JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 159-168

Evaluation of the Antimicrobial Potential of Selected Medicinal Plant Extracts Against Some Plant and Human Pathogens

A.A. Al-Askar[1], Y.M. Rashad[2] and W.M. Abdulkhair[2]

[1]Botany and Microbiology Department, Faculty of Science,
King Saud University, Riyadh, Saudi Arabia.
[2]Department of Science, Teachers College, King Saud University, Riyadh, Saudi Arabia.

(Received: 08 November 2013; accepted: 07 January 2012)

In vitro **antimicrobial activity of methanolic extracts of six medicinal plants (black seed, camphor, cloocynth, clove, ginger and white cedar) were investigated against pathogenic bacteria and fungi at 3, 6 and 9% concentrations. Results obtained in this study showed that the six plant extracts exhibited varied extents of antimicrobial activity against the tested organisms even at low concentration. Among the tested plant extracts, ginger recorded the highest antifungal activity against *Alternaria radicina, Fusarium oxysporum, F. solani, Macrophomina phaseolina, Nigrospora oryzae, Phoma destructiva, Rhizoctonia solani* and *Sclerotium rolfsii* at 9% concentration. Whilst, the maximum antibacterial activity was achieved by black seed extract at 9% concentration against the Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumonia*). Chemical compositions of ginger and black seed methanolic extracts were studied using GC-MS analysis and resulted in the identification of 19 and 17 compounds, respectively. Further studies are needed to investigate the antimicrobial potentiality of the active constituents singly or in mixtures with other antibiotics.**

Key words: Antibacterial, Antifungal, Black seed, Camphor, Cloocynth, Clove, Ginger, Medicinal plant and White cedar.

Medicinal plants are a rich source of many economically important compounds such as phenolic compounds, nitrogen containing compounds, vitamins and minerals which have antimicrobial, antioxidant, antitumor, antimutagenic, anticarcinogenic and diuretic activities[1]. According to World Health Organization 80% of the developed countries use traditional medicine that obtained from medicinal plants. It is cheaper than modern medicine. In fact, essential oils and medicinal plant extracts are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses[2]. So, essential oils and plant extracts are one of the most promising groups of natural compounds for the development of safer antimicrobial agents. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs[3].

Syzygium aromaticum (L.) Merr. and Perry., commonly called clove, which belongs to the family Myrtaceae, is an important aromatic spice. Clove oil is widely used for flavouring pastry, special sauces and condiments. It is also used in medicines, especially in the preparations for gum and teeth. The tinctures, extracts and oleoresins are also used[4]. It has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties[5].

* To whom all correspondence should be addressed.
E-mail: younesrashad@yahoo.com

Citrullus colocynthis (L.) Schrad., commonly known as the colocynth or bitter apple, and belongs to the family Cucurbitaceae. It is a desert wild herb that grows in sandy arid soils. Medicinally root, bark and leaves are used. Colocynth has antimicrobial, antiinflammatory, antidiabetic, and antilithic properties[6].

Nigella sativa L., commonly known as black seed or black cumin, is an annual herb of the family Ranunculaceae. It has a great medicinal importance and is known to include many pharmaceutical properties. In addition to its antimicrobial action, it showed antiinflammatory and antiparasitic effects. Black seed has been reported to have antihelminthic and anticoccidial activities. Moreover, it has an immune stimulating effect[7].

Cinnamomum camphora (L.) Nees and Eberm., commonly known as camphor tree, is an evergreen tree that belongs to the family Lauraceae. Roots, branches, leaves, and wood of *C. camphora* can be used for extracting camphor and camphor oil for pharmaceutical use and as a flavorant. Camphor is used as an anesthetic, antitussive, expectorant, and antipruritic, analgesic, antifungal, antiseptic and antibacterial among many other uses[8].

Zingiber officinale Roscoe, commonly known as ginger, is an herbaceous perennial that belongs to the family Zingiberaceae. It is grown for its edible rhizome (underground stem) which is widely used as a spice. The main pharmacological actions of ginger include immunomodulatory, antimicrobial, antitumorigenic, antiinflammatory, antiapoptotic, antihyperglycemic, antilipidemic and antiemetic actions[9].

Thuja occidentalis L., commonly known as white cedar, is an evergreen coniferous tree, in the cypress family Cupressaceae. White cedar has been reported to have immunostimulating, antiviral, antifungal, antipyretic, antiinflammatory, expectorant activities[10].

To obtain effective and promising substances, many plant extracts have to be assayed. For example, Suffredini *et al*.,[11] assayed 705 plant extracts and found only three extracts with strong antibacterial activity. Furthermore, the screening of plant extracts as antimicrobial agents is necessary to go insight into medicinal flora and get the molecules responsible for this activity[12]. Over the next few years, the study of medicinal plants as antimicrobial agents should be focused in part on ascertaining specific information about the plant's antimicrobial activity, avoiding studies in which researchers use this criterion merely as a complement to a phytochemical study[12].

Therefore, the aim of the present study is (a) to evaluate the antifungal and antibacterial activities of the methanolic extracts of some medicinal plants (black seed, camphor, cloocynth, clove, ginger and white cedar); (b) to examine the chemical composition of the methanolic extracts of the most effective plants by GC–MS/MS.

MATERIALS AND METHODS

Plant samples

Healthy, disease free plant samples of black seed (seeds), camphor (leaves), colocynth (pulp), clove (buds), ginger (rhizome) and white cedar (leaves) were used for the study. The plant samples were washed thoroughly 2-3 times with running water and once with sterile distilled water, then air-dried on sterile blotter under shade. The dried materials were then grinded to powder separately and stored in the airtight containers.

Test organisms

Eight plant pathogenic fungi, kindly provided by Plant Pathology Institute, Agricultural Research Center, Egypt and four human pathogenic bacteria, obtained from the american type culture collection (ATCC), were used for antimicrobial test organisms (Table 1). The bacteria were maintained on nutrient broth (NB) at 37°C and fungi were maintained on Potato dextrose agar (PDA) at 28°C.

Preparation of plant extracts

Fifty grams of each plant material in powder form was homogenized by laboratory blender in 200 ml of methanol (95%) and distilled water (20: 80 v/v) for 10 min, and then left in dark glass bottles for 72h for complete extraction. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 30 min for methanol evaporation. The collected extracts were then stored in a refrigerator at 5°C until needed.

Preparation of inocula

The gram positive (*S. pneumoniae* and *S. aureus*) and gram negative bacteria (*E. coli* and *P.*

aeruginosa) were pre-cultured in NB medium overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inocula (*A. radicina, F. oxysporum, F. solani, M. phaseolina, N. oryzae, P. destructiva, R. solani* and *S. rolfsii*) were prepared from 5 to 10 day old culture grown on PDA medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia/mycelia were scraped using sterile spatula. The suspension density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of 10^5cfu. ml^{-1}.

Antibacterial assay

The methanolic extracts of black seed (seeds), camphor (leaves), colocynth (pulps), clove (buds), ginger (rhizomes) and white cedar (leaves) were tested by the disc diffusion method. Different concentrations of the extracts (3, 6 and 9 %) were prepared by reconstituting with methanol (100 µg. ml^{-1}). The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells. ml^{-1}) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (7 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin sulphate (10 µg. ml^{-1}) used as positive Blank disc impregnated with water-methanol followed by drying off was used as negative control. Three replicates of each treatment were used. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

Antifungal assay

The antifungal activity of the methanolic plant extracts (3, 6 and 9 %) was tested by disc diffusion method. The tested fungi were pre-cultured on PDA medium and adjusted at 10^5cfu. ml^{-1} then added to PDA plates before solidification. The filter paper discs (7 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with water-methanol followed by drying off was used as negative control and Nystatin (10 µg. $disc^{-1}$) used as positive control. Three replicates of each treatment were used. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Gas chromatography–mass spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a DP-5 column (30 m X 0.25 mm i.d. with 0.25 µm film thickness), SHIMADZU Europe, GC-MS-QP 2010 model. Chromatographic conditions are as follows: helium as carrier gas, flow rate of 1 ml. min^{-1}; injector and column oven temperature 220 °C and 40 °C; injection mode, "Split" and Split ratio 1:20. Oven temperature was isothermal at 40 °C for 1 min, then increased to 220 °C, at a rate of 4 °C/min and held isothermal for 40 min. MS conditions are as follows: ionization voltage of 70 eV; ion source temperature of 260 °C; interface temperature of 250 °C [13]. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. The Wiley 7 library (Wiley, New York, Japan) and NIST05 (National Institute of Standards and Technology, Gaithersburg, United Sates) mass spectral database and literature were used for matching the identified components from the plant material.

RESULTS

Antifungal activity

The antifungal activity of the methanolic plant extracts of black seed, camphor, cloocynth, clove, ginger and white cedar were investigated against some pathogenic fungi at different concentrations *i.e.* 3, 6 and 9 % (Tables 2, 3). Results obtained in this study showed that the tested six medicinal plants extracts posses potential antifungal activity against *A. radicina, F. oxysporum, F. solani, M. Phaseolina, N. oryzae, P. destructiva, R. solani,* and *S. rolfsii* even at the least concentration. The inhibition effect on the tested organisms increased with increasing the concentration of plant extracts, while no inhibition was found in the negative control. Comparing with the control treatment, the highest antifungal activity was recorded for the plant extract of ginger followed by black seed extract and the lowest antifungal activity was obtained by the plant extract of colocynth even at the highest concentrations.

When tested by the disc diffusion method, the plant extract of the black seed plant showed antifungal activity against all tested pathogenic fungi with different degrees. At concentration 9% of black seed extract, the highest inhibition (22 mm) was recorded for *N. oryzae* and the lowest activity recorded in *S. rolfsii* measured 15 mm. On the other hand, plant extract of camphor exhibited antifungal activity against all tested fungi at all concentrations. The highest activity was achieved against *N. oryzae* (20 mm) and the lowest with *F. solani* (15 mm). The plant extract of colocynth showed the lowest antifungal activity between the tested plant extracts against all tested fungi even at the highest concentration. At concentration 9% of colocynth extract, the highest inhibition zone was recorded with *F. solani* and *A. radicina* (16 and 16 mm) and the lowest in *S. rolfsii* (1 mm). On the other hand, clove extract showed the maximum activity at 9% concentration against *P. destructiva* and *R. solani* (22 mm) and the lowest zone of inhibition was observed in *F. oxysporum* (15 mm). The plant extract of ginger exhibited the maximum antifungal activity between the tested plant extracts against all tested fungi. Methanolic extract of ginger at 9% concentration showed almost similar or higher activity when compared with the nystatin. The fungus *N. oryzae* was the most susceptible to ginger extract (23 mm) while; *S. rolfsii* was the lowest susceptible (17 mm). On the same time, the plant extract of white cedar at concentration 9% showed varied degrees in the zones of inhibition from 14-19 mm against all the tested fungi. The highest antifungal activity was achieved with *M. Phaseolina* and the lowest with *S. rolfsii*.

Antibacterial activity

The antibacterial activity of the six medicinal plant extracts was investigated against some pathogenic bacteria at different concentrations *i.e.* 3, 6 and 9 %. Results presented in Tables (4, 5) revealed that the tested plants extracts posses potential antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae* even at the least concentration, while no inhibition was found in the negative control. Among the tested plant extracts, black seed extract exhibited the maximum antibacterial activity against all tested bacteria followed by ginger extract while, the colocynth extract showed the lowest antibacterial activity when comparing with control. For all tested plant extracts, the antibacterial activity was directly proportional with the concentration of the extract. When tested by the disc diffusion method, the plant extract of the black seed showed antibacterial activity against all tested pathogenic bacteria with different degrees. At concentration 9%, it recorded better activity against *E. coli* (20 mm) followed by *S. pneumoniae* (19 mm) while, the lowest was against *P. aeruginosa* (17 mm). Even at the lowest concentration, plant extract of camphor exhibited antibacterial potential against all tested bacteria. The highest activity was achieved against *E. coli* (18 mm) and the lowest with both *P. aeruginosa* and *S. pneumoniae* (16 mm). Comparing with the control, colocynth plant extract showed the lowest activity between all tested extracts against all tested bacteria even at the highest concentration. The best activity of colocynth extract was recorded against *E. coli* and *S. aureus* (15 mm) and the lowest with *P. aeruginosa* (12 mm). Plant extract of clove showed varied extents of inhibition zones ranged between 14-19 mm against all the tested bacteria. The most susceptible bacterium was *P. aeruginosa* and the least *S. pneumoniae*. Ginger extract exhibited highest activity against *E. coli* (19 mm) .The lowest activity were observed against *S. aureus* around 16 mm zone of inhibition. Plant extract of white cedar showed highest activity against *S. aureus*

Table 1. Tested organisms used in this investigation

Fungi
Alternaria radicina Meier, Drechsler and Eddy
Fusarium oxysporum (Schl.) emend. Snyd. and Hans.
Fusarium solani (Mart.) App. and Wr. emend. Snyd. and Hans.
Macrophomina phaseolina (Tassi) Goid.
Nigrospora oryzae (Berk. and Br.) petch.
Phoma destructiva Plowr.
Rhizoctonia solani Kühn
Sclerotium rolfsii Sacc.
Bacteria
Gram-negative:
Escherichia coli (Migula) Castellani and Chalmers (ATCC® 13706™)
Pseudomonas aeruginosa (Schroeter) Migula (ATCC® 19429™)
Gram-positive:
Staphylococcus aureus Rosenbach. (ATCC® 33591™)
Streptococcus pneumoniae (Klein) Chester (ATCC® 6301™)

Table 2. Antifungal activity of the plant extracts of black seed, camphor and colocynth against some pathogenic fungi.

Fungal sp.	Zone of inhibition (mm)									
	Control	Nystatin	Black seed			Camphor			Colocynth	
			3%	6%	9%	3%	6%	9%	3%	6%	9%
A. radicina	0.0	19±0.33	7±0.78	14±0.44	20±0.22	4±0.22	10±0.14	18±0.60	1±0.33	10±0.16	16±0.22
F. oxysporum	0.0	16±0.19	5±0.25	12±0.45	18±0.23	3±0.30	9±0.11	17±0.51	1±033	8±0.22	15±0.33
F. solani	0.0	17±0.61	4±0.14	12±0.30	19±0.55	3±0.64	8±0.42	15±0.33	5±0.42	10±0.30	16±0.31
M. Phaseolina	0.0	20±0.66	8±0.15	15±0.66	20±0.45	5±0.66	11±0.15	18±0.26	2±0.31	6±0.33	10±0.42
N. oryzae	0.0	22±0.25	9±0.24	16±0.29	22±0.22	6±0.33	13±0.40	20±0.16	4±0.22	7±0.44	8±0.16
P. destructiva	0.0	19±0.31	5±0.66	13±0.18	20±0.31	5±0.21	12±0.51	19±0.26	0±0.0	0±0.0	5±0.14
R. solani	0.0	21±0.57	7±0.33	14±0.33	21±0.66	5±0.22	12±0.33	19±0.16	3±0.13	8±0.60	15±0.22
S. rolfsii	0.0	15±0.33	3±0.21	10±0.31	15±0.33	4±0.17	11±0.23	16±0.33	0±0.0	0±0.0	1±0.03

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

Table 3. Antifungal activity of the plant extracts of clove, ginger and white cedar against some pathogenic fungi

Fungal sp.	Zone of inhibition (mm)									
	Control	Nystatin	Clove			Ginger			White cedar	
			3%	6%	9%	3%	6%	9%	3%	6%	9%
A. radicina	0.0	19±0.33	7±0.23	12±0.31	19±0.23	10±0.12	16±0.33	21±0.41	6±0.66	10±0.12	17±0.33
F. oxysporum	0.0	16±0.19	5±0.60	9±0.33	15±0.66	8±0.06	14±0.14	19±0.23	7±0.62	11±0.66	18±0.64
F. solani	0.0	17±0.61	6±0.45	10±0.16	17±0.24	8±0.24	14±0.05	18±0.33	5±0.46	9±0.30	16±0.12
M. Phaseolina	0.0	20±0.66	9±0.51	15±0.66	21±0.12	10±0.46	17±0.12	21±0.31	9±0.18	12±0.14	19±0.31
N. oryzae	0.0	22±0.25	8±0.47	14±0.03	20±0.31	11±0.44	18±0.31	23±0.25	5±0.26	9±0.26	16±0.33
P. destructiva	0.0	19±0.31	10±0.29	16±0.21	22±0.66	9±0.21	15±0.22	20±0.36	4±0.33	8±0.31	15±0.57
R. solani	0.0	21±0.57	10±0.45	16±0.16	22±0.15	10±0.33	17±0.15	21±0.41	7±0.33	11±0.22	18±0.17
S. rolfsii	0.0	15±0.33	6±0.33	11±0.41	18±0.33	7±0.68	13±0.36	17±0.26	3±0.01	7±0.44	14±0.43

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

Table 4. Antibacterial activity of the plant extracts of black seed, camphor and colocynth against some pathogenic bacteria.

Fungal sp.	Zone of inhibition (mm)										
	Control	Streptomycin sulphate	Black seed			Camphor			Colocynth		
			3%	6%	9%	3%	6%	9%	3%	6%	9%
E. coli	0.0	18±0.33	8±0.12	14±0.66	20±0.18	7±0.22	12±0.19	18±0.33	5±0.33	9±0.66	15±0.51
P. aeruginosa	0.0	14±0.33	7±0.33	11±0.42	17±0.33	6±0.24	10±0.20	16±0.33	4±0.16	8±0.30	12±0.42
S. aureus	0.0	15±0.33	8±0.33	12±0.88	18±0.33	7±0.33	11±0.88	17±0.88	5±0.22	9±0.63	15±0.86
S. pneumoniae	0.0	16±0.33	9±0.41	13±0.15	19±0.66	5±0.61	10±0.51	16±0.41	4±0.25	8±0.71	14±0.33

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

Table 5. Antibacterial activity of the plant extracts of clove, ginger and white cedar against some pathogenic bacteria

Fungal sp.	Zone of inhibition (mm)										
	Control	Streptomycin sulphate	Clove			Ginger			White cedar		
			3%	6%	9%	3%	6%	9%	3%	6%	9%
E. coli	0.0	18±0.33	4±0.15	9±0.52	15±0.31	7±0.53	12±0.33	19±0.38	4±0.16	9±0.33	15±0.35
P. aeruginosa	0.0	14±0.33	8±0.34	13±0.88	19±0.33	8±0.66	12±0.33	18±0.46	3±0.33	8±0.24	14±0.08
S. aureus	0.0	15±0.33	7±0.27	11±0.33	17±0.64	6±0.88	10±0.60	16±0.33	5±0.64	10±0.16	16±0.66
S. pneumoniae	0.0	16±0.33	4±0.33	8±0.14	14±0.57	7±0.71	11±0.57	17±0.15	4±0.66	8±0.03	14±0.29

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

Table 6. Chemical constituents of the methanolic extract from ginger rhizomes by GC-MS

No.	Retention time (min)	Peak area (%)	Molecular weight	Molecular formula	Compound name
1	13. 65	0.71	204.35	$C_{15}H_{24}$	trans-beta-Farnesene
2	14. 15	8.33	202.33	$C_{15}H_{22}$	Ar-Curcumene
3	14.39	18.82	204.35	$C_{15}H_{24}$	Zingiberene
4	14.67	6.41	222.36	$C_{15}H_{26}O$	Farnesol
5	14.87	10.02	204.35	$C_{15}H_{24}$	β-Sesquiphellandrene
6	15.46	1.35	222.36	$C_{15}H_{26}O$	Peruviol
7	15.97	1.63	222.36	$C_{15}H_{26}O$	1,10-Bisaboladien-3-ol
8	16.53	11.14	204.35	$C_{15}H_{24}$	β-Bisabolene
9	17.10	0.83	222.36	$C_{15}H_{26}O$	Globulol
10	18.64	0.76	326.55	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
11	19.43	1.30	154.25	$C_{10}H_{18}O$	Isoborneol
12	20.17	0.56	150.22	$C_{10}H_{14}O$	Verbenone
13	20.82	11.37	154.24	$C_{10}H_{18}O$	β-Geraniol
14	21.56	0.58	294.47	$C_{19}H_{34}O_2$	Linoleic acid methyl ester
15	22.41	9.46	276.37	$C_{17}H_{24}O_3$	(6)-shogaol
16	24.12	12.65	294.39	$C_{17}H_{26}O_4$	Gingirol
17	26.49	0.37	222.36	$C_{15}H_{26}O$	Elemol
18	27.28	1.63	360.40	$C_{20}H_{24}O_6$	Lariciresinol
19	27.76	2.08	402.65	$C_{27}H_{46}O_2$	*delta* –tocopherol

Table 7. Chemical constituents of the methanolic extract from black seed plant by GC-MS

No.	Retention time (min)	Peak area (%)	Molecular weight	Molecular formula	Compound name
1	12.35	4.24	136.23	$C_{10}H_{16}$	β-Limonene
2	16.71	21.82	164.20	$C_{10}H_{12}O_2$	Thymoquinone
3	17. 66	3.47	150.22	$C_{10}H_{14}O$	Thymol
4	18.03	11.37	154.24	$C_{10}H_{18}O$	β-Terpinen-4-ol
5	22.26	3.11	220.35	$C_{15}H_{24}O$	Butylhydroxytoluene
6	23.18	0.74	166.17	$C_9H_{10}O3$	Acetoguaiacon
7	26.46	0.31	270.51	$C_{17}H_{34}O2$	hexadecanoic acid, methyl ester
8	28.19	0.10	166.21	$C_{10}H_{14}O_2$	3-Methoxy-4,5,6-trimethylphenol
9	30.21	14.21	150.21	$C_{10}H_{14}O$	Carvacrol
10	31.79	3.47	256.42	$C_{16}H_{32}O_2$	Cetylic acid
11	33.86	2.34	226.36	$C_{14}H_{26}O_2$	Citronellyl butyrate
12	35.04	3.48	296.53	$C_{20}H_{40}O$	trans-phytol
13	36.21	11.13	134.22	$C_{10}H_{14}$	p-cymene
14	37.80	18.61	280.44	$C_{18}H_{32}O_2$	Linoleic acid
15	40.01	0.27	155.28	$C_{10}H_{21}N$	Propylhexedrine
16	41.13	0.88	412.69	$C_{29}H_{48}O$	Stigmasterol
17	42.51	0.45	410.71	$C_{30}H_50$	Squalene

followed by *E. coli* and the minimum activity was observed in *S. pneumoniae*. White cedar extract showed highest inhibitory activity against *S. aureus* and least activity observed in *S. pneumoniae*. Among the tested plant extract, black seed extract exhibited higher activity when compared with the streptomycin sulphate. Based on the antimicrobial activity, extracts of ginger and black seed were selected to the next test as a best antifungal and antibacterial extracts, respectively.

Chemical composition by GC-MS analysis

The plant extract of ginger rhizomes was

studied for their chemical composition using GC-MS analysis. Nineteen components were identified in the methanolic extract. The identified compounds are listed in Table (6) according to their elution order. The major compounds were zingiberene (18.82%), gingirol (12.65%), β-geraniol (11.37%), β-bisabolene (11.14%), β-sesquiphellandrene (10.02%), (6)-shogaol (9.46%), Ar-curcumene (8.33%) and farnesol (6.41%). The other components including *delta* -tocopherol, lariciresinol, 1,10-bisaboladien-3-ol, peruviol and isoborneol were found to be less than 3%. Rest of the constituents was found to be insignificant amounts. On the other hand, the chemical constituents of methanolic extract of black seed plant were investigated using GC-MS analysis. Seventeen components were identified and presented in Table (7) according to their elution order. The major compounds were thymoquinone (21.13%), linoleic acid (18.61%), carvacrol (14.21%), p-cymene (11.82%) and α-Terpinen-4-ol (11.37%). The other components including α-limonene, trans-phytol, thymol, cetylic acid, butylhydroxytoluene and citronellyl butyrate were found to be less than 5%. Rest of the constituents was found to be in trace amounts.

DISCUSSION

Medicinal plants represent a rich source of antimicrobial agents. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated[12]. In this study, we investigated the antimicrobial activity of the methanolic extracts of black seed, camphor, cloocynth, clove, ginger and white cedar against some pathogenic bacteria and fungi. Results obtained revealed that all tested plants extracts showed varied extents of antimicrobial activity against the tested fungi and bacteria even at low concentrations. The obtained results are in agreement with that of Al-Askar and Rashad[5], Auta *et al*.,[14], Aisham *et al*.,[15], and Pragadheesha *et al*.,[16]. Diversity in the antimicrobial potentiality of a plant extract against different organisms depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, post-harvest processing, the solvent used for extraction, and the susceptibility of the tested organism. For example, the relatively high temperatures that can be generated during tissue grinding can denature chemical constituents and the extraction solvent, time period, and temperature can affect the level and composition of secondary metabolites extracted from plant tissues[17].

Of all the plants tested in the study, ginger showed the strongest antifungal activity with the maximum activity at 9% concentration. Our results are in accordance with that of Tagoe *et al*.,[18] whom found that ginger extract had the highest antifungal activity among the tested plants against *Aspergillus flavus, Aspergillus niger* and *Cladosporium herbarum*. In the present study, the plant extract of ginger rhizomes was studied for their chemical composition using GC-MS analysis. Nineteen components were identified in the methanolic extract. The major compounds were zingiberene, gingirol, β-geraniol, β-bisabolene, β-sesquiphellandrene, (6)-shogaol, Ar-curcumene and farnesol. The antifungal activity of ginger extract may be attributed to the presence of gingerol, shogaol and Ar-curcumene as active ingredient within methanolic extract of ginger. Since many studies indicated that the antimicrobial potency of ginger mainly caused by the presence of oxygenated mono- and sesquiterpenes, phenolic compounds (shogaol, gingerol) [19, 20]. Another study reports on the bioassay guided isolation of antifungal compounds from an african land race of ginger, *Zingiber officinale* Roscoe, and the identification of 6, 8 and 10 gingerols and 6 gingerdiol as the main antifungal components[21]. Bajpai *et al.*[22] also support our explanation that as a result of the presence of mono- and sesquiterpenoids within plant extract, which consider main cause for their antimicrobial mode of action. Since these compounds have different ways of effect since these compounds not only attack cell walls and cell membranes i.e., affecting their permeability and release of intracellular constituents (e.g. ribose, Na glutamate) but they also interfere with membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, these compounds might have several invasive targets which could lead to the inhibition of fungal pathogens.

On the other hand, black seed extract

exhibited the maximum antibacterial activity against all tested bacteria at 9% concentration. Our results are in agreement with that of Aisham *et al.*,[15] whom recorded a greater inhibition by methanol extract of black seed at the concentration of 100 mg/ml against Gram positive bacteria (*Streptococcus pyogenes*) and Gram negative bacteria (*Pseudomonas aeruginosa, Klebseilla pneumoniae* and *Proteus vulgaris*). Using GC-MS analysis, the chemical constituents of black seed extract was studied in the present study. Seventeen components were identified in the methanolic extract. The major compounds were thymoquinone, linoleic acid, carvacrol, p-cymene and α-Terpinen-4-ol. The antibacterial activity could be attributed to their high percentage of phenolic compounds and, specifically, thymoquinone, carvacrol, p-cymene, thymol, and their precursor c-terpinene[23, 24]. Ultee *et al.*,[25] in previous study has been showed that carvacrol interacts with membrane, where it dissolves in the phospholipid bilayer and is assumed to align between the fatty acid chains. The interaction of lipophilic compounds with the phospholipid membrane components causes dramatic changes in the structure of the membrane. This distortion of the physical structure would cause expansion and destabilization of the membrane, increasing membrane fluidity which, in turn, would increase passive permeability. The amphipathicity of phenolic compounds such as thymol and carvacrol can explain their interactions with biomembranes and thus the antimicrobial activity[26]. Cowan[27] explained the mechanisms thought to be responsible for the phenolics toxicity to microorganisms on the basis of enzyme inhibition by the oxidized compounds, possibly through reaction with sulfohydryl groups or through more nonspecific interactions with the proteins. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. In addition, some investigators have found that the more highly oxidized phenols the more inhibitory effect to a pathogen[28].

CONCLUSION

The results of the present study indicated that black seed extract exhibited the maximum antibacterial activity against all tested bacteria, while ginger recorded the highest antifungal activity between the tested plant extracts against all tested fungi. It can be concluded from this study that these two medicinal plants extracts may be used to find bioactive compounds from natural products that might lead to the development of new antibiotic drugs against human pathogens or biocontrol agents as an alternative to the synthetic fungicides for using in agro-industries. This research raises some interesting possibilities for future research. These include: 1. Testing whether mixtures of plant extracts are more effective than a single one. 2. Testing whether the active ingredients are more effective when applied singly or in mixture (synergistic effect) or it may increase the pathogen susceptibility to another known antibiotic. 3. Whether the active components have any side effects when applied *in vivo*.

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project No. RGP-VPP-327.

REFERENCES

1. Bajpai, M., Pande, A., Tewari, S.K., Prakash, D. Phenolic contents and antioxidant activity of some food and medicinal plants. *Int. J. Food Sci. Nut.*, 2005; **56**(4): 287-291.
2. Deba, F., Xuan, T.D., Yasuda, M., Tawata, S. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from Bidens pilosa Linn. var. Radiata. *Food Control*, 2008; **19** (4): 346–352.
3. Joshi, B., Lekhak, S., Sharma, A. Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. *J. Sci. Eng. Technol.*, 2009; **5**(1): 143-150.
4. Bhuiyan, N.I., Begum, J., Nandi, N.C., Akter, F. Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston). *Afric. J. Plant Sci.*, 2010; **4**(11): 451-454.
5. Al-Askar, A.A., Rashad, Y.M. Efficacy of Some Plant Extracts against *Rhizoctonia solani* on Pea.

J. Plant Protec. Res., 2010; **50**(3): 239-243.
6. Pravin B., Tushar D., Vijay P., Kishanchnad K. Review on *Citrullus colocynthis*. *Int. J. Res. Pharm. Chem.*, 2013; **3**(1): 46-53.
7. Al-Khalaf, M.I., Ramadan, K.S. Antimicrobial and Anticancer Activity of *Nigella sativa* oil. *Aust. J. Basic Appl. Sci*., 2013; **7**(7): 505-514.
8. Djilani, A., Dicko, A.: The Therapeutic Benefits of Essential Oils. In: *Nutrition, Well-Being and Health* (Bouayed J, Bohn T, eds.). In Tech Press, Croatia, 2012; 155- 178.
9. Ali, B.H., Blunden, G., Tanira, M.O., Nemmar, A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food Chem. Toxicol.*, 2008; **46**: 409-20.
10. Naser, B., Bodinet, C., Tegtmeier, M., Lindequist, U. *Thuja occidentalis* (Arbor vitae): A Review of its Pharmaceutical, Pharmacological and Clinical Properties. *Evid. Based Complement Alternat. Med.*, 2005; **2**(1): 69–78.
11. Suffredini, I.B., Sader, H.S., Gonçalves, A.G., Reis, A.O., Gales, A.C., Varella, A.D., Younes, R.N. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. *Braz. J. Med. Biol. Res.* 2004; **37**: 379-384.
12. Ríos, J.L., Recio, M.C. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.*, 2005; **100**: 80-84.
13. Adam, R.P. Identification of Essential Oil Components by Gas Chromatography/ Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, Il., USA., 2001.
14. Auta, K.I., Galadima, A.A., Bassey, J.U., Olowoniyi, O.D., Moses, O.O., Yako, A.B. Antimicrobial Properties of the Ethanolic Extracts of *Zingiber officinale* (Ginger) on *Escherichia coli* and *Pseudomonas aeruginosa*. *Res. J. Biol. Sci*., 2011; **6**(1): 37-39.
15. Aisham H., Mohid, Z.N., Haslinda, M. Antimicrobial activity of *Nigella sativa* seed extract. *Sains Malays.*, 2013; **42**(2):143-147.
16. Pragadheesha, V.S., Sarojc, A., Yadavb, A., Chanotiyaa, C.S., Alamc, M., Samadc, A. Chemical characterization and antifungal activity of *Cinnamomum camphora* essential oil. Ind. *Crop. Prod.*, 2013; **49**: 628-633.
17. Wendakoon, C., Calderon, P., Gagnon, D. Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. *J. Med. Active Plant.*, 2012; **1**(2): 60-68.
18. Tagoe, D.N., Nyarko, H.D., Akpaka, R. A comparison of the antifungal properties of onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) against *Aspergillus flavus, Aspergillus niger* and *Cladosporium herbarum*. *Res. J. Med. Plant.*, 2011; **5**(3): 281-287.
19. Singh, G., Kapoor, I.P., Singh, P., de Heluani, C.S., de Lampasona, M.P., Catalan CA. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food Chem. Toxicol.*, 2008; **46**: 3295–3302.
20. Hasan, H.A., Raauf, A.M., Abd Razik, B.M., Hassan, B.A. Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from *Zingiber Officinale* by Different Solvents. *Pharm. Ann. Acta*., 2012; **3**(9): 184-188.
21. Ficker, C., Smith, M.L., Akpagana, K., Gbeassor, M., Zhang, J., Durst, T., Assabgui, R., Arnason, J.T. Bioassay-guided isolation and identification of antifungal compounds from ginger. *Phytother. Res.*, 2003; 17(8): 897-902.
22. Bajpai, V.K., Al-Reza, S.M., Choi, U.K., Lee, J.H., Kang, S.C. Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of Metasequioa glyptostroboides Miki ex Hu. *Food Chem. Toxicol.*, 2009; **47**: 1876-1883.
23. Halawani, E. Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and their Interaction with some antibiotics. *Advan. Biol. Res.*, 2009; **3**(5-6): 148-152.
24. Paarakh, P.M. *Nigella sativa* Linn.- A comprehensive review. *Indian J. Nat. Prod. Resour.*, 2010; **1**: 409-429.
25. Ultee, A., Kets, E. P. W., Alberda, M., Hoekstra, F. A., Smid, E. J. Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. *Arch. Microbiol.*, 2000; **174**(4): 233–238.
26. Cristiani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija, A., Trombetta, D. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. *J. Agric. Food Chem.*, 2007; **55**: 6300–6308.
27. Cowan, M.M. Plant products as Antimicrobial agents. *Clin. Microbiol. Rev.*, 1999; 12: 564-582.
28. Dai, J., Mumper, R.J. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules.*, 2010; **15**(10): 7313-7352.