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Original Research Paper

Medicinal Plants

EVALUATION OF THE ANTI-MYCOBACTERIAL ACTIVITY OF ALLIUM ASCALONICUM AND ALLIUM SATIVUM

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ABSTRACT Mycobacterium tuberculosis (MTB), the organism causing tuberculosis (TB) remains a major cause of morbidity and mortality. Plant-based drugs have been used worldwide in traditional medicines for the treatment of various diseases including tuberculosis. Medicinal plants are an important source of new antimicrobial agents and remain an attractive alternative strategy. The present study was performed to evaluate anti-MTB activity of two medicinal plants viz., Allium ascalonicum, and Allium sativum. Different concentrations of extracts of these plants were tested for their anti MTB activity against MTBH37Rv strain and the inhibitory activity was expressed as CFU inhibition, % inhibition and IC50. In our study Allium sativum showed higher anti TB activity however this bactericidal property was not significantly different between each groups. The overall anti mycobacterial activity of these extracts might be attributed due to the presence of flavonoids, saponins, steroids, terpenoids, tannins and other phytoconstituents. The extract of the plant also exhibited promising antitubercular activity.

KEYWORDS : Allium ascalonicum, Allium sativum, Anti-Mycobacterial activity, Mycobacterium tuberculosis, MTBH37Rv

INTRODUCTION

Tuberculosis is the major opportunistic infection ofHIV/AIDS in developing countries. MTB developed resistance against both the first line as also the second line drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of MTB all over the world including India [1].Plant-based drugs have been used worldwide in traditionalmedicines for the treatment of various diseases includingtuberculosis. Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases [2].Since last few years several plants have been reported for their Anti-Mycobacterial activity from India.Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material.The clinical efficacy of many existing antibiotics is being threatened by the emergence of MDR pathogens. The increasing incidence of MDR- and XDR-TB worldwide highlighted the urgent need to search for newer anti-tuberculosis compounds/drugs. Only a few plant species have been thoroughly investigated for their medicinal properties [3].Therefore, the aim of the present study was to evaluate the antimycobacterial activity of various extracts of Allium ascalonicum and Allium sativum against MDR and XDR isolates of MTB. In addition the antitubercular activity of the crude extracts against MTB and to correlate the presence of phytochemicals in each extract.

MATERIALS AND METHODS

Preparation Of Plant Extracts

The plant Bulb of A.sativum (Fig: 1) and A.ascalonicum (Fig: 2) were collected and the leaves were dried under. They were powdered with an electric mixer and stored in an air-tight container at 4^0C until usage. 25g of each were pulverized using mixer separately.Successive extraction was done using solvents of varying polarity namely Aqueous, Hexane, Chloroform and Ethanol using soxhlet extractor and homogenized for 1 hour and filtered using Whatman filter paper No. 1. The extracts were concentrated using rotary flash evaporator under reduced pressure and at controlled temperature.

Fig.1 A.sativum (clove)

Fig. 2 A.ascalonicum(shallot)

Anti- Mycobacterial Assay

Antimicrobial assays were performed in LJ medium. The plant extract was incorporated inthe medium at concentration of 2, 4 and 6µg/ml prior to inspissation. Percentage inhibition was calculated bymean reduction in number of colonies on extract containing as compared to extract freecontrols. The number of colonies grown on the LJ media was counted on extract containingand extract free control LJ slants after 42 days of incubation at 37°C were recorded. The percentage inhibition (%I) was determined using the following formula:

$$(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Phytochemical Analysis

All the extracts were subjected to preliminaryphytochemical analysis using standard procedure to identify the various phytoconstituents (Vinayaka K. S, 2020).Accordingly, presence of acidic compounds, alkali compounds, alkaloids, carbohydrates, fats, fixedoils, flavonoids, saponins, steroids, terpenoids and tannins wereinferred using the standard protocolsThe results were reported as (+) for presence, and (−)for absence.

Statistical Analysis

All the data were presented as Mean±SD. The Statistical Analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered as significant. The IC50 values were obtained by linear regression method of plot using Microsoft Excel 2007 software.

RESULTS

The in vitro antimycobacterial activity of two selectedplants against MTB H37Rv and MDR were assayed by L-J proportion method. All extracts of two selected plants showed inhibited activity against MTB H37Rv strain and MDR strain.The intention of this study is to discuss the state of art of plant derivatives and their activity against MTB and other drug resistant mycobacterial strains, Aqueous, Hexane, Chloroform and Ethanol extracts were prepared from each of those plants (Fig:3&4). visible colonies of MTB on LJ medium along with plain control. Minimum inhibitory concentration (MIC) experiment was carried out by using different drug concentrations from 2μg/ml, 4μg/ml, 6μg/ml, respectively.

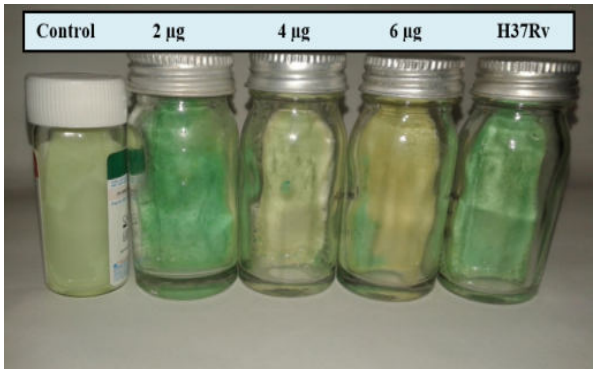

Fig: 3 Allium Sativum- Different Drug Concentrations

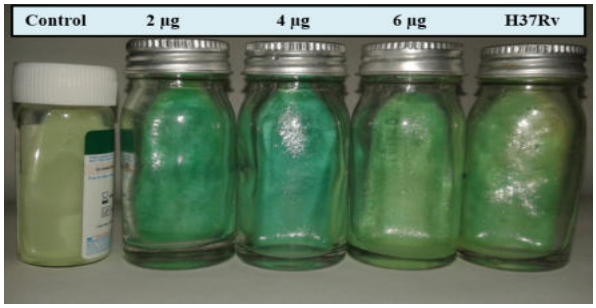

Fig: 4 Allium Ascalonicum- Different Drug Concentrations

Colony Forming Units (cfu) After Drug Treatment:

The plant extracts for their ability to inhibit colony development and found that A.sativum extract outperformed the other plant extracts in inhibiting both MDR-TB and H37RV MTB strains predominantly (Table-1). As shown in the table 1, 6μg/ml of A.sativum showed comparable numbers of colonies (p=<1.0) which were 45.5±2.9 colonies for Aqueous, 39.6±2.1 colonies for Hexane, 35.9±1.2 colonies for Chloroform and Ethanol solvents for 33.6±1.1 colonies by MDR-TB and 39.5±2.9 colonies for Aqueous, 41.7±2.4 colonies for Hexane, 34.8±1.9 colonies for Chloroform and Ethanol solvents for 31.7±1.1 colonies by H37Rv (control bacteria). One interesting aspect is that overallthe inhibitory activity was marginally more with control strain than that the MDR-TB andthese values statistically not significant (p≥0.1). Next to A.sativum exhibited anti-TB activity and these values were significantly higher than A.ascalonicum (p values shown in the table). Throughout the study, A.ascalonicum showed the minimum anti-TB activity on bothstrains. Another important observation with all the extracts was that all of them performed with 6μg/ml and the anti-TB activity was directly proportional to the concentration of the extract.

Table 1: The Table shows values represent the Mean± SD of threeexperiments.

Botanical Name	Local/ Tamil Name	Part Used	Sol-vent	CFU					
				H37Rv MEAN ± SD			MDR-TB MEAN ± SD		
				2 μg	4 μg	6 μg	2 μg	4 μg	6 μg
A. sativum	Puntu	Clove	Aqueous	50.4±3.8	48.6±3.1	39.5±2.9a	52.7±3.8	48.6±2.8	45.3±2.9e
			Hexane	47.5±3.2	45.5±2.9	41.7±2.4b	48.2±2.9	46.6±2.5	39.6±2.1f
			Chloroform	43.5±2.8	39.8±2.1	34.8±1.9c	45.7±2.1	43.5±2.1	35.9±1.2g
			Ethanol	39.7±1.9	35.8±1.8	31.7±1.1d	43.2±1.9	39.4±1.7	33.6±1.1h
A. ascalonicum	Chinnavengayam	Shal-lot	Aqueous	52.2±3.2	47.2±2.9	35.6±2.5a	53.6±3.9	49.8±2.9	47.3±3.1e
			Hexane	49.4±3.1	47.7±2.5	45.2±2.6b	44.3±3.1	46.6±2.9	43.5±2.5f
			Chloroform	45.6±2.9	43.5±2.6	39.7±2.1c	48.6±2.8	45.2±2.5	38.5±1.9g
			Ethanol	40.2±2.1	39.5±1.9	35.4±1.8d	45.6±2.1	41.6±1.9	36.4±1.5h

Analysis of variance (Anova) Methods:
A.sativum - a vs b=0.002, a vs c = 0.002, a vs d=0.002, avs e=0.01, e vs f=0.001, f vs g=0.002, gvs h =0.02, a vs e=0.001, b vs f=0.002,c vs g=0.001,d vsh=0.02.

A.ascalonicum - a vs b=0.002, a vs c = 0.001, a vs d=0.001, avs e=0.02, e vs f=0.01, f vs g=0.001, gvs h =0.01, a vs e=0.001, b vs f=0.002,cvsg=0.002,dvsh=0.02.

Anti-mycobacterial Activity Of Plant Extracts Of Various Plants Were Tested Against Mtb H37rv And Mdr-tb Isolate

Next, we evaluated the percentage inhibition of MTB by these extracts. A.sativum showed a percentage inhibition as it had 43.2±2.9 and 49.2±1.1percentage inhibition againstcontrol strain and MDR strain, respectively. As shown in the table 2, there was a positive correlation withthe percentage inhibition and concentration of the extract used. An inhibition percentage of 45.8±0.9 and 38.5±2.2 was disclosed by A.ascalonicum and remained the poorest in possessing anti-TB activity. These results confirmed the previous data shown above.

Finally, we wanted to further confirm our data on these extracts by evaluating the inhibitoryconcentration 50 (IC50) of each extract against H37Rv strain and MDR-TB strain. This isillustrated in the Fig.5 and as shown in the figure maximum anti-TB activity against both strains was shown by A.sativum as revealed by their IC50 values of 3.6 for control strain and 2.6 for MDR strain (Fig.6). Thus, it may be worth pursuing both extracts for the further evaluation ofthe anti-MTB activity against MDR-TB and XDR-TB.

Table 2: The Table Shows Values Represent The Mean± Sd Of Three Experiments.

Botanical Name	% inhibition of H37Rv MEAN ± SD			% inhibition of MDR-TB MEAN ± SD		
	6μg/ml	4 μg/ml	2 μg/ml	6 μg/ml	4 μg/ml	2 μg/ml
A.sativum	43.2± 2.9a	21.6± 1.3	10.3± 0.4	49.2± 1.1c	25.3± 5.8	13.2± 0.8
A. ascalonicum	38.5± 2.2b	18.8± 1.1	9.5± 0.3	45.8± 0.9d	21.63± 5.1	11.4± 0.5

Analysis of variance (Anova) Methods: a vs b<0.001, b vs c<0.002 , a vs c<0.001, c vs d<0.1,d vs a<0.02

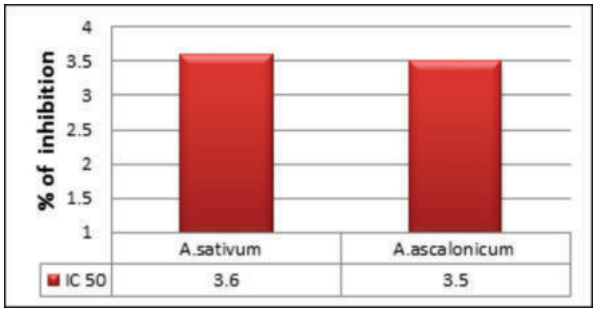

Fig-5 The Inhibitory Concentration 50 (ic50) Of H37rv Mtb Values Were Calculated As Mentioned In The Methods.

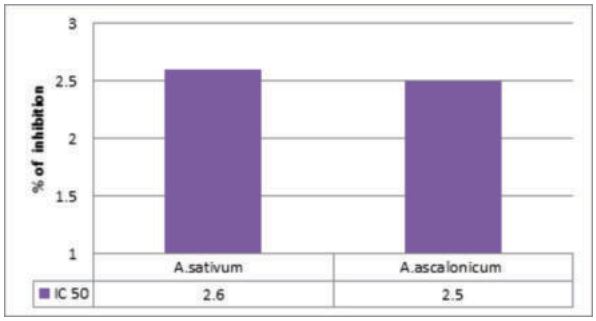

Fig-6: The Inhibitory Concentration 50 (ic50) Of Mdr-tb Values Were Calculated As Mentioned In The Methods.

Phytochemical Screening

Preliminary phytochemical screening of the all the extracts of Allium sativum and Allium ascalonicum revealed the presence of various phytochemical constituents as shown in Table 3.All the phytochemicals are present in aqueous extracts of Allium sativum. Tannins were absent in all the solvents of A.ascalonicum.

Table 3: The Phytochemical Tests Exposed The Presence And Absence. + =Presence; - =Absence

Test plants	Solvent used	Presence of Phyotychemicals						
		Tannins	saponins	Terpenoids	Flavonoids	Glycosides	Alkaloids	Steroids
Allium sativum	Aqueous	+	+	+	+	+	+	+
	Ethanol	-	+	+	+	-	+	+
	Hexane	-	-	+	-	+	-	+
	Chloroform	-	+	-	-	+	-	-
Allium ascalonicum	Aqueous	-	+	+	+	+	+	-
	Ethanol	-	-	+	-	+	-	+
	Hexane	-	-	-	+	-	+	-
	Chloroform	-	+	-	+	-	+	-

DISCUSSION

Due to increase in MDR and XDR strains of MTB, there is an urgent need of finding newer antimycobacterial agents to combat this problem [5]. Rajiniraja et al., 2014 also reported antimicrobial activity of the ethanolic extracts of the whole plant of Acalypha indica, Adhatodavasica and Allium sativum [6]. Even in the presentinvestigation, extracts of Allium sativum proved to be positive for thepresence of metabolites exhibiting antimycobacterial activity. Gupta et al., (2010) showed that the aqueous extracts of plants Acalypha indica, Adhatodavasica and Allium sativum exhibited antimycobacterial activity [5]. Grange et al., (1996) reported that the secondary metabolites isolated from the Adhatodavasica inhibited the growth of MTB. In our study we found that all the four extract of Allium sativum showed good activity against MTB H37Rv strain [7]. In the previous study indicated that the administration of aqueous A. paniculata extracts could minimize the hepatotoxic effect of Rifampicin in rats [8]. These results support the use of Allium sativum plants in traditional medicine andcomplementary medicine.

The aqueous extracts of Allium sativum demonstrated bactericidal effect against MTB H37Rv and MDR strain.Our result consistent with other study that extract of Allium sativum showed antimycobacterial activity against MTB H37Rv standard strain. It is in this backdrop the current study was conducted to evaluate theanti-MTB potential of aqueous extracts derived from two Indian medicinal plants viz. A.sativam and A.ascalonicum on H37Rv and MDR-TB. We found that A.sativam yielded the maximum inhibitory activity with MDR-TB. This was followed by A.ascalonicum with 71% inhibition. Almostsimilar levels of inhibitions were noticed by these plants with H37Rv strain suggesting that A.sativam surpassed all the extracts and showed the maximum anti-TB activity. Basedon the literature we tried 2 different concentrations namely 6, 4, 2 mg/ml and results showed a dose-response effect Alkaloids extracted from several plants have also been reported to exhibit antimycobacterial activity [9]. Those variousfindings indicate that the presence of active compounds such asalkaloids, polyphenols, flavonoids, and terpenoids in medicinalplants were considerably responsible for their activity against Mycobacterium tuberculosis. In our study, we only used different extracts and not any other solvents. With aqueous extracts, we found better anti-TB activity at 6μg/ml itself and that too against both H37Rv and MDR strains. Our study had shown the anti-MTB potential of A.sativum are well-known drugs of traditional medicine systems. However, a study using higherconcentrations of the extract on its anti-TB activities and their toxicity would throw morelight on their anti-TB potentials further. In addition, more studies are warranted to test ourextracts against other forms of MDR and XDR-TB. Overall the study has yielded novel anti-MTB drugs namely A.sativum which are worth pursuing further.

CONCLUSION

This study demonstrated that the two plant extract has showed its effectiveness against clinical isolates of MTB.This study serves to validate traditional knowledge andadds to the growing literature on botanical sources identifiedas providing important novel anti-tuberculosis compounds. The overall results of the antimycobacterial and phytochemical activity of A.sativum justified the traditional uses of plant and suggested that the indigenous traditional medicines could be used as a guide in the continuing search of new natural products with potential medicinal properties. Based on this we concluded that A.sativum is an potent candidate for anti MTB drug preparation however further probing is required.

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