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EVALUATION OF ANTIBACTERIAL AND ANTITUBERCULAR ACTIVITY OF *PHASEOLUS VULGARIS LINN.*, SEEDS

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ABSTRACT

Due to the increasing emergence of drug resistant Tuberculosis (TB), particularly multi-drug resistant TB (MDR-TB), extensive-drug resistant TB (XDR-TB) and also 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. With this aim different extracts of *Phaseolus vulgaris (Linn)* seeds were evaluated for antibacterial and antitubercular activities using ciprofloxacin, norfloxacin and isoniazid as standard drugs, respectively. The alcoholic extract showed significant activity against *Streptococcus faecalis* and *Escherichia coli*. Petroleum ether extract has shown significant antitubercular activity against *M. tuberculosis* strain $H_{37}Rv$ and *Escherichia coli*. The alcoholic and petroleum ether extracts can be considered as a potential candidate for antibacterial and antitubercular activity. The presence of alkaloids in alcoholic extract and steroids, flavonoids in the petroleum ether extract of *Phaseolus vulgaris (Linn)* seeds could be attributed for the antibacterial and antitubercular activities.

KEY WORDS*:* Antibacterial, Antitubercular, *Phaseolus vulgaris (Linn)* seeds, *M. tuberculosis* strain $H_{37}Rv$.

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INTRODUCTION

Tuberculosis (TB) is a contagious, deadly disease that spreads through the air and has become a serious worldwide health problem. It is the greatest cause of mortality and morbidity worldwide[1]. The WHO has reported that one third (nearly 2 billion) of the world's population is already infected with TB bacilli and every second a new case of TB infection is reported [2]. According to the WHO, the increase in the cases of TB during recent years was largely due to the pathogenic synergy with HIV infection[3]**.** The situation has worsened very seriously with the spread of HIV-1, which allows the latent TB to become active and makes the patient more susceptible to re-infection with either drug-susceptible or drug resistant strains. Moreover, the emergence of drug resistant, multi-drug-resistant TB (MDR-TB) [4], extensive drug-resistant TB (XDR-TB) [5] and the latent form of TB are of great threat to mankind and have already caused several fatal outbreaks.[6] Due to the development of resistance in the micro-organisms against available drugs there is a an urgent need to develop novel antibacterial and antitubercular drugs with low toxicity. *Phaseolus vulgaris (Linn)* seeds belong to the family of *Fabaceae*[7]. It is a sub-erect or twining annual herb, native of central and south America and is now grown extensively throughout the warm regions of the world. Vernacular names of the plant *Phaseolus vulgaris* are in Kannada- Tingalavari, Hindi-Rajma, Gujarati-Fansi, Kashmiri-Fraa'sh bean, Marathi-Pharas bee and Punjabi-Fras bean. Literature survey revealed that *Phaseolus vulgaris (Linn)* seeds exhibit different biological activities like enhancement of the bifidogenic effect [8], antioxidant [9], anticarcinogenic [10], anti-inflammatory [11], estrogenic [12], antidepressant [13] effects. *Phaseolus vulgaris (Linn)* seeds have an important place in the folklore throughout the world and in the traditions of many cultures such as pharmacotherapeutic effects [14]. However, *Phaseolus vulgaris (Linn)* has 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 *Phaseolus vulgaris (Linn)* seeds.

MATERIALS AND METHODS

The Seeds of *Phaseolus vulgaris (Linn)* were collected from the local areas of Dharwad in Karnataka and were authenticated by Dr. S. S. Hebbar, Department of Botany, and Government Pre-university College Dharwad. A voucher specimen (No-SETCPD/Pharmacog/Herb/2011/12) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T.'s College of Pharmacy, Dharwad, and Karnataka. The Seeds of *Phaseolus vulgaris (Linn)* were shade dried and finely powdered to particle size (#) 40. About 300 g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether, chloroform 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 rotor evaporator. The yield of petroleum ether, chloroform, ethanol and aqueous extract was found to be 8.36 g (3.12 % w/w), 6.285 g (1.95 % w/w), 24 g (7 % w/w) and 11 g (4.01 % w/w), respectively. All the extracts were kept in a desiccator 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 [15, 16]. 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 µg ml^{-1}. The tubes were inoculated with 10^5 cfu ml^{-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 isoniazid as the standard drug [17]. 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 µg/ml. 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.

Table-1
In vitro antibacterial activity

Extracts	MIC values (µg ml^{-1})					
	Gram-positive organisms[a]			Gram-negative organisms[b]		
	Sa	Sf	Bs	Kp	Ec	Pa
Aqueous Extract	100	25	12.5	100	50	100
Alcohol Extract	25	6.25	100	50	6.25	50
Chloroform Extract	100	50	100	>100	50	>100
Pet-ether Extract	100	50	100	100	25	50
CIP[c]	<5	<5	≤1	≤1	≤1	>5
NOR[d]	<5	<5	≤1	≤1	≤1	>5

The screening organisms.
[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

Extracts	MIC values (µg ml^{-1}) *M. tuberculosis* $H_{37}Rv$
Aqueous Extract	50
Alcohol Extract	50
Chloroform Extract	100
Pet-ether Extract	6.25
Isoniazid	0.25

RESULTS

1) Phytochemical screening

Phytochemical screening revealed the presence of alkaloids in the alcoholic extract and steroids, flavonoids [18] in the petroleum ether extract.

2) Antibacterial and antitubercular activity

The different extracts of *Phaseolus vulgaris (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. Alcoholic extract of *Phaseolus vulgaris (Linn)* seeds has shown significant activity at 25, 6.25 and 6.25 µg ml^{-1} against *Staphylococcus aureus, Streptococuus faecalis and Escherichia coli* respectively. Similarly, this extract showed moderately significant activity in 50, 50, 100 and 50 µg ml^{-1} against *Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis and M. tuberculosis* strain $H_{37}Rv$ respectively. For antibacterial activity ciprofloxacin, norfloxacin and for antitubercular activity isoniazid were used as standard drugs. Petroleum ether extract has shown significant antitubercular activity at 6.25 µg ml^{-1} against *M. tuberculosis* strain $H_{37}Rv$, where as it has shown moderate activity at 50 µg ml^{-1} against both *Pseudomonas aeruginosa and Streptococuus faecalis*. Phytochemical investigation of alcoholic and petroleum ether extracts of seeds of *Phaseolus vulgaris (Linn)* was revealed the presence of alkaloids, flavonoids and steroids respectively. Hence, the presence of alkaloids in alcoholic extract and flavonoids, steroids in petroleum extract could be attributed for observed significant antibacterial [19] and antitubercular activities [20, 21]. 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 an evidence for the antibacterial and antitubercular activities of *Phaseolus vulgaris (Linn)* seeds. Ciprofloxacin and norfloxacin used as standard drugs for screening the antibacterial activity act by inhibiting the enzyme bacterial DNA gyrase [22] and the isoniazid used as standard drug for screening the antitubercular activity act by inhibiting the mycolic acid synthesis [23]. As the MIC values of the extracts (alcohol and petroleum ether) studied are close to those of ciprofloxacin, norfloxacin and isoniazid, 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 the 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.

CONFLICT OF INTEREST

Conflict of interest declared none.

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