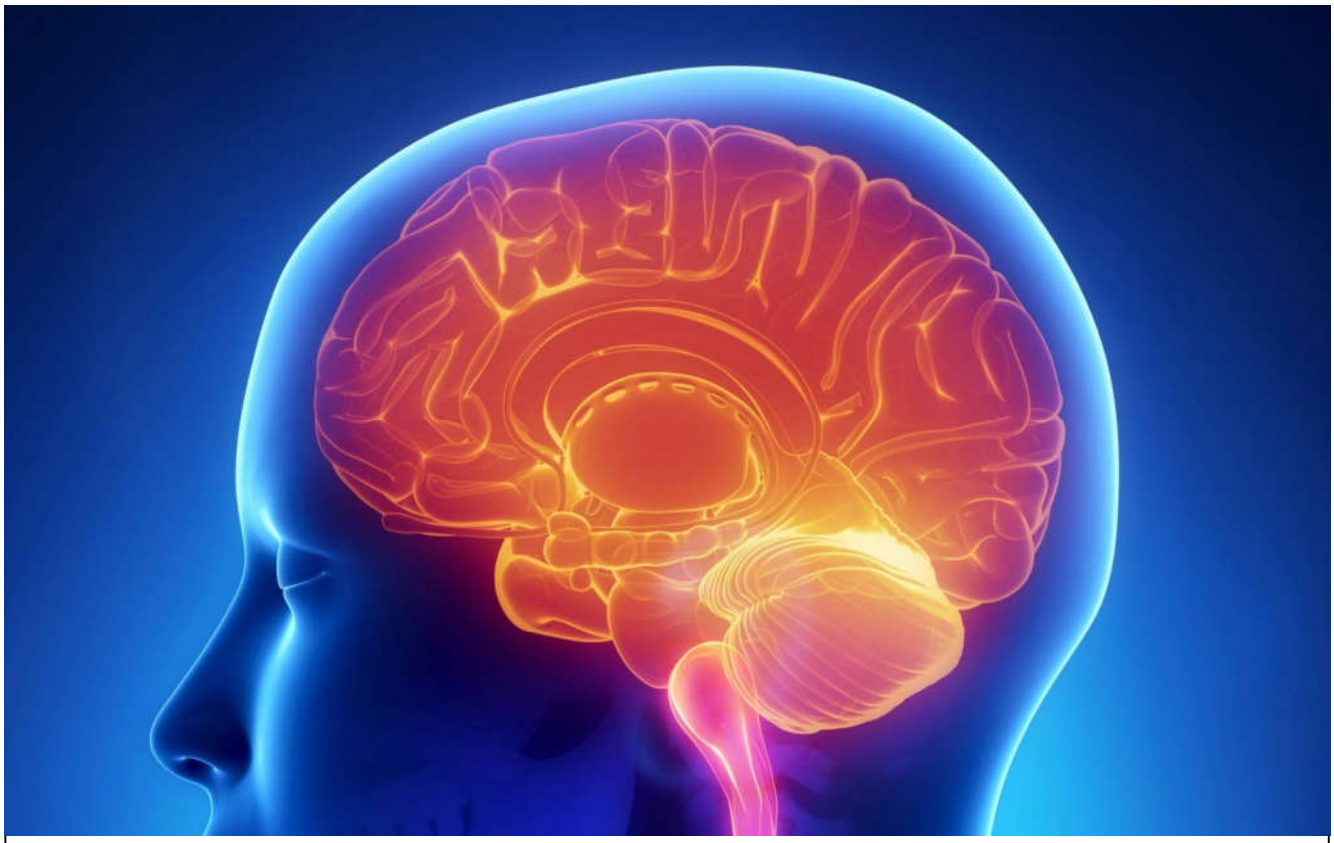

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Research Article

SCREENING OF SELECTED MEDICINAL PLANTS FOR THEIR ANTI-TUBERCULAR ACTIVITY USING NRA METHOD

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ABSTRACT

Our earlier investigations was carried out to determine the possible bioactive components of the several medicinal plants for their Antimycobactrial activity using LJ method. In the present research we are screening these plants which are known for their oil yielding capacity and have potent activities through NRA Methods. Maximum activity was observed in *Allium sativum,, Pinus roxburghii* against *Mycobacterium tuberculosis* and minimum *Nardostachys jatamansi, Trachyspermum ammi against Mycobacterium tuberculosis*

INTRODUCTION

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991). The discovery of medicinal plants in different parts of the world is important both to the agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Some of these plants are commonly used and have been used by people as folk medicine for hundreds of years (Herbal Medicine Research Centre, 2005). The control of bacterial infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents (Mitscher *et al.*, 1987).

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

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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 five local medicinal plant extracts; *Alliium sativum, Terchyspernum ammi, Jatamanshi, Eucalyptus globullus, Pinus roxbergaii*.

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).

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. 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* done using protocol (Golysheyskaia *et al*., 1996)

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 using established protocol of (Godkar and Godkar, 2003).

Principle of the test

Colorimetric methods for detecting drug resistance in *M. tuberculosis* are based on the reduction of an oxidation-reduction indicator added to a liquid culture medium after *M. tuberculosis* has been exposed *in vitro* to different antibiotics. Resistance is detected by a change in colour of the oxidation-reduction indicator, which is directly proportional to the number of viable mycobacteria in the medium.

Preparation of the Middlebrook 7H9-S broth

7H9-Supplemented (7H9-S) = 7H9 broth + 10% OADC+ 0.5 % glycerol. For 200ml preparation of 7H9- media. 0.94 g of 7H9 powder was weighed and dissolved in 180 ml of distilled water; mix until complete solubilization. Further 20 ml of OADC was added (oleic acid dextrose catalase) enrichment and 1 ml of sterile glycerol was mixed. Medium was protected from direct light at 4°C.

Preparation of Stock solution

A 500 mg quantity of the plant extracts was weighed and dissolved separately in 5ml each of the solvent (20% dimethylsulfuroxide) to a concentration of 100 mg/ml. The stock solution was doubly diluted to a concentration of 200 µl /ml in each set of tubes. Further different plant samples having essential oils were dissolved in DMSO and decoction were prepared along with DMSO of some samples as they were not dissolved in DMSO.

RESULT

Table Showing Potency of Selected Medicinal Plants

RESULTS AND DISCUSSION

The use of herbs and other alternative therapies for the treatment of tuberculosis is on the increase. Natural products continue to play a most significant role in the drug discovery

S.N	Plant Drug	Extract	7th Day	10th Day	14th Day	Drug	Media+OADC	Inoculum	Drug+ DMSO
1.	*A. sativum*	Oil				+	200µl	100µl	200µl
2.	*T.ammi*	Oil				±	200µl	100µl	200µl
3.	*N.jatamanshi*	Oil				±	200µl	100µl	200µl
4.	*E.globules*	Oil				-	200µl	100µl	200µl
5.	*P.roxburghii*	Oil				+	200µl	100µl	200µl

Light Pink (Indicates less development of growth of *M. tuberculosis*)
Dark Pink (Indicates development of growth of *M. tuberculosis*)
No colour change (No growth of *M. tubercolosis*)

\+ Indicates drug is effective against *M. tuberculosis*
± Indicates drug is less effective against *M. tuberculosis*
– drug is not showing antitubercular activity.

And development process (Newman and Cragg, 2007). And plants are recognized as a useful source of highly active antimycobacterial metabolites (Gibbons, 2005; Pauli *et al*., 2005).

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

The present study also could pave the way towards possibility to obtain anti *Mycobacterial* moieties against other *Mycobacterial* species

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