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PHYTOPHARMACOGNOSTICAL STUDY OF SOME SELECTED ANTITUBERCULOSIS PLANTS AND STUDY OF ITS CYTOTOXICITY SCREENING USING VERO CELL LINE

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Keywords:

Medicinal plants, Pharmacognostical study, Tuberculosis, Vero cell line, MTT assay, Cytotoxicity

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ABSTRACT: Tuberculosis (TB) is one of the leading infectious diseases and health burdens in the world. One-third of the world's population, including 40% from India, is estimated to be infected with tuberculosis. Current studies have indicated the urgent need for the development of new, safe, and efficacious drugs to help reduce the global burden of tuberculosis. Novel antimycobacterial scaffolds from natural products have recently been reported. Natural products of plant biodiversity have received considerable attention as potential anti-TB agents since they are a proven template for the development of new molecules against tuberculosis. We have selected eleven medicinal plants on the basis of four criteria to choose a plant for antituberculosis activity. The plants were having antituberculosis effect, hepatoprotective effect, immune-modulatory action, and having the ability to enhance bioavailability. All selected eleven plants were reviewed in ancient literature and research article-based review. So here, our objective is to study pharmacognostic, physochemical and cytotoxicity screening of eleven selected medicinal plants. There are three different extracts of eleven selected medicinal plants that were prepared on the basis of phytochemical screening performed for the confirmation of the presence of active constituents. All the parameters of pharmacognostical study and physicochemical parameters compared with standards and results were within the range of limits. Cytotoxicity screening of all extracts of elevn selected plants were performed, and none of the extracts from eleven selected medicinal plants shows any significant cytotoxicity. So further, these all extracts of pants can be taken for antituberculosis screening.

INTRODUCTION: Tuberculosis (TB) is one of the leading infectious diseases and health burdens in the world [1]. One-third of the world's population, including 40% from India, is estimated to be infected with tuberculosis [2]. More than nine million new cases diagnosed and approximately two million people killed annually [3]. Current tuberculosis treatment is a long course of a combination of 3-4 antibiotic drugs, which have one or the other toxic side effects and led to poor patient compliance. Antitubercular drugs such as isoniazid (INH), rifampicin (RIF), pyrazinamide, ethambutol, streptomycin etc., have been a mainstay in the treatment of tuberculosis [4]. Plants are the important source of a diverse range of bioactive principles [5].

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Historically, natural products have proved to be the most prolific and diverse source of antibiotics, including some of those used for the treatment of TB. Current studies have indicated the urgent need for the development of new, safe, and efficacious drugs to help reduce the global burden of tuberculosis. Novel antimycobacterial scaffolds from natural products have recently been reported. Natural products of plant biodiversity have received considerable attention as potential anti-TB agents since they are a proven template for the development of new molecules against tuberculosis.

Many antitubercular compounds that may prove to be useful leads for TB drug discovery have been derived from medicinal plants [6]. Natural products, especially those from the plant biodiversity, have been less intensively investigated in the past even though they are known to contain structurally diverse molecules, many of which are unknown. This has prompted us to investigate medicinal plants for their anti-TB activity.

In this study, we have selected eleven different medicinal plants. Here is the part of a plant used of selected plants is shown in **Table 1**.

TABLE 1: ELEVEN SELECTED MEDICINAL PLANTS

Plant name	Botanical source	Family	Part used
Amla	*Emblica officinalis*	*Euphorbiaceae*	Fruits
Baheda	*Terminalia bellerica*	*Combretaceae*	Fruits
Harde	*Terminalia chebula*	*Combretaceae*	Fruits
Ashwagandha	*Withania somnifera*	*Solanaceae*	Roots
Nagarmoth	*Cyperus rotundus*	*Cyperaceae*	Rhizomes
Rasna	*Alpinia galanga*	*Zingiberaceae*	Rhizomes
Tulsi	*Ocimum sanctum*	*Liliaceae*	Leaves
Vasaka	*Adhatoda vasica*	*Acanthaceae*	Leaves
Long pepper	*Piper longum*	*Piperaceae*	Fruits
Kharkhodi	*Leptedinia reticulata*	*Asclepiadaceae*	Roots
Vevadi	*Cocculus hirsutus*	*Menispermeaceae*	Whole herb

In the present study, eleven different medicinal plants were selected on the basis of ethano-medicinal based review and literature-based review. As tuberculosis is a pathogenic disease mainly connected with hepatotoxicity and immunity of patients. As considered this, there are four criteria selected to choose a plant for antituberculosis activity.

The plants are having antituberculosis effect, hepatoprotective effect, immunomodulatory action, and having the ability to enhance bioavailability. As in the ethnomedicinal-based review, all the selected plants were reviewed from the ancient literature for all mentioned activities. The following **Table 2** shows the selected plant and its ancient literature review.

Ancient Literature Review:

TABLE 2: SELECTION OF PLANTS ON BASIS OF ANCIENT LITERATURE REVIEW

S. no.	Name of plant	Part used	Activity mentioned	Reference
1	Amla	Fruits	Asthma, bronchitis	The Wealth of India, 2002[7]
2	Baheda	Fruits	Asthma, cough	ICMR, 2003[8]
3	Harde	Fruits	Ashma, bronchitis	The Wealth of India, 1982[9]
4	Ashwagandha	Roots	tuberculosis	Nadkarni, KM,1982[10]
5	Nagarmoth	Rhizomes	tuberculosis	Kirtikar KR *et al*., 2007[11]
6	Rasna	Rhizomes	tuberculosis	ICMR, 2003[12]
7	Tulsi	Leaves	tuberculosis	Medicinal plants of Gujarat[13]
8	Vasaka	Leaves	tuberculosis	ICMR, 2005[14]
9	Long pepper	Fruits	Bioavailability enhancer	Kirtikar KR *et al*., 1933[15]
10	Kharkhodi	Roots	tuberculosis	Kirtikar KR *et al*., 1933[16]
11	Vevadi	Whole herb	tuberculosis	Kirtikar KR *et al*., 1933[17]

Research Article Based Review:

TABLE 3: SELECTION OF PLANTS ON RESEARCH ARTICLE BASED REVIEW

Plant name	Antituberculosis Activity	Hepatoprotective activity	Immunomodulatory activity	Bioavailability enhancer
Emblica officinalis	Harpreet Singh Grover *et al.*, 2015[18]	Arish Mohammad Khan Sherwani *et al.*, 2012[19] Deori C *et al.*, 2017[20]	Manish K Singh *et al.*, 2013[21]	---
Terminalia bellerica	Manish Pal Singh, *et al.*, 2018[22]	Deb A *et al.*, 2016[23]	Belapurkar P *et al.*, 2014[24]	---
Terminalia chebula	Min-Kyung Choi *et al.*, 2015[25]	S A Tasduq *et al.*, 2006[26]	Aher V *et al.*, 2011[27] Khan KH *et al.*, 2009[28]	---
Withania somnifera	Periyaka ruppan *et al.*, 2012[29] Sarepaka a *et al.*, [30]	Ranjeet Kumara *et al.*, 2017[31]	Davis L *et al.*, 2000[32] Agarwal R *et al.*, 1999[33]	---
Cyperus rotundus	Vivek Kumar Guptaa *et al.*, 2018[34]	Kumar SS *et al.*, 2005[35]	Aghwan SS *et al.*, 2007[36]	---
Alpinia galanga	Soundhari C *et al.*, 2013[37] Chopra LC *et al.*, 1997[38]	Eram S, *et al.*, 2019[39]	---	---
Ocimum sanctum	Bhatter, P.D. *et al.*, 2016[40] Reddi G *et al.*, 2013[41] Vyas, R.B *et al.*, 2018[42]	Chattopadhyay RR *et al.*, 1992[43]		---
Adhatoda vasica	Patel VK, *et al.*, 1984[44] Narimaian M *et al.*, 1985[45]	Ahmad R *et al.*, 2013[46]	Jinyvar Ghese *et al.*, 2005[47]	
Piper longum	---	Randhawa G.K., *et al.*, 2011[48]	-----	Atal C.K *et al.*, 1985[49]
Leptedinia reticulata	Sonara, G.B *et al.*, 2013[50]	---	---	---
Cocculus hirsutus	Gupta, V.K *et al.*, 2018[51] Tharun Kumar *et al.*, 2012[52]			

MATERIALS AND METHODS:

Collection of Raw Material of Eleven Selected Medicinal Plants: Dried plant materials of nine selected plants [fruits of *Emblica officinalis,* fruits of *Terminalia bellerica,* fruits of *Terminalia chebulla,* roots of *Withania somnifera,* rhizomes of *Cyperus rotundus,* rhizomes of *Alpinia galanga,* leaves of *Oscimum sanctum,* leaves of *Adhatoda vasica* and fruits of *Piper longum*] out of eleven selected plants were procured from Ayurvedic store of Gandhinagar and Fresh plant material of two selected plants [roots of *Leptedinia reticulata* and whole herb of *Cocculus hirsutus*] out of eleven selected plants were collected from Dhandhiya village of Rajkot district, Gujarat, India in the month of January 2015.

Authentication of Raw Material of Eleven Selected Medicinal Plants: The procured material of selected plants was authenticated by a taxonomist and further authenticated by comparing the microscopy with reported literature. Herbarium specimens of selected plant materials (PH/015/001-PH/015/011) were deposited at Pharmacognosy department, K.B.I.P.E.R., Gandhinagar.

Preparation of Samples: Raw material of selected plants were subjected to washing with distilled water and then allowed for drying under shade and powdered to 60# separately and stored in well close container.

Pharmacognostical Study of Raw Material of Eleven Selected Medicinal Plants:

Macroscopical Study of Raw Material of Eleven Selected Medicinal Plants: Raw material of selected nine plants out of eleven [fruits of *Emblica officinalis,* fruits of *Terminalia bellerica, f*ruits of *Terminalia chebulla,* roots of *Withania somnifera,* rhizomes of *Cyperus rotundus,* rhizomes of *Alpinia galanga,* Leaves of *Oscimum sanctum,* Leaves of *Adhatoda vasica and* Fruits of *Piper longum*] were studied and identified by comparing their morphological characters as mentioned in the literature, and raw material of two plants [roots of *Leptedinia reticulata* and the whole herb of *Cocculus hirsutus*] out of eleven selected plants were studied and identified by comparing their morphological characters as mentioned in the literature.

Powder Microscopical Study of Raw Material of Eleven Selected Medicinal Plants: For powder microscopical study, a very little amount of raw material of selected plants was taken on the glass slide. The lignified elements were visualized by staining the powder with a drop of hydrochloric acid and phloroglucinol.

Physicochemical Parameters of Raw Material of Eleven Selected Medicinal Plants: [53] A powder of raw material of selected plants was used for phytochemical analysis. Physicochemical studies of the powdered drug, such as determination of the ash values, extractive values, loss on drying, and foreign organic matter, were performed according to the WHO guidelines.

Determination of Ash Values: Three different methods determined the ash remaining following ignition of medicinal plant materials: total ash, acid insoluble ash, and water-soluble ash. The total ash method was designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which was derived from the plant tissue itself, and "non physiological ash", which was the residue of extraneous matter adhering to plant surface. Acid insoluble ash is the residue obtained after the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter.

This measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the difference in weight between the total ash and residue after treatment of total ash with water.

Determination of Extractive Values: This method determines amount of active constituents extracted with solvents from a given amount of plant materials. Extractive values indicate the nature of the constituents present in a crude drug.

Alcohol Soluble Extractive: Accurately weighed 4 g powdered (60#) of the raw material of selected plants were macerated for 24 h with 100 ml of alcohol of the specified strength in a closed flask, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions against loss of the solvent, and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105 ºC to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug.

Water Soluble Extractive: Accurately weighed 4 g powdered (60#) of raw material of selected plants were macerated with 100 ml of water in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, and 25 ml of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 105 °C to constant weight. The % w/w of water-soluble extractive value was calculated with reference to the air-dried drug.

Determination of Moisture Content (Loss on Drying): About 1 g of the raw material of selected plants was taken and powdered. A glass-stoppered bottle was dried for 30 min under the same conditions to be employed in the determination, and the weight of the bottle was taken. The sample was transferred into the bottle, and the weight of the bottle with the contents was noted. The sample was distributed evenly and was placed in the drying chamber (Oven).

The stopper was removed and left in the chamber. The drying was carried out by heating to 100-105 °C. Then, the bottle was removed from the oven, and the bottle was closed promptly. The bottle was allowed to cool to room temperature and weighed. The experiment was repeated till a constant value was obtained.

Phytochemical Screening of Prepared Extracts of Eleven Selected Medicinal Plants:

Sample Preparation: Three different extracts of eleven selected medicinal plants were prepared to perform phytochemical screening. The dried extracts were then stored in airtight containers until required. All phytochemical tests were performed with these three extracts of eleven selected medicinal plants.

The extracts of eleven selected medicinal plants were subjected to the following tests separately to check the presence of various phytochemicals visually like, alkaloids [54], flavonoids [55, 56], saponins [57, 58], carbohydrates [59], steroids, triterpenoids, carotenoids, amino acids, tannins [60, 61], phenolics [62, 63], coumarins [64, 65] and anthraquinones [66] using standard procedures.

Preparation of Extracts:

Preparation of Alcoholic, Hydroalcoholic and Aqueous Extracts of the Eleven Selected Medicinal Plants: 100 gm of the powder of the eleven selected medicinal plants *i.e.* fruits of *Emblica officinali*, fruits of *Terminalia bellerica*, fruits of *Terminalia chebulla*, roots of *Withania somnifera*, rhizomes of *Cyperus rotundus*, rhizomes of *Alpinia galanga*, leaves of *Oscimum sanctum*, leaves of *Adhatoda vasica* and fruits of *Piper longum*, roots of *Leptedinia reticulata* and whole herb of *Cocculus hirsutus* were taken to prepare its different extracts. Three different extracts, *i.e.*, alcoholic, hydroalcoholic (30:70 water: alcohol) and aqueous extracts were prepared by maceration of raw material of selected plants for 48 hours in respective solvents. It was then refluxed for about 1 h with occasional shaking, consecutively 3 times and filtered. The filtrates were pooled and concentrated to dryness, percentage yield was calculated. The prepared extracts were labelled and stored in an air tight container for further use.

Cytotoxicity Screening:

Cytotoxicity Screening of Prepared Extracts of Elevenselected Medicinal Plants using Vero Cell Line *via* MTT Assay: The Vero cell line was procured from the cell repository of National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India

Chemicals and Instruments: Eagles Minimum Essential Medium (MEM) (HiMedia), Foetal Bovine Serum (FBS) (HiMedia), Antibiotics solution 1% (10000 U Penicillin and 10 mg Streptomycin/ml) (HiMedia), Trypsin–EDTA solution (Trypsin (0.25%)-EDTA (0.2%)) (HiMedia), Dulbeco's Phosphate Buffer Saline (DPBS) (HiMedia), Cryoprotectant DMSO (HiMedia), 70% (v/v) Iso-Propyl alcohol, Bio-safety cabinet, Inverted microscope with phase contrast, CO_2 incubator (Thermo-Fischer), Research Centrifuge (Eltrek), Tissue culture flasks, Micropipettes

Trypan Blue Dye Exclusion Assay: [67, 68, 69]

Chemicals and Instruments: Cell Suspension, Dulbeco's Phosphate Buffer Saline (DPBS) (HiMedia), Trypan Blue Dye 0.4% (w/v) (HiMedia), 70% (v/v) Iso-Propyl alcohol, Haemocytometer and cover slip, Micropipette, Inverted microscope.

Principle of Trypan Blue Dye Exclusion Assay: Trypan blue is a vital stain that leaves nonviable cells with a distinctive blue color when observed under a microscope, while viable cells appear unstained. Viable cells have intact cell membranes and hence do not take in dye from the surrounding medium. On the other hand, nonviable cells do not have an intact and functional membrane and hence do take up dye from their surroundings. This results in the ability to easily distinguish between viable and nonviable cells since the former are unstained, small, and round, while the latter are stained and swollen.

Method of Trypan Blue Dye Exclusion Method: Make a cell suspension in a fixed volume of cells (*e.g.*, 1ml). Although an aseptic technique is not essential in all stages of this procedure, it is good laboratory practice to maintain sterility throughout the procedure [70]. Take 50uL of cell suspension and mix it with an equal volume of trypan blue. Mix solution well using a pipette. Transfer to a hemocytometer and count the live-cell as clear form and dead cell as blue cells. After staining with trypan blue solution, counting should commence in less than 5 min as after that time; the cells will begin to take up the dye. Using a pipette, place some of the cell suspension: trypan blue mixture into the hemocytometer and overlay with a coverslip. The cell suspension will pass under the coverslip by capillary action unless there is an air bubble. Make sure the wells are not overfilled and that the coverslip is not moved once it is placed on the grid and the cell solution is added. Place the hemocytometer on the stage of an inverted microscope. Adjust focus and power until a single counting square fills the field. Calculate the number of cells per ml, and the total number of cells, using the following formula [71]. Calculate percent viability by using the formula:

$$\% \text{ viability} = (\text{live cell count/total cell count}) \times 100$$

Microculture Tetrazolium (MTT) Assay:

Prior to MTT Assay: The Vero cells were maintained by regular sub-culturing of the cells, and every time the cells were sub-culture,d the cell viability, and total number of cells were performed by trypan blue dye exclusion assay method. The medium of the Vero cells in the T-flask was replenished by a freshly prepared growth medium every alternate day.

Principle of MTT Assay: This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water-soluble substrate 3(4, 5dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically [19, 20]. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

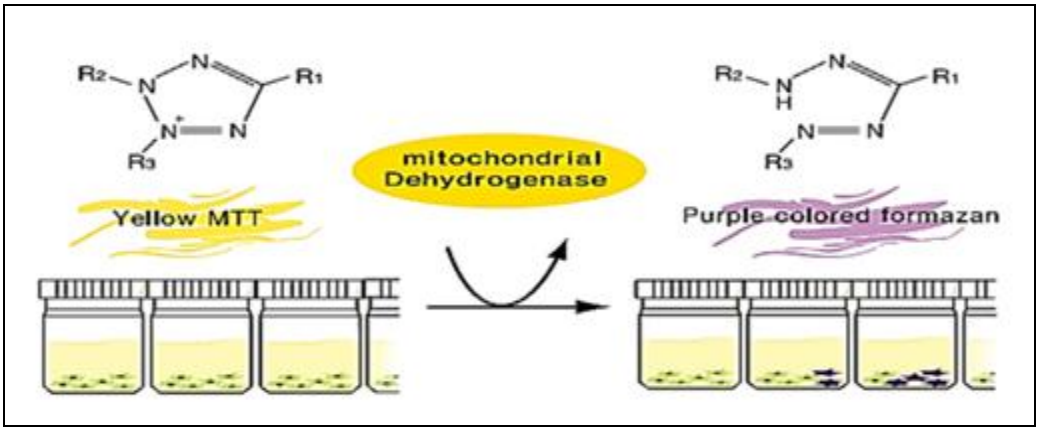


FIG. 1: PRINCIPLE OF MTT ASSAY

Chemicals and Instruments: Complete growth medium, Trypsin–EDTA solution, Dulbeco's Phosphate Buffer Saline, Dimethyl sulfoxide, MTT Reagent (HiMedia), 70% (v/v) Iso-Propyl alcohol, Inverted microscope, Research Centrifuge, ELIZA reader, 96 well plate flat-bottom, Micropipette

Method of MTT Assay: The cells of Vero in the T-flask were cultured until they reached the confluency of 75-80% prior to assay. The cells were harvested, and the cell count and cell viability were performed using Trypan blue dye exclusion method. The Vero cells were seeded at a density of 1×10^4 using 100 µl of cell suspension per well in a flat-bottomed 96-well plate and incubated for 24 h. After 24 h they were checked for their morphology and whether the cells were attached to the well plate. The cells were replenished with 100 µl of fresh media in each well. The population doubling time (PDT) of Vero cell line was 24 h. Hence the cells of Vero cell line were incubated for 24 h at 37 °C and in 5% CO_2. After 24 h, the plant extracts were dissolved using DMSO in the complete growth medium at a safe concentration, and they were sterile passed using a syringe filter. Vero cell line was exposed to different plant extracts at various concentrations (100µg/m, 500µg/ml, and 1000µg/ml) for 48 h. The experiments were conducted in triplicate using three extracts, *i.e.*, alcoholic, hydroalcoholic and aqueous extracts of the eleven selected plants, *i.e.*, fruits of *Emblica officinalis*, fruits of *Terminalia bellerica*, fruits of *Terminalia chebulla*, roots of *Withania somnifera*, rhizomes of *Cyperus rotundus*, rhizomes of *Alpinia galanga*, leaves of *Oscimum sanctum*, leaves of *Adhatoda vasica* and fruits of *Piper longum*, roots of *Leptedinia reticulata* and whole herb of *Cocculus hirsutus*as the test substances. The solvent DMSO treated cells served as control cisplatin was used as a positive standard for Vero cells. Cells were then treated with MTT reagent (0.5 mg/ml as final concentration, *i.e.*, 20µl/well of stock) for 4 h at 37°C. The seeded plates were incubated with MTT reagent (0.5 mg/ml as final concentration, *i.e.* 20µl/well of stock) for 4 h. MTT reagent was prepared by dissolving MTT reagent in DPBS and it was sterile passed using a syringe filter. As MTT is photosensitive, MTT addition was done in dark. After 3 h the media along with the MTT reagent was discarded from the cells in such a way that the monolayer of the adhered cells was not disturbed. The formazan crystals formed were dissolved using DMSO, 200 µl/well and the plate was kept aside for 5-10 min for the formazan crystals to dissolve. The optical density (OD) was recorded at 570 nm using a multi-well plate reader, ELIZA reader, and percentage cell viability was calculated.

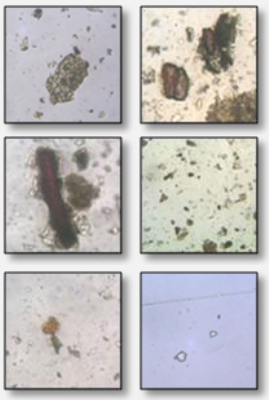
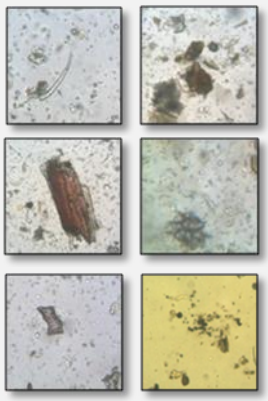
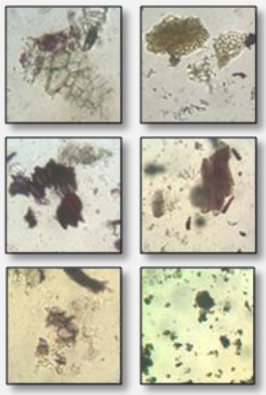
Percentage of cell viability was determined as (Avg. OD of treated cells/Avg. OD of control cells) ×100.

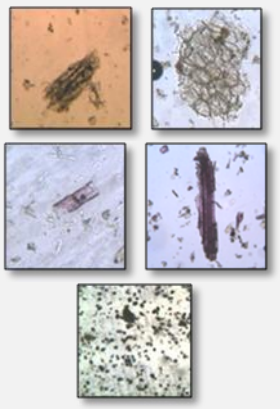
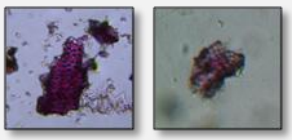
Statistical Analysis: Results are represented as mean ± SEM from at least three separate

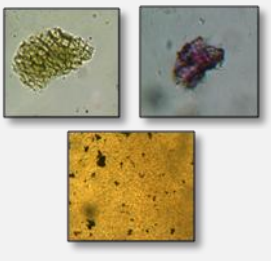
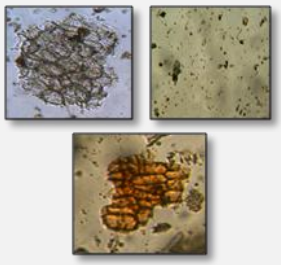
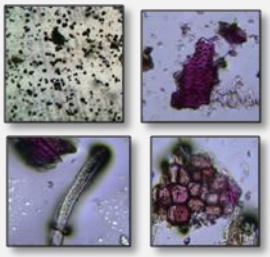
experiments (n=3). Statistical analysis of data was carried out by one-way analysis of variance (ANOVA) followed by Tukey's post hoctest using GraphPad Prism for Windows (version 5). p-values < 0.05 were considered statistically significant.

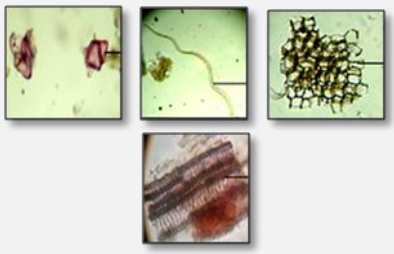
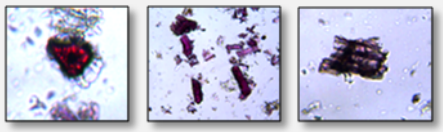
RESULTS: A powder of eleven selected medicinal plants was used for its phytochemical analysis. Macroscopical study and powder microscopical study were performed, and it compared with the standard parameters given in the literature.

TABLE 4: PHARMACOGNOSTICAL STUDY OF ELEVEN SELECTED MEDICINAL PLANTS

Name of the plant	**Amla**	**Baheda**	**Harde**
Botanical name	*Emblica officinalis*	*Terminalia bellirica*	*Terminalia chebula*
Family	*Phyllanthaceae*	*Combretaceae*	*Combretaceae*
Part used	Fruits	Fruits	Fruits
Macroscopy:			
Shape	Globulose	Spherical to ovoid	Ovoid
Surface	Rough, shriveled and wrinkled	Slightly hairy and wrinkled	Wrinkled
Organoleptic Characters:			
Colour	Grey to black	Grey to grayish brown	Yellowish brown
Odour	Characteristic	Aromatic	Characteristic
Taste	Sour and astringent	Astringent	Astringent
Microscopy:			

Name of the plant	**Ashwagandha**	**Tulsi**	**Vasaka**
Botanical name	*Withania somnifera*	*Ocimum sanctum*	*Adhatoda vasica*
Family	*Solanaceae*	*Lamiaceae*	*Acanthaceae*
Part used	Roots	leaves	Leaves
Macroscopy:			
Shape	Cylindrical	Elliptical-oblong	Oblong
Surface	Longitudinally wrinkled	smooth	smooth
Organoleptic Characters:			
Colour	Buff to grayish-yellow	Light greenish	greenish
Odour	Characteristic	pungent	unpleasant smell
Taste	Bitter and acrid	bitter	Bitter
MICROSCOPY:			

Name of the plant	**Long pepper**	**Nagarmoth**	**Rasana**
Botanical name	*Piper longum*	*Cyperus rotundus*	*Alpinia galanga*
Family	*Piperaceae*	*Cyperaceae*	*Zingiberaceae*
Part used	Fruits	Tubers	Rhizomes
Macroscopy:			
Shape	Ovoid	Ovoid, tunicate	cylindrical
Surface	Rough	Rough with striations	wavy annulations of the leaf bases
Organoleptic Characters:			
Colour	Dark black to greenish	Brownish black externally and white internally	Buff yellowish
Odour	Characteristic	Fragrant	characteristics
Taste		starchy	characteristics
MICROSCOPY:			

Name of the plant	**Vevadi**	**Kharkhodi**
Botanical name	*Cocculus hirsutus*	*Leptedenia reticulata*
Family	*Menispermiaceae*	*Asclepidiaceae*
Part used	Whole plant	Roots
Macroscopy:		
Shape	---	Ovoid, tunicate
Surface	---	Rough
Organoleptic Characters:		
Color	Greenish	Brownish black externally and white internally
Odour	Characteristic	Fragrant
Taste		starchy
Microscopy:		

Physicochemical Parameters of Raw Material of Selected Plants: Physicochemical studies of the powdered drug, such as determination of the ash values, extractive values, loss on drying, and foreign organic matter, were performed according to the WHO guidelines.

TABLE 5: PHYSICOCHEMICAL PARAMETERS OF ELEVEN SELECTED MEDICINAL PLANTS

Physico-chemical Parameters	**Ash values (% w/w)**			**Extractive values (%)**		**Loss on drying (%)**
	Total Ash	**Acid Insoluble Ash**	**Water Soluble Ash**	**Water Extractive value**	**Alcohol Extractive Value**	
Embilica officinalis	00.86	00.35	01.80	40.15	44.38	03.05
	NMT	NMT	-	NLT	NLT	
	01.00	0.05		11.0	10.0	
Terminalia bellerica	04.15	00.08	01.45	48.78	51.14	03.25
	NMT	NMT	-	NLT	NLT	
	04.50	00.20		26.0	17.0	
Terminalia chebula	03.67	0.35	0.54	57.32	42.34	04.16
	NMT	NMT	--	NLT	NLT	

	05.05	00.50		56.00	40.00	
Withania somnifera	05.31	00.74	01.13	26.43	15.23	02.34
	NMT	NMT	-	NLT	NLT	
	09.00	02.00		17.00	03.00	
Cyperus rotundus	04.30	02.23	01.63	26.35	24.38	01.26
	NMT	NMT	-	NLT	NLT	
	06.50	02.50		20.00	14.00	
Alpinia galanga	04.89	01.56	02.35	11.45	08.28	01.36
	NMT	NMT	-	NLT	NLT	
	05.50	02.00		20.00	15.00	
Oscimum sanctum	08.80	00.40	03.80	48.26	39.67	02.40
	NLT	NLT	-	NLT	NLT	
	09.50	00.50		15.00	20.00	
Adhatoda vasica	14.09	01.72	03.00	45.92	36.26	07.00
	NMT	NMT	-	NLT	NLT	
	20.00	02.00		22.00	05.00	
Piper longum	05.31	00.41	04.02	45.76	21.52	02.58
	NMT	NMT	-	NLT	NLT	
	04.50	00.20		26.00	17.00	
Leptedinia reticulata	06.50	00.87	02.70	09.40	08.80	06.50
	NMT	NMT	--	NLT	NLT	
	16.50	03.00		22.40	05.20	
Cocculus hirsutus	09.80	02.89	04.60	24.30	15.69	09.80

Phytochemical Screening of Eleven Selected Medicinal Plants: The extracts were evaluated to detect the presence of various phytochemicals like alkaloids, tannins, resins, glycosides, triterpenes, and steroids, *etc*. using the different chemical test to establish its identity. The chemical tests include colour reaction tests; these tests help to determine the identity of the chemical class.

TABLE 6: PHYTOCHEMICAL SCREENING OF ELEVEN SELECTED MEDICINAL PLANTS

Name of the plants	*Emblica officinalis*	*Terminalia bellerica*	*Terminalia chebulla*	*Withania somnifera*	*Cyperus rotundus*	*Alpinia galanga*	*Oscimum sanctum*	*Adhatoda vasica*	*Piper longum*	*Leptedinia reticulata*	*Cocculus hirsutus*
Alkaloids	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	-	+
Triterpenoids	+	+	+	+	+	+	+	+	-	-	+
Cardiac glycosides	-	-	-	-	+		+	+	+	+	-
Tannins	+	+	+	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	+		+	+	+	+	+
Flavonoids	-	-	-	-	+	+	+	+	+	+	+

(+) = present, (-) = absent

Preparation of Extracts (% Yield of Extracts): Alcoholic, aqueous, and 70 % hydro-alcoholic extracts were prepared by maceration of the raw material of selected plants for 48 h in respective solvents. It was then refluxed for about 1 h with occasional shaking consecutively 3 times and filtered. The filtrates were pooled and concentrated to dryness, percentage yield was calculated. The prepared extracts were labeled and stored in an airtight container for further use. The %yield of different plant extracts is shown in **Table 7**.

TABLE 7: % YIELD OF VARIOUS EXTRACTS OF ELEVEN SELECTED MEDICINAL PLANTS

S. no.	Name of the plant	% yield of extracts		
		Alcoholic extract	Hydro-alcoholic extract	Aqueous extract
1	*Emblica officinalis*	44.38%	52.92%	63.52%
2	*Terminalia bellerica*	42.46%	55.16%	66.68%
3	*Terminalia chebulla*	45.08%	50.58%	46.56%
4	*Withania somnifera*	8.91%	15.32%	24.16%

5	*Cyperus rotundus*	8.28%	10.47%	15.46%
6	*Alpinia galanga*	8.26%	5.92%	6.63%
7	*Oscimum sanctum*	17.87%	21.10%	28.21%
8	*Adhatoda vasica*	13.30%	28.56%	36.26%
9	*Piper longum*	21.52%	40.78%	45.76%
10	*Leptedinia reticulata*	8.80%	9.40%	10.39%
11	*Cocculus hirsutus*	16.00%	23.48%	30.12%

Cytotoxicity Screening of the Selected Plants by MTT Assay: All three prepared extracts of selected eleven medicinal plants were screened for their cytotoxicity using Vero cell line.

TABLE 8: INTRODUCTION TO VERO CELL LINE

Organism	African green monkey
Tissue	Kidney
Disease	Normal
Age	Adult
Morphology	fibroblast
Growth Properties	adherent
Complete Growth Medium	Eagle's Minimum Essential Medium (MEM), fetal bovine serum (FBS) 10%. (as per ATCC)
Complete Growth environment	Growth temperature 37º 5% CO_2
Cell growth properties	Population doubling time 24 hours

The Vero cells are the normal cells, *i.e.*, kidney cells of the African Green monkey. Hence, the cytotoxicity of the selected plants was checked on the Vero cell line using MTT assay. The results of the cytotoxicity of the extracts of the eleven selected medicinal plants on the Vero cell line are as shown in **Fig. 2a, 2b,** and **2c**. The results are expressed in terms of the cell viability of the cells. None of the extracts showed significant cytotoxicity on the Vero cells.

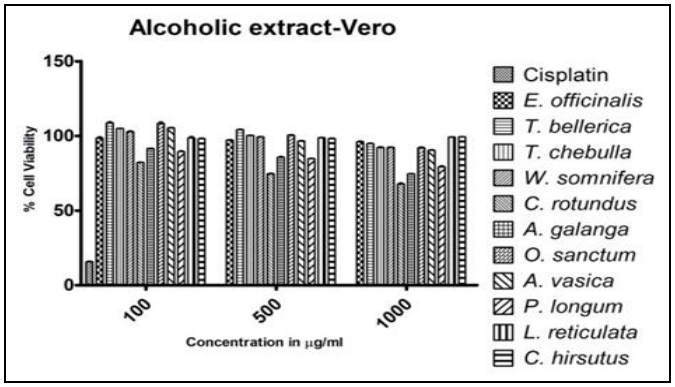


FIG. 2A: PERCENTAGE CELL VIABILITY OF ALCOHOLIC EXTRACTS OF SELECTED PLANTS

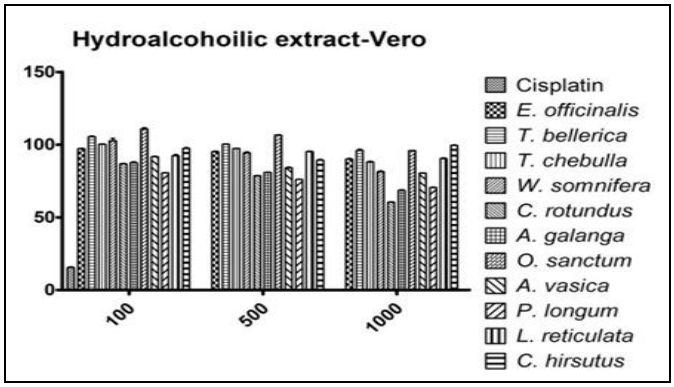


FIG. 2B: PERCENTAGE CELL VIABILITY OF HYDRO-ALCOHOLIC EXTRACTS OF SELECTED PLANTS

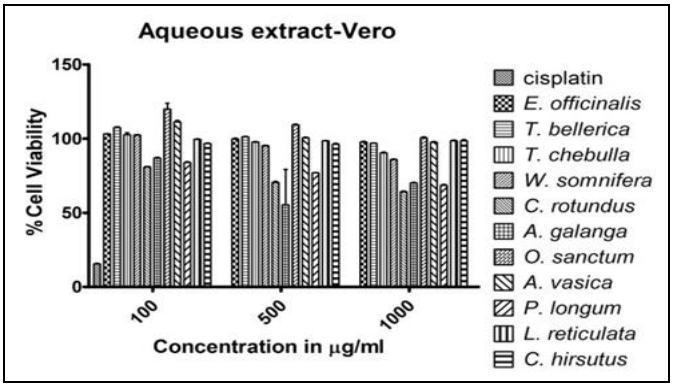


FIG. 2C: PERCENTAGE CELL VIABILITY OF AQUEOUS EXTRACTS OF SELECTED PLANTS

FIG. 2: CYTOTOXICITY SCREENING: PERCENTAGE CELL VIABILITY OF THE EXTRACTS OF THE ELEVEN SELECTED MEDICINAL PLANT USING VERO CELL LINE *VIA* MTT ASSAY

Results are presented as mean ± SD from at least three separate experiments (n=3). Statistical analysis of data was carried out by one-way ANOVA followed by Tukey *post hoc* test using GraphPad Prism for Windows (version 5). Values of $p<0.05$ were considered significant.

DISCUSSION AND CONCLUSION: Tuberculosis (TB) accounts for a significant global burden of disease and substantial investment in research and development. Although it is an ancient and curable disease, TB remains the world's leading cause of death from an infectious agent. According to the World Health Organization (WHO), in 2017, 10 million individuals became ill with TB, and 1.6 million died [72].

The plant is an important source of medicine and plays a key role in world health [73]. Medicinal herbs or plants have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in the health system all over the world. This involves the use of medicinal plants not only for the treatment of diseases but also as potential material for maintaining good health and conditions. Many countries in the world, that is, two-thirds of the world's population, depend on herbal medicine for primary health care. The reasons for this are because of their better cultural acceptability, better compatibility, and adaptability with the human body and pose lesser side effects.

From records, most of the used drugs contain plant extracts. Some contain active ingredients (bioactive components or substances) obtained from plants. Through recent researches, plant-derived drugs were discovered from the study of curative, therapeutic, traditional cures and most especially the folk knowledge of indigenous people and some of these claims and believe of people are irreplaceable despite the recent advancement in science and technology [74].

There has been no anti-TB drug introduced in the past 30 years, and the rapid acquisition of drug resistance to the existing drugs necessitates the development of new, effective, and affordable anti-TB drugs [75]. Plant-derived antimycobacterial compounds belong to an exceptionally wide diversity of classes, including terpenoids, alkaloids, peptides, phenolics, and coumarins. Hence medicinal plants remain an important resource to find new therapeutic agents [76].

Intolerance of anti-TB standard therapy, including isoniazid, rifampicin, and pyrazinamide, is a serious problem in the treatment of tuberculosis. Hepatotoxicity was found to be the most frequent side effect in the patient taking treatment of tuberculosis. Anti-TB drug-induced hepatotoxicity is a serious adverse effect and continues to be a problem worldwide [77].

Selection of eleven plants [fruits of *Emblica officinalis,* fruits of *Terminalia bellerica,* fruits of *Terminalia chebulla,* roots of *Withania somnifera*, rhizomes of *Cyperus rotundus,* rhizomes of *Alpinia galangal,* leaves of *Oscimum sanctum, l*eaves of *Adhatoda vasica and* fruits of *Piper longum,* roots of *Leptedinia reticulata* and the whole herb of *Cocculus hirsutus*] for the study of its antituberculosis activity with hepatoprotective and immunomodulatory activity was the main objective of this invention.

These eleven selected medicinal plants were reviewed for their use in the curing of tuberculosis, helping in the protection of liver disorder and enhance the immunity and bioavailability in the different literature survey as mentioned in earlier **Table 2** and **Table 3**.

Morphological study and powder microscopical study of eleven selected medicinal plants were performed and compared with standards mentioned in Quality standard of Indian medicinal plants and all the plants were comply with the standard limits.

Physicochemical studies of the powdered of all selected eleven plants were determined by ash values, extractive values, and loss on drying according to the WHO guidelines. The total ash method was designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which was derived from the plant tissue itself, and "non-physiological ash", which was the residue of extraneous matter adhering to plant surface. Acid insoluble ash is the residue obtained after the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the difference in weight

between the total ash and residue after treatment of total ash with water [78].

From all eleven selected plants, *Adhatoda vasica* having a maximum amount of ash value (14.09% w/w) as it contains the maximum amount of silica in its dried powder. The water extractive value of *Terminalia chebula* was maximum (57.32 %w/w) compared to all other selected plants it means a higher water-soluble extractive value implies that water is a better solvent for extraction for plant material [79]. The percentage of Loss on drying of all selected eleven plant materials was compared with standard, and it complies in the range of it.

The phytochemical screening carried out on these eleven selected medicinal plants showed that it contained major classes of natural products. Plant extracts are attractive and effective sources of new drugs[80]. Natural products play a significant role in the drug discovery and development of highly active anti-mycobacterial metabolites [81, 82.] Here, our target was isolated phytoconstituent acting on tuberculosis. Our investigation indicates the presence of Flavonoids, saponins, steroids, tannins, terpenes, anthraquinone, and alkaloids. These phytochemicals are responsible for anti-tubercular activity. Similar observations have been made in plants employed for traditional medicines, which were known to contain the said mentioned bioactive components [83].

There are three different extracts of eleven selected medicinal plants prepared on the basis of phytochemical screening. They were extracted with Alcoholic, (30:70) Hydroalcoholic and Aqueous.

Cytotoxicity studies with normal cell culture systems of plant extracts have not been studied extensively, and this is vital for the safety evaluation for any herb or herbal preparation. Therefore, all extracts selected of eleven medicinal plants were screen for their cytotoxicity study using Vero cell line. The alcoholic, hydroalcoholic and aqueous extracts of the selected plants did do not have any significant cytotoxicity on the normal vero cell line.

The study suggests that the extracts might have increased the proliferation of the kidney cells, which can be further studied by cell proliferation assay. Thus, the toxicity of the plant has to be studied for further exploration of various biological activities.

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REFERENCES:

1. Dye C, Scheele S, Dolin P, Pathania V and Raviglione MC: Global burden of tuberculosis: estimated incidence prevalence and mortality by country. Journal of the American Medical Association 1999; 282: 677-86.
2. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD and Katoch VM: Anti-tuberculosis activity of selected medicinal plants against Mycobacterium tuberculosis. Indian Journal of Medicinal Research 2010; 131: 809-13.
3. Gizachew YE, Giday M and Teklehaymanot T: Antimycobacterial activity of selected Ethiopian traditional medicinal plants used for treatment of symptoms of tuberculosis. Global Advanced Research JournalofMedicinal Plants 2013; 2(2): 022-029.
4. Panda VS, Ashar HD and Sharan A: Antioxidant and hepatoprotective effects of *Garcina indica* fruit rind in antitubercular drug-induced liver injury in rats. Botanics: Targets and therapy. 2013; 3: 29-37.
5. Chew AL, Jessica JAJ and Sasidharan S: Antioxidant and antibacterial activity of different parts of Leucasaspera. Asian Pacific Journal of Tropical Biomedicine 2012; 2(3): 176-80.
6. Nguta JM: Journal of Ethnopharmacology 2016; 182: 10-15.
7. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi: Council of Scientific and Industrial Research 2002.
8. Gupta AK: Quality Standards of Indian Medicinal Plants Volume, Indian Council of Medical Research (ICMR), New Delhi 2003.
9. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi Publications and Information DIrectorate: CSIR 1982.
10. Nadkarni KM: Indian Materia Medica. Popular Prakashan Pvt. Ltd. Bombay I: 1982; 1292-94.
11. Kirtikar KR and Basu BD: Indian Medicinal Plants. 2nd ed., Vol. IV. Dehradun: International Book Distributors; 2007: 580-878.
12. Gupta A: Quality Standards of Indian Medicinal Plants. Vol-1, Indian Council of Medical Research (ICMR), New Delhi 2003.
13. Panday CN, Raval SS, Mali S and Salvi H: Medicinal plants of Gujarat - species description and medicinal use, *Oscimum sanctum* 229.
14. Quality standards of Indian medicinal plants, vol II. New Delhi: Indian Council of Medical Research (ICMR); 2OO5: 276-79.
15. Kirtikar KR and Basu BD: Indian Medicinal Plants, 2nd ed., Lalit Mohan Basu Publications, Allahabad, 1933; 2131-33.
16. Kirtikar KR and Basu BD: Indian Medicinal Plants, 2nd ed., Lalit Mohan Basu Publication, Allahabad 1933; I: 1786-89.

17. Kirtikar KR and Basu BD: Indian Medicinal Plants, 2nd ed., Lalit Mohan Basu Publication, Allahabad, 1933; I: 86-88.
18. Grover HS, Deswal H, Singh Y and Bhardwaj A: Therapeutic effects of amla in medicine and dentistry: A review, Journal of Oral Research and Review 2015; 7(2): 65-68.
19. Sherwani AMK, Zulkifle M and Rehmatulla: A Pilot trial of jawarish amla as adjuvant to anti-tubercular treatment drugs for control of adverse reactions in dots regime in pulmonary TB. Journal of Indian Medical Association, 2012; 44: 1-9.
20. Deori C, Das S and Bordoloi SK: Study of hepatoprotective activity of *Emblica officinalis* (AMLA) in Albino rats. Journal of Evidence Based Medicine and Healthcare 2017; 4(54): 3298-3301.
21. Singh MK, Yadav SS, Gupta V and Khattri S: Immunomodulatory role of *Emblica officinalis* in arsenic induced oxidative damage and apoptosis in thymocytes of mice. BMC Complementary and Alternative Medicine 2013; 13(1): 193.
22. Singh MP, Gupta A and Sisodia SS: Ethno and Modern Pharmacological Profile of Baheda (*Terminalia bellerica*): A Review, The Pharmaceutical and Chemical Journal 2018; 5(1): 153-62.
23. Deb A, Barua S and Das B: Pharmacological activities of Baheda (*Terminalia bellerica*): a review. Journal of Pharmacognosy and Phytochemistry 2016; 5(1): 194
24. Belapurkar P, Goyal P and Tiwari-Barua P: Immunomodulatory effects of triphala and its individual constituents: a review. Indian Journal of Pharmaceutical Sciences 2014; 76(6): 467.
25. Choi MK, Kim HG, Han JM and Lee JS: Hepatoprotective effect of Terminalia chebula against t-BHP-induced acute liver injury in C57/BL6 mice. Evidence-Based Complementary and Alternative Medicine 2015; 517350: 1-12.
26. Tasduq SA, Singh K, Satti NK, Gupta DK, Suri KA and Johri RK: *Terminalia chebula* (fruit) prevents liver toxicity caused by sub-chronic administration of rifampicin, isoniazid and pyrazinamide in combination, Human & Experimental Toxicology 2006; 25: 111.
27. Aher V and Wahi A: Immunomodulatory activity of alcohol extract of *Terminalia chebula* retz combretaceae. Tropical Journal of Pharmaceutical Research 2011; 10(5): 567-75.
28. Khan KH: Immunomodulatory activity of *Terminalia chebula* against Salmonella typhimurium in mice. Recent Research in Science and Technology 2009; 1(5): 23.
29. Adaikkappan PR, Kannapiran M and Anthonisamy A: Anti-mycobacterial activity of *Withania somnifera* and *Pueraria tuberosa* against *Mycobacterium tuberculosis* H37Rv. Journal of Academia and Industrial Research 2012; 1(4): 153-56.
30. Sarepaka A, Dhamodaran P, Singh A, Nilani P and Duraiswamy B: *In-vitro* Antitubercular Screening Of Certain Medicinal Plants, Pharmatutor 2013: 1-9.
31. Kumar R, Rai J, Kajal NC and Devi P: Comparative study of effect of *Withania somnifera* as an adjuvant to DOTS in patients of newly diagnosed sputum smear positive pulmonary tuberculosis, Indian Journal of Tuberculosis, 2017: 1-6.
32. Davis L and Kuttan G: Immunomodulatory activity of *Withania somnifera*. Journal of Ethnopharmacology 2000; 71(1-2): 193-200.
33. Agarwal R, Diwanay S, Patki P and Patwardhan B: Studies on immunomodulatory activity of *Withania somnifera* (Ashwagandha) extracts in experimental immune inflammation. Journal of Ethnopharmacology 1999; 67(1): 27-35.
34. Gupta VK, Kaushik A, Chauhan DS, Ahirwar RK, Sharma S and Bisht D: Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms, Journal of Ethnopharmacology 2018; 227: 113-20.
35. Kumar SS and Mishra SH: Hepatoprotective activity of rhizomes of *Cyperus rotundus* Linn. against carbon tetrachloride-induced hepatotoxicity. Indian Journal of Pharmaceutical Sciences 2005; 67(1): 84.
36. Aghwan SS and Al Taei AF: Immunomodulation of aqueous extract of *Cyperus rotundus* Linn against experimental infection with hydatid cysts in mice. Iraqi Journal of Veterinary Sciences 2007; 21(1): 147-57.
37. Soundhari C and Rajarajan S: *In-vitro* screening of lyophilised extracts of *Alpinia galanga* L. and *Oldenlandia umbellata* L. for antimycobacterial activity. International Journal of Bio-Pharma Research 2013; 4(6): 427-32.
38. Chopra LC, Khajuria BN and Chopra CL: Antibacterial properties of volatile principles from *Alpinia galanga* and *Acorus calamus*. Antibiot Chemother 1957; 7: 6.
39. Eram S, Mujahid M, Bagga P, Ahsan F and Rahman MA: Hepatoprotective evaluation of Galanga (*Alpinia officinarum*) Rhizome extract against antitubercular drugs induced hepatotoxicity in rats. Journal of Herbs, Spices & Medicinal Plants 2019; 15: 19.
40. Bhatter PD, Gupta PD and Birdi TJ: Activity of medicinal plant extracts on multiplication of *Mycobacterium tuberculosis* under reduced oxygen conditions using intracellular and axenic assays. International Journal of Microbiology 2016; 8073079: 1-7.
41. Reddi G. Shukla NP and Singh KV: Chemotherapy of tuberculosis--antitubercular activity of *Ocimum sanctum* leafy extract, Fitoterapia 2013; 57(2): 114-16.
42. Vyas RB, Shrivastava P and Parihar G: Evaluation of Anti-mycobacterial Activity of *Ocimum sanctum* and *Lantana camara* Against M. Tuberculosis Standard Strain. International Journal of Multidisciplinary Research 2018; 2.
43. Chattopadhyay RR, Sarkar SK, Ganguly S, Medda C and Basu TK: Hepatoprotective activity of *Ocimum sanctum* leaf extract against paracetamol included hepatic damage in rats. Indian Journal of Pharmacology 1992; 24: 163-65.
44. Patel VK and Venkatakrishna BH: *In-vitro* study of antimicrobial activity of *Adhatoda vasika* Linn. (leaf extract) on gingival inflammation a preliminary report. Indian Journal of Medical Sciences 1984; 38(4): 70-82.
45. Narimaian M, Badalyan M, Panosyan V, Gabrielyan E, Panossian A and Wikman G: Randomized trial of a fixed combination (KanJang) of herbal extracts containing *Adhatoda vasica*, *Echinacea purpurea* and *Eleutherococcus senticosus* in patients with upper respiratory tract infections. Phytomed 2005; 12(8): 539-47.
46. Ahmad R, Raja V and Sharma M: Hepatoprotective activity of ethyl acetate extract of *Adhatoda vasicain* Swiss Albino Rats. International Journal of Current Research and Review 2013; 5: 16-21.
47. Jinyvarghese K, Karpe ST and Kulkarni SR: Immunostimulant activity of *Adhatoda vasica*, *Lawsonia inermis* and *Alkanna tinctoria*. Indian Drugs-Bombay-. 2005; 42(6): 345.
48. Randhawa GK and Kullar RJS: Bioenhancers from Mother Nature and their applicability in modern medicine. International Journal of Applied and Basic Medical Research 2011; 1(1): 5.

49. Atal CK, Dubey RK and Singh J: Biochemical basis of enhanced drug bioavailability by piperine: evidence that piperine is a potent inhibitor of drug metabolism. Journal of Pharmacology and Experimental Therapeutics 1985; 232(1): 258-62.
50. Sonara GB, Saralaya, MG and Gheewala NK: A review on phytochemical and pharmacological properties of *Leptadenia reticulata* (Retz). Pharma Science Monitor, 2013; 4(1): 3408-17.
51. Gupta VK, Kaushik A, Chauhan DS, Ahirwar RK, Sharma S and Bisht D: Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms. Journal of Ethnopharmacology 2018; 227: 113-20.
52. Kumar GT, Murthy JSN, Reddy RR, Vasu K and Choda PK: *Cocculus hirsutus*, a versatile herbal medicine: a review. Asian Journal Pharmacy Life Science 2012; 2(2): 303-08.
53. Anonymous, Physicochemical parameters, WHO Guidelines 1st ed. A.I.T.B.S. Publishers and distributors, Delhi, 2002, 40-43.
54. Geissman A, Peach K and Tracy MV: Modern Methods of Plant Analysis, ed. Heidelberg, Berlin, Springer Verlag, 1955; 3: 471.
55. List PH and Horhammer L: Chemical tests. Hager Hand buch der pharmazeutis chem praxis. Berlin, Springer Verlag Band, 1967; 1: 256.
56. Geissman A, Peach K and Tracey MV: Morden methods of plant analysis, Springer Verlang, Berlin, Gottingen, Heidelberg 1955; 3: 473.
57. Fishcher R: Praktikum der Pharmakognosic, 3rd ed, Berlin, Springer Verlag 1952: 362.
58. Evans WC and Evans D: Trease and Evan's Pharmacognosy, 15th ed. W.B. Saunders Company Ltd., London, 2002: 193.
59. Griffin WJ, Owen WR and Perkin JE: A Phytochemical survey of eastern Australian plants for saponins. Planta Medica 1968; 16(1): 75-81.
60. Simes, JH, Tracey JG, Webb LJ and Dunstan WJ: An Australian phytochemical survey - saponins in eastern australian flowering plants, Australia common wealth scientific Industrial Research Organization Bulletin 1959: 281.
61. Wilson JA and Merill HB: Analysis of leather and material used in making it, 1st ed. The Mcgraw Hill Book Co. Inc., New York 1931: 290-293.
62. Freudenberg K, Weinger K and Geissman A: The chemistry of flavonoid compounds, Pregamon Press. Oxford 1962: 211.
63. Robinson T: The organic constituents of higher plants, their chemistry and interrelationships, Minneapalis 15 Minn., Burgers Publishing Company 1947; 4(1): 1964.
64. Clerk JD, Descamps A and Vander ME: Colorimetric method for determining tannin, Bulletin Association Anciens etud. Brass, University Louvain 1947; 43(4): 68-76.
65. Harborne JB: Chemical test In: Flavonoids, Phytochemical Methods, 2nd ed. Champan and Hall Ltd. London 1973: 42.
66. Feigl F: Identification of individual organic compound. In: Spot Tests in Organic Analysis, 4th ed. Elsevier Publishing Company, London, 1956: 237.
67. Louis KS and Siegel AC: Cell viability analysis using trypan blue: manual and automated methods. Mammalian Cell Viability: Springer 2011: 7-12.
68. Strober W: Trypan blue exclusion test of cell viability. Current Protocols in Immunology 2015; 111(1): A3.
69. Altman SA, Randers L and Rao G: Comparison of trypan blue dye exclusion and fluorometric assays for mammalian cell viability determinations. Biotechnology progress. 1993; 9(6): 671-44.
70. Phillips HJ and Terryberry JE: Counting actively metabolizing tissue cultured cells. Cell Research 1957; 13: 341-47.
71. Masters RW: Trypan Blue Assay sop, Animal cell culture, 3rd ed. 2000, 1-3.
72. Ouben RMGJ and Dodd PJ: The Global Burden of Latent Tuberculosis Infection: A Re-371 estimation Using Mathematical Modelling. PLoS Med 2016.
73. Sandberg F and Corrigan D: Natural Remedies. Their Origins and Uses, Abingdon: Taylor & Francis 2001.
74. Schulz V, Hänsel R and Tyler VE: Rational Phytotherapy, A Physician's Guide to Herbal Medicine. 4th ed. Berlin: Springer-Verlag 2001: 306.
75. Autam R, Saklani A and Jachak SM: Indian medicinal plants as a source of antimycobacterial agents, Journal of Ethnopharmacology 2007; 110(2): 200-34.
76. mushi T, Masoko P, Mdee L, Mokgotho M, Mampuru L and Howard R: Antimycobacterial evaluation of fifteen medicinal plants in South Africa. African Journal of Traditional Complementary Alternative Medicine 2010, 7(1): 34-39.
77. Pirmohamed M, Aithal GP and Behr E: The phenotype standard-ization project: improving pharmacogenetic studies of serious ad-verse drug reactions. Clinical Pharmacolology Therapeutics 2011; 89: 784-85.
78. Mukherjee PK: quality control of herbal drug, 1st edition, 2002, 183-215.
79. Ajazuddin SS: Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, a Unani polyherbal formulation. Pharmacognosy Research 2010; 2(5): 318.
80. Celio TH, Fernando R, Clarice QF, Miriam S, Wagner V and Sergio RA: Triterpenes and antitubercular activity of Byrsonima crassa. QuíNova. 2008; 7: 1719-21.
81. Newman DJ and Cragg GM: Natural products as sources of new drugs over the last25 years. Journal of Natural Product 2007; 70: 461-77.
82. Dolin PJ, Raviglione MC and Kochi A: Global tuberculosis incidence and mortality during 1990-2000. Bull World Health Organization 1994; 72: 213-20.
83. Otshudi AL, Foriers A, Vercruysse A, Van Zeebroeck A and Lauwers S: *In-vitro* antimicrobial activity of six medicinal plants traditionally used for the treatment of dysentery and diarrhoea in Democratic Republic of Congo (DRC) Phytomedicine 2000; 7: 167-72.

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