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Individual Antibacterial Activity of Black Seed, Clove, and Myrrh Various Extracts Against Different Bacterial Strains

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ABSTRACT

Nigella sativa (black seed), Syzygium aromaticum (clove), and Commiphora myrrha (myrrh) are examples of medicinal plants that have gained popularity in the last decades due to their high contents of phytochemical constituents and consequently to their potent biological activities. Different extracts of the aforementioned medicinal plants were prepared, using organic solvents acetone and methanol, as well as a water extract; they were evaluated separately for their antibacterial activities against human pathogenic bacteria Staphylococcus aureus, Bacillus subtilis as Gram-positive bacteria and against Gram-negative Escherichia coli, and Pseudomonas aeruginosa. The experiment was carried out using agar well diffusion techniques. Different inhibition effects were observed, which could be mainly attributed to the presence of various chemical constituents. They can be used as such as good precursors for new antibacterial agents. Data from the present study showed that E. coli was the most sensitive bacterial strain, mostly to the clove methanol extract, followed by the acetone extract on Pseudomonas, Bacillus, and Staphylococcus. Black seed and myrrh solvent extracts showed similar activity against Bacillus more than against all other isolates. All aqueous extracts, except myrrh water extract, demonstrated negligible effect on the tested bacteria, indicating moderate activity against all isolates. This variation in the efficacy could be related to the bacterial structure as well as the major phytoconstituents.

Keywords : *Medicinal plants, Nigella sativa (black seed), Syzygium aromaticum (clove), Commiphora myrrha (myrrh), Antibacterial activity, Phytoconstituents*

INTRODUCTION

The excessive usage of antimicrobial agents has caused a spread of extremely hard-to-medicate microbial infections due to the massive increase in the resistance of microbes against those drugs, as their resistance reflects the natural route the bacteria take in order to adapt to this phenomenon. Consequently, many bacterial isolates become multi-drug resistant, causing a life-threatening medical concern [1]. This complication has led to a search for new, safe, economical antimicrobial agents that have different modes of mechanisms as alternatives for common antibiotics [2]. In addition, more than half of the drugs used to treat infectious diseases are thought to be made from natural resources [3].

Here comes the use of medicinal plants, as they have major roles in the production of therapeutic agents, as well as in developing existing agents for curing various infectious diseases [4].

Diverse medicinal plants possess several bioactive components that act as base materials for drug development, including steroids, carotenoids, alkaloids, and tannins [5], as these antimicrobial agents possess very little harm. Therefore, using specific doses of these components can work as many infectious disease limiters as possible.

Much research has been done to investigate and determine the therapeutic features of plants and their roles in manufacturing common synthetic drug alternatives.

In this research, the antimicrobial activity of clove, black seed, and myrrh was tested against different bacteria due to their promising antibacterial activity, as traditional medicine relies mainly on trials, eventually leading to experience [6].

Clove (*Syzygium aromaticum*) is a spice derived from a dried flower bud [7] used for centuries all over the world, especially in Latin American countries and in Spain [8] for minimizing abdominal pain [7], diarrhea [9] as well as treating many illnesses associated with microbes such as malaria, tuberculosis, and cholera. Black seed (*Nigella sativa*) or black cumin is an annual plant with a capsule-shaped fruit containing small black seeds. It is used extensively in the Middle East, Egypt, and Lebanon as dermal and respiratory medicine [10, 11]. Myrrh is a resinous substance extracted from the abrasion of *Commiphora myrrha* trees. It is composed of essential oils, water-soluble gum, and alcohol-soluble resin. Myrrh is one of the ancient remedies predominantly used by Egyptians [12]. It is a non-specific medicine applied for healing various human health conditions.

Different extracts from the aforementioned medicinal plants were demonstrated in previous studies to possess antibacterial activities with different efficacy against several bacterial isolates, among which multidrug-resistant strains *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [13-15]. Although extensive work has been reported on these medicinal plant extracts, it is essential to re-evaluate their therapeutic properties as promising antibacterial agents confer less resistance and ease of extraction and application. Therefore, the present study was performed to screen the antibacterial activities of acetone, methanol, and aqueous extracts from each of the medicinal plants: clove, black seed, and myrrh against *S. aureus*, *Bacillus subtilis*, *E. coli*, and *P. aeruginosa* using disc diffusion technique in an attempt to offer new opportunities for developing new alternative natural antibacterial agents.

MATERIALS AND METHODS

Plant collection and extract preparation

All plants were purchased from the local herb market in Riyadh, Saudi Arabia. The botany department at King Saud University further identified them.

Each sample was washed, air-dried at room temperature, and crushed into small pieces prior to extraction. 10 g of each crushed sample were extracted separately in 100 mL of absolute acetone and absolute methanol and sterilized distilled water at room temperature, with agitation at 160 rpm for 48 hours. All filtrates were separated from the solid residues using the Whatman No. 1.5 filter papers and were pre-concentrated using the rotary evaporator to yield the crude extracts. The crude extracts were then dissolved in 15 mL Dimethyl sulfoxide (DMSO); they were sterilized using 0.45µm pore size, syringe Millipore (Millipore, USA), and stored at 4 ℃ in sterile tubes until analysis.

Bacterial isolates culture

Four bacterial clinical isolates were acquired from King Khaled University Hospital, Riyadh, Saudi Arabia. They were as follows: two Gram-positive, *Bacillus subtilis* (clinical isolate) and *staphylococcus aureus* ATCC 29213, and the other two were Gram-negative: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. Isolates were pre-cultured on nutrient agar plates (Oxoid, USA). Plates were incubated at 37℃ for 18-24 hrs.

In vitro antibacterial assay

The antimicrobial activity of the extracts against the bacterial isolates in the study was investigated using the agar well diffusion technique. 0.5 MacFarland bacterial suspension of each tested strain was prepared in 5mL nutrient broth. With a sterile cotton swab, each bacterial suspension was spread on the surface of Muller Hinton agar plates (Oxoid, USA). Following inoculation, holes were performed with a sterile cork borer (6 mm), and then 100 μL of each extract, with a final concentration of 66 µg/mL, were loaded into the holes correspondingly. The standard antibiotic Erythromycin (15µg) (Oxoid, USA) was used as a positive control for comparison. All plates were incubated at 37°C for 18–24 hours. The inhibition zones were observed and recorded in mm as average inhibition zones. Experiments were done in triplicate.

RESULTS AND DISCUSSION

These different extracts in the study showed variable efficacy against the four tested bacterial strains; their inhibitory activity was not consistent against all the bacteria. The organic extracts had the highest activity when compared to the aqueous extract. The observed zones of inhibition from different extracts were measured and confirmed using Erythromycin (15µg) (standard antibiotic) as a positive control **(Table 1)**.

Table 1. Antibacterial inhibition zone

Bacterial isolates	Plant Extracts Average inhibition zone (mm) ± SD									Standard antibiotic
	Clove			Black seed			Myrrh			Erythromycin (15µg)
	A	M	Aq	A	M	Aq	A	M	Aq	
Gram-positive										
S. aureus ATCC *29213*	13 ± 0	22 ± 1.41	8 ± 0	10.5 ± 3.53	9.5 ± 2.12	6 ± 0	13 ± 2.82	8.5 ± 4.24	6 ± 0	24 ± 1.41
Bacillus subtilis	12.5 ± 0.70	18.5 ± 2.12	6 ± 0	19.75 ± 9.54	15.75 ± 8.31	6 ± 0	13 ± 7.07	19.25 ± 15.90	11.5 ± 0.70	18.75 ± 3.88
Gram-negative										
E. coli ATCC 25922	24 ± 1.41	25.5 ± 2.21	17.5 ± 2.12	12.5 ± 0.70	9.5 ± 4.94	6 ± 0	16.25 ± 3.81	12 ± 0	7 ± 1.41	6 ± 0
P. aeruginosa ATCC 27853	23 ± 0	19.5 ± 0.70	18 ± 0	6 ± 0	8.5 ± 3.53	6 ± 0	6 ± 0	6 ± 0	6 ± 0	15.5 ± 3.53

A: Acetone extract
M: Methanol extract
Aq: Aqueous or water extract

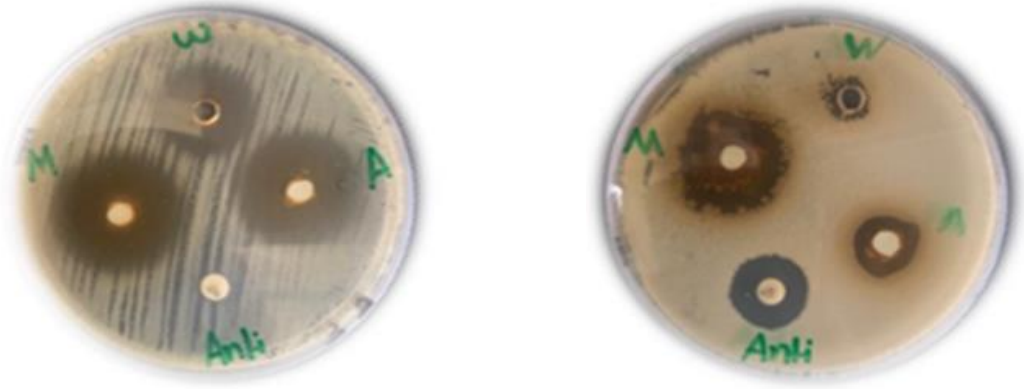
These different plant solvent extracts, as observed in the present study, showed a broad spectrum and significantly different activity, inhibiting both Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *B. subtilis*) **(Figures 1 and 2)**.

Myrrh acetonic extract showed the most efficient antibacterial activity against *E. coli*, followed by *S. aureus*, *B. subtilis*, then *P. aeruginosa* (16.25 ± 3.18 mm, 13 ± 2.82 mm, 13 ± 7.07 mm, 6 ± 0 mm) respectively. Whereas myrrh methanolic extract indicated higher activity against *B. subtilis* (19.25 ± 15.9 mm), then *E coli* (12± 0 mm), followed by *S. aureus* (8.5 ± 4.24 mm), the least activity was observed on *P. aeruginosa* (6 ± 0 mm). The myrrh aqueous extract, on the contrary, showed the least inhibitory effect recorded in the following order: *B. subtilis* > *E. coli*> *S. aureus* > *P. aureus* with inhibition zones of (11.5 ± 0.70 mm, 7 ± 1.41 mm, 6 ± 0 mm, 6 ± 0 mm) respectively.

Similar to the myrrh methanolic extract, black seed acetonic extract was the most effective extract against *B,* then *E coli, S, and P* with inhibition zones 19.75 ± 9.54 mm, 12.5 ± 0.70 mm, 10.5 ± 3.53 mm, 6 ± 0 mm respectively. The methanolic extract inhibited mostly the Gram-positive strains *Bacillus* and *S. aureus* (15.75 ± 8.13 mm, 9.5 ± 2.12 mm); less activity was demonstrated with *E. coli* followed by *Pseudomonas* (9.5 ± 4.94 mm, 8.5 ± 3.53 mm). Again, the aqueous extract showed negligible inhibition with a diameter of inhibition zones equal to 6 ± 0 mm against all tested organisms.

On the other hand, clove methanolic extract showed promising results indicated by the maximal inhibitory activity against *E. coli* (25.5 ± 2.12 mm) followed by *Staphylococcus* (22 ± 1.41 mm), *Pseudomonas (*19.5 ± 0.70 mm), and *Bacillus* (18.5 ± 2.12 mm). Subsequently, clove acetonic extract exhibited the second most effective inhibition against the Gram-negative strains *Escherichia coli* and *Pseudomonas* (24 ± 1.41 mm, 23 ± 0 mm), and then against *Staphylococcus*, ending with *Bacillus* (13 ± 0 mm, and 12.5 ± 0.70 mm) correspondingly. Similarly, the aqueous extract exhibited the least inhibitory effect among the other extracts. A high effect was noted on Gram-negative bacteria *Pseudomonas Escherichia coli,* followed by *Staphylococcus* and *Bacillus* (18 ± 0 mm, 17.5 ± 2.12 mm, 8 ± 0 mm, and 6± 0 mm).

Erythromycin exhibited a considerably high inhibition efficacy, notably against Gram-positive bacteria *S. aureus* and *B. subtilis* with 24 ± 1.41 mm and 18.75 ± 3.88 mm inhibition diameters, respectively, followed by *P. aeruginosa* and *E. coli* (8.5 ± 3.53 mm, and 6 ± 0 mm).



a) b)

Figure 1. Agar well diffusion of various *Syzygium aromaticum* extracts against (a) *Escherichia coli* and (b) *Bacillus subtilis*. The large inhibition zone diameter indicates significant antibacterial activity compared to the standard antibiotic disc Erythromycin. (M) Methanolic extract, (A) acetonic extract, (W) aqueous extract, and Erythromycin disc.

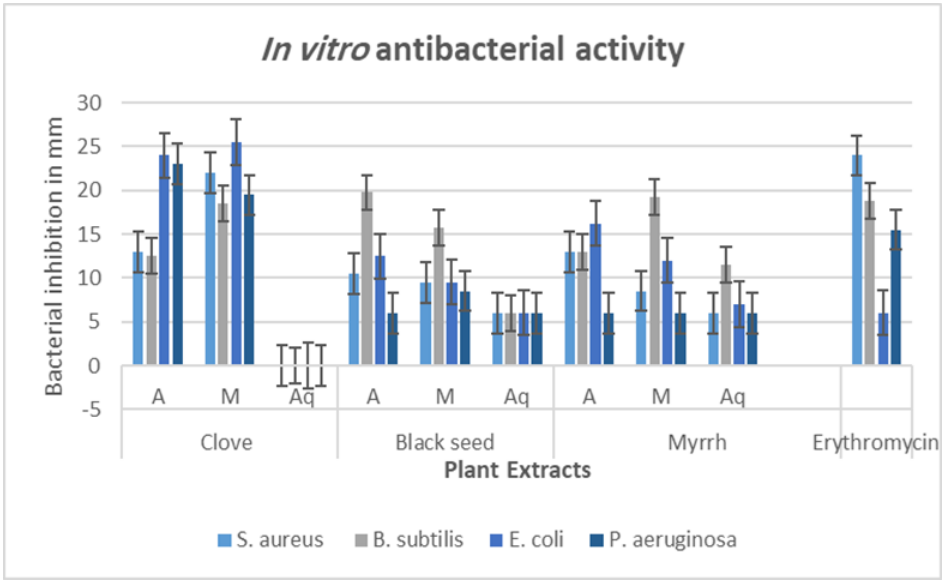


Figure 2. *In vitro* antibacterial assay of the plant extracts against the tested bacteria. Variable antibacterial activity was observed with the different extracts. Clove extracts, acetone, methanol, and aqueous indicated the highest antibacterial activity. Both clove methanolic and acetone extracts inhibited mostly *E. coli*, and its aqueous extract was the only one to reveal a potent antibacterial activity against all tested bacterial strains compared to other water extracts. Data were compared to the positive control antibiotic disc.

This variability in the medicinal extracts' antimicrobial activity was linked to the major phyto-constituents present in varying amounts as well as to the complex interaction between those components and other components present in the extracts. Phytoconstituents, together with the lipophilic products, can interact with the bacterial membranes, causing their disruption to affect membrane fluidity and permeability as well as respiratory rate in all bacterial strains [16]. Literature reports also revealed that the difference in the antibacterial activity depends on either the extraction technique employed or the structure and polarity of the bioactive components in the extracts [17-19]. As evidenced by earlier investigations [20, 21], plant methanol and aqueous extracts contained therapeutic active components such as alkaloids, tannins, phenols, and flavonoids or other phytochemicals recognized as potent antimicrobial agents, and thus, their synergistic activity against pathogenic microorganisms can be a defense.

It is well established that the organic solvent methanol can efficiently extract the most active plant components, such as polyphenols or flavonoids, compared to water, despite its lower polarity [22]. This agrees with the present study, where higher antibacterial activities were observed with the solvent extracts than with water, which demonstrated poor or negligible inhibition, except for the myrrh aqueous extract, which revealed a moderate

inhibition activity. In addition, it was demonstrated that even though water is a universal solvent widely used by traditional healers, organic solvents such as acetone, methanol, and ethanol are known to be more efficient in extracting the major bioactive plant constituents and, hence, have more consistent antimicrobial activity than water. In the present study, clove acetone and methanol extracts were observed with the highest inhibition activity mainly against the Gram-negative bacteria in the following order: *E. coli >P. aeruginosa> S. aureus > B. subitilis*, rendering them as such potent therapeutic agents with their ability to disturb the Gram-negative cell wall known for increasing the bacterial resistance.

CONCLUSION

Evaluation of the medicinal plants extracts *Nigella sativa* (black seed), *Syzygium aromaticum* (Clove), and *Commiphora myrrha* (myrrh) methanolic, acetonic, and aqueous extracts antibacterial activities against S. aureus, B. subtilis, E. coli, and P. aeruginosa revealed different degree of efficacy due to the presence of different phytoconstituents in variable amount. Clove solvent extracts revealed the highest antibacterial activities against the tested bacterial strains. Water extracts showed negligible effect on all bacteria, except for myrrh aqueous extract. Therefore, all these medicinal plant extracts can be used as promising natural therapeutic antibacterial agents alternative to the standard antibiotics available in the market.

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