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The Antimicrobial in Vitro Effects of Different Concentrations of Some Plant Extracts Including Tamarisk, March, Acetone and Mango Kernel

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ABSTRACT

The present study was aimed to assess the plants antimicrobial effects such as Tamarisk, March, acetone and mango kernel. The aqueous and ethanol extract of the plants and also its smoke was prepared in concentrations of 50 and 25 mg/mL. The bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Enterococcus faecalis* were cultured by linear method. Impregnated extract discs were seeded onto the medium and the diameter of inhibition zone was measured following incubated for 24 h. The mango kernel extract by ethanol showed maximum inhibition zone diameter. Despite the weak antibacterial effect of aqueous and ethanol extract of Tamarix, Smoke extract of this plant shows good antimicrobial properties. Different extracts of March showed antimicrobial effects only on *Candida albicans*. Aqueous and ethanol extracts of acetone did not show significant effects.

INTRODUCTION

Microbial diseases are among the most frequent diseases, worldwide and this is due to microbial resistance against antibiotics which is a major issue that trends to increase. This is important in the treatment of infectious diseases. Some of plants need to be protected from infectious microbial agents and antimicrobial substances are synthesized, therefore they can be considered as sources of microbicide (Cha *et al.,* 2012; Gantam *et al.,* 2007). Pseudomonas is a gram-negative bacterium that in immunecompromised patients and burn it acts as an opportunistic pathogen and is resistant to many common antibiotics. The bacteria have multi factors pemmarizaa and can cause various diseases such as bacteremia and septicemia, respiratory tract, gastrointestinal tract (Satari *et al.,* 2005). Staphylococcus aureus is a major cause of nosocomial infections, as its prevalence is increasing. This bacterium causes a range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and etc. (Shopsin and Kreiswirth, 2001). Nosocomial outbreak of staphylococcal and resistance in Staphylococcus aureus is a serious problem. This bacterium is a public health problem due to its increased resistance to antimicrobial agents. Results of research programs performed on six species of medicinal plants showed that four of those plant species which are typically used for treatment of skin and respiratory tract infections are effective against different Staphylococcus strains (Finegold, 1990). To overcome microbial resistance against antibiotics, it seems necessary to approach new antimicrobial compounds (Katzung, 2000). Considering the issue that most of herbs show side effects with lesser extent than synthetic drugs on the human body, they could be considered as good alternatives for these types of drugs. Zareeiyan and colleagues (2007) showed that using *Tamarix aphylla* ointments for healing of wounds are not accelerated but it increased eosinophils within the wound areas and as well as hasten the repair of damage to those or *Ferula asafoetida* ethanol extracts, inhibits the *in vitro* development and growing of more than 90% of the *Trichomonas vaginalis* parasite (Sarkari *et al.,* 2009).

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Peganum harmala is used as an air disinfectant, anti-bacterial and parasite rejection and pain reliever (Astulla *et al.,* 2008). Mangifera indica contains a variety of antioxidant and antimicrobial properties, which has different effects on different types of bacteria (Khammuang and Sarnthima, 2011). Thus based on the above introductory comments regarding roles played by herbal reagents, present study was aimed to examine the antimicrobial effects of ethanol, water and smoke extract of the plants (*Tamarix aphylla, Trichomonas vaginalis, Peganum harmala, Mangifera indica*) at two different concentrations on some bacteria.

MARTIAL AND METHODS

Preparation of extracts

We have used three different types of extracts in the present study, in the first, stems and leaves of each plant (*Tamarix aphylla, Trichomonas vaginalis, Peganum harmala, Mangifera indica*) were dried and turned into powder form.

Preparation of the water extract

A 50 g of the powder prepared from the plants were gently mixed with one litre of distilled water and then were kept at 60 °C for 24 hours. Following incubation the solution was filtered out. Subsequently the water extract was obtained at a concentration of 50 mg/L and the resultant filtered solution was kept at 4 °C. The same procedure was performed for other concentrations (e.g 25mg/L).

Preparation of the ethanol extract

A 50 g of the powder form of each purified plant parts was mixed up in a mixture of ethanol and water (in a ratio of 70/30 mL ethanol/water for 48 to 72 hours at 25 ° C with gentle shaking to dry up completely.

Then the remaining 50 grams of powder were well mixed with one litre of distilled water and filtered through the filter paper and the ethanol extract was obtained at a concentration of 50 mg/L. The same procedure was performed for other concentrations (e.g 25mg/L).

Preparation of the Smoke extraction

To obtain smoke, 50 grams of the dried herb was completely burned by indirect heat in a sealed container with appropriate oxygen level. Smoke was collected by vacuum pump and injected into the chamber with a volume of one litre of distilled water. For mixing the water and smoke, the chamber was put onto a shaker for an hour (Braithwaite *et al.,* 2008). Then the smoke extract was obtained at a concentration of 50 mg/L. Due to the traditional using of Tamarix aphylla and Peganum harmala smoke as having antibiotic properties, only these two plant concentrated smoke of them were prepared. The same procedure was performed for other concentrations (e.g 25mg/L).

Used Bacteria

In this study the *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Enterococcus faecalis and Candida albicans* were purchased from Padtan Teb Company.

Disk diffusion method

The disk diffusion method was used to determine the susceptibility of bacteria to the different extracts. Initially, half of McFarland unite bacterial suspensions were prepared from all bacteria and then 100 ml of the suspension were produced on the surface with Muller-hinton agar medium. Sterilized blank discs (negative control) and antibiotic discs (positive control) and discs impregnated with various extracts were prepared and placed on a medium. Plates were incubated at 37 ° C for 24 h. The zone of growth inhibition is measured by Caliper with an accuracy of 0.1mm. Experiments were performed 4 times for each bacterium. The data were analyzed using SPSS software version 16 at the significance level of $P < 0.05$.

Measurement of MIC & MBC

In order to the positive antibiogram test of extract, serial dilutions were prepared and placed in separate tubes. Half of the bacterial McFarland was added to solution and then the tubes were incubated for 24 hours and finally the growth status of bacteria was studied.

RESULTS

The results of measuring the diameter of inhibition zone plate show that among the extracts, mango seed extract (Mangifera indica) has the most powerful antimicrobial effects. Water and ethanol extract of mango almost have the same effect. The maximum affect was on Streptococcus pneumoniae and its effect on Klebsiella pneumoniae was at minimal level. The concentration of 50 mg/l extract has also an effect of more than 25 mg/L. But this effect is not doubled by doubling the concentrations (Table 2 and 3). Water and ethanol extracts of *Tamarix aphylla* have little impact on various bacteria. The maximum effect of the ethanol extracts was on S. pneumoniae bacteria with an inhibition zone diameter of 3.3 mm.

The minimum its effect was on Gram-negative bacteria while it had no effect on P. aeruginosa (Table 2 and 3). It is interesting to note that *Tamarix aphylla* smoke extract has the highest antimicrobial effect on all the bacteria. The most bacteria-killing properties of this extracts was on Streptococcus pneumoniae with an inhibition zone diameter 14.5mm and the minimumof its effect was on Klebsiella pneumoniae with an inhibition zone diameter of 7.9mm (Table 1). The water and ethanol and smoke extract of *Peganum harmala* only are affected on Candida albicans while the diameter of the inhibition zone size is approximately the same as was in the three extracts (Table 1, 2 and 3).

As shown in the Tables 2 and 3, *Trichomonas vaginalis* extracts had little effect but in the some cases shown limited inhibition zone. Regarding the greatest antimicrobial ethanol extracts of Mangifera indica and smoke extract of *Tamarix aphylla*, MBC and MIC test were applied for these extracts. The results indicate that, the concentration required for killing bacteria and limit the growth at Mangifera indica extract is less than *Tamarix aphylla* smoke extract, except Streptococcus pyogenes and Streptococcus pneumoniae. These ratios are listed in Table 4.

As table 1 shown, *Tamarix aphylla* smoke extract is effective on all bacteria but *Peganum harmala* smoke extract was effective on Candida albicans. Size of the zone of growth inhibition all the extract which the antibiogram result was positive compared to the control group (distilled water) are statistically significant (P <0.05). As table 2 shown Mangifera indica ethanol extract shows the highest effects and Trichomonas vaginalis and Tamarix aphylla ethanol extracts had an effect with a lesser extent. *Peganum harmala* has only effect on the Candida fungus. Size of the zone of growth inhibition all the extract which the antibiogram result was positive compared to the control group (distilled water) are statistically significant (P <0.05). As is clear from table 3 Mangifera indica water extract shows maximum effects and Trichomonas vaginalis and Tamarix aphylla water extracts had the minimum effect.

Peganum harmala has only effect on the Candida fungus. Size of the zone of growth inhibition all the extract which the antibiogram result was positive compared to the control group (distilled water) are statistically significant (P <0.05). Mangifera indica extract in the most cases had more limitations and fatality properties compared to Tamarix aphylla smoke extract.

Table. 1: Demonstrates growth inhibition zone diameter of the different bacteria and different concentration of *Tamarix aphylla* and *Peganum harmala* smoke extract**.**

Smoke	concentration mg/l	Streptococcus pyogenes	Enterococcus faecalis	Klebsiella pneumoniae	Candida albicans	Pseudomonas aeruginosa	Staphylococcus aureus
Tamarix aphylla	50	14 ± 1	8 ± 3.5	7.9 ± 3	8 ± 3.1	11.1 ± 1	13.1 ± 2
	25	11.3 ± 3	5.8 ± 2.3	5.1 ± 1	7.3 ±2.1	8 ± 2.5	9.2 ± 3
Peganum harmala	50	-	-	-	7.8 ± 3	-	-
	25	-	-	-	6.5 ± 2.2	-	-

Table. 2: Demonstrates growth inhibition zone diameter of the different bacteria at the presence of various concentrations of ethanol extracts

Smoke	concentration mg/l	Streptococcus pyogenes	Enterococcus faecalis	Klebsiella pneumoniae	Candida albicans	Pseudomonas aeruginosa	Staphylococcus aureus
Mangifera indica	50	15 ± 1	11 ± 1	13.5 ± 1	12 ± 2	13.1 ± 1.5	14 ± 2.1
	25	12.8 ± 2	9.5 ± 2	11.7 ± 2	11.1 ± 1.2	11.2 ± 2	12.1± 3.1
Tamarix aphylla	50	-	-	-	7.6 ± 1.3	-	-
	25	-	-	-	5.3 ± 1	-	-
Peganum harmala	50	3.1 ± 1	1.3 ± 0.1	0.8 ± 0.1	2.1 ± 0.9	-	1.2 ± 1.2
	25	1.2 ± 0.1	-	-	1.1 ± 0.3	-	0.6 ± 0.1
Trichomonas vaginalis	50	2.1 ± 0.1	-	-	-	-	1.1 ± 0.3
	25	-	-	-	-	-	-

Table . 3: Growth inhibition zone diameter of the different bacteria at the presence of various concentrations of water extracts

Water	concentration mg/l	Streptococcus pyogenes	Enterococcus faecalis	Klebsiella pneumoniae	Candida albicans	Pseudomonas aeruginosa	Staphylococcus aureus
Mangifera indica	50	14.2 ± 1.1	11.2 ± 1	13± 3	11.1 ± 2	14.2 ± 2	14.3 ± 2
	25	10.5 ± 3	9 ± 2	10.2± 1	10 ± 1	10.8 ± 4	11.1± 1
Tamarix aphylla	50	-	-	-	7.1 ± 1	-	-
	25	-	-	-	5.2 ± 1.1	-	-
Peganum harmala	50	2.8 ± 1.1	1.2 ± 0.1	1.1 ± 0.2	2 ± 0.5	-	1.2 ± 0.1
	25	1.2 ± 0.1	-	-	1.2 ± 0.4	-	-
Trichomonas vaginalis	50	2 ± 0.1	-	-	-	-	1.2 ± 0.1
	25	-	-	-	-	-	-

Table. 4: Demonstrates minimum lethal concentration (MBC) and minimum limiting concentration (MIC).

	concentration mg/l	Streptococcus pyogenes	Enterococcus faecalis	Klebsiella pneumoniae	Pseudomonas aeruginosa	Staphylococcus aureus
Mangifera indica	MBC	6.25	50	12.5	25	12.5
ethanol	MIC	3.125	25	6.25	12.5	6.25
Tamarix aphylla smoke	MBC	12.5	25	12.5	6.25	3.25
	MIC	6.25	12.5	6.25	3.125	1.625

DISCUSSION

The chemical components of herbs with fewer side effects than the synthetic antibiotics could be considered as excellent option for treatment of microbial diseases. Using microbiological methods in the present study we have demonstrated that mango kernel extract by ethanol showed maximum inhibition zone diameter. Despite the weak antibacterial effect of aqueous and ethanol extract of Tamarix, Smoke extract of this plant shows good antimicrobial properties. In agreement with our study Sarkari and colleagues also indicated that water extract of *Trichomonas vaginali* at 2 mg/ml concentration after one hour exposure to the parasite *Trichomonas vaginalis* causes 90% death (Sarkari *et al.,* 2009). Some other studies also showed that *Trichomonas vaginalis* is sensitive to *Trichomonas vaginalis* extract (Ramadan and Al Khadrawy, 2003). The cytotoxic effects of *Trichomonas vaginalis* ethanol extract has also been reported (Maraghi *et al.,* 1995). In this study, both types of *Trichomonas vaginalis* water and ethanol extract were examined, However, other types of microorganisms have been used the tested extracts showed weak inhibitory effect on a variety of bacteria. According to these results, it seems that this plant has an inhibitory effect on protozoan parasites. Shahverdi and colleagues showed that the *Peganum harmala* extract has anti-microbial properties and its extract inhibited the growth of *Staphylococcus aureus*, Escherichia coli as well as Candida albicans fungus colonies (Shahverdi *et al.,* 2005). The Harmin, Harmaline and Vazikinoun content of alkaloid compounds in the seeds of *Peganum harmala* shows antifungal, anti-parasitic and anti-bacterial properties (Astulla *et al.,* 2008). In this study, three types of water, smoke and ethanol extract of *Peganum harmala* were performed but all extracts had a cytotoxic effect only on the *Candida albicans*. In all cases by repeated testing, lacks of the other antimicrobial effects of the extracts were observed. The antibacterial *Mangifera indica* extract is also determined by Kabuki and colleagues and shown and reported that this extract has antimicrobial impacts on a broad range of different microorganisms as well as more potent antimicrobial effects on Gram-positive bacteria than Gram-negative ones (Kabuki *et al.,* 2000). In consistent with our study, Engels and colleagues also showed that *Mangifera indica* extract has good antimicrobial properties (Engels *et al.,* 2009). Khammuang and Sarnthima showed that *Mangifera indica* extract has antioxidant and anti-microbial properties with the greatest effect on *Staphylococcus aureus* (Khammuang and Sarnthima, 2011). Our results also indicated that *Mangifera indica* water and ethanol extract has antimicrobial properties and had an inhibitory effect on all tested microorganisms, while the most impact was observed on *Streptococcus pneumoniae*. Research on Tamarix plants identified that the water and ethanol extracts of the seed of *Tamarix gallica* has strong antibacterial effect on *Klebsiella pneumoniae, Staphylococcus aureus* bacteria and *Candida albicans* fungus, Such an inhibition zone diameter with Tamarix water extract and *Candida albicans* medium was 17mm, which the maximum diameter of the inhibition zone among tested microbes (Zaouia *et al.,* 2010). According to the other studies, the methanol extract of the roots of *Tamarix indica* has average effect on Gram-positive and Gram-negative bacteria. This sensitive bacterium was *Shigella sonnine* with an inhibition zone diameter of 16.34mm. The different strains of Shigella and other bacteria such as *Staphylococcus aureus* showed wide variety of sensitivity to the extract (Rahman *et al.,* 2011). In this study, the antimicrobial effects of water, ethanol and smoke extract of *Tamarix aphylla* was investigated. While except the smoke extract, the extracts show little effect. Regarding traditional methods for using the *Tamarix aphylla* indicates that people in desert regions used stems smoke of Tamarix as antibiotic which in a way may probably confirm our results. Finally, results of our study revealed that the *Mangifera indica* extracts and stem smoke extract of *Tamarix aphylla* could possibly be proposed as antimicrobial agents.

CONCLUSION

Overall, it can be concluded that the *Mangifera indica* and *Tamarix aphylla* smoke extracts can be used as antimicrobial agents as well as *Peganum harmala* extract inhibit the growth of *Candida albicans*. While it has no effect on normal flora bacteria, however, most of the side effects of these extracts on the human body deserved to be examined.

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