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ANTITUBERCULAR POTENTIAL OF *DENDROPHTHOE FALCATE* (L.) AND *TRIDAX PROCUMBENS* (L.) PLANTS EXTRACTS AGAINST H37Rv STAIN OF *MYCOBACTERIA TUBERCULOSIS*

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Keywords:

Dendrophthoe falcate, *Tridax procumbens*, HPTLC, GC-MS, MABA, Antituberculosis

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ABSTRACT: The increasing incidence of MDR and XDR tuberculosis worldwide highlight the urgent need to search for newer anti-tuberculosis molecules. This research article presents a phytochemical study and antitubercular potential of methanol: water (MW), ethanol: water (EW) and dichloromethane: methanol (DM) of extracts of *Dendrophthoe falcata* (L.) and *Tridax procumbens* (L.) tested against of H37Rv *Mycobacterium tuberculosis* using microplate alamar blue assay (MABA) method. Preliminary phytochemicals studies and HPTLC fingerprint analysis revealed the presence of phytochemical like alkaloids, flavonoids, saponins, tannins, a phenolic group, glycosides, terpenoids with different R_f values. Fractions of active extracts analyzed by GC-MS shows a finding of probable phytoconstituents. Findings are useful to establishing standards for identification, purity, quality of the plant. The ethanol: water and methanol: water extracts of *Tridax procumbens* and *D. falcata* exhibited significant anti-tuberculosis activity with the MIC values of 0.8 µg/ml, 6.25 µg/ml compared to standard drug Pyrazinamide, Ciprofloxacin and Streptomycin with the MIC values of 3.125 µg/ml 6.25 µg/ml using MABA respectively against *Mycobacterium tuberculosis* (H37Rv strain) ATCC no.-27294. The presence of flavonoids, tannins, the phenolic group may contribute to the observed anti-tubercular activity. The study demonstrated that extract from *T. procumbens* and *D. falcata* could be evaluated further that might provide compounds for developing a new drug to control *M. tuberculosis*.

INTRODUCTION: Infectious diseases represent a critical issue for health and are the major cause of morbidity and mortality worldwide. The impact is even greater in developing countries due to unavailability of medicine.

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Tuberculosis remains a global public health problem in developing countries. Due to the global emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* [1].

There is an urgent need to develop new drugs and strategies to fight TB. The World Health Organisation (WHO) estimated that approximately 80% of the world population relies mainly on traditional medicines, mainly plant drugs in their health care. The last few decades have witnessed a substantial increase in the investigation of

medicinal plants for their biological efficacy in the treatment of various disorders. Over the years, some improved and high throughput techniques towards screening of anti-mycobacterial agents have been developed [2, 3]. Several methods exist for testing the antitubercular potential of plant extracts such as fluorescence-based testing on the Bactec MGIT960 system, use of redox indicator dyes such as alamar blue or resazurin and MTT, using colony forming units (CFU) on solid agar plates. Techniques such as the agar diffusion and broth dilution method have been used [4, 5]. The microplate alamar blue assay (MABA) is a colorimetric oxidation-reduction based assay. It is a non-radiometric, rapid, high-throughput and comparatively low-cost assay producing results with a high degree of confidence [6, 7, 8].

Dendrophthoe falcata (L.f) Ettingsh is highly specialized perennial flowering plant adapted to parasitic life on aerial parts of their hosts. The whole parasitic plant is used in indigenous system of medicine as cooling, bitter tonic, astringent, aphrodisiac, narcotic, diuretic, pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, renal and vesicle calculi and violated conditions of kapha and pitta [9]. *D. falcata* belonging to the family Loranthaceae is an angiosperm hemiparasitic plant was most frequently observed on many host plants comprises of 20 species, and about 7 species are found in India. The medicinal properties of *D. falcata* are generally influenced by the host plant for example when grown on *Calotropus gigantia* (Linn.) (Asclepiadaceae) the parasitic plant is considered useful for improving cognitive function, on *Tamarindus indicus* L. (Fabaceae) it is used to treat impotence and on *S. robusta* (Dipterocarpaceae) it is used to treat paralysis [10]. *D. falcata* bark juice/decoction used in menstrual troubles and asthma while its paste is applied on boils, setting dislocated bones and extracting pus.

The decoction of the whole plant is used to treat joint pains [11]. Quercetin, kaempferol, quercetin are the different flavonoids isolated and reported from the whole plant of *D. falcata* [12, 13]. The reported biological activities of *D. falcata* are antioxidant, anti-inflammatory [14], antitumor [15,16], anthelmintic potential [17], anti-nociceptive and general toxic studies [18], anticonvulsant and muscle relaxant [19], immunomodulatory [20], wound healing and anti-microbial [21], antifertility [22], anticancer studies [23] *D. falcata* (Loranthaceae) a parasite on *Mangifera indica* (Anacardiaceae) potential antidiabetic [24], Anti-diarrhoeal and insecticidal activities [25].

Tridax procumbens Linn. family Compositae commonly known as ‘coat buttons’ because of an appearance of flowers has been extensively used Ayurveda in liver disorders [26]. *Tridax* possesses significant anti-inflammatory, hepatoprotective, wound healing, antidiabetic activity and antimicrobial activity against both gram-positive and gram-negative bacteria [27, 28, 29, 30]. Flavonoids isolated and reported from whole plant [31, 32]. The leaf juice possesses antiseptic, insecticidal and parasiticidal properties and is also used to check hemorrhage from cuts, bruises and wound. Interestingly it also has a hypotensive effect and potent immunomodulating property [33, 34] — i*n-vitro* radical scavaging and *in-vivo* anti-inflammatory potential [35]. The primary reasons to select plants is their known antimicrobial properties, and other pharmacological uses as an antioxidant, immunomodulatory also used for respiratory disorders in reported ethnobotanical surveys was also documented are base for research. The study was undertaken to evaluate a phytochemical analysis and preliminary report on the antitubercular activity of *D. falcata and Tridax procumbens* plant extracts against M. tuberculosis ($H37R_V$ strain) using bio-assay guided fractionation of the extracts of the leaves and fractions thereof.

MATERIALS AND METHODS:

Chemicals: Chemicals used in this study were of analytical grade, and highest purity procured from standard commercial sources Research Lab. and S. D. Fine Lab Mumbai.

Plant Collection and Identification: The *Tridax procumbens* Linn. (Asteraceae) plant and *Dendrophthoe falcata* (L.f) Etting. (Loranthaceae) samples were collected at the flowering stage from the local region, during September - November was collected from Kapurhol Kasurdi on Satara-Pune NH-4 near Pune Maharashtra (India). Both specimens plant were identified and authenticated by Botanical Survey of India (BSI) Pune, Maharashtra India having voucher specimen number VIBTRP2 & VIBDEF3 dated-15/02/2013.

Preparation of Extracts: Extracts were prepared in a sequential manner using ethanol: water, methanol: water, dichloromethane: methanol (order of increasing polarity) as solvents from of shade-dried and coarsely powdered plant material using the maceration and Soxhlet apparatus. Coarsely powdered was defatted with petroleum ether and then exhaustively extracted with a different solvent.

The methanol: water and ethanol: water extract was prepared by maceration by soaking 10g of powdered plant materials in 100 ml of solvent at room temperature for 48 h. The extract was filtered after 48 h, through a sterilized Whatman no. 1 filter paper. The extract concentrated using a rotary vacuum evaporator with the water bath set at 40 °C. Overnight UV-irradiation sterilized the dried extract. The sterile extract was transferred into a sterile lyophilization flask and frozen in a deep freezer. The extract was stored at -20 ºC till bio-evaluation.

Phytochemical Analysis: The phytochemical investigation of the different extracts of *Tridax procumbens* and *Dendrophthoe falcata* was carried out with standard protocol [36, 37]. Extracts were evaluated for physical constants [38].

HPTLC Study: HPTLC is an important analytical tool in the separation, identification, and estimation of various classes of natural phytoconstituents. HPTLC studies were carried out by the method of Harborne [39] and Wagner *et al*., [40, 41]. The extracts were dissolved in the respective solvents (5mg/ml). The 10µl of sample extract was applied with the help of linomat syringe using the linomat applicator 5. Solvent system *n*-hexane: toluene: ethyl acetate (2:4:1). HPTLC silica gel F254 (Merck). The plates were developed in a CAMAG chamber. CAMAG HPTLC densitometer (Scanner) used to measure absorbance mode at 254, 366 nm and 560 nm. Data integration through the software WINCATS Planar Chromatography Manager. The fingerprint so developed and R_f value were noted. Spots were visible without derivatization at 254 nm, 366 nm, and 560 nm wavelengths. A solvent system optimized for TLC study was chosen for HPTLC study.

Anti-tuberculosis Activity:

Microbial Strain for Anti-Mycobacterium tuberculosis Assay: Reference strains H37Rv (ATCC No-27294) of *Mycobacteria tuberculosis* (Vaccine strain) used.

Microplate Alamar Blue Assay: The *M. tuberculosis* (MTB) were cultured in 7H9 medium in the presence of the plant extracts in a 96 well plate was tested at concentration 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100 µg/ml. The anti-mycobacterial activity of compounds was assessed against *M. tuberculosis* using MABA. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. 200µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100µl of the Middlebrook 7H9 broth, and serial dilution of compounds was made directly on the plate. The final drug concentrations tested were 0.2 to 100µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this 25µl 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 color in the well was interpreted as no bacterial growth, and pink color was scored as growth. Interpretations were based on the percent reduction of the dye which is directly proportional to the bacterial growth [42, 43]. The extracts were considered active if the percent reduction value of alamar blue dye was less than that observed for the standard. Triplicate wells were maintained for each variable in every assay, and all the assays were performed thrice.

GC-MS Analysis: Fractions of active extracts analyzed by GC-MS Quadrupole Analyzer at Poona College of Pharmacy, Pune (Maharashtra) India. Shows finding some probable Phyto-constituents in the mass range 300-600 am.

RESULTS:

Physical Constants: The proximate analysis showed a satisfactory result concerning the foreign matter, moisture content, ash value, and extractive values [44]. The physical constants are shown in **Table 1.**

Photochemical Screening: The extracts were screened for phytochemical constituents for the presence of saponins, tannins, alkaloids, flavonoids, a phenolic group, glycosides and reducing sugars [37].

The presence of alkaloids, glycosides, flavonoids, tannins, phenolic, xanthones, quinones, sterols, triterpenoids, *etc* is mostly responsible for the anti-tubercular activity proven in Ayurveda [45]. The results are shown in **Table 2**.

HPTLC Fingerprint Studies: The study revealed that *Tridax procumbens* (Linn.) and *Dendrophthoe falcata* (Linn.) showed best results in *n*-hexane: toluene: ethyl acetate (2: 4: 1) solvent system for TPEW, DFMW, and DFDM extracts.

TABLE 1: EVALUATION OF PHYSICAL CONSTANTS OF POWDERED *TRIDAX PROCUMBENS* (L.) AND *DENDROPHTHOE FALCATA* (L.)

S. no.	Evaluation parameter	Value (%)	
		Tridax procumbens (L.)	*Dendrophthoe falcate* (L.)
1	Foreign matter	1	1
2	Moisture content	10.6	9.5
3	Total ash value	12.7	11.9
4	Water soluble ash value	3.5	3.2
5	Acid-insoluble ash value	8.0	9.1
6	Water soluble extractive value	4.89	3.87
7	Chloroform soluble extractive value	0.4	0.5
8	Methanol soluble extractive value	6.66	6.73
9	Ethanol soluble extractive value	4	5.2

TABLE 2: PRELIMINARY PHOTOCHEMICAL SCREENING OF EXTRACTS OF *T. PROCUMBENS* (L.) AND *D. FALCATA* (L.)

Phyto-constituents	*Tridax procumbens* extracts			*Dendrophthoe falcata* extracts		
	Methanol: Water	Ethanol: Water	Dichloromethane: Methanol	Methanol: Water	Ethanol: Water	Dichloromethane: Methanol
Alkaloids	-	+++	++	+	-	+
Flavonoids	++	+++	+++	++	+++	+++
Phenolic groups	+	+	-	-	-	-
Saponin Glycosides	++	+++	+	++	+	-
Tannins	+++	+++	+++	+++	+++	+++
Steroids	++	+++	++	++	++	++
Carbohydrates	+	+	+	+	+	+
Terpenoids	+	++	+	+	++	+
Cardiac Glycosides	-	-	-	+	+	+
Reducing sugar	+	-	++	+	-	+
Anthroquinone Glycosides	-	-	-	-	-	+

+ indicates presence - indicates absence.

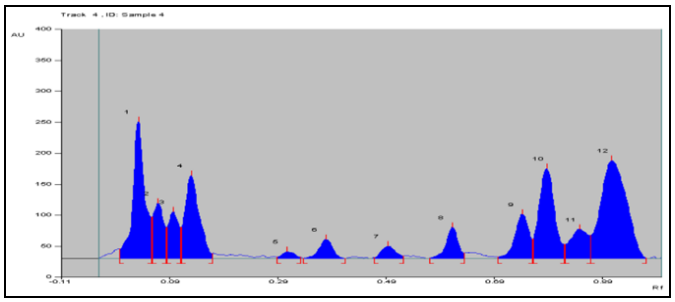

FIG. 1: CHROMATOGRAM OF ETHANOL: WATER EXTRACT OF *TRIDAX PROCUMBENS* MEASURED AT 366nm

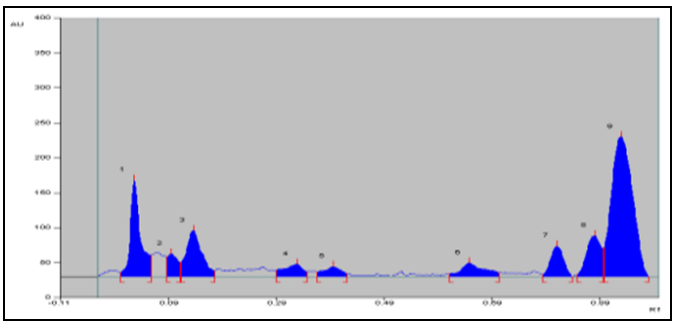

FIG. 2: CHROMATOGRAM OF METHANOL: WATER EXTRACT OF *D. FALCATA* (L.) MEASURED AT 366 nm

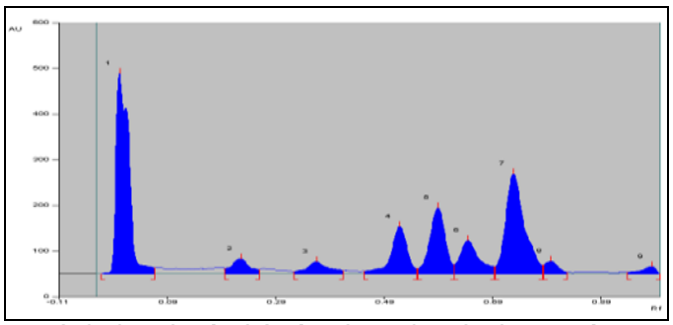

FIG. 3: CHROMATOGRAM OF DICHLOROMETHANE: METHANOL EXTRACT OF *D. FALCATA* (L) MEASURED AT 560 nm

After scanning and visualizing the plates in absorbance mode at both 366 nm, 560 nm. The HPTLC images shown that all sample constituents were separated without any tailing and diffuseness. As a shown in **Fig. 1**, **Fig. 2** and **Fig. 3**.

Anti-Tuberculosis Evaluation: The anti-microbial effects of the extracts evaluated by using microplate alamar blue assay (MABA) against *M. tuberculosis* (H37Rv strain) ATCC No-27294, were evaluated at the Department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum - 590010, India.

The method applied is similar to that reported by Maria and Lourenco [43]. Pyrazinamide (MIC- 3.125 µg/ml), Ciprofloxacin (MIC-3.125 µg/ml) and Streptomycin (MIC-6.125 µg/ml) were used as reference standard to evaluate the potency of the extracts. As shown in **Table 3** and **Fig. 4**, **Fig. 5** and **Fig. 6**.

TABLE 3: SHOWING COMPARATIVE ANTI-TUBERCULOSIS SCREENING RESULTS BY MIC METHOD

Code no.	Compounds / Extracts	MIC values µg/ml	Code no.	Compounds / Extracts	MIC values µg/ml
A1	DFDM	12.5	Std 1	Pyrazinamide	3.125
A2	DFEW	25	Std 2	Ciprofloxacin	3.125
A3	TPMW	25	Std 3	Streptomycin	6.25
B1	TPDM	25			
B2	TPEW	0.8			
B3	DFMW	6.25			

T.P -*Tridax procumbens* and D.F- *Dendrophthoe falcata*

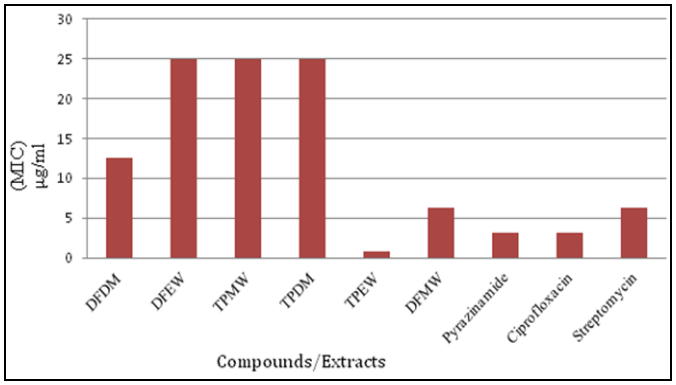

FIG. 4: MIC µg/ml OF TESTED EXTRACTS AGAINST H37Rv OF *MYCOBACTERIA TUBERCULOSIS*

*DFDM- *Dendrophthoe falcata* dichloromethane-methanol, DFEW - *Dendrophthoe falcata* ethanol-water, TPMW- *Tridax procumbens* methanol-water, TPDM- *Tridax procumbens* dichloromethane-methanol, TPEW- *Tridax procumbens* ethanol-water, DFMW- *Dendrophthoe falcata* methanol-water

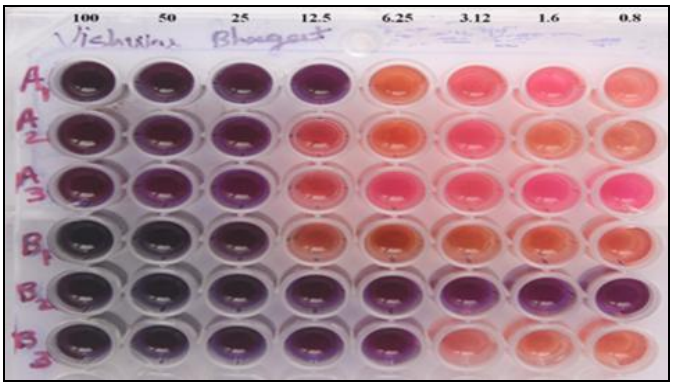

FIG. 5: MICROPLATE ALAMAR BLUE ASSAY 96 WELL PLATE FOR ACTIVITY OF PLANT EXTRACTS

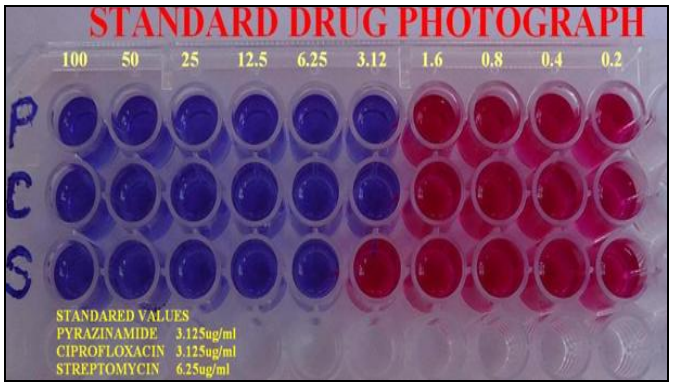

FIG. 6: MICROPLATE ALAMAR BLUE ASSAY 96 WELL PLATE FOR ACTIVITY OF STD

GC-MS Analysis: GC-MS analysis of *Tridax procumbens* (L.) active ethanol-water extract shows finding number of probable phytoconstituents in Mass range 300-600 amu **Fig. 7 - 12**.

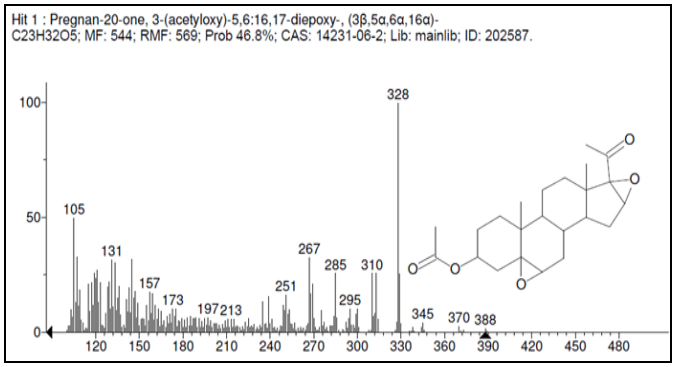

FIG. 7: GC-MS SPECTRA OF PHYTOCONSTITUENT-1

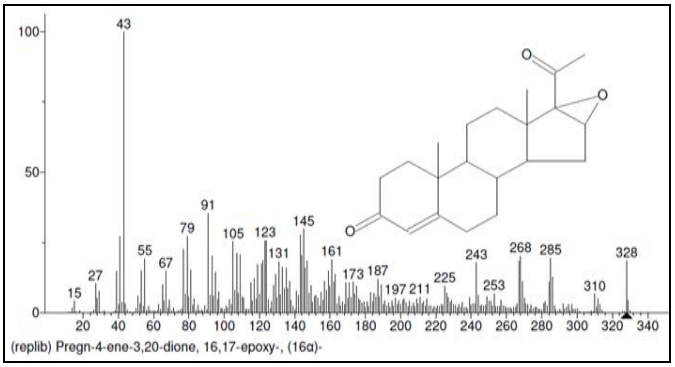

FIG. 8: GC-MS SPECTRA OF PHYTOCONSTITUENT-2

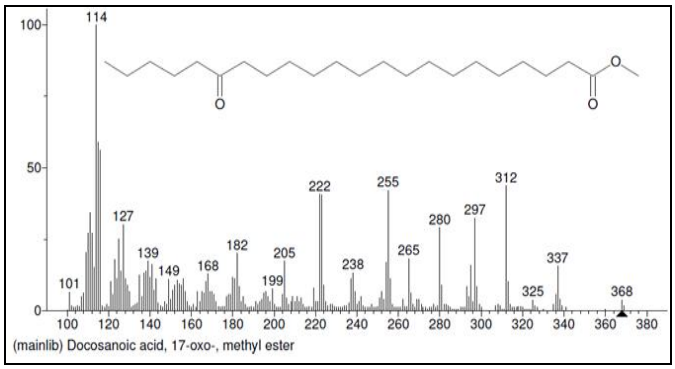

FIG. 9: GC-MS SPECTRA OF PHYTOCONSTITUENT-3

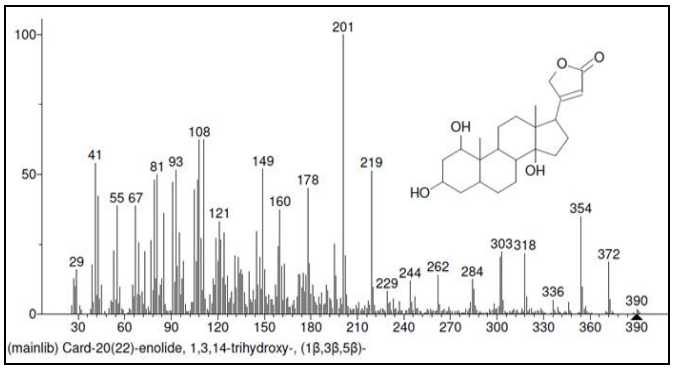

FIG. 10: GC-MS SPECTRA OF PHYTOCONSTITUENT-4

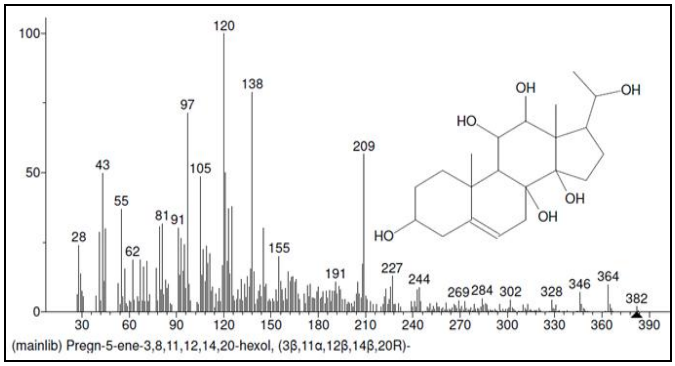

FIG. 11: GC-MS SPECTRA OF PHYTOCONSTITUENT-5

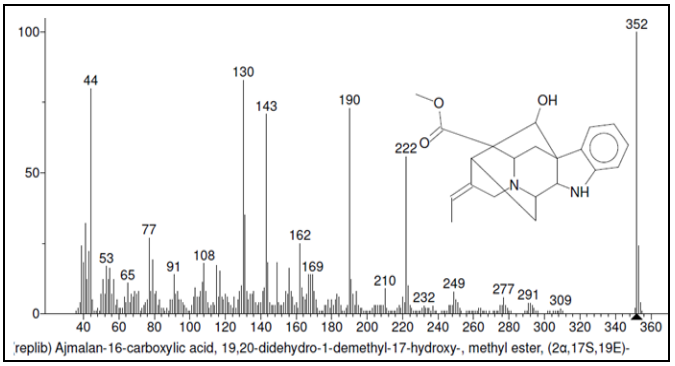

FIG. 12: GC-MS SPECTRA OF PHYTOCONSTITUENT-6

DISCUSSION: The Antimycobacterial activities of extracts were evaluated by using microplate alamar blue assay (MABA). In the present study, the TPEW extract exhibited significant anti-tuberculosis activity with the MIC values of 0.8µg/ml against H37Rv of *M. tuberculosis*. The DFMW extract exhibited significant anti-tuberculosis activity with the MIC values of 6.25µg/ml against H37Rv of *M. tuberculosis*.

DFDM extract shows moderate anti-tuberculosis activity with the MIC values of 12.50 µg/ml. The DFEW extract and TPMW, TPDM extract displayed a weak activity against H37RV of *M. tuberculosis* strain compare to standard drugs as Pyrazinamide, Ciprofloxacin, and Streptomycin with the MIC values of 3.125 µg/ml, 3.125 µg/ml and 6.25 µg/ml using microplate alamar blue assay (MABA). As many different methods are available to evaluate antituberculosis activity, no specific cut-off value has been established for reference to analyze the anti-tuberculosis activity [46]. Values show that out of the six, two extracts was found to be more potent than std - drugs as Pyrazinamide, Ciprofloxacin, and Streptomycin.

The preliminary phytochemical screening of various extracts shows the presence of alkaloids, flavonoids, saponins, tannins, a phenolic group, glycosides, terpenoids, reducing sugars. On investigation of extracts having significant anti-tuberculosis activity is revealed the presence of alkaloids, flavonoids, saponins, tannins, a phenolic group, glycosides, terpenoids. Flavonoids may act by depolarization of membrane and inhibition of DNA, RNA and proteins synthesis.

It may be reduced the bacterial cell density rapidly and caused lysis [47]. The efficacy of extracts could be due to the interplay of active constituents present, leading to better activity. It has been demonstrated that different constituents of crude extracts act through different mechanisms. Tannin is used as antimicrobial growth-promoting factor (AGP) in the sub-therapeutic dose for long periods is particularly favorable for the selection of antimicrobial resistant microorganism. Chalcones as a flavonoids, 1-(2-hydroxyphenyl)-3-(3-chlorophenyl)-2-propane-1-one and 1-(2 hydroxyphenyl)-3-(-iodophenyl)-2-propane-1-one, showed inhibition (90%) of *Mycobacterium tuberculosis* H37Rv. Some chalcone like a compound with heterocyclic ring showed even higher inhibition (95%) as anti-tubercular agent [48].

The HPTLC fingerprint analysis chromatograms developed are particular with the finalized solvent system, *n*-hexane: toluene: ethyl acetate in the ratio of (2: 4: 1). HPTLC profiling of the extract confirms about the presence of various phytochemicals. R_f value % area can serve as an improved tool for the extract/subfraction standardization.

The present study gives enough information regarding various phytoconstituents present in the methanol: water, ethanol: water, dichloromethane: methanol extract of *Tridax procumbens* (Linn.) and *Dendrophthoe falcata* (Linn.) also helps in generating the basis for the quality control, correct identification, and standardization.

The HPTLC finger print of ethanol: water extract of *Tridax procumbens* (L.) clearly showed signified existence of 12 phytoconstituents whose R_f values ranged from 0.06 to 0.87 eluting out at 366 nm With R_f values 0.87, 0.82, 0.76,0.70 were found to be leading as the percentage area was more, *i.e*. 25.74%, 5.32%, 14.18%, and 6.34% respectively. Fractions of active extracts analyzed by GC-MS shows finding a number of probable phytoconstituents. The study revealed that *T. procumbens* and *D. falcata* was rich in secondary metabolites particularly tannins and flavonoids which are may be responsible for the antitubercular activity. It identifies *T. procumbens* and of *Dendrophthoe falcata* have a promising antitubercular activity.

CONCLUSION: The study indicated that selected *Tridax procumbens* (L.) and of *Dendrophthoe falcata* (L.) leaf extracts exhibited potential anti-tubercular activity. The present investigation may be the focus of further phytochemical research of *Tridax procumbens* and *Dendrophthoe falcata* to identify and isolate the constituents responsible for antitubercular activity are being undertaken, along with the exploration of mechanisms of action and contribute greatly to the development new phytomolecule against tuberculosis.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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