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In-vitro antimicrobial activity of *Cymbopogoncitratus*Stem extracts

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ABSTRACT
Essential oils have always been a field of interest attributed to their strong antibacterial properties. Present study was intended to determine the in-vitro antimicrobial and antioxidant activities of *Cymbopogoncitratus*stems. Studyinvolved successive extraction of*Cymbopogoncitratus*stems by Soxhlet extraction using different solvents such as dichloromethane, acetone, ethanol and methanol in increasing order of polarity to yield different solvents extracts. The extracts were investigated for their antimicrobial potential.Among all extracts, dichloromethane extract exhibited highest antimicrobial activity followed by ethanolic extract, acetone extract and lastly methanolic extract. Present study concludes that dichloromethane extracts of *Cymbopogoncitratus* stem possess highest inhibitory action over the growth of bacterial strains of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Acinetobacterbaumanni and Neisseria gonorrhoeae.* The high antimicrobial potential of dichloromethane extract of *Cymbopogoncitratus* stem may be due to presence flavonoidal constituents.

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Keywords: Antimicrobials, Cymbopogoncitratus, bacteria, stem, extract

1. Introduction

The human body microbiota is known to possess a ratio of 1:1 human cells and bacteria [1]. A slight disturbance in this ratio manifest in various infections and diseases [2,3]. The development of microbial resistance and patient tolerance have gradually reduced the efficacy of commercially available antibiotics on one hand and increased the demand for alternative antibiotics [4,5]. Essential oils have always been a field of interest attributed to their high medicinal value in humans and animals[6,7]. Plants are known to possess high medicinal value [8-18]. Medicinal plants are the good source of natural antimicrobial agents attributed to their defence mechanism against microorganism [19-25].*Cymbopogoncitratus*is a frost-tender clumping perennial grass that is popularly used as a lemony flavouring in Thai, Vietnamese, Laotian and Cambodian cooking and is widely cultivated in Southeast Asia. *C. citratus* is believed to be a native of Malaysia [24]. In English, the herb is commonly known as lemongrass, citronella grass or fever grass. More recently, the aromatic oils of the plant have been extracted and exported for use in perfumes [23]. Lemon grass is common and widespread within its natural range, andalso occurs in cultivation. The leaves of *Cymbopogoncitratus* have been used in traditional medicine and are often found in herbal supplements and teas. Many effects have been attributed to both their oral consumption and topical use, with modern research supporting many of their alleged benefits. Laboratory studies have shown cytoprotective, antioxidant, and anti-inflammatory properties in vitro as well as antifungal properties [24]. A total of 16 chemical constituents accounting for 93.69 % of the oil, were identified in *C. citratus* among which, geranial (27.04 %), neral (19.93 %) and myrcene (27.04 %) were the major constituents.Lemongrass should be harvested at the appropriate level of maturity in order to achieve high quality essential oil and low production cost. Only 13 compounds were present at each of the maturity stage. Among 13 compounds, only 7 compounds (β-myrcene, 3-undecyne, neral, geranial, nerol, geranyl acetate and juniper camphor) had a concentration of greater than 1% [25].Development of bacterial resistance to conventional antibiotics necessitates the search for new antibacterial agents[26-31]. Hence the present study was carried out to find out the antibacterial activity of lemongrass oil against the selected pathogenic bacteria.

2. Material and methods

The *Cymbopogoncitratus* were collected from Laguna Merbok in Sungai Petani, district of Kedah state of Malaysia. The collected plant stems were dried using the oven at 40˚C for three consecutive days. The chemical and reagents used in the study were of laboratory grade and were procured from Merck, SD fine, and Sigma Aldrich. For example: dichloromethane, acetone, ethanol, methanol were used for extraction of *Cymbopogoncitratus*. Muller Hinton Agar and Nutrient Broth were used as media and Gentamicin as standard for antimicrobial test.

2.1. Authentication of plant materials (Preparation of herbarium)

The specimen including stems was collected from *Cymbopogoncitratus*plant. The specimen was washed with water and rinsed with 70% ethanol in order to remove dirt particles. The specimen was kept between the newspapers and which was then pressed under the wooden press. The specimen was kept air-dried for one month. The dried specimen was mounted onto an acid-free paper. The herbarium was labeled with its common name, geographical situation, place and date of collection, and other features of plant [32]. The herbarium was then submitted to the Pharmaceutical Chemistry Unit, Faculty of Pharmacy, AIMST University. (Herbarium voucher specimen Accession number AIMST/FOP/15 *Cymbopogoncitratus*)

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2.2. Collection and drying of *CymbopogonCitratus* stems

The *Cymbopogoncitratus*stems were collected, cleaned with distilled water and dried using oven at 40˚C for three days. The dried stems were then crushed into coarse powder using blender. Later on, the obtained powder was subjected to different solvent extraction using Soxhlet apparatus [33-44].

2.3. Preparation of *Citrus citratus*stem extracts

The *Cymbopogoncitratus*dried stems powder was subjected to successive extraction with dichloromethane, acetone, ethanol and methanol in an increasing order of polarity.About 15 g *Cymbopogoncitratus*dried stem powder was placed into the Soxhlet extraction tube. The Soxhlet apparatus was set up and made ready for extraction. Extra care was needed while setting up the apparatus. A few pieces of porcelain chips were placed to reduce the bumping during extraction. The dried stems powder was successively extracted using different solvents such as dichloromethane, acetone, ethanol and methanol. The reaction was started once the solvent was heated for 25 siphonic cycles. After about 25 siphonic cycles (approximately for 8 hours), a change in the colour of the extraction solvent from yellowish/green to colourless was observed in the Soxhlet extracting tube. Lastly, the solution was collected and evaporated using evaporating dish to obtain a concentrated pure extract [45-51].The experiment was repeated by using different extraction solvent successively in their order of increasing polarity such as: dichloromethane, acetone, ethanol, followed by methanol.

2.4. Determination of Antibacterial potential

2.4.1. Preparation of bacterial culture

Six bacterial strains such as: *Staphylococcus aureus*, *Staphylococcus pyogenes, Bacilus cereus:* Gram positive bacteria, *Escherichia coli*, *Acinetobacterbaumannii*, and *Neisseria gonorrhoeae*were used for the antimicrobial study.The microorganism stock cultures were maintained and stored at 4°C in the refrigerator. The Nutrient Broth and apparatus used in the transfer of microorganisms were previously autoclaved to ensure absence of other organisms. Subcultures were prepared by transferring a loopful of colonies of microorganisms from the stock culture into Nutrient Broth and incubated at 37˚C for 24 hours in the shaking incubator. Turbidity of the broth indicated the growth of microorganism [52-60].

2.4.2. Agar Well Diffusion method

Antibacterial potential of the extracts of *Cymbopogoncitratus*stems was determined using well diffusion method. Mueller Hinton agar (MHA) medium was used in this test. Approximately 100 µl bacteria suspension was pipetted on the MHA plate and spreaded over the agar surface evenly using a sterile L-shaped glass rod. Study involved gentamicin (0.1 mg/ml), dichloromethane extract (1 mg/ml), acetone extract (1 mg/ml), ethanol extract (1 mg/ml), and methanol extract (1 mg/ml). Holes with diameter of 8-10 mm were punched aseptically with a sterile cork borer on the agar containing bacteria suspension. Volume of approximately 100-200 µl of the gentamicin solution, extracts and phosphate buffer at desired concentration were introduced into the well. The agar plates were incubated at 37^{o}C for 24 hours. The diameters of the zones of inhibition with different extracts were measured in mm. Each test was performed in triplicate and the mean values were recorded [61-68].

2.4.3. Agar Disc Diffusion method

Antibacterial potential of the extracts of *Cymbopogoncitratus*stems was determined using disc diffusion method. Mueller Hinton agar (MHA) medium was used in this test.

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Approximately 100 µl bacteria suspension was pipetted on the MHA plate and spreaded over the agar surface evenly using a sterile L-shaped glass rod.Study involved gentamicin (0.1 mg/ml), dichloromethane extract (1 mg/ml), acetone extract (1 mg/ml), ethanol extract (1 mg/ml), and methanol extract (1 mg/ml). Dried and sterilized filter paper discs with 6 mm diameter were suspended into the gentamicin solution and extracts solution with the help of forceps. Discs with absorbed extracts were placed on the surface of the agar containing bacteria suspension with sterile forceps. The plates were incubated at 37°C for 24 hours. The diameters of the zones of inhibition by discs with different extracts were measured in mm.Each test was performed in triplicate and the mean values were recorded [69-78].

3. Results

3.1. Determination of antimicrobial potential

In present study, the prepared extracts of lemon grass (*Cymbopogoncitratus*) stem were evaluated for their antimicrobial potential against various bacterial strains such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Acinetobacterbaumanni*and *Neisseria gonorrhoeae*, using Agar well diffusion and disc diffusion methods for measurement of zone of inhibition.The prepared extracts of lemon grass (*Cymbopogoncitratus*) stem were evaluated for their antimicrobial potential against various bacterial strains using well diffusion and disc diffusion methods. The results so obtained are given in table 1 and table 2.

Table 1: Zone of inhibition of the lemon grass (CymbopogonCitratus) extracts using well diffusion method

Microorganism	**Component**	**Zone of inhibition (mm)**			
		Reading 1	**Reading 2**	**Reading 3**	**Average value**
Staphylococcus aureus	Positive control *Gentamicin (1mg/ml)*	16	20	14	16.7
	Dichloromethane	-	-	-	-
	Acetone	-	-	-	-
	Ethanol		2	-	-
	Methanol	-	-	-	-
	Negative control	-	-	-	-
Streptococcus pyogenes	Positive control *Gentamicin (1mg/ml)*	18	15	13	15.3
	Dichloromethane	8	-	4	6
	Acetone	-	3	3	3
	Ethanol	-	6	2	4
	Methanol	-	-	3	-
	Negative control	-	-	-	-
Bacillus cereus	Positive control *Gentamicin (1mg/ml)*	19	16	14	16.3
	Dichloromethane	8	5	10	7.67
	Acetone	-	3	2	2.5
	Ethanol	-	3	-	-

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	Methanol	2	1	2	1.67
	Negative control	-	-	-	-
Escherichia coli	Positive control *Gentamicin (1mg/ml)*	18	18	15	17
	Dichloromethane	19	15	-	17
	Acetone	-	10	2	6
	Ethanol	-	8	2	5
	Methanol	-	2	-	-
	Negative control	-	-	-	-
Acinetobacterbaumanni	Positive control *Gentamicin (1mg/ml)*	14	19	15	16
	Dichloromethane	6	8	-	7
	Acetone	2	8	2	4
	Ethanol	-	3	-	-
	Methanol	2	-	2	2
	Negative control	-	-	-	-
Neisseria gonorrhoeae	Positive control *Gentamicin (1mg/ml)*	17	18	13	16
	Dichloromethane	14	13	-	13.5
	Acetone	-	9	7	8
	Ethanol	-	10	-	-
	Methanol	-	2	3	2.5
	Negative control	-	-	-	-

Table 2: Zone of inhibition of the lemon grass (CymbopogonCitratus) extracts using disc diffusion method

Microorganism	**Component**	**Zone of inhibition (mm)**		
		Reading 1	**Reading 2**	**Average value**
Staphylococcus aureus	Positive control *Gentamicin (1mg/ml)*	9	10	9.5
	Dichloromethane	-	-	-
	Acetone	-	-	-
	Ethanol	-	1	-
	Methanol	2	3	2.5
	Negative control	-	-	-
Streptococcus pyogenes	Positive control *Gentamicin (1mg/ml)*	15	8	11.5
	Dichloromethane	-	-	-
	Acetone	3	-	-
	Ethanol	3	8	5.5
	Methanol	4	3	3.5
	Negative control	-	-	-

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Bacillus cereus	Positive control *Gentamicin (1mg/ml)*	18	15	16.5
	Dichloromethane	-	-	-
	Acetone	7	4	5.5
	Ethanol	7	2	4.5
	Methanol	4	3	3.5
	Negative control	-	-	-
Escherichia coli	Positive control *Gentamicin (1mg/ml)*	17	23	20
	Dichloromethane	-	-	-
	Acetone	6	2	4
	Ethanol	7	11	9
	Methanol	5	4	4.5
	Negative control	-	-	-
Acinetobacterbaumanni	Positive control *Gentamicin (1mg/ml)*	16	18	17
	Dichloromethane	-	-	-
	Acetone	7	-	-
	Ethanol	8	2	5
	Methanol	2	2	2
	Negative control	-	-	-
Neisseria gonorrhoeae	Positive control *Gentamicin (1mg/ml)*	18	16	17
	Dichloromethane	2	-	-
	Acetone	10	2	6
	Ethanol	10	3	6.5
	Methanol	3	2	2.5
	Negative control	-	-	-

4. Discussion

There is an increasing demand on evaluation of alternative antimicrobial due to development of resistant to currently available antimicrobial. As per the literature survey, *Cymbopogoncitratus* is reported as a herbal medicine that is useful in treating various diseases and it is majorly due to its strong antimicrobial activity. Therefore, the investigators planned to work over In-vitro antimicrobial activity of *Cymbopogoncitratus* stem extracts. Antimicrobial study was conducted as per standard reference [11,15, 16].The antimicrobial results of the present study revealed dichloromethane extracts showed inhibitory effect over growth of *Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Acinetobacterbaumanni, Neisseria gonorrhoeae* in well diffusion method. Overall, dichloromethane showed more activity against Gram negative bacteria on well diffusion. Acetone extracts showed inhibitory effect on *Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Acinetobacterbaumanni*and *Neisseria gonorrhoeae.* As in disc diffusion, it showed inhibitory effect on *Bacillus cereus, Escherichia coli,Neisseria gonorrhoeae* except on *A. baumanni.* Overall, it showed more antimicrobial activity in well diffusion method and Gram-negative bacteria.Ethanolic extracts showed inhibitory effect on S. pyogenes and *E. coli* in well

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diffusion. In disc diffusion, it showed inhibitory effect against *B. cereus, E. coli, A. baumanni,* and *N. gonorrhoeae*. To summarize, ethanolic extracts showed more antimicrobial activity in disc diffusion method and Gram-negative bacteria.Methanolic extracts showed inhibitory effect against *B. cereus* and *N. gonorrhoeae*in well diffusion method. Whereas in disc diffusion method, it showed inhibitory effect in all studied bacteria. To summarize, methanolic extracts showed more antimicrobial activity in disc diffusion method and Gram-negative bacteria.Based on the present antimicrobial study conducted, the study showed that dichloromethane extract has the highest antimicrobial activity followed by ethanolic extract, acetone extract and lastly methanolic extract. For three microorganisms (*S. pyogenes*, *A. baumanni*, and *N. gonorrhoeae*), so far, no study has been done, it is reported for the first time in our study. Extracts used in present antimicrobial study have shown overall good antimicrobial activity against these three bacteria in both disc and well diffusion.

5. Conclusion

Based on antibacterial potential of different solvent extracts of *Cymbopogoncitratus*stems, it is hereby concluded that dichloromethane extract of *Cymbopogoncitratus*stems exerts highest inhibitory action against growth of bacterial strains of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Escherichia coli, Acinetobacterbaumanni*and *Neisseria gonorrhoeae.* The high antimicrobial potential of dichloromethane extract of *Cymbopogoncitratus* stems may be due to presence of flavonoidal constituents. Present study concludes high antibacterial potential of *Cymbopogoncitratus* plant. To obtain deeper insight into the mechanism of action of extracts of stems of *Cymbopogoncitratus* plant, further investigation on isolates of extracts should be done.

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