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In vitro antibacterial and synergistic effects of some plant extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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Abbreviations:
S.: Syzygium, C.: Commiphora, A.: Allium, S.: Staphylococcus, K.: Klebsiella, K.K.H.: KingKhalid Hospital, NCCLS: National Committee for Clinical Laboratory Standards, IZD: Inhibition Zone Diameter, mm: Millimeter, ANOVA: Analysis of Variance, SPSS: Statistical Package for the Social Sciences

Keywords:
Antibacterial, Synergism, Plant extract, *Syzygium aromaticum, Commiphora molmol, Allium sativum, Staphylococcus aureus, Klebsiella pneumoniae*

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Abstract
Discovery of new antibacterial agent represents a revolution in the world of antimicrobials to face increase of bacterial resistance to conventional antibiotics. Plants considered an important potential source of new antibacterial agents. There fore, the growth inhibitory effect of water and ethanolic extracts of *Syzygium aromaticum, Commiphora molmol* and *Allium sativum* and their synergism with antibiotics against *Staphylococcus aureus* and *Klebsiella pneumoniae* were evaluated. Agar well diffusion method and disk diffusion technique were used for antimicrobial assay and evaluation of synergistic effect respectively. Ethanolic extracts of the three tested plants exhibited growth inhibitory effect against tested bacteria. The results indicated that water extract of *Syzygium aromaticum* presented greater inhibitory effect on *Staphylococcus aureus* than water extract of *Allium sativum* whereas water extract of *Commiphora molmol* did not show any antibacterial activity. The growth of *Klebsiella pneumoniae* was not inhibited by the water extracts of all tested plants. Synergistic effect against tested bacteria was showed when water and ethanolic plant extracts combined with antibiotics. We are of the opinion that ethanolic extracts of *Syzygium aromaticum, Commiphora molmol* and *Allium sativum* alone and in combination with antibiotics presented a new choice for treatment of microbial infection especially the tested bacteria. Further work is needed to isolate, identify and characterize the active principles from plants and test their antibacterial and synergistic effects with antibiotics against microorganisms.

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1. Introduction

Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major challenge for the healthcare industry (Neethu et al., 2013). Indiscriminate use of antibiotics promotes antibiotic resistance leading to multiple drug resistance and makes it difficult (Khanuja et al., 2007). *Staphylococcus aureus* can induce many diseases, such as mastitis, skin disease and gastroenteritis due to the ingestion of enterotoxins (Reinhardt et al., 2013; Moran et al., 2013; Hyeon et al., 2013) and are often associated with antibiotic resistance. Another species that has been shown to be antibiotic resistant is *Klebsiella pneumoniae* (Tenover, 2006). The appearance of new antibiotic-resistant bacteria is a societal problem that requires the development of new alternative treatments (Willer et al., 2013). For developing a cheap broad-active agent that can be applicable against different pathogens, it is necessary to develop an alternative source for normal antibacterial agents (Neethu et al., 2013). Herbs and spices have been used for generations by humans as food and to treat ailments. Scientific evidence is accumulating

that many of these herbs and spices do have medicinal properties that alleviate symptoms or prevent disease (Lai and Roy, 2004). Herbal medicine is still the most common source for primary health care of about 65-80% of the world's population, mainly in developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects (Amal et al., 2010). Clove (*Syzygium aromaticum*) constitutes one of the major spices. Cloves are dried unopened floral buds of an evergreen tree, *Syzygium aromaticum* belonging to the family Myrtaceae (Shyamala et al., 2003). Clove is used as flavoring agent and as spice for scenting, chewing tobacco. It is aromatic, stimulant & carminative, used for dyspepsia and gastric irritations. Clove buds and their essential oils have been known to possess various antimicrobial and antioxidant properties (Fu et al., 2007). Garlic (*Allium sativum*) is a common spice used for flavoring and has been traditionally popular with strong folkloric awareness. It is the edible bulb of lily family, Liliaceae. It contains aromatic sulphur based compounds, which contribute to the characteristics odour and taste. Antimicrobial activity of garlic is attributed to its key component allicin, which is a volatile molecule, gives garlic its characteristic odour. Allicin is unstable; once it is generated it readily decomposes to produce diallyl sulphide, dialyl disulphide, diallyl trisulphide, allyl methyl trisulphide, dithiins and ajoene (Jabar and Al-Mossawi, 2007). *Commiphora molmol* (myrrh) has been in use in traditional medicine in many Arab countries for ages. *C. molmol* is originally found in Northern Africa, Arabia and Northern Somalia (Hanus et al., 2005). Early Muslim writers recorded many medicinal uses for *C. molmol*. It has been used to treat wounds, intestinal disorders, diarrhoea, cough and chestaliments (Ghazanfar, 1994). One of the possible ways to reduce drug dosage is synergism between two therapeutic agents that is combination therapy (Khanuja et al., 2007). However, few studies on synergism have been carried out (Cruz et al., 2007).

2. Objectives of Research

The aim of this study was the evaluation of antibacterial activity and synergistic effects of three plant extracts with eight antibiotics on *Staphylococcus aureus* and *Klebsiella pneumoniae*.

3. Materials and Methods

There was an immense need to search for new antibacterial agents from natural sources, hence the water and ethanolic extracts of 3 plants were evaluated for their antibacterial and synergistic activity on *S. aureus* and *K. pneumoniae*. The plant extracts were prepared in laboratory of Pharmacognosy, Pharmacy College, Najran University. Confirmation of isolates identification, antibacterial assays, antimicrobial susceptibility pattern and evaluation of the synergistic effect of plant extracts with antibiotics on *Staphylococcus aureus* and *Klebsiella pneumoniae* were conducted in Microbiology laboratory, Department of Applied Medical Sciences, Community College, Najran University during January to November 2013.

3.1 Source of bacterial isolates and culture media

In this study, pathogenicbacterial isolates, *S. aureus* and *K. pneumoniae* were obtainedfrom the Microbiology laboratory, King Khalid Hospital (K.K.H), Najran region, Saudi Arabia. Najran is a region of Saudi Arabia, located in the south of the country along the border with Yemen. It has an area of 119,000 km². Its capital is Najran. The organisms were identified by an automated system (Micro Scan Walkaway, Siemens) and the results were confirmed (Koneman et al., 1992). The isolates were maintained onagar slant at 4° C and subcultured on afresh appropriate agar plates 24 hrs. prior toany antimicrobial test. Brain Heart Infusion broth (Oxoid, England), Mueller Hintonagar (Oxoid, England) and Nutrient agar (Oxoid, England) were used in this study. All media were prepared according tomanufacture recommendations.

3.2 Plant materials

The plant samples used in this study were flowers of *Syzygium aromaticum* (clove), unorganized part of *Commiphora molmol (myrrh)* and bulbs of *Allium sativum* (garlic). Samples were purchased from markets distributed in Najran region, Kingdom of Saudi Arabia, during October 2012. The collected plant materials were dried in an incubator at 37° C and stored at room temperature before the experiments.

3.3 Preparation of extracts

The three plant samples were ground into fine powders using an electric blender and the extracts were prepared by soaking 125 gm. of each sample separately into 500 ml of solvents (distilled water and 80% ethanol) using conical flasks plugged with cotton plugs, the mixtures were kept at room temperature for 72 hrs. under discontinuous shaking. The crude extracts were filtered through sintered

glass funnel (500 ml) under vacuum, the filtrates were evaporated for dryness by rota-vapour (Buchi, R-215, Switzerland), the rotary water bath was adjusted to 55° C, then the extracts were kept overnight under vacuum fume hood to obtain a constant dry weight and the extracts stored in closed amber vessels at 4° C in refrigerator for further use. The dried extracts were weighed and dissolved according to the solvent type (distilled water and 80% ethanol) at a concentration of 50 mg/ml.

3.4 Antimicrobial assays the growth inhibitory effect was determined by the agar well diffusion method according to NCCLS (1993). The bacterial cultures were grown in Brain Heart Infusion broth at 37° C. After 4 hrs of growth, each microorganism, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated by streaking the swab over the entire surface of Mueller Hinton agar plates. After allowing the inocula to dry at room temperature, the medium was punched with six millimetres diameter wells. Each extract was checked for growth inhibitory effect by introducing 10 µl into the well and allowed to diffuse at room temperature for 20 minutes. The plates were incubated aerobically at 37° C for 24 hrs. and inhibition zone diameter (IZD) formed around the wells were measured (mm) using a ruler. All tests were done in triplicate and the growth inhibitory effect of plant extracts was recorded.

3.5 Bacterial isolates resistance

Antimicrobial susceptibility pattern was performed using disk diffusion method (Bauer et al., 1966). The method measures microbial growth inhibition at the surface of an inoculated medium around paper disks of various antibiotics. Eight antibiotics were tested in this investigation (Table 1). Each microorganism, at a concentration of 1.5×10^6 cells/ml (adjusted to the 0.5 McFarland turbidity standards) was inoculated by streaking the swab over the entire surface of Mueller Hinton agar plates. The plates were allowed to dry for 5 minutes. Thereafter, all discs were placed on the surface of the plates and pressed gently to ensure complete contact with agar. A distance of at least 15 mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. After 15 minutes of placing disks, the plates were incubated for 24 hrs at 37° C. The assessment of bacterial resistance was based on the measurement of the diameter of the inhibition zone (mm) formed around the antibiotic disk. The experiment was repeated in triplicates for each isolate.

3.6 Evaluation of the synergistic effect

The isolates at a concentration of 1.5 × 106 cells/ml (adjusted to the 0.5 McFarland turbidity standards) were inoculated onto the surface of Mueller Hinton agar plates. Subsequently, the antibiotic disks of 6 mm in diameter saturated with 10 µl plant extract were placed on the surface of each inoculated plate. The plates were incubated for 24 hrs. at 37° C. The diameters of clear zones were measured and compared with that of the antibiotic alone (Betoni et al., 2006).

3.7 Statistical analysis

All assays were performed in triplicate in three independent experiments. Data analysis results were expressed as means ± S.E. (Standard Error) and differences between means were analyzed statistically using an analysis of variance (ANOVA) according to Tukey, s test through SPSS 15.0 software package in Microsoft Windows 7.0 operating system. Differences are considered significant when $p \leq 0.05$.

4. Results

4.1 The antibacterial and synergistic effect of water plant extracts on S. aureus

The results of growth inhibitory effect of the three plant extracts and their synergism with different antibiotics on *S. aureus* were presented in Table 2. Our results showed that the water extracts of *Syzygium aromaticum* (clove)and bulbs of *Allium sativum* (garlic) exhibited growth inhibitory effect against *S. aureus* with IZDs ranged from 16.00±0.58 mm-9.00±0.58 mm. Water extract of *Commiphora molmol (myrrh)* did not show any inhibitory effect against the tested microorganism with IZD 00.00±.00 mm. *S. aureus* was sensitive tosulphamethoxazole + trimethoprim, levofloxacin, amikacin, amoxicillin + clavulanic acid and nitrofurantoin with IZDs ranged from 29.67±0.88 mm -11.00±0.58 mm. It was observed that *S. aureus* was resistant to imipenem, pipracillin + tazobactam and erythromycin. The results revealed that the water extracts of *Syzygium aromaticum* and *Allium sativum* had a synergistic effect with different antibiotics and were able to suppress the *S. aureus* growth. Water extract of *Commiphora molmol* (myrrh) with imipenem, pipracillin + tazobactam and erythromycin had no synergistic effect against *S. aureus* with IZD 00.00±.00 mm and synergism was showed with the remaining antibiotics with

Table 1: List of antibiotics

Antibiotic	Symbol	Potency(µg)	Company
Imipenem	IPM	10	Oxoid
Sulphamethoxazole (23.75 µg)+ Trimethoprim(1.25 µg)	SXT	25	Oxoid
Levofloxacin	LEV	5	Oxoid
Amikacin	AK	30	Oxoid
Amoxicillin + Clavulanic acid (2:1)	AMC	30	Oxoid
Pipracillin (30 µg) + Tazobactam (10 µg)	TZP	40	Oxoid
Erythromycin	E	15	Oxoid
Nitrofurantoin	F	50	Oxoid

Table 2: Antibacterial and synergistic effect of 3 water plant extracts against *S. aureus* (all values in mm)

Antibiotic	Antibiotic alone*	*S. aromaticum*		*C. molmol*		*A. Sativum*	
		Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*
Imipenem	00.00 ±.00** [a]	16.00[a]±0.58	16.67[a]±0.67	00.00±.00	00.00[a]±.00	9.00[a]±0.58	18.00[b]±0.58
Sulphamethoxazole+ Trimethoprim	17.00 ±0.58 [c]	16.00[a]±0.58	22.00[b]±0.58	00.00±.00	23.00[c]±0.58	9.00[a]±0.58	25.00[c]±0.58
Levofloxacin	11.00 ±0.58 [b]	16.00[a]±0.58	18.00[a]±0.58	00.00±.00	11.67[b]±0.88	9.00[a]±0.58	10.33[a]±0.88
Amikacin	17.00 ±0.58 [c]	16.00[a]±0.58	24.67[b]±0.88	00.00±.00	23.00[c]±0.58	9.00[a]±0.58	19.33[b]±0.88
Amoxicillin + Clavulanic acid	29.67 ±0.88 [d]	16.00[a]±0.58	33.33[c]±0.67	00.00±.00	31.00[d]±0.58	9.00[a]±0.58	40.33[d]±0.67
Pipracillin + Tazobactam	00.00 ±.00 [a]	16.00[a]±0.58	16.67[a]±0.88	00.00±.00	00.00[a]±.00	9.00[a]±0.58	8.33[a]±0.33
Erythromycin	00.00 ±.00 [a]	16.00[a]±0.58	17.33[a]±0.88	00.00±.00	00.00[a]±.00	9.00[a]±0.58	8.67[a]±0.33
Nitrofurantoin	18.67 ±0.33 [c]	16.00[a]±0.58	16.33[a]±0.88	00.00±.00	24.67[c]±0.88	9.00[a]±0.58	23.33[c]±0.33
F- value	507.43	0.00	60.54	-	517.86	0.00	307.29

* Values are the mean of three replicates ± S.E.
** In the same column, means followed by the same letters are not significantly different (p≤0.05) as analyzed by Tukey's HSD test. F-value is significant at p≤0.001.

Table 3: Antibacterial and synergistic effect of 3 ethanolic plant extracts against *S. aureus* (all values in mm)

Antibiotic	Antibiotic alone*	*S. aromaticum*		*C. molmol*		*A. Sativum*	
		Extract alone*	Extract +Antibioti*	Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*
Imipenem	00.00 [a] ±.00**	21.00 ±0.58 [a]	32.00d±0.58	17.67 ±0.33 [a]	18.33 [a] ±0.33	10.33 ±0.33 [a]	13.00[ab]±0.58
Sulphamethoxazole + Trimethoprim	17.00 [c] ±0.58	21.00 ±0.58 [a]	25.67c±0.88	17.67 ±0.33 [a]	22.00[ab] ±1.15	10.33 ±0.33 [a]	22.33[c]±0.33
Levofloxacin	11.00 [b] ±0.58	21.00 ±0.58 [a]	22.00ab±0.58	17.67 ±0.33 [a]	19.67 [a]±0.88	10.33 ±0.33 [a]	14.67[b]±0.88
Amikacin	17.00 [c] ±0.58	21.00 ±0.58 [a]	21.00a±0.58	17.67 ±0.33 [a]	25.00[b]±0.58	10.33 ±0.33 [a]	23.67[c]±0.88
Amoxicillin + Clavulanic acid	29.67 [d] ±0.88	21.00 ±0.58 [a]	36.00e±0.58	17.67 ±0.33 [a]	35.00[c]±0.58	10.33 ±0.33 [a]	33.00[d]±0.58
Pipracillin+ Tazobactam	00.00 [a] ±.00	21.00 ±0.58 [a]	24.67bc±0.88	17.67 ±0.33 [a]	19.33 [a] ±0.88	10.33 ±0.33 [a]	13.00[ab]±0.00
Erythromycin	00.00 [a] ±.00	21.00 ±0.58 [a]	23.00abc±0.58	17.67 ±0.33 [a]	20.33 [a] ±0.88	10.33 ±0.33 [a]	11.33[a]±0.33
Nitrofurantoin	18.67 [c] ±0.33	21.00 ±0.58 [a]	39.67f±0.88	17.67 ±0.33 [a]	34.67[c]±0.88	10.33 ±0.33 [a]	30.67[d]±0.88
F- value	507.43	0.00	97.91	0.00	71.17	0.00	178.36

* Values are the mean of three replicates ± S.E.
** In the same column, means followed by the same letters are not significantly different (p≤0.05) as analyzed by Tukey's HSD test. F-value is significant at p≤0.001.

Table 4: Antibacterial and synergistic effect of 3 water plant extracts against *K. pneumoniae* (all values in mm)

Antibiotic	Antibiotic alone*	*S. aromaticum*		*C. molmol*		*A. Sativum*	
		Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*
Imipenem	31.00[e]±0.58**	00.00±0.00	36.00[d]±0.58	00.00±0.00	36.33[d]±0.88	00.00±0.00	37.00f±0.58
Sulphamethoxazole +	00.00[a]±.00	00.00±0.00	00.00[a]±0.00	00.00±0.00	00.00[a]±0.00	00.00±0.00	00.00[a]±0.00

Trimethoprim							
Levofloxacin	10.00^{b}±0.58	00.00±0.00	12.00^{b}±0.58	00.00±0.00	11.00^{b}±0.58	00.00±0.00	11.00^{b}±0.58
Amikacin	20..67^{d}±0.33	00.00±0.00	21.33^{c}±0.33	00.00±0.00	21.00^{c}±0.00	00.00±0.00	23.00^{d}±0.58
Amoxicillin + Clavulanic acid	17.33^{c}±0.33	00.00±0.00	22.00^{c}±1.15	00.00±0.00	20.00^{c}±0.58	00.00±0.00	25.00^{e}±0.58
Pipracillin + Tazobactam	17.33^{c}±0.33	00.00±0.00	20.00^{c}±0.58	00.00±0.00	21.00^{c}±0.58	00.00±0.00	19.00^{c}±0.58
Erythromycin	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00
Nitrofurantoin	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00	00.00±0.00	00.00^{a}±0.00
F- value	1061.69	-	574.94	-	790.00	-	927.00

*Values are the mean of three replicates ± S.E.
** In the same column, means followed by the same letters are not significantly different (p≤0.05) as analyzed by Tukey's HSD test. F-value is significant at p≤0.001.

Table 5: Antibacterial and synergistic effect of 3 ethanolic plant extracts against *K. pneumoniae* (all values in mm)

Antibiotic	Antibiotic alone*	*S. aromaticum*		*C. molmol*		*A. Sativum*	
		Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*	Extract alone*	Extract +Antibiotic*
Imipenem	31.00^{e}±0.58**	14.00^{a} ±0.58	35.00^{d}±0.58	11.33^{a} ±0.33	37.00^{d}±0.58	10.67^{a} ±0.33	34.67^{d}±0.33
Sulphamethoxazole + Trimethoprim	0.00^{a}±.00	14.00^{a} ±0.58	14.00^{a}±0.58	11.33^{a} ±0.33	11.67^{a}±0.33	10.67^{a} ±0.33	12.33^{a}±0.33
Levofloxacin	10.00^{b}±0.58	14.00^{a} ±0.58	13.33^{a}±0.33	11.33^{a} ±0.33	13.67^{a}±0.88	10.67^{a} ±0.33	12.00^{a}±0.58
Amikacin	20..67^{d}±0.33	14.00^{a} ±0.58	23.67^{c}±0.88	11.33^{a} ±0.33	24.00^{c}±0.58	10.67^{a} ±0.33	23.33^{c}±0.88
Amoxicillin + Clavulanic acid	17.33^{c}±0.33	14.00^{a} ±0.58	20.33^{b}±0.88	11.33^{a} ±0.33	20.67^{b}±0.33	10.67^{a} ±0.33	22.00^{b}±0.58
Pipracillin + Tazobactam	17.33^{c}±0.33	14.00^{a} ±0.58	18.00^{b}±0.58	11.33^{a} ±0.33	23.00^{c}±0.58	10.67^{a} ±0.33	20.00^{b}±0.58
Erythromycin	00.00^{a}±0.00	14.00^{a} ±0.58	14.00^{a}±0.58	11.33^{a} ±0.33	11.67^{a}±0.33	10.67^{a} ±0.33	13.00^{a}±0.58
Nitrofurantoin	00.00^{a}±0.00	14.00^{a} ±0.58	14.00^{a} ±0.58	11.33^{a} ±0.33	11.67^{a}±0.33	10.67^{a} ±0.33	11.33^{a}±0.33
F- value	1061.69	0.00	132.91	0.00	286.63	0.00	215.56

* Values are the mean of three replicates ± S.E.
** In the same column, means followed by the same letters are not significantly different (p≤0.05) as analyzed by Tukey's HSD test. F-value is significant at p≤0.001.

IZDs ranged from 31.00±0.58 mm - 11.67±0.88 mm.

4.2 The antibacterial and synergistic effect of ethanolic plant extracts on S. aureus

The ethanolic extracts of *Syzygium aromaticum*, *Commiphora molmol (myrrh)* and *Allium sativum* exhibited growth inhibitory effect against *S. aureus* with IZDs ranged from 21.00 ±0.58 mm - 10.33 ±0.33 mm (Table 3). It was observed that the three ethanolic plants extract exerted synergistic effect with the various antibiotics against *S. aureus*.

4.3 The antibacterial and synergistic effect of water plant extracts on K. Pneumonia

The growth inhibitory effect of the three water plant extracts and their synergism with different antibiotics against *K. pneumonia* were presented in Table 4. The present findings revealed that the three water extracts did not show any inhibitory effect against *K. pneumoniae* with IZD 00.00±0.00 mm. *K. pneumoniae* was sensitive toimipenem, levofloxacin, amikacin, amoxicillin + clavulanic acid and pipracillin + tazobactam with IZDs ranged from 31.00±0.58 mm - 10.00±0.58 mm. The present study recorded that *K. pneumoniae* was resistant to sulphamethoxazole + trimethoprim, erythromycin and nitrofurantoin. Water extracts of tested plants exerted synergistic effect against *K. pneumoniae* with imipenem, levofloxacin, amikacin, amoxicillin + clavulanic acid and pipracillin + tazobactam with IZDs ranged from 37.00±0.58 mm -11.00±0.58 mm.

4.4 The antibacterial and synergistic effect of ethanolic plant extracts on K. pneumoniae

The results showed that the ethanolic extracts of *Syzygium aromaticum*, *Commiphora molmol* and *Allium sativum* had inhibitory effect against *K. pneumoniae* with IZDs ranged from

14.00±0.58 mm -10.67±0.33 mm. Ethanolic extracts of the all tested plants exhibited synergistic effect with different antibiotics on *K. pneumoniae* with IZDs from 37.00±0.58 mm to 11.33±0.33 mm (Table 5).

5. Discussion

According to the World Health Organization, infectious diseases are a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries (WHO, 2003). Plants have always played a central role in traditional systems of medicine for the prevention and treatment of disease around the world (Mahady, 2001). In this study, extraction was done using water and 80% ethanol. Chemical content of plant extracts differs depending on the nature of the solvent used in the extraction procedure (Jules et al., 2011). The ethanolic extracts of the tested plants exhibited the highest antibacterial activity against *S. aureus*. Similar to our results, Anna et al. (2013) found that ethanol extracts of clove showed relatively strong antimicrobial activities against all bacteria tested. In the present study, water extract of *Syzygium aromaticum* had greater growth inhibitory effect against *S. aureus* than *Allium sativum*. Our findings agree with other observations (Gislene et al., 2000; Joyce et al., 2006; Neogi et al., 2007; Priscila et al., 2007; Vuddhakul et al., 2007; Lee et al., 2011; Jehan et al., 2011). *S. aureus* was resistant to water extract of *Commiphora molmol (myrrh)*. This result contradicted with those reported by (Emad et al., 2009). They reported that water extract of *Commiphora molmol (myrrh)* exhibited antibacterial activity against *S. aureus* and MRSA strains. The growth of *S. aureus* was inhibited when clove andgarlic water extracts were combined with all tested antibiotics and the highest synergistic effect was observed when *Allium sativum* water extract associated with amoxicillin + clavulanic acid. These results were consistent with the results previously cited by (Gislene et al., 2000; Gupta et al., 2009) and disagree with the findings reported by (Betoni et al., 2006). No synergistic effect was observed when myrrh water extract associated with non-effective antibiotics against *S. aureus*. In the present investigation, ethanolic extract of clove exerted the highest growth inhibitory effect against *S. aureus* followed byethanolic extract of *Commiphora molmol (myrrh)* then *Allium sativum* ethanolic extract. This result was supported by the results previously reported by (Ram et al., 2010). The growth of *S. aureus* was inhibited when ethanolic extracts of tested plants were combined with effective and non-effective antibiotics. Synergistic effects resulting from the combination of antibiotics with extracts were documented by (Ikram and Inamul, 1984; Tarek et al., 2011). Our findings indicated that *K. pneumoniae* was resistant to water extracts of the all tested plants. Anna et al. (2013) revealed that water extracts displayed little or no antimicrobial activity against the tested microorganisms. These results contradicted with those reported by (Jehan et al., 2011; Meriga et al., 2012) who reported that aqueous extract of *A. sativum* exhibited antibacterial activity against *K. pneumoniae*. The growth of *K. pneumoniae* was inhibited when water extracts of the tested plants combined with imipenem, levofloxacin, amikacin, amoxicillin + clavulanic acid and pipracillin + tazobactam. In contrast, no synergistic effect was detected when water extracts of the three plants associated with sulphamethoxazole + trimethoprim, erythromycin and nitrofurantoin. These findings were supported by the results previously recorded by (Gislene et al., 2000). The present study showed that the growth of *K. pneumoniae* was inhibited by ethanolic extracts of all tested plants. Our findings agree with other observations (Sulieman et al., 2007; Ram et al., 2010; Jehan et al., 2011; Omer et al., 2011). Combination of ethanolic extracts of clove, myrrh and garlic with various antibiotics exerted synergistic effect against *K. pneumoniae*. Synergism between plant extracts and antibiotics was documented in previous studies (Stephen et al., 2012; Neethu et al., 2013). Statistical analysis revealed that there was significant difference in the values ($p \leq 0.05$) between the different plant extracts either alone or in combination with antibiotics.

Highlights of Research

Our study aimed to search for new effective antibacterial agents. The ethanolic extract of three tested plants exhibited more promising antibacterial activity against tested bacteria as compared to water extracts. The combination of ethanolic extracts of the tested plants with effective and non-effective antibiotics inhibited the growth of *S. aureus* and *K. pneumoniae*.

Limitations

However, plant extracts exerted antibacterial and synergistic effect with antibiotic on *S. aureus* and *K. pneumoniae*. Further investigations are needed for isolation, identification and characterization of active

principles to obtain compound with more potent antibacterial and synergistic activity.

Justification

This study was designed to explore for new antimicrobial agents from natural sources to obstacle the increase of bacterial resistance to conventional antibiotics. Therefore the water and ethanolic extracts of 3 plants were evaluated for their antibacterial and synergistic activity against *S. aureus* and *K. pneumoniae*.

Conclusion

The obtained data concluded that extracts of *Syzygium aromaticum* (clove), *Commiphora molmol (myrrh)* and *Allium sativum* (garlic) possesses growth inhibitory effect against *S. aureus* and *K. pneumoniae*. The synergism between the tested plant extracts and antibiotics presented a new choice for treatment of microbial infection especially the tested bacteria.

Authors' Contribution

The authors helped in refining and designing of this study. Hani Gouda collected plant materials and prepared Extracts. Elsayed Masoud confirmed identification of bacterial isolates, determined the antibacterial assays, performed antimicrobial susceptibility pattern, evaluated the synergistic effect of plant extracts with antibiotics, drafted and revised the manuscript.

Competing Interest

The authors declare that they have no competing interests.

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