Chapter

Cymbopogon Nardus Essential Oils: Extraction, Chemical Composition and Biological Activities

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Abstract

Essential oils (EOs) have attracted considerable scientific interest due to their ecological role in plants and their broad biological applications. This chapter presents an overview of extraction techniques, chemical composition, and biological properties of EOs, with emphasis on *Cymbopogon nardus* (L.) Rendle. Conventional and modern extraction methods are discussed, highlighting factors that influence oil yield and quality. The genus *Cymbopogon* is recognized for its medicinal relevance and high EO content, particularly in monoterpenes such as citronellal, citronellol, and geraniol, which are responsible for diverse biological activities. The chemical profile of *C. nardus* oil underpins its applications in pharmaceutical, cosmetic, and agricultural industries. Its biological potential includes antioxidant, antimicrobial, and insecticidal properties, which reinforce its value as a natural alternative to synthetic products. The compilation of studies presented in this chapter demonstrates that *C. nardus* EO is a promising candidate for environmentally friendly applications and human health protection.

Keywords: essential oils, *Cymbopogon nardus*, extraction methods, monoterpenes, antioxidant activity, antimicrobial activity, insecticidal potential

1. Introduction

In recent decades, the study of essential oils (EOs) has garnered mounting attention due to their therapeutic properties and industrial applications. These volatile, aromatic compounds are synthesized from various plant parts, including flowers, leaves, seeds, and roots. These substances are the result of secondary

metabolism. In contrast to primary metabolites, which are directly involved in plant growth and development, secondary metabolites, such as EOs, have adaptive functions. As demonstrated in the literature, these functions include defense against herbivores and pathogens, as well as the attraction of pollinators [1–4].

EOs are natural, liquid, and highly volatile substances. EOs are produced and stored in specialized structures called secretory cavities, glandular trichomes, and idioblasts. These structures are located in plant organs such as leaves and flowers, and they play a fundamental ecological role for the plant [5, 6]. EOs have a complex chemical composition consisting mainly of low-molecular-weight volatile organic substances and may contain more than 300 different compounds, such as terpenes and phenylpropanoids [7]. The concentrations of these compounds usually depend on the specific characteristics of each plant.

The terpene class constitutes the most substantial and varied category of natural organic compounds, with a total of 80,000 terpenoids identified to date [1, 8]. Terpenes fulfill pivotal biological functions in living organisms, including defense against biotic and abiotic stresses. These compounds are classified as cyclic or acyclic hydrocarbons, with some exhibiting oxygenation [9]. The classification of these compounds is determined by the number of isoprene units (C_5H_8) and the length of the carbon chains [10]. The chemical composition of these substances varies according to the number of isoprene units in their structure. Monoterpenes, with 10 carbon atoms ($C_{10}H_{16}$), and sesquiterpenes, with 15 carbon atoms ($C_{15}H_{24}$), are the main constituents [11, 12].

During the process of obtaining EOs, a variety of factors have the capacity to impact their chemical composition, which, in turn, affects their properties. The variability of these factors, both intrinsic and extrinsic, directly influences the yield and quality of the oil. Among the intrinsic factors, the interaction between species and environmental conditions, such as the time of collection and water stress, stands out. Extrinsic factors, such as the extraction method, precipitation conditions, and postharvest care, have been identified as influential elements in this regard. These factors have the potential to influence not only the composition of EO but also its efficacy [5, 13–15].

The EOs have demonstrated applications in various domains due to their biological activities, including antioxidant, insecticidal, antimicrobial, and antifungal properties [16, 17]. These oils have a variety of applications, including their use as natural food preservatives, in aromatherapy for the treatment of depression and the improvement of sleep quality, and in agriculture as an alternative to synthetic products for mosquito control [18–20].

2. Extraction methods

The production of EOs is a complex process whose efficiency depends on numerous factors, such as the handling of the plant material, increased control and quality of equipment, and improved extraction methods, especially techniques with lower energy, solvent, and time costs [21]. These extraction techniques are classified into two categories: traditional (conventional) and modern [22].

Conventional methods for EO extraction involve the use of water distillation by heating, such as hydrodistillation and steam distillation. Conversely, innovative

techniques are predicated on enhancing the extraction process with respect to time criteria for isolating EOs, as well as the yield and quality of these EOs. Among these techniques are supercritical fluid extraction and solvent-free microwave extraction (SFME) [23].

2.1 Hydrodistillation

Hydrodistillation is recognized as one of the most widely used techniques for extracting EOs. In this method, the plant material is immersed in water and brought to a boil, usually at atmospheric pressure. The steam generated carries the volatile compounds and is cooled in a condenser, thus separating the EO from the water. A notable benefit of this approach is that the distillation process takes place at relatively low temperatures, a factor that aids in the preservation of compounds that are particularly sensitive to heat. Despite its prevalence as a technique for distilling botanical material, the method has certain limitations. These include the necessity for greater time and water input, in addition to extended heating of the plant material, which can result in the generation of an undesirable odor in the EO. Moreover, prolonged exposure to boiling water can trigger chemical reactions, such as ester hydrolysis, which involves the transformation of alcohols and acids [24, 25].

2.2 Steam distillation

Steam distillation extraction is a method employed for the extraction of EOs from plant material. This method is regarded as a straightforward and financially feasible process, with the added benefit of not employing toxic solvents that could potentially harm the environment [26]. The duration of distillation can vary from one to 10 hours, depending on factors such as the type of plant, the temperature applied, the exposure time to steam, and the pressure conditions [27]. In this method, the plant material is exposed directly to steam, causing the heat from the steam to break down the plant cells, thereby allowing the release of volatile compounds. Subsequently, the steam is condensed, yielding water and EO. In conclusion, the mixture of EO and water is separated [28].

2.3 Supercritical fluid extraction

This technique is one of the most innovative and sustainable, providing protection to the environment. Furthermore, this method has demonstrated its efficacy in the extraction of EOs from diverse plant matrices. The use of low temperatures during the extraction process ensures the preservation of the active compounds present in EOs, a feature that distinguishes this method from conventional extraction processes. The gas most commonly used in this method is carbon dioxide (CO_2), which is inert and reduces the pressure at the extractor outlet during its elimination [29]. This gas possesses a number of advantageous properties, including its capacity to reach the critical point (with a low critical pressure of 72.9 atm and temperature of 31.2°C) with ease. It is also nonaggressive to plant essence molecules, nonflammable, and available in high purity at a relatively low cost. Additionally, it exhibits a polarity similar to pentane, making it suitable for the extraction of lipophilic compounds [23].

2.4 SFME

This extraction method is regarded as innovative, clean, and environmentally friendly. It is employed for the purpose of obtaining EOs from herbs or aromatic plants. The isolation and concentration of volatile compounds are carried out in a single stage [30]. In addition to being regarded as an environmentally sustainable method, this technique has been shown to yield high levels of EOs while exhibiting selectivity and a reduced extraction time. This is achieved by integrating two techniques: first, microwaves are used to heat the samples, and then atmospheric pressure dry distillation is employed, without the need for solvents [23]. The utilization of these techniques offers several key benefits, including a reduction in energy consumption, a decrease in extraction time, and the elimination of hazardous solvents. At SFME, the chemical constituents of interest are extracted using microwaves that heat and extract the EO from the plant material [31].

In the context of the extraction of the EO from the *Cymbopogon nardus* plant, studies show that the above extraction techniques directly influence the quality of this EO. In addition, there are no statements about the best method used to obtain the volatiles of *C. nardus*. However, it should be noted that extraction methods involving heating can favor the triggering of significant thermal degradations or the hydrolysis of thermosensitive constituents.

3. Genus *Cymbopogon*

The genus *Cymbopogon*, which is part of the Poaceae family, comprises approximately 180 species. These species are native to the tropical and subtropical regions of Asia. Presently, they are cultivated in countries in South and Central America, Africa, and other tropical regions [32].

In Brazil, the genus comprises eight recognized species and 10 synonyms, including *C. caesius*, *C. citratus*, *C. densiflorus*, *C. flexuosus*, *C. iwarancusa*, *C. marginatus*, *C. martini*, *C. natus*, *C. schoenanthus*, and *C. winterianus*. The species of the genus were introduced to Brazil and currently have geographical distributions across the North, Northeast, Midwest, Southeast, and South regions [33].

The species of the genus *Cymbopogon* have been observed to employ a variety of reproductive strategies, including vegetative propagation by means of seeds or rhizomes. However, the process of reproduction by seeds is less efficient due to the rarity of flowering and fruiting in many species. The method of reproduction is contingent upon the species and environmental conditions. The genus's capacity for versatile reproduction has been demonstrated to contribute to its wide distribution and facilitate cultivation [32]. *Cymbopogon* species have been observed to interact with other plants in the environment, engaging in competition for resources such as nutrients, light, and water. Furthermore, they are utilized as livestock feed in agroecological systems [34].

Cymbopogon, a genus of significant medicinal importance, is employed in traditional medicine for the treatment of various ailments. In Brazil, the leaves of *C. marginatus* are utilized in the treatment of epilepsy and anxiety [35]. In East Asia, the leaves of *C. citratus* and *C. flexuosus* have been utilized by traditional peoples for the treatment of digestive problems and fevers [36, 37]. In Egypt, *C. schoenanthus* is utilized in traditional medicine [38]. These traditional applications reflect the

empirical knowledge of traditional peoples in different countries, demonstrating the importance of the genus in folk medicine [39].

The genus *Cymbopogon* is distinguished by its capacity to produce substantial amounts of EO in its secondary metabolism, a characteristic that confers upon it significant commercial value. These oils find application in the cosmetic and pharmaceutical industries, as well as in biological control practices [40, 41]. The EOs of the genus are characterized by a high content of terpenes, including hydrocarbons and oxygenated monoterpenes, such as citral, geraniol, myrcene, limonene, and linalool [42–44]. On the other hand, the minority compounds present in the EO, such as β-myrcene, α-terpinolene, 4-terpineol, and other compounds, through synergism, can enhance its application and pharmacological potential.

Finally, it is important to note that the amount and content of volatile compounds present in the EO of the genus *Cymbopogon* during the extraction process are impacted by several factors, both biotic, such as stress caused by fungi and bacteria, and abiotic, such as temperature, soil type, plant nutrition, and sunlight.

4. *Cymbopogon nardus* (L.) Rendle

Cymbopogon nardus (L.) Rendle, commonly known as lemongrass, is a species widely distributed throughout tropical and subtropical regions globally. This species is native to South Asia, particularly India and Sri Lanka, and it has demonstrated a high degree of adaptability to diverse climatic conditions, a factor that has contributed to its successful dispersal and establishment in a variety of environments [45, 46].

C. nardus is cultivated in various regions worldwide, including South America, Africa, India, and China, due to the high commercial interest in its EO, which is produced in the plant's secretory cavities. These specialized structures have been shown to be responsible for both the synthesis and storage of EOs [47, 48].

The species *C. nardus* is notable for its economic potential in industries such as pharmaceuticals, cosmetics, and agriculture. The chemical composition of the oil is widely recognized for the presence of monoterpenes, such as citronellal, geraniol, and citronellol (**Figure 1**). These monoterpenes have been shown to confer antimicrobial, antifungal, antiinflammatory, and insect-repellent properties [2, 47, 49, 50].

Despite the prevalence of monoterpenes in *C. nardus* EO, sesquiterpenes, encompassing both oxygenated and hydrocarbon compounds, are present in trace

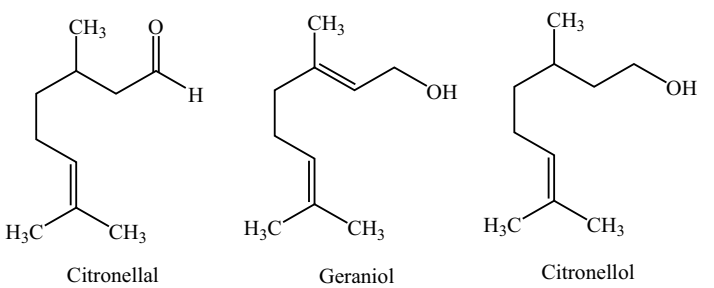


Figure 1.
Two-dimensional structures of citronellal, geraniol, and citronellol.

amounts within the EO of this species. The minimal concentration of these compounds in *C. nardus* oil indicates that their contributions to the oil's overall biological properties are comparatively negligible when compared to monoterpenes [49–52].

The prevalence of oxygenated monoterpenes, predominantly geraniol and citronellal, distinguishes *C. nardus* EO as a promising candidate for applications involving insect repellency, antimicrobial, and antioxidant activities. However, the presence of low concentrations of sesquiterpenes may impose limitations on the utilization of the EO in contexts requiring biological activities associated with these compounds, such as sustained antiinflammatory effects. This chemical profile renders *C. nardus* oil particularly well-suited for formulations aimed at environmental protection and human health.

5. Antioxidant activity

Plants have been identified as a promising source of molecules with antioxidant properties. In this context, the antioxidant activity of EOs can serve as a natural alternative to synthetic antioxidants. EOs are natural, nontoxic, and have fewer side effects, whereas synthetic antioxidants can pose potential risks to human health. Antioxidants are defined as substances with the ability to delay or prevent oxidative damage by neutralizing free radicals. Free radicals are unstable molecules that cause damage to cells and DNA, which can lead to degenerative diseases in the body [53, 54].

There are several methods employed to assess the antioxidant capacity of a substance. The $ABTS^+$ assay is a particularly noteworthy method that is used to assess the antioxidant capacity of different substances based on the neutralization of the stable radical cation ($ABTS^+$). The $ABTS^+$ radical is distinguished by its blue-green hue and demonstrates efficacy for both hydrophilic and lipophilic compounds. The $DPPH^•$ test is predicated on the neutralization of the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^•$) by electron donation, where the color change indicates antioxidant activity [55].

The antioxidant activity of *C. nardus* EO was examined using two radical-based assays: $DPPH^•$ and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$). The findings of the study demonstrated that the EO manifested antioxidant properties by impeding the action of $DPPH^•$ radicals and the $ABTS^+$ radical cation. In the $DPPH^•$ assay, the results indicated 102.19 ± 4.2 μg extract/μg compared to 0.11 ± 0.04 μg for gallic acid, which was used as a control. In the $ABTS^+$ assay, EO demonstrated activity of 0.009 ± 0.0004 μM TE/g, in comparison to 2.66 ± 0.31 μM TE/g for the control group. The authors attribute the antioxidant potential to the presence of geraniol, with a concentration of 28.40% in the oil. Conversely, the EO exhibited reduced activity in comparison to gallic acid, which was utilized as a standard reference [50].

The antioxidant potential of *C. nardus* oil was investigated using the $DPPH^•$ method. The study's findings indicated that the oil exhibited a high antioxidant capacity, with values reaching 93.86 μg TEAC/mL. This outcome may be associated with the bioactivity of compounds such as geraniol and limonene [56].

The difference observed in the results of the oxidizing activity of these EOs is related to the different methods and the mediums in which they react, for example,

ethanol for the DPPH assay and phosphate-buffered saline for the ABTS assay. In addition, there is another factor, such as reaction time (5 minutes for the ABTS assay and 30 minutes for the DPPH assay).

6. Biological potential

6.1 Insecticide and toxicity

The term "insecticidal activity" refers to the capacity of certain compounds, including EOs, to repel, control, or eliminate insects. This activity offers effective solutions for pest control in agriculture and can help combat insect resistance to synthetic insecticides. These compounds are generally nontoxic to mammals and act as fumigants and repellents. Consequently, EOs have emerged as a promising alternative for the development of safer insecticides. A notable example is *Cymbopogon nardus* oil, which contains active compounds such as geraniol, citronellal, and limonene. These compounds have the capacity to interfere with the neural pathways of insects through various mechanisms of action, making it a highly effective insecticide [2, 57–60].

Boonyuan et al. [61] evaluated the insecticidal potential of the EO from *C. nardus* leaves against the *Aedes aegypti* mosquito. The researchers found a median lethal concentration (LC_{50}) of 4.02%, indicating low toxicity of the oil. This result suggests that *C. nardus* is less effective at killing mosquitoes compared to other EOs tested, such as *C. citratus* oil.

In a related study, the larvicidal activity of *C. nardus* EO was examined against *A. aegypti* larvae, demonstrating effective larvicidal action with an LC_{50} of 75.85 mg/mL. The chemical composition of the EO was characterized by citronellal (21.8%), citronellol (18.1%), and geraniol (11.3%) [62]. Studies by Manimaran et al. [63] and Kurniasih et al. [64] demonstrated that *C. nardus* EOs exhibited toxicity against *A. aegypti* larvae at 47.21 mg/L and 35.133 mg/L, respectively, over a 24-hour period. Consequently, the utilization of EO as an alternative to pesticides is a viable proposition.

The insecticidal potential of *C. nardus* EO was investigated against larvae of two genotypes of *Anopheles gambiae*, a mosquito that hosts and transmits malaria [65]. The EO exhibited high larvicidal activity, with LC_{50} values of 36.83 mg/L and 97.33 mg/L in two distinct strains. According to the authors, citronellal (37.87%) and nerol (19.88%) may be responsible for the larvicidal activity of the oil [65].

The insecticidal and repellent activity of the EO was evaluated against two insect pests, *Oryzaephilus surinamensis* (L.) and *Sitophilus zeamais* Motsch. The repellent activity values after four hours of exposure were determined to be LC_{50} = 0.03 μL/cm^2 (*O. surinamensis*) and LC_{50} = 0.04 μL/cm^2 (*S. zeamais*). For fumigant activity, the EO of *C. nardus* ranged from 46.9 to >604 μL/L. Although the EO of *C. nardus* exhibited low toxicity in relation to fumigant activity against the two insect pests, it was observed that it has repellent action [49]. Loko et al. [66] evaluated the repellent activity of *C. nardus* EO against *Dinoderus porcellus*, and the results demonstrated that even low concentrations of the EO can act as a repellent.

The activity of EO against *Artemia salina* larvae was examined, revealing that the oil exhibited no toxicity, with LC_{50} values greater than 1,000 μg/mL. The oil contained concentrations of citronellal (27.34%), geraniol (23.21%), and geranial

(13.37%) [67]. In contrast, in the study by Cansian et al. [68], citronella EO was evaluated against *A. aegypti* and *A. salina* larvae. The activity values were LC_{50} = 111.84 μg/mL and LC_{50} = 5.29 μg/mL, respectively, with concentrations of citronellal (38.98%), geraniol (20.56%), and citronellol (17.54%).

6.2 Antimicrobial

The antimicrobial activity of EOs has been highlighted in several studies due to their potential to combat the action of microorganisms. These oils contain complex bioactive compounds that possess antifungal and antibacterial properties. The antibacterial efficacy of essential oils can vary depending on the plant from which they are derived and the type of target bacteria, being influenced by the chemical structure of the compounds present, their functional groups, and synergistic interactions. Research indicates that the primary antimicrobial action occurs through the inhibition of cell growth or the death of bacterial cells. Furthermore, these oils have been the focus of increased research as potential alternatives to combat bacterial resistance, demonstrating their value in the development of novel treatments [54, 69, 70].

The antifungal activity of *C. nardus* EO was evaluated against *Candida albicans* in vitro and in vivo, with the minimum inhibitory concentration (MIC) determined using the microdilution technique. The oil was found to contain citronellal (27.34%), geraniol (23.21%), and geranial (13.37%), which are known to exhibit antifungal activity. The MIC values for the standard strain (CAATCC) and the clinical strain (CAV) were determined to be 500 μg/mL and > 500 μg/mL, respectively. The oil's efficacy was demonstrated in an in vivo study [67]. Prado et al. [71] evaluated the antifungal activity of an EO from *C. nardus* with a high citronellal (44.44%) and citronellol (16.97%) content against *C. albicans*, and the substance demonstrated efficacy against two strains (planktonic and biofilm). The authors reported that the EO contains constituents that aid in the antifungal effect and that it is an alternative for the control of fungal infections.

The EO of *Cymbopogon nardus*, collected in Piraquara, Brazil, was evaluated for its antifungal potential *in vitro* against mycotoxin-producing strains of *Aspergillus niger*, *A. nomius*, *A. flavus*, and *Fusarium graminearum*. The EO, which is abundant in geraniol (35.52%), citronellal (24.39%), and citronellol (10.73%), demonstrated antimicrobial activity against all fungi tested through contact and volatilization methods, with MIC values of 0.75%, 0.75%, 0.5%, and 0.25% [72].

A number of studies have demonstrated the antibacterial properties of *C. nardus* EO in combating pathogens. The antibacterial activity of *C. nardus* EO was evaluated against *Escherichia coli* strains, with MIC determined using the microdilution technique. The oil exhibited no substantial effects against the 22 *E. coli* strains, with MIC of ≥6,400 μg/mL [73]. As demonstrated in studies conducted by Millezi et al. [74], the oil exhibited antibacterial properties against *Staphylococcus aureus* and *E. coli* bacteria. This effect was attributed to the presence of elevated concentrations of citronellal.

In other studies, the EO was evaluated for its antibacterial action against 10 species of *Pseudomonas* isolated from freshwater fish. Utilizing disc diffusion and MIC methodologies, the oil exhibited favorable properties for impeding the proliferation of these species, which are frequently linked with the deterioration of foodstuffs such as fish [56].

7. Conclusions

This chapter has therefore presented a significant compilation of information about EOs, also demonstrating the main techniques for obtaining them. The chapter has considered both conventional methods, such as hydrodistillation and steam distillation, and modern methods, such as supercritical fluid extraction and microwave extraction. Furthermore, the text underscores the significance of the genus *Cymbopogon* and the species *Cymbopogon nardus* in the production of EO, a matrix distinguished by the presence of oxygenated monoterpenes, including citronellal, geraniol, and citronellol. Moreover, it elucidates numerous pertinent studies that document the antioxidant potential of *C. nardus* EO, along with its antimicrobial and insecticidal properties.

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