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Antimycobacterial activity of some medicinal plants used in traditional medicine in North of Iran

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Abstract—**This study was designed to screen the antimycobacterial activity of some medicinal plants which have been used traditionally in Golestan province, north of Iran. The medicinal herb practiced in traditional medicine for the treatment of infectious diseases, Tuberculosis, or its sign and symptoms are selected through ethno pharmacology. The plants were collected in their natural habitats in our province. Hydro-alcoholic extract of plant organs were obtained . Antimycobacterial activities were determined by Disc Diffusion (DD) and Micro dilution methods against M.smegmatis and M.bovis BCG strain.**

Six species (11.5%) from 52 plants showed antimycobacterial activity. Citrus limon extract with 27 mm inhibitory zone and MBC 32µg/ml demonstrated the strongest effect on M.smegmatis. The peel extract of Punica granatum L. with 32 mm inhibitory zone and MBC 16µg/ml showed the best effect on M.bovis BCG strain. We also found that Peganum harmala L. extract showed good effect on both species.

The findings of this investigation revealed that at least some part of the plants used in traditional medicine can show in vitro antimycobacterial activity. We suggest designing protocols similar to traditional medicine for in-vitro assessment of synergistic effect of these plant extracts against Mycobacteria.

Key words: Antimycobacterial effect, Herbal Medicine, Golestan Province, Iran

I. INTRODUCTION

According to the World Health Organization, the estimated incidence rate of TB in Iran is 28 cases per 100,000 populations[1], Pakistan and Afghanistan are the two countries neighboring Iran with high incidence of TB which put our country in high risk.In recent years, strains of MDR (multi drug resistant) and XDR (Expanded Drug Resistant) M.tuberculosis are being increased in the world. Among of 1284 isolated Mycobacterium tuberculosis which were referred to the National Research Institute of Tuberculosis and Lung Diseases (Tehran, Iran) from 2003 - 05, 150 (11.7%)isolates were identified as multidrug-resistant M. tuberculosis and a total of 12 (10.9%) of113 MDR strains were resistant to all 8 second line drugs tested and, therefore, were denoted as XDR M. tuberculosis[2].

More generally, medicinal plants remain important resources to find original active drugs or new therapeutic agents. Due to these facts various studies for screening the herbal plants with antimycobacterial effect are being carried out in all over of the world[3].Over 350 natural products have been evaluated for their antimycobacterial activities by Newton[4], Billo and McCutcheon worked on new Caledonian and Vanuatu and British Columbian Medicinal Plants, respectively [5,6].Their findings demonstrated that some of plants practiced in traditional medicine showing antimycobacterial effect in vitro.

Golestan Province located in the south east of Caspian Sea has the second highest incidence of TB in Iran [7]. This Province is fortunate to have such varied climate and heterogeneous ecological condition that almost many herbs, especially medicinal plants can grow wild and be cultivated economically.The aim of the present study was, screening the plant species for antimycobacterial activities.

II. MATERIAL AND METHOD

A. Selection of plants and study area

The herbal medicine in this study were collected over 7 months from May to December 2006, chosen based on their usage in traditional medicine for the treatment of TB, Pulmonary disease or symptoms of these diseases. Most of the plants were collected in three regions; Ziaret, Deraznoo and Charbagh in south of Golestan province, with ranges height as 650-2250m. In total of 52 plant speices belongs to 26 families were collected.

B. Preparation of plant extracts

Fresh plant parts were collected- identified and their voucher specimens were preserved in the herbarium ofFaculty of Plant Science, Islamic Azad University of Gorgan branch, subsequently, were shadow dried and grounded into fine powder using electric blender. Plant hydro-alcoholic extracts were prepared by cold percolation method, with final concentration of 4,2,1,0.5 mg/ml, for the Disc Diffusion method and 2000, 1000, 500, 250, 125, 62, 31 µg/ml, in the Micro dilution Broth method.

C. Tested Mycobacteria

M.smegmatis (PTCC 1307) and slow growing Mycobacteria, M.bovis BCG strain (Pasteur Institute of Iran, Vaccinal strain) were used for this study.

III. Result and Discussion

Our findings demonstrated that out of 52 plant species hydro-alcoholic extracts of only 6 plants (11.6%) and 5 (9.7%) showed to have antimycobacterial activity against M.smegmatis and M.bovis BCG, respectively. In Disc Diffusion method, the inhibitory zone around Citrus Limon pulp, against M.smegmatis (22mm) was more than other extracts .The MBC of the later extract for this bacterium was 31µg/ml (Table 1).

The highest inhibitory zone against M.bovis BCG strain belongs to hydro-alcoholic extract of Punica granatum peel (32mm) and its MBC was equal to 16 µg/ml. Peganum harmala show good inhibitory effect against both mycobacterial strains.(figure 1).

Punica granatum L., belongs to Punicaceae family, is native to Iran. Traditionally, the yellow fruit rind and the membranous separators have been given in gastrointestinal infections [8], Its peel is a powerful astringent and cure for diarrhea, oral aphthae and suppression of inflammation and also has antioxidant activity [9]. In addition its antibacterial effect on Helicobacter pylori [10], E.coli O157:H7 [11]. The investigation on Punica granatum peel show that it contains both flavonoids and tannins abundantly but the presence of alkaloids (e.g., pelletierine) is equivocal [9]. On the bases of above findings and our results in this present study, we guess that the anti mycobacterium effect of Punica granatum peel extract might be related to the above chemical substances.

Peganum harmala seeds are commonly named “Esphand” in Iran. Since ancient times it has been considered an important medicinal plant. Several reports in the literature indicate a great variety of pharmacological activities of P. harmala such as antimicrobial, antitumor, antinociceptive and MAO (monoamine oxidase inhibitor A) -inhibiting activities. Its smoke is widely used in Iran and Turkey for all kinds of rituals against evil eye and bad luck, and as a disinfecting agent. The seed smoke of this plant was found to be effective against S. aureus, S. epidermidis, E. coli and P. aeroginosa [12] .it also has "antibacterial activity against drug-resistant bacteria.[13] Although many researches indicate that the Esphand demonstrate antimicrobial effect, we could not find any work on its antimycobacterial efficacy in the literature, but our findings in the present study indicated its inhibitory effect on both M.smegmatis and M.bovis BCG strains.

Citrus lemon is belongs to Rutaceae family, native to India, It was later introduced to Iran . Essential oil of the outer fruit rind contain flavonoids, it has been used traditionally as antibacterial, anti-inflammatory, anti scorbutic, antiseptic, astringent, disinfectant, expectorant and sedative. It is traditionally used in Europe to fend off major epidemic diseases and is ideal home remedy to relieve colds and flu[14].

In addition to these three plants, we found that Digitaria sp, Berberis vulgaris and Rosa canina hydroalchoholic extracts have limited effect on tested Mycobacteria (MBC1000, 2000µg/ml)

In our study only 11.6% of plant extract demonstrated antimycobacterial efficacy, but Billo showed among 20 plants that used in traditional New Caledonian and Vanuatu medicine for treatment of symptoms potentially related to Tuberculosis only either extracts or essential oil have good antimycobacterial activity [9], and Newton found that only three (5.8%) among 43 plants extract have suitable antimycobacterial effect against M.aurum but not against M.smegmatis.

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Table1: The plant extracts with antimycobacterial effect, according to Disc diffusion method and their Minimum Bactericidal Concentration (MBC)

Method /Type of Mycobacteria / Plant(Family)	Disc diffusion								MBC	
	M.smegmatis				M.bovis(BCG)				M.smegmatis	BCG
	*4	2	1	0.5	4*	2	1	0.5		
Citrus limon(Rutaceae)	**22	15	14	9	9	R	R	R	#31	500
Peganum harmala (Zigophyllaceae)	18	15	11	R	16	14	8	R	250	125
Punica granatum (Punicaceae)	14	11	9	R	32	28	22	19	250	16
Rosa canina (Rosaceae)	13	R	R	R	11	9	R	R	2000	1000
Berberis vullgaris (Berberidaceae)	16	11	R	R	R	R	R	R	1000	1000
Digitaria sativa (Ranunculaceae)	15	10	R	R	11	R	R	R	2000	2000

*concentration hydro-alcoholic extract, mg/ml
**inhibitory zone around Disc, mm
Minimum Bactericidal Concentration, µg/ml

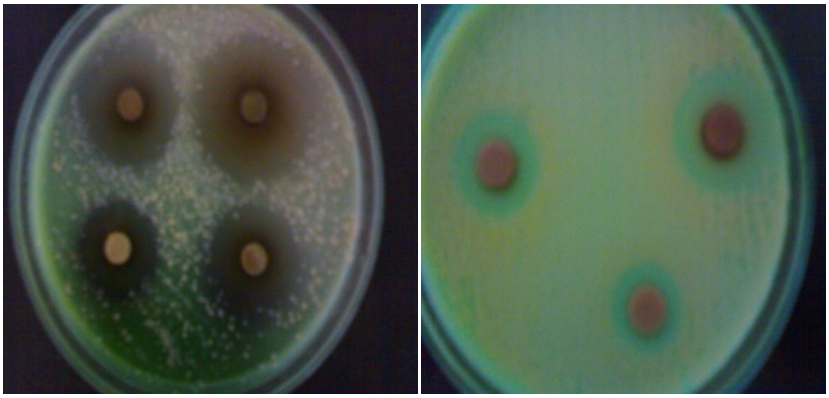

Figure 1: The inhibitory zone of the peel of Punica granatum extract against M.bovis BCG strain (A) and the inhibitory zone of Peganum harmala seeds extract against M.smegmatis(B)