A
Ph.D. SYNOPSIS / WORK PLAN

on

PHYTOCHEMICAL EVALUATION AND HPTLC FINGER PRINTING OF SOME MEDICINAL PLANTS IN RELATION TO ANTIMICROBIAL ACTIVITY

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Ph.D. SYNOPSIS / WORK PLAN

Abstract of proposed work plan/ problem:

PHYTOCHEMICAL EVALUATION AND HPTLC FINGER PRINTING OF SOME MEDICINAL PLANTS IN RELATION TO ANTIMICROBIAL ACTIVITY

1 INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country (Ahmed, 2001). Phytochemicals are chemical compounds synthesized during the various metabolic processes. Various phytochemical possess a variety of pharmacological and antimicrobial activities. They are known to posses various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which are therefore, should be utilized to combat the disease causing pathogens. Some of these serve as plant defense mechanisms against pathogenic organisms. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for control of pathogens. The purpose of this study is to investigate the active principles of selected medicinal plants. This may lead to the discovery of an alternative form of treatment other than pesticides or antibiotics being used at present, to which many of the microorganisms are developing resistance. Many plants have been used traditionally in the treatment of diseases and researchers have noted that further investigations on these plants might lead to the development of antimicrobial drugs for the control of diseases. Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David 2001) i.e. any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that
are often associated with synthetic antimicrobials, hence the present problem is undertaken for study.

2. REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT
(Previous work done in the relevant area):

The use of plant extracts and Phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as in tannin (Saxena et al, 1994)

In the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem (Mahesh & Satish, 2008). Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Ahmad & Aqil, 2007, Barbour et.al.2004). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et.al.1999). Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds (Tomoko, 2002). Analysis of different species of medicinal plants for biological active components known to have pharmacological properties have been conducted and most of the studied plants have shown antimicrobial property (Rabe & Vanstaden, 1997 Ongsakul, 2002). The medicinal properties of the plants could be credited to the presence of one or more of the active constituents of the plant. It has been reported that the antimicrobial activities of medicinal plants can be due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids. In recent years in order to discover novel antimicrobial drugs, screening of plants has been accelerated. Therefore the preliminary screening of medicinal plants for antimicrobial activity and for the presence of phytochemicals can establish a flat form for further development on the research of this area. It is believed that plants which are rich in a wide variety of secondary metabolites belonging
to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in their anti-microbial activities (Cowan, 1999). This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Flavonoids are known to be synthesized by plants in response to microbial infection (Dixon et al. 1983). They have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes [Tsuchiya et. al.]. In many cases, antimicrobial effects of various plant extracts have been attributed to their flavonoid contents (Tsao et.al.,1982, Cafarchia et.al.,1999).

The plant has also exhibited the presence of tannins, which could be the one of the contributing factor of antimicrobial activity shown by the plant. Their mode of antimicrobial action may be related to their ability to inactivate microbial enzymes, cell envelope transport proteins, etc. Scalbert A (1991) reviewed the antimicrobial properties of tannins and listed 33 studies, which had documented the inhibitory activities of tannins. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity(Jones,1994).

Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004); some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). The results of this indicate the need for further research into traditional health systems (Romero et al., 2005).

HPTLC offers better resolution and estimation of active constituents and can be done with reasonable accuracy in a shorter time (Sethi P D 1996). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics, due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases, has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical
structures, which overcome the above disadvantages [Okemo et al, 2003, lakshmi et al, 2006].

The medicinal plants around the world contain many compounds with antibacterial activity (Marjorie 1999). Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds (Tomoko et al. 2002). The use of botanical medicines is generally on the rise in many parts of the world (Bbosa et al. 2007). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents (Amani et al. 1998; Salvat et al. 2001; Costa et al. 2008). Numerous experiments have been carried out to screen natural products for antimicrobial property (Martinez et al. 1996; Ateb and Erdourul 2003; Nair and Chanda 2006; Nair et al. 2007a; Ndhlala et al. 2009). Considering the above, it can be stated that plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future (Shahidi Bonjar et al. 2004; Aslim and Yucel 2008).

3. OBJECTIVES OF RESEARCH/ PROPOSED HYPOTHESIS:
The objectives of the present study are:

- To detect the phytochemicals from selected medicinal plants
- To evaluate the active principles from medicinal plants by HPTLC
- To evaluate the antimicrobial activities of the extracts of some plants used in Indian traditional healthcare system, against a group of pathogenic bacteria and fungi.

4. METHODOLOGY TO BE ADOPTED:
Collection and identification of plants
Medicinal Plants will be selected from Nanded district and will be authenticated from University department/Research institutes

Preparation of plant extract
The aqueous and organic solvent extracts will be prepared for evaluation of different chemicals
Preliminary phytochemical Analysis

Preliminary phytochemical Analysis will be carried out for the identification of the active chemical constituents such as alkaloids, glycosides, terpenoids, flavonoids, reducing sugar, saponins and tannins by the following methods.

**Alkaloid**
Alkaloid solution produces brown colour precipitate when a few drops of Wagner reagent (Dissolve 2 gm of Iodine and 6 gm of Potassium Iodide in 100 ml water) are added.

**Glycoside**
To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

**Terpenoid**
About 0.5 gm of each extract in 2 ml of chloroform. Conc. H$_2$SO$_4$ carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoid.

**Flavonoid**
About 0.2 gm of each plant extract was dissolved in dil. NaOH and HCl was added. A yellow solution that turns colourless indicates the presence flavonoids.

**Tannins**
To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution were added. The Blue color was observed for gallic tannins and green black for catecholic tannins [Okemoet.al., 2003].

**Reducing sugar**
To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling’s solution were added at hot and observed for brick red precipitate.

**Saponins**
About 0.2 gm extract was shaken with 5 ml of water, then heated to boil. Frothing (appearance of a creamy mass of some bubbles) shows the presence of saponins.

**HPTLC Profile of selected plant parts**
HPTLC analysis will be carried out using the method described by Misra et al. (2008) with some modification.
Antimicrobial activity

Antibacterial activity
The antibacterial activity of different plant extracts will be evaluated by using the agar well diffusion technique/Paper disc method (Bauer A W, Kriby, 1966).

Antifungal activity
The Antifungal activity will be determined by poisoned food technique (Grover and Moore, 1962; Mishra and Tiwari, 1992; Nene and Thapliyal, 2000). Percentage inhibition of mycellial growth will be evaluated by comparing the colony diameter of poisoned plate (with plant extract) and nonpoisoned plate (with distilled water) and calculated using the formula given below (Verma and Kharwar, 2008);

\[
\frac{\text{Mycellial growth (control)} - \text{Mycellial growth (treatment)}}{\text{Mycellial growth (control)}} \times 100
\]

Spore germination assay
In addition to the above mentioned assays, antifungal activity of plant extracts will be evaluated by spore germination assay using the slide technique (Nair, et al., 1991)

5. IMPORTANCE OF STUDY/ SOCIETY APPLICATION:

INTERNATIONAL STATUS:
The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc (Krishnaraju et al. 2005).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances.
NATIONAL STATUS

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times (Bhattacharjee 1998). India is one of the 12-mega biodiversity centers having about 10% of the world’s biodiversity wealth, which is distributed across 16 agro-climatic zones (Shiva 1996). In India around 20,000 medicinal plant species have been recorded recently (Dev 1997), but more than 500 traditional communities use only about 800 plant species for curing different diseases (Kamboj 2000). With a view to strengthen the medicinal plants sector all over the country as well as to conserve the wild stock, the NMPB (National Medicinal Plants Board) was set up by the Government of India in 2000.

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro et al 2000). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds.

SIGNIFICANCE OF THE STUDY

Bacterial resistance to antimicrobial drugs is a worldwide problem that has emerged even among the common poultry pathogens. Nowadays, the use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs. Herbal medicines are in great demand in both developed and developing countries as a source of primary healthcare owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and will overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell.

HPTLC profile is useful as a phytochemical marker. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the
medicinally important plant. HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various colored bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds. Therefore the current work is to be undertaken with an aim to provide a fingerprint for authentication of the material used for herbal preparation and also evaluate its biological activity against plant pathogens and multi drug resistance organisms.

6. PROPOSED WORK PLAN/ FORMULATION AND STRUCTURE OF STUDY:
Year-wise Plan of work and targets to be achieved

First Year
Reference work related to the problem
Collection and identification of Medicinal Plants
Phytochemical tests of different plant parts

Second Year
HPTLC analysis of plant extract prepared in different solvents
Preparations of different herbal formulations from medicinal plants

Third Year
Antimicrobial activity of Plant extracts
Antimicrobial activity of Herbal formulations
Compilation of data of work done in the form of thesis
Submission of thesis

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