A COMPARATIVE STUDY ON THE PHARMACOLOGICAL EVALUATION OF *ACACIA SINUATA*(LOUR.)*, CASSIA ANGUSTIFOLIA*(L) AND *PSORALEACORYLIFOLIA*(L)

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Abstract: 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 to in the Rigveda (3500-1800 BC).

Beginning with 1800 AD there was continuous activity in this area and many of the well known medicinal plants were chemically analyzed and their pharmacological active principles were characterized. Soon after their isolation and characterization, these compounds either in pure state or in the form of well characterized extracts, became part of pharmacopeias of several countries. This is where plant medicine and modern medicine have a common link (Handa, 1991; Kokate*et al.,* 2000).In modern medicine, medicinal plants occupy a very significant place as raw materials for some important drugs, although synthetic drugs brought about a revolution in controlling various human and animal diseases. But these synthetic drugs are out of reach to millions of people. Those who live in remote places depend on traditional healers, whom they know and trust. Judicious use of medicinal herbs can even cure deadly diseases. The medicines that come under Ayurveda, Siddha and Unani system of treatment are called as Indian System of Medicines (ISM). The drug and Cosmetic Act defines the ISM as "Ayurvedic, Siddha and Unani drug includes all medicines, intended for internal or external use in diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals" (Sampath*et al*., 2001, Chatopadhyaya P. K, 2013)

Introduction: 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 to in the Rigveda (3500-1800 BC).

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Importance and Uses of Medicinal Plants: Medicinal plants are natural and more accessible than manufactured drugs as they were used in treating a wide spectrum of diseases (Ahmad *et al*., 2014). Plants are used in traditional medicine for their antibacterial, antifungal, antioxidant and anticancer activities (Schinella*et al*., 2002; Vander Jagt*et al*., 2002; Pushpa*et al*., 2015).

Objective and Scope of Work: Assay methods vary depending on what bioactivity is targeted and these may include antimicrobial, antioxidant,

antidiabetic and anthelminthic activity. The assay method however should be as simple, specific, and rapid as possible

Selection of Plant Species: The author has considered ethnopharmacological uses for the selection of three important medicinal species belonging to Fabaceae family such as *Acacia sinuate* (lour.) merr,*Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) .

Plan of the Proposed Work: 1. To investigate the phytochemistry and pharmacological activities of *Acacia sinuate* (lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) in a scientific manner.

Systemic Position of Selected Plants:

Systemic position of *Acacia sinuata*:

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Sub family:Mimosaceae
Genus: *Acacia*
Species: *sinuata*
Common Names: Chikaka, Shikakai, Banritha, Reetha, Kochi, Ritha, Sige, Shikai, Shikaya
Habitat: Throughout India, grows wildly in forests especially in Peninsular region.
Useful Parts: Leaves & Fruits Pods
Description: Shikakai is a climbing, most well-known for the natural shampoo derived from its fruit. Thorny branches have brown smooth stripes -thorns are short, broad-based, flattened. Leaves with caducous stipules are not thorn-like. Pods are thick, somewhat flattened, stalked, 8 cm long, 1.5-1.8 cm wide.

***Fig: 1 Acacia sinuate* (Lour.) merr. Plant**

Medicinal Uses:

- It is popularly referred as "fruit for the hair" as it has a naturally mild pH that gently cleans the hair without stripping it of natural oils.
- Shikakai is used to control dandruff, promoting hair growth and strengthening hair roots.
- Preventing premature greying of hair.
- Extracts of the ground pods have been used for various skin diseases.
- An extract of the Shikakai leaves is used to cure malarial fever.
- A decoction of the pods relieves biliousness and acts as a purgative.

Systemic position of *Cassia angustifolia:*

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinioideae
Tribe: Cassieae
Genus: Cassia
Species: Angustifolia
Common names: Seena, Indian Senna, Tinnervelly Senna, Cassia Senna.
Habitat: Cultivated in dry lands of Southern & Western India, and indigenous to Arabia.
Parts Used: Pods, Stems and leaves
Description: A small erect shrub, Indian senna attains a height of about 2 to 3 feet. Its stem is pale green, smooth and erect. The spread out branches possess around 4 to 5 pairs of leaves. The plant surface is shiny green and dorsal surface is yellowish green. The plant has small yellow flowers. The brown pod contains 5 to 7 seeds that are dark brown in color.

***Fig: 2 Cassia angustifolia* (Linn.) Plant**

Uses & Benefits of Indian Senna

- Indian senna decreases pita and allows free movement of vata in the body.
- The herb stimulates liver for proper secretion of enzymes in the body.
- It helps in lowering bowels and increasing the peristaltic movement of the intestines.
- Indian senna purifies blood and restores the metabolic imbalance lost due to indigestion.
- The powder made from leaves and fruit is helpful in treating constipation and indigestion.
- It is useful in the treatment of osteoarthritis, gout and rheumatoid arthritis.
- The herb is used as an expectorant, wound dresser, antidysentric, carminative and laxative.
- Indian senna is handy in treating loss of appetite, hepatomegaly, spleenomegaly, malaria, skin diseases, jaundice and anemia.
- The herb has purgative, anthchiintic, antipyretic, laxative, vermifuge and diuretic properties.

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, Ku Tzu Malaysia, Scurf-pea, Malaysian Scurfpea. In India it is commonly called 'Babchi'. In Telugu it is called Bavanchalu.

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 and they are black, roundish or oblong, closely pitted, one seeded, smooth. It is a slow growing species mainly cultivated by seeds.

***Fig:.3 Psoralea corylifolia* Plant**

Medicinal Uses:

- According to Ayurveda, root is useful in carries of teeth and leaves are good for diarrhoea.
- Fruit is diuretic, and causes biliousness.
- It is useful in treatment of vomiting, piles, bronchitis, inflammation, anaemia etc. It improves hair growth and complexion.
- Seeds are refrigerant, alternative, laxative, antipyretic, antihelmintic, alexiteric and good for heart troubles.
- Seed oil is used externally for the treatment of elephantiasis.
- The seed is antihelmintic, antibacterial, aphrodisiac, astringent, cardiac, cytotoxic, , diaphoretic, diuretic, stimulant, stomachic and tonic.
- The seed and fruit contain Psoralen. The root is used for treating dental caries.
- The plant yields a useful medicinal oleoresin, it treats kidney disorders, impotence, lumbago.
- It is also used externally 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.

Phytochemistry and Pharmacology of Selected Plants:

Genus: *Acacia:* The genus Acacia, one of the important genera of the Fabaceae family (Sub family Mimosaceae), which includes approximately 1350 species and is abundant in India, Africa, America and Australia. The Acacia species are of immense value for reforestation and reclamation of wastelands (Skolmen, 1986), for fuel wood, timber, shelter belts and soil improvement (Palmberg, 1981). Most of its species yield excellent firewood and some are rich sources of protein, tannin, paint, ink, flavouring agents, pulpwood and gum. A number of secondary metabolites have been reported from various Acacia species including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non-protein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003).

Free radical scavenger and reducing power assays of methanol and aqueous extracts of *Acacia sinuate* (Lour.) merr leaf revealed that the methanol extract of *Acacia sinuate* (Lour.) merr possessed highest content of phenol and flavonoid while aqueous extract had the lowest content (Anand*et al.*, 2014). Free radical scavenging properties of phenolic compounds is an important property underlying their various biological and pharmacological activities. Methanol as well as aqueous of *A. sinuata*, exhibited an antioxidant activity in a dose-dependent manner. *A. Sinuate* methonolic extract at different doses exhibited significantly highest antioxidant activity as compared to aqueous extract of *A. sinuata.* The extracts of *A. sinuata* possessed free radical scavenging activities, but to varying degrees, ranging from 12.87 to 76.12%. DPPH scavenging, Methanol extract showed immense DPPH scavenging activity.

Genus: *Cassia:* Cassia is the largest genus in subfamily Caesalpinioideae of the Fabaceae. It comprises about 600 species (Brenan, 1967; Singh, 2001). Members of the genus Cassia consists of annual or perennial herbs, shrubs and trees which have been differentiated on the basis of number of leaflets, fertile and sterile stamens in single flower and glands present on the leaves (Cooke 1902).

Anti-Inflammatory Activity of Flower Extract of *Cassia angustifolia* results indicate that the its Acetone flower extract possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavanoids, tannins, steroids and phenols. Protective effect on heat and hypotonic saline, induced erythrocyte lysis is known to be a good index of anti-inflammatory activity of any agent. Since the membrane of RBC is structurally similar to the lysosomal membrane the effect of any substance on stabilization of RBC membrane may be extrapolated to the stabilization of lysosomal membrane. Purification of bioactive compound is necessary which may show increased activity. Hence, this study gives an idea that the compound of plant *C. angustifloia* can be used as a lead compound for designing a potent anti-inflammatory drug which can be used to cure inflammation (Anitha Rani *et al*., 2014).

Genus: *Psoralea:* *Psoralea* genus is a legume that has 150 species (Hooker and Jackson, 1960). 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 (scurf pea) has a wide geographic distribution including the New World, Eurasia, India and South Africa. These plants can be herbs or shrubs with 1-5 foliolate leaves, and variously colored flowers in spikes or racemes. Some of the species of plants plays an important role in the treatment of

various disease like antipyretic, anthelmintic, alexiteric, bronchitis, inflammation, anaemia etc.,

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 phytochemical and TLC analysis of the methanol extract followed by GC- MS screening confirmed the presence of various phytochemicals. Major identified phytochemicals in methanol extract of *P. corylifolia* seeds are phenol derivatives and coumarin, psoralen, isopsoralen which may be accountable for its antifungal activity (Borate *et al*., 2014).

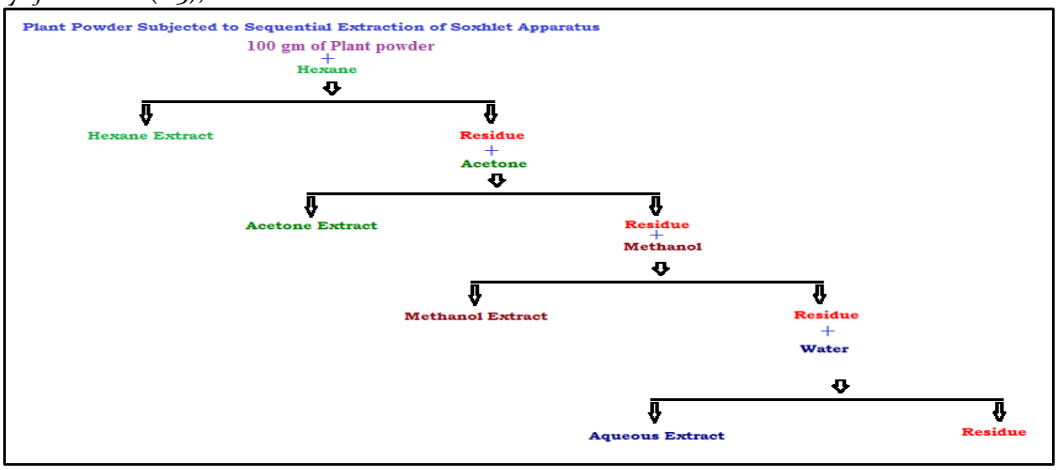
The in vitro antibacterial activity of *Psoralea corylifolia* leaf and its corresponding callus extracts were studied against pathogenic bacteria causing periodontitis. Leaves and corresponding calli were extracted using petroleum ether, chloroform, acetone, methanol and distilled water. Among the five solvents used, leaf and callus extracted in methanol were found to be more effective against pathogenic bacteria (Archana Moon *et al*., 2012).

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* (Sheng *et al*.,2004).

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). PriyankaPandey*et al* (2013) investigations found that alkaloids, phenols, tannins, flavanoids andsaponins are present in seeds of plants. TLC and HPLC analysis also confirmed these results. The TLC results of ethanol extract and aqueous extract shows that at least two different bioactive phyto constituents are present in each extract of *Psoralea corylifolia (Linn.).*

Phytochemistry and Pharmacology of Selected Plants:

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 *Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.)* and *Psoralea corylifolia (Linn.)* were collected and washed with running water followed by distilled water. The dried material was stored in a sterilized polythene bags for further study.



Sequential Extraction of Soxhlet Apparatus

Phytochemical Analysis:

Preliminary Screening of Phytochemicals (Qualitative Analysis): Standard screening tests of four extracts *of Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.) and 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 using standard procedures (Satheesh Kumar *et al.,*2012).

Physicochemical Evaluations: The physicochemical characteristics of the *Acacia sinuate* (Lour.) merr., *Cassia angustifolia*(Linn.)

and *Psorelea corylifolia* (Linn.) extracts showed the preliminary information of such plant extracts. The successive extracts of plant material with non polar to polar solvents results were tabulated in table No: 4.1.1 to 4.1.3 and fig.4.1.1 to 4.1.3. Variation in the colour of the extracts showed the variability of the presence compounds in the solvent extracts, and it also proved that variation in the dissolution of bioactive compounds from non polar to polar solvents. The tabulated results and graphical representation showed that yield of extract has been increasing from non polar solvents to the polar solvents (Sultana *et al.*, 2009).

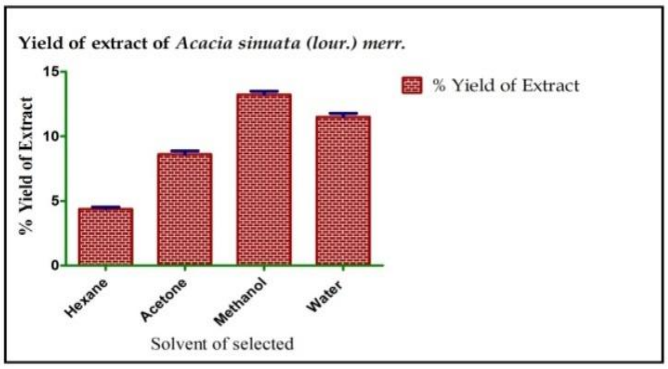


Fig: 4: Analysis of Physicochemical Characteristics of *Acacia sinuata* (Lour.) merr.Extracts

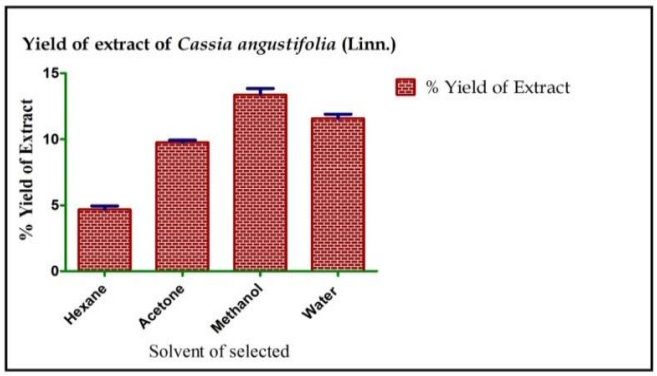


***Fig.* 5:** Analysis of Physicochemical Characteristics of *Cassia angustifolia* (Linn.)Extracts

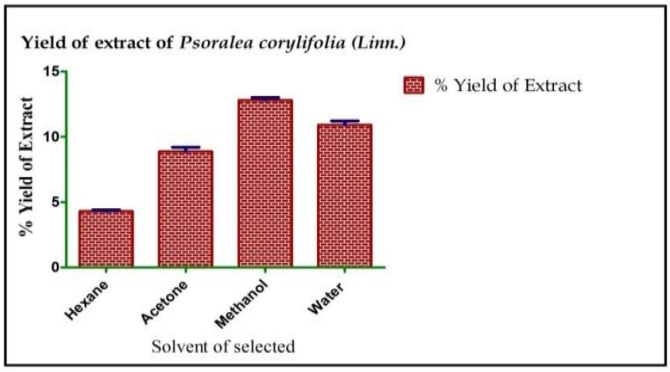


***Fig.* 6: Analysis ofPhysicochemical characteristics of *Psorelea corylifolia* (Linn.) Extracts Total Phenol Content of selected plants**

The total phenol content of the *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) extracts were determined by folinciocalteu method where Gallic acid was used as a standard control (Karim*et al.,* 2011).The quantitative analysis results of above said extracts were tabulated in table 4.1.7 to 4.1.9.These results showed that *Acacia sinuata*(Lour.) merr extracts posses high amount of phenols than other two plant extracts. Hexane extracts contain less amount of phenol when compared with polar solvent extracts, where as methanol extract of above said plants contain high quantity of phenol followed by water and acetone extracts.

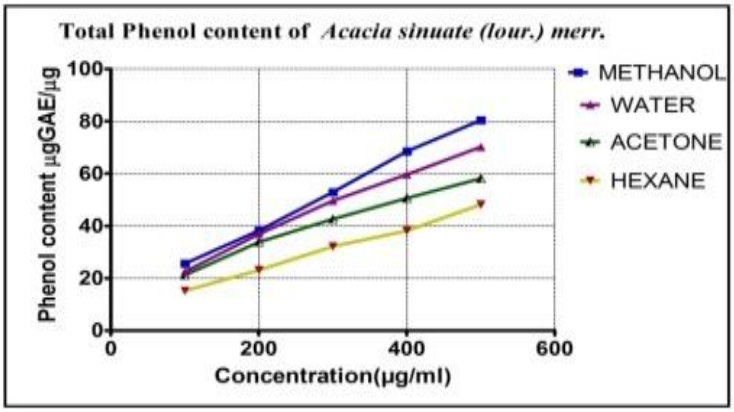


Fig: 7 Studies on Total phenol content of *Acacia sinuate* (Lour.) merr.

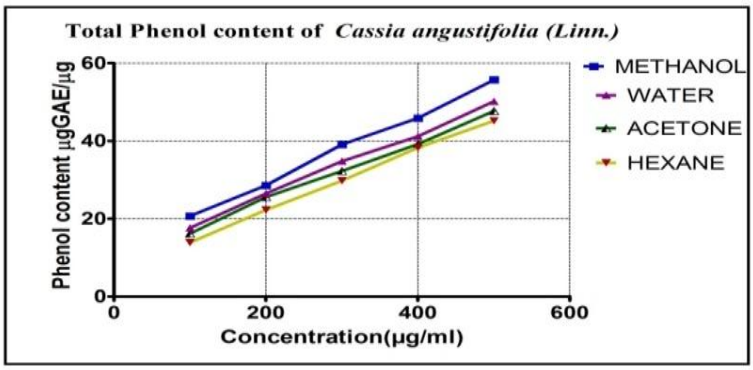


Fig. 8 Studies onTotal phenol content of *Cassia angustifolia* (Linn.)

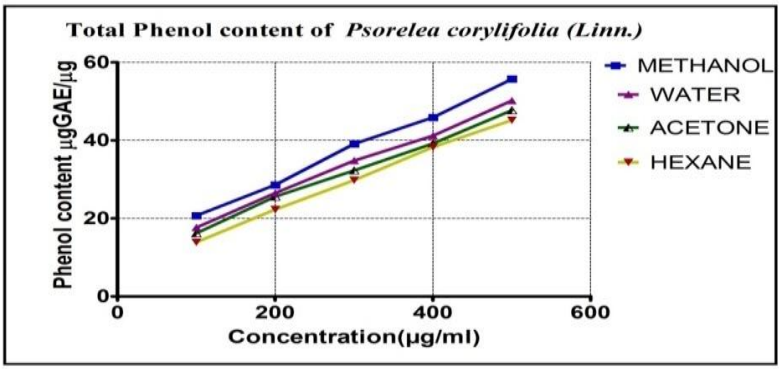


Fig: 9 Detection of Total phenol content of *Psorelea corylifolia* (Linn.)

Total Flavonoid content of Selected Medicinal plants

In the present study the quantification of flavonoids in the extracts of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) *was* determined by aluminum chloride colorimetric method where rutin was used as a positive control. The results of all these plant extracts were tabulated in table 4.1.10 to 4.1.12. Out of above said plant extracts *Acacia sinuata*(Lour.) merr extracts possess high amount of flavonoids followed by *Psoralea corylifolia* (Linn.) and *Cassia angustifolia (Linn.).*The results showed that the flavonoids were higher in methanol extract as compared to other three extracts. Methanol extract showed dose dependent activity i.e. by increasing the concentration, the amount of flavonoids increased gradually. Aqueous extract contained significantly more amount of flavonoids as compared to acetone and hexane extracts.

Antibacterial Activity on gram +ve strains: Generally, the antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed. Also this antibacterial actions could be related to their chemical components in the crude extracts (Barile *et al.*, 2007; Ayoola*et al.*, 2008; Blois *et al.*,1958;

Akharaiyi*et al*., 2011;Varahalarao *et al*., 2012; Sekar*et al*., 2012).

The antimicrobial activity of four extracts of*Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) were tested against five gram positive bacteria- *Staphylococcus aureus, Streptococcus mutans, Lactobacillus casei, Lactobacillus acidophilus, and Bacillus megaterium.* After proper incubation the results were recorded and represented in Fig: 4.1.10 to Fig: 4.1.12. These recorded results said that *Acacia sinuata*(Lour.) merr has most potential antimicrobial activity against gram positive organism followed by *Psoralea corylifolia* (Linn.) *and Cassia angustifolia (Linn.).*

Among the above extracts methanol extract of the *Acacia sinuate* (Lour.) merr showed a grater activity than the other extracts. 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.*

Antibacterial Activity for gram +ve strains:

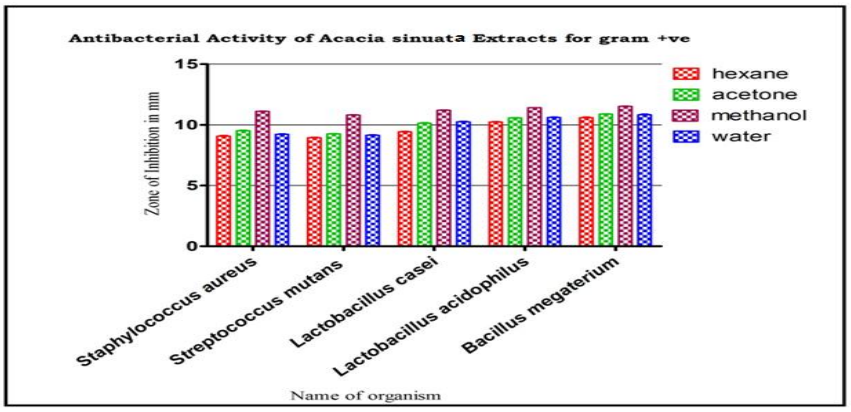


Fig : 10Studies on Antimicrobial activity of *A.sinuata*(Lour.) merr. extracts for gram $^{+ve}$

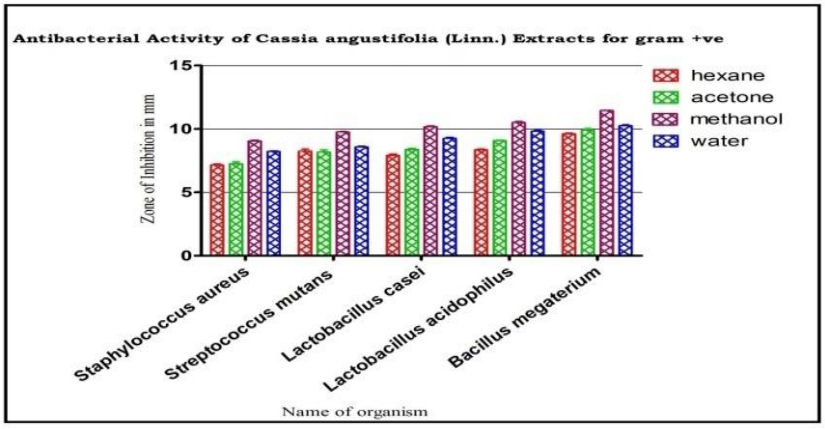


Fig : 11: Detection ofAntimicrobial activity of *C.angustifolia*(Linn.)extracts for gram $^{+ve}$

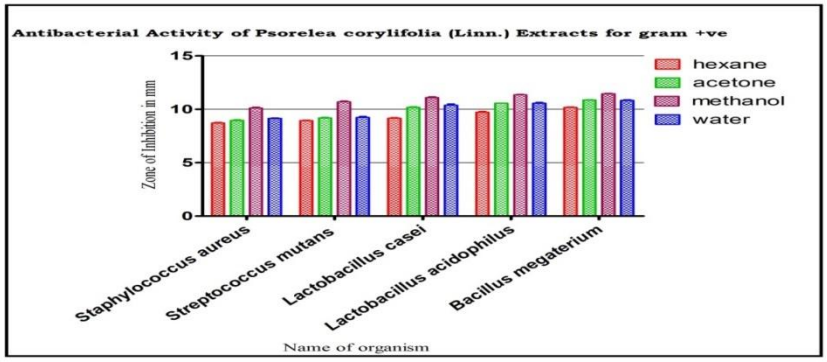


Fig. 12: Studies on Antimicrobial activity of *Psoreleacorylifolia*(Linn.)extracts for gram $^{+ve}$ Anti fungal Activity

In the present study all the extracts of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) showed antifungal activity and the results were recorded and represented in Fig 4.1.16 to 4.1.18. From these results it was proved that Methanol extracts of above said plants showed highest antifungal activity, followed by water and acetone. Whereas hexane extract found lesser antifungal activity than other extracts. Out of three experimented plants, *Acacia sinuate* (Lour.) merr extracts found to possess highest antifungal activity against *Candia albicans, Aspergillusniger, Rhizopusoryza and Candida rogasa*. The extracts of *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) showed moderate antifungal activity. The methanol extract of *Acacia sinuate* (Lour.) merr showed most potential activity against tested fungal species. Antifungal activity of tested plant extracts also proved that methanol extract of *Acacia sinuate* (Lour.) merr found to contain most antimicrobial activity on *Candida albicans*.

Detection of Anti-fungal Activity of *Acacia sinuate* (Lour.) merr.: In the present study all the extracts of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) showed antifungal activity and the results were recorded and represented in Fig 4.1.16 to 4.1.18. From these results it was proved that Methanol extracts of above said plants showed highest antifungal activity, followed by water and acetone. Whereas hexane extract found lesser antifungal activity than other extracts. Out of three experimented plants, *Acacia sinuate* (Lour.) merr extracts found to possess highest antifungal activity against *Candia albicans, Aspergillusniger, Rhizopusoryza and Candida rogasa,* .The extracts of *Cassia angustifolia*(Linn.) and *Psoralea corylifolia*(Linn.) showed moderate antifungal activity. The methanol extract of *Acacia sinuata* (Lour.) merr showed most potential activity against tested fungal species. Antifungal activity of tested plant extracts also proved that methanol extract of *Acacia sinuate* (Lour.) merr found to contain most antimicrobial activity on *Candida albicans*.

Antioxidant Activity: DPPH Radical Scavenging Antioxidant Activity: The DPPH activity results of three plant extracts were tabulated and calculated the IC_{50} for these results. From the results, it was proved that the extracts of *Acacia sinuata*(Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) possess hydrogen donating capabilities and it will act as an antioxidant. The results from this experiment revealed that the methanol extracts of screened plants showed highest antioxidant capacity followed by water and acetone extract. Whereas hexane extract showed lowest antioxidant activity. Out of all the extracts *Acacia sinuate* (Lour.) merr methanol extract showed the maximum antioxidant potentiality and lower IC_{50} value.

FRAP Radical Scavenging Antioxidant Activity: The FRAP assay is a simple and inexpensive procedure that measures the total antioxidant levels in a sample. The method measures the reducing ability of antioxidants against the oxidative effects of ROS. The higher the absorbance, the higher is the antioxidant activity which is indicated by the high FRAP value. The reducing power of a compound is related to its electron transfer ability and may therefore, serve as a significant indicator of its antioxidant activity (Ajila*et al* ., 2007). In the reducing power assay, the extracts of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) showed a concentration dependent antioxidant potential. Among all the tested fractions, methanol extract of the *Acacia sinuate* (Lour.) merr exhibited highest FRAP Value followed by water, acetone and hexane extracts. All the fractions of *Cassia angustifolia* (Linn.) showed lower FRAP values.

From the antimicrobial studies, it was proved that methanol extracts possess most potential solvent to extract the antimicrobial substances which are found in the plant material. This is because, methanol solvent is an ideal solvent to extract the antimicrobial possessing compounds from the plant source. Out of all the extracts of selected plats, methanol extract of *Acacia sinuate* (Lour.) merr, possess most potential antimicrobial activity. The potential antimicrobial activity of the methanol extract of *Acacia sinuate* (Lour.) merr, due to the presence of a detectable amount of alkaloids, flavonoids, terpenes and saponins.

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 this context medicinal plants have the most efficient antioxidant compounds and are being used traditionally. Nonetheless, these plants need further screening to assess their medicinal properties. Investigations on medicinal properties of these plants have been enhanced globally in recent years owing to their low toxicity, wide pharmacological activities and economic viability (Auddy*et al.,* 2003). In the Indian traditional practice of Ayurveda rasayana of medicinal plants, extensive antioxidant properties were identified since they are being used in curing diseases (Aqil*et al.,* 2006).

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 *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The IC50 was calculated for each extract as well as ascorbic acid as standard. The results from this experiment revealed that the methanol extracts of screened plants showed highest antioxidant capacity followed by water and acetone extract. Whereas hexane extract showed lowest antioxidant activity. Out of all the extracts *Acacia sinuate* (Lour.) merr methanol extract showed the maximum antioxidant potentiality and lower IC_{50} value. DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. 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. Substances capable of donating electrons/hydrogen atoms are able to convert DPPH (Purple) into their non-radical form 1, 1-diphenyl-2- picrylhydrazine (Yellow), a reaction which can be followed spectrophotometrically. 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 *Acacia sinuata*(Lour.) merr, *Cassia angustifolia*(Linn.) and *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; Moura*et al.,* 2007).

FRAP Radical Scavenging Antioxidant Activity: Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003; Hsu et al., 2006). 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 *Acacia sinuata* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) showed the concentration dependent antioxidant potential. When we compare the three plant species, *Acacia sinuate* (Lour.) merr, has highest antioxidant activity than others. Among the extracts methanol extract of selected plant species exhibited highest FRAP Value followed by water, acetone and hexane extracts. The results indicated that methanol is the ideal solvent to extract the plant metabolites which are having antioxidant activity.

Summary: The present investigation is aimed at evaluate the phrmacological activity of selected Fabaceae plants and describe the suitable media for micro propagation of those plants. Based on previous reports I have selected three Fabaceae plants namely- *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoreleacorylifolia* .

To achieve this entitled work we, have designed the work with two main objects.

1. Investigation of the phytochemistryand pharmacological activities of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) in a scientific manner. It includes following steps.
2. Standardization of the Micro propagation protocol for the selected plants by using following steps in scientific manner.

After 25-30 days of first subculture, established cultures were transferred to culture jars having respective media combinations. Multiplication of shoot cultures was carried out by culturing nodal segments/clusters excised from *in vitro*-raised plants. Observations were taken for evaluating the growth of explants by taking parameters like inter nodal distance average shoot length and number of nodes (15 shoots randomly selected per medium). From these results it was observed that *Acacia sinuat a*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea corylifolia* (Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium, while MS medium showed lowest number of shoots. The texture of leaf was succulent and fleshy. During each passage, the number of leaves/shoots has increased substantially along with the height of shoots. The leaf size was approximately 0.5-0.6 cm in *Acacia sinuate* (Lour.) merr, 0.4-0.6 cm in *Cassia angustifolia (Linn.), and* 0.6-0.7 cm in *Psoralea corylifolia (Linn.)*.

After three cycles of multiplication subculture, elongated shoots of 2 cm in length were excised and cultured on MS basal medium having different combinations of sugar and agar with MS basal (MS+ Sugar 30 gm/l +Agar-0. 8 gm/l) as control. The experiments were conducted twice, with 3 replications (with 3 shoots per bottle). Rooted shoots were taken after 2 weeks, shoot length, root length and no of roots per explants (total 9 explants per treatment each time), were measured and tabulated. Initiation of rooting took place after 5-6 days of inoculation. Single and multiple roots were formed from the base and the nodal portions and the length of the roots were 1-2 cm within 8-10 days. During multiplication the rooting in plants in vitro culture is fairly spontaneous and no addition of growth regulators is further necessary. Hence the experiment is designed to study the rooting response with different treatment combination with two variations of agar (7 and 8 gm/l) and sugar (0, 10, 20, 30 gm/l). The results were postulated that highest shoot and root ratio (S/R) and biomass accumulation may indicate positive responses. It was observed in RT 5 (MS+agar 7gm/l+sugar 20gm/l) recorded highest S/R in all explants of *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea corylifolia (Linn.)* (3.01, 3.10 and 3.06 respectively). RT6 of three plants showed highest length of root. Minimal media (RT 1 and RT 2) i.e. having 0% sugar and lower concentration of agar also showed positive results so this combination can also be used for rooting of explants. Our results indicate 100% root formation in all the mediums (MS basal without any additional growth regulator). Ex-vitro rooting was also carried out for *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea corylifolia* (Linn.) using single shoots of 1-2 cm in height derived from MS medium and then transplanted in Soil: agro peat mixture in the ratio of 4:1which gave 100% survival rate.

Transplantation was done by using different soil mixtures in protrays and polybags. These trays and bags were then kept inside the poly house, where humidity maintained is approximately 80% and temperature was 28 –30°C and kept there for 15 days. The observation was taken 30 days after transplantation. However in terms of root length and shoot length, HM_8 potting mixture (4 soil: 1agropeat) was found to be the best combination. Number of roots was found to be highest in case of HM_6, soil and farmyard manure mixture (4:1), (HM_1) was found to be the better option with significantly higher number of roots and high shoot/root ratio. It was observed that across four parameters two best hardening mixtures were HM_6 and HM_1. The current micropropagation protocol is quite successful and recommended for further necessary work.

Conclusion: Basing up on the observations in the present study, it can be concluded that all the extracts of *Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.), Psorelea corylifolia*, posses a detectable amount of phytometabolites. Quantitative analysis revealed that, phenols and flavonoids concentrations increase from non polar to polar solvents. Out of these selected plants

extracts *Acacia sinuate* methanol extract found highest concentration of flavonoids and phenols. Pharmacological studies revealed that all the extracts of selected plants showed the antibacterial, antifungal and antioxidant activity, their activities increases from non polar to polar solvents. This work also proved that *Acacia sinuate* (Lour.) merr extracts posses most potent anti microbial and antioxidant activities than *Cassia angustifolia (Linn.) and Psorelea corylifolia*. 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 *Acacia sinuate* in BM_2, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) in BM_3, were 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.
- *Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea corylifolia* (Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium. Whereas MS medium showed lowest number of shoots.
- RT $_5$medium is the best media to get highest S/R in all explants of *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea 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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