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ORIGINAL ARTICLE

Antimicrobial Properties of Clove (*Syzygium aromaticum* L.) Extracts

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ABSTRACT

Background: *Clove (Syzygium aromaticum) is an abundant source of bioactive chemicals. This study aimed to evaluate several clove extracts regarding their phenolic content, antioxidant capacity, and antibacterial efficacy against pathogenic microorganisms. Objective: This study aimed to identify the phytochemical content and antibacterial activity of Syzygium aromaticum L. extracts, with a particular focusing on their effects against Acinetobacter baumannii and Streptococcus pneumoniae.* ***Methodology:*** *Cloves extracts were obtained from the plant by using a Soxhlet apparatus with petroleum, ether, chloroform, acetone, industrial methylated spirit (IMS), and hot water. Acid hydrolysis was performed on the acetone, IMS, and hot water extracts to liberate free phenolic chemicals.* ***Results:*** *These compounds including chlorogenic acid, caffeic acid, rutin, gallic acid, and ferulic acid were measured by using high-performance liquid chromatography (HPLC). The IMS extract displayed the highest content of ferulic acid, while the acetone extract demonstrated the lowest concentration of rutin. The extracts were tested for antibacterial activity against S. pneumoniae (Gram-positive) and A. baumannii (Gram-negative), both of which are clinically important human pathogens. Antibacterial effects of phenolic-rich extracts were variable. Interestingly, the hot water extract showed the best inhibition of A. baumannii (41.65mm) by 100% and the least inhibition (13.54mm) at 25% concentration was found in the IMS extract. In the same way, the best inhibition (36.63 mm) of S. pneumoniae was provided by hot water extract at the 100% concentration, while the worst inhibition (10.26 mm) was produced by acetone extract at 25% concentration.* ***Conclusion:*** *Syzygium aromaticum L. extracts exhibited variable antibacterial activities depending on solvent type and concentration, with the hot water extract showing the most potent effect against both tested pathogens.*

INTRODUCTION

Medicinal plants have a history of use for their healing properties since ancient times. Recent scientific studies have been shown that many of these plants offer a wide variety of bioactive compounds which can interact with microbial systems; and can be considered as potential sources of new antimicrobial agents [1]. Clove (Syzygium aromaticum L.) is an aromatic herbaceous plant and one of the many medicinal plants that shows antimicrobial activity. Flower buds grow on the branches of the plant and change color pale, to green, and then bright red when they mature and are ready to be harvested. The buds are usually 1.5 to 2 cm long, have a long calyx with 4 flattened sepals, and 4 petals fused into a central ball [2].

The anti-inflammatory antimicrobial analgesic and antifungal effects of clove oil are well documented. Clove is a traditional remedy for many diseases, including gastrointestinal infections, peptic ulcers, gingival diseases and dental pain[3]. The bulk of these therapeutic effects are due to the essential oils and phenolic constituents of the plant. According to Zheng *et al.*[4], mucilage from clove buds, the top bio-active compounds are eugenol, eugenyl acetate, heptanone, salicylate, pinene and caryophyllene, the highest bioactive ingredient is eugenol, it is one of the major ingredients in clove bud oil, which is about 81.1% in clove bud oil. In another study, Cai and Wu [5] found that phenolic compounds from clove such as ellagic acid, kaempferol, myricetin, gallic acid and oleanolic acid have shown notable antimicrobial activity against different strains of microorganism.

The rise in antibiotic-resistant infections across the world makes the identification of new antimicrobial agents crucial. Natural products represent a pivotal source of bioactive molecules with the potential to function as lead compounds for the development of novel antimicrobial therapeutics [6].

Acinetobacter baumannii is a Gram-negative, aerobic, non-motile bacillus which is also one of the most common pathogens in hospital infections, particularly in Intensive care units. It has become an important healthcare-associated pathogen with its remarkable capacity of acquiring resistance to multiple classes of antibiotics[7]. It is well known that this

opportunistic pathogen is a causative agent of many severe infections such as sepsis, bacteremia, meningitis, urinary tract infections, pneumonia, wound infections, burn infections, osteomyelitis, and keratitis[8]. *A. baumannii* pathogenesis is related to numerous virulence factors including outer membrane proteins, lipopolysaccharides, and capsular polysaccharides all contributing to tissue adherence, immune evasion, and biomfilm development [9]

Streptococcus pneumoniae, or pneumococcus, is a Gram-positive, nonmotile, facultatively anaerobic bacterium. It is a fastidious organism needing enriched media, i.e., blood agar, and high carbon dioxide levels (5–7%) for optimal growth [10]. *S. pneumoniae* still one of the Top 10 morbidity and mortality causes worldwide for diseases like pneumonia, meningitis, and sepsis, especially among children, the elderly, and immunocompromised individuals [11]. An explanation of its mechanism is polysaccharide capsule, which prevents phagocytosis and complement activation, the bacterium also expresses myriad virulence factors, pneumolysin, hemolysin, neuraminidase, autolysin, and choline-binding proteins. Together these contribute to tissue destruction, immune evasion and colonization of the host[12]. This study aims to study the phytochemical content and antibacterial activity of Syzygium aromaticum L. extracts, with a particular focusing on their effects against *Acinetobacter baumannii* and *Streptococcus pneumoniae*.

METHODOLOGY

Collection of the Flower Buds

Clove (Syzygium aromaticum L.) flower buds were collected, cleaned from dust, and ground into fine particles. The powdered buds were then placed in a paper bag and stored in a dry, dark place away from sunlight until further use.

Preparation of Plant Extracts Using a Continuous (Soxhlet) Extraction Apparatus

The flower buds of Syzygium aromaticum L. were dehydrated and ground by using grinder. A total of 25 grams of the powdered buds were placed in a Soxhlet extractor, and 200 mL of petroleum ether (60–80 °C) was used as the initial solvent for oil extraction. The extraction process was conducted for 6 to 7 hours daily until the solvent in the apparatus turned colorless[13]. A serial extraction was performed by using a petroleum ether (60–80 °C), chloroform, acetone, industrial methylated spirit (IMS), and finally, hot aqueous extraction[14].

Acid Hydrolysis Process

Measurements of 10 mL of each crude induced acetone, IMS, and hot water extract were performed separately by adding 25 mL of 1N hydrochloric acid (HCl) and after they kept refluxing at 100 °C for 60 min. After several steps, samples analysis by high-performance liquid chromatography (HPLC) [15, 16]. The identified phenolic compounds were indicated by using equation [17].

Identification of Phenolic Compounds Using HPLC-UV

Phenolic compounds identification was accomplished in Baghdad, in the laboratories of the Ministry of Science and Technology, Department of Environment and Water Resources after acid hydrolysis.

The analysis was performed on a high performance liquid chromatography (HPLC) system (Sykam, Germany)[18]. Flow rate was kept as 1.3 mL/min.

The mobile phase (A) consisted of methanol, distilled water, and formic acid in a ratio of 70:25:5. Separation was achieved using an ODS-18 column (25 cm × 4.6 mm), and detection was carried out at a wavelength of 360 nm using a UV detector.

Sensitivity Test Method (Well Diffusion Technique)

The antimicrobial activity of the plant extracts was assessed using the well diffusion method. Extract solutions were prepared at concentrations of 100, 75, 50, and 25 mg/mL following acid hydrolysis [19, 20].

RESULTS

Identification of Phenolic Compounds

Table 1 summarizes the retention times and spectra of the phenolic compounds from the chromatographic analysis by HPLC-UV obtained from the developed HPLC-UV system. The retention times were compared with that of standard compound. Retention times for chlorogenic acid (2.80 min), caffeic acid (4.28 min), rutin (5.89 min), gallic acid (7.92 min), and ferulic acid (11.92 min).

The presence of these phenolic compounds in the extracts of Syzygium aromaticum (dimethoxyphenol of clove) supports the capacity of this plant to be used as a rich natural source of bioactive phenolics. This confirms the phenolic compounds found in flower bud extracts of Syzygium aromaticum L. that Contents of chlorogenic acid determined after acid hydrolysis were 0.009785, 0.0105949 and 0.01021168 mg/g in all three extracts, respectively. Likewise, caffeic acid was detected at the concentrations of 0,0213056, 0,0227443 and 0,0219689 mg/g.

Rutin was present in the concentrations of 0.0060416, 0.0068040, and 0.0065379 mg/g, respectively, while the amounts of gallic acid (GA) were in the concentrations of 0.0136663, 0.01692592, and 0.0141728 mg/g (Figure 3); presence of ferulic acid (FA) was also detected in all extracts in the concentrations of 0.0332024, 0.03681176, and 0.03593208 mg/g, respectively.

Table 1: The standard retention times and the concentration of some phenolic compounds by using HPLC technique

n	standard phenolic Compounds	Standard retention times	Acetone extract		IMS extract		Hot aqueous extract	
			Reten time (min)	concentration Mg/g	Reten time (min)	Conc.	Reten time (min)	Conc.
1	**Chlorogenic acid**	2.80	0.009785	2.78	0.0105949	2.72	0.01021168	2.74
2	**Caffeic acid**	4.28	0.0213056	4.26	0.0227443	4.24	0.0219689	4.26
3	**Rutin**	5.89	0.0060416	5.88	0.0068040	5.88	0.0065379	5.84
4	**Gallic acid**	7.92	0.0136663	7.90	0.01692592	7.99	0.0141728	7.99
5	**Ferulic acid**	11.92	0.0332024	11.90	0.03681176	11.93	0.03593208	11.98

Antibacterial Activity of Clove Flower Bud Extracts against *Acinetobacter baumannii* and *Streptococcus pneumoniae* using the Well Diffusion Method

As shown in Table 2 and Figure (1,2,3,4), acid hydrolysis was conducted for each four dilution rates (25%, 50%, 75%, and 100%) that were then subjected to three different types of extracts (acetone, intermediate solvent [IMS], and hot aqueous) to test the potential to extract a compound.

The hot aqueous extract showed the highest inhibitory activity against *A. baumannii*, more than that of the standard antibiotic ciprofloxacin. The inhibition zone was measured at 100% (41.65 mm), 75% (40.04 mm), 50% (36.01 mm), and 25% (27.54 mm). Ciprofloxacin, on the other hand, formed only an inhibition zone of 21.62 mm.

The IMS extract also showed high antibacterial activity. The inhibition zones for the sludge were 28.54 mm and 23.87 mm at 100% and 75% concentrations respectively and both greater than ciprofloxacin 20.09 mm. For 50% and 25% lower activity have been found at (20.12 mm and 13.54 mm) inhibition zone, both were less than antibiotic control.

The acetone extract also had an inhibition zone of 29.04 mm at 100% level. Counter wise, a 75% zone (20.55 mm) was closest to that of ciprofloxacin (21.41 mm), with 50% (16.35 mm) and 25% (15.02 mm) yielding lower activity.

Similarly, the hot aqueous extract exhibited the highest antibacterial activity for *S. pneumoniae* but not at even the lowest tested concentrations (Table 2). Inhibition zones include 36.63 mm (100%) and 33.97 mm (75%), and 30.41 mm (50%), and 27.25 mm (25%) all higher from inhibition zone of ciprofloxacin 24.94 mm.

Only the IMO extract was effective at 100%, and the inhibition zone of 30.42 mm was higher than that of ciprofloxacin (24.07 mm). The activity started to drop at lower concentrations (75%, 50%, and 25%) with inhibition zones of 22.84 mm, 18.63 mm, and 11.22 mm, respectively.

The antibacterial activity of acetone extract was shown at all concentrationsa in moderate manner. Fieldwork tests yielded inhibition zones of 21.00 mm (100%), 17.14 mm (75%), 14.59 mm (50%), and 10.26 mm (25%), all lower than indictor ciprofloxacin (23.95 mm).

Table 2: Antimicrobial Activity

A. baumannii

Extract	25% (mm)	50% (mm)	75% (mm)	100% (mm)	Control (CIP)
Hot Water	27.54	36.01	40.04	41.65	21.62
IMS	13.54	20.12	23.87	28.54	20.09
Acetone	15.02	16.35	20.55	29.04	21.41

S. pneumoniae

Extract	25% (mm)	50% (mm)	75% (mm)	100% (mm)	Control (CIP)
Hot Water	27.25	30.41	33.97	36.63	24.94
IMS	11.22	18.63	22.84	30.42	24.07
Acetone	10.26	14.59	17.14	21.0	23.95

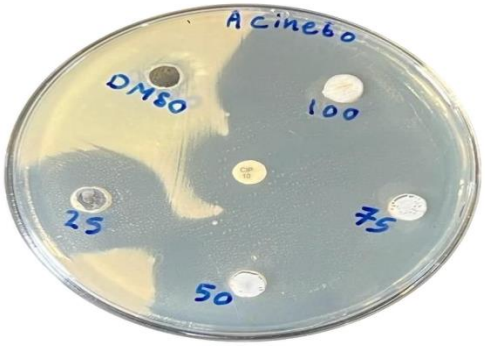

Fig. 1: The effect of hot water extract on *A. baumannii*

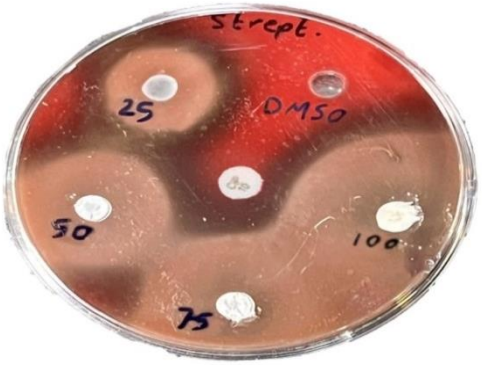

Fig. 2: The effect of hot water extract on *S.pneumoniae*

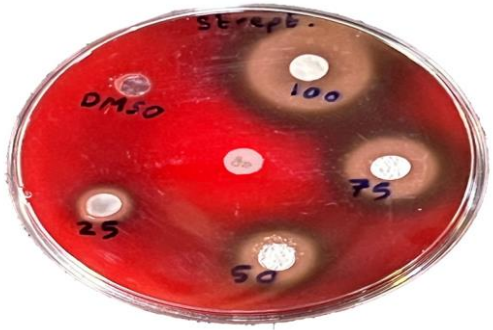

Fig. 3: The Effect of the Phenolic IMS Extract on *S.pneumoniae*

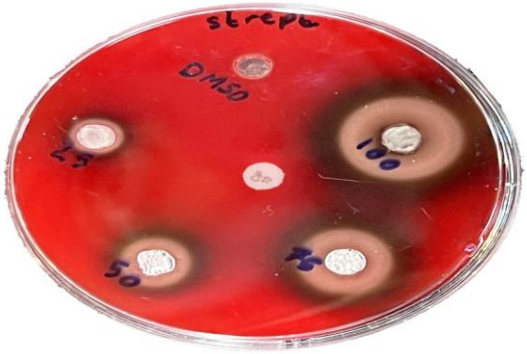

Fig. 4: The Effect of the Phenolic Acetone Extract on *S.pneumoniae*

DISCUSSION

The extracts showed an inhibitory effect against *Acinetobater Streptococus* and *Pneumoniae basmaeil*. The hot aqueous extract was the most effective, exhibiting the highest inhibition across all tracers, surpassing the standard antibiotic ciprofloxacin. This is attributed to the water-soluble phenolic compounds, such as ferulic and caffeic acids, which play a key role in the antibacterial activity exhibited by carnation flower buds.

These results can be explained by the fact that the high temperature of the hot aqueous extract facilitates the extraction of the plant's active compounds and breaks down the complex bonds between phenols and other compounds. Water, being a polar compound, damages phenolic compounds, tannins, and flavonoids, which are key in inhibiting bacterial growth by disrupting biofilm synthesis or weakening bacterial cell wall structure. The results of the current study are consistent with previous findings Abdul Aziz *et al* [21]indicating that phenolic compounds are the main component responsible for bacterial inhibition. This supports its potential use as a natural disinfectant and a safe and effective source of antibacterial agents.

Other study showed antimicrobial activity of aqueous extract of against *E.coli*[22], *K. pneumoniae*[23, 24], *P. aeruginosa*[25]. One of the phytoconstituents that may have greatly aided the antibacterial activities is eugenol. So, clove as natural product may be a useful adjuvant, especially in the treatment against certain pathogens. Herbal medicine is currently gaining popularity as a secure and reliable method of treating a wide range of medical issues[22].

CONCLUSION

The present study demonstrated contain Clove (Syzygium aromaticum L.) Extracts high content of phenolic compounds as chlorogenic acid, caffeic acid, rutin, gallic acid, and ferulic acid. In addition the findings showed that these phenolic compounds had significant antibacterial effect on *Acinetobacter baumannii* and *Streptococcus pneumoniae*. The hot aqueous extract, especially, showed a potent inhibitory activity against all test microbes at all concentrations.

Conflict of interests: Non
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Author contribution: Authors contributed equally in the study.

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