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Research Article

Phytochemical screening and evaluation of antibacterial, antitubercular activities of *Peganum harmala linn*., seeds

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ABSTRACT

The objective of this research work is to carry out the phytochemical screening and evaluate the antibacterial, antitubercular activities of *Peganum harmala* Linn., seeds. Due to the development of resistance in the micro-organisms against available drugs there is a need to develop new, potent, fast-acting antibacterial and antitubercular drugs with low toxicity. In this study different extracts of *Peganum harmala (Linn)* seeds were evaluated for antibacterial and antitubercular activities against different gram-positive, gram-negative bacterial strains and *Mycobacterium tuberculosis* strain $H_{37}Rv$ using ciprofloxacin, norfloxacin and pyrazinamide as standard drugs respectively. Ethyl acetate extract showed significant activity against *Streptococuus faecalis*, *Klebsiella pneumoniae, M. tuberculosis* strain $H_{37}Rv$ and *Pseudomonas aeruginosa*, while this extract was found to show moderate activity against *Escherichia coli.* Alcoholic extract has showed significant activity against *M. tuberculosis* strain $H_{37}Rv$, *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*, while this extract has shown moderate activity against *Bacillus subtilis* and *Streptococuus faecalis*. The ethyl acetate and alcoholic extracts can be considered as a potential candidates for antibacterial and antitubercular activities. The presence of alkaloids, flavonoids in alcoholic extract and alkaloids, steroids, flavonoids in ethyl acetate extract of *Peganum harmala (Linn)* seeds could be attributed for the antibacterial and antitubercular activities.

Keywords: Ciprofloxacin; Pyrazinamide; MABA method; *M. tuberculosis* strain $H_{37}Rv$; *Peganum harmala*; Zygophyllaceae

INTRODUCTION

Tuberculosis (TB) is one of the oldest and most pervasive diseases in the history (Okumade A. L et al., 2004; Yves L. J., 2007). According to the WHO, 2 million people die every year and at least 9 million are getting infected, which provides a pool for the development of new active form of tuberculosis (WHO report. 2008**)**. The present chemotherapy DOTS (Directly Observed Treatment Short-course) for TB and DOTS-Plus (DOTS and Second line anti-TB drugs) for MDR-TB has a cure rate of about 95% if patient given compliance (Loddenkemper R et al., 2002; Perri G.D et al., 2004). Despite of the fact that TB is curable and also preventable, the disease has been spreading at a steady rate over the past decade (Bishai W.R et al., 1997). Commonly used drugs for treating this are isoniazid and rifampicin. Multi-drug resistant strains of *Mycobacterium tuberculosis*, which are resistant to two major drugs isoniazid and rifampicin have made the situation still worst. The AIDS pandemic has lead to the development of HIV/ TB co-infection for patients living with AIDS. Tuberculosis is a leading cause of death among HIV-positive patients (13% of AIDS deaths worldwide). Due to the development of resistance in the micro-organisms against available drugs there is an urgent need to develop novel antibacterial and antitubercular drugs with low toxicity.

Plants and plant extracts have been used since the dawn of civilization by mankind. The uses of ethnobotanical preparations for various reasons justified or not, are still continued by various cultures all over the world. Considering structural and biological diversity of terrestrial plants, they offer a unique renewable resource for the discovery of potential new drugs and modern medicine has developed a rational strategy for drug discovery which involves the study of plants and plant materials based on their ethnobotanical usage (Cordell G.A et al., 1991). Natural products are sources of active compounds that may be useful in the development of new drugs.

Peganum harmala L. belongs to the family of Zygophyllaceae (Qazan W.S., 2009). It is a wild growing flowering plant. It is also called Syrian rue, African-rue, wild

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rue. The plant is widely distributed in predesertic regions of south-east Morocco, North Africa and the Middle East (EL-Bahri L et al., 1991). It is the only species found growing wild in the middle and northen parts of Iraq (Muhi-eldeen Z et al., 2008). Literature survey revealed that *Peganum harmala* L., shows different pharmacological activities like antioxidant (Dickson R.A et al., 2006), antileishmanial (Di Giorgio C et al., 2004), antihemosporidian (Fan B et al., 1997), antihistaminic (Gholamreza Asghari et al., 2002), vasorelaxant (Hicham Berrougui et al., 2006), antinociceptive (Hamid Reza Monsef et al., 2004), antitumor (Lamchouri F et al., 1999), wound healing (Derakhshanfar A et al., 2010), antiplasmodial (Adil Astulla et al., 2008), MAO inhibition (Herraiz T et al., 2010), DNA topoisomerase 1 inhibition (Armin Madadkar Sobhani et al., 2002), myeloperoxidase inhibition (Sihem Bensalem et al., 2014) etc. However, *Peganum harmala (Linn)* seeds have not been investigated for antibacterial and antitubercular activities. Hence, this study was carried out to evaluate the potent bioactive constituents for antimicrobial and antitubercular activities in *Peganum harmala (Linn)* seeds.

MATERIALS AND METHODS

The Seeds of *Peganum harmala (Linn)* were collected from the local areas of Dharwad in Karnataka and were authenticated by Dr. S. S. Hebbar, Department of Botany, Government Pre-university College Dharwad. A voucher specimen (No- SETCPD/Pharmacog /Herb/ 2013/14) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T.'s College of Pharmacy, Dharwad, Karnataka. The Seeds of *Peganum harmala (Linn)* were shade dried and finely powdered to particle size (#) 40. About 300g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether (60-80), chloroform, ethyl acetate and ethanol (95%). Aqueous extract was also obtained by cold maceration of the drug (300 g) with 2% chloroform water. After the extraction, the extracts were filtered and concentrated under reduced pressure using a rota evaporator. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract was found to be 9.45 g (3.15 % w/w), 8.4 g (2.8 % w/w), 16 g (5.33 % w/w), 22 g (7.33 % w/w) and 20 g (6.66 % w/w) respectively. All the extracts were kept in a dessicator for drying.

Evaluation of Antibacterial activity

The MIC determination of different extracts were carried out simultaneously in comparison with ciprofloxacin, norfloxacin against Gram-positive (*Staphylococcus aureus, Streptococuus faecalis, Bacillus subtilis*) and Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*) by broth microdilution method (Sunil J et al., 2012: National committee., 1985). Serial dilutions of the all extracts and reference drugs were prepared in Mueller-Hinton broth. Standard drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 ml). Further progressive dilutions were done to obtain final concentrations of 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 μgml^{-1}. The tubes were inoculated with 10^5 $cfuml^{-1}$ (colony forming unit/ml) and incubated at 37° C for 18 h. The MIC was the lowest concentration of the extracts that yield no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and DMSO had no effect on the micro-organisms in the concentrations studied. The MIC values are given in µg/ml. Ciprofloxacin and norfloxacin were used as standard drugs. The preliminary results of antibacterial activities are shown in Table-1.

Evaluation of Antitubercular activity

MIC values were determined for the different extracts against *M. tuberculosis* strain $H_{37}Rv$ using the Microplate Alamar Blue assay (MABA) using pyrazinamide as the standard drug (Franzblau S.G et al., 1998). The 96 wells plate received 100 µL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 μgml^{-1}. Plates were covered and sealed with parafilm and incubated at 37ºC for 5 days. Then, 25 µL of freshly prepared 1:1 mixture of almar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented colour change from blue to pink. The result of antitubercular activity depicted in Table-2.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids in the alcoholic extract and alkaloids, flavonoids and steroids in the ethyl acetate extract. The results are shown in Table-4. Physico chemical parameters for the *Peganum harmala* Linn., seeds are shown in Table-3.

Antibacterial and antitubercular activity

The different extracts of *Peganum harmala (Linn)* seeds were screened for antimicrobial activity against Gram-positive bacteria: *Staphylococcus aureus* ATCC 11632, *Streptococcus faecalis* ATCC 14506 and *Bacillus subtilis* ATCC 60511. Gram-negative bacteria: *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 10536 *Pseudomonas aeruginosa* ATCC 10145 and results are shown in Table-1. Ethyl acetate extract of *Peganum harmala (Linn)* seeds showed significant activity at 50 μgml^{-1} against *Staphylococcus aureus, Streptococuus faecalis and Klebsiella pneumonia*. Similarly this extract showed moderately significant activity at 100 μgml^{-1} against *Pseudomonas aeruginosa* and *Escherichia coli*. Alcoholic extract also showed good activity at 50 µg ml^{-}

Table 1: *In vitro* antibacterial activity

Extracts	MIC values (µgml^{-1})					
	Gram-positive organisms[a]			Gram-negative organisms[b]		
	Sa	Sf	Bs	Kp	Ec	Pa
Pet-ether Extract	100	>100	>100	100	>100	50
Chloroform Extract	100	50	100	>100	50	>100
Ethyl acetate Extract	50	50	>100	50	100	100
Alcoholic Extract	50	100	100	50	>100	50
Aqueous Extract	100	>100	>100	100	>100	100
CIP[c]	<5	<5	≤1	≤1	≤1	>5
NOR[d]	<5	<5	≤1	≤1	≤1	>5

[a]Gram-positive bacteria: *Staphylococcus aureus* ATCC 11632 (Sa), *Streptococuus faecalis* ATCC 14506 (Sf), *Bacillus subtilis* ATCC 60511 (Bs); [b]Gram-negative bacteria: *Klebsiella pneumoniae* ATCC 10031 (Kp), *Escherichia coli* ATCC 10536 (Ec) *Pseudomonas aeruginosa* ATCC 10145 (Pa); Reference drugs: [c]Ciprofloxacin, [d]Norfloxacin.

Table 2: In vitro antitubercular activity of *Peganum harmala* Linn., seeds

Extracts	MIC values (µg ml^{-1}) *M. tuberculosis* $H_{37}Rv$
Petroleum ether Extract	>100
Chloroform Extract	50
Ethyl acetate Extract	12.5
Alcohol Extract	50
Aqueous Extract	>100
Pyrazinamide	3.125

Table 3: Physico-chemical parameters of *Peganum harmala* Linn., seeds

S.No	Parameter	Determined values in %w/w
1	Alcohol soluble extractives	7.88
2	Hydro-alcoholic extractives	14.11
3	Water soluble extractives	18.73
4	Ether soluble extractives	6.98
5	Total ash value	7.64
6	Acid insoluble ash	1.52
7	Water soluble ash	3.59
8	Sulfated ash	8.33
9	Moisture content	2.4

Table 4: Preliminary phytochemical analysis of various extracts of *Peganum harmala* Linn.,

Phytoconstituent	Petroleum ether	Chloroform	Ethyl acetate	Alcohol	Aqueous
Alkaloids	-ve	+ve	+ve	+ve	-ve
Steroids	-ve	-ve	+ve	-ve	-ve
Carbohydrates	-ve	-ve	+ve	+ve	+ve
Phenolic	-ve	-ve	-ve	-ve	-ve
Flavonoid	-ve	-ve	+ve	+ve	-ve
Glycoside	-ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	-ve	-ve

+ve= Present -ve= Absent

[1] against *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa.* Similarly this extract showed moderate activity at 100 µgml^{-1} against *Streptococuus faecalis, Bacillus subtilis.* For antibacterial activity ciprofloxacin, norfloxacin were used as standard drugs.

Ethyl acetate extract has shown significant antitubercular activity at 12.5 µgml^{-1} against *M. tuberculosis* strain $H_{37}Rv$. For antitubercular activity pyrazinamide was used as standard drug.

Phytochemical screening revealed the presence of alkaloids, flavonoids in the alcoholic extract and alkaloids, flavonoids and steroids in the ethyl acetate extract.

Hence, the presence of alkaloids, flavonoids in the alcoholic extract and alkaloids, flavonoids and steroids in the ethyl acetate extract could be attributed for observed significant antibacterial (Mahesh B et al., 2008) and antitubercular activities (Wu M.C et al., 2011: Saludes J.P et al., 2002). However, research work is under

progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principle for the claimed antibacterial and antitubercular activities.

CONCLUSION

The present study provides evidence for the antibacterial and antitubercular activities of *Peganum harmala (Linn)* seeds. Ciprofloxacin and norfloxacin were used as standard drugs for screening the antibacterial activity act by inhibiting the enzyme bacterial DNA gyrase (Tripathi K.D., 2008) and the pyrazinamide used as standard drug for screening the antitubercular activity act by inhibiting the mycolic acid synthesis similar to isoniazid but by interacting with a different fatty acid synthase encoding gene (Tripathi K.D., 2008). As the MIC values of the extracts (ethyl acetate and alcohol) studied are close to those of ciprofloxacin, norfloxacin and pyrazinamide, the bioactive principles present in the extracts may be having the mechanism of action similar to that of the tested standard drugs. However research is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed antibacterial and antitubercular activities. The present study may form the basis for the selection of plant species for further investigation in potent bioactive compounds for antibacterial and antitubercular activities.

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