Chemical Composition and Antibacterial Activity of Ethanol Extract of Lemon Grass (*Cymbopogon citratus*) on Pathogenic Bacteria: A review

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บทคัดย่อ

Cymbopogon citrates เป็นที่รู้จักกันในปัจจุบันคือตะไคร้ นอกจากการใช้เป็นยายังสามารถนำไปใช้ในอุตสาหกรรมอาหาร รวมทั้งอุตสาหกรรมเครื่องสำอางและอุตสาหกรรมยา สารสกัดเอทานอลของตะไคร้พบว่ามีฤทธิ์ในการต้านแบคทีเรียก่อโรคบางอย่ างชนิด เช่น *Staphylococcus aureus* และ *Salmonella typhi* ฤทธิ์ในการต้านแบคทีเรียของสารสกัดเอทานอลของตะไคร้ (*Cymbopogon citrates*) อาจจะขึ้นอยู่กับสารปฐมภูมิ (Primary metabolite) และสารทุติยภูมิ (Secondary metabolite) ที่เป็นองค์ประกอบในสารสกัด สารปฐมภูมิในสารสกัดเอทานอลของตะไคร้ประกอบด้วยโปรตีน ไขมัน และคาร์โบไฮเดรต โดยเฉลี่ยการพบสารปฐมภูมิในการวิเคราะห์คือ ไขมัน < โปรตีน < คาร์โบไฮเดรต ในขณะที่สารทุติยภูมิของสารสกัดจากใบของตะไคร้ประกอบด้วยแทนนิน, ฟลาโวนอยด์, เทอพีนอยด์, ฟีนอล และกรดแอสคอบิก จากรายงานเกี่ยวกับคุณสมบัติของสารสกัดเอทานอลของตะไคร้สามารถสรุปได้ว่าสารสกัดเอทานอลของตะไคร้น่าจะสามารถนำมาใช้เป็นสารต้านแบคทีเรียในอนาคตต่อไป

คำสำคัญ: ส่วนประกอบทางเคมี กิจกรรมการต้านแบคทีเรีย สารสกัดด้วยเอทานอล ตะไคร้ (*Cymbopogon citratus*) แบคทีเรียก่อโรค

Abstract

Cymbopogon citratus species, is currently known as lemon grass. Besides the medicinal use, lemon grass can be also used in the food industry, as well as in cosmetics and pharmaceutical industries. Ethanol extracts of lemon grass showed antimicrobial activity to certain some pathogenic bacteria, for example, *Staphylococcus aureus* and *Salmonella typhi*. The antibacterial activities of ethanol extract of lemon grass (*Cymbopogon citratus*) could be based on the primary and secondary metabolite in these extract. Primary metabolites of the ethanol extract of lemon grass were proteins, lipid and carbohydrates. On average, the increasing order of the analyzed primary metabolites was: lipid < protein < carbohydrates. While secondary metabolites of leaves extracts of lemon grass were tannins, flavonoids, terpenoids, phenolic compounds and ascorbic acid. From reviewed papers related to the properties of lemon grass extract, it is concluded that ethanol extract of lemon grass was able to be used as antibacterial agents in future.

Keyword: Chemical composition; Antibacterial activity; Ethanol extract; Lemon grass (*Cymbopogon citratus*); Pathogenic bacteria

1. INTRODUCTION

Since ancient times, medicinal plants have been used for antimicrobial activity [1,2,3]. Crude plant extracts (e.g. infusion, tincture, decoction or others) are traditionally used by populations all over the world for medicinal purposes. Although their effectiveness and mechanisms of action have not been scientifically tested in the majority of the cases, they often mediate beneficial responses due to their bioactive chemical components such as alkaloids, phenolic compounds, flavonoids, tannins, terpenoids, and steroids [4,5]. Moreover, medicinal plants have been used as the sources of medicine because most of microorganisms have become resistant to the first line antibiotics such as chloramphenicol, tetracycline, and even combine drugs like penicillin-streptomycin. Similarly, orthodox drugs have adverse effect on those who use them [6,7]. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents [8]. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant [9]

Cymbopogon citratus, commonly known as lemon grass, is one of medicinal plant which has been shown potential as a source of chemotherapeutic compounds. This aromatic plant has also been reported by its antimicrobial activity against a variety of microorganisms such as *Aspergillus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* [10,11]. *Aspergillus* causes the aspergillosis, particularly, *A. fumigates*, *A. flavus* and *A. niger* [12]. *Escherichia coli* is a normal inhabitant of the gut flora; however, extended spectrum beta-lactamase (ESBL)-producing *E. coli* is also a major public health issue [13,14,15,16].

Pseudomonas aeruginosa is one of the most commonly nosocomial infections and is a multidrug resistance [17]. *Streptococcus pneumoniae* is a primary pathogen of otorhino-laryngological infections, respiratory tract infections in children, sepsis and purulent meningitis after infancy [18,19]. Moreover, penicillin-intermediate *S. pneumoniae* (PISP) and penicillin-resistant *S. pneumonia* (PRSP) is increased [20]. *Streptococcus pyogenes* or group A *streptococcus* is a etiologic agent for a variety of diseases, including acute pharyngitis, impetigo, rheumatic fever and the streptococcal toxic shock syndrome. Moreover, *S. pyogenes* also causes a nosocomial infection [21].

Therefore, there are several scientific studies that confirm the antimicrobial properties and chemical components of these plants. This is in pursuance of the efforts to search for drugs from plants to be use as an antimicrobial drug for the treatment of the test microbes. In this reviewed papers, chemical composition and antibacterial activity of ethanol extract of lemon grass (*Cymbopogon citratus*) extract on pathogenic bacteria was evaluated.

2. ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF LEMON GRASS (*CYMBOPOGON CITRATUS*) ON PATHOGENIC BACTERIA

The comparative antibacterial activity of lemon grass (*Cymbopogon citratus*) extract on pathogenic bacteria was evaluated by Nyarko et al. [22]. They demonstrated that only ethanol extracts of *Cymbopogon citratus* (Lemon grass) showed antimicrobial activity to *Staphylococcus aureus* but not *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Table 1 and Table 2).

Table 1 Antimicrobial activity of 20% (w/v) ethanol extract of *Cymbopogon citratus* (Lemon grass) using agar well diffusion method (modified from Nyarko et al. [22])

Extracts	Diameter of Zones of Inhibition (mm)			
	P. mirabilis	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*
Ethanol	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00	0.00 ± 0.00
Choramphenicol (Control)	16.00 ± 2.08	25.33 ± 2.33	21.33 ± 1.85	14.67 ± 1.33

Values expressed as mean ± standard deviation

Table 2 Minimum Inhibition Concentration (MIC) recorded for the ethanol and aqueous extract of *Cymbopogon citratus* (Lemon grass) against the test bacteria (modified from Nyarko et al. [22])

Extract	Tested bacteria	Minimum Inhibition Concentration (mg/mL)
Ethanol	*Staphylococcus aureus*	0.78
	Klebsiella pneumoniae	-
	Proteus mirabilis	-

(-) = No activity on tested bacteria

Table 2 showed that ethanol extract of lemon grass presented the MICs against only *S. aureus* but not against to *K. pneumoniae* and *P.mirabilis*. The MIC against *S. aureus* was 0.78 mg/mL. While Rathabai & Kanimozhi [23] presented the efficacy of the ethanol extract of lemon grass as antibacterials against *P.mirabilis*, *S. typhi* and *E. faecalis* as shown in Table 3. The ethanol extract was more effective against *S. typhi* than *P.mirabilis* and *E. faecalis*. The results of antibacterial activity are clearly showed that the ethanol extract of lemon grass has antibacterial activity against all tested organisms. Table 3 [24] showed the inhibition zone of ethanol extract of lemon grass by disk diffusion method as shown in Table 3.

Table 3 Inhibition zone of diameter of ethanol extract of lemon grass by disk diffusion method (modified from Rathabai & Kanimozhi, [13])

No.	Tested bacteria	Concentration (µg) zone of inhibition in mm.					Streptomycin
		1,000	500	250	125	62.5	
1	*Proteus mirabilis*	14.00 ± 0.34	12.00 ± 0.24	9.00 ± 0.21	-	-	26.00 ± 0.33
2	*Salmonella typhi*	25.00 ± 0.37	16.00 ± 2.08	10.00 ± 0.18	6.00 ± 0.11	-	18.00 ± 0.26
3	*Enterococcus faecalis*	3.00 ± 0.28	1.00 ± 0.14	-	-	-	22.00 ± 0.28

Values expressed as mean ± standard deviation, (-) = No activity on tested bacteria

These finding indicated that ethanol extract of lemon grass possesses antibacterial activity against all tested bacteria. *S. typhi* was the most sensitive to the extract, the less extent were *P. mirabilis* and *E. faecalis*, respectively.

There were different results on the efficacy of ethanol extracts as antibacterials. Nyarko et al. [22] demonstrated that the ethanol extract showed the anti-*S. aureus* which was a Gram positive bacterium but not against any tested Gram negative bacteria (*P. mirabilis*, *K. pneumoniae* and *P. aeruginosa*). However, the ethanol extract of lemon grass [23] showed the antibacterial activity against all tested Gram negative bacteria (*P. mirabilis* and *S. typhi*) higher than Gram positive bacteria (*E. faecalis*). This phenomenon could be explained due to the differences in extraction techniques, antimicrobial test as well as tested concentration leading to the differences in results of antimicrobial efficacy [25,26,27]. For the other reason, the edaphic and climatic conditions may promote differences in the synthesis of secondary metabolites which may contribute to the different in antimicrobial property [28]

3. CHEMICAL COMPOSITION OF ETHANOL EXTRACT OF LEMON GRASS (*C. CITRATUS*)

In an aspect of chemical compositions of ethanol extract, the primary and secondary metabolites in ethanol extract were reported [23]. Soares et al. [28] demonstrated the primary metabolites of ethanol extract of lemon grass as summarized in Table 4.

Primary metabolites of the leaves of lemon grass consisted of the increasing order of the analyzed nutrients were: lipid < protein < carbohydrates. Crude protein and lipid levels were 19.79% and 4.98%, respectively. Total carbohydrate was account for approximately 60% of lemon grass leaves. The secondary metabolites of ethanol extract of lemon grass (*C. citratus*), [23] were shown in Table 5. The secondary phytochemical substances in ethanol extract of lemon grass were tannins, saponins, proteins, anthraquinones and steroids [23] while Soares et al. [28] found tannins, flavonoids and terpenoids in the qualitative aspect (Table 6). Flavonoids were found with the highest amount (6.62 ± 0.65 mg GE/mL). Phenolic compounds and ascorbic acid were measured as 6.11 ± 0.11 mg GE/mL and 3.05 ± 0.12 mg AA/mL respectively. The least amount in the extract was tannins, found at 2.32 ± 0.16 mg GE/mL (Table 7)

Table 4 Primary metabolites of lemon grass (modified from Soares et al. [28])

No.	Primary metabolites	Percentage (%)
1	Proteins	19.79
2	Lipids	4.98
3	Carbohydrates	62.60

Table 5 Secondary metabolites of ethanol extract of lemon grass (modified from Rathabai & Kanimozhi [23])

No.	Secondary metabolites	Lemon grass
1	Alkaloids	-
2	Saponins	+
3	Tannins	+
4	Anthraquinones	+
5	Steroids	+

(+) = Presence of phytochemical compounds, (-) = Absence of phytochemical compounds

Table 6 Secondary metabolites of leaves extracts of lemon grass (modified from Soares et al. [28])

Plant extract	Tannins	Flavonoids	Alkaloids	Steroids	Terpenoids
Ethanol	+	+	-	-	+

(+) = Presence of phytochemical compounds, (-) = Absence of phytochemical compounds

Table 7 Secondary metabolites analysis of the ethanol extract of lemon grass leaves (modified from Soares et al. [28)

Secondary metabolites	Lemon grass extract
Phenolic compounds (mg GE/mL)	6.11 ± 0.11^{a}
Flavonoids (mg GE/mL)	6.62 ± 0.65^{a}
Tannins (mg GE/mL)	2.32 ± 0.16^{a}
Ascorbic acid (mg AA/mL)	3.05 ± 0.12^{ab}

Values expressed as mean ± standard deviation obtained from 2 measurements. For each group of compounds (within each table line), different letters (a, b or c) represent significant differences at $p < 0.05$. The same letter indicates that there are no significant differences ($p < 0.05$) between results.

Table 8 Comparison of secondary metabolites of ethanol extract of lemon grass [23,28]

No.	Secondary metabolites	Ethanol extract of lemon grass (*Cymbopogon citratus*)		
		Rathabai and Kanimozhi (2013)	Soares et al. (2013)	
			Quality	Quantity
1	Alkaloids	-	-	-
2	Saponins	+	ND	ND
3	Tannins	+	+	2.32 ± 0.16 (mg GE/mL)
4	Anthraquinones	+	ND	ND
5	Flavonoids	ND	+	6.62 ± 0.65 (mg GE/mL)
6	Terpenoids	ND	+	ND

ND = Not detected, (+) = Presence of phytochemical compounds, (-) = Absence of phytochemical compounds

Table 8 summarized the comparison between the obtained results of secondary metabolites in ethanol extract of lemon grass by the different research groups [23,28]. Only tannins were found in the experiments both reported by Rathabai & Kanimozhi [24] and Soares et al. [28]. In nature, plant produces the polyphenols which can combine with protein or the other polymers for forming tannin. Up to date, tannin was investigated for its use as the ailments in humans, including diarrhea, gastric ulcers, snake bites and wounds [29]. There are a variety of antibacterial mechanisms of tannin, for example, hindrance of extracellular microbial enzymes, blockage of microbial metabolism through suppression of oxidative phosphorylation and dismantling the microbial substrate [30]. Flavonoids and phenolic compounds were the major components of lemon grass ethanol extracts [31]. Flavonoids are the secondary metabolite and are commonly found in fruit, vegetables, nuts, seeds, stems and flowers and have been used as the active compound for curing human diseases [32]. Many reports demonstrated that compounds containing high flavonoid content revealed the antibacterial activity [33,34] and the anti-infective compound for human and plant pathogens. Phenolic compounds are a group of secondary metabolites widely consisted of one or more

hydroxyl derivatives of benzene rings. These compounds are commonly produced by plants and revealed the antiviral, antibacterial and antiparasitic activities [35,36,37,38,39,40,41]

Ascorbic acid is another common compound found in fruits and vegetables [42,43]. Ascorbic acid showed an antibacterial activity against *Mycobacterium tuberculosis* [44,45] and many other microorganisms [46,47,48,49]. Ascorbic acid also represented the antimicrobial activities against food and plant pathogens as well as enteroviruses [25,50,51,52]

Steroids are also generally found in plants and animals and these compounds showed the antibacterial activity [53]. Theirs antibacterial activities are reactions in membrane lipids and causing leakages from liposomes [54].

Saponins are the natural glycosides and the secondary metabolites were extensively produced by higher plants. These compounds have a variety of pharmacological activities and act as detergent which could enhance the permeability of bacterial cell membrane [24,55]. These compounds can also cause the leakage of proteins and some type of enzymes from the cell [56]

4. CONCLUSIONS

From reviewed reports, the results suggested that lemon grass extract is useful as the antimicrobial activity against some pathogenic bacteria, for example, *Staphylococcus aureus* and *Salmonella typhi* and could be applied in the medical, industrial, cosmetic and other aspects. However, the efficacy of these extracts depends on the extraction techniques, the sources of used medicinal plants, and the other processes which effect on the shelf-life and mode of action of the active ingredients in the extract. Therefore, the further information related to the useful lemon grass extract should be required to investigate.

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