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In Vitro Antimicrobial Activity of Essential Oils from *Cymbopogan* against *Cutibacterium acnes*

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Abstract
Acne is a chronic, common disease that poses a significant therapeutic, psychological and social problem. Cutibacterium acnes (C. acnes) is an anaerobic Gram-positive bacterium generally considered as a human skin commensal, but is also involved in different infections, such as acne and surgical infections. Although there are a variety of treatments, the side effects and the problem of bacterial drug resistance still limit their clinical usage.The study was to evaluate the antibacterial activity of essential oils (EOs) against Cutibacterium acnes and to analyze the chemical compositions of the effective oils. The experiment was conducted using a completely randomized design with duplications. The EOs obtained from Cymbopogan citratus and Cymbopogan nardus (Lemon grass) by hydro-distillation process, cultivated in North eastern Indian State Assam were tested for antibacterial activity using an agar well diffusion assay and the macro-dilution method.The results showed that both species essential oil distilled from hrdro-distillation method had the highest potent inhibitory zone on C. acnes, according to the findings. As a bactericidal, essential oil of both species of Cymbopogan citratus and Cymbopogan nardus had MIC and MBC values of 0.47 and 0.69 mg/mL, and even a MIC index (MBC/MIC) of Citral (52.16%), geranial (30.28%), neral (26.53%), caryophyllene-oxide (4.9%) geraniol (4.1%), linalool (2.6%) and limonene(2.40%) were the major components of lemon grass oil. The results suggest that lemon grass oil might be used to develop topical anti-acne treatments.These observations suggest the potential application of this synergy in the development of innovative topical formulations useful in the management of acne.

Keywords: *Cutibacterium acnes; essential oils; antibacterial activity; lemon grass*

Introduction

Skin diseases are a significant therapeutic, psychological and social problem nowadays. The importance of these conditions is regularly underestimated due to their chronic nature and low mortality, but their frequency can be very high, with estimates ranging from 21% to 87% of the population suffering from some skin disease. They account for a quarter of consultations in primary care due to the significant physical and mental impairments associated with it [1]. The most common skin disease is Acne vulgaris, which affects 80% of the teenage population; it is even believed that 100% of the population suffered from a more or less severe form of this disease at various times in their lives [2]. Acne is a chronic inflammatory disease, and its pathogenesis is very complex and multifactorial; there are no strict guidelines that would define specific reasons for the occurrence of this skin disease. The development of acne is mainly due to increased sebum production, increased proliferation and decreased shedding of keratinocytes, inflammation, and hyper-colonization of *Cutibacterium acnes* (formerly *Propionibacterium acnes*) [3,4].

Cutibacterium acnes (C. acnes) is considered to reside in the sebaceous glands of the skin, and plays an important role in maintaining skin pH by decomposing skin triglycerides and producing fatty acids [5, 6]. However, it is also a conditional pathogenic bacterium that is able to cause invasive infection of organs and tissues under specific cases, such as surgery or trauma [7]. In addition, studies have shown that C. acnes is one of the main culprits of acne [8]. As one of the most common skin diseases with high incidence in young

adulthood, acne can cause physical discomfort, face skin defects, or disfigurement as the long-term chronic inflammations persist. It will even cause a tremendous psychological burden to the patients, causing anxiety and even severe mental illness [9-11].

In the past, the antibiotics, such as macrolides, clindamycin, and tetracyclines, were the most common medications prescribed for acne [12]. Although they are still largely active against the majority of C. acnes, the emerging of drug resistance becomes an urgent problem (Leyden, 1976) [13]; therefore, it is not appropriate to treat acne with a single antibiotic. Instead, a variety of antibiotics or the combination of antibiotics and other drugs are used, but there are still many problems with these drugs in clinical use, like irritation of the skin and mucous membranes [14-17]. In recent years, more and more attention has been paid to natural products derived from plants due to their higher efficacy and lower toxicity, which can replace or assist antimicrobial agents in inhibiting the proliferation of C. acnes [18-20]. Accumulating evidence showed that many essential oils from plants such as cloves, cactus, tea tree, and so on possessed an inhibitory effect on C. acnes [21-24]. However, there is a lack of research on the mechanism underlying the antibacterial activity.

Essential oils (EO) are produced from plant derivatives and contain between 20 and 60 constituents, the most being part of the terpene family such as hydrocarbons or oxygenated derivatives, esters, and phenols. Research has shown that essential oils have an antimicrobial and antioxidant effect and are often used in alternative medicine [25].The antimicrobial activity of essential oils has also been extensively studied in late years and it has been shown that some essential oils can inhibit the growth and multiplication of antibiotic-resistant pathogenic microorganisms.Due to their antimicrobial activity, essential oils might be considered an alternative to antibiotic treatment.

Cymbopogon citrates (DC.) Stapf, commonly known as lemongrass, is a coarse grass with a strong lemon taste. It is a perennial herb widely cultivated in the tropics and subtropics [26]. Researchers have found that lemongrass holds antidepressant, antioxidant, antiseptic, astringent, nervine and sedative properties [27]. Furthermore, the antibacterial activity of lemongrass oil against a diverse range of species comprising Gram-positive and Gram-negative organisms, along with yeast and fungi, was formerly established [28-30]. The major component of lemongrass EO is citral, consisting of the two geometric isomers citral A (geranial) and citral B (neral) [31]. Other compounds identified in lemongrass EO include limonene, citronellal, -myrcene and geraniol [32], along with trace compounds [33]. On the compound level, citral, the major component of lemongrass EO, is known to be antibacterial, but other compounds have not yet been studied in detail from this perspective. Furthermore, an earlier study has also reported about the capacity of thyme EO, cinnamon EO and lemongrass EO to reduce viable spores [34], which could be an important feature of these EOs, as B. thuringiensis, one aetiological agent of pitted keratolysis, is a typicalspore-forming bacterium species, and spore fomation can influence survival and therefore therapeutic success [35].

The objectives of the study were to evaluate the in vitro antibacterial activity against C. acnes from the essential oils derived from two species of *Cymbopogan citratus* and *Cymbopogan nardus* cultivated in Indian North-estern state Assam. Furthermore, the minimum inhibitory concentration of the most effective oil from lemon grass plant cultivars in vitro activity is a potential new basic guideline for acne therapies in terms of lotions, solutions, and washes.

Materials and Methods

Plant Materials

The leaves of *Cymbopogan citratus* and *Cymbopogan nardus* were collected from East Siang, Arunachal Pradesh India, in 2022. All plants tested were identified and authenticated by the Prof. Gireesh Chand, College of Agriculture (Central Agricultural University), East Siang, Arunachal Pradesh, India.

Hydro-distillation

Three (3.0) kg of each plant was cleaned and cut into small pieces before even being packed into a round-bottom flask in a Clevenger-type apparatus for 3 h separately. Following that, the oil was extracted with water, filtered, and the droplets were removed with sodium sulfate anhydrous. A rotary evaporator was then used to condense the oil. After that, each essential oil was weighed and placed in a fraud paper-wrapped

container for oxidation resistance (kept at 0–4 °C) (Dziri et al. 2014) [36].

Bacterial Strain and standard component

C. acnes were supplied by the Faculty of Pharmaceutical Science, Apex Professional University, Arunachal Pradesh, India. Citral (95 %), geranial and neral mixture; were purchased from Yucaa laboratories Private Limited, Mumbai.

Antibacterial Assay

The antibacterial activity was conducted using the agar diffusion assay [37]. Briefly, the bacterial inoculum was maintained in nutrient agar (NA) at 37 °C for 24 h in anaerobic conditions. After that, 2–3 colonies were chosen for transferring to be cultured in nutrient broth (NB) under anaerobic conditions at 37 °C for 24 h, and the number of cells was counted at an absorbance of 595 nm of 1.5 x 108 CFU/mL (McFarland No. 0.5) standardization and adjusted by 0.85% w/v sterile sodium chloride solution. The NAs (19 mL for each tube) had been melted and combined with the adjusted cell suspension (1 mL) and then poured onto the test plates, allowing them to set. The test plates were made with a 5-mm cork borer and loaded with 55 µL of essetial oils of *Cymbopogan* with a final concentration of 10 mg/mL (dissolved in 10% Dimethyl Sulfoxide, DMSO) in such wells, compared with 10% DMSO (negative control) and 1 mg/mL erythromycin (positive control). The plates were incubated in anaerobic conditions overnight at 37 °C, and the inhibition zones were measured.

Determination of the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The NCCL's procedure was used to assess the susceptibility of macro-dilution broth. The chosen oil was dissolved in 10% DMSO. Serial dilutions of the isolated both species of *Cymbopogan* essential oils, from 100 mg/mL to 0.00 mg/mL were generated 10-fold in 10 experiment tubes with sterile NBs (1 mL) as diluents. Each dilution was piped with 1 mL of test bacteria at the standard concentration (1.5 x 108 CFU/mL). The 1% erythromycin and the DMSO (10%) were employed as positive and negative controls, respectively. The conduits were incubated at 37 °C for 24 h. The MIC was comprised of the lowest oil extract concentration, which demonstrated no significant visible growth. After 24 h of incubation, the MIC values were determined. Duplicates of the bacterium were carried out, even with results expressed as quantitative variables. Each MIC broth's test media (10 mL) was disseminated over NB panels. The plates were incubated in anaerobic conditions for 24 h at 37 °C. A MBC of the extract that indicated no bacterial growth in agar plates was observed (Singh *et al.* 2011). The MIC indices were calculated using MBC/MBC values to indicate the mode of action, including bactericidal (MIC index < 4) and bacteriostatic (MIC index ≥ 4) [39].

Statistical Analysis

The data set was analyzed using the SPSS software for Windows version 11.0. A comparison with the DMRT was performed, and significance was observed at the $p<0.05$ level. The experiment was designed as a general linear model with duplication within a completely randomized design.

Results

Percentage Yields

The percentage yield (%w/w) of distilled essential oils of both species of *Cymbopogan citratus* and *Cymbopogan nardus* gave 1.02, and 1.07, respectively, are represented in Table 1.

Table 1. Yields of isolated essential oils.

Plant species	% yield	Color Characteristics
Cymbopogan citratus	1.02	Light yellow color
Cymbopogan nardus	1.07	Light yellow color

Physical-chemical parameters of isolated essential oils of lemon grass

The physical-chemical properties of the investigated essential oils are used as quality parameters according to

recommendations established by the Indian Pharmacopoeia and International Standards. Physical-chemical parameters of the investigated essential oils are presented in Table 2.

Table 2. Physical-chemical properties of obtained essential oils

Physico chemical parameters	*Cymbopogan citratus*	*Cymbopogan nardus*
Relative density [g/cm3]	0.92	0.91
Refractive index, ʎ [nm]	1.456	1.458

Antibacterial Activity

The antibacterial activity of essential oils performed against *C. acnes* is presented in Table 2. The size of inhibition zones to indicate relative antibacterial activity is not typically quantitative and sufficient. Both species of *Cymbopogan* essential oil at 10 mg/mL displayed the significantly highest inhibition zone, followed by gardenia oil and hydechium rhizome oil. Therefore, the garlic oil was selected for the MIC/MBC values and subjected to chemical constituents. The MIC values were determined in NB using the macro dilution technique. The garlic oil and 1% erythromycin had MIC (MBC) values of 0.39 (0.78) and 0.01 (0.01) mg/mL, respectively (Table 3). Due to its bactericidal properties, garlic oil was suitable for subjecting to phytochemical investigations.

Table 3. Antibacterial activity of essential oils isolated from *Cymbopogan* against *C. acnes*

Samples (10 mg/ml)	Inhibition zone (mm[a]±SD)
Cymbopogan citratus	62.49±2.90
Cymbopogan nardus	57.25±3.73
10% DMSO(-Ve control)	-
1% erythromycin (+Ve control)	37.79±3.07

Values for inhibition zones are represented as mean ± SD ofduplications and mean values with different superscript letters in each column are significantly different (p <0.05, DMRT.

Table 4. MIC and MBC plus MIC index of essential oils isolated from *Cymbopogan* against *C. acnes*

Treatment	Concentration (ml)			
	MIC	MBC	MIC Indices	Indication
Cymbopogan citratus	0.49	0.81	2	Bactericidal
Cymbopogan nardus	0.43	0.79	2	Bactericidal
1% erythromycin	>0.01	>0.01	1	Bactericidal

Indication based on the MIC indices – MBC/MIC; bactericidal – MIC indices < 4 and bacteriostatic ≥ 4.

DISCUSSION

Plants produce a large and diverse array of organic compounds thatappear to have no direct function in growth and development, and these are named as secondary metabolites. They differ from primary metabolites of plants such as chlorophyll, amino acids, nucleotides, simple carbohydrates etc. in having a restricted distribution in the plant kingdom. Plant secondary metabolites can be divided mainly into three chemically distinct groups: Terpenes, Phenolic compounds and Nitrogen-containingsecondary products, such as alkaloids, which are primarily biosynthesized from amino acids. The biological properties displayed by plants have been attributed to their ability to synthesize such compounds [40]. The ability of these compounds to form complexes with certain enzymes or directly inhibit enzymes, toxic effects on membrane structure and integrity, quenching of free radicals, stimulation of natural killer cells in the humans, modulation of steroid concentrations etc. could be among important mechanisms underlying their biological activity. However, quite often, exact mechanisms in particular cases remain to be clarified [41-43]. One of the important biological properties of these essential

oils is their antimicrobial nature, and in many cases this activity was found to be due to the presence of active monoterpene constituents [44]. Another property of the plant products that is generating considerable interest is their ability to control plant diseases. Till now chemical control remains the main measure to reduce the incidence of plant diseases. Two serious problems, development of resistance by plant pathogenic fungi & bacteria and the presence of high level toxic residues in agricultural products, hamper against the effective use of the chemical fungicides and bactericides in controlling plant pathogenic microbes.Acne is a common chronic inflammatory skin disease with a high occurrence rate. If it is not treated properly, it is easy to leave scars, which will have a serious detrimental influence on the quality of life and psychosocial function of patients. Antibiotics are a successful option for treating acne in the case of microbial infections. However, the prolonged and widespread adoption of antibiotics has ultimately caused a high level of drug resistance globally [45]. And a rising number of studies have reported resistance to a variety of antibiotics in C. acnes, which limits its use as a therapy [46, 47]. In order to avoid drug resistance, natural extracts, including essential oils and total flavonoids, have been widely used in many cosmetics or supplements to promote acne in recent years [48, 49]. As herbal skincare has a long history in skincare, it gains more and more attention and recognition.

In the present study, we have studied the antimicrobial properties of essential oils isolated from the two species of Cymbopogon, and their major components. Initially we have screened the antimicrobial activity of the test essential oils and their major components using paper disc assays. However, using the size of inhibition zone to indicate relative antimicrobial activity of essential oil and its constituents is not adequate. The zone of inhibition may be altered by the solubility and rate of diffusion of the test compounds in agar medium. Moreover, evaporation of the essential oils and their constituents can affect the doses applied to paper discs and thus the results. Hence we have determined the relative antimicrobial activity of the testcompounds using liquid culture assay by determining their minimum inhibitory concentrations [50]. Based on paper disc assay the most effective antibacterial essential oil is lemongrass.Among the three major components, citral showed the best antibacterial activity. The results from the disc diffusion assay and liquid culture assay indicate that citral could be the main antibacterial component ofthe lemon grass essential oil.After establishing the antibacterial activity and time course of lethal action of the essential oils and the major components, their activity against the drug resistant pathogenic bacteria isolated from human patients was studied. As expected lemon grass oil was found to be the most potent against all the pathogenic drug resistant bacteria tested. However the studies clearly demonstrate antibacterial properties of some of the test compounds and suggest their potential use as chemotherapeutic agents, disinfectants or food preservative.

Conclusions

The antimicrobial effects of essential oils distilled from the leaves of two species of lemon grass were confirmed on a group of bacteria responsible for the superficial skin infection. Lemongrass proved to be the most effective EO as it was most able to actively inhibit spore formation. From among the compounds, the antibacterial effect of citral was confirmed on all the known aetiological agents, geranial, and neralwas revealed to possess a similar effect. Results of this study have demonstrated the potential of therapeutics containing lemongrass EO in the treatment of acne.

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