

THE ANTIBACTERIAL ACTIVITY OF TRADITIONALLY USED *SALVADORA PERSICA* L. (MISWAK) AND *COMMIPHORA GILEADENSIS* (PALSAM) IN SAUDI ARABIA

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*E-mail: mmalsiny@hotmail.com**Abstract****Background:** Nowadays there is a need to find naturally occurring substances from plants with antimicrobial activity as an alternative to available used antibiotics.**Materials and Methods:** *Salvadora persica* (miswak) and *Commiphora gileadensis* were collected, dried and extracted with either methanol or warm water and the obtained extracts were assessed for their antibacterial activity against 5 different genera of bacteria using agar well diffusion method. The tested bacteria included some human pathogens.**Results:** The obtained extracts exhibited considerable inhibitory effects against all the tested bacteria with various degrees of growth inhibition. It was shown that methanol extract was more effective compared to water extracts. The minimum inhibitory concentrations (MIC) of the methanol extracts ranged from 50-100 µg/ml. No toxicity was found using *Artimia salina* as test organism and no antitumor activity against Ehrlich ascites carcinoma.**Conclusion:** *S. persica* and *C. gileadensis* showed moderate to high inhibitory activity on pathogenic bacteria with no toxicity and can be used traditionally as alternative medicine**Key words:** plant extracts, antibiotic, *Salvadora indica*, miswak, *Commiphora gileadensis*, toxicity, MIC and antitumor**Introduction**

Recently, a number of human pathogenic bacteria develop resistance to commonly used antibiotics, due to the indiscriminate use of antibiotics (Aly, and Bafeel, 2010). Furthermore, many antibiotics with different undesirable side effects have forced many scientists to look for new antimicrobial substances from various sources, e.g. medicinal plants (Ushimaru *et al.*, 2007). More so, according to World Health Organization, more than 80%, of the world's population use traditional medicine for their primary healthcare needs (WHO, 1997). Plants used in traditional medicine contain a wide range of substances. These includes; favenoids, polyphenls. and alkaloids. The screening of plants grown in Saudi Arabia, for antimicrobial activity showed that these plants or their extracts are potential sources for new antibiotics (Abbas *et al.*, 2007, Aly and Bafeel, 2008, Bokhari, 2009, Omer *et al.*, 2011).

The toothbrush tree, *Salvadora persica* L., commonly named miswak or chewing sticks, belong to the family known as Salvadoraceae. It has been used by many Islamic communities as chewing sticks, and has been scientifically proven as being very useful in the prevention of tooth decay, even when used without any other tooth cleaning methods (Salehi and Momeni Danaie, 2006). Chewing sticks gotten from the roots, twigs, or stems of *S. persica* are commonly used in the Middle East, as a means of maintaining oral hygiene. Studies show that *S. persica* extracts can be somewhat compared to other oral disinfectants, and anti-plaque agents, such as triclosan, and chlorhexidine gluconate, if used at a very high concentration (Almas, 2002, Almas *et al.*, 2005). It has been reported that extracts from miswak, posses various biological properties, containing significant antifungal (Al-Bagieh *et al.*, 1994), and antibacterial effects, especially against bacteria considered important for the development of dental plaque (Al-Lafi and Ababneh, 1995).

Commiphora gileadensis (syn. *Commiphora opobalsamum*) communally referred to as Balm of Mecca, belongs to the Burseraceae family, and is widely known in the Mediterranean Basin, within the dry stony hills around the Red Sea, especially within the borders of Saudi Arabia, Yemen, Oman, and Eritrea (Miller and Morris, 1988; Wood, 1997). It is also known as balsam, and well known for the expensive perfume, produced from it, as well as for exceptional medicinal properties that were attributed to its sap, wood, bark, and seeds. It was recognized in ancient times as a perfume and incense plant, commonly found in specific ecological areas (Groom, 1981). It yields a fragrant of oleo-gum-resin, following the damage of the bark (Steyn, 2003). The crude methanolic extract of *Commiphora* show a significant antimycobacterial activity, with a minimum inhibitory concentration of 62.5 µg/ml (Newton *et al.*, 2002). *C. gileadensis* was also active against *E. coli*, and *Bacillus cereus* (Iluz *et al.*, 2010). The aim of this study is to determine the antimicrobial activity of *Salvadora indica*, and *Commiphora gileadensis* against some pathogenic bacteria. Their MIC, antitumor, and toxicity were also recorded.

Materials and methods**Source of microorganisms**

Some pathogenic bacteria were used as test organisms and were obtained from the culture collection of Dr. R. Bonally, Laboratoire de Biochimie Microbienne, Fac. De Pharmacie, Nancy, France.

Collection of plant materials

Salvadora persica, and *Commiphora gileadensis* (Figure 1, Voucher number :H-KAU / 05-40, H-KAU/06) were collected during year, 2010 summer from the Al Baha region of Saudi Arabia. Their identification took place at the Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. Stem parts of collected plants were washed separately with distilled water, and air dried at room temperature for a week, followed by oven-drying for 6 hours, at 60°C.

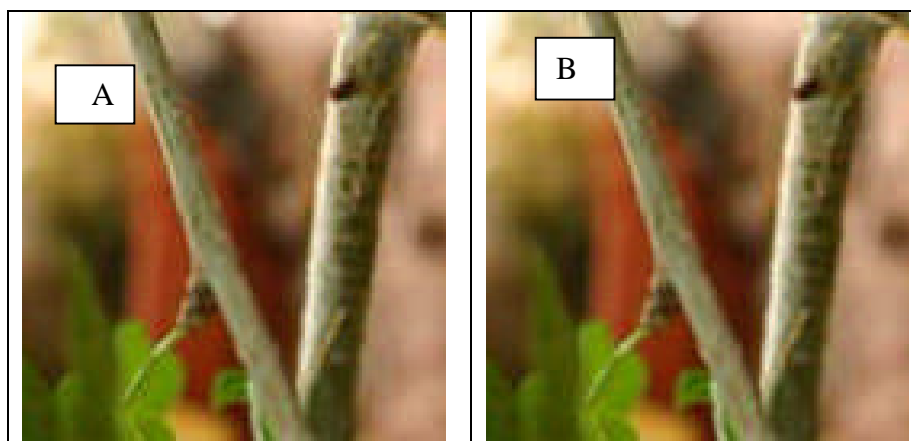


Figure1: The collected fresh plants, *Salvadora persica* (A) and *Commiphora gileadensis* (B)

Preparation of plant extracts

Stem parts were cut into small pieces, and grinded into fine powder using electrical blinder. About 10 g of each dried plant was extracted using 100ml of water, or 95%, methanol for 24hrs (Aly and Bafeel, 2008). The slurry obtained was left in clean sterile glass container, and shaken vigorously for enhanced extraction. The slurry was filtered using a sterile filter paper, and the extract obtained, concentrated to dryness at 40°C, under vacuum or lyophilized, dissolved in dimethyl sulphoxide (DMSO), stored at 4°C, and used within one week.

Antibacterial activity of the plant extracts

The sensitivity of some pathogenic bacteria (test organisms), to the plant extracts was determined using agar well diffusion method (Holder and Boyce, 1994). Preculture of each test organism was prepared using nutrient broth medium. Each Muller Hinton agar plate containing 15ml, of the cooled medium was inoculated with 0.1ml of the pre-culture from the tested bacterium (4×10^6 CFU/ml), and using sterile cork borer wells of 7mm diameter, was filled with 100 μ l, of the tested extract. The zone of inhibition was measured in (mm), after incubation for 24hr at 37°C. DMSO and Ampicillin were used as negative and positive control, respectively (Agwa et al., 2000).

Detection of Minimal Inhibitory Concentration:

Minimal Inhibitory Concentration (MIC) was determined for each extract using Fluorescein diacetate (FDA) method (Chand *et al.*, 1994), in ELISA plate.

Toxicity of the prepared plant extracts

Artemia salina (brine shrimp), was used to investigate the toxicity at different concentration levels of each plant extract from 0.0-400mg/ml, and LD₅₀ was recorded (Adoum, 2009). LD₅₀ values were calculated as the geometric mean of the highest non-lethal dose (with no deaths), and the lowest lethal dose (where deaths occurred).

Antitumor activity of the plant extract

Ehrlich Ascites carcinoma cells, obtained from National Cancer Institute, Egypt were treated with different doses of the plant extract for 24 hours and the cells, centrifuged, counted after staining with trypan blue (Sigma, USA), and the percentage of cell viability assessed, to determine LD₅₀ (Aly and Gumgumgi, 2011). The results obtained was compared with control antitumor agent (Cisplatin).

Statistical analysis

Mean and Standard deviations were recorded for all reading and student t- test was carried out to detect any significant differences

between the results of control and the treated sample.

Results and Discussion

The use of plants, their extracts inclusive for secondary bioactive metabolites (tannins, terpenoids, alkaloids, and flavonoids), in traditional medicine, increased significantly (Fatope, 1995, Crown, 1999). The flexible, strong, young stems or roots of *Salvadora persica* (miswak) and *Commiphora gileadensis* (Balasm) are common in the Saudi Arabian region, and the Middle East. They are inexpensive, and traditionally used to clean teeth. *S. persica* and *C. gileadensis* were collected, and extracted with methanol or water and screened for their antimicrobial activities against different bacteria (*Fusobacterium nucleatum*, *Lactobacillus casei*, *Staphylococcus epidermidis*, *Streptococcus mutans* and *Streptococcus salivarius*). The antibacterial activity of the two selected plants is recorded in Table 1. The diameter of inhibition zone ranges from 23-29mm, with mean antibacterial index of 25mm, and from 15-21mm, with mean index of 18mm for methanol and water extract of miswak respectively. The aqueous and methanolic extracts of *C. gileadensis* showed moderate antibacterial activity, the diameter of inhibition zone ranged between 14-23mm, for methanolic extract and from 14-20mm, for water extract. The lowest antibacterial activity was obtained by water extract of the two tested plants. The diameter of inhibition zone ranged between 26-34mm for ampicillin (positive control). From the previous results, it is clear that the antibacterial activity of the tested extracts was in the following manner; the activity of methanolic extract of *S. persica* was, > methanolic extract of *C. gileadensis*, which was, > the activity of aqueous extract of *S. indica* which was, > aqueous extract of *C.*

Table1: The antibacterial activities (diameter of inhibition zone, mm) of methanol and water extracts of the two plants against some pathogenic bacteria.

Plant used	Salvadora Persica		Commiphora gileadensis		Control antibiotic (Ampicillin)
Test organisms	Methanol extract	Water extract	Methanol extract	Water extract	
<i>Fusobacterium nucleatum</i>	23±4.1	20±0.2	21±1.1	20±0.2	28±0.12
<i>Lactobacillus Casei</i>	25±9.1	20±0.2	21±1.0	20±0.2	26±0.35
<i>Staphylococcus aureus</i>	25±3.2	20±0.2	21±2.2	19±0.2	28±0.54
<i>Staphylococcus epidermidis</i>	27±11.0	20±0.2	22±1.1	19±0.2	34±0.44
<i>Streptococcus mutans</i>	29±6.2	21±0.2	23±1.2	20±0.2	30±0.60
<i>Streptococcus salivarius</i>	23±4.4	15±0.2	19±2.0	16±0.2	30±0.31
Bacterial index**	25.5*	18.8*	20.17*	15.0*	30.5*

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

Table 2: Minimal inhibitory concentration (MIC) expressed in µg/ml of methanol extracts of the rhizomes of the selected plants against different bacteria compared to a standard antibiotic (Ampicillin).

Test organisms	Salvadora persica	Commiphora gileadensis	Control antibiotic (Ampicillin)
<i>Fusobacterium nucleatum</i>	50±5.1	50±12.1	2±0.3
<i>Lactobacillus casei</i>	50±6.2	50±3.0	2±0.1
<i>Staphylococcus aureus</i>	50±4.2	50±5.2	2±0.1
<i>Staphylococcus epidermidis</i>	50±5.0	50±4.1	5±0.1
<i>Streptococcus mutans</i>	75±3.2	100±9.1	5±0.1
<i>Streptococcus salivarius</i>	75±2.3	100±7.4	5±0.6

Table 3: Toxicity and antitumor activity of the methanolic plants extracts

Tested plant	Toxicity against <i>Artimia salina</i> (LD50, mg/ml)	Antitumor activity (LD50, µg/ml)
<i>Salvadora persica</i>	300*	>400**
<i>Commiphora gileadensis</i>	100*	>400

* No significant results at $p < 0.05$ compared to control

** No antitumor activity was recorded up to 400 µg/ml

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gileadensis. The most inhibited bacterium was *Streptococcus mutans*, followed by *Streptococcus salivarius*. Jenkinson and Lamont (2005), reported that mouth bacteria are responsible for some of the most common bacterial diseases in humans especially gum disease and tooth decay caused specially by *S. mutans*, and similar related species through digestion of sugars and starches in foods and production of acids which dissolve tooth enamel. Several studies have shown the antibacterial effects of *S. persica* on cariogenic bacteria, and periodontal pathogens, particularly bacteroides species (Wolinsky and Sote, 1983, Sote and Wilson, 1995, Al-Lafi and Ababneh, 1995), and inhibitory action on dental plaque formation (Ezoddini-Ardakani, 2010). The comparison of antimicrobial activity in aqueous, and alcohol extracts has been made (Al-Bagieh and Almas, 1997). The therapeutic and prophylactic effects of *S. persica* may be due to mechanical cleaning, and the potential release of biologically active chemicals when used, and/or a combination of both (Almas and Al-Zeid, 2004). The results of Almas and Al-Zeid (2004), showed that there was a significant reduction of *Streptococcus mutans* counts using *S. persica* or its extract but the reduction in lactobacilli count was not significant. They concluded that *S. persica* has an immediate antimicrobial effect and *Streptococcus mutans* were more susceptible than lactobacilli. The use of *S. persica* as anti-plaque and in many pharmacological agent in Saudi Arabia, the Arab world, and Iran have been reported (Abderahim and Jurner, 1983, Guile et al., 1996, Ezoddini-Ardakani, 2010). The preliminary studies described by Iluz et al. (2010), revealed that *C. gileadensis* possesses antibacterial activities that validate its usage in the local treatment of wound Infections. Moshi et al. (2007) indicated the use of both *S. persica*, and *Commiphora* in many countries in Africa, like Tanzania as cheap and alternative medicine to manage opportunistic fungal infections.

MICs for the selected plant extracts were calculated using flurocin diacetate method, and compared with that of Ampicillin that is a β -lactam antibiotic that has been used extensively to treat bacterial infections. The MIC of Ampicillin against different tested bacteria ranged between 2-10 μ g/ml, and 50-100, for methanolic extracts of *Salvadora* whereas, it was 75-100 μ g/ml, for methanolic extracts of *C. gileadensis* (Table 2). It can be concluded that, MICs for the two selected plants were greater than those obtained for Ampicillin. Further studies are needed to isolate the active compound(s) in each plant extract, as well as its formulation to be applicable as alternative methods in treating mouth pathogenic bacteria. Therefore, such results are of significant value, required for the confirmation of the therapeutic potency of some plants, used in traditional medicine. It should also form a good basis for further phytochemical and pharmacological investigations. Useful phytochemical antimicrobial agents are polyphenols (simple phenols, and phenolic acids, quinones, flavones, flavonoids, and flavonols, tannins, coumarins); terpenoids; essential oils; alkaloids; lectins; polypeptides and other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisms vary, but depend on these compounds (Aly and Bafeel, 2008). Their mechanism of actions may include enzyme inhibition by the oxidized compounds that act as sources of stable-free radical, and often leading to inactivation of the protein and loss of function (Aly and Bafeel, 2010, Aly and Gumgumgi, 2011). Plant extracts may contain active component, with the ability to complex with extracellular and soluble proteins of the microbial cell, and/or to complex with bacterial cell walls and disrupt microbial membranes (Ali, 1999). Some extracts may have ability to intercalate with DNA, formation of ion channels in the microbial membrane, and competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Cowan, 1999; Bokhari, 2009).

In the course of the search for antitumor agents, our results showed that, there is no antitumor activity against Ehrlich Ascites Carcinoma cell line, up to 400 μ g/ml, of the plant extract (table 3). On the contrary, many authors reported that some plant extracts were shown to possess anticancer activity (Chuang et al., 2000, Skrzypezac-Jankun et al., 2000, Bala et al., 2010), and antitumor activities (Unnikrishnan and Rao, 1995). Amiel et al (2012) report that *C. gileadensis* stem extract contained an apoptosis inducer material that act, in a selective manner, against tumor cell lines, and not against normal cells. The methanolic extract of *Curcuma longa* exhibited excellent antitumor activity with LC₅₀ of 100 μ g/ml (Aly and Gumgumgi, 2011). Laboratory animal model studies have suggested that plant extracts may play an important role in inhibiting the process of carcinogenesis and may be effective in inducing apoptosis or programmed cell death in human myeloid leukemia cells (HL-60), due to active compounds that act as antitumor agents (Kuo et al., 1996, Cui et al., 2006).

Biological testing including brine shrimp toxicity evaluation of some plants used traditionally have played an important role in toxicity studies of plant extracts (Adoum et al., 1997, Araújo and Leon, 2001, Moshi et al., 2007, Aly and Gumgumgi, 2011). Screening of plant extracts for toxicity effects have been carried out but never exhausted. In our study, no toxicity was recorded using *Artimia salina* as test organisms for methanolic extracts of *Salvadora* and *Commiphora gileadensis* with LD₅₀ of 100, and 300mg/ml (Table 3). Toxicity studies of several local plant extracts on insects and fish must be carried out before being applied on animals (Aly and Bafeel, 2010). Some plant extracts including *Mentha arvensis*, *Eugenia caryophyllus*, and *Decaspermum montanum* exhibited 100% mortality whereas extracts of *Cymbopogon citratus* exhibited about 30% mortality at the same concentration (Sukari, 1992). The results of Moshi et al. (2007) indicate that 9, out of 44, plant species exhibited high toxicity with LC₅₀ values below 20 μ g/ml, also, 11, plants gave low toxicity (LC₅₀ values of 50-100 μ g/ml), and 18, plants gave LC₅₀ values greater than 100 μ g/ml.

In conclusion, the crude extracts of *S. persica* and *C. gileadensis* exhibited useful alternatives, or auxiliary antibacterial agent with no toxicity to improve mouth hygiene and treat uncomplicated superficial mouth infections that caused especially by some clinically important bacteria and the two plants should be evaluated further in-depth to isolate the active component(s).

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