

Antibacterial Activity of Some Medicinal Plant Extracts

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Abstract—Medicinal plants are now gaining attractiveness in treatment of bacterial infections and food preservation. The objective of this study was to assess antibacterial activity of some medicinal plants on pathogenic bacteria. Screening of antibacterial activity of aqueous and methanol extracts of some plants: Jojoba, Ginger, Sage, Thyme and Clove against *Bacillus cereus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli* were investigated. Antibacterial activity was performed by agar diffusion and disc diffusion method. Jatropha, Jojoba, Clove and Ginger extracts showed notable bacterial activity in the first screening step then selected to be tested against *Bacillus cereus* (Gram+), *Staphylococcus aureus* (Gram+) and *Salmonella typhimurium* (Gram-) and their effect was compared using antibiotics as control. Screening results showed potential antibacterial activity of the tested plant extracts against the screened bacterial strains. It was found that methanol extracts exhibited higher antibacterial activity than aqueous extracts. Methanol extract of Jatropha showed the highest inhibition zone against *Staphylococcus aureus* (Gram+) with 24.00 mm diameter, compared to the other plant extracts followed by clove. Meanwhile, the inhibition zones of methanol extracts of Jojoba and Ginger were the same (12mm). The Gram-positive bacteria were found to be more sensitive to aqueous and methanol extracts than Gram-negative bacteria.

Keywords—Antibacterial activity, Food-borne pathogenic bacteria, Medicinal plants, Plant extracts.

I. INTRODUCTION

FOOD can be subjected to contamination by microorganisms. Microbial growth is a major concern because some microorganisms can potentially cause food-borne illness [1]. Incidences of food borne-illnesses are still a major problem. In fact, food spoilages are causing the dramatic economic loss in the food industry. In fact, food spoilages are causing the dramatic economic loss in the food industry. In the meantime, consumers are concerned about the safety of foods containing preservatives. Therefore, there has been growing interest in new efforts and effective techniques in order to decrease those losses [2].

Currently there is intensely scientific awareness in the food industry for using plants in processed foods as natural antibacterial agents, e.g. essential oils and extracts of various species of edible and medicinal plants, herbs and spices which have long been used as alternative source of synthetic antimicrobials for preservation in food and beverages due to

the presence of antimicrobial compounds [2], [3]. Natural products have gained a special attention in the recent years because of increasing the phenomena of acquiring antibiotic resistance by different bacterial species [4].

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times as reported by [5]. Medicinal plants represent a rich source of antimicrobial agents. These plants possess potent medicinal value that is due to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body [6]. They are used medicinally in different countries as well a source of many potent and powerful drugs [7]. Phytotherapy (herbal medicine) have a long-standing history in Egypt.

Scientific investigations for medicinal plants have been initiated in many countries because of their contributions to health care. It was cleared that the primary benefits of using plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. A lot of supplementary treatment strategies were tried. Current social trends in health care showed a definite movement towards the use of natural remedies like medicinal plants away from chemotherapeutic regimens [8].

Spices and herbs have been used as food additives since ancient times, as flavoring agents and as natural food preservatives. Some spices showed antimicrobial activity against different types of microorganisms. Antimicrobial activity depends on the type of spice or herb, type of food and microorganism, as well as on the chemical composition and content of extracts and essential oils. Herb extracts are widely used in the food industry and are generally regarded as safe (GRAS). Hence, they may be considered as natural preservatives acceptable by the food industry [9]. Spices are defined as any dried, fragrant, or aromatic vegetable or plant substance that contributes flavor in a whole, broken, or ground form. Spices include all parts of herbaceous plants except the leaf which is considered as an herb [10].

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Also, use of plant essential oils in both food and pharmaceutical industries has been developed interestingly; systematic examination of plant extracts for these properties has become increasingly important. The use of natural plant antimicrobial compounds is important not only in the preservation of food but also in the control of microbial growth in the diseases condition [11].

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[13]. In recent studies, extracts of various parts of medicinal plants were found to have broad spectrum antimicrobial activities against pathogenic organisms [14].

Antimicrobial agents are effective in curing diseases because of their selective toxicity against pathogenic microbes without causing any harm to the cells of the host [6]. The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms as cleared by [15]. Some studies concluded that the spices may be very valuable because bacteria develop resistance to conventional antibiotics [16]. Antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [17].

Simmondsia chinensis (Link) C. Schneider commonly is known as jojoba and belongs to family *Simmondsiaceae*. It is cultivated in different parts of the world including Egypt due to its high economic value. This plant extract has been reported to be useful as a dietary supplement for use in a weight control regiment in humans, a component of functional food, a food additive, a medical food, or as a therapeutic agent. Different extracts from jojoba plant are widely used in many folk medicinal uses [18], [19]. Also, it has been reported that the essential oil and various extracts from seeds of *Z. jujuba* can be used as natural preservatives in food against the well-known causal agents of food-borne diseases and food spoilage [20].

Jatropha curcas is a nut belonging to the *Euphobiaceae* family. Recently, the tree of this plant has been successfully cultivated in Upper Egypt [21]. *Jatropha curcas* is used for its medicinal properties. This plant has played a major role in the treatment of various diseases including bacterial and fungal infections [22].

Clove (*Syzygium aromaticum*) is the aromatic dried flower buds of a tree in the family *Myrtaceae*. Cloves are antimutagenic, antiinflammatory, antioxidant, antiulcerogenic and antiparasitic [23]. Several studies have demonstrated the potent antifungal, antiviral and antibacterial effects of clove [24], [25].

Ginger (*Zingiber officinale*), belonged to the family *Zingiberaceae*, is a perennial herb with thick tuberous rhizomes. The gingerols (an essential oil) have antibacterial properties [26]. It has been showed that ginger extracts have medicinal properties, antibacterial activity [27]. Also, Ginger inhibited the growth of *Staphylococcus aureus* and *Streptococcus pyogenes* [28].

Thyme (*Thymus vulgaris*), locally known as zaatar, belonged to family *Lamiaceae*; Thymol oil derived from thyme has demonstrated biological properties such as antimicrobial, antioxidant and antiseptic activities [29]. Generally, thyme species are commonly used as herbal tea, flavoring agent and medicinal plants [30].

Sage (*Salvia officinalis* L) *Lamiaceae* - [leaf] is one member of the *Lamiaceae* family and their antimicrobial activities have been reported previously by [31]. Addition of

sage extract to dry fermented sausage was effective for reducing the formation of the biogenic amine (putrescine) due to its antimicrobial activities [32].

Thus, the objectives of the present study were conducted with the aim to screen the antimicrobial activity of some natural medicinal plants/herbs or spices extracts and to evaluate the antibacterial activity of the selected aqueous and methanol extracts of Jojoba, *Jatropha*, ginger and clove against some food-borne pathogenic bacteria and compared to the antibiotics used as control.

II. MATERIALS AND METHODS

A. Medicinal Plants

Jojoba (*Simmondsia chinensis*), *Jatropha* (*Jatropha curcas*), Ginger (*Zingiber officinale*), Sage (*Salvia officinalis* L.), Thyme (*Thymus vulgaris*) and Clove (*Syzygium aromaticum*) were used as sources of antimicrobial agents.

Jojoba (pericarp and leaves) was purchased from the Egyptian Natural Oils Company, Cairo, Egypt. *Jatropha curcas* (leaves and roots) were obtained from the Ministry of Agriculture and Reclamation Land, Egypt. Jojoba and *Jatropha* were air-dried, powdered and kept in tightly closed glass containers.

Sage, Ginger, Thyme and Clove in dry form were purchased from the local market then powdered and kept in tightly closed glass containers.

B. Extraction of the Medicinal Plants

Each sample of the tested medicinal plants was prepared into aqueous and methanol extracts. Aqueous extracts were prepared by dissolving 20gm of fine powder of each plant separately in 100 ml distilled water. The contents were kept on a shaker for 48 h. Then the extract was filtered and dried in air-oven at 40 °C. The extracts were stored under refrigeration at 4°C for further studies. Methanolic extracts of the tested plants were prepared according to [33]. The extracts were obtained using soxhlet extraction of 20 g of each sample for 6 h in about 250 ml methanol then concentrated to dryness under reduced pressure using rotatory evaporator and the residues were stored at 4°C.

C. Microorganisms

The following Gram negative and Gram positive bacterial species were used: *Bacillus cereus* (ATCC 10907), *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 29213) *Clostridium perfringens* (ATCC 13124) and *Escherichia coli* (ATCC 25922). Bacteria strains were obtained from Naval Medical Research Unit 3 (NAMRU-3) Cairo, Egypt. Bacteria were maintained on nutrient agar slant and Sabouraud's Dextrose Agar (SDA) respectively then stored at 4°C. Bacteria were sub-cultured onto fresh media at regular intervals until used.

D. Antibacterial Activity Screening

The first screening step, in this study, was carried out to prop the antibacterial activity of aqueous and methanol extracts of Jojoba, *Jatropha*, Ginger, Sage, Thyme and Clove

plants against *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens* as Gram-positive, *Salmonella typhimurium* and *Escherichia coli* as Gram-negative bacteria species. All experiments were duplicated. The diameter in mm of the clear zone indicated the inhibition activity.

In the second screening step, the antibacterial activity of the four selected extracts of Jojoba, Jatropha, Ginger and Clove against *Bacillus cereus* (Gram+), *Staphylococcus aureus* (Gram+) and *Salmonella typhimurium* (Gram-) was measured by the inhibition zones produced

Antibacterial activity of aqueous and methanol extracts of the tested plant samples was determined according to [34] using disk diffusion assay. The tested bacteria strains were suspended in 5ml 0.1% peptone water and 100µl of suspension were swabbed on entire surface of Plate Count Agar (PCA) for bacteria. Sterile 6-mm filter paper discs (Whatman, Kent, UK) immersed with aqueous and methanol extracts of the tested samples individually were aseptically placed on the center of the inoculated plates. The diameters of inhibition zones were measured in mm after incubation at 37°C for 24 h. Each sensitivity test was performed by using an antibiotic drug as a control. The antimicrobial activity was measured by the inhibition zones produced. All the testes were performed in duplicate.

The antimicrobial activities of the selected plant extracts against the tested bacteria were compared with the available antibiotics. The antibiotic discs such as Chloramphenicol, Penicillin, Gentamycin and Deoxytetracycline were placed on the surface of the plates. The plates were incubated at 37°C for 24 h and after incubation the diameter of the inhibition zones were measured in mm and recorded [35].

III. RESULTS AND DISCUSSION

The current work was designed to investigate the antimicrobial activity of some medicinal plant extracts: Jojoba, Jatropha, Ginger, Sage, Thyme and Clove against some food-borne and spoilage pathogenic bacteria (*Salmonella typhimurium*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus* and *Escherichia coli*). The antibacterial activity of the tested plant extracts was qualitatively assessed by the presence or absence of inhibition zones and the results are depicted in (Table I).

In the first preliminary screening step, the aqueous and methanol extracts of Jojoba showed antibacterial activity with clear zone (+) against *Salmonella typhimurium* and *Staphylococcus aureus*. Meanwhile, methanol extract only of this plant displayed the same inhibition zone against *Bacillus cereus* and *Escherichia coli*. On the other hand, the methanol extract of Jatropha showed antibacterial activity with clear zone (+) against *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus*; whereas aqueous and methanol extracts of this plant showed the same activity and inhibition zones against *Clostridium perfringens* and *Escherichia coli*. Ginger methanol extract showed activity with clear zone against both *Bacillus cereus* and *Staphylococcus aureus*. While, Ginger aqueous and methanol extracts exhibited marked activity against *Escherichia coli*. Sage aqueous and

methanol extracts showed antibacterial activity with clear zone against *Bacillus cereus*. Meanwhile, its aqueous extract only was of the same activity against *Escherichia coli*. Thyme aqueous and methanol extracts gained noticeable activity with clear zone against *Bacillus cereus* and *Clostridium perfringens* bacteria species. Clove aqueous and methanol extracts were noticed for their antibacterial activity against *Salmonella typhimurium* and *Bacillus cereus*. The methanol extract only was of same activity against *Staphylococcus aureus*.

Thus, in the present study the obtained screening results showed potential antimicrobial activity of the tested medicinal plant extracts against all the screened bacterial strains. It was also observed that the methanol extracts exhibited higher antibacterial activity than aqueous extracts. Various publications have been documented for the antibacterial activity of essential oil constituents and plant extracts [36]–[38]. This activity could be attributed to the presence of phytochemicals in the tested medicinal plant extracts. Medicinal plants are a rich source of antimicrobial agents due to the secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids that are found in these plants [7], [39]. The medicinal plant extracts exhibited remarkable activity against some of the representative food-borne and spoilage pathogenic bacteria such as *Staphylococcus aureus* KCTC 1916, *L. monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* KCTC 2004, *Salmonella typhimurium* KCTC 2515 and *Escherichia coli* ATCC 8739. This activity could be attributed to the presence of oxygenated mono- and sesquiterpene hydrocarbons such as eucalyptol, caryophyllene, caryophyllene oxide. The results of this work were compared with the antibacterial effect of various solvent extracts to the standard antibiotics [20].

According to the results given in (Table I), Jatropha, Jojoba, Clove and Ginger plant extracts showed high bacterial activity in the first screening step. Therefore, the antibacterial activity of aqueous and methanol extracts of these four medicinal plants were selected to be tested against *Bacillus cereus* (Gram+), *Staphylococcus aureus* (Gram+) and *Salmonella typhimurium* (Gram-) and their antibacterial effect was comparable to the antibiotics used as control.

In the second screening step, disk diffusion test on agar diffusion method was used for determination of the antimicrobial activity of aqueous and methanol extracts for the selected four medicinal plants (Jojoba, Jatropha, Ginger and Clove) against *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium*. The inhibitory activity was measured by zone diameter (mm) of inhibition.

Bacillus species are common microbes found in most natural environments including soil, water, plant and animal tissues. Most of these species are regarded as having little pathogenic potential. Both *Bacillus cereus* and *Bacillus subtilis* were known to act as primary attacker or secondary infectious agents in a number of diseases and implicated in some cases of food poisoning [40]. Many of the plants in the food and medicine of the indigenous people may have helped to combat these microbes [41].

TABLE I
ANTIBACTERIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS ON FIRST SCREENING STEP

Medicinal Plants	<i>Salmonella typhimurium</i>		<i>Bacillus cereus</i>		<i>Clostridium perfringens</i>		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
Jojoba	+	+	+	–	–	–	+	+	+	–
Jatropha	+	–	+	–	+	+	+	–	+	+
Ginger	–	–	+	–	–	–	+	–	+	+
Sage	–	–	+	+	–	–	–	–	–	+
Thyme	–	–	+	+	+	+	–	–	–	–
Clove	+	+	+	+	–	–	+	–	–	–

(+) = with clear zone, (–) = no clear zone

TABLE II
ANTIBACTERIAL ACTIVITY OF THE PLANT EXTRACTS AGAINST *BACILLUS CEREUS* ON SECOND SCREENING STEP

Medicinal plants	Aqueous Extract		Methanol Extract	
	Test 1	Test2	Test 1	Test 2
	Dia.of zone (mm)	Dia.of zone (mm)	Dia. of zone (mm)	Dia. of zone (mm)
Jojoba	9	9	13	13
Jatropha	10	11	17	18
Ginger	8	8	9	9
Clove	11	11	15	15
Chloramphenicol	31	31	31	31

Dia. = Diameter of clear zone

TABLE III
ANTIBACTERIAL ACTIVITY OF THE PLANT EXTRACTS AGAINST *SALMONELLA TYPHIMURIUM* ON SECOND SCREENING STEP

Medicinal Plants	Aqueous Extract		Methanol Extract	
	Test 1	Test 2	Test1	Test 2
	Dia .of zone (mm)	Dia. of zone (mm)	Dia. of zone (mm)	Zone (mm)
Jojoba	8	8	8	8
Jatropha	9	9	12	12
Ginger	6*	6*	6*	6*
Clove	10	10	12	12
Penicillin	13	13	15	13

Dia. = Diameter of clear zone.

* = no clear zone and report as R resistant

Data predicted in (Table II) showed antibacterial activity of the four potential medicinal plants against *Bacillus cereus* (Gram+). Jatropha (methanol extract) was found to be of the largest zones of inhibition against *Bacillus cereus* followed by Clove then Jojoba came in the third order. Upon using aqueous extracts it was observed that Clove exhibited the largest zones of inhibition while, Jatropha and Jojoba extracts were in the second and third order respectively. Noticeably, Ginger (aqueous and methanol extracts) came in the last order. Thus, the tested plants showed antimicrobial effect against *Bacillus cereus* in both aqueous and methanol extracts. Methanol extracts exhibited higher antibacterial activity than aqueous extracts. Concerning to the antibiotic Chloramphenicol, which used as control, it was of the strongest effect against *Bacillus cereus* (inhibition zone: 31mm).

With regard to the antibacterial activity of the tested plant extracts against *Salmonella typhimurium* (Gram-) in (Table III), it was noticed that all plants except Ginger showed activity with diameter of inhibition zones in the range of 8-12 mm in aqueous and methanol extracts. Ginger showed the lowest activity with diameter of (6 mm) inhibition zones in both aqueous and methanol extracts. Worthy to note that medicinal plant extracts (aqueous and methanol) had the same pattern of antibacterial activity but with slight decrease in zone diameter (mm) of inhibition in aqueous extracts.

It was also observed that the inhibition zones of jojoba against *Salmonella typhimurium* were (8 mm) either in aqueous or methanol extracts. Moreover, the tested methanol extracts exhibited somewhat higher antibacterial activity than aqueous extracts. Worth mentioning to observe that the antibiotic penicillin (as control) showed higher antibacterial activity against *Salmonella typhimurium* either in methanol or aqueous extracts (inhibition zone: 15 and 13 mm) than the activity of all of the tested plant extracts. The Gram-positive bacteria were found in the present study to be more sensitive to aqueous and methanol extracts than Gram-negative bacteria this agreed with [20]. This is probably due to the cell membrane of Gram-positive bacteria, which can interact directly with hydrophobic compounds of essential oils whereas, the external cell wall around the cell membrane of Gram-negative bacteria is hydrophilic and blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membrane as reported by [42].

Data in (Table IV) illustrated that all aqueous extracts of the medicinal plant showed antibacterial activity against *Staphylococcus aureus* (Gram+). Also, it was noticed that the activity of Jojoba aqueous extract was the highest inhibition zone (16 mm); while Ginger in the same extract was the lowest inhibition zone (6mm). Worth to note that the tested methanol extracts exhibited maximum antibacterial activity than aqueous extracts. Methanol extract of Jatropha exhibited the highest inhibition zone against *Staphylococcus aureus* (Gram+) with 24.00 mm mean diameter compared to the tested medicinal plants extracts and followed by Clove. Meanwhile, the inhibition zones of methanol extracts of Jojoba and Ginger were the same and equal (12mm). Concerning, antibacterial activity of the antibiotic Gentamycin against, *Staphylococcus aureus* had inhibition with 15mm diameter in either aqueous or methanol extracts. In some cases

oil and organic extracts (chloroform, ethyl acetate and methanol) exhibited higher antibacterial activity compared to streptomycin, while tetracycline showed higher activity in some other cases than the essential oil and solvent extracts [20].

TABLE IV
ANTIBACTERIAL ACTIVITY OF THE PLANT EXTRACTS AGAINST
STAPHYLOCOCCUS AUREUS ON SECOND SCREENING STEP

Medicinal plants	Aqueous Extract		Methanol Extract	
	Test 1	Test2	Test 1	Test2
	Dia. of zone (mm)	Dia. of zone (mm)	Dia. of Zone (mm)	Dia. of zone (mm)
Jojoba	16	16	12	12
Jatropha	13	13	24	24
Ginger	6*	6*	12	12
Clove	14	15	18	19
Gentamycin	15	15	15	15

Dia. = Diameter of clear zone.

* = no clear zone and report as R resistant

The confirmatory step was performed using the Cup Plate Agar Diffusion Method and showed nearly same results as in the second step (Table V). It was also found that the methanol extracts exhibited higher antibacterial activity than aqueous extracts. Jatropha, Jojoba, Ginger and Clove showed high potential antimicrobial activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus*. Among the different types of antibiotics used in the study it was observed that Deoxetetracycline had wide range of impact on the species of pathogenic bacteria and had highest antibacterial activity on the selected test organisms as seen in (Table V).

IV. CONCLUSION

It can be generally concluded that the obtained results indicated the possibility of using the tested medicinal plants and spices (traditional food ingredients) as natural sources and their extracts were recommended to be used as natural preservatives in food against the screened bacterial species which cause food-borne diseases and food spoilage.

TABLE V

ANTIBACTERIAL ACTIVITY OF THE PLANT EXTRACTS AGAINST *BACILLUS CEREUS*, *SALMONELLA TYPHIMURIUM* AND *STAPHYLOCOCCUS AUREUS* ON CONFIRMATORY STEP

Plants Medicinal	<i>Bacillus cereus</i>		<i>Salmonella typhimurium</i>		<i>Staphylococcus aureus</i>	
	Methanol Extract	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract	Aqueous Extract
Jojoba	24	16	18	12*	32	20
Jatropha	21	19	12*	12*	21	19
Ginger	20	19	12*	12*	18	12*
Clove	25	25	27	25	28	24
Deoxetetracycline	41	41	55	55	44	44

• = no clear zone and report as R resistant

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