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ANTI-TUBERCULAR ACTIVITY OF DIFFERENT ORGANIC EXTRACTS OF LEAVES OF *CAPPARIS DIVARICATA* LAM

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ABSTRACT

Tuberculosis is a highly infectious disease with about one third of the world's population estimated to be infected by it. Plants contain numerous biological active compounds, many of which have been shown to have antimicrobial activity. The search for biologically active extracts based on traditional use of plants is relevant due to the appearance of microbial resistance to many antibiotics and the occurrence of fatal opportunistic infections. The present study was carried out to study antitubercular activity of *Capparis divaricata* Lam leaves. The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).The leaves extracts of Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Water was used and prepared different concentrations 100 to 0.2µg/ml. The maximum sensitive extract at 12.5µg/ml was shown by the ethanol extract and aqueous extract as compared to other extract and standard drug used Isoniazid.

KEYWORDS: *Capparis Divaricata* Lam, Anti-tubercular activity, *M. tuberculosis* ATTC 27294, Alamar Blue assay.

INTRODUCTION

There is a major global health problem attributable to diseases, such as tuberculosis(TB), which are complicated due to drug resistance. This is coupled with the problem of mycobacterial persistence, thus highlighting the need to develop novel TB drugs that are not only active against drugresistant bacteria, but more importantly, kill persistent bacteria and shorten the length of treatment[1].The aim of the present study was to determine the antitubercular activity of the *Capparis divaricata* Lam leaves of different solvent extracts.

Capparis divaricata Lam commonly known as caper bush, belonging to the genus *Capparis* of family *Capparidaceae*, found throughout the India especially in the Deccan Peninsula from Maharashtra southwards to Tamil Nadu[2].*Capparis divaricata* Lam species exhibit pharmacological activities like locomotor, diuretic, analgesic, antipyretic and synergistic activity of *Capparis divaricata* lam and *caesalpinia bonducella* L.

Tuberculosis (TB) is principally a disease of poverty, with 95 per cent of cases and 98 per cent of deaths occurring in developing countries. Tuberculosis (TB) is a bacterial infection caused mainly

by *Mycobacterium tuberculosis* (MTB) [3].Tuberculosis (TB) is second among the leading infectious diseases in the world. In 2008 the World Health Organization (WHO) reported 9.4 million cases of TB, including 500,000 cases of multi-drug resistant TB (MDR-TB).[4] As a result of TB infection in 2008 approximately 1.8 million peoples died. TB has killed more adults than any other single infectious agent, including AIDS and malaria. TB is responsible for more than a quarter of unnecessary deaths in adults.[5] One of the main problems in the persistence and severity of TB is drug resistance. In 1993, TB was declared a global emergency by the WHO. In 2006 WHO, in conjunction with the Stop TB Partnership developed a Stop Tuberculosis strategy, with the aim to "dramatically reduce the global burden of tuberculosis by 2015" and thereby eliminating TB as a global health problem by 2050. India accounts for nearly one-third of the global burden of tuberculosis and the disease is one of India is most significant public health problems. In India, approximately 2 million people acquire TB every year. The currently used drugs to treat the TB infections are rifampicin, ethambutol, isoniazid and pyrazinamide.[6] Medicinal plants proposal a great hope to achieve these needs and have been used for curing diseases for several centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties[7].

This study was explored the anti-tubercular activity of different solvent extract like Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Water of leaves against Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Capparis divaricata* Lam. (Capparidaceae) were collected at the flowering stage in the month of August from Sangli district, Maharashtra State, India. It was authenticated and taxonomically identified and approved by Botanist, Botanical Survey of India Collection voucher No.RVP 01.

Preparation of crude drug for extraction

The authenticated fresh leaves were dried under shade and used for the preparation of extract. These leaves were coarsely powdered with the help of mechanical grinder and passed through sieve no.60.The powder was stored in an air tight container for further use[8].

Method of extraction

Continuous hot percolation (successive solvent extraction) process by using soxhlet apparatus and cold maceration method. Extraction of dried leaves with different solvent extraction like Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Water.

Phytochemical screening

Tests for alkaloids, glycosides, flavonoids, fixed oil and fats, phenolic compounds, protein, tannins, gum and mucilage and carbohydrates, saponins and terpenoids were performed for the extracts[9].

METHOD

The anti-TB activity of compounds were measured against M. tuberculosis using microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows

good correlation with proportional and BACTEC radiometric method. About 200µl of sterile deionized water was added to all outer border wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The serial dilutions of compounds were made directly on plate. The 96 wells plate received 100µl of the Middlebrook 7H9 broth. The final drug concentrations tested were 100 to 0.2µg/ml. Plates sealed with parafilm and incubate at 37ºC for five days. Then 25µl of freshly prepared 1:1 mixture of 10% tween 80 and Alamar Blue reagent added to it and incubated for 24 hrs. A blue color in the well indicates no bacterial growth whereas, pink color indicates there is growth of bacteria. MIC is the minimum drug concentration that prevented the color change from blue to pink[10-12].

RESULTS AND DISCUSSION

Table: Anti-TB activity of *Capparis divaricata* Lam by using Alamar Blue Dye for preliminary extract

Sample code	Concentrations									
	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.56 µg/ml	0.78 µg/ml	0.39 µg/ml	0.19 µg/ml
Ethanol Extract	S	S	S	S	R	R	R	R	R	R
Aqueous Extract	S	S	S	S	R	R	R	R	R	R
Ethyl acetate Extract	S	S	S	R	R	R	R	R	R	R
Acetone Extract	S	S	S	R	R	R	R	R	R	R
Pet ether Extract	S	S	R	R	R	R	R	R	R	R
Chloroform Extract	S	S	R	R	R	R	R	R	R	R
STD drug (Isoniazid)	S	S	S	S	S	S	S	S	S	S

Note: S- Sensitive, R-Resistant

Different organic solvent extract of *Capparis divaricata* Lam was prepared by differentially extracting the dried leaves of the plant with different organic solvent with increasing polarity in a soxhlet apparatus and anti-tubercular activity of different organic extracts tested by Alamar Blue Dye assay.

From the table it is evident that maximum sensitive extract at 12.5µg/ml was shown by the ethanol extract and Aqueous extract as compared to other extract and standard drug used Isoniazid.

CONCLUSION

The Phytochemical constituents were extracted by successive solvent extraction like Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol, Waterand identified by chemical tests. These tests showed the presence of Alkaloids, Carbohydrate, tannins, glycosides, phenolic compounds and flavonoids. The ethanol extract and aqueous extracts were shown maximum sensitivity at a concentration of 12.5 µg/ml as compared to other compounds and standard drug used Isoniazid.

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