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A Therapeutic Approach for Study of Anti-tubercular Activity of Selected Medicinal Plants using NRA Methods

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ABSTRACT

The present investigation was carried out to determine the possible bioactive components of the several medicinal plants for their Antimycobactrial activity and GC-MS. The chemical compositions of the ethanol and methanol extracts of the five plants and five essential oil plants were investigated using Hewlett-Packard Gas chromatography–Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the Wiley and National Institute of Standards and Technology (NIST) library or with the published mass spectra. Maximum activity was observed in alcoholic extracts of Tinospora cordifolia, Sida rhombifolia, Morinda citrifolia, Pithecellobium dulce, Allium sativum,, Pinus roxburghii against Mycobacterium tuberculosis and minimum Nardostachys jatamansi, ,Trachyspermum ammi, Lantana camara against Mycobacterium tuberculosis.

Introduction

Herbal medicines are prepared from a variety of plant materials as leaves, stems, roots, bark etc. They may usually contain biologically active ingredients and are used primarily for treating mild or chronic ailments. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries. Viral warts are extremely common and most people suffer from one or more at some point during their life (Kokate et al, 2008 and Anand et al, 1997)

In recent years, attention has been devoted to novel molecules derived from natural sources that exhibit a range of clinical and pharmacological activities. These bioactive have been extra nutritional constituents occur in small quantities and diverse in chemical structure and function such as phenols, alkaloids, steroids, lignins, tannins etc. Initially on the basis of their function and biosynthesis these were divided into primary and secondary metabolites. But now, it is well established that secondary metabolism is an integral part of the plant cell metabolism wherein specialized proteins and cells are involved. There is a thin line between primary and secondary metabolites as they are linked together and integrated in various metabolic pathways (Seigler, 1998).

Tuberculosis (TB) is an infectious disease, caused by the bacterium called Mycobacterium tuberculosis. It was first isolated by Robert Koch in 1882 (Ait- Khaled and Enarson, 2003). At the time, TB was rampant, causing 1/7 of all deaths in Europe and 1/3 of deaths among productive young adults (Prescott et al, 2005). Today TB remains a problem of global importance. Among communicable diseases, TB is the second leading cause of death worldwide killing 2 million people each year (Frieden et al, 2003).

The upsurge of TB cases has been noticed in developing countries (WHO, 2003). In India, like in most other developing countries, the tuberculosis situation has worsened over the past few years. Several factors have been associated with the TB upsurge which have a distinct difference in symptoms from earlier out-breaks of the disease.

Since no anti-TB drugs have been introduced in past 30 years, there is an urgent need to search for and develop new, effective and affordable anti-TB drugs. In this scenario, the plant kingdom with enormous chemical diversity may be looked as an important source of new anti-TB agents. Among 17,500 higher plant species occurring in India only about 365 species have been evaluated so far for antimycobacterial activity which has been mentioned in ethnomedicine and Ayurveda. In recent years, there has been target interest in biologically active constituents, isolated from plant species for the elimination of pathogenic micro-organisms, because of the resistance that micro-organisms have built against antibiotics or because they are ecologically safe compounds.

A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literatures (Salvat et al, 2001; Geyid et al, 2005). The present study was to screen the anti-mycobacterial activities of ten local medicinal plant extracts; Alliium sativum , Tinospora cordifolia , Sida rombifolia, Terchyspernum ammi ,Morinda cordifolia, Jatamanshi, Eucalyptus globullus ,Pinus roxbergaii, Lantana camara Pithelobium dalci.

The medicinal plants investigated in the family Malvaceae are largely represented and some of these plants such as Sida rhombifolia L. (Malvaceae). A decoction of this Malvaceae is locally used in the treatment of coughs, rheumatic and abdominal pain, and diarrhea while the leaf decoction is used in the treatment of fever and to prevent miscarriage (Holm et al, 1997, Howard, 1989, Liogier, 1994).

Sida stems are used as rough cordage, sacking, and for making brooms. The stems have a high quality fiber and were once exported from India and elsewhere as “hemp” Chemical analysis revealed that the leaves contain respectable amounts of

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nutrients: 74,000 to 347,000 ppm protein, 94,000 to 475,000 ppm carbohydrates, 33,000 to 167,000 ppm fiber, 14,000 to 71,000 ppm fat, and 16,000 to 81,000 ppm ash. However, it was reported that the root contained 450 ppm alkaloids and the presence of ephedrine and saponin (Southwest School of Botanical Medicine 2002). Another source reports alkaloid content in the root of 0.1 percent and the presence of choline, pseudoephedrine, betaphenethylamine, vascin, hipaphorine and related indole alkaloids (Shaman Australis Ethnobotanicals 2002). Perhaps because of these chemicals, the plant is unpalatable to cattle (Kuniata and Rapp, 2001). Sida has significant medicinal applications for which it is cultivated throughout India.

Tinospora cordifolia commonly named as "Guduchi" in Sanskrit belonging to family Menispermaceae is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude (Rana et al, 2012; Parthipan et al, 2011). A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides (Upadhyay et al, 2010) have been isolated from the different parts of the plant body, including root, stem, and whole plant. Recently, the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. In the present investigation, we focus on its clinical importance of selected medicinal plants for their biocompatibility.

T. cordifolia has inhibited the in vitro growth of Mycobacterium tuberculosis at 1:50,000 dilution (Gupta and Viswanathan,1956) Its ethanolic extract has exhibited significant antipyretic activity in experimental rats. 'Septilin' syrup, a compound preparation containing T. cordifolia (7.82% in 5 ml of syrup) was found to elicit good clinical response in children suffering from upper respiratory tract infection and chronic otitis media (Vedavathy and Rao, 1991)

Pithecellobium A medium sized tree with compound leaves and spinous stipules, a good fodder tree for camel and goats. Saline extract of seeds shows hemolytic agglutinating reactions with human blood, yields a edible oil from seeds. Dr. S.D. Shanmugakumar has recently indicated that the leaves extract of this plant is very efficacious against M. tuberculosis and could go a long way in eradicating tuberculosis. Pithecellobium dulce (P. dulce) Benth (Fabaceae) is a small to medium sized, evergreen, spiny tree, up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil .

(http://www.merinews.com/article/manila-tamarind-could-help-treat-tuberculosis/15765533.shtml)

The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also used in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifacient and antidiabetic properties. The presences of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported in the seeds. The bark contains 37% of tannins of catechol type. Quercetin, kaempferol, dulcitol and afezilin have been reported from the leaves (Nigam et al, 1997).

The Verbenaceae family comprises about 100 genera and 2000 species distributed in tropics and subtropics, mainly in temperate zone of southern hemisphere (Joly, 1993) Lantana is a genus of about 150 species that are very popular as popular ornamental garden plant (Ghisalbarti, 2000) Lantana species are known to are virtually immune to herbivory owing to the presence of a wide array of photochemical of diverse groups (Ravindar et al, 2006) The plants have been used in many parts of the world to treat a wide variety of disorders as antirheumatic, stimulant, sudoriparous, to treat bronchitis and asthma and in biologic control (Dua et al ,1996).

Various uses of L. camara have been reported. It is mainly used as herbal medicine but other uses like providing a source of firewood, mulch, making hedges, and use as a source of microbicides, fungicides and nematicides, insecticides have also been reported. Chemical compounds isolated from extracts of leaves of L. camara are reported to have shown to exhibit antimicrobial, fungicidal, insecticidal and nematicidal activity. There are also reports that lantana compounds isolated from the extracts can be applied as weed killers verbacoside, a compound isolated from lantana extract has been demonstrated to possess anti microbial immunosuppressive and anti tumour activities. Use of lantana oil in treatment of skin itches and as an antiseptic for wounds and externally for leprosy and scabies is also reported along with role in cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera (Jimenez-Arellanes et al , 2003). The plant Morinda citrifolia Linn commonly known as noni belongs to the Rubiaceae family. Reports of the medicinal properties of this plant have been found in ancient literature as well as in traditional folklore (Marderosian ,1999) The flowers, leaves, fruits, barks and roots of the plant have been reported to have antibacterial, anti-viral, anti-fungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory and immune-enhancing effects (Wang et al, 2002)

An exhaustive survey of the reported uses of Morinda citrifolia revealed in the treatment of tuberculosis, diabetes, heart ailments and high blood pressure. In addition, its immunostimulant potential has led to its use in the prevention of AIDS, prevention of Epstein-Barr virus activation and as an anticancer agent6. The present investigation was carried out to study the physicochemical and phytochemical characteristics of M. citrifolia fruits. Additionally, chromatographic fingerprints of the extracts were also obtained using suitable solvent systems (Burt, 2004; Celiktas et al, 2006; Skocibusic et al, 2006).

Essential oils are generally liquid, aromatic and possess pleasant odour and essence. The term "essential oil" is often used in cosmetics and perfume industries as synonymous with perfume oil, base or, "compound". Chemically, the essential oils are a complex and highly variable mixture of constituents that belong to two groups: terpenoids and aromatic compounds. The name terpene is derived from the English word "Turpentine" (Guenther, 1952; Guenther,1985). The terpenes are the unsaturated hydrocarbons which have a distinct architectural and chemical relation to the simple isoprene molecule (CH2==C(CH3)—CH==CH2).

E. globulus is a tall tree more than 60m high, volatile oil derived from leaves highly medicinal, especially in diseases of lungs and respiratory tract. The leaves and oils of many Eucalyptus species are especially used for respiratory ailments such as bronchitis and croup (Kasper et al ,1994) and the dried leaves are smoked like tobacco for asthma in some countries. Some of the Eucalyptus species are also used for feverish conditions e.g.(malaria, typhoid, cholera) and skin problems like burns, ulcers and wounds. Aqueous extracts are used for aching joints, bacterial dysentery, ringworms, tuberculosis, etc.

Table 1. Formulation of Various Extracts and Oils

S.no	Plant Name	Extracted in	Part use	Volume	Sample (Nature)	Boiling point
1	Sida rhombifolia	Methanol	Whole plant	900 gm+6 lt.	Decoction	65°c
2	Tinospora cordifolia	Ethanol	Stem	800gm +5 lt.	Decoction	105°c
3	Pithecellobium dulce	Methanol	leaves	600gm+ 5 lt.	Decoction	100°c
4	Morinda citrifolia	Ethanol	leaves	700gm+ 5lt.	Decoction	100-125°c
5	Lantana camara	Methanol	Leaves	900gm+ 6lt.	Decoction	60-240°c
6	Allium sativum	water	Bulbs	4kg	oil	248°c
7	Eucalyptus globules	water	leaves	3kg	oil	176-177°c
8	Turpentine	water	Stem bark	4kg	oil	155-185°c
9	Jatamanshi	water	Roots	5kg	oil	288-292°c
10	Ajwain	water	Seeds	2kg	oil	176-177°c

Table 2. Showing Potency of Selected Medicinal Plants

	Plant drug	Extract	7^{th} Day	10^{th} day	14^{th} Day	Drug	Media+ OADC	Inoculum	Drug+ DMSO
1	Garlic	Oil	No color	No color	No color	Effective	200µl	100µl	200µl
2	Ajwain	oil	Light pink	Light pink	Light pink	Less Effective	200µl	100µl	200µl
3	Jatamanshi	oil	Light pink	Light pink	Light pink	Less Effective	200µl	100µl	200µl
4	Eucluptus	oil	Dark pink	Dark pink	Dark pink	No effect	200µl	100µl	200µl
5	Terpentine	oil	No color	No color	No color	Effective	200µl	100µl	200µl
6	Giloy	Decoction	No color	No color	No color	Effective	200µl	100µl	200µl
7	Bala	Decoction	No color	No color	No color	Effective	200µl	100µl	200µl
8	Lantana	Decoction	Light pink	Light pink	Light pink	Less Effective	200µl	100µl	200µl
9	Noni	Decoction	No color	No color	No color	Effective	200µl	100µl	200µl
10	Jungle jalebi	Decoction	No color	No color	No color	Effective	200µl	100µl	200µl

Table 3. GC-MS of the Compounds Identified in Garlic Oil.

peak	R. time	Area	Area %	Name
1.	8.637	8834851	1.28	Diallyl sulfide
2.	12.265	45545708	6.61	Dithiane-1,3
3.	12.454	2310631	0.34	Dimethyl phosphonodithioite
4.	15.357	22195293	3.22	Methanesulfonothioic acid
5.	15.519	19576268	2.84	Methyl methanethiolsulfonate
6.	17.866	6777214	0.98	Allyl disulphide
7.	18.613	261315454	37.91	Diallyl tetrasulphide
8.	18.705	41470825	6.02	1,4-Diallyltetrasulfane
9.	19.854	3277575	0.48	Dimethylthioacetamide
10.	21.919	17284930	2.51	Methyl allyl sulfide
11.	26.059	3123641	0.45	Allicin
12.	27.800	251119137	36.43	Allyl trisulfide
13.	39.895	3299502	0.48	Durophenol
14.	44.495	3245647	0.47	Spathulenol
		689376676	100.00	

Table 4. Garlic oil GC Table

Peak	Ret. Time	Area	Area %	Name
1.	8.843	2550771	2.2860	Diallyl sulfide
2.	12.291	7648279	6.8544	Dithiane-1,3
3.	12.501	391415	0.3508	Dimethyl phosphonodithioite
4.	15.301	1786352	1.6009	Methanesulfonothioic acid
5.	15.501	833000	0.7465	Methyl methanethiolsulfonate
6.	17.790	1450312	1.2998	Allyl disulphide
7.	18.501	57770001	51.7733	Diallyl tetrasulphide
8.	18.701	5808419	5.2055	1,4-Diallyltetrasulfane
9.	19.924	793179	0.7108	Dimethylthioacetamide
10.	21.780	6054259	5.4258	Methyl allyl sulfide
11.	26.047	688592	0.6171	Allicin
12.	27.644	24588894	22.0365	Allyl trisulfide
13.	39.668	440760	0.3950	Durophenol
15.	44.353	778415	0.6976	Spathulenol
Total		111582648	100.0000	

They are applied for similar reasons in both western and eastern medicine. The Eucalyptus oils and their main component (1,8-sineole) are largely used in the preparation of liniments, inhalants, cough syrups, ointments, toothpaste and also as pharmaceutical flavours in veterinary practice and dentistry. While being used as fragrance component in soaps, detergents and toiletries, they have little use as perfumes. The oils of Eucalyptus species have also antioxidant properties (Grassmann et al, 2000) and anti-inflammatory effects because of 1,8-cineole.(Juergens, 2003).

Nardostachys Jatamansi DC, is a perennial herb, belongs to the family Valerianceae and commonly found in Himalayas. The plant has a rich history of medicinal use and has been valued for centuries in Ayurvedic and Unani systems of medicine. It is used as a stimulant, antiseptic, insect repellent and for the treatment of epilepsy, hysteria, convulsive affections, stomachache, constipation and cholera. The essential oil of Jatamansi also has medicinal properties. In combination with cold water, the oil is considered to be effective against nausea, stomachache, liver problems, jaundice, kidney complaints, insomnia and headache. Externally, the oil is added to a steaming bath to treat inflammation of the uterus. The oil is also used in eye compounds and as poison antidotes. Oil is reported to be useful in the treatment of atrial flutter (Amritpal et al, 2009; Chatterjee et al ,2005).

Allium sativum L., commonly known as garlic, is a species in the onion family Alliaceae. Its close relatives include the onion, shallot, leek, and chive. Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking.A bulb of garlic, the most commonly used part of the plant, is divided into numerous fleshy sections called cloves. The cloves are used as seed, for consumption (raw or cooked), and for medicinal purposes. The leaves, stems , and flowers (bulbils) on the head (spathe) are also edible and are most often consumed while immature and still tender. The papery, protective layers of "skin" over various parts of the plant and the roots attached to the bulb are the only parts not considered palatable.

The widespread use of garlic (Allium sativum L.) as a flavoring agent in food is well known. Garlic is also known to have medicinal properties. Recent studies have shown that garlic contained active components which plays major role in lowering the blood glucose and lipid levels in humans (Bordia et al, 1975) and in animals. The essential oil of garlic prevented lipid accumulation in the aorta and showed protective effects against atherosclerosis in rabbits fed an atherogenic diet (Jain et al, 1973). Oral administration of garlic to humans depressed platelet aggregation (Munday et al, 1999). The garlic oil supplements in human subjects lead to the increased resistance of low density lipoprotein to oxidation and may be one of the powerful mechanisms accounting for the antioxidative and anti-atherosclerotic properties of garlic (Lau, 2001).

The inhibitory effect of garlic on M. tuberculosis has been mentioned in clinical reports for nearly 100 years; however, the only previous laboratory evaluation was performed by Rao et al. in 1946 for a single strain of M. tuberculosis. In this study we were able to confirm their quantitative determination of the concentration required to inhibit M.tuberculosis and to establish evidence of the inhibitory nature of garlic extract on species of Mycobacteria.

Trachyspermum ammi The present invention is the consequence of planned experimentation through specific activity bio-evaluation assays. The intent of the investigation has been to ascertain and evaluate the potential of plant compound thymol from the oil of `Ajwain` as the advanced generation antibiotic and development of a herbal antibiotic formulation with enhanced activity particularly the activity of killing drug resistant bacteria. T.ammi belongs to the family umbelliferae and is known as a popular aromatic herb and spices. The use of herbs as complementary and alternative medicine has been increased dramatically in the last 20-25 years (Rios and Recio, 2005). The fruit of T.ammi has been used in cooking and as medicine to control in digestion and flatuene. It is used for colic, diarrhea, antimicrobial and other bowel disorder and in the treatment of asthma (Kaur and Arora, 2009).

T.ammi tested positive for sterols and terpenes. T. ammi also used as spice and condiments in foods for their flavor, aroma and preservation and their dried ripe fruit and essential oils have aromatic carminative, stomach and diuretics properties. It is also traditionally known as a digestive aid, relieves abdominal discomfort due to indigestion and antiseptics. Many assume that it relives colic in babies and for improves digestion and appetite. Antimicrobial potential of different medicinal plants was being studied all over the world (Ahmed et al, 1998).

Pinus roxburgii is a tall tree, with a basal trunk, about 10-15m in height, with a pyramidal crown of branches with needle-shaped leaves. The wood, stem, cortex and leaves contain resin canals. The resin consists of a solid substance rosin consists and the volatile oil, called turpentine oil which is the medicinal components .The most volatile components of turpentine are two terpenes: alpha (α) and beta (β) pinenes. They are the dominant odorous compounds emitted by trees, shrubs, flowers and grasses (Ennifar et al , 2001).

Materials and Methods

The shade dried plant parts of all experimental plants were collected from Botanical garden Department of Botany, University of Rajasthan Jaipur. Various plant parts (1 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (940 g). The powdered plant sample was packed into a Soxhlet apparatus (5L) and extracted exhaustively with 2L Alcoholic products (Ethanol/ Methanol) for 72 hours on water bath. The Ethanol/ methanol extract was concentrated using a rotary evaporator at 45°C and left on the laboratory bench for 2 days to obtain a dark-brown liquid, further the compounds were separated by column chromatography. Finally isolated compounds were characterized by GC-MS.

Oil Isolation procedure

Samples of the plants (50-70 g, three times) were subjected to hydro-distillation for 2.5h using a Clevenger-type apparatus. The oils separated from water and dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

Evaluation of anti-tubercular activity

Anti-tubercular testing against the standard strain of M. tuberculosis H37RV (Padma et al, 2012).

Inoculation and Cultivation of Mycobacterium tubercle

Pure culture of standard strain M. tuberculosis (H37RV) was inoculated in freshly prepared Loweinstein-Jensen (LJ) slant and incubated at 37°C for 2-6 weeks for cultivation until growth of M. tuberculosis was observed. Identified and confirmed the colonies of MTB by colony characteristics, Ziehl Neelsen staining and biochemical reactions (Godkar and Godkar, 2003).

Gas Chromatography Mass Spectroscopy (GC-MS)

The extract and the standard samples were analyzed by GCMS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with a HP-5. Capillary column

(phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40 °C/min; detector temperature, 250 to 280°C; carrier gas, Helium (0.9 ml/min). Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectrain Table no.2

Gas Chromatography (GC) for Oil

Quantitative analysis of the essential oil of Allium Sativum was carried out using a Shimadzu GC-2010. Nitrogen was used as carrier gas at 10 psi inlet pressure with FID and AB inno-wax column (60 m x 0.25 mm id, film thickness 0.25 µm). Injector and detector temperatures were 270°C and 280°C respectively. Column temperature programmed from 70°C to 230°C at 3°C/min with hold time of 3 min and 14 min. respectively. The flow rate of carrier gas was 1.21ml/min and split ratio was 1:80. The data were processed on GC solutions software for oil composition.

GC-MS Analyses for oil

GC-MS data was obtained on a Shimadzu GCMS-QP-2010 plus system using AB inno-wax column (60 m x 0.25 mm id, film thickness 0.25 m). Injector, mass detector and Ion source temperatures were 260°C, 280°C and 250°C respectively. Column temperature programmed from 50°C to 230°C at 3°C/min with hold time of 2 min and 20 min. respectively. The flow rate of carrier gas was 1.2 ml/min and split ratio was 1:80. Helium was used as carrier gas. EI source and mass range were 70 eV and 40-650 amu. Compounds were identified by using Willey, NIST and Perfumery libraries.

Result

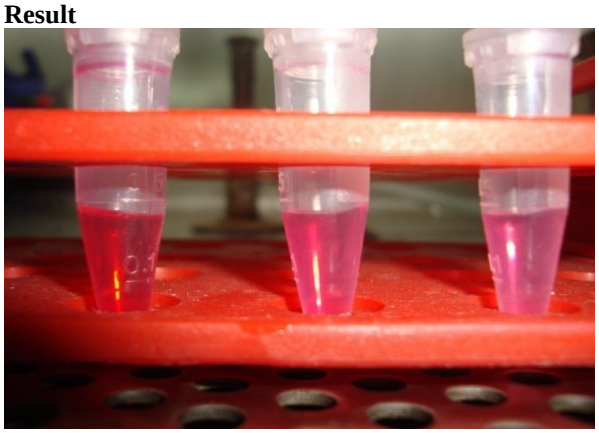

Fig 1. Showing Control For Samples Without Drug

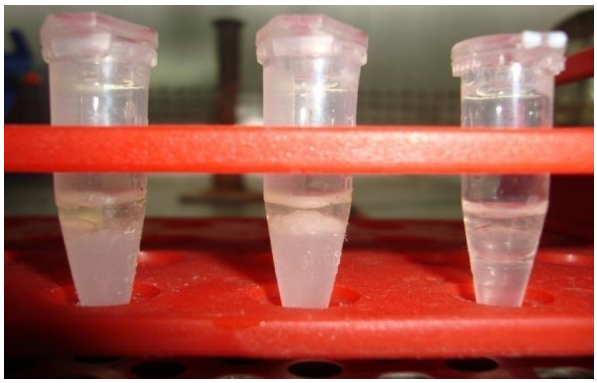

Fig 2. Garlic oil showing transparent color denoting potent activity against the strain

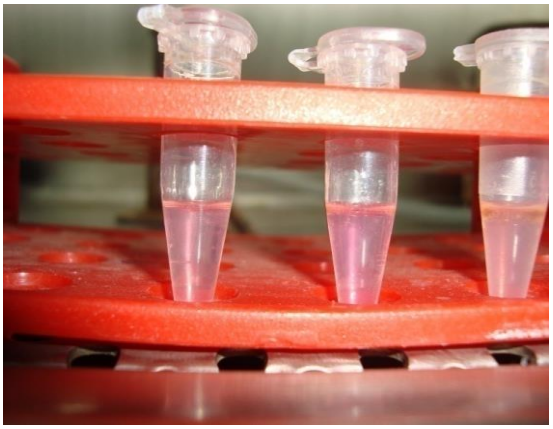

Fig 3. Jatamansi oil Denotaing Slightly Pinkish Colour Showing Less Effective Drug

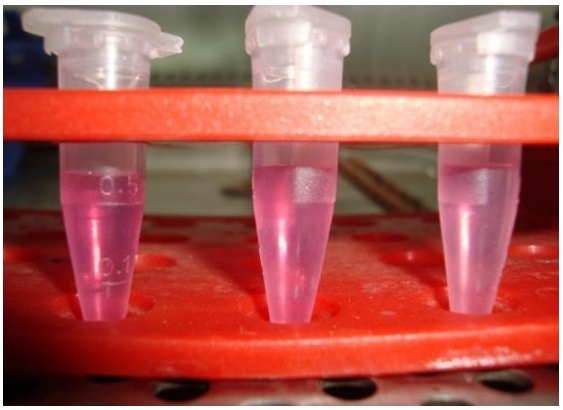

Fig 4. Eucalyptus Oil Showing No Activity

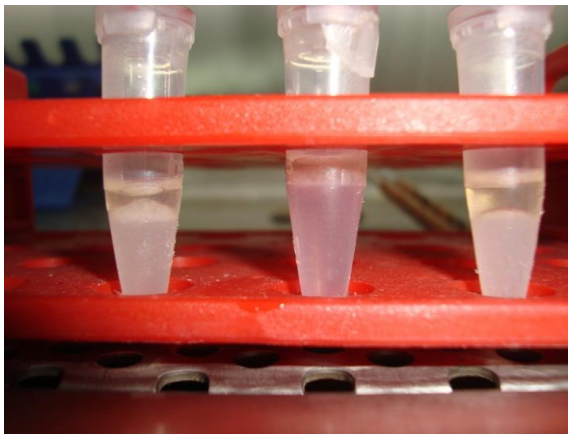

Fig 5. Terpentine Show Little Resistance of the Colony

Fig 6 . Ajwain oil Show Less Resistance of the Colony

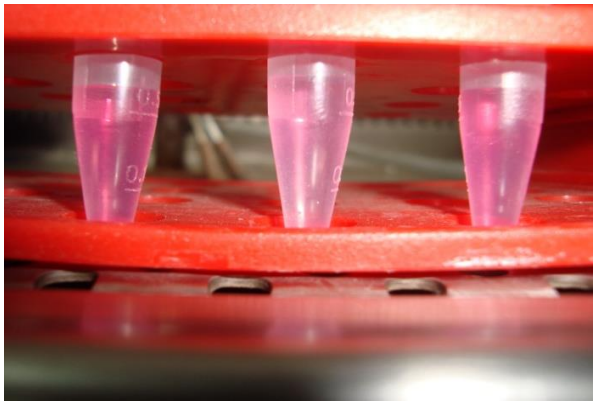

Fig 7. Lanata camara extract show less Resistance of the colony

Fig 8. Tinospora cordifolia extract show potent effect on the colony

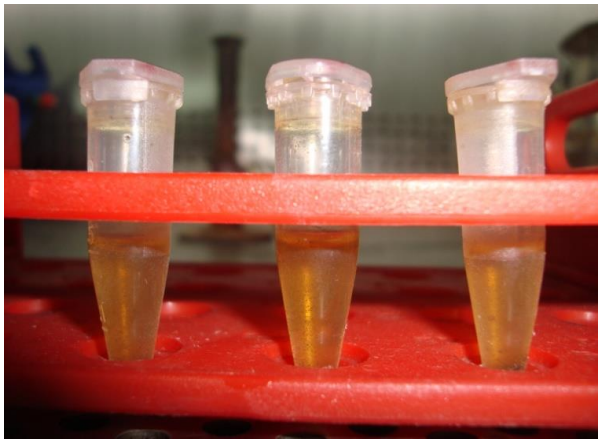

Fig 9. Pithecelobium Dulce Extract Show Potent Effect on the Colony

Fig 10. Sida Rhombifolia Extract Show Potent Effect on the Colony

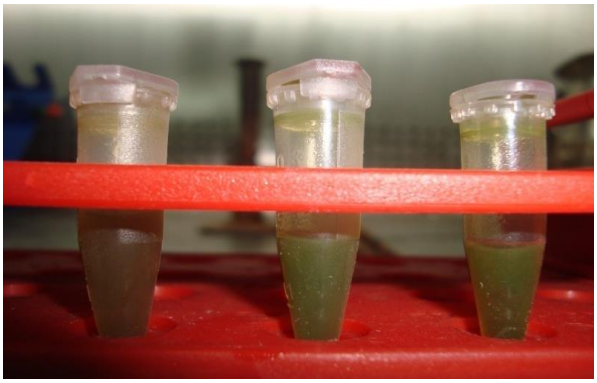

Fig 11. Morinda Citrifolia Extract Show Potent Effect on the Colony

Results and discussion

It is clearly evident from the data presented in above tables that the Indian medicinal plants have great potential to be used as anti-TB agents. The data illustrates that extracts of plant species from wide range of families and genera have exhibited significant in vitro antimycobacterial activities and a number of active plant-derived compounds belonging to different chemical classes have been isolated. There is a strong positive correlation between the antimycobacterial activity results and the traditional knowledge on plants used for TB and TB-related diseases in Ayurveda and the ethnomedicine. In the light of modern science, the efforts should be made to identify and characterize the active constituents from these plants. As mentioned earlier, globally around 2 million people die annually due to TB, these findings may help the scientists to undertake the project in the search of novel natural product leads useful for new anti-TB drug discovery and development. The anti mycobacterial activity showed that extracts and oils has the potential to cure tuberculosis and is a promise for future therapeutic interventions. The results assume significance and throw some light on the basis of the ancient use of the tree in our traditional systems of medicine and in folklore. Further detailed phytochemical screening and bio activity studies need be carried out using crude solvent extracts as well as further purified constituents to comprehend their role in anti-tuberculosis activity and develop suitable drugs so that the most deadly disease in the world can be combated. The present study also could pave the way towards possibility to obtain anti mycobacterial moieties against other Mycobacterial species

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