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Original Article

BIOAUTOGRAPHY GUIDED SCREENING OF SELECTED INDIAN MEDICINAL PLANTS REVEALS POTENT ANTIMYCOBACTERIAL ACTIVITY OF ALLIUM SATIVUM EXTRACTS- IMPLICATION OF NON SULFUR COMPOUNDS IN INHIBITION

MUNIYAN RAJINIRAJA AND GURUNATHAN JAYARAMAN*

School of Bio-Sciences and Technology, VIT University, Vellore 632014, Tamilnadu, India.
Email: gjayaraman@vit.ac.in

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ABSTRACT

Objective: The purpose of this study is to investigate the antimycobacterial activity of selected medicinal plant extracts and to screen the class of phytochemicals responsible for mycobacterial inhibition.

Methods: Serial extraction, antimicrobial assay and *Mycobacterium tuberculosis* assay by agar well diffusion method, MIC by MABA assay, qualitative phytochemical test, contact bioautography, identification of active metabolites by spray reagents.

Results: In the present investigation, extracts of six medicinal plants were tested for antibacterial property using selected gram-positive and gram-negative organisms. Of these, extracts of *Acalypha indica, Adhatoda vasica* and *Allium sativum* exhibited substantial inhibition of the microbial growth. Activity guided serial extraction was performed to identify potential fractions responsible for antimicrobial activity, including the inhibition of *M. tuberculosis* H37Ra. Among the fourteen extracts prepared, plate assays indicated that petroleum ether and ethyl acetate extracts of *Allium sativum* displayed substantial inhibition of *M. tuberculosis* growth. Phytochemical analysis indicated the absence of sulfur containing compounds but could be either fats and fixed oils or phenol and aryl amine derivatives.

Conclusion: The studies, for the first time, reveal the presence of a non–thio metabolite in *Allium sativum* with potential antimycobacterial activity.

Keywords: Antimicrobial activity, Plate assay, Antimycobacterial activity, Phytochemical analysis, Fractionation, Contact bioautography.

INTRODUCTION

Tuberculosis (TB) is a pandemic disease, caused by *Mycobacterium tuberculosis* - the most prolific infectious agent affecting human health. It is one of the leading causes of mortality worldwide and it is estimated that a third of world population is infected by it [1]. 'Global Tuberculosis Control 2011' states that WHO registered about 8.8 million people worldwide, of which 2.5 million infections are from India [2]. The advent of first line drugs (FLD) such as isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin decreased the number of TB cases, especially in developed countries [3]. However, due to the prolonged duration of the therapy (for at least six months) using FLD, led to the emergence of Multi-Drug Resistant-TB (MDR-TB) [4]. MDR-TB is resistant to isoniazid and rifampicin with or without resistance to other FLD's. The intolerant MDR-TB patient is treated with second line drugs (SLD) such as p-aminosalicylic acid, amikacin, ofloxacin, ethionamide, kanamycin, capreomycin which further resulted in the emergence of Extensively Drug Resistant -TB (XDR-TB). XDR-TB is resistance to at least isoniazid and rifampicin, and to any of the three second-line injectables (amikacin, capreomycin, and kanamycin) [5]. Although this regimen is effective in treating active TB, it is associated with many adverse drug reactions (ADRs) and poses a significant challenge to completion of treatment [6]. Therefore, there is a need to discover and develop new lead molecules from natural sources [7]. Plant-based drugs have been used worldwide in traditional medicines for the treatment of various diseases including tuberculosis [8]. Approximately 80% of world's populations still depend on medicinal plants for their primary healthcare (WHO, 2002). According to a survey by NCI (National Cancer Institute), (USA), small-molecules are introduced as drugs worldwide during 1981–2002. It is documented that 70% of 365 plant species in India have shown antimycobacterial activity [9, 10, 11, 12]. In a recent study by Gupta *et al* 2010, aqueous extracts of *Acalypha indica, Adhatoda vasica, Allium cepa, Allium sativum and Aloe vera* were shown to be susceptible with MDR-TB strains [13]. In order to obtain further insights on the antimycobacterial property of the metabolites from these plants, we have performed activity guided fractionation to narrow down to fractions containing the active molecules. The potential compound types identified might further be used as lead molecules for the development of drug against resistant strains of *Mycobacterium tuberculosis*.

MATERIALS AND METHODS

Collection and identification of the plant materials

Based on the available literatures, six plants (*Acalypha indica, Adhatoda vasica, Allium cepa, Allium sativum, Aloe vera* and *Heliotropium indicum)* were selected for the current investigation. All the plants were identified by either Dr. G.V.S. Murthy (Botanical Survey of India, Southern Regional Centre, Coimbatore, India) or Dr. K. Madhava Chetty (Department of botany, Sri Venkateswara University, Tirupati, India).

Preparation of plant extracts

Fresh leaves of *Acalypha indica, Adhatoda vasica* and *Heliotropium indicum* were collected and shade-dried at room temperature for one week, and then powdered. For *Allium* sp., outer skin was pealed and washed with distilled water and chopped into small pieces, shade-dried at room temperature for two weeks, and then ground into a fine paste. The gel from the leaf of *aloe vera* was collected by removing the outer layer of the leaves. All the samples were stored in cold (4°C) until use [14].

Extracts for preliminary screening

Aqueous extracts of all the six plants were prepared by mixing 10 g of powdered leaves/paste/gel in 100 ml water (1:10). The mixture was heated at 60ºC for 1 h with continuous stirring and cooled to room temperature. The extracts were filtered through Whatman (No.1) filter paper. The filtrates were evaporated to dryness and stored in cold (4ºC) until use [15].

Serial extraction of active components

The plant samples were subjected to serial extraction using Soxhlet apparatus (HiMedia). Extraction was performed sequentially with

250 ml of solvents, ranging from low to high polarity. Serial extraction with petroleum ether (40°C), chloroform (61°C), ethyl acetate (76.5°C), methanol (64.7°C) (all purchased from SDF-Chem. Ltd) was performed for approximately 7-9 hours. The extracts were then evaporated to dryness using rotary evaporator (40°C). The extracts were stored in sterile bottle (covered with aluminium foil) [16].

Bacterial strains

The bacterial strains *Mycobacterium tuberculosis* H37Ra (MTCC 300), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 452), *Salmonella typhi* (MTCC 734), *Klebsiella pneumonia (*NCIM 2707) and *Pseudomonas aeruginosa* (MTCC 1688) were purchased from IMTECH, Chandigarh (India). The strains were sub-cultured periodically as per the specifications and were maintained as glycerol stocks.

Culture medium

Solid culture

M. tuberculosis H37Ra (MTCC 300) was sub cultured by suspending 6.2 gm of Loewenstein-Jensen medium base (Fluka- 63237) in 100 ml water containing 2 ml glycerol (Sigma-Aldrich) and autoclaved (15 lbs for 15 min). The egg emulsion (50 ml) was mixed with the base to obtain uniform mixture. The medium was aliquoted (10 ml) in sterile screw capped bottles and was made to coagulate by heating to 85-90ºC for 45 min (NCIM). After solidification, *M. tuberculosis* was streaked and incubated at room temperature for four weeks.

Liquid culture

The pure culture was isolated from the slant (LJ medium) and inoculated into 5 ml Middlebrook 7H9 broth base supplemented with 10% (v/v) ADC (Accumix™) and 0.2% (v/v) glycerol (Sigma-Aldrich). The inoculated media was maintained at 37 ºC for 72 hours on a shaker until an OD_{600} of 0.6–0.8 was reached; (equivalent to 3×10^7 cfu/mL). The culture was used for antimycobacterial assay [17, 18]. For other bacterial strains, inoculum was prepared by using a loop full of test organism in 5ml of Nutrient broth and incubated at 37 ºC for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards [19].

Assay

General antimicrobial assay

0.5g/ml of each extracts were prepared by dissolving 500mg of the plant extract in 1 ml of DMSO. Agar medium was prepared by dissolving 28 g of the nutrient agar (HiMedia) in 1000 ml of distilled water and autoclaved (121°C for 15 min). The agar was allowed to cool to room temperature and 25ml of the agar was dispensed into each plates. The nutrient agar was allowed to solidify. Pure culture was isolated; inoculated into 5ml nutrient broth and were then incubated for 24 h at 37°C. The inoculum was swabbed on to the plate using sterile cotton. For S1 and S2, 50 µl of the extract (0.5g/ml in DMSO) was loaded in each well (10 mm dia). For S4, 50 µl of the extract (0.1g/ml in DMSO) was loaded in each well (10 mm dia). The plates were incubated for 24 h at 37°C and the zone of inhibition was measured. 30 µg gentamicin disc (HiMedia) was used as the positive control while the DMSO was used as the negative control for each extracts [20, 21].

***Mycobacterium tuberculosis* assay**

The antimycobacterial activities of the extracts were assessed by the agar well diffusion assay. Agar medium was prepared by dissolving 2.35 g of Middlebrook 7H9 broth base in 450 ml of distilled water and added 2ml of glycerol and 2% agar (2g/100ml medium); the mixture was autoclaved at 121°C (15 lbs pressure) for 10 min. The agar was allowed to cool to room temparature and 25 ml of the medium was dispensed into Petri plates. The medium was allowed to solidify [22]. The plates were pre-seeded with *M. tuberculosis.* About 50 µl of the samples were loaded in each well (10 mm dia). The plates were incubated for 72 hours at 37°C. The diameter of the zone of inhibition was measured [23, 24]. Rifampicin (5µg/ml) served as a positive control whereas dimethyl sulfoxide (DMSO) was used as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using MABA (Microplant Alamar Blue Assay) in a 96 well micro titer plates. To measure the MIC values, various concentrations (range of 0.1 – 50 mg/ml) of the extracts were used. The minimum inhibitory concentration was defined as the lowest concentration of the extract that could prevent the color of Alamar Blue to change from blue to pink (visual determination). This indicates that the inhibition of the growth of the indicator organism, as inferred from the values of the zone of clearance [25, 26].

Qualitative analysis of the phytochemicals

Phytochemical tests were performed for extracts that responded significantly to antimicrobial assay. Accordingly, presence of acidic compounds, alkali compounds, alkaloids, carbohydrates, fats, fixed oils, flavonoids, saponins, steroids, terpenoids and tannins were inferred using the standard protocols [27, 28, 29, 30].

Contact bioautography

The extracts were resolved using thin layer chromatography, using suitable developing solvent. The plates were exposed to UV light (365 or 254 nm). The TLC plate was flipped and placed onto the inoculated agar layer and left for 45 min to enable diffusion of the separated compounds [31, 32]. Subsequently, the TLC plate was removed and the plate was incubated for 72 h at 37°C. The zone of clearance on the agar surface indicated the presence of the compound with antimycobacterial activity.

Spray reagents

Alkaloids

Presence of alkaloids were inferred either by using Dragendorff regent (1.5 g bismuth nitrate and 16.7 g tartaric acid and 13.3 g of potassium iodide in 100 ml water) or with 37% formaldehyde in conc H_2SO_4 (1:10) [33].

Fats and fixed oils (Fatty acid test)

Presence of fats and fixed oils were inferred by using copper acetate-potassium hexacyanoferrate(II) reagent. 10 ml aqueous copper acetate solution with 240 ml water contains 1.5% aqueous potassium hexacyanoferrate(II) [33].

Phenol and aryl amines

Inference in the presence of phenol and aryl amine were obtained by spraying 1% amino antipyrine (4-aminophenazone) dissolved in 80% ethanol followed by treatment with 4% potassium hexacyanoferrate (III) dissolved in 80% ethanol [33].

Sulfur containing compounds

Iodoplatinate reagent was used to testify the presence of sulfur containing compounds (0.15gm potassium chloroplatinate and 3.0 g potassium iodide in 100 ml dilute hydrochloric acid [34].

RESULTS

Table 1 summarizes the medicinal plants and the part used in the current study. Medicinal uses of the plant part along with the voucher specimen number are also given for reference. The aqueous extract of these plants were initially screened for antimicrobial activity using the agar-well diffusion assay.

It is evident from Figure 1 that extracts of *Acalypha indica, Adhatoda vasica* and *Allium sativum* exhibit significant zone of clearance indicating the presence of compound(s) with antimycobacterial activity.

Table 2 indicates that the MIC of *Acalypha indica*, *Adhatoda vasica* and *Allium sativum* was 5, 10 and 1.25 mg/ml respectively. This confirms that the *Allium sativum* extract contains plant metabolites with potent antimycobacterial activity.

Table 1: Consolidated information on the selected medicinal plants used in the present study and its traditional uses

S. No.	Botanical name/ Family (Common name)	Label	Plant parts used	Traditional uses and Reference	Voucher specimens number
1	*Acalypha indica* Linn/ Euphorbiaceae (Kuppaimeni)	S1	Leaves	Diuretic, purgative, bronchitis, asthma, pneumonia, scabies anthelmintic, [35]	839
2	*Adhatoda vasica* Nees/ *Acanthaceae* (Vasaka)	S2	Leaves	Cold, cough, bronchitis and asthma [35]	840
3	*Allium cepa* Linn/ Alliaceae (Onion)	S3	Bulbs	Cough [35]	2015
4	*Allium sativum* Linn/ Alliaceae (Garlic)	S4	Bulbs	Arthritis, tuberculosis, stimulant, carminative, expectorant, diuretic, hypotensive [35]	2011
5	*Aloe vera* / Aloaceae (Indian aloe)	S5	Leaf Gel	Asthma and cough [35]	2025
6	*Heliotropium Indicum* Linn/ Boraginaceae (Thel Kodukku)	S6	Leaves	Cough, hypertension, candidiasis. [35]	811

Table 2: MIC of aqueous extracts of selected medicinal plants against *M. tuberculosis* strain using visual MABA and mean values of triplicate of plate assay are tabulated along with its standard deviation (mean±SD)

Botanical name/ Common name	MIC (µg/ml) against *M. tuberculosis*	Zone of inhibition (mm in dia)
Acalypha indica	5×10^3	15 ± 1.0
Adhatoda vasica	10×10^3	14.3 ± 0.5
Allium sativum	1.25×10^3	29.3 ± 0.5
Rifampicin	2	38 ± 1.0

Based on this preliminary result, these three plants were chosen for serial extraction. The extracted metabolites were quantitated and the color and consistency were recorded in Table 3. In addition to antimycobacterial activity, antimicrobial activity of these fractions with specific indicator organism was studied in order to evaluate the specificity of inhibition. Table 4 depicts the quantitative information on the zone of clearance obtained for all the indicator organisms by plate assay; it could be observed that the petroleum ether and ethyl acetate extracts of *Allium sativum* displayed the presence of metabolites with potential inhibitors of *M. tuberculosis*. This indicates that these extracts contain metabolites that are specific for inhibiting more the growth of *M. tuberculosis.*

The petroleum ether and ethyl acetate extracts of *Allium sativum* were then assayed for the type of phytochemicals present, using standard protocols. Table 5 indicates that both the extracts were positive for the presence of acidic compounds, alkaloids, fats and saponins, where as alkali type compounds, carbohydrates, flavonoids, steroids and tannins were absent. Additionally petroleum ether extract seemed to contain terpenoids, which was found absent in the ethyl acetate extract.

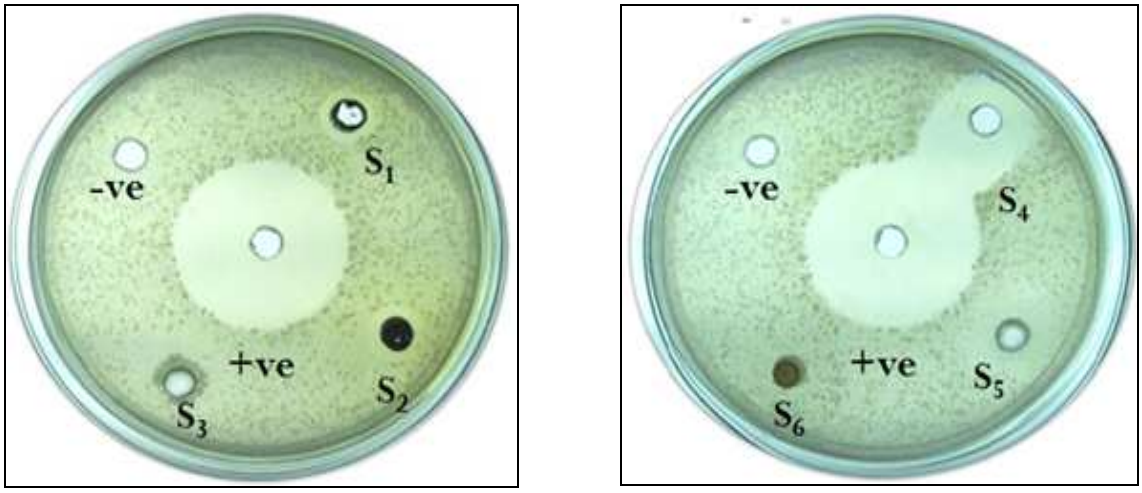

Fig. 1: Agar well diffusion assay for the aqueous extract of selected plants- *Acalypha indica* (S1), *Adhatoda vasica* (S2), *Allium cepa* (S3), *Allium sativum* (S4), *Aloe vera* (S5) *and Heliotropium indicum* (S6). Rifampicin (5µg) and DMSO (50 µl) were used as the positive and negative controls respectively.

Table 3: The extraction for S1, S2 and S4 with solvents of various polarities

Plant	Solvents	Weight of the sample (g)	Weight of dried extract (g)	Yield (%)	Color	Consistency
Acalypha indica (S1)	Petroleum ether	500	7	1.4	Greenish yellow	Paste
	Chloroform	493	12	2.4	Dark green	Paste
	Ethyl acetate	481	3	0.6	Green	Paste
	Methanol	478	5	1.0	Brown	Paste
	Water	473	3	0.6	Dark brown	Paste
Adhatoda vasica (S2)	Petroleum ether	500	6	1.2	Greenish yellow	Paste
	Chloroform	495	9	1.8	Dark green	Paste
	Ethyl acetate	486	3	0.6	Green	Paste
	Methanol	483	5	1.0	Brown	Paste
	Water	478	3	0.6	Pale brown	Paste
Allium sativum (S4)	Petroleum ether	500	1	0.2	Pale yellow	Paste
	Chloroform	499	1	0.2	Yellow	Paste
	Ethyl acetate	498	0.6	0.1	Yellowish brown	Paste
	Methanol	497	14	2.8	Pale brown	Paste
	Water	483	63	13	Brown	Paste

Table 4: Antimycobacterial /Antimicrobial Assay by agar-well diffusion method of S1, S2 and S4 plants. The average data obtained from triplicate experiments are tabulated along with its standard deviation (mean±SD)

Organism Plant		*M. tuberculosis*	*P. aeruginosa*	*S. aureus*	*S. typhi*	*E. coli*	*K. pneumoniae*
***Acalypha indica* (25mg)**	PE	15 ± 1.0	16.3 ± 0.5	13 ± 1.0	-	-	-
	C	21 ± 1.7	14 ± 0.0	13 ± 1.0	12.3 ± 0.5	-	-
	EA	14 ± 2.0	19 ± 2.0	18 ± 1.0	15 ± 2.6	16 ± 1.0	12 ± 1.0
	M	-	16 ± 1.0	13 ± 0.0	12 ± 1.0	-	-
	W	-	14 ± 2.0	12.3 ± 0.5	-	-	12.3 ± 0.5
	+ve	35.3 ± 0.6	20 ± 1.0	19 ± 0.0	18 ± 1.0	15 ± 0.0	12 ± 1.0
	-ve	-	-	-	-	-	-
***Adhatoda vasica* (25mg)**	PE	-	12 ± 1.0	-	-	-	-
	C	-	17.3 ± 0.5	13.3 ± 0.5	-	-	-
	EA	16 ± 2.0	-	-	-	-	-
	M	15.3 ± 0.5	13 ± 1.0	13 ± 2.0	14 ± 1.0	14.3 ± 0.5	18 ± 2.0
	+ve	35 ± 1.0	20 ± 0.0	19 ± 1.0	18 ± 1.7	15.3 ± 0.5	12.3 ± 0.5
	-ve	-	-	-	-	-	-
***Allium sativum* (5mg)**	PE	**30.3 ± 0.5**	13 ± 1.0	12.3 ± 0.5	16 ± 1.0	-	16 ± 1.7
	C	19 ± 1.0	12.3 ± 0.5	12 ± 1.0	14 ± 1.7	-	15 ± 1.0
	EA	**26.3 ± 0.5**	14 ± 1.0	13 ± 2.0	18 ± 2.0	16 ± 1.0	18 ± 1.7
	M	12 ± 1.0	-	-	-	-	-
	W	-	-	-	-	-	-
	+ve	32.3 ± 0.5	22.3 ± 0.5	21 ± 1.0	17 ± 1.0	14 ± 0.0	18 ± 1.0
	-ve	-	-	-	-	-	-

The above table concise PE= Petroleum ether, C= Chloroform, EA= Ethyl acetate, M= Methanol, W= Water, +ve control= Gentamicin disc (30µg) and Rifampicin (5µg), -ve control= DMSO, Well size = 10 mm dia. The PE and EA extracts of *Allium sativum* (S4) reveals higher zone of inhibition were highlighted in Bold.

Table 5: Phytochemical tests for the petroleum ether and ethyl acetate extracts of *Allium sativum* (S4)

Phytochemicals	Name of the test	PE	EA
Acidic compounds	Litmus paper	+	+
Alkali compounds	Litmus paper	-	-
Alkaloids	Mayers test	+	+
	Tannic acid test	+	+
Carbohydrates	Molisch's test	-	-
Fats and Fixed oils	Glycerin test	+	+
Flavonoids	Shinoda test	-	-
	Alkaline test	-	-
Saponins	Froth test	+	+
Steroids	Salkowski test	-	-
Terpenoids	Salkowski test	+	-
Tannins	Ferric chloride test	-	-

+ve: Present, - ve: Absent

In order to get further insights on the active components present in these two extracts, thin layer chromatography analysis followed by contact bioautography was performed. Petroleum ether extract seemed to contain multiple components of lower polarity than compared to the ethyl acetate fraction. The R_f values of the spots is summarized in Table 6 and 7.

Clear zones (Figure 2) were observed for the spots with low Rf value indicating that the compounds with inhibition activity is moderately polar in nature. The result thus indicated that both the petroleum ether and ethyl acetate fractions of the *Allium sativum* extract contain more than one compound which is responsible for the antimycobacterial activity.

Table 6: Thin layer chromatography analysis of petroleum ether extract of *Allium sativum*. Petroleum ether:Chloroform (4:1) was used as the mobile phase

Detection method	Number of spots	Rf value
UV-Visible (365nm absorption)	1	0.12
Short-UV (254nm absorption)	6	0.12, 0.33, 0.48, 0.64, 0.76, 0.94

Table 7: Thin layer chromatography analysis of ethyl acetate extract of *Allium sativum*. Petroleum ether: Ethyl acetate (2:3) and Petroleum ether :Methanol (2:3)was used as the mobile phase

Detection type	Mobile phase	Number of spots	Rf value
UV-Visible	PE:EA (2:3)	2	0.01, 0.99
Short-UV	PE:EA (2:3)	2	0.01, 0.99
UV-Visible	PE:M (2:3)	2	0.32, 0.89
Short-UV	PE:M (2:3)	1	0.32

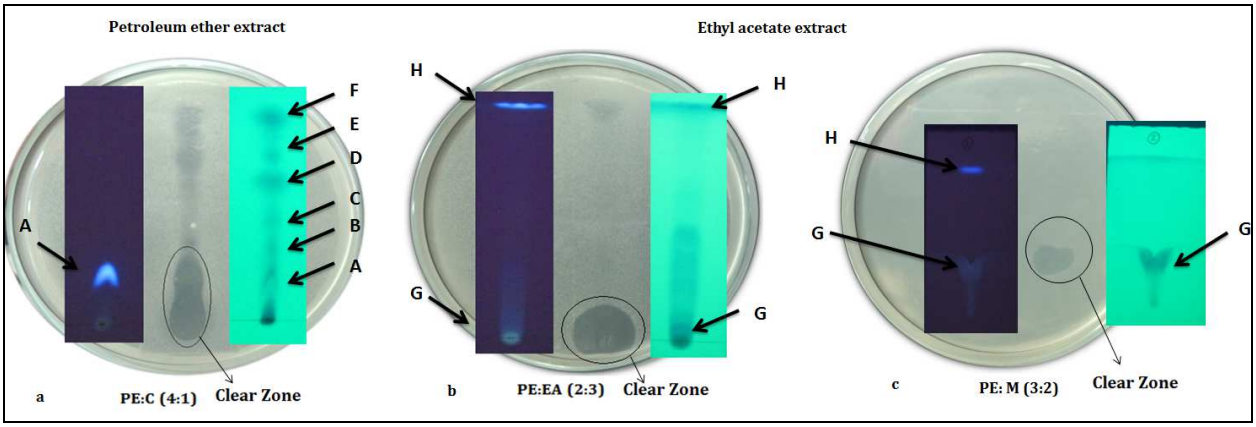

Figure 2: Contact bioautography for Petroleum ether (PE) and Ethyl acetate (EA) extracts of *Allium sativum*, a) PE extract with mobile phase PE: EA (4:1), b) EA extract with mobile phase PE: EA (2:3), c) EA extract with mobile phase Petroleum ether: Methanol (3:2).

Spray reagent test for alkaloids, phenol and aryl amine, fats and fixed oils and sulfur containing compounds were preformed for the chromatogram to identify the type of compounds associated with the active spot. The results were positive for fats and fixed oil, phenol and aryl amine, where as alkaloids and sulfur containing compounds proved to be negative. The developed plates were dipped into $KMnO_4$ solution for further confirmation. Appearance of pale white indicated the probable oxidation of the said phytochemical types.

DISCUSSION

Tuberculosis is one of the leading causes of mortality in human worldwide. It is estimated that, one third of world population infected by *M. tuberculosis*. These statistical data reflects on severity of the disease in human population and combating with *M. tuberculosis*. In this study we aimed at screening extracts of selected medicinal plants against *M. tuberculosis*. All the medicinal plants used in the study were collected based on their use against *M. tuberculosis* by traditional healers and ayurvedic practioner in the region of Vellore district (India). Till date, extracts of several plants were shown to exhibit antimycobacterial activity. The plant extracts used in the present study displayed broad range of antibacterial activity, including the inhibition of mycobacterial growth. There was differential antibacterial activity of various extracts, indicating the absence of non-specificity in the inhibition process. Connell (1994) [36] and Jayakumari et al., (2010) [37] also reported antimicrobial activity of the ethanolic extracts of the whole plant of *Acalypha indica, Adhatoda vasica and Allium sativum.* Even in the present investigation, extracts of *Allium sativum* proved to be positive for the presence of metabolites exhibiting antimycobacterial activity. Gupta *et al*., (2010) [13] showed that the aqueous extracts of plants *Acalypha indica, Adhatoda vasica and Allium sativum* exhibited antimycobacterial activity. Grange *et al*., (1996) [38] and Gupta *et al*., (1954) [39] reported that the secondary metabolites isolated from the *Adhatoda vasica* inhibited the growth of *M. tuberculosis*. The recent studies by Mohamad *et al*., (2011) [40], Hannan *et al*., (2011) [41], Dibua *et al*., (2009) [42] and Gautam *et al*., (2007) [43] revealed that the methanolic and ethanolic extracts of *Allium sativum* had inhibitory role against *M. tuberculosis.* The minimal concentration to inhibit the *M. tuberculosis* growth was found to be the minimum for *Allium sativum* extract. This could be either due to the low concentration of the active inhibitory molecules or the presence of molecules with high inhibition constant. Spray reagents were used to identify specific class of biologically active phytoconstituents present. The results proved to be positive for either of two classes of compounds: fats and fixed oils or phenol and aryl amine derivative. Though the antimycobacterial compounds were reported for the metabolites from *Allium sativum*, majority of the compounds are sulfur derivatives (with the exception of

geranial). However, in the present study, it was proved by spray reagents that the active components are non-sulfur compounds. Evidently, the chromatogram with $KMnO_4$ solution also produce pale white color in the active region that could reflect the presence of either fats and fixed oils or phenol and aryl amine derivatives.

CONCLUSION

It should be pointed out that this is the first study on the serial extraction of the active components through bioactivity guidance from the specified plant parts. Though these extracts have general antibacterial activity, the antimycobacterial activity appears to be more pronounced in certain fractions of *Allium sativum*. This gives positive clues for the identification of lead molecules for the development of drugs to combat *M. tuberculosis* infection.

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