solubility	Soluble in mineral oil, freely soluble in propylene glycol but insoluble in water and glycerine, and dissolves readily in alcohol	Soluble in mineral oil, freely soluble in propylene glycol but insoluble in water and glycerine, and yields cloudy solution with alcohol	Food Chemical Codex (2014)

when the citronella oil was obtained by the hydrodistillation method. The appearance of red color when using other extraction methods may also be caused by lipid oxidation occurring in the extraction system. Essential oil obtained from *C. citratus* by microwave-assisted hydrodistillation had the following characteristics: refractive index at 20°C, density (g/ml) at 27°C, color parameters are 1.483, 0.873 g/ml, and color as (L* = 97, a* = –2.44, b* = 6.29), respectively (Vazquez-Briones et al. 2015).

6.7 General Uses of Lemongrass Oil

Lemongrass oil is one of the 20 most important essential oils that are traded globally (Lawrence 1993). Citronella oil is highly demanded because of its wide usage in perfumes and the soap manufacturing, cosmetics, and flavoring industries, and because it is effective as an insect repellant. This essential oil is characterized by a high content of citral, which is used as a raw material for the production of ionone, vitamin A, and β-carotene. Due to its appealing citric flavor and strong antimicrobial potential, it has been used as flavoring agent in several food products and as a natural preservative for extending the shelf life of food products. In addition to food and cosmetics, this oil is also used in many traditional medicines and has great potential in various pharmaceutical applications. The general uses of lemongrass oil are in food, cosmetics and personal care products, traditional medicine, and pharmaceuticals.

6.7.1 Food

A recent consumer trend toward a preference for products with a lower salt and sugar content presents a greater need for efficient food preservatives. However, an increasingly negative consumer perception of synthetic food additives has spurred an interest in finding natural alternatives. Essential oils like lemongrass oil are natural compounds that have shown promising properties, such as antifungal, antibacterial, and antiviral activities. Moreover, essential oils have also been proven to have other diverse beneficial functions, such as antidiabetic, antiradical, and antioxidant effects.

Apart from its appealing flavor, lemongrass (*C. citratus*) essential oil has been shown to have antimicrobial potential. This makes it susceptible for incorporation in food products. The oil of lemongrass could suppress the growth of mesophiles and psychrophiles in fresh-cut apples (Raybaudi-Massilia et al. 2008). Tzortzakis and Economakis (2007) reported that the essential oil of lemongrass oil inhibited the growth of *Botrytis cinerea.* Essential oil lemongrass controls food spoilage and shows antibacterial activity against *Listeria monocytogenes* (Nguefack et al. 2004) and *Staphylococcus aureus* (Baratta et al. 1998). Although this essential oil has shown to be a promising alternative to chemical preservatives against foodborne pathogens, it presents special limitations that preclude its use in food products. Low water solubility, high volatility, strong odor, and toxicological effects at high doses make it difficult for food application. In addition to these drawbacks, the incorporation of oil–based compounds like essential oil in aqueous food products is a big challenge since it shows physical and chemical instability when it is applied in food systems (McClements et al. 2004). Therefore, several studies have shown that the use of nanoemulsion can be a great choice for the application of essential oil in a food matrix. Kim et al. (2013) studied the plum coatings of lemongrass oil incorporating carnauba wax–based nanoemulsion to evaluate antimicrobial properties and physical and chemical changes in plums. The nanoemulsion was able to inhibit the *Salmonella* and *Escherichia coli* population without changing the flavor,

fracturability, or glossiness of the product, and it reduces the ethylene production and retards the changes in lightness and the concentration of phenolic compounds.

Essential oils as natural sources of phenolic components attract investigators to evaluate their activity as antioxidants or free radical scavengers. Vazquez-Briones et al. (2015) studied the antioxidant properties of essential oil obtained from *C. citratus* by the microwave-assisted hydrodistillation extraction method. In this study, the phenolic content and antioxidant capacity were reported to the tune of 149.2 ± 6 mg gallic acid equivalent (GAE) per 100 ml of oil and 44.06 ± 0.20 mg Trolox per ml of essential oil, respectively. Different antioxidant capacity values were reported by different researchers (Selim 2011; Mirghani et al. 2012). The difference in values is attributed to factors such as climate, soil composition, season, part of the plant, age, and stage of growing of the plant (Angioni et al. 2006). *C. citratus* could be of great interest in the food industry to be used as a natural additive for flavoring.

6.7.2 Cosmetics and Personal Care Products

The redolence of the oil enables its use in soaps, detergents, and the application in the perfumery. Wuthi-udomlert et al. (2011) evaluated the antifungal activity of lemongrass oil against *Malassezia furfur,* an opportunistic yeast associated with dandruff. Two percent lemongrass oil shampoo provided the required qualities necessary for commercial use. After being kept for 6 weeks at 28°C–30°C and 45°C, this formulated shampoo gave minimum fungal concentrations (MFCs) against *M. furfur* of 75 and 18.75 µl/ml, respectively. The 2% concentration of lemongrass oil was selected because of the smell, consistency, and stability of the shampoo.

6.7.3 Traditional Medicine

In traditional medicine, the oil has been used as an aromatic tea, vermifuge, diuretic, and antispasmodic. Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia, and vascular disorders. Studies have shown that lemongrass has antibacterial and antifungal properties. The traditional use includes treatment of fever, intestinal parasites, and digestive and menstrual problems. Mixed with pepper, it is a homeotherapy for menstrual troubles and nausea. Lemongrass is a good cleanser that helps to detoxify the liver, pancreas, kidney, bladder, and digestive tract. It cuts down uric acid, cholesterol, excess fats, and other toxins in the body, while stimulating digestion, blood circulation, and lactation; it also alleviates indigestion and gastroenteritis. It is said that lemongrass also helps improve the skin by reducing acne and pimples and acts as a muscle and tissue toner. Also, it can reduce blood pressure. A recent study by the Food and Nutrition Research Institute of the Department of Science and Technology (DOES) showed that lemongrass can help prevent cancer. It has many uses in aromatherapy (Karkala and Bhushan 2014). It can be used as massage oil for aching joints and muscles. When mental illness has to be treated, citronella can be clarifying and balancing. Combining it with lemon oil can bring an even greater brightening effect to the mind.

6.7.4 Pharmaceutical Uses

A vast array of ethnopharmacological applications of lemongrass exist today. Its health restorative capacity may be ascribed to the diverse secondary metabolites it produces. The pharmacological activity of lemongrass oil is summarized in Table 6.4. Batubara et al. (2015) confirmed the ability of β-citronellol, the major component of lemongrass

TABLE 6.4

Pharmacological Actions of Lemongrass Oil (*Cymbropogon citratus*)

Serial Number	Biological Activity	Constituents	Observation	Potential Use	Reference
1	Antimicrobial activity	Ethanolic extracts of the leaves Flavonoids and tannins	Antibacterial property against *Staphylococcus aureus*	Potential antibacterial property against *Staphylococcus aureus*	Danlami et al. (2011)
2	Antifungal activity	Lemongrass oil and citral	The antifungal activity of lemongrass and citral against *Candida* species	Formulating herbal drugs for oral healthcare	Taweechaisupapong et al. (2012)
3	Antiprotozoan activity	Citral and major constituents of lemongrass oil	The promasigotes of *Leishmania infantum* undergo programmed cell death upon exposure to citral and constituents of lemongrass oil	Lemongrass may be foreseen as an antiprotozoan drug of the future	Machado et al. (2012)
4	Antioxidant activity	Phenolic acids present in the plant	Showed the antioxidant profile		Garg et al. (2012)
5	Antidiarrheal activity	Citral	Relief in diarrhea		Tangpu and Yadav (2006)
6	Anticancerous activity	Emulsion of citral and lemongrass oil	Anticancerous properties on cervical cell lines	The constituents of lemongrass may be used to form potent anticancer drugs in the future	Ghosh (2013)
7	Antiviral activity	Lemongrass oil and citral	Reduced viral infectivity by coating the viral capsid and preventing it from binding to the host cell	Can be used to sanitize food and surfaces to prevent viral infection	Gilling et al. (2014)

oil, to bring about a reduction in weight of rats fed a high-fat diet. Inhalation of vapors of β-citronellol enhances the sympathetic nerve activity of the rats, which leads to increased activity in the adipose tissue, resulting in weight loss without affecting the concentration and activity of the liver enzymes. The essential oil of lemongrass is also used to maintain oral health. The antagonistic activity of lemongrass against the planktonic and biofilm forms of *Candida dubliniensis*, a common oral pathogen, has been reported. Therefore, lemongrass may be used in formulating herbal drugs for oral healthcare (Taweechaisupapong et al. 2012). The essential oil of *C. citrus* has been shown to have anti-inflammatory, anticonvulsant, analgesic, and anxiolytic effects (Blanco et al. 2009; Sforcin et al. 2009; Gbenou et al. 2013). It has been reported that the lemongrass is bestowed with hypolipidemic, hypocholesterimic, and hypoglycemic properties. The antagonistic activity of lemongrass toward different pathogenic bacteria, protozoa, and fungi has also been reported. Research on antimicrobial and anti-inflammatory activities, along with GC-MS analysis of lemongrass oil, revealed that the major constituents, like limonene, nerol, gerianal, geraniol, and myrcene, may be responsible for its microbicidal and anti-inflammatory effects. It has been reported that the promastigotes of *Leishmania infantum* undergo programmed cell death upon exposure to citral, the major component of lemongrass oil (Machado et al. 2012).

The combination of silver nanoparticles and the oil has synergistic inhibitory action on the growth of pathogens like *E. coli*, *Staphylococcus*, *Moraxella*, *Enterococcus*, and *Candida* sp. Citronella oil also exhibits antifungal activity against *Aspergillus niger.* The anti-inflammatory effect is through inhibition of production of IL-1β by bioactive compounds of lemongrass oil (citral, neral, and geranial) (Perez et al. 2011). An antiviral effect of lemongrass against an enveloped murine novovirus has been reported (Gilling et al. 2014). The bioactive compounds in citronella oil are studied for their anticancerous properties. An emulsion of citral and lemongrass oil exhibited anticancerous properties on cervical cell lines by reducing cell proliferation and initiating apoptosis (Ghosh 2013). Hence, it is envisaged that the constituents of lemongrass may be used to form potent anticancer drugs in the future.

6.8 Insecticidal Activity of Lemongrass Oil

The development of natural products for pest control is increasingly important, since some synthetic pesticides are associated with environmental concerns or are being withdrawn for economic and regulatory reasons. In addition, pesticides sometimes lose their effectiveness due to the difficulty of managing pest resistance, and the search for new synthetic compounds is increasingly time-consuming and expensive. Plant secondary metabolites play an important role in plant–insect interaction, and such compounds may have insecticide, hormonal, or antifeedant activity against insects (Bernays and Chapman 1940). The essential oil compounds and their derivatives are considered to be an alternative means of controlling many harmful insects because these compounds are very specific to harmful insects but do not affect the beneficial insects, and they degrade rapidly unlike synthetic compounds. Essential oils of plants contain a number of bioactive compounds that may exert regulatory or inhibitory influence on insect life processes, such as growth and development, reproduction, and orientation. Recent research has demonstrated their larvicidal and antifeedant activity, capacity to delay development, adult emergence and fertility, deterrent effects on oviposition, and arrestant and repellent action.

6.8.1 Insecticidal Activity

Among the compounds present in essential oils, monoterpenes are usually the main component, and are consequently regarded as a candidate for insecticidal activity. These natural compounds have been proposed as lead compounds for the development of safe, effective, and fully biodegradable insecticides. Most of the monoterpenes are cytotoxic to plants and animal tissue, causing a drastic reduction in the number of mitochondria and Golgi bodies, impairing respiration and photosynthesis and decreasing cell membrane permeability (Tripathi et al. 2009). At the same time, they are volatile and may serve as chemical messengers for insects. The doses of essential oils needed to kill insects or pests and their mechanism of action are potentially important for the safety of humans and other vertebrates. Therefore, the target sites and mode of action need to be understood and well elucidated. Although a little is known about the physiological actions of essential oils on insects, treatment with various essential oils and their constituents causes symptoms that suggest a neurotoxic mode of action. A monoterpenoid linalool has been demonstrated to act on the nervous system, affecting ion transport and the release of acetylcholine esterase in insects (Re et al. 2000).

Sudiarta et al. (2013) studied the effect of lemongrass oil extracted from *C. citratus* on *Plutella xylostella*, which causes club root disease in cabbage. The phytotoxicity test was conducted in the field with several concentrations of lemongrass (5%, 2.5%, 1%, 0.5%, 0.25%, and 0.1%). The results showed that a high concentration of lemongrass (10%) was effective as a phytotoxic, with burn symptoms of the cabbage leaf. However, low concentrations (1% and 0.5%) of lemongrass oil can control the population of *P. xylostella* without any phytotoxicity. The essential oils of *Cymbopogon martini* have been studied and found to display high anthelmintic activity against *Caenorhabditis elegans* at an ED_{50} value of 125.4 μg/ml. Essential oils of *C. citratus* in West Africa displayed about a 100% mortality rate against adult *Anopheles gambiae* (Nonviho et al. 2010). The essential oil from *C. winterianus* has caused a dose-dependent mortality of *Culex quinquefasciatus.*

6.8.2 Larvicidal Activity

To minimize and eradicate the occurrence of mosquito-borne diseases, many steps have been taken to prevent their spread to different extents, for example, mosquito eradication at an early stage, disease prevention via prophylactic drugs and vaccines, and the prevention of mosquito bites using repellants. Out of these, larviciding has the greatest impact on the mosquito population because the larvae are concentrated, immobile, and accessible. Nazar et al. (2009) studied the larvicidal effect of *C. citratus* essential oil against *Cx. quinquefasciatus* larva and reported an LC_{50} value of 24 mg/L. The essential oil from *C. citratus* had a larvicidal activity against *Aedes aegypti*, causing 100% mortality at a concentration of 100 ppm (Cavalcanti et al. 2004).

6.8.3 Lemongrass Oil as a Repellant

Very little is known about the receptors responsible for the repellent responses in cockroaches. Oleic acid and linoleic acid have been indicated in death recognition and death aversion in cockroaches, and the term *necromone* has been proposed to describe the compound responsible for this type of behavior (Rollo et al. 1995).

Citronella oil has repellency activity against *Ae. aegypti* mosquitoes. The extracted oil was microencapsulated (1.5% gelatin and 1.5% arabic gum by a complex coacervation method). This citronella oil was treated on cotton fabrics using gelatin and gum acacia microcapsules by the pad dry method, in which 15%, 30%, and 50% repellency effects were studied. The 50% concentrated repellents gave the best mosquito repellency. However, the microencapsulated oil gave a better repellant effect for a longer time (Murugan et al. 2012).

6.9 Advantages of Lemongrass Oil as a Pesticide

The constituents of lemongrass oil are selective and have little or no harmful effect on the environment and the nontarget organism. Due to the multiple sites of action through which the constituents can act, the probability of developing a resistant population is very low. These botanical insecticides degrade rapidly in air and moisture, and detoxification enzymes break them readily. Due to rapid breakdown, they are less persistent in the environment (Koul et al. 2008).

6.10 Constraints of Lemongrass Oil as a Pesticide

The efficacy of these materials falls short when compared with synthetic pesticides. Essential oils also require somewhat greater application rates (as high as 1% active ingredients) and may require frequent reapplication when used outdoors. The commercial application of plant essential oil–based pesticides has challenges, like having sufficient quantities of plant material, the standardization and refinement of pesticide products, the protection of technology, and regulatory approval. In addition, as the chemical profile of plant species can vary naturally, depending on geographic, genetic, and climatic annual or seasonal factors, pesticide manufacturers have to take additional steps to ensure that their product will perform consistently. All this requires substantial costs, and smaller companies are not willing to invest the required funds unless there is a high probability of recovering the costs through some form of market exclusivity. Finally, once all these issues are addressed, regulatory approval is required (Mohan et al. 2011).

6.11 Lemongrass Oil–Based Insecticides

The commercial plant product based on lemongrass oil, available as the trade name Green Match EX™, contains essential oils of lemongrass from *C. nardus*, *C. citratus*, and *Cymbopogon flexiosus* containing citronellal and citral as the main bioactive compounds. This formulation is used as an insecticide (Fischer et al. 2013).

6.12 Conclusion

Lemongrass oil (*Cymbopogon* sp.) consists of a diverse array of bioactive compounds and exhibits a wide range of activities, such as antimicrobial, antioxidative, anticarcinogenic, antiviral, and insecticidal activities. Therefore, this oil has great potential not only in food, pharmaceuticals, and cosmetics, but also as an insect repellant. It is expected that the innovative formulation of pesticides based on this essential oil will find their greatest commercial application in urban pest control, vector control vis-à-vis human health, and pest control in agriculture, and will help in organic food production systems, where a few alternative pesticides are available.

References

Abena, A.A., Gbenoub, J.D., Yayib, E., Moudachiroub, M., Ongokac, R.P., Oumbac, J.M., Siloud, T. Comparative chemical and analgesic properties of essential oils of *Cymbopogon nardus* (L.) Rendle of Benin and Congo. *Afr. J. Tradit. Complement. Altern. Med.* 2007; 4(2): 267–272.

Andrade, E.H., Zoghbi, M.D., Lima, M.D. Chemical composition of the essential oils of *Cymbopogon citratus* (DC.) Stapf cultivated in north of Brazil. *J. Essent. Oil Bear. Plants* 2009; 12: 41–45.

Angioni, A., Barra, A., Coroneo, V., Dessi, S., Cabras, P. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and flowers. *J. Agric. Food Chem.* 2006; 54: 4364–4370.

Bagheri, R., Mohamadi, S., Abkar, A., Fazlollahi, A. Essential oil components of *Cymbopogon parkeri* STAPF from Iran. *Pak. J. Biol. Sci.* 2007; 10: 3485–3486.

Baratta, M.T., Dorman, H.J.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G., Ruberto, G. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour Fragr. J.* 1998; 13: 235–244.

Batubara, I., Suparato, I.H., Sadiah, S., Matusuoka, R., Mitsunaga, T. Effect of inhaled citronella oil and related compounds on rat body weight and brown adipose tissue sympathetic nerve. *Nutrients* 2015; 7: 1859–1870.

Bernays, E.A., Chapman, R.F. *Host Plant Selection by Phytophageous Insects.* Chapman & Hall, New York, 1994.

Blanco, M.M., Coasta, C.A.R.A., Freire, A.O., Santosh, J.G., Costa, M. Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. *Phytomedicine* 2009; 16: 265–270.

Baratta, M.T., Dorman, H.J.D, Deans, S.G., Figueiredo, A.C., Barroso, J.G. and Ruberto, G. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour Fragr J.* 1998; 13: 235–244.

Bassolé, I.H., Lamien-Meda, A., Bayala, B., Obame, L.C., Ilboudo, A.J., Franz, C., Novak, J., Nebié, R.C., Dicko, M.H. Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine* 2011; 18: 1070–1074.

Cavalcanti, E.S., Morais, S.M., Lima, M.A., Santana, E.W. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem. Inst. Oswaldo Cruz* 2004; 99: 541–544.

Chisowa, E.H., Hall, D.R., Farman, D.I. Volatile constituents of the essential oil of *Cymbopogon citratus* Stapf grown in Zambia. *Flavour Fragr. J.* 1998; 13: 29–30.

Chowdhury, S.R., Tandon, P.K., Chowdhury, A.R. Chemical composition of the essential oil of *Cymbopogon flexuosus* (Steud) Wats. growing in Kumaon region. *J. Essent. Oil Bear. Plants* 2010; 13: 588–593.

Danlami, U., Rebeca, A., Machan, D.B., Asuquo, T.S. Comparative study on the antimicrobial activities of the ethanolic extracts of lemon grass and polyalthia longifolia. *J. Appl. Pharm. Sci*. 2011; 1(9): 174–176.

Dubey, V.S., Mallavarapu, G.R., Luthra, R. Changes in the essential oil content and its composition during palmarosa (*Cymbopogon martini* (Roxb.) Wats. var. motia) inflorescence development. *Flavour Fragr. J.* 1999; 15: 309–314.

Evans, W.C. *Trease Evans' Pharmacognosy*, 13th ed. Bailliere Tindall, London, 1989.

Farhang, V., Amini, J., Javadi, T., Nazemi, J., Ebadollahi, A. Chemical composition and antifungal activity of essential oil of *Cymbopogon citratus* (DC.) Stapf. against three *Phytophthora* species. *Greener J. Biol. Sci*. 2012; 3: 292–298.

Fischer, D., Imholt, C., Pelz, H.J., Wink, M., Prokopc, A., Jacoba, J. The repelling effect of plant secondary metabolites on water voles, *Arvicola amphibious. Pest Manag. Sci.* 2013; 69: 437–443.

Food Chemical Codex. 9th ed. National Academy Press, Washington, DC, 2014.

Gbenou, J.D., Ahounou, J.F., Akakpo, H.B., Laleye, A., Yayi, E., Gbaguidi, F., Baba-Moussa, L. et al. Phytochemical composition of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils and their anti-inflammatory and analgesic properties on Wistar rats. *Mol. Biol. Rep.* 2013; 40: 1127–1134.

Guenther, E. *The Essential Oils 4*. Van Nostrand, New York, 1950.

Garg, D., Muley, A., Khare, N., Marar, T. Comparative analysis of phytochemical profile and antioxidant activity of some Indian culinary herbs. *Res. J. Pharm. Biol. Chem. Sci.* 2012; 3(3): 845–854.

Ghosh, K. Anticancer effect of lemongrass oil and citral on cervical cancer cell lines. *Pharmacogn. Commun.* 2013; 3: 41–48.

Gilling, D.H., Kitajima, M., Torrey, J.R., Bright, K.R. Mechanism of antiviral action of plant antimicrobials against murine norovirus. *Appl. Environ. Microbiol.* 2014; 80: 4898–4910.

Ha, H.K.P., Maridable, J., Gaspillo, P.D., Kawasaki, J. Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation. *Philippine Agric. Sci.* 2008; 91(1): 36–41.

Harjeet, S., Gupta, V.K., Rao, M.M., Sannd, R., Mangal, A.K. Evaluation of essential oil composition of *Cymbopogon* spp. *Int. J. Pharma Recent Res.* 2011; 3(1): 40–43.

Hazwan, M.H., Man, H.C., Abidin, Z.Z., Jamaludin, H. Comparison of citronella oil extraction methods from *Cymbopogon nardus* by ohmic-heated hydrodistillation, hydrodistillation and steam distillation. *Bioresources* 2014; 9(1): 256–272.

Jensen, W.B. The origin of Soxhelt Extractor. *J. Chem. Educ*. 2007; 84(12): 1913.

Jirovetz, L., Buchbauer, G., Eller, G., Ngassoum, M.B., Maponmetsem, P.M. Composition and antimicrobial activity of *Cymbopogon giganteus* (Hochst.) Chiov. essential flower, leaf and stem oils from Cameroon. *J. Essent. Oil Res.* 2007; 19: 485–489.

Jowitt, I.F. Annals of the Royal Botanical Gardens, Peradeniya 4: 185. In Gildemiester, A., Hoffman, A. (eds.), *Volatile Oils*. 2nd ed. Longmans, London, 1908.

Karkala, M., Bhushan, B. Review on pharmacological activity of *Cymbopogon citratus*. *Int. J. Herbal Med*. 2014; 1(6): 5–7.

Kasali, A.A., Oyedeji, A.O., Ashilokun, A.O. Volatile leaf oil constituents of *Cymbopogon citratus* (DC.) Stapf. *Flavour Fragr. J.* 2001; 16: 377–378.

Kim, I.H., Lee, H., Kim, J.E. Plum coatings of lemongrass oil–incorporating carnauba wax–based nanoemulsion. *J. Food Sci*. 2013; 78: E1551–E1559.

Koul, O., Walia, S., Dhaliwal, G.S. Essential oils as green pesticides: Potential and constraints, *Biopestic. Int*. 2008; 4: 63–84.

Kumar, B.S. Essential oil of *Cymbopogon citratus* against diabetes: Validation by *in vivo* experiments and computational studies. *J. Bioanal. Biomed*. 2013; 5: 194–203.

Lawrence, B.M. A planning scheme to evaluate new aromatic plants for the flavor and fragrance industries. In Janick, J., Simon, J.E. (eds.), *New Crops*. Wiley, New York, 1993, pp. 620–627.

Leite, B.L.S., Souza, T.T., Antoniolli, A.R., Guimarães, A.G., Siqueira, R.S., Quintans, J.S.S., Bonjardim, L.R. et al. Volatile constituents and behavioral change induced by *Cymbopogon winterianus* leaf essential oil in rodents. *Afr. J. Biotechnol.* 2011; 10: 8312–8319.

Leung, A.Y. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. John Wiley & Sons, New York, 1980.

Machado, M., Pires, P., Dinis, A.M., Santos-Rosa, M., Alves, V., Salgueiro, L., Caveleiro, C., Sousa, M.C. Monoterpenic aldehydes as potential anti-*Leishmania* agents: Activity of *Cymbopogon citratus* and citral on *L. infantum*, *L. tropica* and *L. major. Exp. Parasitol.* 2012; 130: 223–231.

Mahboubi, M., Kazempour, N. Biochemical activities of Iranian *Cymbopogon olivieri* (Boiss) Bor. essential oil. *Indian J. Pharm. Sci.* 2012; 74: 356–360.

Marie, E.L., Farid, C., Jacqueline, S. Solvent free microwave extraction of essential oil from aromatic herbs: Comparision with conventional hydrodistillation. *J. Chromatogr. A*. 2004; 1043(2): 323–327.

Masango, P. Cleaner production of essential oils by steam distillation. *J. Cleaner Prod.* 2005; 29(1): 171–176.

McClements, D.J. *Food Emulsions: Principles, Practices and Techniques*. CRC Press, Boca Raton, FL, 2004.

Matasyoh, J.C., Wagara, I.N., Nakavuma, J.L., Kiburai, A.M. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. *Afr. J. Food Sci.* 2011; 5: 138–142.

Menut, C., Bessiére, J.M., Samaté, D., Djibo, A.K. Aromatic plants of tropical west Africa. XI. Chemical composition, antioxidant and antiradical properties of the essential oils of three *Cymbopogon* species from Burkina Faso. *J. Essent. Oil Res.* 2011; 12: 37–41.

Mirghani, M.E.S., Liyana, Y., Parveen, J. Bioactivity analysis of lemongrass (*Cymbopogon citratus*) essential oil. *Int. Food Res. J.* 2012; 19(2): 569–575.

Mohamed, H.R., Sallam, Y.I., el-Leithy A.S., Aly, S.E. Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. *Ann. Agric. Sci.* 2012; 57: 113–116.

Mohan, M., Haider, S.Z., Andola, H.C., Purohit, V.K. Essential oils as green pesticides; for sustainable agriculture. *Res. J. Pharm. Biol. Chem. Sci.* 2011; 2(4): 100–106.

Murugan, V.K., Masthan, M.K., Vediappan, V.K. Potential and controlled repellent activity of microencapsulated citronella oil treated textile cotton fabrics against *Aedes aegypti. Hitek. J. Bio. Sci. Bioengg.* 2012; 1(1): 1–8.

Nazar, S., Ravikumar, S., Prakash, W.G., Syed, A.M., Suganthi, P. Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus. Indian J. Sci. Technol.* 2009; 2: 24–27.

Nidia, A. de. B., Rabson, R.R., Andre von, R. de. A., Marisa, F.M. Extraction of basil oil (*Oscimum basilicum* L.) using supercritical fluid. III iberoamerican Conference on Supercritical Fluids Cartegena de Indias (Columbia). 2013; pp. 1–8.

Nguefack, J., Leth, V., Amvam Zollo, P.H., Mathur, S.B. Evaluation of essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *Int. J. Food Microbiol*. 2004; 94: 329–334.

Nonviho, G., Wotto, V.D., Noudogbessi, J., Avlessi, F., Akogbeto, M., Sohounhloué, D.C. Original research paper insecticidal activities of essential oils extracted from three species of *Poaceae* on *Anopheles Gambiae* Spp., major vector of malaria. *Sci. Study Res.* 2010; 11: 411–420.

Nur Ain, A.H., Zaibunnisa, A.H., Halimahton Zahrah, M.S., and Norashikin, S. An experimental design approach for the extraction of lemongrass (*Cymbopogoan citratus*) oleoresin using pressurised liquid extraction. *Int. Food Res. J.* 2013; 20: 451–455.

Padalia, R.C., Verma, R.S., Chanotiya, C.S., Yadav, A. Chemical fingerprinting of the fragrant volatiles of nineteen Indian cultivars of *Cymbopogon* Sprenz. (Poaceae). *Rec. Nat Prod*. 2011; 5(4): 290–299.

Perez, G.S., Zavala, S.M., Arias, G.L., Ramos, L.M. Anti-inflammatory activity of some essential oils. *J. Essential Oil Res.* 2011; 23: 38–44.

Quintans-Júnior, L.J., Souza, T.T., Leite, B.S., Lessa, M.N., Bonjardim, L.R., Santos, M.R., Alves, P.B., Blank, A.F., Antoniolli, A.R. Phythochemical screening and anticonvulsant activity of *Cymbopogon winterianus* Jowitt (Poaceae) leaf essential oil in rodents. *Phytomedicine* 2008; 15: 619–624.

Ranaweera, S.S., Dayananda, K.R. Mosquito-larvicidal activity of Ceylon citronella (*Cymbopogon nardus* [L.] Rendle) oil fractions. *J. Nat. Sci. Council* 1996; 24: 247–252.

Ranitha, M., Abdurahman, H.N., Ziad, A.S., Azhari, H.N., Thana Raj, S. A comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation and conventional hydrodistillation method. *Int. J. Chem. Eng. Appl.* 2014; 5(2): 104–108.

Ranitha, M., Nour, A.H., Sulaiman, A.Z., Nour, A.H., Thani, R.S. A comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) method. *Int. J. Chem. Eng. Appl.* 2014; 5: 104–108.

Ranjitha, J., Vijiyalakshmi, S. Facile methods for the extraction of essential oil from the plant species—A review. *Int. J. Pharm. Sci. Res.* 2014; 5(4): 1107–1115.

Raybaudi-Massilia, R.M., Rojas-Grau, M.A. Mosqueda-Melgar, J., Martin Belloso, O. Comparative study on essential oils incorporated in to an alginate based edible coating to assure the safety and quality of fresh cut Fuji apples. *J. Food Prot.* 2008; 71: 1150–1161.

Re, L., Barocci, S., Sonnino, S., Menacarelli, A., Vivani, C., Paolucci, G., Scarpantonio, A., Rinaldi, L., Mosca, E. Linalool modifies the nicotinic receptor-ion channel kinetics at the mouse neuro muscular function. *Pharmacol. Res.* 2000; 42: 177–181.

Rollo, C.D., Borden, J.H., Caey, I.B. Endogenously produced repellant from American cockroach (Blattaria: Blattidae) function in death recognition. *Environ. Entomol.* 1995; 24: 116–124.

Selim, S.A. Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass *Cymbopogon proximus stapf. Grasas Aceites* 2011; 62(1): 55–61.

Sforcin, J.M., Amaral, J.T., Fernades, A., Sousa, J.P.B., Bastos, J.K. Lemongrass effects on IL-1β and IL-6 production by macrophages. *Nat. Prod. Res.* 2009; 23: 1511–1519.

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., Mann, A.S. Scientific basis for the therapeutic use of *Cymbopogon citratus* stapf (lemongrass). *J. Adv. Pharm. Technol. Res.* 2011; 2: 3–8.

Shahi, A.K., Sharma, S.N., Tava, A. Composition of *Cymbopogon pendulus* (Nees ex Steud) Wats, an elemicin-rich oil grass grown in Jammu region of India. *J. Essent. Oil Res.* 1997; 9: 561–563.

Shams, K.A., Abdel-Azim, N.S., Saleh, I.A., Hegazy, M.-E.F., El-Missiry, M.M., Hammouda, F.M. Green technology: Economically and environmentally innovative methods for extraction of medicinal & aromatic plants (MAP) in Egypt. *J. Chem. Pharm. Res.* 2015; 7(5): 1050–1074.

Sidibé, L., Chalchat, J.-C., Garry, R.-P., Lacombe, L., Harama, M. Aromatic plants of Mali (IV): Chemical composition of essential oils of *Cymbopogon citratus* (DC.) Stapf and *C. giganteus* (Hochst.) Chiov. *J. Essent. Oil Res.* 2001; 13: 110–112.

Soares, M.O., Vinha, A.F., Barreira, S.V., Coutinho, F., Aires-Goncalves, S., Oliveira, M.B., Pires, P.C., Castro, A. *Cymbopogon citratus* EO antimicrobial activity against multi-drug resistant Gram-positive strains and non-*albicans-Candida* species. *FORMATEX* 2013; 1081–1086.

Sudiarta, P., Sumiartha, K., Antara, N.S. Utilization of essential oil of lemongrass (*Cymbopogon citratus*) as a bio-pesticide to control *Plutella xylostella* (Lepidoptera: Plutellidae). *E-Jurnal Agroeteknologi Tropika* 2013; 2(1): 1–5.

Tangpu, V., Yadav, A.K. Antidiarrhoeal activity of *Cymbopogon citratus* and its main constituent, citral. *Pharmacologyonline* 2006; 2: 290–298.

Taweechaisupapong, S., Ngaonee, P., Patsuk, P., Pitiphat, W., Khunkitti, W. Antibiofilm activity and post antifungal effect of lemongrass oil on clinical *Candida dubliniensis* isolate. *South Afr. J. Bot.* 2012; 78: 37–43.

Taylor, L.T. *Supercritical Fluid Extraction.* John Wiley & Sons, New York, 1996.

Tripathi, A.K., Upadhyay, S., Bhuiyan, M., Bhattacharya, P.R. A review on prospects of essential oils as biopesticide in insect pest management. *J. Pharmacogn. Phytother.* 2009; 1(5): 53–63.

Tzortzakis, N.G., Economakis, C.D. Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key post harvest pathogens. *Innov. Food Sci. Emerg. Technol.* 2007; 8: 253–258.

Vazquez-Briones, M.C., Herandez, L.R., Guerrero-Beltran, J.A. Physicochemical and antioxidant properties of *Cymbopogon citratus* essential oil. *J. Food Res.* 2015; 4(3): 36–45.

Vilkhu, K., Mawson, R., Simons, L., Bates, D. Application and opportunities for ultrasound assisted extraction in food industry: A review. *Innov. Food Sci. Emerg. Technol.* 2008; 9: 161–169.

Wany, A., Jha, S., Nigam, V.K., Pandey, D.V. Chemical analysis and therapeutic uses of citronella oil from *Cymbopogon winterianus*: A short review. *Int. J. Adv. Res.* 2013; 1: 504–521.

Wei, L.S., Wee, W. Chemical composition and antimicrobial activity of *Cymbopogon nardus* citronella essential oil against systemic bacteria of aquatic animals. *Iran. J. Microbiol.* 2013; 5: 147–152.

Wijesekara, R.O.B. The chemical composition and analysis of Citronella oil. *J. Natl. Sci. Counc. Srilanka*. 1973; 1: 67–81.

William B.J. *Journal of Chemical Education*. 2007; 84(12): 1913.

Wuthi-udomlert, M., Chotipatoomwan, P., Panyadee, S., Gritsanapan, W. Inhibitory effect of formulated lemongrass shampoo on *Malassezia furfur*: A yeast associated with dandruff. *Southeast Asian J. Trop. Med. Public Health* 2011; 42(2): 363–369.