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ANTIMICROBIAL ACTIVITIES OF *SOLANUM INCANUM, ELETTARIA CARDAMOMUM* AND *ZINGIBER OFFICINALE,* USED TRADITIONALLY TO TREAT PATHOGENIC MICROBES

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ABSTRACT: The use and search for antibiotics derived from plants have been accelerated in recent years. Three plants, used traditionally as medicine and as food additives were collected and extracted with hot water, methanol, diethyl-ether, ethyl acetate and chloroform. The used plants were *Solanum incanum*, *Elettaria cardamomum* and *Zingiber officinale*. The plant extracts were screened for their inhibitory effects on eight bacterial and seven fungal pathogens using agar well diffusion method.

It was shown that methanol extract was more effective as compared to hot water, diethyl-ether, ethyl acetate and chloroform extracts. Inhibition zone diameters of the methanol extracts of the used plants ranged from 10 to 29 mm and minimum inhibitory concentrations (MIC) from 50 to 150 µg/ml. No toxicity was found using *Artimia salina* as test organism. Antitumor activity against Ehrlich ascites carcinoma and Lymphoma cell line was recorded only for *S. incanum* extract.

Key words: *Solanum incanum*, *Elettaria cardamomum, Zingiber officinale*, antimicrobial activities, plant extracts.

INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 1978; Aibinu *et al.*, 2003 and Aibinu *et al.,* 2004). Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Davis, 1995). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki *et al.*, 1999). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. The indiscriminate use of antibiotics has led to drug resistance of many bacterial strains. The development of multidrug-resistant pathogens has been related to the occurrence of over and under-dosage of antimicrobials (Vargas *et al.*, 2004; Schelz *et al.*, 2006). The emergency of antibiotic resistance by bacteria has become a medical catastrophe and we may be entering a 'post-antibiotic' era where antibiotics are no longer effective. Development of new microbial compounds for resistant organisms is becoming critically important (Martini and Eloff, 1998). Herbal medicine is the oldest and most tried and tested form of medicine. In a sense it forms the basis of all medicine. It is the original medicine, the mother of all remedies used today. It has been used by all cultures for centuries and is still the main form of medical treatment. Herbal medicine is the most important medicine for the majority of people on the planet, especially those who cannot afford expensive drugs (McKenna, 1996). *Solanum incanum*, commonly known as bitter garden egg belongs to the family Solanaceae. It is a delicate perennial often cultivated as an annual crop. It is a shrub, growing 1-3 m high. The leaves are simple, ovate, elliptic, 2.5-12 cm long and 2.5-8 cm wide. The fruit is fleshy, less than 3 cm in diameter on wild plants but much larger in cultivated forms. Fruit is spherical, green, often striped with white, turning yellow to orange-brown when ripe (Denston, 1951). *Solanum* species are the most potent plants against pathogenic microorganisms. *Solanum incanum* (L) is one of the important traditional medicinal plants belongs to Solanacae family. Antibacterial activity of *Solanum incanum* was studied (Britto and Senthilkumar, 2001 and Pavitra *et al*., 2012) and presence of analysis of phytochemicals were also studied (Pavitra *et al*., 2012). Other S*olanum* species, *S. torvum* (leaf, stem and roots) showed antibacterial and antifungal activity (Bari *et al*., 2010) and antibacterial activity of *Solanum surattense* whole plant extract (Patil *et al*., 2009) and leaf extract (Sheeba, 2010) were studied. Analysis, presence of phytochemicals and potent antibacterial activity of leaf, root and seed extracts were studied in *S. nigrum* (Sridhar *et al*., 2011). Cardamom is a dried fruit of *Elettaria cardamomum* belonging to family Zingiberaceae. It is commonly known as queen of spices for the versatile use in culinary practice. Cardamom is a perennial shrub with thick, fleshy, lateral roots which can grow to a height of eight feet (Kapoor, 2000). Soriful *et al*. (2010) studied the antimicrobial activity of *Elettaria cardamomum* on some Gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, and *Sarcina lutea* as well as Gram negative bacteria: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae*, and *Shigella sonnei*. Methanolic extract inhibited the growth of all the tested bacteria having various degrees of inhibition zones. Ginger known as rhizomes of *Zingiber officinale* (Zingiberaceae) is one of the spices valued for its aroma and pungency characteristic. It grows in the tropical region, especially in the southern and eastern part of Asia. That spice is commercialized in the dry form. Ginger is a stimulant, carminative and frequently used for dyspepsia, gastroparesis, constipation and colic (Wood, 2013). Ginger oil has been proved to prevent skin cancer in mice (Oyagbemi *et al*., 2010). Some studies have demonstrated that gingerols can be used to fight against ovarian cancer (Park *et al*, 2008; McGee, 2004). [10]-gingerol and [12]-gingerol isolated from ginger rhizome have been reported to show antibacterial activity against periodontal bacteria (Bartley and Jacobs, 2000). Previous phytochemical investigations on the rhizome of *Zingiber officinale* yielded 6-gingerol, zingerone, shogaol, butyl hydroxyl toluene, butyl hydroxyl anisole (Imadia *et al*., 1983; Miri *et al*., 2008). The aim of the present study was detection of antimicrobial activities and toxicity of *Solanum incanum, Elettaria cardamomum* and *Zingiber officinale.*

MATERIALS AND METHODS

Plant materials:

Aerial parts, fruits, roots and leaves of some plants were collected from local markets in Jeddah, Saudi Arabia during summer 2012. The collected plant materials were put in clean plastic bags and transferred directly to the lab. The plants under investigation were *Solanum incanum, Elettaria cardamomum* and *Zingiber officinale* (the families and common names of these plants are shown in Table, 1).

Tested organisms:

Bacterial and fungal isolates were obtained from Biology Department, Faculty of Science, King Abdul-Aziz University (KAU), Jeddah, Saudi Arabia. The bacterial strains were *Escherichia. coli* ATCC 25922,

Table 1. The selected medicinal plants, their families and common names.

Scientific name	Family	Common name	Used part
Solanum incanum	Solanaceae	Bitter apple	fruits
Zingiber officinale	Zingiberaceae	Ginger	Root
Elettaria cardamomum	Zingiberaceae	Cardamom	fruits

Pseudomonas aeruginosa ATCC 27853, *Klebsiella pneumoniae* (ESBL) ATCC 700603 , *A. baumannii* ATCC 1656-2, *P. mirabilis* ATCC 12453, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (MRSA) ATCC 33591, and *Enterococcus faecalis* (VRE) ATCC 51299. The tested fungi were *Aspergillus flavus*, *Aspergillus niger, Epidermophyton floccosum, Trycophyton mentagrophytes* and *Microsporum canis.* Two strains of yeast have been investigated in this study; *Candida albicans* and *Cryptococcus neoformans.*

Preparation of plant extracts:

Organic solvents used in this experiment were obtained from Sigma-Aldrich Company. The dried roots, seeds or leaves were ground into fine powder with an electric blender. Fifty grams were suspended in hot water or organic solvents (methanol, diethyl-ether, ethyl acetate and chloroform) in sterile 250 ml conical flasks and kept at 4°C overnight. After overnight incubation, the supernatant was filtered through Whatman No.1 filter paper and the filtrate was concentrated by evaporation in a rotary evaporator at 40°C. The residue was weighed, dissolved in 5% dimethyl sulfoxide (DMSO) and stored in the refrigerator at 4°C prior to use.

Antimicrobial activity:

This test was carried out using agar well diffusion method according to Joshi *et al.* (2009). Bacteria or yeast were taken and shaken in the sterile distilled water corresponding to 10^8 CFU/ml for bacteria and 10^6 CFU/ml of yeast (Mihajilov-Krstev *et al*., 2010). Fungal inocula were prepared by flooding Petri dish with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was 1.5×10^4 spore/ml (Adigüzel *et al*., 2005). Minimal inhibitory concentration was determined by the method recommended by Ter-Laak *et al*. (1991). Each antimicrobial agent was serially diluted by transferring 100 µl of the antimicrobial agent through sterilized microtitre plate containing 100 µl media (nutrient broth for bacteria, Sabouraud dextrose broth for fungi and yeast). Freshly prepared standard number of cells (1.5×10^8 CFU/ml for bacteria or yeast and 4×10^4 spore/ml for fungal isolates) was added to the media that contained some drops of phenol red. Glucose metabolisms were measured by a change of the color of phenol red indicator from red to yellow. MIC was determined at the concentration with no color change and DMSO was used as a control.

Toxicity and antitumor activity of the plant extracts:

Cytotoxicity is performed by Brine Shrimp Test (BST). Different dilutions of each plant extract made in DMSO were prepared (100, 200, 300 and 400 µg/ml) and 0.5 ml of each dilution was added to 4.5 ml of brine solution and maintained at room temperature for 24 h under the light (Krishnaraju *et al*., 2005). The surviving larvae were counted by light microscope and the number of dead shrimps in each vial was recorded. The concentration at which 50% of the larvae were killed (LD_{50}) was determined as the toxic concentration (Lachumy *et al*., 2010). The antitumor activity of the tested plants was determined against Ehrlich carcinoma and Lymphoma cell line. Cells were grown in RPMI 1640 medium (Sigma, USA) with 10% fetal calf serum (FCS) (Gibco, USA) at 37°C under a humidified atmosphere consisting of 95% air and 5% CO_2 for 48 h. The percentage of cell viability was assessed to determine the 50 % lethal dose by which 50% of cells are killed (LD_{50}).

RESULTS AND DISCUSSION

The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al*., 2003).

In this study, three plants were collected and extracted using hot water, methanol, ethyl acetate, N-butanol, diethyl ether and chloroform. The tested plants were *S. incanum, E. cardamomum* and *Z. officinale.*

The tested plants were selected based on traditional medicine knowledge used by Saudi Arabian people. All the obtained extracts were screened for their antibacterial activity against *E. coli.*

It was clear that all of the extracts have inhibitory activity against *E. coli* (Table, 2). The inhibitory activity varied according to the solvent used. The methanol extracts for all of tested plants showed significant antibacterial activities against *E. coli* compared to the activity of the other solvents which almost have similar effect. Previous studies provide similar results for methanol as a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents such as water and ethanol (Ahmad *et al*., 1998), hot water, N- butanol, diethyl-ether, ethyl acetate and chloroform (El Sayed and Aly, 2014).

Since methanol extract was the most active solvent, its activity was tested against the other pathogenic organisms including Gram positive bacteria (*S. aureus, S. aureus* MRSA and *E. faecalis* VRE), Gram negative bacteria (*P. aeruginosa, K. pneumonia, A. baumannii* and *P. mirabils*), yeast (*C. albicans*, and *C. neoformans*) and molds (*A. flavus*, *A. niger, E. floccosum.,T. mentagrophytes* and *M. canis*) (Tables, 3 and 4).

S. incanum has an effect on Gram negative and Gram positive isolates, but zones of inhibition are greater in Gram negative organisms (17-29 mm.). It has the best activity against *P. aeruginosa.* MIC values were 50-150 µg/ml. The most affected Gram negative organism was *E. coli*, while *E. faecalis* was the most resistant organism.

In accordance, Omwenga *et al*. (2012) found that the methanolic extract of *S. incanum* has better activity on *P. aeruginosa* producing a wide zone of inhibition compared to *S. aureus* and *B. subtilis*. Such results are promising since *P. aeruginosa* is hard to be controlled by most antibiotics due to its cell wall properties (Omwenga *et al*., 2009).

Z. officinale is exceeding the effect of *S. incanum* against Gram negative bacteria (inhibition zones, 22-24 mm., MIC 50-100 µg/ml.). Significant antimicrobial activity of *Z. officinale* against *E. coli* has been reported by Yahaya *et al*. (2012). Gull *et al*. (2012) reported antimicrobial activity of the plant methanolic extract on both Gram positive and Gram negative bacteria. The antibacterial activities of the extracts are expected perhaps due to the compounds like flavonoids and volatile oil which were dissolved in organic solvents. It is reported that sesquiterpenoids are the main component of ginger which attributes its antibacterial activity (Malu *et al*., 2008).

Table 2. The antibacterial activity of some plants extracts using water and organic solvents against *E. coli.*

Plant \ Solvent	Diameter of inhibition zone mm					
	Hot water	Methanol	N- butanol	Diethyl-ether	Ethyl acetate	Chloroform
S. incanum	19 ± 0.07	39 ± 1.0	19 ± 0.9	17 ± 0.2	15 ± 0.9	18 ± 0.4
Z. officinale	18 ± 0.17	39 ± 1.0	19 ± 0.6	17 ± 0.2	15 ± 1.0	18 ± 2.0
E. cardamomum	17±1.30	26 ± 1.0	18 ± 0.3	17 ± 0.4	19 ± 2.0	13 ± 1.0
Bacterial index*	54	104	56	51	49	49

*** Bacterial Index: Total Activity (mm).**

Table 3. Antibacterial Activities (Diameter of the Inhibition Zone, mm) of the methanolic plant extracts against different pathogenic bacteria.

Bacteria \ Plants	*S. incanum*	*E. cardamomum*	*Z. officinale*	Control (DMSO)
P. aerugenosa	29±1.0	27±0.2	23±3.0	ND
K. pneumoniae	16±1.0	16±0.2	22±2.8	ND
P. mirabilis	17±2.1	16±0.4	24±0.5	ND
A. baumannii	19±0.8	26±0.4	12±0.6	ND
E. faecalis	11±0.5	14±0.6	11±0.4	ND
***S. aureus* 33591**	14±0.9	12±0.4	18±0.4	ND
***S. aureus* 25923**	14±1.0	18±0.8	14±0.8	ND
Bacterial index*	120	129	124	ND

***Bacterial Index: Total Activity (mm).**

Table 4. The minimal inhibitory concentration (µg/ml) of methanolic extracts against different pathogenic bacteria.

Bacteria \ Plants	*S. incanum*	*E. cardamomum*	*Z. officinale*	Control (DMSO)
P. aerugenosa	50	50	100	> 200
K. pneumoniae	150	150	50	> 200
P. mirabilis	150	100	50	> 200
A. baumannii	150	50	150	> 200
E. faecalis	150	150	150	> 200
***S. aureus* 33591**	100	150	100	> 200
***S. aureus* 25923**	100	100	100	> 200
E. coli	100	50	100	> 200

The greater antimicrobial activity for the methanolic extracts of the three tested plants was *E. cardamomum*. It has relative increasing in inhibition zones compared to *Z. officinale* with exception of *K. pneumonia, P. mirabilis* and *S. aureus* 33591 as *Zingiber officinale* had a stronger effect.

The inhibitory effect on Gram negative strains was greater than Gram positives. The markedly increase in the antimicrobial activity against *P. aeruginosa,* Gram negative bacteria and *E. faecalis* (VRE) among Gram positive bacteria is contributing to the production of drugs effective against multi-drug resistant bacteria. In accordance, Jemal *et al*. (2011) observed that complete (100%) growth inhibition was at 15% cardamom hydrosol concentration against *E. coli*, *S. aureus* and *S. typhi*, but in contrast, he found *P. aeruginosa* as the most resistant organism.

Antifungal activity of the selected plants has also been investigated and represented in Tables (5) and (6). *E. cardamomum* had the highest values of inhibition on filamentous fungi with exception of *A. niger* compared to methanolic extracts of other plants. *S. incanum* has the highest effect on such strain. Inhibition zones of *E. cardamomum* ranged from 11 to 17 mm, the former was recorded for *A. niger*. MIC were 50-100 µg/ml. Obvious antimicrobial action on dermatophytes (*E. floccosum, T. mentagrophytes* and *M. canis*) was observed. It also recorded considerable effect against *C. albicans*. A similar result was obtained by Ağaoğlu *et al*. (2005). According to Aneja and Sharma (2010), *E. cardamomum* displayed good to moderate activity against *C. albicans*. *E. cardamomum* is widely used in various parts of the world's traditional medicine system and it has been used in India since ancient times (Dhulap *et al*., 2008). The antimicrobial potential of this plant extracted in different solvents (e.g. aqueous, methanol, ethanol, acetone, chloroform, hexane, ethyl acetate and diethyl ether) had been evaluated against different bacterial and fungal human pathogens and had reported variable activities in different parts, seeds, pods and fruits in different solvents (Dhulap *et al*., 2008; Agaoglu *et al*., 2005; Kaushik *et al*., 2010; Singh *et al*., 2008 and Aneja and Joshi, 2009).

Cytotoxic effect using brine shrimp and antitumor activity using Erlich cell line and lymphoma cell line of the plants under investigation were studied and represented in Table (7). The highest cytotoxic effect was recorded for the methanolic extract of *S. incanum* (LD_{50} is 600), while the minimal effect was recorded for *E. cardamomum* (LD_{50} is $\geq$ 600). Antitumor activity of *E. cardamomum,* and *Z. officinale* showed LD_{50} $\geq$ 600 µg/ml on both Lymphoma cell line and Erlich cell line, while it was 400 µg/ml on *S. incanum* µg/ml on both Erlich cell line and lymphoma cell line.

CONCLUSION

On the basis of the experimental results and discussion, it can be postulated that methanol is the best solvent for all of the plants under investigation. *S. incanum* and *Z. officinale* methanolic extracts can be used effectively against *E. coli.* Moreover, *S. incanum* was the best plant extract against *P. aeruginosa*. Methanolic extract of *Z. officinale* provides the best action against *S. aureus* (MRSA) and *K. pneumonia* (ESBL). So, it is considered to be as promising antibiotic against multidrug resistant bacteria. *E. cardamomum* has broad spectrum antimicrobial activity against Gram negative bacteria, Gram positive bacteria, yeast and filamentous fungi.

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Table 5. Effect of the methanolic extracts on fungi and yeast.

Fungi \ Plants	*S. incanum*	*E. .cardamomum*	*Z. officinale*	Control (DMSO)
A. flavus	11±1.0	17±0.23	13±1.3	ND
A. niger	17±0.8	11±0.08	10±0.5	ND
E. floccosum	11±1.2	14±0.22	11±0.8	ND
T. mentagrophytes	11±1.1	13±0.43	11±1.5	ND
M. canis	11±0.18	16±0.14	11±0.16	ND
C. albicans	10±0.15	16±0.16	11±0.10	ND
C. neoformans	13±1.4	11±0.8	13±0.18	ND
Fungal index*	84	98	80	ND

*Fungal Index: Total Activity (mm).

Table 6. The minimal inhibitory concentration (µg/ml) of methanolic extracts against different pathogenic fungi and yeast, compared with that of control.

Fungi \ Plants	*S. incanum*	*E. cardamomum*	*Z. officinale*	Control (DMSO)
A. flavus	50	100	100	>200
A. niger	50	100	100	>200
E. floccosum	100	100	50	>200
T. mentagrophytes	100	50	50	>200
M. canis	50	100	50	>200
C. albicans	50	50	100	>200
C. neoformans	150	150	150	>200

Table 7. Toxicity against *Artimia salina* (% mortality) and antitumor activities of the different concentrations of plant methanolic extracts.

Plants	Toxicity against *Artimia salina* (% of mortality) at different concentrations (µg/ml)					Antitumor activity (LD_{50}, µg/ml)	
	Control	200	400	600	LD_{50}	Lymphoma cell line	Erlich cell line
S. incanum	0	10	20	59	500	400	400
E. cardamomum	0	0	0	10	≥ 600	≥ 600	≥ 600
Z. officinale	0	0	10	26	≥ 600	≥ 600	≥ 600

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النشاط الضد ميكروبي لنباتات الحنظل و الهيل و الزنجبيل المستخدمة عادة في علاج الميكروبات الممرضة

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تسارع استخدام المضادات الحيوية المشتقة من النباتات في السنوات الأخيرة. تم الحصول على مستخلصات من ثلاث نباتات باستخدام الماء الساخن والميثانول والايثير ثنائى الايثيل و أسيتات الايثيل و الكلوروفورم. النباتات المستخدمة هي الحنظل (*Solanum incanum*) و الهيل (*Elettaria cardamomum*) و الزنجبيل (*Zingiber officinale*). و قد تم إختبار التأثير المثبط لهذه النباتات على ثمانية أنواع من البكتيريا و سبعة أنواع من الفطريات الممرضة. و تبين أن مستخلص الميثانول هو الأكثر تأثيرا إذا ما قورن بمستخلصات الماء الساخن و الايثير ثنائى الايثيل و أسيتات الايثيل

والكلوروفورم. تراوح معدل قياسات مناطق تثبيط النمو الميكروبي لمستخلصات الميثانول من ١٠ إلى ٢٩ مم و التركيز الأدنى لتثبيط النمو الميكروبي (MIC) من ٥٠ إلى ١٥٠ميكروجرام/مل. لم يُسجل أي تأثير سام على الخلايا باستخدام الأرتيميا (*Artimia salina*) ككائن تجارب. سجل مستخلص نبات الحنظل نشاطا مضادا للأورام عند اختباره على خلايا سرطان ايرلخ الاستسقائى (*Ehrlich ascites* carcinoma cell line) و خلايا سرطان الغدد الليمفاوية (Lymphoma cell line).