



ORIGINAL ARTICLE

Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms

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Abstract In the present study aqueous and methanol extracts of *Zingiber officinale*, *Curcuma longa*, *Commiphora molmol* and *Pimpinella anisum* were investigated for antimicrobial activity. The microorganisms employed were *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The susceptibility of bacteria strains against the two extracts was determined using the disk diffusion method. The most susceptible micro organisms were *S. pyogenes*, *S. aureus*, while the least susceptible was *E. coli*. Highest antibacterial activity was observed with methanol extract of *C. longa* and *C. molmol* against *S. pyogenes* and *S. aureus* (19 mm) respectively while minimum activity was observed with aqueous extract of *P. anisum* against *E. coli* and *P. aeruginosa* (7 mm). Methanolic extracts of almost all samples dominated aqueous extracts in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to those of kanamycin used as positive controls. Phytochemical analyses revealed the presence of carbohydrates and saponins in all samples. Alkaloids were found in *Z. officinale* and *C. myrrha* whereas flavonoids in *C. longa*, and *P. anisum*. Steroids and tannins were found only in *Z. officinale* and *C. longa*, respectively.

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1. Introduction

For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world. Use of traditional medicine among the tribal local people and medicinal healers (*Hakim*) is

a significant part of Saudi Arabia's tradition and it is widely practiced till date (Al-Essa et al., 1998).

Zingiber officinale, *Curcuma longa*, *Commiphora myrrha* and *Pimpinella anisum* have been used as herbal drugs by local inhabitants from ancient time and even today (Amal, 2010). *Z. officinale* and *C. longa* belonging to the family Zingiberaceae, are most commonly used rhizomes for their medical values. In Ayurveda, *Z. officinale* has been used in the treatment of inflammation. Gingerols and diarylheptanoids present in *Z. officinale* are powerful inhibitors against prostaglandin biosynthesizing enzyme and arachidonate 5-lipoxygenase which results in inhibition of prostaglandin and leukotriene biosynthesis (Kiuchi et al., 1992). *Z. officinale* is known for pain relief (Black et al., 2010), soothing and antibacterial properties and is very helpful in constipations (Hara et al., 1998). *Z. officinale* is very helpful in pregnancy to cure nausea and morning sickness, also it is useful for treating nausea caused by chemotherapy (Ernst and Pittle, 2000), inflammation, rheumatism, cold, heat cramps, and diabetes (Amin-Al, 2006; Afshari, 2007). *C. longa* commonly known as turmeric possesses many therapeutic properties and is used as a remedy for various problems especially in lowering cholesterol and triglyceride levels, inhibiting platelet aggregation, as anti-septic and also possesses anti-inflammatory and antioxidant properties (Luer et al., 2012). It is full of essential oils, yellow pigment, starch and oleoresin (Leung and Foster, 2003). As a strong anti oxidant it employs various protective effects on the gastrointestinal tract, and is very good for the cardiovascular system (Luthra et al., 2001).

C. myrrha commonly known as myrrh is a tree belonging to Burseraceae family. It has been used as a traditional remedy in Arab countries for long time. Originally it was found in Northern Africa, Arabia and Northern Somalia (Hanus et al., 2005). Early Muslim scholars reported many medicinal uses of *Commiphora molmol*. It has been found helpful in treating intestinal disorder, diarrhea, wound healing (Al-Said, 2010), respiratory conjunction (Ghazanfar, 1994). Also found effective for treating fascioliasis (Massoud et al., 2001). It is also used to treat gum diseases (Serfaty and Itid, 1988) and inflammations (Kimura et al., 2001).

P. anisum is a flowering plant belonging to Apiaceae family, originated in India and Southwest Asia. *P. anisum* has been used as a traditional aromatic herb in stimulating digestion and is known for its antiparasitic, antifungal (Soliman and Badea, 2002) and antipyretic (Afifi et al., 1994) antibacterial (Parasa et al., 2012) properties. Its essential oil has been reported for treatment of some disease like seizures and epilepsy (Avicenna, 1988; Abdul-Ghani et al., 1987). Commonly *P. anisum* is used for the treatment of constipation (Chicouri and Chicouri, 2000) and as a muscle relaxant (Albuquerque et al., 1995). Also, its oil is used as an antibiotic substitute in rations for broilers (Mehmet et al., 2005).

The current investigation was carried out to screen the antibacterial activity of four medicinal plants used for herbal treatment by local communities against some pathogenic bacterial strains.

2. Material and methods

2.1. Collection of plant materials

Samples of dry *Z. officinale*, *C. longa*, *C. molmol* and *P. anisum* were collected from a local market in Riyadh. All the samples

were washed and rinsed with distilled water. Samples were dried and crushed using mortar pestle and finally reduced to fine particles using Waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before being used for analysis.

2.2. Microorganisms

Reference bacterial strains were obtained from the Botany Department of King Saud University, which included *Staphylococcus Aureus*, *Streptococcus Pyogenes*, *Escherichia coli* and *Pseudomonas Aeruginosa*. (Clinical isolate). The strains were kept at 4 °C on agar slant and sub cultured at 37 °C for 24 h on nutrient agar (Sigma–Aldrich, Germany) before any susceptibility test.

2.3. Extraction of material

2.3.1. Aqueous extraction

10 g of powdered sample was dissolved in 100 ml of distilled water and boiled for 2 h on slow heat. The residue was removed by filtering through 8 layers of muslin cloth; the filtrate was then centrifuged at 5000g for 10 min. The supernatant was collected and further boiled till the volume was reduced to one-fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml. (Harborne, 1973). It was then autoclaved at 121 °C and at 15 lbs pressure and stored at 4 °C (Jigna and Sumitra, 2007).

2.3.2. Methanol extraction

Ten grams of powdered sample was dissolved in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190–220 rpm for 24 h. The supernatant was collected slowly and evaporated in wide mouthed evaporating bowls at room temperature for 2–3 days till the final volume was reduced to one fourth of the original volume of the solvent used [that was 100 ml] giving the concentration of 400 mg/ml. (Harborne, 1973) and stored at 4 °C in airtight bottles.

2.4. Phytochemical analysis

Phytochemical analysis of all the samples was determined as follows:

2.4.1. Molisch's test for Carbohydrates

Five hundred milligram of powdered sample was taken and dissolved in 5 ml of distilled water and then filtered. Filtrate was added with few drops of Molisch's reagent, followed by addition of 1 ml of conc. H₂SO₄ by the side of the test tube. After two minutes, 5 ml of distilled water was added. Red or dull violet color formation at the interphase of the two layers was taken as positive test (Sofowora, 1993).

2.4.2. Test for alkaloids

100 mg of powdered sample was dissolved in 5 ml of methanol and then filtered. Then 2 ml of filtrate was mixed with 5 ml of 1% aqueous HCl. One milliliter of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent

was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids (Sofowora, 1993).

2.4.3. Liebermann–Burchard test for steroids

200 mg of powder sample was dissolved in 2 ml of acetic acid separately; solutions were cooled followed by the addition of few drops of conc. H₂SO₄. Color development from violet to blue or bluish-green was taken as positive test steroidal ring (Sofowora, 1993).

2.4.4. Test for saponins

One gram of powdered sample was boiled in 10 ml of distilled water and then filtered. 3 ml of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins (Sofowora, 1993).

2.4.5. Shinoda's test for flavonoids

Five hundred milligram of sample was dissolved in 5 ml of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of conc. HCl. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids (Trease and Evans, 2002).

2.4.6. Test for tannins

500 mg of powdered sample was mixed with 10 ml of distilled water and then filtered followed by the addition of few drops of 1% ferric chloride solution. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

2.5. Media preparation

Twenty three grams of nutrient agar (Sigma–Aldrich, Germany) was dissolved in 1000 ml of distilled water and bring to boil.

Agar was then autoclaved for 15 min at 121 °C and left to cool at room temperature. Once the LB medium was cooled (about 45 °C), it was poured into Petri dishes. Each Petri dish was left on the flat surface for 30–40 min until completely set.

2.6. Antimicrobial assay

Antibacterial activity was assayed by disc diffusion method. For all bacteria strains, overnight culture grown in broth was adjusted to an inoculum's density of 100 µl: 0.1A600 culture containing 3.2×10^8 colony forming unit. Further, 20 µl was spread onto 20 ml of sterile agar plates by using a sterile cotton swab. The surface of the medium was allowed to dry for about 3 min. Sterile filter paper discs (5 mm in diameter) impregnated with 100 µl of different test extracts (40 mg/disc) were then sited on the surface of these agar plates. Kanamycin (30 µg/disc) was used as positive control. The plates were then incubated at 37 °C for 24 h for bacteria after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a transparent scale. Each extract was analyzed in triplicate, the mean values are presented. Kanamycin disc (30 µg/disc) was used for comparing the bioassay.

3. Results

Antibacterial activities of the extracts obtained from *Z. officinale*, *C. longa*, *C. molmol* and *P. anisum*, against the tested organisms are shown in Table 1. All the plant extracts tested showed antibacterial activity; however, the plants differ in their activities against the micro-organisms tested. Extracts of *Z. officinale*, *C. longa* and *C. molmol* showed antimicrobial activity against *S. pyogenes*, *S. aureus* and *P. aeruginosa* than against *E. coli*. Highest antibacterial activity was observed with methanol extract of *C. longa* and *C. molmol* against *S. pyogenes* and *S. aureus* (19 mm) respectively while minimum activity was observed with aqueous extract of *P. anisum* against *E. coli* and *P. aeruginosa* (7 mm) (Table 1). Results obtained in the

Table 1 Antimicrobial activity of methanol and aqueous extracts of selected medicinal plants against different microorganisms.

Plant extract	Zone of inhibition			
	Micro organism	AQ	ME	KA
<i>Z. officinal</i>	<i>S. pyogenes</i>	10 ± 0.20	12 ± 0.58	28 ± 0.57
	<i>S. aureus</i>	10 ± 0.33	12 ± 0.70	26.5 ± 0.33
	<i>E. Coli</i>	9 ± 0.88	10 ± 0.23	20 ± 0.33
	<i>P. aeruginosa</i>	14 ± 0.50	12 ± 0.00	25 ± 0.10
<i>C. longa</i>	<i>S. pyogenes</i>	11 ± 0.80	19 ± 0.20	
	<i>S. aureus</i>	11 ± 0.80	15 ± 0.10	
	<i>E. Coli</i>	11 ± 0.55	12 ± 0.65	
	<i>P. aeruginosa</i>	14 ± 0.55	12 ± 0.30	
<i>C. molmol</i>	<i>S. pyogenes</i>	12 ± 0.88	8.5 ± 0.36	
	<i>S. aureus</i>	14 ± 0.66	19 ± 0.41	
	<i>E. Coli</i>	9 ± 0.33	9 ± 0.55	
	<i>P. aeruginosa</i>	12 ± 0.90	13 ± 1.10	
<i>P. anisum</i>	<i>S. pyogenes</i>	10 ± 0.80	8 ± 0.70	
	<i>S. aureus</i>	-	12 ± 0.10	
	<i>E. coli</i>	7 ± 0.88	8 ± 0.55	
	<i>P. aeruginosa</i>	7 ± 0.44	14 ± 0.20	

Values are mean inhibition zone (mm) ± S.D of three replicates.

[AQ-aqueous, ME-methanol and KA-Kanamycin(30 µg/discolor)] [- = no inhibition].

Table 2 Phytochemical analysis of selected plant samples.

Constituents	<i>Z. officinale</i>	<i>C. longa</i>	<i>C. molmol</i>	<i>P. anisum</i>
Carbohydrates	+	+	+	+
Alkaloids	+	–	+	–
Steroids	+	–	–	–
Saponins	+	+	+	+
Flavonoids	–	+	–	+
Tannins	–	+	–	–

Key: + = present; – = absent.

current investigation revealed that studied herbal extracts possess potential antibacterial activity against entire tested organisms, albeit methanol extract was found to have shown the strongest and broadest spectrum. Phytochemical analysis of selected plant samples is shown in Table 2. Carbohydrates and saponins were found in all samples. Alkaloids were found in *Z. officinale* and *C. myrrha* whereas flavonoids in *C. longa*, and *P. anisum*. Steroids and tannins were found only in *Z. officinale* and *C. longa*, respectively (Table 2).

4. Discussion

Antibacterial activity obtained in this study varied with solvents used for extraction. *Z. officinale* extracts showed moderate inhibition activity with the zone range of 9–14 mm. Maximum inhibition was observed against *P. aeruginosa* (14 mm) and minimum inhibition against *E. coli* (9 mm). Malu et al. (2009) reported anti bacterial activity of various extracts of *Z. officinale* against *C. bacillus*, *S. epidermidis* and *S. viridians*. Bele et al. (2009) also confirmed that the methanol extract of *Z. officinale* showed a significant zone of inhibition against *E. coli*, *S. aureus* and *Z. officinale* is known to contain resins and volatile oils such as borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine and zingiberol phenols (Ahmad et al., 2008; Hirasa and Takemasa, 1998) which may be responsible for its potent antimicrobial activities.

The crude extracts of *C. longa* were active against all bacterial strains showing maximum zone of inhibition (19 mm) against *S. pyogenes* from methanol extract. This may be due to the presence of tannins (Table 2). Tannins are known for their astringent property and antimicrobial activity (Cowan, 1999). The antibacterial activity of extracts of *C. longa* may be attributed to the presence of active ingredients p-tolymethyl-carbinol, curcumin (Lutomoski et al., 1974; Ramprasad and Sirsi, 1956; Huhtanen, 1980) and essential oils (Banerjee and Nigam, 1978). Methanol extract of *Z. officinale* and *C. longa* displayed effective antimicrobial activity against selected pathogens with the inhibition zone in the range of 7–19 mm. These results are in parallel to the finding of previously reported study by Anbu Jeba et al. (2009).

Oleo-resin of *C. molmol* is likely of same potential as of ciprofloxacin and tetracycline against various strains of *S. aureus* and has also shown antibacterial activities against *S. enterica* and *K. pneumonia* (Rahman et al., 2008). Phytochemical analysis by Emad et al. (2009) revealed the presence of the carbohydrates in *C. molmol* extracts which is in agreement with our results (Table 2). Alkaloids and saponins detected in *C. molmol* may be responsible for the antibacterial activity of the plant species (Table 2).

Among all the extracts, *P. anisum* was found to have least antibacterial activity which has also been reported in previous study by Akhtar et al. (2008). Now a day's its oil is being used as antibiotic substitute in rations for broilers (Mehmet et al., 2005).

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. Against all the tested bacterial strain, we observe methanol extract of all the samples showing much better antibacterial activities in contrast to aqueous extract, which may be because of organic nature of methanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds (Cowan, 1999). The antimicrobial action of the aqueous extracts could be ascribed to the anionic components such as thiocyanate, nitrate, chlorides and sulfates besides other water soluble components which are naturally occurring in the plant material (Darout et al., 2000). These results confirmed the substantiation of previous studies which have reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water (Ahmad et al., 1998; Eloff, 1998; Lin et al., 1999; Karaman et al., 2003; Emad et al., 2009; Parekh et al., 2005; Mothana and Lindequist, 2005).

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals (Talaly and Talaly, 2001). As a result, sterilization is needed especially for aqueous extracts before use to get rid of these contaminations. In present study aqueous extracts were autoclave-sterilized before use as autoclaving is reported to cause less damage to the antibacterial activities of the aqueous extract (Hashemi et al., 2008).

5. Conclusion

Our results suggest that *Z. officinale* and *C. longa* can serve as potential source of bioactive healthy compounds in the diet and their consumption could be useful in the prevention of diseases. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

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