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Phytochemical evaluation and anti-tuberculosis activity of selected nine ethnomedicinal plants

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Abstract
Tuberculosis is one of the leading infectious disease and health burden in the world. It has been estimated that, one third of world's population including 40% from India estimated to be infected with tuberculosis. As plants are the important sources of diverse range of bioactive principles. The revival of interests in plant derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than expensive synthetic drugs, which have adverse side. In this study nine healthy plants with medicinally potential were collected and different extracts were prepare to evaluate its phytochemical study and anti-tubercular activity on *Mycobacterium smegmatis* using two different models. There are many similarities between *Mycobacterium smegmatis* and the much more virulent obligate pathogens that are *Mycobacteria*. On the basis of both the models, form nine selected plants three plants *A. vasika, O. sanctum* and *A. galanga* shows maximum anti-tuberculosis activity at the MIC 100 µg/ml, 250 µg/ml and 250 µg/ml respectively. The present study has revealed the importance of plant extracts to control *Mycobacterial* infections which are being a threat to human health and for the development of alternate, safe and effective medicines.

Keywords: Tuberculosis, phytochemical evaluation, *M. smegmatis*, anti-tuberculosis activity

1. Introduction
Tuberculosis (TB) is one of the leading infectious disease and health burden in the world [1]. It has been estimated that, one third of world's population including 40% from India estimated to be infected with tuberculosis [2]. More than nine million new cases diagnosed and approximately two million people killed annually [3].There are a number of new factors that make people more susceptible to tuberculosis infection worldwide, the important of which is Human Immunodeficiency Virus (HIV) infection and the corresponding development of AIDS. The association of tuberculosis with HIV infection is so dramatic that in some cases, nearly two third of the patients diagnosed with the tuberculosis are also HIV-1 seropositive [4]. Current tuberculosis treatment is a long course of 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 [5].The global emergence of multidrug resistance (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* and more recently the reports of totally drug resistant tuberculosis [6] has become a common phenomenon, which cause drugs to be ineffective.

Mycobacterium smegmatis is an acid-fast bacterial species in the phylum *Actinobacteria* and the genus *Mycobacterium*. It is 3.0 to 5.0 µm long with a bacillus shape and can be stained by Ziehl-Neelsen method. The bacteria will be finely wrinkled and creamy white while it is growing on accessible nutrients. When *Mycobacterium smegmatis* has been growing for quite some time (generally after 48 hr. growth) and is abundant, the color will turn from white to a non-pigmented creamy yellow. It will also be waxy because of the high amount of unique Gram-positive cell wall coated with mycolic acids. The bacteria also range in textures, being seen as smooth, flat and glistening or coarsely folded or finely wrinkled [7].

Mycobacterium smegmatis is very useful for the research analysis of other species in the genus *Mycobacteria* in cell culture laboratories. There are several *Mycobacterial species* that are common, harmful like *Mycobacterium leprae, Mycobacterium tuberculosis and Mycobacterium bovis*. *Mycobacterium smegmatis* is so important because it is fast growing and non-pathogenic compared to these species. There are many similarities between *Mycobacterium smegmatis* and the much more virulent obligate pathogens that are *Mycobacteria*. The most significant is the complementary uses of mycothiol biosynthesis of *Mycobacterium* for making an essential thiol that is responsible for life. If it is knocked out, the species will be terminated and a treatment will be found [8].

Plants are the important source of diverse range of bioactive principles [9]. The revival of interests in plant derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than expensive synthetic drugs, which have adverse side [10]. In addition herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of new potential drugs effects [11].

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 [12]. 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

2. Materials and Methods

2.1 Collection and authentication of plants: The healthy plants with medicinally potential were collected and shade dried. The accuracy of the plant species was ascertained with Department of pharmacognosy and phytochemistry, KBIPER, Gandhinagar, Gujarat. The names of plants and parts of plant used are shown in Table -1.

Table 1: Selected ethnomedicinal plants and part used

Plant name	Botanical source	Family	Part used
Amla	*Emblica officinalis*	Euphorbiaceae	Fruits
Baheda	*Terminalia bellerica*	Combretaceae	Fruits
Harde	*Terminalia chebula*	*Combretaceae*	Fruits
Ashwagandha	*Withaniasomnifera*	Solanaceae	Roots
Long pepper	*Piper longum*	Piperaceae	Fruits
Tulsi	*Ocimum sanctum*	Liliaceae	Leaves
Vasaka	*Adhatoda vasica*	Acanthaceae	Leaves
Nagarmoth	*Cyperus rotundus*	Cyperaceae	Rhizomes
Rasna	*Alpinia galanga*	Zingiberaceae	Rhizomes

2.2 Preparation of extracts

Preparation of aqueous, hydro-alcoholic and alcoholic extracts of selected plant parts were prepared by separately dried in shade in well ventilated enclosures. Dried plant materials were powdered by using mixer and fine powder of plant parts were obtained through sieving.20 gm. of powder of selected plants were taken to prepare its different extracts. Extracts were prepared by maceration of powder material for 48 hours. Solvents were removed by Rota evaporator. This method was repeated thrice and all extracts were pooled together. The extracts were filtered through a 0.22µm pore sized milli pore filter. Percentage yield were calculated [13].

2.3 Phytochemical screening [14]

Qualitative phytochemical screening of selected plants:

The different qualitative chemical tests were performed for establishing profile of the extract for its chemical composition. The following standard tests were performed on extracts to detect various phytoconstituents present in them.

2.3.1 Test of phytosterols: The extract was dissolved in 2ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tubes colour change shows the presence of phytosterols.

2.3.2 Test for terpenoids: One ml of sample was taken (100µl of compound in 900µl of methanol). 1ml of Conc H2SO4 was added Observed for appearance of red ring.

2.3.3 Test for tannins: One mg of the extract was dissolved in 1ml of sterile distilled water and few drops of 0.1% ferric chloride was added and observed for blue colourization/brownish green.

2.3.4 Test for alkaloids: One mg of the extract was dissolved in 1ml of sterile distilled water. To that 1 ml of dragendorff's reagent was added and observed for prominent yellow colour precipitate.

2.3.5 Test for caumarins: evaporate 5ml of ethanol solution, dissolved the residue 1-2 ml of hot distilled water and divide it in to two parts. Take half the volume as witness and to another half volume add NH4OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of caumarins.

2.3.6 Test for flavonoids: About 0.5 gm of extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium turnings was added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration will indicate the presence of flavonoids.

2.3.7 Test for anthraquinones: One mg of the extract was dissolved in 1ml of sterile distilled water. To that aqueous ammonia was added and observed for change in colour of aqueous layer.

2.3.8 Test for proteins: One mg of the extract was dissolved in 1ml of sterile distilled water. Add 1% of NaOH followed by few drops of CuSO4. If the solution turns purple indicates the presence of proteins.

2.3.9 Test for carbohydrates: Molisch's test: Few drops of molisch's reagent were added to the extract, dissolved in distilled water; this was then followed by addition of 1 ml of concentrated H2SO4 by the side of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers indicates presence of carbohydrates.

2.3.10 Test for saponins: The extract was diluted with distilled water and made up to 20ml. The suspension is shaken in a graduated cylinder for 15 mins. Observed for foam indicates the presence of saponins.

2.4 In-Vitro Anti-Tuberculosis activity [15]

2.4.1 Material required

Bacterial strain: *Mycobacterium smegmatis (MTCC 6)*

Medium used: Lowenstein Jensen Medium (LJ Medium)(Hi-media)

Medium base: Middle Brooke 7H10 Agar Base (Hi-media)

Medium base: Middle Brooke 7H9 Broth (Hi-media)

2.5 Preparation of medium

2.5.1 Lowenstein-Jensen medium (LJ medium)

Suspend 37.24 grams in 600 ml distilled water containing 12 ml glycerol. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lb pressure (121 °C) for 15 minutes. Meanwhile prepare 1000 ml of whole egg emulsion. Aseptically add and mix egg emulsion base in prepared LJ medium. Autoclave the prepared medium at 85°C for 45 minutes. Then add freezed dried culture of *Mycobacterium smegmatis* (MTCC 6) in LJ medium. Incubate it at 37 ºC for seven days for the growth of bacteria [16].

2.5.2 Preparation of bacterial Inoculum

Add one loopful of fresh cultured *Mycobacterium smegmatis* in freshly prepared LJ medium. Incubate prepared medium with bacteria for 24 hrs at 37 ºC. Check the growth of bacteria similar as 0.5 McFarland standards dilution for the In-Vitro assay. If the growth is more than 0.5 McFarland standards dilution then again serial dilute the bacterial culture. Purity of prepared culture was confirmed by Ziehl-Neelsen Staining specific for confirmation of acid fast bacilli.

2.6 The Agar Diffusion Cup Method

This method is used to screen the anti-tuberculosis activity of selected ethnomedicinal plants. Agar plates were seeded with 0.5 McFarland standards bacterial culture of *M. smegmatis*. Agar plates were then bore holed using 6 mm diameter cork bore.100, 500, 1000 µg/ml concentration of extracts were prepared to perform this assay. 0.3 ml each of the extract concentration was introduced into the hole and allowed to diffuse for 5-10 minutes before incubation. The Petri dishes used for antitubercular screening were incubated at 37 ^{0}C for 48 hours. All the concentration was done in triplicate to minimize the error. The inhibition zone diameters (IZD) were determined and recorded for further analysis. Isoniazid and Rifampicin were used as a standard.

2.7 MTT assay [16]

MTT assay, which measures the reduction of the tetrazolium salt MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide; Roche) into a blue formazan product, mainly by the activity of the mitochondrial enzymes, cytochrome oxidase and succinate dehydrogenase.

Freshly prepared bacterial culture (0.5 McFarland standards) were seeded in a flat-bottomed 96-well plate and incubated in a 5% CO2 humid incubator for 24 hours at 37 °C. *M. smegmatis* was treated with different concentrations of plant extracts (1000µg/ml, 500µg/ml and 250µg/ml) and the incubation was continued for 48 hours, followed by adding 20 µl (0.5 mg/ml as final concentration, i.e. 20µl/well of stock) of MTT dye solution to each well for 4 hours at 37 ºC. After removal of the MTT dye solution, bacterial suspension were treated with 200 µl DMSO and the absorbance at 570 nm was quantified using ELISA reader. The cytotoxicity was calculated after comparing with the control (treated with 0.1% DMSO) and Isoniazid + Rifampicin was used as a Positive Standard. All tests and analysis were run in triplicate and mean values recorded

Percentage of bacterial viability was determined as (Avg. OD of treated bacterial count/Avg. OD of control bacteial count) × 100.

3. Result

3.1 Collection and authentication of plants

Fresh plant material was collected from different places. Raw material was subjected to washing with distilled water and then allowed for drying for 5 days under shade and powdered to 60# separately and stored in well close container.

The procured materials were authenticated by taxonomist and further authenticated by comparing the microscopy with reported literature. Herbarium specimen were deposited (PH/15/001 to PH/15/009) at Pharmacognosy department, K.B.I.P.E.R., Gandhinagar.

3.2 Preparation of extracts

Alcoholic, aqueous and 70% hydro-alcoholic extracts were prepared to screen its anti-tubercular activity using different model. % yield of prepared extracts are shown in Table no. 2.

Table 2: % Yield of prepared extracts of selected plants

Sr. No	Name of plant	%Yield of extracts		
		Aqueous	Hydro-alcoholic	Alcoholic
1	Amla	52.92%	63.52%	44.38%
2	Baheda	66.68%	55.16%	42.46%
3	Harde	46.56%	50.58%	45.08%
4	Ashwagandha	24.16%	15.32%	8.91%
5	Long pepper	45.76%	40.78%	21.52%
6	Tulsi	28.21%	21.10%	17.87%
7	Vasaka	13.30%	28.56%	36.26%
8	Nagarmoth	15.46%	10.47%	8.28%
9	Rasna	6.63%	5.92%	8.26%

3.3 Phytochemical screening of selected plants

The extracts were evaluated to detect the presence of various phytochemicals like alkaloids, tannins, resins, glycosides, triterpenes, and steroids etc. using different chemical test to establish its identity. The chemical tests include color reaction test, these tests help to determine the identity of the chemical class. Phytochemical screening of selected plant extracts were shown in Table no. 3.

Table 3: Phytochemical screening of selected plants

Name of plant	Different extracts	phytoconstituents										
		Steroids	Terpenoids	Tannins	Phenols	Alkaloids	Caumarins	Flavonoids	Anthraquinone	Proteins	Carbohydrates	saponins
Amla	Aqueous	-	-	+	+	+	-	-	+	-	+	+
	Hydro-alcoholic	+	+	+	+	+	-	-	+	-	+	+
	Methanolic	+	+	+	+	+	-	-	+	-	+	+
Baheda	Aqueous	-	-	+	+	+	-	-	+		+	+
	Hydro-alcoholic	+	+	+	+	+	-	-	+		+	+
	Methanolic	+	+	+	+	+	-	-	+		+	+
Harde	Aqueous	-	-	+	+	+	-	-	+		+	+
	Hydro-alcoholic	+	+	+	+	+	-	-	+		+	+
	Methanolic	+	+	+	+	+	-	-	+		+	+
Ashwagandha	Aqueous	-	-	+	+	+	-		+		+	-
	Hydro-alcoholic	+	+	+	+	+	-		+		+	-
	Methanolic	+	+	+	+	+	-		+		+	-
Long pepper	Aqueous	-	-	+	+	+	+	-	-	+	+	-
	Hydro-alcoholic	-	-	+	+	+	+	-	-	+	+	-
	Methanolic	-	-	+	+	+	+	-	-	+	+	-

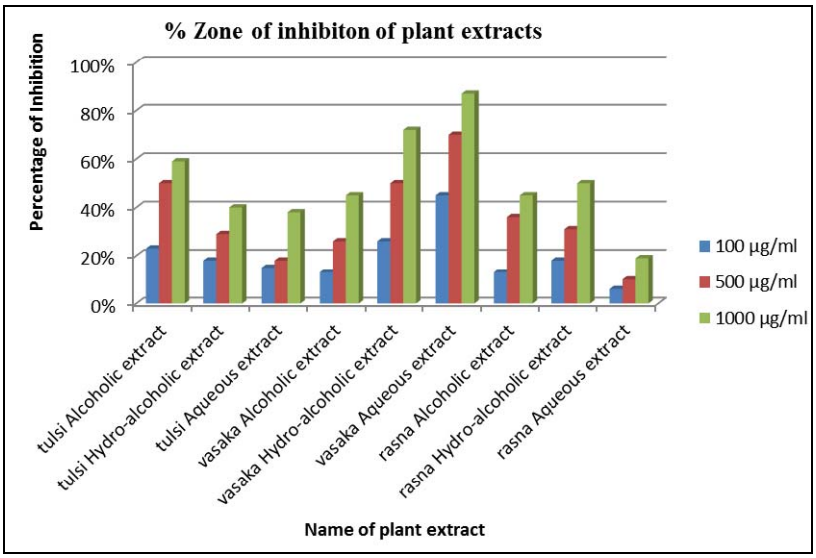
Tulsi	Aqueous	-	-	+	+	+	-	+	-	+	+	+
	Hydro-alcoholic	+	+	-	+	+	-	+	-	+	+	-
	Methanolic	+	+	-	+	+	-	+	-	+	+	-
Vasaka	Aqueous	-	-	+	+	+	-	+	-	-	+	+
	Hydro-alcoholic	-	-	+	+	+	-	+	-	-	+	+
	Methanolic	+	+	+	+	+	-	+	-	-	+	+
Nagarmoth	Aqueous	-	-	+	+	+	-	+	-	+	+	-
	Hydro-alcoholic	-	-	+	+	+	-	+	+	+	+	-
	Methanolic	+	+	+	+	+	-	+	+	+	+	-
Rasna	Aqueous	-	-	+	+	-	-	-	-	-	+	-
	Hydro-alcoholic	-	-	+	+	-	-	+	+	+	+	-
	Methanolic	+	+	+	+	-	-	+	+	+	+	-

3.4 *In vitro* anti-tuberculosis activity of selected plants:

The present study was conducted to investigate the antituberculosis activity of9 medicinal plant extracts against *Mycobacterium smegmatis* species.

3.4.1 The Agar Diffusion Cup Method

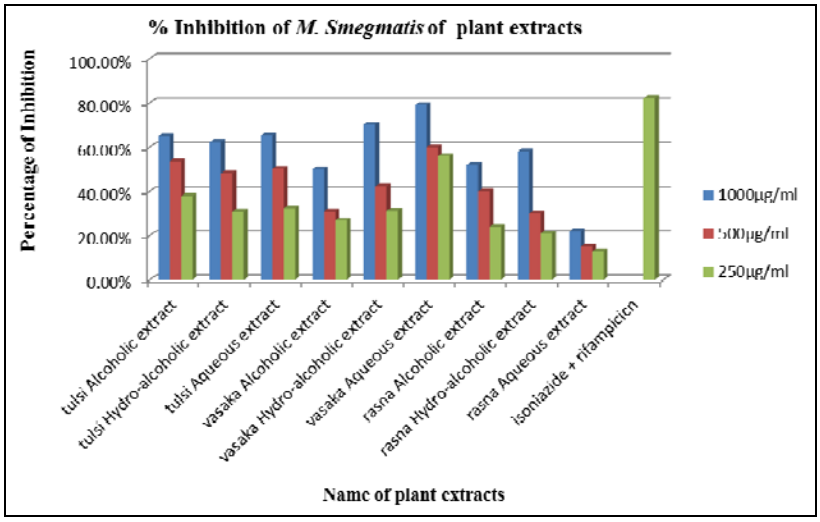
Plants shows significant percentage of zone of inhibition on *Mycobacterium smegmatis* at different concentration is shown in Graph 1. From these plants *Adhatoda vasika* shows maximum anti-TB activity comparing with standard drug.



Graph 1: percentage of Zone of Inhibition of *M. smegmatis* by plant extracts

3.4.2 MTT assay

Plants shows significant percentage of inhibition on *Mycobacterium smegmatis* at different concentration is shown in Graph 2. On the basis of this assay, *Adhatoda vasika, Alpinia galanga and Ocimum sanctum* shows good anti-TB activity. From these all three plants *Adhatoda vasika* shows potent anti-TB activity comparing with standard drug.



Graph 2: Percentage of inhibition on *Mycobacterium smegmatis* of palnt extracts

4. Discussion

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 development of new, effective and affordable anti-TB drugs [17]. 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 [18]. The advantages of using antimicrobial compounds from medicinal plants include fewer side effects, better patient acceptance due to long history of use, reduced costs and cultivability rendering them renewable in nature [19].The present study was conducted to investigate the antituberculosis activity of 9 medicinal plant extracts against *Mycobacterium smegmatis*. The activity of plant extracts against bacteria have been studied for over a century, but work in this area has intensified in the last 3 decades The results obtained showed the strong antimycobacterial activity of some of the extracts, namely, those from *Adhatoda vasika, Ocimum sanctum* and *Alpinia galanga*. The results were comparable to those of the standard drug (Isoniazid and Rifampicin). This may be due to the bioactive constituents, such as Alkaloids, steroids and tannins in Adhatoda vasika [20] and *Ocimum sanctum* [21] and Flavonoids, alkaloids and saponins in *Alpinia galanga* [22]. *A. galanga* is a known wide spectrum antibacterial agent. It has reported the antimycobacterial activity of *galanga* against isoniazid resistant isolates at minimum inhibitory concentration (MIC) of 250µg/ml [23].In our study, three different extracts of *A. galanga* was found to be antituberculosis to *M. smegmatis.* From them hydro alcoholic extracts of *A. galanga* shows strong anti-TB activity at 250 µg/ml. The variation in the active concentrations could be due to differences in the method of extraction and the assay used. The antimycobacterial activity of the essential oils from *A. galanga* has been reported since 1957 [24]. A leaf extract of *A. vasika* was investigated for antibacterial activity using the paper disc and dilution methods. In-vitro screening showed a strong activity of *Adhatoda*'s alkaloids against the bacteria Pseudomonas aeruginosa [25]. A chemical constituent of Adhatoda alkaloids, vasicine, produces bromhexine and ambroxol – two widely-used mucolytics. Both of these chemicals have a pH-dependent growth inhibitory effect on *Mycobacterium tuberculosis* [26]. In our study different three extracts of A. vasika has been screen for their anti-TB activity and aqueous extract of *A. vasika* showed highest anti-Tb activity at the concentration of 100µg/ml. *O. sanctum* is also reported to possess antituberculosis substance [27] and because of these it shows MIC at 250 µg/ml.

5. Conclusion

The present study has proved that all three extracts are bactericidal to *M. smegmatis* at the MIC value. Hence all plants need further evaluation by isolation of molecules that might provide lead compounds for developing a drug to control virulent *M. tuberculosis* inclusive of resistant strain isolates. The present study has revealed the importance of plant extracts to control virulent strains of *M. tuberculosis* which are being a threat to human health and for the development of alternate, safe and effective medicines.

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7. References

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: estimated incidence prevalence and mortality by country. JAMA, 1999; 282:677-686.
2. Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM *et al*. Anti-tuberculosis activity of selected medicinal plants against Mycobacterium tuberculosis. Ind J Med Res. 2010; 131:809-813.
3. Gizachew YE, Giday M, Teklehaymanot T. Antimycobacterial activity of selected Ethiopian traditional medicinal plants used for treatment of symptoms of tuberculosis. GloAdv Res J Med Plants, 2013; 2(2):022-029.
4. Ilango K, Arunkumar S. Synthesis, antimicrobial and antitubercular activities of some novel trihydroxybenzamido azetidin-2one derivatives. Trop J Pharm Res. 2011; 10(2):219-229.
5. Panda VS, Ashar HD, Sharan A. Antioxidant and hepatoprotective effects of Garcinaindica fruit rind in antitubercular drug-induced liver injury in rats. Botanics: Targets and therapy. 2013; 3:29-37.
6. Singh MM. XDR-TB-danger ahead. Ind J Tuberc. 2007; 54:1-2
7. Gordon R, Smith M. Rapidly Growing Acid Fast Bacteria I. Species Descriptions of Mycobacterium phlei Lehmann and Neumann and Mycobacterium smegmatis (Trevisan) Lehmann and Neumann, Journal of Bacteriology. 1953; 66:41-48.
8. Newton G, Fahey R. Mycothiol biochemistry Arch Microbiol, 2002; 178:388-94.
9. Chew AL, Jessica JAJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of Leucasaspera. Asian Pac J Trop Biomed. 2012; 2(3):176-180.
10. Jigna P, Sumitra C. In-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), Vitisvinifera L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr J Biomed Res., 2006; 9:8993.
11. Vinayaka KS, Nandini KC, Rakshitha MN, Martis R, Shruthi J, Hegde SV *et al*. Proximate composition, antibacterial and anthelmintic activity of *Capsicum frutescens* (L.) var. longa (Solanaceae) leaves. Pharmacognosy Journal. 2010; 2(12):486-491.
12. Nguta JM. Journal of Ethnopharmacology 2016; 182:10-15.
13. Jethva Khushboo, Bhatt Dhara, Zaveri Maitreyi. in-vitro Cytotoxicity Activity of Some selected ethnomedicinal Plants Against Vero Cell Line, Int. J Pharm. Sci. Rev. Res. 2016; 37(2):130-133
14. Soundhari C, Rajarajan S. / International Journal of Biological & Pharmaceutical Research. 2013; 4(6):427-432.
15. Khushboojethva, Dhara Bhatt, Maitreyi Zaveri. I*n- vitro* anti-tuberculosis activity of selected ethnomedicinal plants, International Journal of Herbal Medicine. 2016; 4(4):126-128.
16. Sadeghi I. *In vitro* Cytotoxic and Antimicrobial Activity of Essential Oil from Satureja Intermedia, Iran Red Crescent Med J 2013; 15(1).
17. Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of antimycobacterial agents. J Ethnopharmacol 2007; 110(2):200-234, 5.
18. Mmushi T, Masoko P, Mdee L, Mokgotho M, Mampuru L, Howard R Antimycobacterial evaluation of fifteen medicinal plants in South Africa. Afr J Tradit Complement Altern Med 2010; 7(1):34-39
19. Gur S, Turgut-Balik D, Gur N. Antimicrobial activities and some fatty acids of turmeric, ginger root and linseed used in the treatment of infectious diseases. J Agr Sci. 2006; 2:4
20. Gaurav Kumar Sharma. Studies on Phytochemical Constituents of Medicinal Plants, AJPPS. 2014; 1(4):61-74
21. Ashish Ranjan Singh. Phytochemical estimation and

Antimicrobial activity of Aqueous and Methanolic extract of *Ocimum Sanctum*, J. Nat. Prod. Plant Resource. 2013; 3 (1):51-58
22. Thokcom Sharatchandra Singh. Preliminary phytochemical screening and ditermination from rhizome and flower of *Alpinia galanga*, wjpps, 3(11):1354-1361.
23. Soundhari C, Rajarajan S. *In vitro* screening of lyophilised extracts of *Alpinia galanga* L. and *Oldenlandia umbellata* L. for antimycobacterial activity. Int J Bio Pharma Res 2013; 4(6):427-432.
24. Chopra LC, Khajuria BN, Chopra CL. Antibacterial properties of volatile principles from *Alpinia galanga* and Acorus calamus. Antibiot Chemother. 1957; 7:6.
25. Patel VK, Venkatakrishna BH. *In vitro* study of antimicrobial activity of *Adhatoda vasika* Linn. (leaf extract) on gingival inflammation a preliminary report. Indian J Med Sci. 1984; 38(4):70-2.
26. Narimaian M, Badalyan M, Panosyan V, Gabrielyan E, Panossian A, 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. Phytomedicine. 2005; 12(8):539-47.
27. Gupta KC, Vishwanathan R. Antibiotics and Chemotherapy. 1955; 5:22-23.