A STUDY ON PHARMACOLOGICAL EVALUATION AND MICROPROPAGATION OF *PSORALEA CORYLIFOLIA*

V.E.VIJAYA SEKHAR, T. SUJATHA

Abstract: Medicinal plants have been identified and used throughout human history. Medicinal plants are origined even before human being. Man's existence on this earth has been made possible only because of the vital role played by plant kingdom in sustaining life (Bhushan Patwardhan*et al*., 2009). Medicinal plants have been regarded as sacred and used by early civilizations to treat sickness and to embellish man's wellbeing (Dickson *et al*., 2004). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Tapsell*et al*., 2006). In India the earliest records referring to curative properties of certain herbs are referred in Rigveda (3500-1800 BC).
According to Huang *et al*. (2008), medicinal plants have been screened for their potential uses as alternative remedies. Medicinal plants are considered as a source of biologically active biochemicals like secondary metabolites, used for various applications in food, medicines, and industry (Justin *et al*., 2014).

Introduction: Medicinal plants have been identified and used throughout human history. Medicinal plants are origined even before human being. Man's existence on this earth has been made possible only because of the vital role played by plant kingdom in sustaining life (Bhushan Patwardhan*et al*., 2009). Medicinal plants have been regarded as sacred and used by early civilizations to treat sickness and to embellish man's wellbeing (Dickson *et al*., 2004). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Tapsell*et al*., 2006). In India the earliest records referring to curative properties of certain herbs are referred in Rigveda (3500-1800 BC).

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Traditionally, plants have been collected for medicinal use from wild areas. This has prompted industries, as well as scientists to consider the possibilities of using cell cultures as an alternative supply for the production of plant natural products (Dicosmo and Misawa, 1995). Plant cell cultures have the potential of providing a low cost route to numerous plant derived natural products that are very expensive to synthesize chemically or that occur naturally at very low concentration.

During the past few decades, tissue culture techniques have been manipulated for many purposes such as for the improvement of plants growth, secondary metabolites production and biological activities and transformation (Mulabagal and Tsay, 2004).

Presently there is great demand for the use of plant based medicines in place of synthetic drugs. As a result of non-scientific exploitation, most of the medicinal plant resources are being endangered and are on the verge of extinction.

Tissue culture is the technique of maintaining plant tissue in an artificial medium in vitro under control condition. Propagation of plants through tissue culture has become an important and popular technique. Tissues from various organs such as stem and leaf of the axenic plantlets can be induced to form callus. Callus tissue can serve as an experimental system to investigate the biological activities using specific bioassays. However, many factors contribute to the ability of a specific tissue to form callus such as medium and plant growth regulators.

At present, researchers aim to produce plants possessing substances having antitumor, antiviral, hypoglycaemic, anti-inflammatory and antimicrobial capability, through tissue culture technology. Organogenesis is a suitable method for obtaining a large quantity of genetically homogenous and healthy plant material, which can be used for planting (Pierik, 1987). In massive cultivation of medicinal plants, the first step is production of high quantities of healthy and genetically homogeneous plant material, which can be propagated at low cost. To date we can speed up the production rate of the average plant by approximately 10,000 times and a large number of productive plants can be multiplied routinely through tissue culture (Rao*et al*., 1996).

Cellular totipotency has led to the concept of tissue culture studies. The ability of a single cell develop into a complete individual organism is called Cellular totipotency. Tissue culture is also called Micro-propagation or *Invitro* culture. Plant tissue culture (PTC)which is also called has advanced the knowledge of fundamental plant studies and industrial production of plant secondary metabolites etc.

Micropropagation of *Psoralea corylifolia:* The usual methods of regeneration through tissue culture are callus mediated organogenesis and somatic embryogenesis. Media used in plant tissue culture are composed of several components e.g. salts, vitamins, amino acids, sugars, growth regulators, gelatin or agar and water. Plant growth regulators (PGR) added to plant tissue culture media are taken up and enhance the endogenous level of plant hormones. PGRs induce cell division, cell growth, tissue differentiation and organ formation. Murashige and Skoog medium has been variously modified and extensively used for micropropagation of several plant species very successfully. Agrotechnology or protocol for the micropropagation of several medicinal plants is available (Debnath, 2007; Rao*et al*., 2010; Kaur and Malik, 2009).

Systemic position of *Psoralea corylifolia*

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Genus: *Psoralea*
Species: *corylifolia*

Common Names: Psoralea, Malay Tea, Cot Chu,Scurfpea Ku Tzu Malaysia, Scurf-pea. In India it is called 'Babchi'. In Telugu it is called Bavanchalu, Kalangiya, Karobogi and Koriasthan.

Useful Parts: All parts are useful particularly seeds are more useful.

Description: It is an endangered herbaceous medicinal plant distributed in the tropical and subtropical regions of the world. It grows mainly in winter season. It is an erect annual herb with an average height of 150 cm, with densely gland-dotted branches. Leaves are round, dotted with black glands on both surfaces. Flowers are small, bluish purple, 10-30 in a bunch, arising in axillary racemes. Fruits (pods) are 4 mm x 2.5 mm in size, black, round, one seeded and smooth. Its hard Seed coat is the cause of low germination percentage (5-7%) (Chand and Sahrawat 2002). It is a slow growing species mainly cultivated by seeds.

***Psoralea Corylifolia* Plant**

Medicinal Uses:

- In Ayurveda, its root is useful to treat the carries of teeth and leaves are good for diarrhoea.
- It is useful in treatment of vomiting, piles, bronchitis, inflammation, anaemia etc. It improves hair growth and complexion.
- The seed and fruit contain Psoralen that is used as a laxative, antipyretic, antihelmintic, alexiteric and for heart troubles.
- Seed oil is used externally for the treatment of elephantiasis.
- It is used in the treatment of febrile diseases, premature ejaculation, impotence, lower back pains, frequent urination, incontinence, bed wetting etc.
- The plant yields a useful medicinal oleoresin, it treats kidney disorders, impotence, lumbago.
- It is also used to treat various skin ailments including leprosy, leucoderma and hair loss.
- The antibacterial action of the fruit inhibits the growth of Mycobacterium tuberculosis.
- According to Unani system of medicine, its seed is purgative, stomachic, anthelmintic,

vulnerary, stimulant, aphrodisiac and cures blood related troubles. It is applied externally in treatment of skin.

Phytochemistry and Pharmacology of *Psoralea Corylifolia:*

- *Psoralea* genus is a legume that has 150 species. The name *Psoralea* is origined from the Greek word 'Psoraleos' meaning warty or scurfy, in reference to the dots or warts on the bark. Psoralea has a wide geographic distribution including the New World, Eurasia, India and South Africa.
- The antifungal studies on *Psoralea corylifolia* revealed that the seed oil and methanol seed extract of *P. Corylifolia* were explored specifically against common fungal skin pathogens. The results clearly demonstrate that, the methanol seed extract of *P. Corylifolia* comprise a promising antifungal activity against common fungal pathogens as compare to seed oil.
- The in vitro antibacterial activity of *Psoralea corylifolia* leaf and its corresponding callus extracts were studied against pathogenic bacteria causing periodontitis. Leaves and corresponding calli were extracted using petroleum ether, chloroform, acetone, methanol and distilled water.
- Ethno botanic study, phytochemical screening and antioxidant activity of the methanol and aqueous extracts of *Psoralea plicata* (Cullen plicatum) Delile revealed that they contain alkaloids, flavonoids, ellagic acid, tannins, saponins and proanthocyanidins.
- The aqueous, alcohol, petroleum ether extracts and essential oil obtained from the seeds of *P. corylifolia* has significant antibacterial activity and moderate antifungal activity. The oleoresins have been proved to have antimutagenicity on *Salmonella typhimurium*TA98 and can treat leucoderma.
- Sheng *et al.*, isolated three new prenyiflavonoids, namely *corylifols* A-C (1-3), from the seed of *P. corylifolia* showed antibacterial activity against *Staphylococcus aureus*and *S.epidermidis*.
- Antioxidants from *Psoralia corylifolia* L. Phyto-therapy research studies showed that potent inhibitory activity against 4 species of fungi viz. *Trichophyton rubrum Trichophyton mentagrophytes, Epidermophyton floccosum* and *Microsporum gypseum* (Jiangning*et al.*, 2004).
- Previous studies revealed that the chloroform extract of *P. corylifolia* seed at a close of 400 mg/kg is effective against carageenin induced paw oedema in rat and mouse ear inflammation (Khatune*et al.*, 2002). Forestieri*et al.*, 1996 investigated that the alcoholic extracts of its seeds evaluated for antihelmintic activity for *Ascaridia galli.*
- Pharmacological studies on seed extracts of *Psorelea corylifolia* revealed that they had synergistic action on anti-bacterial and anti-psoriatic studies (Anusha*et al.*, 2013). TLC and HPLC analysis also confirmed these results.
- Kiran*et al.* (2010) employed various invitro standard assay to assay the antioxidant properties of aqueous and solvent extract of seeds of *P. corylifolia* L. All the extracts tested were effective in quenching superoxide anion.
- Various in vitro standard assays on the *P. corylifolia* revealed that, all the extracts tested were effective in quenching superoxide anion. The results suggest strong antioxidant potential of alcohol and water (1:1) extract of seeds of *P. corylifolia* that could play an important role in the modulation of oxidative stress (Kiran*et al.*, 2010).

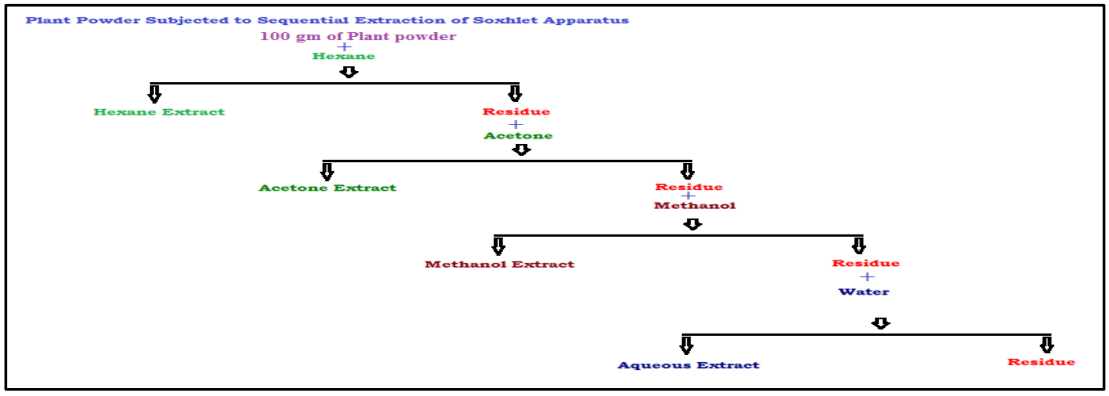
Micropropagation:

- The father of tissue culture is Gottlieb Haberlandt (1902), who gave the idea of totipotency. Still it did open up new vistas in morphogenesis.
- The advantage over the other techniques stem from the fact that it allows a living system to be studied under controlled environmental conditions. Tissue culture has now become a well-established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even organelles under precisely controlled physical and chemical conditions. During the last few decades micropropagation technique has emerged as a promising technique for rapid, and large scale propagation of selected plants. A small piece of tissue can be used to raise hundreds or thousands of plants in a continuous process.

Collection of Plant Material: The plant material was collected from the Seshachalam forest. The authentication was checked by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh. Required quantity of plant raw material i.e. leaves of *Psoralea corylifolia (Linn.)* were collected and

washed with running water followed by distilled water. Chopping process was carried out by separating the leaves from stems and they were allowed to dry under shade (Mehrotra, 1976). The dried material was stored in a sterilized polythene bags for further study.

Extraction Technique: The dried powder of the leaves was extracted sequentially (Wiart*et al.*, 2004) by soxhlet apparatus (Lin *et al.,* 1999), using different solvents depending upon their polarities like Hexane, Acetone, Methanol and water (Flow chart 1). The extracts were concentrated and solvent was removed under reduced pressure, using rotary evaporator. The dried crude concentrated extracts were weighed to calculate the extractive yield and stored in air tight bottles, until used for analysis.



Sequential Extraction of Soxhlet Apparatus

Phytochemical Analysis:

Preliminary Screening of Phytochemicals (Qualitative analysis): Standard screening tests of four extracts *of Psoralea corylifolia*(Linn.) were carried out to know the presence or absence of various secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones.

1. Detection of Alkaloids: Extract was dissolved individually in dilute Hydrochloric acid and the resultant solution was clarified by filtration. *Mayer's Test,Wagner's Test, Dragendroff's Test* are used for this purpose.

Detection of Phenols: It is carried out by *Ferric Chloride Test.*

Detection of Flavonoids: It is carried out by *Alkaline Reagent Test and Lead Acetate Test:***Detection of Anthraquinones:** It is carried out by *Free Anthraquinones Test (Borntrager's test).*

Detection of Phytosterols: It is carried out by *Salkowski's Test:*

Detection of Tannins: It is carried out by *Ferric Chloride Test, Lead Acetate Test and Potassium Dichromate Test:*

Detection of Saponins: It is done by *Froth Test:*

Detection of Anthocyanins: The extract was added to 2 ml of 2 N HCl and Ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanins.

Quantitative Estimation of Phyto Constituents: The quantity refers to the intrinsic value of the drug *i.e.*, the amount of medicinal principles present. The active constituents were glycosides, tannins, flavonoids, phenolic compounds, alkaloids, proteins and vitamins. The biological activity of a plant was influenced by the presence of various phyto-constituents. Natural antioxidants such as Vitamin C and Vitamin E directly influence the activity.

3.1.4.1 Determination of Total Phenol Content: The amount of total phenol content, in different solvent extracts of *Psorelea corylifolia* was determined by Folin- Ciocalteu's reagent method.

3.1.4.2 Determination of Total Flavonoid Content: The amount of flavonoid content in different solvent extracts of *Psoralea corylifolia*(Linn.) was determination by aluminium chloride colorimetric method (Chang *et al.*, 2002). Rutin was used as positive control.

Antimicrobial Study: Antimicrobial activity is expressed as zone of inhibition in millimeters, which is measured with a zone reader. The Hexane, Acetone, Methanol and Aquoues extracts of *Psoralea corylifolia* (Linn.) were screened for antimicrobial activity against a wide spectrum of microorganisms and the activity of extracts was compared with appropriate reference standards

(Streptomycin for both gram positive and gram negative organisms and fluconazole for fungal strains

Test Organisms: The microorganisms used for the experiments were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Gram-Positive Organisms: *Staphylococcus aureus*(MTCC 3160), *Streptococcus mutans*(MTCC 497), *Lactobacillus casei*(MTCC 1423),*Lactobacillus acidophilus* (MTCC 495) &*Bacillus megaterium*(NCIM 2187)

Gram-Negative Organisms: *Enterococcus faecalis (MTCC 439), Xanthomonas campestris (MTCC 2286), Escherichia coli (ATCC 35218) & Pseudomonas aeruginosa (ATCC 9027.*

Fungal Strains: *Candida albicans* (ATCC 10231) *Aspergillus niger*(ATCC 1015) *Rhizopus oryzae (MTCC 262) &Candida rugosa(ATCC 96275).*

Culture Medium: The following media were used for the present antimicrobial studies.

Nutrient Broth for Bacteria: Beef extract - 0.35% , Sodium chloride - 0.5% , Peptone - 0.5%. These ingredients, weighing 37 g were dissolved in distilled water (1000 ml) and the pH was adjusted to 7.2 - 7.4 and sterilized by autoclaving at 15 lbs/ $inch^2$ for 20 minutes.

Sabouraud's Dextrose Agar Medium For Fungi (SDA): Dextrose - 4.0%, Peptone - 1.0% & Agar- 2.5%. They were dissolved in distilled water and pH was adjusted to 5.6 ± 0.2 and then sterilized by autoclaving at 15 lbs//$inch^2$ for 20 min.

Sterilization : Sterilization of the media, water etc., were carried out by autoclaving at 15 lbs/$inch^2$ for 20 minutes. The glassware like syringes, petridishes, pipettes, empty test tubes were sterilized by dry heat in an oven at a temperature of 160 °C for one hour.

Antimicrobial activity of *Psoralea corylifolia*(Linn.): *In vitro* testing of the sensitivity bacterial and fungal isolates to antimicrobial agents using the disc diffusion assay, according to the guidelines set by the National Committee for Clinical Laboratories Standards (NCCLS, 1997). The extracts were tested for antimicrobial activity against and gram positive, gram negative bacteria and fungi.

All the plates were kept in a refrigerator at 2 to 8 °C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity.

The solvent control was run simultaneously to assess the activity of Dimethyl Sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

Anti Oxidant Activity: Free radicals can also be produced by many cells as a protective mechanism. Neutrophils produce free radicals to attack and destroy pathogens, while the liver uses free radicals for detoxification (Lunec*et al*., 2002). However, the presence of free radicals within the body can also have a significant role in the development and progression of many disease processes like heart disease, congestive heart failure, hypertension, cerebrovascular accidents and diabetic complications (Chen *et al*., 2002). As free radicals play an important role in the diseases scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance. The literature revealed that some of the selected herbal drugs are known to possess either superoxide, or hydroxyl, or DPPH radical scavenging and lipid peroxidation inhibition activities. There is no detailed study on free radical scavenging activity on each plant. Hence, a detailed study was carried out on Hexane, Acetone, Methanol, Aqueous extracts of *Psoraleacorylifolia*(Linn.) for scavenging activity of DPPH and FRAP radical.

DPPH : The DPPH radicals are widely used to investigate the scavenging activity of some natural compounds. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured, diphenylpicrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of non-radical form DPPH-H by the reaction. Resulting colour change from purple to yellow, the absorbance decreased when the DPPH was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH molecule.

FRAP: Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

At low pH, reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form (which has thick blue colour) canbe monitored by measuring he change in absorption at 593 nm.

DPPH (2,2-diphenyl-l-picryl hydrazyl) Radical Scavenging Assay : A stock solution of Hexane, Acetone, Methanol, aqueous extracts of *Psoraleacorylifolia*(Linn.) and standard ascorbic acid were prepared in the concentration of 100 mg/100ml (1mg/ml). From each stock solution 1ml, 2ml, 3ml, 4ml & 5ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10 ml whose concentration was then 100µg/ml, 200µg/ml, 300 µg/ml, 400 µg/ml & 500µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 517 nm against the blank. For the control, 1 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation (Oliver BEP *etal.,*1960; Trease GE *etal.,* 2002; Khan SS *et al.,*1979).

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Micropropagation of Selected Medicinal Palnts:

3.2.1 Media Preparation: The nutritional requirements for *in-vitro* micropropagation vary with species to their optimal growth. As such, no single medium composition is suggestible for all types of plant tissues and organs.

The stock solutions of the major salts, minor salts and growth regulators were prepared as per the composition and stored under refrigeration. Auxins were prepared by dissolving in 1N Potassium hydroxide and cytokinins in 1 N Hydrochloric acid before making up the final volume with distilled water. Auxins are generally used in plant tissue culture at a concentration range of 0.01-10.0 mg/l.

Cytokinins are generally used in plant tissue culture at a concentration range of 0.1-10.0 mg/l. When added in appropriate concentrations they may promote shoot proliferation

3.2.3 Explant Selection and Sterilization: Nodal explants were selected from disease free, young and healthy plants for carrying out our study as young and healthy cells are supposed to have retained their totipotency.

Explants Sterilization: The leaves were removed from the explants and washed under running tap water for 20- 30 minutes. Then explants were soaked in an aqueous solution containing 0.2 % Bavistin and 0.03% Streptomycin for 10 minutes in Laminar flow hood. This was followed by gentle wash with sterile double distilled water for 5 minutes for two cycles.

After that the explants were immersed in aqueous solutions of Savlon(1.5% v/v chloro-hexidinegluconate solution and 3.0% w/v cetrimide) for 10 minutes and were shaked regularly..

Composition & Different Combinations of multiplication media (Medium code: BM-BM 5)

Medium Code	Medium details
MS	MS basal as control
BM	MS + 0.1 mg/l BAP + 0.1 mg/l NAA
BM 1	MS + 0.5 mg/l BAP + 0.5 mg/l NAA
BM 2	MS + 1.0 mg/l BAP + 0.2 mg/l NAA
BM 3	MS + 4.0 mg/l BAP + 0.4 mg/l NAA
BM 4	MS + 0.5 mg/l BAP + 0.5 mg/l Kn
BM 5	MS + 1.0 mg/l BAP + 1.0 mg/l Kn

Rooting Protocol: In the laminar flow, under sterile conditions the Klin film and cap was removed from the culture bottles in sterile condition (laminar flow chamber) and with the help of sterile forceps the multiplied shoots were removed from the medium and placed on the sterile glass plate. With the help of sterile scalpel elongated shoots up to 1-2 cm in length were cut and placed into the rooting medium. The culture bottles were then capped and placed in the growth room under the same condition. The time required for *in vitro* rooting of shoots may vary from 10 – 15 days.

Table: 3.2.2: Different types of Rooting Media(Medium code: RT_1 – RT_7)

MS	MS + Sucrose (30 gm/l)+ Agar (8gm/l)
RT_1	MS + Sucrose (0 gm/l)+ Agar (7gm/l)
RT_2	MS + Sucrose (0 gm/l)+ Agar (8gm/l)
RT_3	MS + Sucrose (10 gm/l)+ Agar (7gm/l)
RT_4	MS + Sucrose (10 gm/l)+ Agar (8gm/l)
RT_5	MS + Sucrose (20 gm/l)+ Agar (7gm/l)
RT_6	MS + Sucrose (20 gm/l)+ Agar (8gm/l)
RT_7	MS + Sucrose (30 gm/l)+ Agar (7gm/l)

Protocol Followed: Plantlets were taken out of the culture bottles (multiplication subculture) with the help of forceps and washed thoroughly with sterilized water to remove any remaining of the medium.

0.1% Bavistin treatment was given to the plantlets in order to protect them from the fungal attack in the near future. Plantlets were separated into single shoots by cutting their bases gently with the help of blade. Single shoots were dipped in IBA solution (200 ppm) before planting into a hardening mixture. After this the single shoots are carefully planted in the trays containing soil and agropeat (M/s Varsha Enterprises, Bangalore, India) mixture in 1:1 ratio.

3.2.8 Transplantation and Acclimatization of the Plantlets: The transfer of plants from the culture vessel to the soil requires a careful, stepwise procedure. The roots of the plants are gently washed to remove the agar sticking to them. The most essential requirement for the successful transplantation is to maintain the plants under a very high humidity (90 – 100%).For the first 10 – 15 days by keeping them under moist or covering them with clear plastic. Some small holes may be poked in the plastic for air circulation. Inside the culture vessel the humidity is high and thus, the natural protective covering of cuticle is not fully developed. During this time plant attain ability to synthesize more food and develop cuticular covering. Plants are maintained under shade and are then ready to be used in open nursery.

Protocol Followed: After 10 – 14 days of culture on rooting media, the rooted plantlets were transplanted to pots or trays for hardening prior to their final transfer to soil.

Rooted plantlets were taken out of the culture bottles with the help of forceps and washed thoroughly with water to remove any remaining of the medium.0.1% Bavistin treatment was given to the plants in order to protect them from the fungal attack in the near future.

After this the plants are carefully planted in the polybags containing different soil mixtures in different ratios as shown in the table.

Different Combinations of Soil and VAM Based Potting Mix used in the Present Investigation (Mixture code: HM 1 – HM 8)

HM_0	Soil
HM_1	Soil + VAM based potting mix(10:1)*
HM_2	Soil + VAM based potting mix (10:2)*
HM_3	Soil: Vermicompost (2:1)*
HM_4	Soil: Vermicompost (4:1) *
HM_5	Soil: Flyash: Farmyard Manure (1:1:1) *
HM_6	Soil: Farmyard Manure (4:1) *
HM_7	Soil: Agropeat (2:1) *
HM_8	Soil: Agropeat (4:1) *

* parameter measured in v/v.

After planting, plantlets are thoroughly watered and kept in polyhouse under humidity range of approximately 80%. These plantlets should be sprinkled with water time to time as per the requirement and after two weeks should be transferred to shade house having humidity range of approximately 60%.

The plantlets are then transferred to open area after 9-10 days and kept there for ten days prior transferring them to the field.

Results:

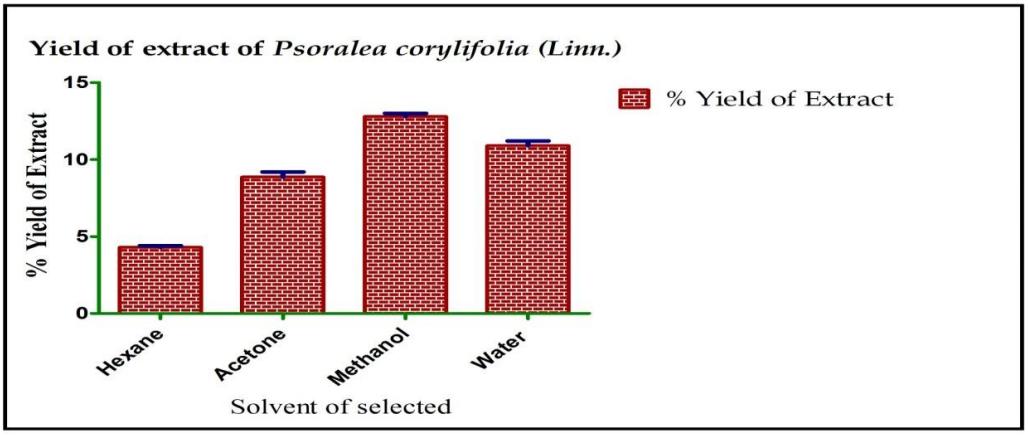


Fig: AnalysisofPhysicochemical characteristics of *Psoreleacorylifolia*(Linn.)Extracts

Table :4.1.3 Analysis of Physicochemical characteristics of *Psoraleacorylifolia*(Linn.)

Solvent	Initial Weight of the Powder (g)	Final Weight of the Powder (g)	Weight of the Crude Extract (g)	Crude Extract %	Colour of the Extract
Hexane	100	95.7	4.3	4.3	Dark Brown
Acetone	100	90.9	9.1	9.1	Dark Green
Methanol	100	87.2	12.8	12.8	Dark Green
Water	100	89.1	10.9	10.9	Dark Red

Discussion:

Total Flavonoid Content of *Psoralea Corylifolia:* Flavonoids are the largest group of polyphenolic compounds having benzo-γ-pyrone structure and ubiquitous in plants. These are directly involved in the human dietary habituates and health, hence it is logical to evaluate its functional and structural relationship.

From the results it was proved that moderate amounts of flavonoids presentin*Psoreleacorylifolia.* More over polar extracts possess more amounts of flavonoids than the non-polar ones. Flavonoids have protective activities on humans which include coronary heart disease prevention, anti-inflammatory, antioxidant activity, hepatoprotective and anticancer activities. Flavonoids are being produced in bulk in the pharmaceutical industry with the aid of microbial biotechnology.

5.1.3 Antimicrobial Activity : Antibacterial Activity on Gram + ve Strains: The antimicrobial activity of *Psoraleacorylifolia* extracts were tested against five gram positive bacteria - *Staphylococcus aureus, Streptococcus mutans, Lactobacillus casei, Lactobacillus acidophilus, and Bacillus megaterium.* The results said that *Psoralea corylifolia* (Lour. has most potential antimicrobial activity against gram positive organisms. These results also proved that *Bacillus megaterium* was more sensitive to all the plant extracts followed by *Lactobacillus acidophilus, Lactobacillus casei, Staphylococcus aureus and Streptococcus mutans.* Antimicrobial activity of the above plant extracts against gram positive organisms also proved that methanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. Water and acetone extracts also showed the moderate antimicrobial activity, but hexane extract showed lesser activity. The inhibitory zones of different extracts varied with the type of microorganism involved in the work.

Antibacterial Activity on Gram - ve Strains: Most of the pathogenic bacteria belong to gram negative, they causes several diseases like sexually transmitted diseases, respiratory diseases,

gastrointestinal problems, nosocomial infections etc. Owing to these problems, researchers show more interest to isolate potential drugs against gram negative organisms from plant origin. For evaluating antimicrobial activity of selected plant extracts against gram negative organisms, the four gram negative pathogenic microorganisms were selected - *Enterococcus faecalis, Xanthomonascampestris, Escherichia coli and Pseudomonas aeruginosa.* After proper incubation the results proved that methanol extract of *Psoralea corylifolia* (Linn.) showed good activity against above said gram negative organisms.Methanol extracts of above said plant has most potential activity followed by water, and acetone extracts, whereas hexane extract of respective plant showed less potentiality against gram negative organisms. The results of our research also highlights the fact that the methanol solvent extracts exhibited greater antimicrobial activity. So the present observation suggests that the methanol solvent extraction was suitable to verify the antimicrobial properties of medicinal plants which are also supported by many other investigators (Krishna *et al*., 1997; Singh *et al*., 2000)..

Anti Fungal Activity: Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (CSIR Wealth of India, publications & Information Directory, 1998). Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (Portillo A*et al.,* 2001). The results of this experiments proved that all the four extracts of *Psoraleacorylifolia*(Linn.) possess antifungal activity and its methanol extract showed highest antifungal activity, followed by water and acetone. The antifungal activity of this plant makes them potential source of antifungal agents and may be of economic importance as source of antifungal natural plant products.

5.1.4 Antioxidant Activity: Antioxidant activity is indispensable for cure of many diseases. Antioxidant based drug productivity is used for prevention and treatment of very cognisant diseases like cancer, diabetes, Alzheimer's stroke and atherosclerosis (Devasagayam, 2004). Reactive oxygen species involves antioxidant activity that include hydroxyl radicals, superoxide radicals, hydrogen peroxide and singlet oxygen and these are the alternative products of biological reactions or they may be enclosed by exogenous factors (Kikuzaki*et al*., 1993).

In the past decade, researchers have been searching for efficient antioxidant compounds. Recent studies have also showed several plant products including terpenes, polyphenols and several plant extracts contains an antioxidant action (Zhou *et al*., 1991; Quinn *et al*., 1996). The risk of cancer and cardiac disease is low when individual consumes diet rich in fruits and vegetables (Salah *et al.,* 1995; Hertog*et al*., 1997). The data on antioxidant activities of food plants have been generated globally and there is enough evidence to predict the natural antioxidants and their role in nutrition and human health (Aruoma*et al.,* 1994; Cao *et al.,* 1996; Kaur*et al.,* 2002).In the Indian traditional practice of Ayurveda rasayana of medicinal plants, extensive antioxidant properties were identified since they are being used in curing diseases.

DPPH Radical Scavenging Antioxidant Activity: One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index. In the DPPH Free radical scavenging activity, the four extracts of *Psoraleacorylifolia*(Linn.) were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. Whereas hexane extract showed lowest antioxidant activity. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up. Free radical scavenging activity of the extract is concentration dependent, as the concentration of the test compounds increases, the radical scavenging activity increases and lower IC50 value reflects better protective action.

Water and methanol extracts of *Psoralea corylifolia* (Linn.) exhibited excellent antioxidant activities. These results can be attributed to the presence of phenols, flavonoids, tannins, alkaloids, saponins and terpenoids and this is in agreement with studies in which these compounds have been associated with high antioxidant activities (Dangles *et al.,* 2000; Gulcin *et al.,* 2004)

FRAP Radical Scavenging Antioxidant Activity: Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are

electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). For the measurement of the reductive ability, we used the FRAP assay which was developed to determine the ferric reducing ability of biological fluids and aqueous solutions of pure compounds and can be applied to study the antioxidant activity of plant extracts. In this study, it was proved that all the extracts of *Psoraleacorylifolia*(Linn.) showed the concentration dependent antioxidant potential.

Conclusion: Basing up on the observations in the present study, it can be concluded that the extracts of *Psoreleacorylifolia*, possess a detectable amount of phytometabolites. Quantitative analysis revealed that, phenols and flavonoids concentrations increase from non-polar to polar solvents. Pharmacological studies revealed that all the extracts of *Psorelea corylifolia* showed the antibacterial, antifungal and antioxidant activity, their activities increases from non polar to polar solvents. This work also proved that polar solvent i.e methanol is the suitable solvent to extract pharmacological active compounds.

From the Micro propagation studies we, have concluded that -

- Explants of *P.corylifolia* (Linn.) inBM_3were found to be the best initiation medium in terms of bud breakage, and lower contamination.
- All explants of three plant species, showed highest shoot length with higher percentage of cluster in BM_1 medium.
- *P.corylifolia* (Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium and MS medium showed lowest number of shoots.
- RT_5medium is the best media to get highest S/R in all explants of *P. corylifolia (Linn.).*RT6 medium is suitable to get highest length of root.
- HM_1 medium is best hardening mixtures to higher number of roots and high shoot/root ratio.

In this entitled work we have successfully completed the pharmacological and micropropagation studies of selected plants of Fabaceae. But further pharmacological, and purification studies are needed to know the complete medicinal properties and to prepare new natural drugs from these plants.

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V. E.Vijaya Sekhar
Lecturer in Botany A. C. College, Guntur, A.P.
Email: ezravsekhar @gmail.com
T. Sujatha
Lecturer in Chemistry, Sri Sarathi Institute of Engineering & Technology,
Nuzividu, Krishna Dist, A.P.