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Evaluation of selected Indian traditional folk medicinal plants against *Mycobacterium tuberculosis* with antioxidant and cytotoxicity study.

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ABSTRACT

Objective: To evaluate different solvent extracts of selected Indian traditional medicinal plant against *Mycobacterium tuberculosis*, its antioxidant potential and cytotoxicity. **Methods:** *Acacia catechu* (L.) Willd (Root extract) and *Ailanthus excelsa* Roxb., leaf extracts of *Aegle marmelos* Corr., *Andrographis paniculata* Nees. and *Datura metel* L. were sequentially extracted in water, ethanol, chloroform and hexane and evaluated for their anti-tuberculosis (TB) activity against *Mycobacterium tuberculosis* using agar diffusion assay. The zone of inhibition (at 20 and 40 mg/ml) was measured and MIC were calculated. The results were compared with Rifampicin as a standard anti TB drug. The extracts were also evaluated for DPPH and OH radical scavenging activities to understand their antioxidant potential. MTT based cytotoxicity assay was used for evaluating cytotoxicity of the selected samples against Chang liver cells. **Results:** The selected botanicals were sequentially extracted in water, ethanol, chloroform and hexane and tested for growth inhibition of *M. tuberculosis*. The hexane extract of *A. catechu* root and ethanol extract of *A. paniculata* leaf showed promising activity against *M. tuberculosis* while remaining extracts showed moderate anti TB activity. The samples were found to possess considerable DPPH and OH radical scavenging activities with no demonstrable cytotoxicity against Chang liver cells. **Conclusions:** Five traditional medicinal plants were selected for the present study. The selection of medicinal plants was based on their traditional usage for the treatment of tuberculosis, asthma and chronic respiratory diseases. Herein we report for the first time, the anti TB activity of root extracts of *Acacia catechu* and *Ailanthus excelsa* while leaf extract of *Andrographis paniculata*, *Aegle marmelos* and *Datura metel*. The study holds importance in the midst of multi drug resistance (MDR) crisis in the TB management, since it unravels the scientific basis of use of these plant species for the management of TB and related disorders which will be useful for searching the lead compounds from natural products as potential antimycobacterial agents.

1. Introduction

Tuberculosis (TB) is known since antiquity and evidence of spinal TB in the form of fossil bones dates back to around 8000 BC [1, 2]. In 1993, the World Health Organization (WHO) announced this chronic disease to be a 'global emergency' [3]. Still today TB remains to be the most prevalent cause of death in developing countries, due to a single infectious agent [4]. In India, TB kills 14 times more people than all tropical diseases. There are more than 4.5 million TB cases with 1.8 million new cases being reported each year.

Approximately 50% of India's population is reported to be tuberculin test positive [5], and one person dies from TB every minute [6].

TB requires a lengthy treatment period of six months with the cocktail of first-line drugs rifampicin, isoniazid, ethambutol and pyrazinamide [7]. If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as para-aminosalicylate (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide and cycloserine, that are generally either less effective or more toxic with serious side effects [8]. The use or in most cases misuse of these drugs over the years has led to an increasing prevalence of multiple-drug resistance (MDR) strains. MDR-TB resistance to rifampicin and isoniazid, now exceeds 0.5 million cases per year and in some states accounts for up to 22% of TB

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cases^[9]. Extensively–drug resistant (XDR) strains of *M. tuberculosis*, resistant to both first and second–line drugs, were first reported in the United State, Latvia and South Korea in 2006 ^[10] but are now present in 57 countries ^[9] including India ^[11].

The increasing problem of MDR/XDR–TB has focused attention on developing new drugs that are not only active against MDR–TB, but also shorten the length of therapy. Thus, there is an urgent need of an hour to expand significant interest in developing new TB drugs because; no new class of TB drugs has been developed in the past 40 years. Medicinal plants offer a great hope to fulfill these needs because natural products are a proven template for the development of new scaffolds of drugs ^[12,13] they have received considerable attention as potential anti–TB agents. In Indian traditional Ayurvedic system of medicine, the history of TB also dates back to 600 BC where in ‘Sushruta Samhita’, the disease is known as ‘Kshya Kasha’ means ‘wasting disease’ or ‘Raja Yaksha’ means ‘the king of diseases’ because it exceedingly difficult to treat. A considerable number of plant species have been mentioned in Ayurveda for the treatment of TB, leprosy and related disorders. Sharma ^[14] has listed 60 plant species for TB and 91 for leprosy. In ‘Ayurvedic Formulary of India’ ^[15], 60 Indian medicinal plant species have been reported to be used in different Ayurvedic formulations for TB. Other works on Indian medicinal plants like ‘Indian Medicinal Plants, vol. I–IV’ by Kirtikar and Basu ^[16] also describe several species for TB and related diseases in various Indian systems of medicine. Recently, reviews on Indian medicinal plants treated against *Mycobacterium* sp. ^[17] have been published. Taking into consideration the above facts and extensive literature survey it has been observed that there is an ample scope to explore folk traditional medicinal plants being used by local traditional healers against *Mycobacterium tuberculosis* and for the management of other respiratory disorders. Therefore, the present study was aimed to establish the scientific basis of usage of selected plants claimed by the traditional healers for the management of tuberculosis

2. Materials and Methods

2.1. Materials

The *Mycobacterium tuberculosis* (MTCC 300) was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), India and was sub cultured and maintained onto Lowenstein Jensen media as described previously ^[18]. Chang Liver cell line was requested from National Centre for Cell Science (NCCS: a National Cell Line Facility) Pune (MS), India. DPPH (1,1–diphenyl–2–picryl hydrazyl), MTT (3–(4,5–dimethylthiazol–2–yl)–2, 5–diphenyl tetrazolium

bromide) were procured from Sigma–Aldrich Co. (St. Louis MO, USA)., Other chemicals like Rifampicin (Himedia Laboratories Pvt. Ltd. Mumbai), Ascorbic acid, 1–10 phenanthroline, solvents, reagents used were of AR grade and were obtained from commercial sources.

2.2. Collection, identification and authentication of the selected medicinal plants

Five medicinal plants were selected for the present study. The selection of plants was based on their traditional use for the treatment of tuberculosis and asthma by the local traditional practitioners. The selected plants *Acacia catechu* (L.) Willd. (Mimosaceae), *Aegle marmelos* Corr. (Rutaceae), *Ailanthus excelsa* Roxb. (Simaroubiaceae), *Andrographis paniculata* Nees. (Acanthaceae) and *Datura metel* L. (Solanaceae) were collected from the nearby regions of Nanded district (MS), in the month of October 2010. The plants were identified and authenticated with the help of regional Flora ^[19]. Voucher specimens (B1–B5) of the collected plants were deposited in the herbarium centre of Department of Botany, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded. The shade dried and powdered plant samples were preserved for further investigations.

2.3. Sequential Extraction of the plant samples

The shed dried powdered plant samples (~10gm) were sequentially extracted in water, ethanol, chloroform and hexane up to 8 hours using Soxhlet’s apparatus. The extracted samples were evaporated by using rota evaporator. The dried extracts were preserved for further analysis.

2.4. Determination of antimycobacterial activity of selected plant samples

Stock solutions of the individual plant extracts were prepared in 0.5 % dimethyl sulfoxide (DMSO) and diluted to the final concentrations of 20 and 40 mg/ml in sterile distilled water. As a part of experimental standardization, initially 1mg/ml concentration of plant extract was used for antimycobacterial study and it was further extended up to 20 mg/ml; however no clear zone of inhibition was observed under experimental conditions. Considering the respective results of the optimization experiments, the concentrations of plant extract were kept on higher side (20 and 40 mg/ml) in order to have clear demonstrable antimycobacterial effects in terms of zone of inhibition ^[20].

The sensitivity of *Mycobacterium tuberculosis* strain to the various extracts of the selected botanicals was demonstrated by agar diffusion method ^[21]. In short, a sterile cork borer of 7 mm diameter was used to bore holes into the inoculum seeded solidified nutrient agar. A 50 μ l volume of each (20 and 40 mg/ml) of the plant extracts was loaded into

the labeled well in the prepared media plate using sterile pipette. The test was performed in triplicates. The plates were kept in refrigerator for prediffusion of the sample and incubated at 37°C for 48 hours. Growth of *M. tuberculosis* was observed after the incubation of 48 hours and the diameter of inhibition zone was measured subtracting the well size. Rifampicin (10 µg/ml) was used as a reference standard.

2.5. Determination of the Minimum Inhibitory Concentration (MIC) of the plant extracts

The MIC of the various plants extracts were performed using the broth microdilution assay against the test *Mycobacterium tuberculosis*. Tests were performed in sterile 96-well microplates by dispensing into each well a total volume of 200 µl, comprising 100 µl of standardized suspension of *M. tuberculosis* (1x10⁶ cells/ml) and 100 µl of different concentrations of plant extracts and incubated up to 48 h at 37°C. Microbial growth was determined by absorbance measurement at 620 nm using Thermo make Automatic Ex-Microplate Reader (M 51118170). The MIC of Rifampicin was also calculated for comparison.

2.6. MTT Cytotoxicity assay

The MTT cytotoxicity assay was performed by the previously reported method [22, 23, and 24]. The Chang liver cells were harvested (4.5 × 10⁴ cells/well) and inoculated in 96 well microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the individual plant extract (1mg/ml). After 72 hrs incubation, the medium was aspirated followed by addition of 150 µL of MTT solution (5 mg/mL in PBS, pH 7.2) to each well and the plates were reincubated for 4 hrs at 37 °C. After incubation time, 800 µL of DMSO was added to the wells followed by gentle shaking to solubilize the formazan crystal for 15 min. Absorbance was read at 540 nm using Thermo make Automatic Ex-Microplate Reader (M 51118170) and the % cell viability was calculated. The H₂O₂ (1mM) was used as a standard cytotoxic agent.

2.7. DPPH radical scavenging assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay was carried out as per reported method with slight modifications [25,26]. In brief, 1 ml of test solution (individual plant extract) was added to equal quantity of 0.1 mM solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1mM) was used as reference compound.

2.8. OH radical scavenging assay

The OH radicals scavenging activity was demonstrated

with Fenton reaction [27]. The reaction mixture contained, 60 µl of FeCl₂ (1mM), 90 µl of 1–10 phenanthroline (1mM), 2.4 ml of phosphate buffer (0.2M, pH 7.8), 150 µl of H₂O₂ (0.17M) and 1.5 ml of individual plant extract (1mg/ml). The reaction was started by adding H₂O₂. After 5 min. incubation at room temperature, the absorbance was recorded at 560nm. Ascorbic acid (1mM) was used as reference compound.

The cell viability inhibition (%), DPPH and OH radical scavenging activity (%) was calculated by using following formula.

$$\text{Activity (\%)} = 1 - T/C \times 100$$

T = Absorbance of the test sample

C = Absorbance of the control sample

3. Results

3.1. Area of collection and selection of plant materials

The area of Nanded district lies between 180 16' and 190 55' North latitude and 760 56' and 780 19' East longitude. The Kandhar and the area of Bhokar forest constitute one of the major floristic regions of Marathwada region of Maharashtra state [19]. The traditional healers of this area are well known for their traditional knowledge of the use of medicinal plants. This was the main reason of selection of plants from this region for the present investigations. The total five plants from different five families were selected for the present investigation. Table 1 presents the botanical names, local names and their traditional uses as known according to information collected by Dr. Mahesh M. Pund (Head Dept. of Botany, Indira (Sr.) College, CIDCO, Nanded) through interviews with local traditional healers. Questionnaires were asked about the use of medicinal plant species for the traditional herbal treatment for treating tuberculosis, asthma and chronic respiratory diseases. The plants selected for this study were selected based on the outcome of interviews with local traditional healers and the people who have otherwise experience with traditional medicine from this region. The plants used alone, i.e. not mixed with other plant or inorganic materials were considered further.

3.2. Antimycobacterial activity and MIC profile of the selected plant extracts

The selected samples extracted in different solvents were tested at concentration of 20 and 40 mg/ml for their antimycobacterium activity against *M. tuberculosis* using agar diffusion assay with rifampicin as a standard drug and efficacy of the selected plant extracts were calculated in terms of MICs. The results of antimycobacterial activity are presented in the form of zone of inhibition and MIC in Table 2.

Amongst the different solvent extracts of five selected

Table 1.

Ethnobotanical information of selected medicinal plants.

Sr. No.	Name of the Plant	Local name	Family	Part used	Traditional use	Literature review
1	<i>Acacia catechu</i> (L.) Willd	Kala khair, Kattha	Mimosaceae	Root	Two spoon full root paste taken orally with empty stomach daily for 60 days for tuberculosis.	No literature on anti TB activity
2	<i>Ailanthus excelsa</i> Roxb.	Maharukh, Ghodlimb	Simaroubaceae	Root	Two spoon full root paste taken orally with empty stomach daily for 50 days for chronic asthma.	The allied species <i>Ailanthus altissima</i> (Mill.) Swingle syn. <i>Altissima glandulosa</i> Desf. for asthma [34]
3	<i>Aegle marmelos</i> Corr.	Bel	Rutaceae	Leaf	Two spoon full shed dried leaf powder taken orally for 2 months for asthma and other respiratory complaints.	Bronchitis, asthmatic complaints [30,32,33]; cough, tuberculosis [16]
4	<i>Andrographis paniculata</i> Nees.	Bhuilimb, Kalmegh	Acanthaceae	Leaf	Take two glass water, add two spoon full shed dried leaf powder and allow for boiling until it will remain only half glass. This decoction take orally twice in a day for 2 months to treat TB.	Cough with thick sputum [35]
5	<i>Datura metel</i> L.	Kala Dhotra	Solanaceae	Leaf	One spoon full shed dried powder of leaf taken orally for the period of 3 months for the treatment of asthma.	No literature found on <i>M. tuberculosis</i>

Table 2.

Anti-Mycobacterium activity of selected medicinal plants in different extract.

Name of the Plant	Zone of Inhibition (mm)		MIC (mg/ml)
	20 mg/ml	40 mg/ml	
Water extract			
<i>Acacia catechu</i>	NR	NR	NR
<i>Ailanthus excelsa</i>	7.67 ±0.58	11.60 ±0.53	20 ±0.81
<i>Aegle marmelos</i>	8.17 ±0.67	12.10 ±0.46	20 ±0.26
<i>Andrographis paniculata</i>	NR	NR	NR
<i>Datura metel</i>	20.50 ±0.50	24.93 ±0.90	10 ±1.13
Ethanol Extract			
<i>Acacia catechu</i>	11.60 ±0.53	15 ±0.70	10 ±1.94
<i>Ailanthus excelsa</i>	7.57 ±0.59	15 ±0.85	10 ±1.14
<i>Aegle marmelos</i>	7.80 ±0.98	9.90 ±0.85	40 ±0.77
<i>Andrographis paniculata</i>	21.63 ±0.55	30.17 ±1.46	2.5 ±1.45
<i>Datura metel</i>	26.93 ±0.90	31.87 ±0.81	05 ±1.24
Chloroform Extract			
<i>Acacia catechu</i>	11.07 ±0.70	16.10 ±0.95	10 ±1.68
<i>Ailanthus excelsa</i>	NR	NR	NR
<i>Aegle marmelos</i>	9.00 ±1.00	13.40 ±0.78	20 ±1.35
<i>Andrographis paniculata</i>	19.97 ±1.05	25 ±1.00	05 ±1.56
<i>Datura metel</i>	17.26 ±0.56	23.20 ±0.62	20 ±0.95
Hexane Extract			
<i>Acacia catechu</i>	30.63 ±0.55	35.67 ±0.59	2.5 ±0.55
<i>Ailanthus excelsa</i>	8.93 ±0.40	15.17 ±0.96	10 ±0.79
<i>Aegle marmelos</i>	7.27 ±1.42	12.20 ±0.62	40 ±1.60
<i>Andrographis paniculata</i>	NR	NR	NR
<i>Datura metel</i>	9.03 ±0.35	10.30 ±0.61	40 ±0.86
Rifampicin (10 ¹⁰ g/ml)		32.50 ±0.56	0.28 ±1.34

Results presented here are the mean values from three independent experiments ± S.D., NR = No reaction under experimental condition

medicinal plants, most of the plant extracts exhibited potential antimycobacterium activity. No antimycobacterial activity was exhibited by water soluble fractions of *A. catechu* and *A. paniculata*; chloroform fraction of *A. excelsa* and hexane fraction of *A. paniculata*. The ethanol extracts of all the selected five medicinal plants possess potent antimycobacterium activity. The maximum zone of inhibition was observed with hexane extract of *A. catechu*

(35.67 ± 0.59 mm) followed by ethanol extract of *D. metel* (31.87 ± 0.81 mm) and *A. paniculata* (30.17 ± 1.46 mm) as compared with the standard rifampicin (32.50 ± 0.56 mm). Most potent antimycobacterium activity shown by ethanol extracts of *A. paniculata* and *A. catechu* with MIC value 2.5 ± 1.45 mg/ml followed by chloroform extract of *A. paniculata* and ethanol extract of *D. metel* (05 ± 1.24 mg/ml). Remaining plant extract showed moderate but interesting

Table 3.
Effect of selected plant extracts on the viability of Chang Liver cells (Human).

Sr.No.	Name of the Plant	Inhibition of Cell Viability (%) at 1mg/ml			
		Hexane	Chloroform	Ethanol	Water
1.	<i>Acacia catechu</i>	-17.32 ± 1.54	-42.09 ± 2.90	-29.43 ± 2.32	NR
2.	<i>Ailanthus excelsa</i>	-10.31 ± 1.90	NR	-39.98 ± 1.43	-28.90 ± 0.81
3.	<i>Aegle marmelos</i>	-18.90 ± 0.67	-26.98 ± 1.76	-91.90 ± 3.71	23.09 ± 1.32
4.	<i>Andrographis paniculata</i>	NR	-14.65 ± 3.21	-92.48 ± 0.65	NR
5.	<i>Datura metel</i>	-21.89 ± 0.54	-20.98 ± 2.89	-43.03 ± 1.09	-32.75 ± 2.54
6.	H ₂ O ₂ (1 mM)		3.13 ± 0.49		

Results presented here are the mean values from three independent experiments ± S.D., NR = No reaction under experimental condition

Table 4.
Antioxidant potential of selected traditional medicinal plants.

Sr. No.	Name of the Plant	Antioxidant activity (%)							
		Water		Ethanol		Chloroform		Hexane	
		DPPH	OH	DPPH	OH	DPPH	OH	DPPH	OH
1	<i>A. catechu</i>	NR	NR	NR	1.95 ± 0.05	74.64 ± 0.06	NR	NR	97.33 ± 0.83
2	<i>A. excelsa</i>	09.77 ± 0.04	64.80 ± 0.05	20.19 ± 0.59	NR	NR	NR	21.84 ± 0.07	60.46 ± 0.45
3	<i>A. marmelos</i>	39.65 ± 0.11	91.33 ± 0.60	52.64 ± 0.03	NR	46.50 ± 0.10	NR	35.85 ± 0.96	NR
4	<i>A. paniculata</i>	18.50 ± 0.08	NR	NR	88.37 ± 0.25	03.18 ± 0.04	NR	NR	NR
5	<i>D. metel</i>	68.90 ± 0.06	66.19 ± 0.34	47.48 ± 0.05	92.59 ± 1.66	45.65 ± 0.13	NR	01.07 ± 0.15	74.90 ± 0.70
6	<i>Ascorbic acid</i>	78.59 ± 0.04	2.78 ± 0.13						

Results presented here are the mean values from three independent experiments ± S.D., NR = No reaction under experimental condition.

anti TB activity with MIC range 10 to 40 mg/ml.

It is interesting to note that only ethanol fraction of *A. paniculata* exhibited promising activity and other solvent fractions showed no inhibitory activity against *M. tuberculosis*. Results summarized in Table 2 also reveals interesting fact that except hexane extract of *A. catechu* all the selected medicinal plants exhibit their promising anti TB activity by water and ethanol fractions. Therefore, it can be firmly concluded that the present study fully agreed with the rationale of use and mode of drug delivery employed by traditional healers because most of the healers use either paste or decoction of medicinal plant parts to treat tuberculosis and asthma.

3.3. Cytotoxicity Evaluation of Selected Medicinal Plants

Plant extracts in different solvents of the selected plant samples were evaluated for their cytotoxic effects on Chang Liver cells using MTT assay. The results of the cytotoxicity of crude extracts of these plants are summarized in (Table 3). At 1mg/ml concentration, most of the plant samples were found to be nontoxic except the negligible cytotoxicity demonstrated by water extract of *Aegle marmelos* (23.09 ± 1.32 %). H₂O₂ (4.92 ± 0.021 %) shows a most cytotoxic activity towards the cell lines which was used as reference standard.

3.4. Antioxidant activity of selected medicinal plants

3.4.1. DPPH radical scavenging activity

The DPPH radical scavenging assay has been used for preliminary screening of the plant extract for their antioxidant activity. The proton radical scavenging action

is known as an important mechanism of antioxidants. The results of DPPH reduction are summarized in (Table 4). It is clear from the results that all the four solvent extracts of *Aegle marmelos* and *Datura metel* plant samples (1mg/ml) were found to interact with the stable free radical DPPH, which indicates their potent radical scavenging ability. It is interesting to note that only chloroform extract of the *Acacia catechu* display the potent radical quenching ability as compared with other solvent extracts. The overall range of DPPH radical scavenging activity was found to be 01.07 ± 0.15 to 74.64 ± 0.06 %. The water extracts of all the samples except, *A. catechu* showed significant DPPH radical scavenging activity in a range of 9.77 ± 0.04 to 68.90 ± 0.06 % as compared to ascorbic acid (78.59 ± 0.04 %). It was observed that, the hexane extracts of selected plant samples were found to interact weakly with DPPH radicals.

3.4.2. OH radical scavenging assay

The OH radicals are most hyper reactive amongst the relative oxygen species and that affect every type of molecule found in living system. Physiologically important biomolecules such as sugar, amino acids, phospholipids, DNA bases, organic acids may undergo reaction with OH radicals and may change normal physiological function of cells [28].

The summary of OH radical scavenging activities has been shown in (Table 4). It was observed that water, ethanol and hexane extracts (1mg/ml) of almost all selected medicinal plants showed effective OH radical stabilizing potentials in a range of 1.95 ± 0.05 to 97.33 ± 0.83 % as compare to standard ascorbic acid (02.78 ± 0.13 %) while chloroform extracts

did not interacted with OH radicals. The hexane extract of *A. catechu* (97.33 ± 0.83 %) was found to be hyper reactive towards OH radicals whereas the ethanol extract (01.95 ± 0.05 %) showed minimum effect on OH radicals. It was interesting to note that only water fraction of *A. marmelos* and ethanol fraction of *A. paniculata* are able to scavenge OH radical while remaining solvent fractions are unable to show OH radical scavenging activity.

4. Discussion

The present study clearly shows the strong positive correlation between the selection of medicinal plants and the results obtained. Total five medicinal plants were selected for the present study with an aim to validate the conventional folk phytotherapy on scientific background. The selection of medicinal plants was based on their use by traditional healers in this region for the treatment of tuberculosis, asthma and chronic respiratory disorders.

A considerable number of plant species have been described in Ayurveda for the treatment of TB, leprosy and TB related disorders. Recently in a review published by Gautam et al.,^[29] on Indian medicinal plants active against TB reported total 255 plant species mentioned in Ayurveda. Out of five selected traditional medicinal plants in the present study, only *Aegle marmelos* and one allied species of *Ailanthus* were found to be mentioned in Ayurveda for the treatment against tuberculosis. Newton et al.,^[30] reported methanol extract of fruit of *A. marmelos* showed MIC of >500 μ g/ml for *M. avium* and *M. smegmatis*. In another study Taylor et al.,^[31] showed that methanol extract root of was found to be active against *M. phlei* at a concentration of 300 mg/ml of dried plant material in disc diffusion assay with chloramphenicol as positive control. Dastur^[32] and Wren^[33] reported decoction of the leaves is a febrifuge and expectorant and is particularly used for asthmatic complaints, for the treatment of acute bronchitis, fever and dysentery. The present study first time reporting the activity of *A. marmelos* leaf extract in polar and non-polar solvent on *M. tuberculosis*.

It is interesting to note that only hexane extract of *A. catechu* root and ethanol extract of *A. paniculata* leaf showed promising activity against *M. tuberculosis*. However, the anti TB activity of *A. excelsa* and *A. marmelos* was comparatively weak because they have been widely used traditionally to treat asthma but there is no report of its use in treating TB infection. Interestingly enough the ethanol extract of *D. metel* showed potent inhibitory activity against *M. tuberculosis* besides its traditional use for treating chronic respiratory disorders and asthma. Historically the natural products have served as a starting material in the search for new antimycobacterial agents. Gautam et al.,^[29] shows that extracts of plant species from wide range of families and genera have exhibited significant in vitro antimycobacterial

activities and this efficacy is interestingly compatible with the ethnomedicinal knowledge on plants. The results of the present study forms a rational basis for the selection of candidate plant species for further phytochemical and pharmacological investigations for the lead identification.

Conclusively it is to stated that the present study first time reporting the anti TB activity of root extracts of *Acacia catechu* and *Ailanthus excelsa* while leaf extract of *Andrographis paniculata*, *Aegle marmelos* and *Datura metel*. One drawback of the current anti TB literature of medicinal plant is that almost no indication of selectivity (anti-TB vs. mammalian cytotoxic activity) is provided. The present investigation carefully studied the cytotoxicity of selected plant extracts with their antioxidant potential. *Andrographis paniculata* and *Acacia catechu* both were equally potent against *Mycobacterium tuberculosis* followed by *Datura metel*. These traditional medicinal plants appeared to be promising candidates for further investigation into our study of new lead compounds from natural products as potential antimycobacterial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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