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Studying the Effect Echinophora Platyloba Extract on Bactira (*Staphilococus aureus* and *Pseudomonas aeroginosa*) and Fungi (*Candidia albicans*, *Aspergilus flavus* and *Aspergilus niger*) *In Vitro*

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Abstract: With daily increasing of medicinal herbs consumption in medical treatment, this branch and field of medicine is find the complementary of especial position in diseases treatments. Some herbs are consuming in traditional treatments but are not considered much yet which one of them is Echinophora platyloba. These herbs made us to study the effect of herbal essences on two spices bacteria and three types of fungi. From the picked herbs in growing season, its methanol essence achieved by the percolation method. In the next phrase, methanol essences of Echinophora Platyloba separately tested against two bacteria and three fungi and after study of the culture the results recorded. The results showed that the anti-bacterial effect on the growth of two bacteria (*Staphilococus aureus* and *Pseudomonas aeroginosa*) of this essences are methanol and even in high concentration the growth essences reach to zero. It is in case that there was no observation of prevention from growth for three considered funguses such as *Candida albicans, Aspergillus flavus* and Fluse and *Aspergillus niger*. Anti-septic components obtained from plants are more effective in treating the diseases. They not only are effective to treat septic diseases but also can eliminate more side effects resulted from other chemical antibiotics.

Key words: *Echinophora platyloba* · Essence · *Staphilococus aureus* and *Pseudomonas aeroginosa*

INTRODUCTION

Medical herb is a branch of traditional medicine of countries such as Iran, which had been played vital role in treatment of disease till a century ago [1]. With appearance of chemical medicines, these types of medicine place for the herbal ones and played main role in diseases treatments. Fortunately in recent years using of medical herbs is increasing in case that now about one third to half of existing medicines in USA has herbal resources [2]. Also in England, herbal productions and herbal supplements are increases [3]. In addition to Micro biologists are so intended to uses in comparative with herbal drugs, significantly [4]. Genus Echinophora, of family Apiaceae, having 4 species in Iran distributed in the west and western north of Iran [7]. Species E.platyloba is known by local names of Khosharize, Tigh Touragh, Tigh Masti, Koshander, Kouzang, Tanghez or Khousharouze. This plant is a native in Iran and is being studied for the first time [6]. This plant is used for scenting the foods in Kermanshah, so it is of pasture plants [5]. Recently, regarding to its side effects and its strength on pathogenic microorganisms against antibiotics, so in medical field, its extracts has been attended more with its biological properties. Plant components with anti microbial properties are accounted as valuable resources in medicine and they can be beneficial in treating the septic diseases. Anti-septic components obtained from plants are more effective in treating the diseases. They not only are effective to treat septic diseases but also can eliminate more side effects resulted from other chemical antibiotics. This study is

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going to examine the anti bacterial properties of methanolic extract of Khosharizeh plant against two species of bacteria and three species of fungi.

MATERIAL AND METHODS

In this interventional study, the plant was collected in summer 2007 from Lashkarak hill located in eastern north of Tehran. Taxonomy of plant materials was verified by data of the biological part of medical college of Azad University of Tehran and assisting from the book of Dr. Vali-allah Mozafarian. Collected plat materials were dried under the shadow and its leaves removed from the stem and then they were powdered separately. Powdered samples of stem and leaves were extracted by floating in methanol and then by hot water. Nearly 200 gr of powder for each sample (leaves and stems) solved in 600 ml of methanol and then left for about 48 h in the ambient temperature (25°) while shaking slowly. Each extract filtered by Watman paper filter No.1. Filtered extracts were dried in the room temperature by air flow [14,6,8]. Extracts dried by UV ray were sterilized at night and their sterility was studied on nutrient agar medium by culturing the plant extract. All dried extracts stored in room temperature until examination. Later, methanolic extracts of Khosharizeh were tested separately against two species of bacteria and 3 species of fungi. Microorganisms were provided in the microbiology section of medical college of Islamic Azad University of Tehran. The identity of test microorganisms was verified by standard microbial identification system of this sector.

To Evaluate the Anti Bacterial Activity, Serial Dilution Assay Was Conducted as Following: Using turbidity method, there was prepared 10 dilutions from studied bacteria culture with the first dilution based on Macfarland 0.5 standard. Of these dilution tubes were cultured on the Muler Hinton mediums of agar and incubated for about 24 h in 37°C. the best plate was selected based on their colony [15]. Then dried plant extracts solved in 10cc of serum physiology 0.09% and its concentration attained to 300 gr/l and sterilized by 0.45 µ filters. Then fluid incubation was prepared from selected bacterial culture and then 1cc of it solved in 1cc of dissolved extract and added to culture medium of Muler Hinton Agar with 6 treatment to be ensured of its result (for each species of bacteria). Two plates were considered as control (without extract). There was no bacterial growth in any plates, while it was seen in two control plates. The minimum inhibition concentration (MIC) then determined for both species of bacteria sensitive to the plant extract as following. Of dissolved extract with 300 gr/l, there was prepared 20 successive dilutions then it was added about 1cc to each tube and incubated for about 24h in 37°C. Afterward, 1cc of each tube added to each plate and finally melted Muler Hinton Agar medium was added and incubated again for 24h in 37°C. And finally plated were examined for colonial growth (for each species of bacteria)[15]. To study the anti fungal effects of plant extract, there was used Disk Diffusion Paper method and Punched Hole Test. To attain the suitable concentration of fungi, there was used of spectrophotometery and selected a dilution with OD of 0.09 to 0.11. This dilution was cultured for any kind of fungi on the Saborad dextrose Agar (SDA) medium[15]. Disks with 6mm in diameter were smeared with 10 µl of previous dissolved extract; and after fixation, the concentration of extract on disk was 5mg/disk. Then in some plates there were located disks smeared with above mentioned extract and in some plates there was bored some holes on them and extract added to them.

Then they left in 37°C for 48h to be fermentized and then incubated in 25°C for about 72 h(each assay with two repeats) and then the corona of growth inhibition was measured to study the anti fungi activity [15].

RESULTS

Results indicated that methanolic extract can inhibit the growth of two bacterial species of S. aureus and P. aeroginosa and even it can be zero in higher concentrations. While there was non indicated considerable growth prevention corona for three species of fungi, *Candidia albicans*, *Aspergilus flavus* and *Aspergilus niger*.

DISCUSSION

This herb is one of the four species in Iran which is the only species in Iran [9,10] which is used also as the food species [5]. Till now there are reports of scientific evidences about the anti-microbial effect of E.sibthorpiana. [5]. In study which is carried on the Echinophora Platyloba it is cleared that this herb has elements such as Saponin, Alkaloids and Flavonoids. [11]. Saponines are elements with toxic effects. [12]. Saponins are glycosides with paide terpenoeied with Surface active properties. One of the existing saponin elements in Capsicum SP., is CAY-I which is in lower level of toxic dozes for man kind cells[13]. CAY-I, is a saponin which

Table 1: Antibacterial activity of methanolic extract of Khousharizeh plant against species of bacteria *Pseudomonas areruginosa* (left) and *Staphylococcus aureus* (right) based on serial dilution method

Extract concentration (gr/l) of Khousharizeh	The number of *Pseudomonas areruginosa* colony	Extract concentration (gr/l) of Khousharizeh	The number of *Staphylococcus* aureus colony
300	-	300	-
300	-	30	-
3	-	3	3
3×10^{-1}	-	3×10^{-1}	9
3×10^{-2}	16	3×10^{-2}	17
3×10^{-3}	23	3×10^{-3}	39
3×10^{-4}	19	3×10^{-4}	51
3×10^{-5}	27	3×10^{-5}	42
3×10^{-6}	39	3×10^{-6}	64
3×10^{-7}	58	3×10^{-7}	67
3×10^{-8}	46	3×10^{-8}	95
3×10^{-9}	79	3×10^{-9}	93
3×10^{-10}	86	3×10^{-10}	125
3×10^{-11}	82	3×10^{-11}	142
3×10^{-12}	98	3×10^{-12}	186
3×10^{-13}	107	3×10^{-13}	302
3×10^{-14}	139	3×10^{-14}	285
3×10^{-15}	137	3×10^{-15}	604
3×10^{-16}	Very high	3×10^{-16}	800
3×10^{-17}	Very high	3×10^{-17}	838
Control (mean)	152	Control (mean)	973

Table 2: Analysis of leave and stems essence of Echinophora platyloba

No.	Component Name	percentage	Retention Index
1	2-butenal	1.8	870
2	myrcene	6.0	989
3	ñ-cymene	1.2	1021
4	limonene	1.5	1025
5	cis-β-ocimene	2.3	1034
6	trans-β- ocimene	67.9	1047
7	unknown	1.4	1095
8	linalool	3.1	1098
9	ñ-mentha-1,5,8-triene	1.5	1127
10	unknown	2.2	1207
11	cis-3-hexenyl 2-methyl butanoate	2.0	1229
12	unknown	2.9	1271
13	2-furanone	6.2	1466

seems to have demolition effect on bacteria and is one of the components of Alkaloid Echinophora Platyloba herbal essences. Alkaloids comprise elements of some herbs which have toxic effects for animal organisms [12].

Also in the carried out research, they get to this conclusion that it can use such herbs in mentioned concentration against dermatophytes, trichophyton shoenlini and verrucosum.In Trichophyton mentagrophytes and microsporum canis and epidermophyton flocosum also depend on patient condition and infection can use such herb [16]. In this study, there was collected the antibacterial components of leaves and stems of Khosharizeh from Lashkarak hills and examined by three methods of disk diffusion paper, punched hole test and serial dilution. Based on Enayat-Allah Kalantar, studied on the antibacterial effects of Kharkhasak fruit in Arak, the extract of current planthad the same results and occasionally better results than traditional antibiotics on bacteria *Streptococcus fecalis*, *Staphilococcus aureus*, *E. coli* and *Pseudomonas aeroginosa* [17].

Another study by Roussis indicated the change in the influence of plant essences on the activity of different microorganisms and the variability of effects of different microorganisms is depended on the type and size of effective molecules and the power of penetrability to the microorganism [18]. Methanolic extract inhibits both species of bacteria while it had co considerable effects on three species of fungi. It was also seen that in all three methods, the effects of methanolic extract of leaves were more than stems. Different components Antibacterial existing in this plant were previously studied and the most important ones include trans-β-ocimene (67/9%), 2-furanone (6.2%), myrcene (6%), linalool (3.1%) and cis-β-ocimene (2.3%).[14]

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