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Antibacterial activity of five medicinal plant extracts against some human bacteria

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ABSTRACT

The antimicrobial effect of ethanol extracts of five plant extract against human bacteria namely Streptococcus pyogenes ATCC® 19615™, Streptococcus pneumoniae ATCC 49619, Staphylococcus saprophyticus ATCC®15305, Hafnia alvei ATCC 51873, Acinetobacter baumannii ATCC 19606,Enterococcus faecalis ATCC 29212,Proteus mirabilis ATCC 35659,Serratia marcescens ATCC 274, Staphylococcus aureus ATCC® 2592 were determined using broth microdiluation method. The results showed that Maximum Inhibition Concentration was observed with ethanol extract of Sesamum indicum against S.aureus, S. pyogenes, S. pneumonia, *A. baumannii, E. faecalis and P. mirabilis (100 ppm). Minimum Inhibition Concentration was observed with ethanol extract of Satureja hortensis L. against S. pyogenes,* S. pneumonia, *S. saprophyticus, A. baumannii and P.mirabilis (25ppm).*

Keywords: Antibacterial activity, medicinal plants, human bacteria, MIC.

INTRODUCTION

During the last several decades, natural products with antimicrobial effect were investigated in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health. Aromatic plants are known for a very long time and they are used in phototherapy and food preservation [1]. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies [2, 3, 4]. Savory (*Satureja hortensis* L.) from the *Lamiaceae* family is a species frequently cultivated in south-eastern Poland. In folk medicine, *S. hortensis* is used as stomachic, stimulant, carminative, expectorant, aphrodisiac, antispasmodic and antidiarrhoeal [5]. *Sesamum indicum* (Sesame) is an ancient spice, one of the first recorded plants used for its seeds. It has been used for thousands of years and is still an oil seed of worldwide significance. Several beneficial effects of sesamol were reported including antioxidation, chemoprevention, antimutagenic, and antihepatotoxic properties [6, 7, 8]. Traditionally, sesame seed oil was used to remove wrinkles and prevent aging, when applied in a facial massage to the skin [9]. *Zataria multiflora* is a plant belonging to the Lamiaceae family that geographically grows mainly in Iran, Pakistan and Afghanistan [10]. The plant consists of small ovate or nearly round dotted, leathery leaves mixed with numerous minute flowers [11]. *Cassia angustifolia* (syn *Cassia senna*) in commerce is known as Indian or Tinnevelly Senna, is a well know traditional medicinal plant belonging to family Leguminosae [12]. *Cassia angustifolia* is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of

constipation [13, 14]. Bell peppers (*Capsicum L.*) are the most important vegetable spice grown in the tropical and sub-tropical regions of the world because of their color, taste, pungency, flavor and aroma [15, 16, 17].

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacterial strains were obtained from standard laboratory of veterinary department Zabol University, Zabol, Iran. To evaluate the antibacterial activity the plant extracts were investigated using strain of bacteria *Streptococcus pyogenes* ATCC® 19615™,*Streptococcus pneumoniae* ATCC 49619,*S. saprophyticus* ATCC®15305, *Hafnia alvei* ATCC 51873, *Acinetobacter. baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659,*Serratia marcescens* ATCC 274, *Staphylococcus aureus* ATCC® 25923. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4^{o}C until required for study.

Agar disk diffusion assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar as recommended by CLSI (CLSI, 2002). The procedure followed is briefly described here. *S. pyogenes* ATCC® 19615™, *S. pneumoniae* ATCC 49619, *S. saprophyticus* ATCC®15305, *H. alvei* ATCC 51873, *A. baumannii* ATCC 19606, *E. faecalis* ATCC 29212, *P. mirabilis* ATCC 35659, *S. marcescens* ATCC 274, *S. aureus* ATCC® 25923 plates were grown overnight on blood agar, Nutrient agar and colony suspension was prepared using the sterile salin water equivalent to a 0.5 McFarland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media plate. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis viz. ceftazidim (30 µg), erythromycin (15 µg), ceftazidime (30 µg) and tetracyclin (30 µg).

Plant materials

The leaves of Z. *multiflora, C. angustifolia, S. hortensis* and fruits of C. *annuum* and seed of *S. indicum* were collected in the different regions of Iran (Zahedan and Kerman, SE of Iran) and plants were deposited in herbarium of Zabol University. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts

Plants were properly dried and pulverized into a coarse powder. Each of 20 g grinded powders was soaked in 60 ml ethanol 95 %, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper) .Then the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 4^{0}C in air tight screw-cap tube.

Minimum Inhibitory Concentration (MIC) of plant extracts

The broth microdilution method was used to determine MIC. All tests were performed in Mueller Hinton Broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 200ppm to 25 ppm. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^{6} CFU/ml) was added to each well to achieve a concentration of 10^{4} CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

RESULTS AND DISCUSSION

The study showed that the levels MIC observed ranges from 25 to 100 ppm. Maximum Inhibition Concentration was observed with ethanol extract of *S.indicum* against *S.aureus, S. pyogenes, S. pneumonia, A. baumannii, E. faecalis* and *P. mirabilis* (100 ppm). Minimum Inhibition Concentration was observed with ethanol extract of *S.hortensis* against *S. pyogenes,* S. pneumonia, *S. saprophyticus, A. baumannii* and *P.mirabilis* (25ppm) (Table 1).

Plant extracts are a potentially useful source of antimicrobial compounds. In the study maximum inhibition was observed with ethanol extract of *S.indicum* against *S.aureus, S. pyogenes, S. pneumonia, A. baumannii, E. faecalis* and *P. mirabilis* (100 ppm). Minimum Inhibition Concentration was observed with ethanol extract of *S.hortensis* against *S. pyogenes, S. pneumonia, S. saprophyticus, A. baumannii* and *P.mirabilis* (25ppm). The results of Razzaghi-Abyaneh showed *S. hortensis* L. as strong inhibitor of aflatoxin production by *A. parasiticus* [18]. In the

study, the high concentrations of oil *S. hortensis* processed greater antimicrobial effects against all microbial species than other low concentrations. 10, 20, 30 dose of oil were partly ineffective on *S. salivarius*, *L. monocytogenes*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *P. aeruginosa* and *S. Mutans* [19]. The results of Gulluce, revealed that the maximal inhibition zones and minimum inhibition concentration values for bacterial strains, which were sensitive to the essential oil and nonpolar extract of *S. hortensis*, were in the range of 5-29 mm and 31.25-500 µL/mL and 9-16 mm and 250-500 µL/mL, respectively [20]. Savory with all its antibacteriostatic and fungistatic properties resulting from biologically active substances, particularly secondary metabolites and essential oils including carvacrol and phenolic compounds, is known to be affected by phytopathogens [21, 22]. According to the study of Rao aqueous extract of Sesamol had antimicrobial activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *K. pneumoniae*, and *Candida albicans* [23]. The study of Ahmed revealed the methanolic extract of Sesame exhibited a mild antimicrobial activity against *S. aureus* and *C. albicans*, but no inhibitory effect on S. *pneumoniae*. *C. albicans* was mildly inhibited by the aqueous extract [24].

Table1: Antimicrobial susceptibility, MIC plant extracts for Standard bacteria

Bacteria	Z.multiflora	C.angustifolia	S.hortensis	S.indicum	C.annuum L	Antibiotic resistance
Staphylococcus aureus	100	50	Any growth	100	50	E,CE,TE
Streptococcus pyogenes	100	50	25	100	100	-
Streptococcus pneumoniae	50	50	25	100	100	E,CE,CF
Hafnia alvei	50	100	50	25	25	E,TE
S. saprophyticus	50	50	25	25	50	E,CF,TE
***Acinetobacter**. baumannii*	50	50	25	100	50	CE,TE
Enterococcus faecalis	50	25	50	100	Any growth	E,CE
Proteus mirabilis	100	25	25	100	100	E,TE
Serratia marcescens	100	25	100	50	50	CE

E= Erythromycin, CE= Cefixime, CF= Ceftazidime, TE= Tetracyclin

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