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Antibacterial Activities of Six Medicinal Plants Used Traditionally by Saudi People to Treat Common Diseases

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Authors' contributions

This work was carried out in collaboration between both authors. Authors HEAE and MMA designed the study, wrote the protocol, initiated the experiments, collected the data, performed the statistical analysis, managed the literature review and wrote the final draft of the manuscript. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Antibiotic resistance is increasing and dangerous problem resulting in a decrease in the used number and fully active antimicrobial agents available to treat infections with multi-drug resistant (MDR) bacteria. Herbal medicines may be an alternative treatment options. This study aimed to evaluate the antibacterial activities of some medicinal plants used traditionally in Saudi Arabia against some pathogenic and MDR bacteria. The antimicrobial activities of water and organic crude extracts were prepared. Out of the 6 plants tested, 5 showed antimicrobial activities against one or more of the tested genera using paper disc diffusion assay. The most active antimicrobial plants were the extract of *Azadirachta indica* (neem), *Zingiber officinale* (ginger) *Eucalptus globules*, *Rosmarinus officinalis* and *Lawsonia inermis* with minimal inhibitory concentration (MICs) values ranged from 50-150µg/ml. *Lepidium sativium* have very weak activity with MIC of ≥200 µg/ml. At 600µg/ml, no toxicity was recorded for all tested extracts except of *A. indica* extract that showed toxicity (% of mortality ≥50). No antitumor activities for the different plant extracts were recorded against Human gastric cancer SGC-7901 cells. The

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biological activities may be due to some photochemical compounds including Anthocyanin, Butacyanin, Phytobutanins, Flavonoides, Stetoides and/or Saponins.

Keywords: Antimicrobial activity; plant extract; azadirachta; eucalyptus; zingiber; rosmarinus; escherichia; lepidium.

1. INTRODUCTION

For many years, antibiotics have been used to treat many bacterial diseases, however, antibiotic resistance has been increasing in prevalence [1] and this add an urgency to the search for new infection-fighting strategies [2]. To overcome the problem of resistance of antibiotic, medicinal plants have been extensively studied as alternative treatments for diseases [3] and many efforts have been made to discover new antimicrobial compounds from various kinds of plants. Screening of traditionally used plants may result in the discovery of novel effective compounds [4,5]. Chemical studies of medicinal plants provide valuable materials with enormous therapeutic potential and heal many infectious diseases. particularly emerging enteropathogens with multiple antimicrobial resistances [6]. Nowadays, it is well known that plant extracts of natural origin are safer, contrary to the synthetic drugs which are associated with many side effects [7]. These plant compounds have different structures and mode of actions when compared with conventional antibiotics used to control the microbial growth and survival. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism.

There have been documented evidences that rosemary (*Rosmarinus officinalis*) extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Azcan and Chalchat, [8] studied the antimicrobial properties of rosemary plant extract against different pathogenic microorganisms while Moreno et al. [9] reported that rosemary plant is a rich source of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. The antimicrobial activity is attributed to carnosic acid and carnosol. Rosemary leaf extracts were found to be effective in reducing germination of *Phytophthora* zoospores and based upon high performance liquid chromatography (HPLC) analyses, the active compound in these extracts was determined to be caffeic acid, rosmarinic acid or some simple derivatives [10].

Azadirachta indica (neem) is used for a long time in agriculture and medicine. The medicinal and the antimicrobial properties of the plant were studied by several workers. Neem leaf extract significantly reduced and inhibited the growth of *Curvularia lunata* [11]. Bachir and Benali [12] documented that essential oil of the leaves of *Eucalptus globulus* has antimicrobial activity against Gram negative bacteria (*E. coli*) as well as Gram positive bacteria (*Staphylococcus aureus*). Similarly, Abd-El-Khair and Haggag [13] found that the leaf extract of *E. globules* reduced the growth and percentage of spore germination of two plant pathogenic fungi. The use of *Eucalyptus* for controlling the mycelia growth of many plant pathogenic fungi was recommended [14,15]. This may be due to the presence of gallic acid and phenolic compounds which has strong antimicrobial activity against bacterial growth and spore germination of the pathogenic fungi.

Lawsonia inermis leaves are used both as a cosmetic and in wounds and mycotic infections. Habbal et al. [16] have concluded that henna possess high antibacterial activities against *Pseudomonas aeruginosa*. Moreover, Satish et al. [17] screened a large number of plants for antimicrobial activity and found that the aqueous extract of *Lawsonia inermis* was among the

most active extracts. On the other hand, Auta et al. [18] reported excellent antibacterial activity of *Zingiber officinale*. Recently ginger was also reported for treatment of non-alcoholic fatty liver diseases [19]. *Lepidium sativum* belongs to the family Brassicaceae and seeds of the plant contain volatile oils which are used in treating dysentery, bone fracture healing in human and migraine [20,21]. Phytochemical screening of *L. sativum* seeds revealed the presence of flavonoids, alkaloids, sterols and/or triterpenes, tannins and glucosinolates [22]. Tannins may form irreversible complexes with proline-rich proteins [23] resulting in the inhibition of the cell protein synthesis. With the increasing awareness of population toward natural therapies, spices can be considered as obvious alternate medication [24].The aim of the present study was to find out the antibacterial activities, toxicity and antitumor activity of six common plant extracts which were used traditionally by Saudi people.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

The plant materials used were collected from the markets and bazaars of Jeddah, Saudi Arabia. The roots of *Zingiber officinale* and the leaf of *Eucalptus globules, Lawsonia inermis and Rosmarinus officinalis* were collected and shade -dried at room temperature (28–30°C) for 14 days. Seeds of *Lepidium sativium* and *Azadirachta indica* were washed and air dried for two days at room temperature. Samples of the plants were identified and authenticated by the Department of Biology, King Abdulaziz University, Faculty of Science, Saudi Arabia see Aly and Baffeel [5].

2.2 Source of the Tested Bacteria

The tested bacteria that usually cause a serious risk to human were obtained from the culture collection of Dr. R. Bonally, Laboratoire de Biochemie Microbienne, Fac. De Pharmacie, Nancy, France. The used tested bacteria were either Gram negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeuroginosa* and *Shigella dysenteriae*) or Gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus roseus*).

2.3 Preparation of Soluble 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, 50g were suspended in hot water or organic solvents (Methanol, Diethyl-ether, Ethyl acetate and Chloroform) in sterile 250ml 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 evaporating 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.

2.4 Antibacterial Activity

The antibacterial activity of the different plant extracts was carried out using the disc diffusion agar method described by Irobi and Daramola [25]. About 0.1ml of bacterial suspension ($2x10^6$cfu/ml) was mixed with 10ml of the Mueller Hinton agar in sterile Petri dishes and allowed to solidify. Sterilized filter paper discs (5mm) were soaked in each of the

crude extracts. Five soaked discs were transferred on the surface of each inoculated plate. Discs soaked in DMSO or Ampicillin (A 5354 Sigma**,** 5µg/ml) were used as negative and positive controls, respectively. All plates were then incubated at 37°C and the mean diameter of the inhibition zones were measured after 24hr. of incubation. The minimal inhibitory concentration of each plant extract was calculated using fluorescein diacetate method Chanda et al. [26] and modified by Aly and Gumgumjii [27].

2.5 Toxicity and Antitumor Activity of the Plant Extracts

The toxic effect of different concentration of plant extracts was determined at the cell level using *Artimia salina* as the test organism [28] and the toxic compound must increase mortality up to 50% [29]. The antitumor activity of the six tested plants was determined against Human gastric cancer SGC-7901 cells which were supplied by the National Cancer institute, Egypt. The cells were grown in RPMI 1640 medium (Sigma, USA) with 10% fetal calf serum (Gibco, USA) at 37°C under a humidified atmosphere consisting of 90% air and 10% CO_2 for 48 hr. Human gastric cancer SGC-7901 cells (cell number $8x10^8$/ml) were treated with different doses of the plant extract for 24hr. Cells were centrifuged for 2 min at 1600 g and counted after removing the supernatant using hemacytometer and trypan blue (Sigma, USA) in normal saline (1:1 v/v). The percentage of cell viability was assessed to determine the lethal dose by which 50% of cells were killed (LD_{50}).

2.6 Preliminary Phytochemical Analysis

The plant extracts were analyzed for the presence of any active constituent. Preliminary studies were carried out on the chemical analysis of those extracts using methods described by Fadeyi et al. [30] and Varadharajan et al. [31]. The prepared plant extracts were analyzed for the presence of anthocyanins, butacyanins, flavonoids, saponins, steroids and tannins. For Tannins, one ml of the tested plant extract was mixed with 2ml of 5% ferric chloride and formation of greenish black color indicated the presence of tannins. To detect saponins, to 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins. In case of flavonoids detection, 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid and appearance of yellow coloration indicated the presence of flavonoids. Alkaloids was detected after the addition of 2ml of plant extract to 2ml of concentrated hydrochloric acid + few drops of Mayer's reagent were added and presence of green color indicated the presence of alkaloids. In case of Anthocyanin and betacyanin detection, 2ml of plant extract was added to 1ml of 2N sodium hydroxide and the mixture was heated for 5minutes at 100°C and yellow color formation indicated the presence of betacyanin. Quinones were detected by the addition of 1ml of extract to1ml of concentrated sulphuric acid and formation of red color indicated the presence of quinones. In case of Glycosides detection, 2ml of plant extract, 3ml of chloroform and 10% ammonia solution were mixed. Pink color formation indicated the presence of glycosides. To test for steroids, 2ml of the extract was added to 5 ml of chloroform and the mixture was filtered. Acetic anhydride (2ml) was added to 2ml of the obtained filtrate, followed by addition of 2ml of sulphuric acid. Color changes from violet to blue or green, indicated the presence of steroids.

2.7 Statistical Analysis

The triplicate data were subjected to an analysis of variance and comparison of means was analyzed using SPSS package program version 20, differences were considered significant when $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Tremendous use of antibiotics has developed multiple drug resistance in many bacterial pathogens. Similarly, preservatives like sulfites, nitrates, nitrites and antibiotics, are harmful for human health and have many side effects including headache, nausea, weakness, mental retardation, seizures, cancer and anorexia [3]. Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades.

In the present study, six plants were collected and extracted using hot water, methanol, ethyl acetate, n-butanol, diethyl ether and chloroform. The tested plants were *Azadirachta indica, Rosmarinus officinalis, Lawsonia inermis, Eucalptus globules, Zingiber officinale* and *Lepidium sativium* Table 1.

Table 1. The Selected Medicinal Plants, Their Families and Common Names

Medicinal Plant	Family	Name	Used part
Azadirachta indica	Meliaceae	Neem	Leaves
Rosmarinus officinalis	Labiatae	Rosemary	Leaves
Lawsonia inermis	Lythraceae	Henna	Leaves
Eucalptus globules	Myrtaceae.	Blue Gum	Leaves
Zingiber officinale	Zingiberaceae	Ginger	Rhizome
Lepidium sativium	Brassicaceae	Garden cress	Seeds

The tested plants were selected based on traditional medicine knowledge used by Saudi Arabian people. Plants were extracted using hot water and 5 organic solvents including methanol, n-butanol, diethylether, ethyl acetate and chloroform. All the obtained extracts were screened for their antibacterial activity against *Escherichia coli* Table 2.

This study showed that methanol extract n-butanol and diethyl ether showed significant antibacterial activities against *E. coli* compared to the activity of the water extract and methanol extract was the most active, thus, its activity was recorded against a number of pathogenic bacteria including *Klebsiella pneumonia*, *Pseudomonas aeuroginosa, Shigella dysenteriae* (Gram negative) and *Bacillus subtilis, Staphylococcus aureu, Micrococcus roseus* (Gram positive) as shown in Table 3. The present study correlated with the previous studies that methanol was a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents such as water and ethanol [32]. The results of Aly and Gumgumjii [27] explained that optimal extracts of antimicrobial compounds from various medicinal plants may require different solvents.

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

Medicinal Plant Extracted	Solvent Used					
	Hot water	Methanol	n-butanol	Diethyl ether	Ethyl acetate	Chloroform
Azadirachta indica	14±0.07	29±1.0	19±0.0	14±0.0	12±0.0	15±0.4
Rosmarinus officinalis	13±0.9	27±3.5	14±0.0	20±0.0	18±0.04	14±0.01
Lawsonia inermis	11±0.7	29±2.0	19±0.0	11±0.0	12±0.0	19±2.0
Eucalptus globules	12±1.1	22±1.4	14±0.0	13±0.0	13±0.0	19±1.0
Zingiber officinale	12±1.0	23±2.0	18±0.0	18±0.0	15±0.0	10±1.0
Lepidium sativium	8 ±0.9	11±2.0	14±0.5	12±0.0	11±3.0	10±1.0
Bacterial index*	70	141**	98**	98**	81	87

**Bacterial Index: Total Activity (mm), **: Significant results at $P \leq 0.05$ compared to control (hot water)*

Table 3. The antibacterial activities (Diameter of the Inhibition Zone, mm) of the six plant extracts against different pathogenic bacteria

Tested Pathogenic Bacteria	Diameter of the Inhibition Zone (mm)±SE					
	Azadirachta indica	***Rosmarinus officinalis***	***Lawsonia inermis***	***Eucalptus globules***	***Zingiber officinale***	***Lepidium sativium***
Gram negative bacteria						
Escherichia coli	29±1.0	27±0.2	22±2.00	23±3.0	18±0.01	11±0.40
Klebsiella pneumoniae	16±1.0	16±0.2	14±1.40	22±2.8	15±2.20	08±0.80
Pseudomonas aeuroginosa	17±2.1	16±0.4	12±0.80	24±0.5	18±1.00	08±0.02
Shigella dysenteriae	19±0.8	26±0.4	22±2.00	12±0.6	18±0.61	08±0.03
Gram positive bacteria						
Bacillus subtilis	11±0.5	14±0.6	12±0.40	11±0.4	10±1.0	6±1.04
Staphylococcus aureus	14±0.9	12±0.4	12±0.90	18±0.4	11±0.8	5±0.04
Micrococcus roseus	14±1.0	18±0.8	18±0.01	14±0.8	11±2.0	8±0.04
Bacterial index*	121	129	112	124	101	46

**Bacterial index: Total activity (mm)*

Our results showed that five out of the 6 tested methanolic extracts showed excellent activities against all the tested bacteria. Gram negative was more sensitive compared to Gram positive. Ampicillin was used as positive control. Aly and Bafeel [5] reported similar activities against *Candida albicans* and some human pathogenic fungi and they added that the methanolic plant extracts had fewer side effects in comparison to the formulated drugs and were also inexpensive, showed better patient tolerance and were available for low socioeconomic population. *A. indica* and *R. officinales* were the most active plant extracts to inhibit bacterial growth especially *E. coli* (diameter of the inhibition zone, 27-29mm). On the other hand, *L. sativium* extract showed the weakest activity with inhibition zone diameter 5-11mm while *L. inermi*s, Z. officinale and *E. globules* had moderate activities (10-24mm). This observation is in the line with the work of Ajaiyeoba et al. [33] on *Ritchiea capparoides*. Extract of *E. indica* inhibited four pathogenic bacteria of fish (i.e. *Aeromonas hydrophila, Pseudomonas fluorescens, Escherichia coli* and *Myxobacteria* spp.) which had maximum reduction percentage about 63.90% [34]. Similarly, ginger aqueous extract showed moderate activity against all tested bacteria with MIC of different bacterial species varied from 0.05 to 1.0mg/ml [35]. 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 [36,37].

The results obtained in our study corroborate with the report of Roy et al. [38], which explains that bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and antimicrobial activity of ginger extract decreases with storage. The plant extracts might be specific in their antibacterial activity since zones of inhibition varied for each bacterium and there was no inhibition on some bacteria. Aly and Bafeel [5] reported that*A. indica* showed excellent antifungal activities against both *C. albicans* and *C. tropicals* and weak activity was recorded against plant pathogenic fungi. Bhatnagar and Zeringue, [39] used *A. indica* to inhibit the growth of two species of the genus *Aspergillus*. The different rates of inhibition may probably be due to the quantity of the photochemical compounds present in the extracts. The minimal inhibitory concentrations (MICs) of the plant extract were calculated and compared with that of Ampicillin. It was ranged from 50-150µg/ml. The MIC for *L. sativium* was >200µg/ml Table 4. At 600µg/ml, no toxicity was recorded for all tested extracts except of *A. indica* extract that showed toxicity (% of mortality ≥50).

In the course of searching for antitumor agents, our results showed no antitumor activities against Human gastric cancer SGC-7901 cells Table 5. In contrast, *Curcuma longa* exhibited excellent antitumor activity against Ehrlish ascites carcinoma cell line and no toxicity was found using *Artimia salina* as test organism [27].

The antimicrobial activity of the tested plants may be due to many detected photochemical compounds Table 6. The phytochemical tannin and flavonoids have antimicrobial activities at low concentration and tannins can inhibit the growth of pathogenic microorganisms and act as an antibacterial agent at higher concentration by coagulating the protoplasm of the microorganism [40]. Traditionally, the aqueous extracts of *L. inermis* and *A. indica* are used topically to cure skin diseases [41]. Natarajan and Lalithakumar [42] reported the use of *Lawsonia* to treat fungal diseases. Ficker et al. [43] found that several plant extracts, notably those from *Z. officinale* and *Juglans cinerea*, had pronounced antimicrobial activity. This report supports the use of some plant extracts to control plant and animal pathogenic bacteria. The bioactive constituents of *A indica, Z. officinale*, *E. globules*, *L. inermis*, *R. officinalis* and *L. sativium* may be due to anthocyanins, flavonoids, and tannins, since the presence of these phytochemicals in other plants have been reported by Barnabas and Nagarajan [44]; Barapedjo and Bunchoo [45]; Adekunle and Ikumapayi [46].

Table 4. The minimal inhibitory concentration (µg/ml) of the 6 selected extracts against different pathogenic bacteria and compared with that of ampicillin

Pathogenic Bacteria	Minimal Inhibitory Concentration (µg/ml)						
	Azadirachta indica	***Rosmarinus officinalis***	***Lawsonia inermis***	***Eucalptus globules***	***Zingiber officinale***	***Lepidium sativium***	**Ampicilin**
Escherichia coli	50	100	100	150	100	>200	15
Pseudomonas aeuroginosa	50	150	100	150	150	>200	10
Shigella dysenteriae	50	100	150	150	150	>200	10
Klebsiella pneumoniae	100	100	150	150	150	>200	15
Bacillus subtilis	100	150	100	150	150	>200	5
Staphylococcus aureus	100	150	150	150	150	>200	5
Micrococcus roseus	100	150	150	150	150	>200	5

Table 5. Toxicity against *Artimia salina (%* of mortality) and antitumor activities of the different concentrations of the six selected plant extracts

Medicinal plant	Toxicity against *Artimia salina* (% of mortality)				Antitumor activity (LD_{50},µg/ml)
	0µg/ml (control)	200µg/ml	400µg/ml	600µg/ml	
Azadirachta indica	0.0±0.0	0.0±0.0	10 ± 0.0	54*± 0.0	≥600
Rosmarinus officinalis	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	≥600
Lawsonia inermis	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	≥600
Eucalptus globules	0.0±0.0	0.0±0.0	10 ± 0.0	16 ± 0.0	≥600
Zingiber officinale	0.0±0.0	0.0±0.0	0.0 ± 0.0	8 ± 0.08	≥600
Azadirachta indica	0.0±0.0	0.0±0.0	0.0± 0.0	0.0 ± 0.0	≥600

**: Toxic Effect (% Of Mortality ≥ 50)*

Table 6. The active materials detected in different plant extracts

Medicinal plant	Photochemical Compounds					
	Anthocyanin	**Butacyanin**	**Phytobutanins**	**Flavonoides**	**Stetoides**	**Saponins**
Azadirachta indica	+	-	-	+	+	-
Rosmarinus officinalis	+	-	-	+	+	-
Lawsonia inermis	+	-	-	-	-	-
Eucalptus globules	+	+	-	+	-	-
Zingiber officinale	+	+	-	-	-	-
Lepidium sativium	+	-	-	-	-	-

(+): The material present (-) : The material absent

The detected phytochemical compounds may have a role to inhibit cell wall formation leading to the bacterial cell death. This current study provides some scientific justification for the utilization of extracts from these plants to treat bacterial diseases including unary tract infection, pneumonia and dysentery. However, it is important to point out that crude extracts need to be further purified to isolate and identify the active compounds responsible for biological activity. Thus, from this study, it could be concluded that plant extracts are a promising antimicrobial agent which showed strong inhibitory activity against *E. coli, K. pneumonia, P. aeuroginosa, S. dysenteriae and S. aureus*. They may act to lyse cell wall and inhibite protein synthesis. Further study is required to determine whether they could be used in the inhibition of pathogenic bacteria, with less toxicity. In conclusion, the crude methanolic extracts of *A. indica*, *Z. officinale*, *E. globules*, *R. officinalis* and *L. inermis* exhibited good antibacterial activities with no toxicity and the extracts should be evaluated further in-depth to isolate the active component(s) to be used as an alternative drug.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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