

The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria

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Abstract: Increasing the use of antibiotic is harmful to human health, ecosystem and environment and enhancing incidences of drug-resistant pathogens. Medicinal plants have recently received the greatest attention to find naturally occurring substances with therapeutic value. *Coriandrum sativum*, *Crocus sativus* and *Nerium oleander* were collected, dried and extracted with methanol, ethanol, n-butanol, chloroform, ethyl acetate or water. The obtained extracts were assessed for antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA). The methanolic extracts were the most active, thus they were screened for their antimicrobial activity against some different genera of bacteria using agar well diffusion method. The tested bacteria included resistant bacteria to one or more antibiotics. The obtained extracts exhibited considerable inhibitory effects against all the tested bacteria and the less inhibited bacterium was *Salmonella*. The minimum inhibitory concentrations (MIC) of the methanol extracts ranged from 50-100 µg/ml. No toxicity for *C. sativum*, *C. sativus* was found using *Artimia salina* as test organism whereas acute toxicity was recorded for *N. oleander*. The extract of *C. sativus* showed excellent antitumor activity against Ehrlich ascites carcinoma.

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1. Introduction

Antibiotics are miracle drugs, curing all sorts of formerly incurable infectious diseases. Unfortunately, humans have used these drugs unwisely, allowing the bacteria to quickly evolve ways to get around them. The heavy use of antibiotics applies enormous selection pressure on bacterial populations to evolve resistance and the incorrect use of antibiotics gives the bacterial populations the opportunity to quickly evolve resistance (Al Masoudi *et al.*, 2013). The continuous evolutions of bacterial resistance to currently available antibiotics are increasing problems. Drug-resistant bacteria create additional cases of illness, longer recuperation times and unnecessary deaths that necessitated the search for novel and effective antimicrobial compounds (Bokhari, 2009, Aly and Bafeel, 2010). Plants have been shown to be a potential source for multiple antimicrobial agents, as they produce a wide variety of secondary compounds as natural protection against microbial attack (Mahmoud *et al.*, 2004, Amer *et al.*, 2007). The use of plants to treat infectious diseases has been a common practice in virtually all culture for a long time (Ushimaru *et al.*, 2007). It is well known that more than 400.000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than chemotherapeutic agents (Odugbemi, 2006). Moreover according to World Health Organization

(WHO, 1997), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used for traditional medicine contain a wide range of substances including flavonoids, polyphenols and alkaloids. *Coriandrum sativum*, *Crocus sativus* and *Nerium oleander* were among the most used plants in traditional medicine in Saudi Arabia.

Coriandrum sativum (Umbelliferae) is called "coriander" and the countries of its origin are the shores of the Mediterranean and Central Asia. Now, it is cultivated all over the world and has been commonly used for thousands of centuries as spices. Both the leaves and seeds are rich in volatile oils that act mainly on the digestive system, stimulating the appetite, relieving irritation and act as an expectorant (<http://en.wikipedia.org/wiki/-Coriander>).

Medicinally, coriander is used for minor digestive problems and externally for hemorrhoids and painful joints where it is rich in vitamins, decanal, nonanal, linalool and many fine substances (Du, 1999, Li *et al.*, 2002, Song and Wang, 2003). Unfortunately, there are relatively few research reports about the antimicrobial efficiency of coriander (Dai *et al.*, 2009). It was active against almost all Gram positive and negative bacteria with the exception of *Bacillus cereus* and *Enterococcus faecalis* (Guo *et al.*, 2005). They added that the primary mechanism of action of Coriander oil is

membrane damage, which leads to cell death. However acetone extract of Coriander showed the highest inhibitory activity against *Escherichia coli* whereas methanol extracts showed highest activity against *Pseudomonas* spp. (Silva *et al.*, 2011). Furthermore, the antibacterial activity of coriander extracts was stable under heating and had the best antibacterial effects at pH 6 with 2.0% NaCl concentration (Dash *et al.*, 2011).

Crocus sativus L., commonly known as saffron, is a plant cultivated in various parts of the world such as Iran, China, Spain, Italy and Greece. Chemical analysis of its stigmas has shown the presence of water-soluble carotenoids, small amounts of monoterpene aldehyde and its glucoside (safranal and picrocrocin) and flavonoids (quercetin and kaempferol) as described by Cao *et al.* (2012). The pistils of *C. sativus* are used in traditional medicine as an antispasmodic, eupeptic, nerve sedative and emmenagogue (Tarantilis *et al.*, 1995). Its crude extract and purified chemicals have been demonstrated to prevent tumour formation (Pitsikas *et al.*, 2007, Nair *et al.*, 1991, Salomi *et al.*, 1991), atherosclerosis (Gainer and Jones, 1975) or hepatic damage (Wang *et al.*, 1991).

Nerium oleander L. belongs to family Apocynaceae and commonly known as Gandeera which is a large glabrous evergreen shrub with milky juice. The antibacterial activity of *Nerium* leaf on certain Gram positive and negative bacteria was determined (Fridous, 1990, Hussain and Gors, 2004). The results obtained are encouraging as the methanolic, ethanolic and chloroformic extracts have shown considerable antimicrobial activity. The antibacterial activity of the plant is appreciable. The aim of this study was to determine the antimicrobial activity of *Coriandrum sativum*, *Crocus sativa* and *Nerium oleander* against some pathogenic bacteria. MIC, antitumor and toxicity of the previous plant extracts were also recorded.

2. Material and Methods

Source of pathogenic bacteria

Pathogenic bacteria used as test organisms were obtained from the culture collection King Fahad General Hospital, Jeddah, Saudi Arabia.

Collection of plant materials

Coriandrum sativum (leaves), *Crocus sativus* (flowers) and *Nerium oleander* (leaves) were collected from Jeddah market, Saudi Arabia during summer 2009 and identified in Biology Department, Faculty of Sciences, KAU, Jeddah, Saudi Arabia.

Preparation of plant extracts.

Plant materials were washed individually with distilled water and oven-dried for 6 hours at 60°C. Each plant was grinded into fine powder. About 50 g

of each plant sample was extracted by 500 ml of methanol, ethanol, n-butanol, chloroform or water under shaking for 24 hrs. The obtained extract was filtered using a sterile filter paper and the obtained extract was concentrated to dryness at 50°C under vacuum, dissolved in dimethyl sulphoxide (DMSO), sterilized using 0.45 µm bacterial filter and stored at 4°C until used (Aly and Bafiel, 2008).

Screening of the plant extracts for antimicrobial activity

All the prepared plant extracts were screened to determine their antimicrobial activities using agar well diffusion method as described (Holder and Boyce, 1994). Some pathogenic bacteria were used as test organisms. Preculture of each test organism was prepared using nutrient broth medium and 0.1ml of the preculture (4×10^6 CFU/ml) was used to inoculate each agar plates, containing 15 ml of the cooled Muller Hinton agar medium. Wells of 7 mm diameter were made in the seeded agar using sterile cork borer and about 100 µl of the tested plant extract were added to each well. The diameter of the resulting inhibition zones were measured in mm. DMSO was used as negative control whereas ampicillin was used as standard antibacterial agent (Agwa *et al.*, 2000).

Minimal inhibitory concentration:

MIC was determined using 0.2% w/v solution of Fluorescein diacetate (FDA) in acetone (Chand *et al.*, 1994) The resulting green color, from the hydrolysis of FDA, was measured at 490 nm (MR7000 automatic ELISA tray reader) against control well, containing microbial cultures only. MIC was detected by the plant extract concentration that did not increase the FDA absorbance more than control (Aly and Gumgumjee, 2011).

Toxicity test

Toxicity of the plant extracts was determined using Brine shrimp (*Artemia salina*) lethality bioassay. In sterile seawater, 50 mg of Brine shrimp eggs were added. After incubation at room temperature for 48 hr under illumination using inflorescent lamp, the hatching shrimp larvae were collected. Different concentrations of the tested plant extracts, 400, 300, 200 and 100 µg/ml were prepared and their toxic effects on shrimp larvae were determined. Each dosage was tested in triplicate and the mean value was recorded. % of cell inhibitory of the shrimp larvae for each plant extract was recorded and the concentration which gives 50% inhibition considered toxic (Aly and Gumgumjee, 2011).

Antitumor activity of the plant extract

The antitumor activity of the methanolic plant extract was determined using Ehrlich Ascites carcinoma cell line (National cancer institute, Egypt), grew in 10% fetal calf serum in RPMI 1640 medium (Sigma, USA) at 37°C and 5% CO₂ for 48 hours. The

percentage of cell viability and LD₅₀ were assessed for each extract ((Aly and Gumgumjee, 2011).

Statistical analysis

Three replicates for each experiment were carried out and the mean value ± standard deviation was recorded. Student t- test using SPSS version 16 was applied to detect any significant differences between the control and the treated samples.

3 Results and Discussion:

Higher plants can be a very good source of many novel drugs and antibiotics that made significant contribution towards human health (Anesini and Perez, 1993, Adoum, 2009). The presence of antifungal and antibacterial substances used for the treatment of many diseases in the higher plants is well established^{22,31}(Fridous *et al.*, 1990 Didry *et al.*, 1998). Plant extracts with enormous therapeutically potential can serve as a medicine without any side effects that are often associated with synthetic antibiotics and continued search for these plants is needed today. In this study, *Coriandrum sativum* (leaves), *Crocus sativus* (flowers) and *Nerium oleander* (leaves) were collected and extracted using different organic solvents. The antimicrobial activity was determined against Methicillin-resistant *Staphylococcus aureus* (MRSA) which is resistant to the most common antibiotics β-lactams which include methicillin, oxacillin, penicillin and amoxicillin. In the community, most MRSA infections are skin infections and more potentially life-threatening MRSA infections occur among patients in healthcare settings. While 25- 30% of people are nose colonized *S. aureus*, less than 2% are colonized with MRSA (Gorwitz *et al.*, 2008). Anti-methicillin-resistant *S. aureus* activity of ethanolic extracts of four medicinal plants were detected. Similarly, Palombo and Semple (2002) screened the ethanolic extracts of five traditional Australian medicinal plants for their abilities to inhibit clinical isolates of methicillin-resistant *S. aureus* and vancomycin-resistant enterococci.

Our results showed that, the most active organic solvent was methanol followed by ethyl acetate (table 1). Plant extracts have very complex structure and the active ingredients present in the form of natural organic compounds and the process of extraction for a particular compound is dependent on the solubility of the component in the solvent (water or organic solvent). The process and the extraction system are invariably different from product to product and component to component. It is very difficult to generalize a process to extract active compounds from various plants. The methanolic extracts of Fenugreek and Coriander were effective in inhibiting the growth of *Pseudomonas spp.*, *Escherichia coli*,

Shigella dysenteriae, and *Salmonella typhi* (Dash *et al.*,2011). Using organic solvents for extraction is more inducible than water that may due to insolubility of the active material in water. The highest activity was recorded for the methanolic extract of *Nerium oleander* followed by *Crocus sativus* and finally by *Coriandrum sativum*. The antibacterial activity of *Nerium odorum*, *N. oleander* and *Ocimum sanctum* on certain Gram positive and Gram negative bacteria were studied and considerable antimicrobial activity were found (Fridous *et al.*, 1990, Hussain M.A. and Gorski, 2004). The antimicrobial activity may due to a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids that have antimicrobial activities (Crown, 1999).

The methanolic extract of *C. sativum*, *C. sativus* and *N. oleander* (the most active extracts) were screened for their antimicrobial activities against different bacteria as test organisms (table 2). The diameter of inhibition zone ranged from 10-24 mm with mean antibacterial index of 14.5 mm, from 15-20 mm with mean index of 18.7 mm and from 23-27 with mean index of 25 mm for the extracts of *C. sativum*, *C. sativus* and *N. oleander*, respectively. The lowest activity was recorded for methanolic extract of *C. sativum*. The results of Bakhiel *et al.* (2008) showed no activity for Petroleum ether extract of *C. sativum* while the ethanolic extract was active against *S. aureus*, *E. coli*, *Ps. aeruginosa* and *Salmonella typhi* but the water extract was only active against *S. aureus*, *Ps. aeruginosa* and *S. typhi*. Moreover, using agar diffusion method, the ethyl acetate, alcohol, chloroform and acetone extracts of *C. sativum* were active against 13 bacterial species and strains including *Ps. aeruginosa* and *S. aureus* (Elgayyar *et al.*, 2001, Larran *et al.*, 2001). Excellent antibacterial activity (inhibition zones 11-24 mm and MIC 0.026-0.33 µg/ml) against some bacterial isolates was reported for *N. oleander* extract (Bidarigh *et al.*, 2012).

Using flurocin diacetate method, MICs of the three selected plant extracts were calculated and compared with that of Ampicillin which is a β-lactam antibiotic, used to treat bacterial infections. The MIC of Ampicillin against different tested bacteria was 2-5 µg/ml while it was 50-100, 50-75 and 50 µg/ml for the methanolic extract of *C. sativum*, *C. sativus* and *N. oleander*, respectively (Table 3). It can be concluded that, MICs for the three selected plants were greater than that obtained for Ampicillin. Further studies are needed to isolate the active compound(s) in each plant extract as well as its formulation to be applicable as alternative medicine for bacterial infection. The mechanism of actions of the active compound(s) may include enzyme

inhibition that leading to inactivation of the protein and loss of function, complex formation with extracellular and soluble proteins of the microbial cell, complex formation with bacterial cell walls and/or disruption of microbial membranes (Adoum,

2009). Some extracts may have ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999, Bidarigh *et al.*, 2012).

Table 1. The antibacterial activity (diameter of inhibition zone, mm) of the three tested plants extracted using either organic solvents or water against Methicillin-resistant *Staphylococcus aureus*

Tested plant	Mean diameter of the inhibition zone±SD					
	Methanol	Ethanol	Ethyl acetate	n-butanol	Cholor-oform	Water
<i>Coriandrum sativum</i>	24±0.11	18±0.11	17±0.0	14±0.3	12±0.23	10±0.1
<i>Crocus sativus</i>	23±0.12	17±0.12	19±0.0	10±0.5	12±0.54	11±0.2
<i>Nerium oleander</i>	22±0.51	17±0.21	24±0.4	18±0.7	11±0.14	10±0.0
Mean value	23	17.3	20	14	11.6	10.3

Table 2. The antibacterial activities (diameter of inhibition zone, mm) of methanol extracts of three plants against some pathogenic bacteria

	<i>Coriandrum sativum</i>	<i>Crocus sativus</i>	<i>Nerium oleander</i>	Control (Ampicillin)
<i>Bacillus subtilis</i>	24±0.12	20±2.1	27±4.1	35±0.0
<i>Escherichia coli</i>	16±0.35	21±3.0	26±9.1	34±0.2
<i>Klebsiella sp.</i>	16±0.54	20±2.2	25±3.2	34±0.1
<i>Pseudomonas aeuropinosa</i>	14±0.44	22±6.1	23±11.0	32±0.1
<i>Salmonella</i>	10±0.60	17±3.2	27±6.2	30±0.0
<i>Shigella dysentiriae</i>	11±0.31	16±2.4	26±4.4	30±0.1
<i>Staphylococcus aureus</i>	11±0.18	15±2.2	25±2.3	21±0.1
Bacterial index **	14.5*	18.71*	25.6*	30.8*

**Bacterial index : Total activities against bacteria divided by the number of the tested bacteria, *: significant results at $p \leq 0.05$ compared to control (DMSO)

Cancer is one of the major causes of mortality throughout the world and cancer patients are increasing, which indicate that by the year 2020, it is predicted that cancer will be causing seven out of 10 deaths (American Cancer Society, 2008). To reduce this high mortality rate, medical research now turns to the discovery of new molecules that will help to develop natural anticancer drugs. Our results showed that *N. oleander* and *C. sativum* extracts showed excellent antitumor activity against Ehrlich Ascites Carcinoma cell line (table 4) with LC_{50} of 50 and 75 $\mu\text{g/ml}$, whereas *C. sativum* showed no activity up to 400 $\mu\text{g/ml}$. Similar to our results, the methanolic extract of *Curcuma longa* exhibited excellent antitumor activity (Chuang *et al.*, 2000), however the extracts of *Rheum palmatum* or *Alpinia officinarum* up to 400 $\mu\text{g/ml}$ were without activity (Aly and Gungumjee, 2011). In a study carried in Morocco, out of 55 plants, *Aristolochia longa*, *Trigonella foenumgraecum*, *Cassia absus*, *N. oleander*, *C. sativum* and *Nigella sativa* have been cited to be effective as antitumors in patients regularly use these medicinal plants before or during medical treatment (Kabbaj *et al.*, 2012). Similar antitumor activity was reported for *N. oleander* and *C. sativus* (Luay *et al.*, 2011, Akshi *et al.*, 2009).

All the plant extracts before applied on animals must be screening for toxicity effects on insects or fish (Adoum, 2009, Aly and Bafeel, 2010). Biological testing played an important role in detection of toxicity. Brine-shrimp bioassays offer a quick, simple and cost-efficient way of testing the toxicity of plant extracts, and allow a high throughput (McLaughlin *et al.*, 1991, Cupleanu *et al.*, 1994, Coe *et al.*, 2010).

This is of particular importance in developing countries where a large percentage of the population relies on the use of crude medicinal plant extracts to meet their health care need^o. Toxicity values with LC_{50} values of 1000 $\mu\text{g/ml}$ are considered to be toxic and need further screening (Meyer *et al.*, 1982). The brine shrimp lethality assay (BSLA) was among the used methods to determine the toxicity of medicinal plant extracts. It was used to detect the toxicity of 501 aqueous and ethanolic extracts of plant species belonging to 218 genera of 91 families used in Peruvian traditional medicine (Bussmann *et al.*, 2011). *Mentha arvensis*, *Eugenia caryophyllus* and *Decaspermum momtanum* exhibited 100% mortality whereas extract of *Cymbopogon citratus* exhibited about 30% mortality using Brine-shrimp bioassays (Sukari *et al.*, 1992). Furthermore, the aqueous extracts of 55 plant extract showed high toxicity values ($LC_{50} < 249 \mu\text{g/ml}$), 18 extract showed median

toxicity (LC₅₀: 250-499 µg/ml) and 18 low toxicity (LC₅₀: 500-1000 µg/ml) using brine shrimp assay and alcoholic extracts proved to be much more toxic (Bussmann et al., 2011). In our study, the toxicity was recorded only for *Nerium* extract. The acute toxicity of *Nerium* may due to two potent compounds, oleandrin and nerine (Sukari et al., 2011).

In conclusion, the crude extracts of the tested plants exhibited good potential antibacterial and

antitumor activities and the potential for developing antimicrobial agents from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against multidrug resistant microbes. The active extracts should be evaluated further in-depth to isolate active component (s), can be used as medicine and/or natural food preservative.

Table 3. Minimal inhibitory concentration (MIC) expressed in µg/ml of methanol extracts of the three selected plants against different bacteria compared to a standard antibiotic (Ampicillin).

	<i>Coriandrum sativum</i>	<i>Crocus sativus</i>	<i>Nerium oleander</i>	Control (Ampicillin)
<i>Bacillus subtilis</i>	50±11.2	75±12.1	50±5.1	0.2±0.3
<i>Escherichia coli</i>	75±15.2	75±3.0	50±6.2	4.0±0.1
<i>Klebsiella sp.</i>	75±13.2	50±5.2	50±7.2	2.0±0.1
<i>Pseudomonas aeruginosa</i>	75±8.0	50±4.1	50±1.0	2.1±0.1
<i>Salmonella typhi</i>	100±11.2	75±9.11	50±9.2	2.3±0.1
<i>Shigella dysenteriae</i>	75±12.4	75±7.4	50±4.3	0.5±0.6
<i>Staphylococcus aureus</i>	75±10.2	75±10.2	50±13.3	>5

Table 4. Toxicity and antitumor activity of the three selected methanolic plant extracts

Tested plant	Toxicity against <i>Artimia salina</i> (% of cell inhibition)					Antitumor activity (LC ₅₀ , µg/ml)
	Control 0 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	
Control (DMSO)	0	10	10	10	10	ND
<i>Coriandrum sativum</i>	0	8	10	14	20	>400
<i>Crocus sativus</i>	0	11	14	20	20	75*
<i>Nerium oleander</i>	0	14	22	34	52*	50*

*significant result at p≤0.05

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