

Qualitative and quantitative analysis of biologically active principles, baicalein, luteolin and psoralen from *Oroxylum indicum*, *Premna integrifolia* and *Aegle marmelos* respectively and its allied species



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**Qualitative and quantitative analysis of
biologically active principles, baicalein,
luteolin and psoralen from *oroxylum
indicum*, *premna serratifolia*, *aegle
marmelos* and their allied species**

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Title of the project : **KFRI/486/05**, Qualitative and quantitative analysis of biologically active principles, Baicalein, Luteolin and Psoralen from *Oroxylum indicum*, *Premna serratifolia*, *Aegle marmelos* and their allied species

Objectives :

- Isolation and characterisation of *baicalein*, *luteolin* and *psoralen* from *Oroxylum indicum*, *Premna integrifolia* and *Aegle marmelos* respectively through HPLC/GC.
- Comparative analysis of the principle with its allied species.
- Screening of biological activities by *in vitro/in vivo* assay system.

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Abstract

In the present study, analyses were made to detect the presence of baicalein, in *Scutellaria colebrookiana* and *S. violacea* (Lamiaceae); *Oroxylum indicum*, *Stereospermum colais*, *Stereospermum suaveolens*, *Dolichandrone arcuata*, *Radermachera xylocarpa* and *Millingtonia hortensis* (Bignoniaceae). *Premna serratifolia* (Verbenaceae) was analysed for the presence of luteolin. *Aegle marmelos*, *Clausena indica*, *Glycosmis pentaphylla*, *Murraya koenigii* and *Paramignya monophylla* (Rutaceae) were also subjected to phytochemical analysis to detect the presence of Psoralen.

Among the plants studied, the baicalein content was detected in *Scutellaria colebrookiana*, *S. violacea* and *Oroxylum indicum*. As per the procedure followed, luteolin was not detected in *Premna serratifolia*. Among the five Rutaceae species screened, psoralen was detected in *Aegle marmelos* and *Murraya koenigii*. The presence of baicalein and psoralen was confirmed through Column chromatography, Spectrophotometry, Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) analyses.

Antioxidant properties of the selected species were analysed by scavenging the free radicals such as superoxide, hydroxyl radicals and lipid peroxidation generated by *in vitro* assay systems. Among the four species studied for antioxidant properties, *Scutellaria colebrookiana* and *S. violacea* showed significant free radical scavenging activities. Further, the stable free radical 1,1-Diphenyl-2 Picrylhydrazyl (DPPH) was effectively scavenged by chloroform and acetone extracts of the roots. The data obtained from *in vitro* antioxidant studies indicate that *Scutellaria colebrookiana* and *S. violacea* possess significant antioxidant properties. This preliminary data suggests the wide spectrum biological potentials of the two *Scutellaria* species studied. Though luteolin content in *Premna serratifolia* was reported earlier, it was not detected in the present study as per the procedures followed. However, the plant extracts showed significant antioxidant properties in *in vitro* assays.

1. Introduction

India is enriched with vast resources of medicinal plants that have been extensively used in various Indian systems of medicine. Because of many adverse effects of synthetic products in therapy, the interest and usage of medicinal plants have increased tremendously. In most Indian systems of medicine, the source of a drug is often attributed to only one species, and its continuous extraction will lead to rarity (Patwardhan, 2000). In the absence of the preferred species, the general trend is to meet the demand with substitutes consisting of related or unrelated species without proper evaluation (Patwardhan *et al.*, 2004). Hence, the identification of plants, chemical constituents, and biological property analysis in the phytochemical industry are very important criteria.

Recently, considerable attention has been paid to utilise eco- or bio-friendly plant based products for the prevention and cure of different human diseases. The identification of biologically active compounds is an essential requirement in plant-based drug preparation. The compounds, responsible for biological activity, are secondary metabolites. Complete phytochemical investigations of the medicinally important herbs have not been carried out so far. National Cancer Institute (NCI) has started a major screening programme to sort out the biologically active principles from natural resources since 1960. Over half a century after launching therapy for treatments, phytochemicals have become an important part of drugs. Actually, 70% of drugs approved between 1940 and 2002 are either natural products or developed based on knowledge gained from natural products.

The development of therapeutic drugs involves screening of natural products. During the past decades, a large number of compounds like various types of alkaloids, flavonoids glycosides, terpenes, phytoalexins, coumarins, polyphenols etc. were isolated, identified and their mechanism of action tested against various diseases.

Among plant-derived chemicals, the flavonoids are the most effective group of compounds for general ailments because of their strong antioxidant efficacy. According to modern medicine, a number of the diseases are caused by the overproduction of free radicals. These highly reactive radicals may damage the biological macro molecules including oxidation of enzymes, modification of amino acids, fragmentation of proteins, nucleic acids, etc. Even though the body system has several 'safeguard' mechanisms, these may not be sufficient to overcome the insult produced by excess stress. In such circumstances, supplementation of non-toxic antioxidants may have a chemoprotective role in the body (Logani *et al.*, 1979).

The development of molecular technique has led to the identification of key molecule that regulates normal biological process and, in some instances, has elucidated the cellular pathway responsible for diseases. This information is now exploited for molecular design of therapeutic drugs that specifically target the key pathways. Among these, a considerable interest has been focused on the biological efficacy of various alkaloids, flavonoids glycosides and polyphenolic groups. Most of these are widely distributed in nature mainly as secondary metabolites in all plants. Some of these flavonoids act as vital links in the electron transport chain playing important role in the biochemistry of energy production in their hosts. While many others show pronounced cytotoxic and allergenic actions that might enable the hosts to defend themselves against invading pathogen. A number of natural flavonoids as well as their synthetic analogues have been found to possess significant medicinal properties. Among these the 2 flavonoids, baicalein, luteolin and a phytoalexin, psoralen are found to be considerable focus with its various biological activities.

Baicalin (Figure 1), is a trihydroxy flavonoid glycoside, originally isolated from *Scutellaria baicalensis*. This herbaceous plant belonging to the family Lamiaceae, is well known in Chinese medicine. The plant is generally called 'Skullcap' and is widely used

for the treatment of various diseases such as hepatitis, bronchitis as well as tumors, contemporarily. Based on Chinese medicine, this drug is considered as a detoxification and dampness removing agent and unblocks the lung and stomach meridians. Therefore, it can be used to treat the diseases related to respiratory and digestive systems. Various phytochemical as well as pharmacological studies have been reported its antioxidant, anti-tumour, anti-genotoxic, anti-inflammatory and anti-mutagenic properties. Apart from above properties, *Scutellaria* alone or in combination with other herbs has recently been shown to possess cytostatic effect on several cancer cell lines in *vitro* as well as *in vivo* (Yano *et al.*, 1994). The bioactive components of *Scutellaria* have been confirmed to be flavones including wogonin, baicalin and its aglycon form baicalein. It is concluded that baicalein had the strongest biological properties. In particular, baicalein is a specific inhibitor of reverse transcriptase, the enzyme responsible for the multiplication of RNA of retroviruses including HIV. This glycoside can induce apoptosis in cancer cells *via* mitochondria-mediated pathways and inhibit reverse transcriptase enzyme in retroviruses. Importantly, they show very little toxicity to normal epithelial and normal peripheral blood and myeloid cells (Li-Weber, 2008). It also inhibits the proliferation of various transformed cell lines, induced apoptosis and inhibits COX-2, which may occur via the down-regulation of NF-kB.

The presence of flavonoid glycosides and its aglycon forms as well as some biological properties reported in *Scutellaria baicalensis* have also been reported from other species like *S. lateriflora* (American Skullcap), *S. radix* and *S. barbata*. The presence of baicalein, its variable glycoside and aglycon form have been recently reported from the unrelated *Oroxylum indicum* (Uddin *et al.*, 2003). Gastro-protective effect (Zaveri and Jain, 2007), immunostimulant activity (Zaveri *et al.*, 2006; Gohil *et al.*, 2008), cytotoxic, anti-tumor effects (Roy *et al.*, 2007), anti-inflammatory, anti-mutagenesis, antibiosis, anticancer effects (Yin *et al.*, 2007) of *Oroxylum indicum* are also

reported. Considering the presence of baicalein in a number of *Scutellaria* spp. and an unrelated Bignoniaceae species, *Oroxylum indicum*, the qualitative and quantitative analytical studies were carried out in *Scutellaria* spp. found in the Western Ghats of Kerala and allied species of *Oroxylum indicum* such as *Stereospermum colais*, *S. suaveolens*, *Dolichandrone arcuata*, *Radermachera xylocarpa* and *Millingtonia hortensis* for the baicalein content.

During aerobic metabolism, reactive oxygen species (ROS) are constantly generated by cells as normal byproducts. Under physiologic conditions, free radicals such as superoxide ($\cdot O_2$), hydroxyl radicals ($\cdot OH$) and the non-radical hydrogen peroxide (H_2O_2) are minor products of the mitochondrial oxidative respiratory chain. This occurs mostly in the form of $\cdot O_2$ which is metabolized to non-radical H_2O_2 via a dismutation reaction, catalyzed by the superoxide oxidoreductase dismutase (SOD), a cytoplasmic enzyme that defends against oxidative stress (Curtin *et al.*, 2002). Although ROS plays an important role in defense against pathogens and in the regulation of diverse cellular functions, including intracellular signaling, high levels of ROS induce oxidative stress that can lead to different types of diseases (Storz, 2005). Free radicals are chemically highly reactive molecules that indiscriminately attack human tissue and cause all kinds of problems including ageing, inflammation, autoimmune diseases, cancer, cardiovascular diseases, chronic fatigue, depression, etc. Flavonoids belong to phenolic antioxidants (PhOH) which are capable to transfer electron free radicals and also act as chelators of redox-active metal ions (Havsteen, 2002; Rice-Evans *et al.*, 1996; Valko *et al.*, 2006). Thus, these compounds interfere with the oxidation of lipids and other molecules by the rapid donation of hydrogen atom to radicals $ROO\cdot + PhOH \rightarrow ROOH + PhO\cdot$, and by take the redox-active metal ions away. Unlike the hydroxyl ($\cdot OH$) and superoxide ($\cdot O_2$) radicals, which are highly active due to a very short half-life, the phenoxy radical intermediates are relatively stable. So they do not initiate (propagate) further radical

reactions and thereby stopping the radical reaction chain. Thus, the association of flavonoids intake with reduced risks of cancer can be largely explained by the anti-ROS activities of these compounds. Investigation of the bioactivity and structure relationships of different flavonoids has revealed that the antioxidant properties of flavonoids involves the presence of 2,3-unsaturation in conjugation with a 4-oxo group in the C-ring, the hydroxyl groups in the B-ring and the 5-hydroxy group in the A-ring. Baicalein and baicalin possess the 2, 3-unsaturation and the 4-oxo in the C-ring and 5-hydroxyl group in the A-ring.

As in the case of baicalein, luteolin (Figure 2) is also an important flavonoid considering its antioxidant activities. Luteolin shows strong inhibitory activity in several models of oxidative stress and in Trolox test, it is twice stronger than that of vitamin E. Luteolin shows strong scavenging properties for superoxide radicals, singlet oxygen and inhibits single strand break in DNA induced by singlet oxygen. When ranked in order of potency, luteolin is more effective than vitamin C and seven other flavonoids in reducing DNA oxidative damage.

The body regulates ROS by means of enzyme systems and producing the antioxidant glutathione, superoxide dismutase and catalase. Usually these enzymes are less efficient in neutralizing ROS as a body ages and as a result, the body needs dietary sources of antioxidants. Furthermore, the enzyme called Gamma Glutamyl Transpeptidase (GGT) degrades glutathione and GGT is up regulated when the liver attempts to eliminate toxins from our diet, lifestyle, or infections. Dietary compounds that reduce GGT levels therefore increase glutathione and restore the proper levels of glutathione. These include bioflavonoids such as luteolin and its analogs such as quercetin, silymarin, which decreased GGT levels up to 23.79% in a double-blind placebo controlled study.

Thus, luteolin is such a good antioxidant that can help the body to withstand the adverse effects of radiation and chemotherapy. In a

study from Japan, researchers went looking for the factor in Rooibos tea that was protecting DNA from radiation-induced free radicals. They discovered that the protective factor is luteolin and gave dramatic protection to the bone marrow and spleen against radiation. Luteolin also provided dramatic protection against this drug-induced free radical damage. Bone marrow peroxidation decreased 91% and CPK levels (an indication of heart damage) were normalized by luteolin. Importantly, luteolin did not interfere with the therapeutic effects of doxorubicin, an anticancer flavonoid. Together, the actions of luteolin and other bioflavonoids can provide powerful cancer-fighting benefits.

The luteolin from the artichoke has shown to prevent LDL-cholesterol oxidation, which may reduce risk of atherosclerosis (Morton *et al.*, 2000). The anti-inflammatory and anti allergic properties of luteolin are also reported (Ueda *et al.*, 2002; Sartor *et al.*, 2002). Luteolin in low micromolar range inhibits the proliferation of normal and tumor cells, as well as *in vitro* angiogenesis and inhibits tyrosinekinase, an enzyme involved in tumor cell proliferation (Chowdhury *et al.*, 2002). Moreover, recent reports suggest that luteolin is strong chemopreventive agent and strong anti-carcinogen (Bagli *et al.*, 2004). Dietary supplementation with luteolin and 20-methylcholanthrene showed significant reduction on tumor expression, up to 60%. The tumor growth and tumor volume decreased to 85% compared with the control at the end of the experimental period. The ability of luteolin to inhibit N-acetyltransferase is relevant also for its antineoplastic properties. Luteolin inhibited the arylamine N-acetyltransferase activity and DNA-2-aminofluorene adducts formation in human and mouse leukemia cells for 24 h. Luteolin also displays potent antimutagenic action against some dietary carcinogens (e.g. polychlorinated biphenyl).

The presence of luteolin is reported in various plants including parsley, artichoke leaves, celery, peppers, olive oil, rosemary, lemon, peppermint, sage, thyme etc. Recently luteolin content was detected

in one of the Dasamoola plant, *Premna integrifolia* (Gokani *et al.*, 2007; Dasgupta *et al.*, 1984). Considering the presence of luteolin in a number of plants, qualitative analysis as well biological property studies were carried out in the allied species of *Premna serratifolia* (Figure 3).

The phototoxic phytoalexin, psoralen an effective ingredient originally extracted from the Chinese herb, *Psoralea corylifolia* L., is used to increase melanin in the skin. It is also used in the treatment of vitiligo, bald patches of hair and psoriasis. Recent studies reported that psoralen induces interstrand cross links in DNA when activated by UV light (Ashwood-Smith *et al.*, 1980). The presence of psoralen content in *Aegle marmelos* has been reported recently (Dhalwal *et al.*, 2007; 2008).

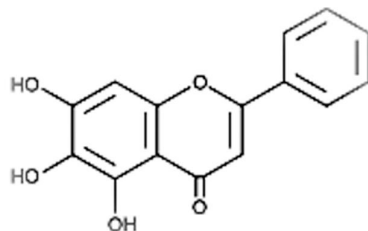


Figure 1. Structure of baicalein

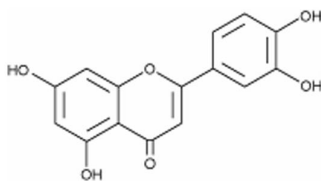


Figure 2. Structure of luteolin

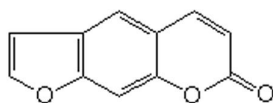


Figure 3. Structure of psoralen

2. Materials and methods

2.1. Phytochemical analysis

2.1.1. Screening for baicalein

Allied species of *Oroxylum indicum* such as *Stereospermum colais*, *S. suaveolens*, *Dolichandrone arcuata*, *Radermachera xylocarpa* and *Millingtonia hortensis* (Bignoniaceae); *Scutellaria colebrookiana* and *S. violacea* (Lamiaceae) were collected from various parts of Kerala.

Selected species and their profile

Oroxylum indicum (L.) Benth. ex Kurz (**Plate 1a**)

Local names: Aralu, Palakapayyani, Vellapathiri

Medium-sized deciduous trees. Leaves opposite, compound, 2-3 pinnate, pinnae 5-9; opposite; rachis 60-100 cm, stout, glabrous; leaflets 3-5 in each pinnae,; lamina ovate, base cordate, oblique or truncate; apex acuminate. Flowers 10 cm long, in lax terminal racemes, reddish-purple outside, pinkish-yellow within, racemes to 30-50 cm long. Fruit a capsule 40-75 x 5-8 cm, 2 valved, compressed, tapering at both end; seeds 5-6 cm long, winged all around except at base.

Stereospermum colais (Buch.-Ham. ex Dillw.) Mabb. (**Plate 1b**)

Local names: Pathiri, Poopathiri

Large deciduous trees. Leaves opposite, imparipinnate; rachis 10-21 cm long,; leaflets 7-13, opposite; lamina elliptic-lanceolate, elliptic-oblong, ovate or obovate; base oblique, acute or obtuse; apex acuminate or caudate acuminate; margin entire or serrate, glabrous above and puberulent beneath. Flowers bisexual, yellow

veined with red, 2 cm long, in terminal drooping panicles. Fruit a capsule, to 35 x 0.7 cm, subtetragonus, tapering at apex and base, epicarp thin, spirally splitting; seeds 8 mm long, wings obtuse at both ends.

Stereospermum suaveolens (G. Don) DC. (**Plate 1c**)

Local names: Kalagari, Paadal

Deciduous trees. Leaves opposite, compound, imparipinnate, rachis 150-450 mm long, slender; leaflets 5-11, lamina elliptic or ovate; base oblique, acute or unequally rounded; apex acuminate; margin entire or serrulate on young trees, shiny and glabrous above and pubescent beneath, coriaceous. Flowers bisexual, 3-4 cm long, dull crimson, in drooping pubescent panicles. Fruit a capsule, 30-60 x 0.5 cm, nearly terete, grey, lenticellate; seeds 3 cm long, trigonus, wedge shaped with a membranous wing with obtuse ends.

Dolichandrone arcuata (Wight) Clarke (**Plate 1d**)

Medium-sized deciduous trees. Leaves compound, opposite, estipulate; rachis 7-20 cm long; leaflets 5-11, opposite; petiolule to 5-30 mm, slender, tomentose; lamina 4-7.5 x 2-4 cm, orbicular or elliptic-ovate; base oblique or acute; apex acute or obtuse. Flowers bisexual, white, few in terminal corymbs or panicles. Capsule 2 valved, upto 45 x 1.5 cm, linear, terete, pubescent, speckled with white dots, curved.

Radermachera xylocarpa (Roxb.) K. Schum. (**Plate 1e**)

Local names: Kattumuringa, Pannimuringa

Medium sized deciduous trees. Leaves opposite, bipinnate or tripinnate; rachis 24-50 cm long; pinnae 7-9, leaflets 3-9 in each of the pinnae; lamina elliptic-lanceolate, ovate-lanceolate or ovate; base oblique or acute; apex acute or acuminate; margin

entire, membranous, glabrous. Flowers bisexual, 4.5 cm long, white, tinged with yellow, fragrant, in terminal paniced cymes. Fruit a capsule, elongate, 30-75 x 1.5-3.7 cm, warty tubercled, woody, bivalved, dissepiments spongy, slightly curved; seeds 1.5 cm long with membranous wings at both ends.

***Millingtonia hortensis* L.f. (Plate 1f)**

Local names: Akasaveppu, Maramalli

Medium-sized trees. Leaves opposite, 2-3 pinnate, rachis 45-70 cm long, stout; pinnae 11-17 pairs; leaflets 3-5, opposite; lamina 2.5-8 x 1.5-5 cm, ovate or elliptic-ovate, base oblique, truncate or acute, apex acuminate, margin entire or coarsely dentate-crenate, glabrous except midrib and lateral nerves beneath. Flowers bisexual, white, in terminal corymbose panicles; corolla 2.5 cm across; tube narrow, cylindric, 7 cm. Fruit an elongated capsule, 30x2 cm, 2-valved; seeds many, winged.

***Scutellaria colebrookiana* Benth. (Plate 2a & b)**

Herbs, puberulus. Leaves opposite, 2-4 cm across, deltoid, apex acute, base truncate, dentate, nerves 4 pairs; petiole 2.5 cm long. Racemes to 12 cm long. Flowers paired, calyx 5 x 5 mm, upper lip and lower lip equal, white, glabrous; corolla 12 mm long, pale purple, lower lip broader; filaments glabrous.

***Scutellariaia violacea* Heyne ex Benth. (Plate 2c)**

Local name: Kattuthulasi

Erect herbs, stem hispid. Leaves opposite, 4-7 x 3-5 cm, ovate, apex acute, base cordate, crenate, hispid; petiole 4-5 cm long. Racemes to 15 cm long, glandular hispid; bracts ovate, 3 mm long. Flowers paired, long-pedicelled; calyx glabrous, upper lobe saccate; corolla pale blue, 15 mm long, lateral lobes of lower lip shorter, glabrous; filaments fimbriate at base.



a. *Oroxylum indicum*



b. *Stereospermum colais*



c. *Stereospermum suaveolens*



d. *Dolichandrone arcuata*



e. *Radermachera xylocarpa*



f. *Millingtonia hortensis*

Extraction of baicalein from *Oroxylum indicum* and related species

The roots of the plants were dried and powdered. Approximately 15 g of sample was extracted with 300 ml ethyl acetate with a soxhlet extraction system. The solvent of extract was evaporated in a rotary evaporator (Buchi) at 60 °C. The ethyl acetate extract was again fractionated through medium pressure liquid silica gel (60 meshes) column chromatography system with successive elution with hexane, hexane and ethyl acetate (1:1) and ethyl acetate. The collected elutes were subjected to Thin Layer Chromatography (TLC) with various solvent system and the baicalein glycosides content was detected by spraying Benedict's Reagent and by spectrophotometry.

Solvent system used for chromatographic separation

1. Chloroform: acetic acid (9:1)
2. Ethyl acetate: benzene (9:11)
3. Benzene: ethyl acetate (19:1)
4. Petroleum benzene: acetone: methanol (4:4:2)
5. Acetone: methanol: water (4:3:3)
6. Petroleum benzene: acetone: methanol: water (2:4:3:1)

Column chromatography

Approximately 1 ml ethyl acetate was added in the sample, mixed and loaded on silica gel column (15 cm length) and eluted successively with 50-74 ml each n-hexane, n-hexane & ethyl acetate (3:1), n-hexane & ethyl acetate (2:2), n-hexane & ethyl acetate (1:3) and ethyl acetate. Elutes 50 ml of each extract was collected and evaporated to dryness.

Extraction from *Scutellaria*

The plants were dried at 50 °C and powdered. Approximately 10 g of sample was successively extracted with 300 ml of petroleum benzene, chloroform, acetone and methanol with soxhlet extraction system. The extracts were recycled for 6 times. The samples were transferred to 500 ml beakers and allowed to evaporate at room temperature.

Spectrophotometric analysis

Baicalein purchased from Sigma Chemical Co., USA was used as standard. The standard baicalein or isolated samples (1 mg/1 ml) were dissolved in ethanol and the absorbance from 200 nm to 900 nm was measured using a spectrophotometer (P.G. Instruments, USA) and the data were analysed with UV win5 software.

HPLC analysis

The presence of baicalein in *Scutellaria* species detected by medium pressure liquid chromatography was confirmed by High Pressure Liquid Chromatography (HPLC, Shimadzu, Japan) using standard baicalein.

2.1.2. Screening for psoralen

Fruits of *Murraya koenigii*, *Glycosmis pentaphylla* and *Aegle marmelos* were collected from the plains and *Paramignya monophylla* and *Clausena indica* from forests of Sholayar in Thrissur district.

Selected species and their profile

***Aegle marmelos* (L.) Correa (Plate 2d)**

Local names: Koovalam, Vilvam

Small deciduous trees. Leaves alternate, trifoliate; leaflets membranous and glabrous. Flowers yellowish-white in an axillary panicle, regular, actinomorphic and bisexual. Sepals 5, small. Petals 5, imbricate. Stamens numerous (60-80) inserted round the disc, anthers elongate. Fruit a berry, 5-10 cm across; seeds numerous, embedded in fleshy pulp.

***Murraya koenigii* (L.) Spreng. (Plate 3a)**

Local names: Kariveppila, Karivepu

Shrubs or small trees. Leaves pinnately 7-15 foliolate; leaflets, ovate, acuminate. Flowers regular, actinomorphic, bisexual in axillary and terminal cymes. Sepals ovate, acute. Petals greenish-white, clawed, imbricate. Stamens 10; anthers ovate. Ovary bilocular. Fruit a berry, subglobose, 7-10 mm across, black when ripe.

***Glycosmis pentaphylla* (Retz.) DC. (Plate 3b)**

Local names; Kuttipannel, Kurum panel, panal.

Shrubs. Leaves alternate, imparipinnate, acute; leaflets 3-5, subopposite. Flowers creamy white, regular, actinomorphic, bisexual in racemose panicles. Sepals minute, ciliolate. Petals 4-5. Stamens 10. Ovary 2-3 carpellary. Fruit a berry, globose, 8-10 across, pink when ripe; seeds 1-2.

***Clausena indica* (Dalz.) Oliver (Plate 3c)**

Local names: Kattukariveppila

Small trees. Leaves imparipinnate, 9 foliolate; leaflets alternate. Flowers regular, actinomorphic, bisexual in a panicle. Sepals ovate, acute. Petals greenish- white, ovate, glabrous. Stamens 10. Ovary



a. *Scutellaria colebrookiana*



b. *Scutellaria colebrookiana*



c. *Scutellaria violacea*



d. *Aegle marmelos*





a. *Murraya koenigii*



b. *Glycosmis pentaphylla*



c. *Clausena indica*



d. *Paramignya monophylla*



e. *Premna serratifolia*



tri-tetra carpellary. Fruit a berry, globose, 7-8 mm across, creamy yellow when ripe; seeds 1 or 2.

***Paramignya monophylla* Wight (Plate 3d)**

Climbing spiny shrubs. Leaves alternate, entire. Flowers regular, axillary. Sepals cupular 4-5 lobed. Petals 4-5 free. Stamens 8, free. Ovary 3-5 celled, style elongate. Fruit globose or obovoid berry, 25-3 cm long; seeds large.

Extraction of psoralen

The collected fruits were washed thoroughly with water and air dried for few days. The air dried fruits were further dried in the oven at 50 °C. The dried fruits were powdered in a mixer grinder. Approximately 1 g fruit powder was dissolved in 5 ml of methanol and stirred for 1 hour by a magnetic stirrer. Approximately 1 g of fruit powder was also dissolved in 5 ml of ethanol, chloroform and acetone. All these were subjected to magnetic stirring for 1 hour and left overnight. Each extract was filtered using Whatman filter paper and this filtrate was used for further TLC analysis.

TLC solvent systems used

1. toluene: dioxin: acetic acid (6 : 3.5 : 0.5)
2. chloroform: acetone: formic acid (7.5 : 1.5 : 1)
3. chloroform: ethyl acetate (6 : 4)
4. hexane: ethyl acetate: methanol: water (2.5 : 2.5 : 3:2)
5. ethyl acetate: methanol: water (3.5 : 3 : 3.5)
6. chloroform: acetic acid (5.5 : 4.5)
7. hexane: ethyl acetate: chloroform: ethanol (2.5 : 2.5 : 2.5 : 2.5)
8. toluene: petroleum benzene: acetic acid (4.4 : 4.4 : 1.2)

The most suitable solvent system was found to be toluene: petroleum benzene: acetic acid (4.4: 4.4: 1.2).

Spray reagents

1. Lead acetate
2. Aluminium chloride
3. Benedict's Reagent

The most suitable spray reagent for coumarin was Benedict's Reagent.

The powder of each sample was extracted with methanol, ethanol, chloroform and acetone, fractioned through thin layer chromatography with various solvent systems and analyzed for psoralen. These plates were sprayed with Benedict's Reagent.

Spectrophotometric analysis

The fractions eluted from TLC plates were used for the absorbance wavelength using a spectrophotometer. The results obtained were analysed with the software.

HPLC analysis

The presence of psoralen detected by medium pressure liquid chromatography in *Aegle marmelos* and *Murraya koenigii* was confirmed by High Pressure Liquid Chromatography (HPLC, Shimadzu, Japan) using standard psoralen (Sigma Aldrich).

2.1.3. Screening for luteolin

Selected species and their profile

***Premna serratifolia* L. (Plate 3e)**

Local name: Munja, Kozhichedi

Small trees or large shrubs. Leaves simple, opposite, lamina 2.5-8.5 x 2-7.2 cm, elliptic, elliptic-oblong, base acute, obtuse,

subcordate or rounded, apex acuminate, mucronate, obtuse, margin entire or subserrate. Flowers bisexual, greenish-white, in terminal corymbose paniced cymes. Corolla tube short, hairy inside. Fruit a drupe, seated on the calyx, globose, purple; seeds oblong.

Extraction of luteolin

The bark of *Premna serratifolia* was washed thoroughly with water and air dried for a few days. The air-dried bark was placed inside the oven at 50 °C for further drying and powdered in a mixer grinder. Approximately 5 gm of powder was dissolved in 20 ml of methanol and subjected to stirring for 1 hour by a magnetic stirrer. The extracts were filtered using Whatman filter paper and this filtrate was used for further chromatographic analysis including medium pressure silica gel and thin layer chromatography.

2.2. Biological property analysis

Male Swiss albino mice (25-30 g size) were purchased from Small Animal Breeding Station, College of Veterinary, Agricultural University, Thrissur, Kerala. The animals were maintained under standardized environmental conditions (22-28 °C, 60-70 % relative humidity, 12 h. dark/light cycle) and fed with standard rat feed (Lipton, India) and water *ad libitum*. All the animal experiments were carried out at Amala Cancer Research Centre with the prior permission of Institutional Animal Ethics Committee (IAEC).

Preparation of extracts

The dried bark and leaves were powdered and successively extracted with the solvents of different polarity like petroleum ether, chloroform, acetone and methanol using sohxlet extraction system.

The extracts were filtered, concentrated and evaporated to dryness. The dried extracts were dissolved in phosphate buffer saline (PBS) and used for further biological studies.

***In vitro* antioxidant activities**

The antioxidant properties of the plants were analysed by determining the scavenging effects of free radicals such as superoxide, hydroxyl radical and lipid peroxidation generated with *in vitro* assay systems.

Superoxide radical scavenging activity

The scavenging activity of extracts on superoxide anion radicals was determined by light induced superoxide generation with riboflavin and subsequent reduction of nitro blue tetrazolium (NBT) (McCord and Fridovich, 1969). Different concentrations of extracts ranging from 10 to 1000 μg were added to the reaction mixture containing 3 μg NaCN in 0.1 M EDTA; 0.12 mM riboflavin and 0.6 M phosphate buffer (pH 7.8) in a final volume of 3 ml. The tubes containing the reaction mixture were continuously illuminated with incandescent lamp for 15 minutes. The optical density measurements were taken at 530 nm before and after illumination. The effect of test material to inhibit superoxide generation was evaluated by comparing the Optical Density (OD) of control and treated samples.

$$\% \text{ of inhibition} = (\text{OD of Control} - \text{OD of treated} / \text{OD of Control}) \times 100$$

Hydroxyl radical scavenging activity

The scavenging activity of extracts on the hydroxyl radical (OH \cdot) was measured by the thiobarbituric acid reacting substances (TBARS) method. The scavenging activity was measured by studying the competition between scavenging and test compounds for hydroxyle

radical generated by the Fe³⁺/ascorbate/H₂O₂ system (Fenton reaction). The hydroxyl radical attacks deoxyribose eventually resulting in the formation of thiobarbituric acid reacting substances (Elizabeth and Rao, 1990). The reaction system contained deoxyribose (2.8 mM), FeCl₃ (0.1 mM), KH₂PO₄ KOH buffer (20 mM; pH 7.4) and from 10 µg to 1000 µg/ml of the test material in a final volume of 1 ml. The reaction mixture was incubated for 37 °C for 1 hour. The scavenging activity of hydroxyl radicals was expressed as:

$$\% \text{ of inhibition} = (\text{OD of Control} - \text{OD of treated} / \text{OD of Control}) \times 100$$

Lipid peroxidation assay

Lipid peroxidation induced in rat liver homogenate by the method of Bishayee and Balasubramonian (1971) and was estimated by thiobarbituric acid reactive substances by the method of Ohkawa *et al* (1979). Different concentrations of the extracts were incubated with 0.1 ml of rat liver homogenate (25%) containing 30 mM KCl, Tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM), ferrous ion (0.16 mM) in a total volume 0.5 ml for 1 hour at 37 °C. After incubation, 0.4 ml of the reaction mixture was treated with 0.2 ml SDS (8.1%), 1.5 ml thiobarbituric acid (0.8%) and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4 ml by adding distilled water and kept in water bath at 95 °C for 1 hour. After cooling, 1 ml distilled water and 5 ml of butanol-pyridine mixture (15:1, v/v) was added. After vigorous shaking, the tubes were centrifuged and the upper layer containing the chromophore was measured at 532 nm. The lipid peroxidation inhibition was calculated as:

$$\% \text{ of inhibition} = (\text{OD of Control} - \text{OD of treated} / \text{OD of Control}) \times 100$$

Anti-inflammatory activity

Acute and chronic anti-inflammatory assay systems were performed. To induce acute and chronic inflammation on right hind paw, carrageenan and formalin were used, respectively.

Carrageenan induced paw edema

Animals were divided into different groups comprising 6 animals in each group. Acute inflammation was induced through sub-plantar injection of 0.02 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of mice. One group with carrageenan alone served as positive control. The second group was administered with diclofenac (4 mg/kg body weight) intraperitoneally as standard reference drug. The other groups received extracts at the dosage of 50 and 100 mg/kg body weight, orally, 1 hour prior to the injection of carrageenan. The paw thickness was measured using vernier caliper before and 3 hours after carrageenan injection.

Formalin induced paw edema

Animals were divided into different groups comprising 6 animals in each group. Chronic inflammation was produced by injection of 0.02 ml of 1% formalin in the right hind paw of mice. One group was kept as control, the second group received standard reference drug diclofenac (4 mg/kg body weight) intraperitoneally, while other groups received 50 and 100 mg/kg body weight of extracts, orally one hour prior to formalin injection. The drug administration was continued for six consecutive days. The paw thickness was measured using vernier caliper before and 6 days after formalin injection.

2.3. Statistical analysis

The values are presented as mean \pm SD. Differences between group's means were estimated using a one way analysis of variance followed by Students' *t*-test. The results were considered statistically significant when $P < 0.05$.

3. Results

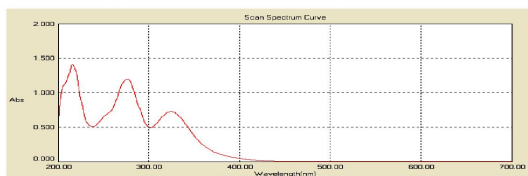
3.1. Detection of baicalein, psoralen and luteolin

Baicalein content

The presence of baicalein, its variable glycoside and aglycon form was reported from *Oroxylum indicum*. Preliminarily, the baicalein content was isolated using standardized protocol from *Oroxylum indicum*. The sample isolated from *O. indicum* and baicalein purchased from Sigma Chemical Co. USA was used as standard. Three peaks such as 324, 276 and 216 were observed in the absorption spectra of baicalein (Figure 4). Weight of the fractions

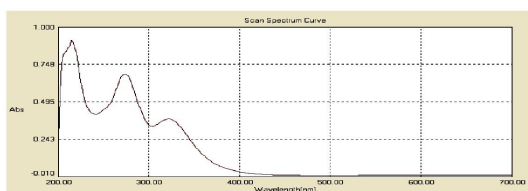
obtained in ethyl acetate are shown in Table 1. The quantity and percentage yield of elutes obtained by silica gel column are shown in Table 2. As shown in the table, most of the fractions loaded on column were recovered by hexane: ethyl acetate solvent system and the percentage yield ranged from 10-20%.

Extraction for the baicalein content was carried out in *Stereospermum colais*, *S. suaveolens*, *Dolichandrone arcuata*, *Radermachera xylocarpa* and *Millingtonia hortensis* belonging to the family Bignoniaceae and *Scutellaria colebrookiana* and *S. violacea* (Lamiaceae). The quantity and percentage yield of elutes obtained by silica gel column are shown in Table 2. As shown in the Table, most of the fractions loaded on column were recovered by the hexane: ethyl acetate solvent system and the percentage yield ranged mostly from 10-20%.



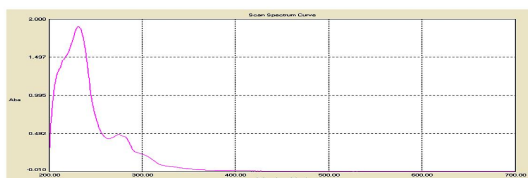
Peak 1: 324.00	0.725
Peak 2: 276.00	1.200
Peak 3: 216.00	1.410

Baicalein Standard (2ug/ml)



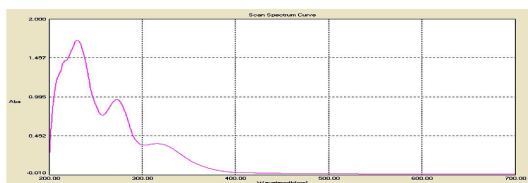
Peak 1: 321.00	0.379
Peak 2: 274.00	0.684
Peak 3: 215.00	0.913

Oroxyllum elution 3 (2ug/ml)



Peak 1: 275.00	0.479
Peak 2: 232.00	1.904

Oroxyllum elution 1 (2ug/ml)



Peak 1: 316.00	0.392
Peak 2: 273.00	0.965
Peak 3: 230.00	1.730

Oroxyllum elution 2 (2ug/ml)

Figure 4. Absorption spectra of various solvent extracts of *Oroxyllum indicum* isolated through column chromatography

The different fractions eluted from the column were then subjected to spectrophotometric analysis. The wavelength ranging from 200–900 nm was selected for generating the spectra (Figure 5-8). Among the fractions screened, the fraction obtained from *Scutellaria* spp. Alone showed the peaks identical with standard baicalein. The

collected elutes were also subjected to TLC with various solvent systems and the baicalien content was detected by spraying Benedict's reagent (Figure 9). The Rf. value of standard baicalein and samples from *Scutellaria* was equal.

Table 1. Weight of the fractions obtained in ethyl acetate by soxhlet extraction

No	Sample	mg.l
1	<i>Stereospermum suaveolens</i>	451
2	<i>Stereospermum colais</i>	599
3	<i>Dolichandrone arcuata</i>	499
4	<i>Radermachera xylocarpa</i>	428
5	<i>Millingtonia hortensis</i>	211
6	<i>Scutellaria colebrookiana</i>	227
7	<i>Scutellaria violacea</i>	271

Table 2. The quantity and % yield of different elutes obtained through silica gel column

No	Sample (1mg)	1 st Elute	2 nd Elute	3 rd Elute	4 th Elute	5 th Elute
1	<i>Stereospermum suaveolens</i>	0.166 mg (16.6%)	0.151 mg (15.1%)	0.199 mg (19.9%)	0.140 mg (14%)	0.140 mg (14%)
2	<i>Stereospermum colais</i>	0.166 mg (16.6%)	0.153 mg (15.3%)	0.155 mg (15.5%)	0.145 mg (14.5%)	0.133 mg (13.3%)
3	<i>Dolichandrone arcuata</i>	0.124 mg (12.4%)	0.170 mg (17.0%)	0.154 mg (15.4%)	0.258 mg (25.8%)	0.187 mg (18.7%)
4	<i>Radermachera xylocarpa</i>	0.163 mg (16.3%)	0.121 mg (12.1%)	0.189 mg (18.9%)	0.173 mg (17.3%)	0.239 mg (23.9%)
5	<i>Millingtonia hortensis</i>	0.063 mg (6.3%)	0.160 mg (16%)	0.149 mg (14.9%)	0.243 mg (24.3%)	0.181 mg (18.1%)
6	<i>Scutellaria colebrookiana</i>	0.056 mg (5.6%)	0.102 mg (10.2%)	0.156 mg (15.6%)	0.122 mg (12.2%)	0.098 mg (9.8%)
7	<i>Scutellaria violacea</i>	0.121 mg (12.1%)	0.217 mg (21.7%)	0.176 mg (17.6%)	0.162 mg (16.2%)	0.133 mg (13.3%)

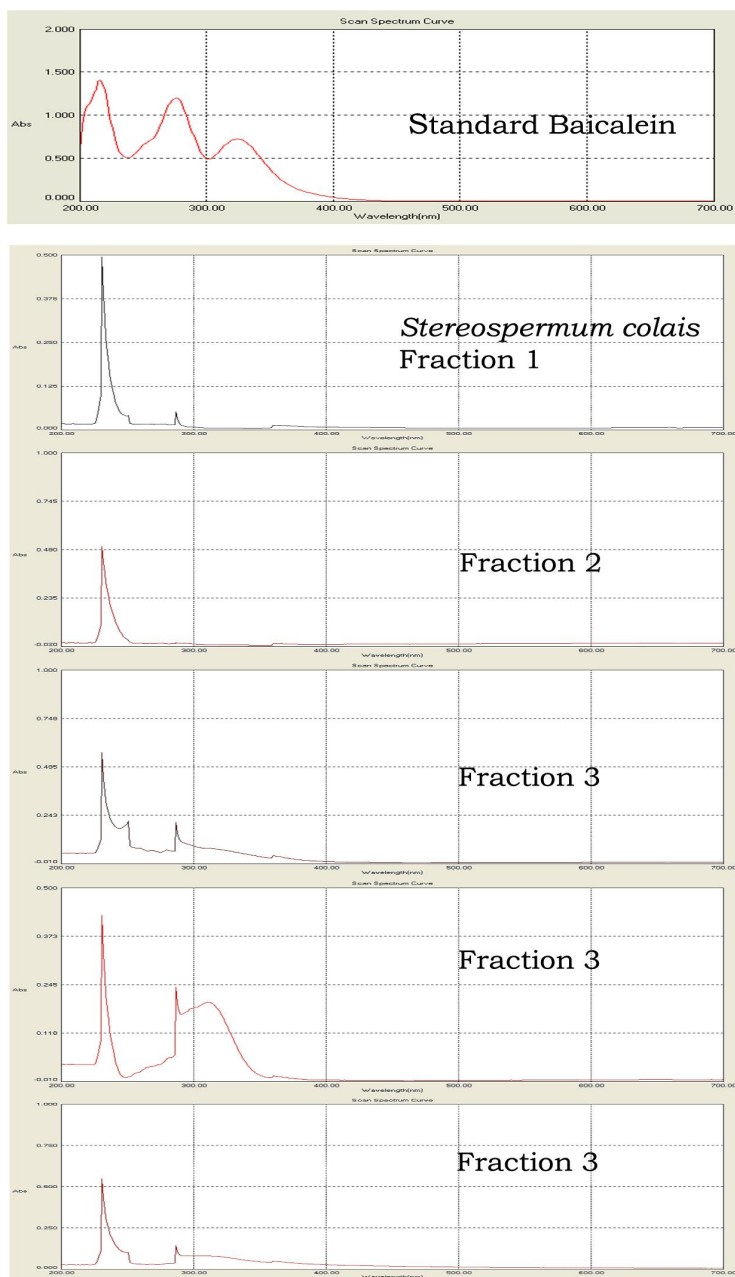


Figure 5. Absorption spectra of *Stereospermum colais* solvent fractions isolated through medium pressure liquid chromatography

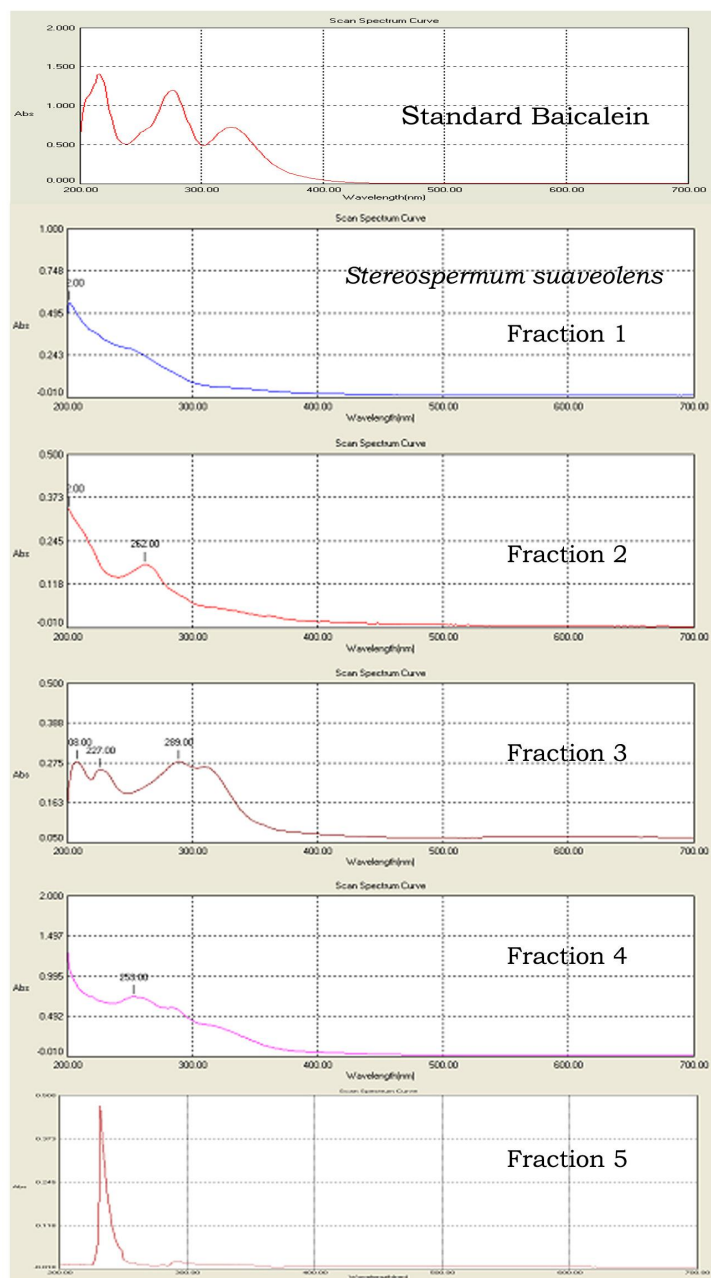


Figure 6. Absorption spectra of *Stereospermum suaveolens* isolated through medium pressure liquid chromatography

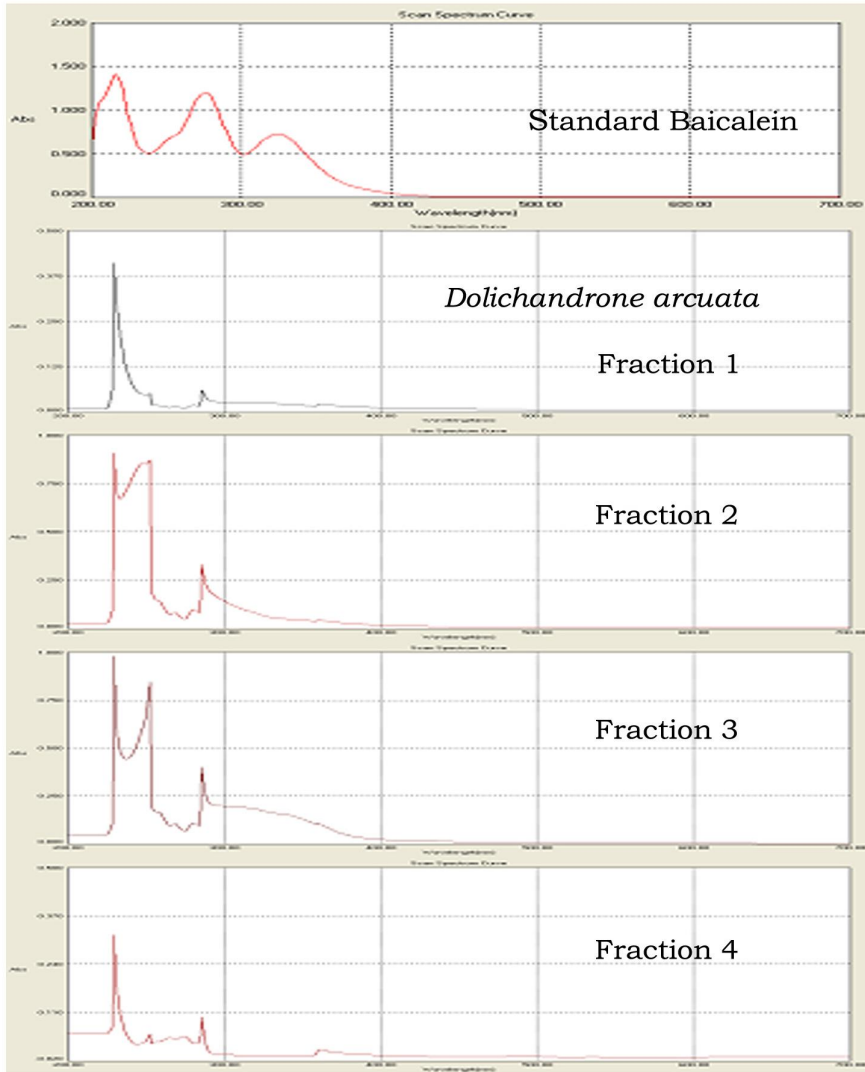


Figure 7. Absorption spectra of *Dolichandrone arcuata* isolated through medium pressure liquid chromatography

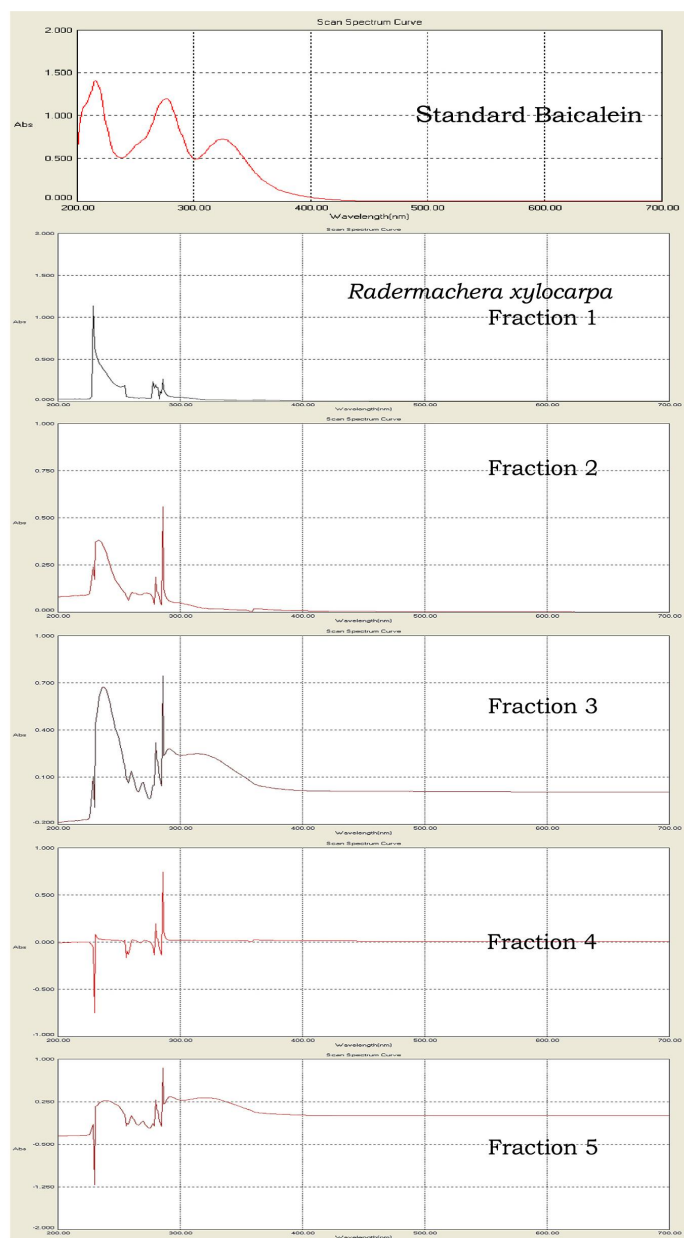
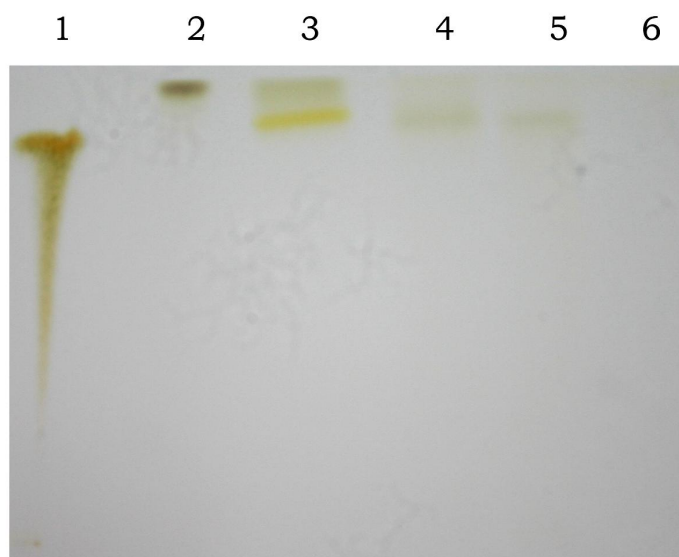
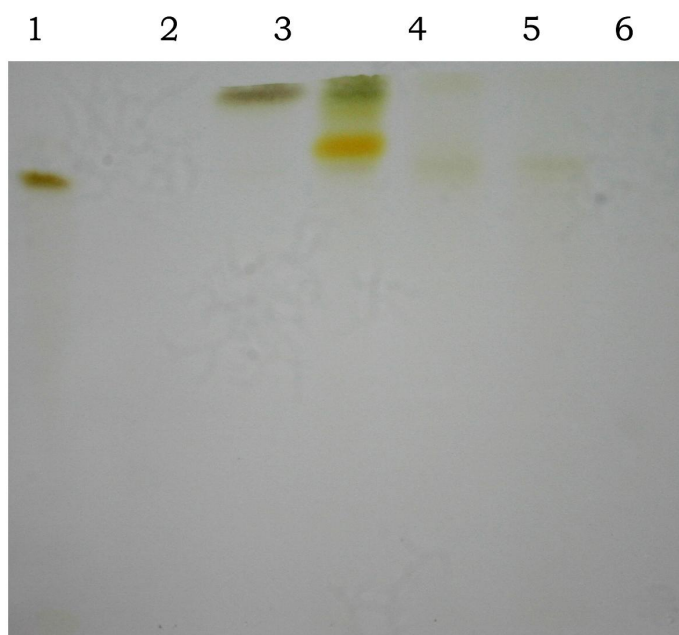


Figure 8. Absorption spectra of *Radermachera xylocarpa* solvent fractions isolated through medium pressure liquid chromatography



A. *Scutellaria colebrookiana*



B. *Scutellaria violacea*

Figure 9. TLC analysis of *Scutellariaia* extracts from column chromatography

The presence of baicalein in *Scutellaria colebrookiana* and *S. violacea* detected by medium pressure liquid chromatography was confirmed by HPLC.

For the confirmation of baicalein, the chloroform and acetone extracts of *Scutellaria* were analysed with HPLC (Shimadzu, Japan). The separation was performed using reverse-phase C18 column at 25 °C with a flow rate of 1 ml/min of the solvent acetonitrile. The absorbance for the baicalein was monitored at 274 nm with a UV detector SPD-10A and CLASS VP software. The retention time of standard baicalein (Sigma) was 3.3 min (Figure 10 A). Peaks in the same retention time (3.3 min) were also detected in chloroform as well as acetone extracts of both species of *Scutellaria*. The concentration of baicalein in the extracts was also calculated using standard. In *Scutellaria colebrookiana*, the concentration of baicalein was 0.229 and 0.210 mg/g dry weight (root) in acetone and chloroform extracts, respectively (Figure 10 B and 10 C). The concentration was 0.134 and 0.150 mg/g dry weight (root) in acetone and chloroform extracts, respectively in *S. violacea* (Figure 10 D and 10 E).

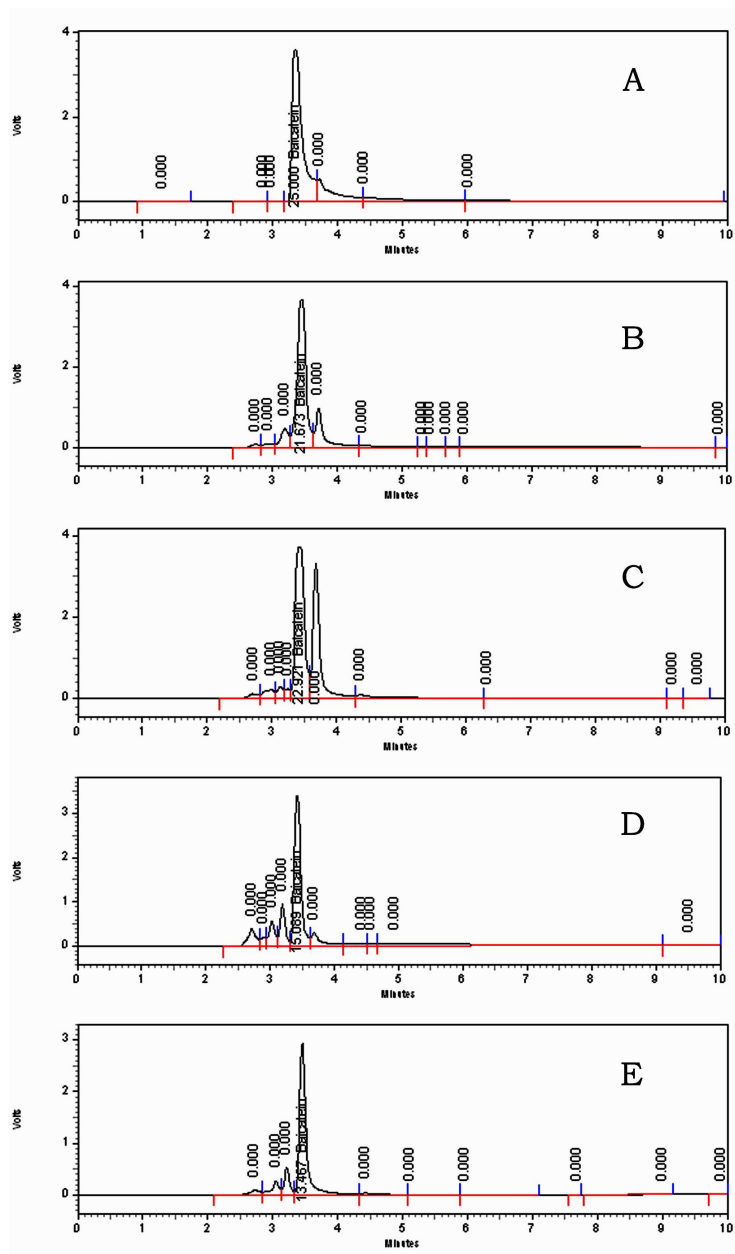


Figure 10. HPLC analysis of standard baicalein (Sigma) and *Scutellaria* extracts with acetonitrile through C18 column. Extracted in acetone (B) and chloroform (C) obtained from *S. colebrookiana* and in acetone (D) and chloroform (E) *S. violacea*

Psoralen content

TLC analysis

The methanolic, ethanolic, chloroform and acetone extracts of fruits along with control were subjected to thin layer chromatography using various solvent systems. The most suitable solvent system was toluene: petroleum benzene: acetic acid (4.4: 4.4: 1.2).

Profiles obtained for samples show more or less similar patterns of bands with control (Table 3). When ethanolic extract was used, separation and intensity of bands were more.

Table 3. Detection based on intensities of brownish bands

Plants		Samples taken for study				
		Standard	Methanol	Ethanol	Chloroform	Acetone
<i>Aegle marmelos</i>	Intensities of brownish bands	Dark brown	Light brown	Dark brown	Brown	Brown
<i>Murraya koenigii</i>		Dark brown	Light brown	Dark brown	Brown	Brown
<i>Clausena indica</i>		Dark brown	Light greenish brown	Greenish brown	Greenish brown	Light greenish brown
<i>Paramignya monophylla</i>		Dark brown	Fleshy	Dark fleshy	Dark fleshy	Fleshy
<i>Glycosmis pentaphylla</i>		Dark brown	Light greenish brown	Greenish brown	Greenish brown	Light greenish brown

By spraying Benedict's Reagent, different banding patterns were obtained in all the samples used (Table 4). *Aegle marmelos* and *Murraya koenigii* (Figure 11) showed more or less same number of bands. When *Paramignya monophylla* and *Clausena indica* compared in TLC analysis, the results were entirely different (Figure 12). *Aegle marmelos* and *Glycosmis pentaphylla* in TLC analysis showed difference in the number of bands (Figure 13). *Murraya koenigii* and *Glycosmis pentaphylla* did not show any resemblances (Figure 14).

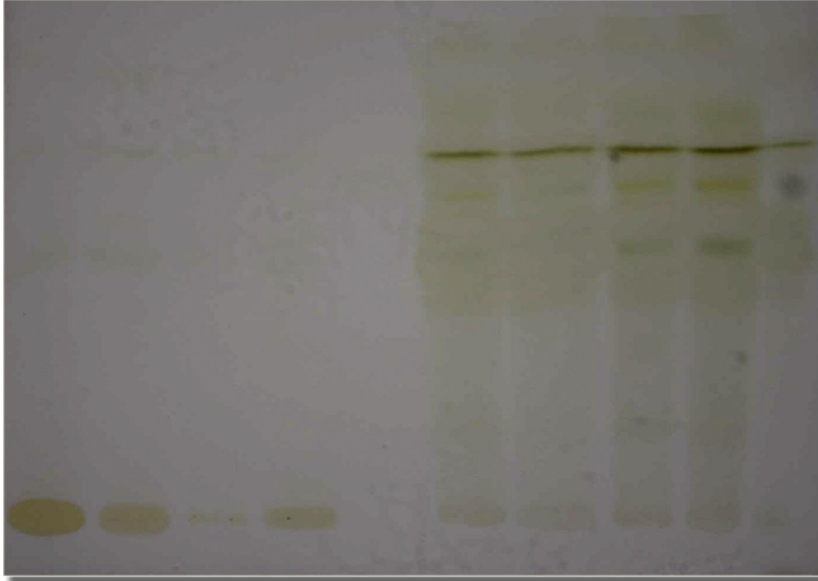


Figure 11. TLC profile of *Aegle marmelos* and *Murraya koenigii*



Figure 12. TLC- comparison of *Paramignya monophylla* and *Clausena indica*



Figure 13. TLC- comparison of *Aegle marmelos* and *Glycosmis pentaphylla*

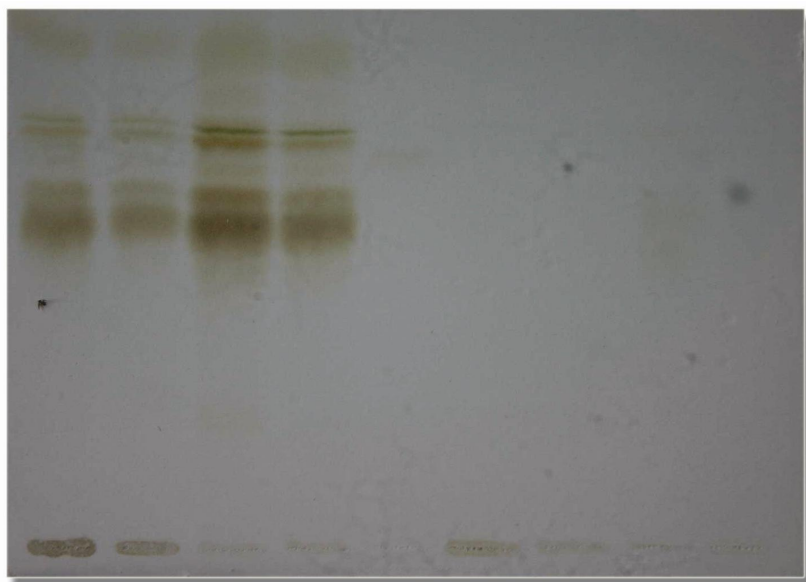


Figure 14. TLC- comparison of *Murraya koenigii* and *Glycosmis pentaphylla*

Table 4. Detection based on R_f values of bands

Plants		Samples taken for study			
		Methanol	Ethanol	Chloroform	Acetone
<i>Aegle marmelos</i>	No. of bands appeared after spraying	4	4	4	4
<i>Murraya koenigii</i>		6	6	6	6
<i>Clausena indica</i>		3	3	3	3
<i>Paramignya monophylla</i>		2	2	2	2
<i>Glycosmis pentaphylla</i>		1	1	1	1

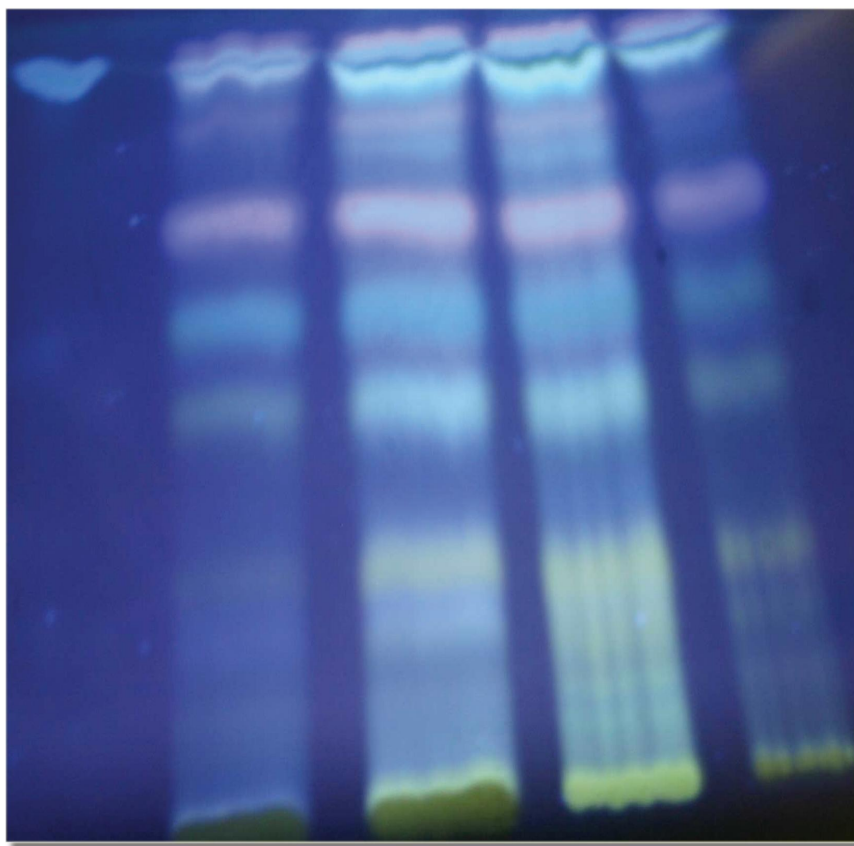


Figure 15. *Aegle marmelos* TLC plate under UV rays

Aegle marmelos and *Murraya koenigii* have the same R_f value at 0.07. *Aegle marmelos* showed numerous bands under UV rays (Figure 15). The remaining plants *Clausena indica*, *Paramignya monophylla* and *Glycosmis pentaphylla* gave similar R_f value at 0.06. When *Clausena indica* and *Glycosmis pentaphylla* were compared the bands were entirely different. Here both the plant extracts after TLC analysis under UV rays showed much resemblance with each other (Figure 16).

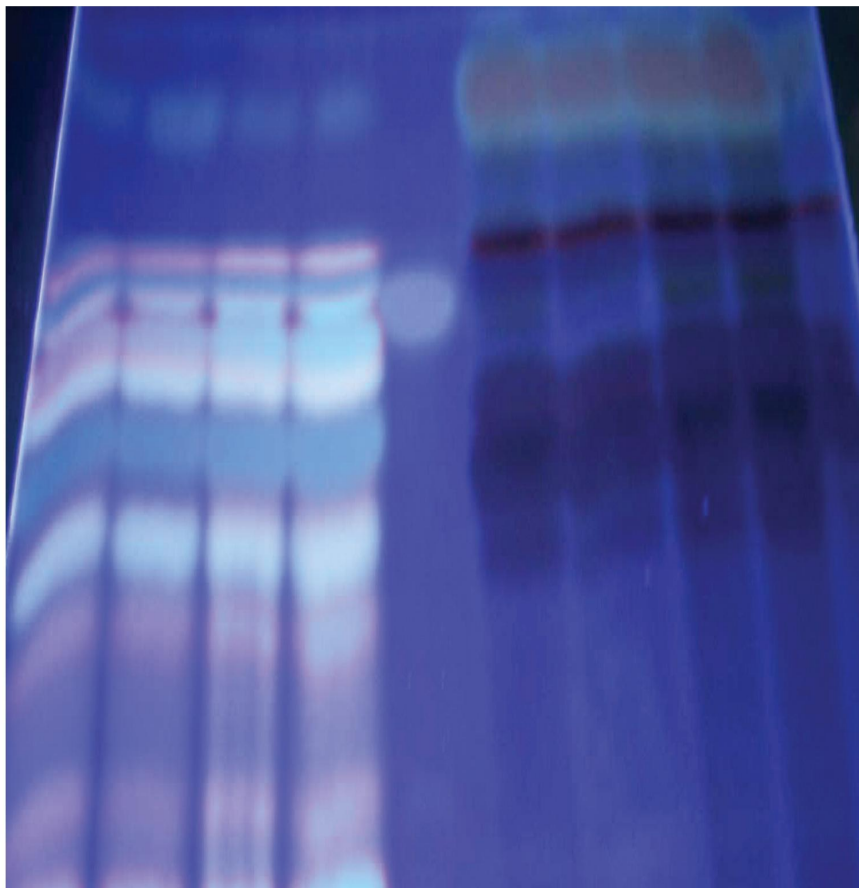


Figure 16. TLC- Comparison of *Clausena indica* and *Glycosmis pentaphylla* under UV rays

Spectrophotometric assay

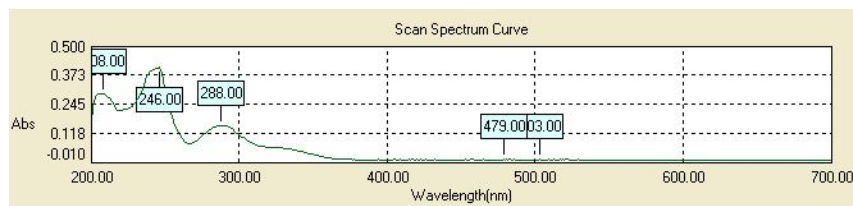
The maximum absorbance (O.D) between the ranges of 200 nm to 700 nm was analyzed by spectrophotometer. Here optical density of different extracts was analyzed (Table 5).

Table 5. Absorbance values of different extracts

Plants	Optical density	Control	Methanol	Ethanol	Chloroform	Acetone
<i>Aegle marmelos</i>		246λ, 292λ, 331λ	242λ, 294λ	241λ, 290λ	248λ, 297λ	241λ, 290λ
<i>Murraya koenigii</i>		246λ, 292λ, 331λ	236λ, 288λ, 331λ	248λ, 288λ, 330λ	239λ, 289λ, 331λ	239λ, 288λ, 330λ
<i>Clausena indica</i>		246λ, 292λ, 331λ	402λ, 663λ	266λ, 667λ	266λ, 667λ	263λ, 357λ
<i>Paramignya monophylla</i>		246λ, 292λ, 331λ	314λ, 346λ, 548λ	314λ, 364λ, 548λ	533λ	355λ, 381λ, 548λ
<i>Glycosmis pentaphylla</i>		246λ, 292λ, 331λ	323λ, 548λ	314λ, 364λ, 548λ	368λ, 548λ	372λ, 548λ

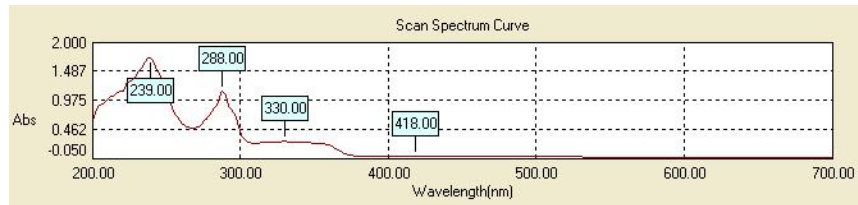
Figure 17. Absorbance profile of different plant extracts

Control (Standard psoralen)

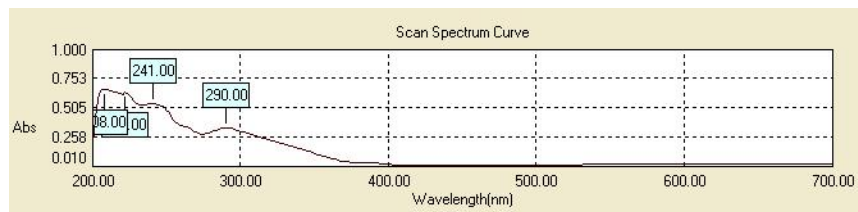


A. Comparison of acetone extracts

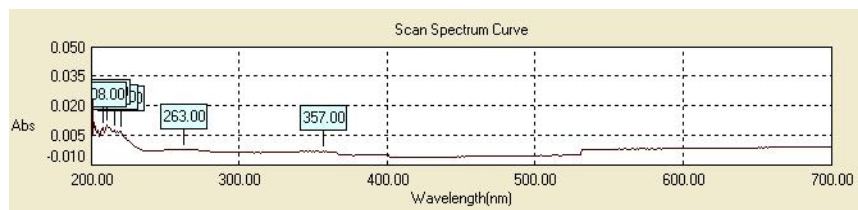
Murraya koenigii



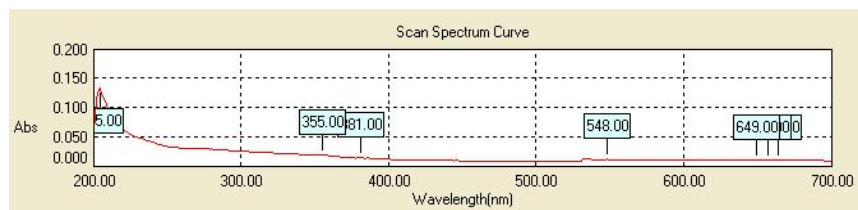
Aegle marmelos



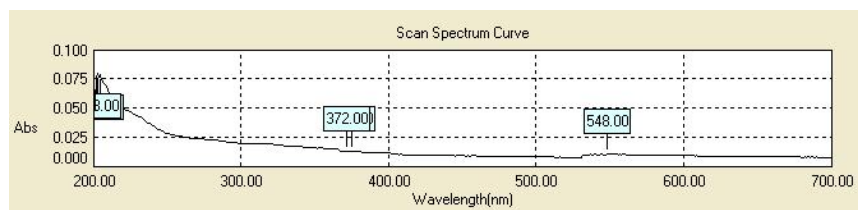
Clausena indica



Paramignya monophylla

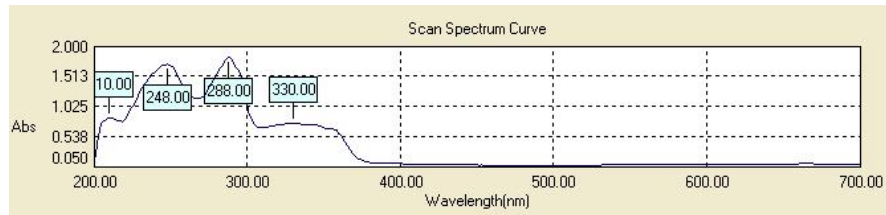


Glycosmis pentaphylla

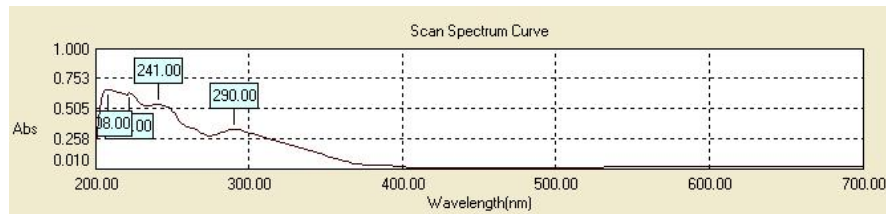


B. Comparison of ethanol extracts

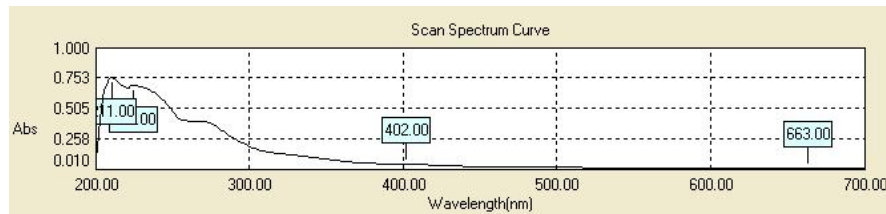
Murraya koenigii



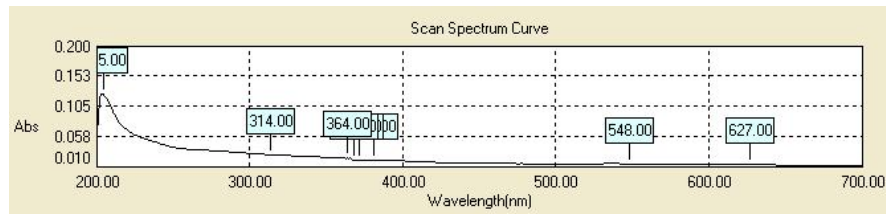
Aegle marmelos



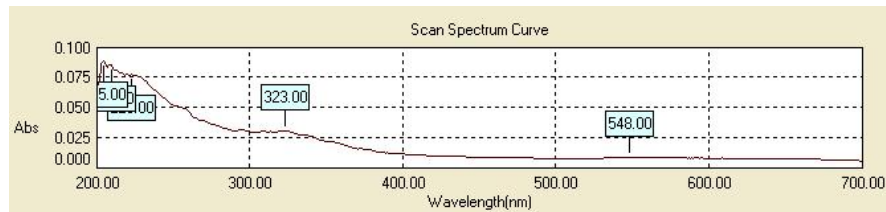
Clausena indica



Paramignya monophylla

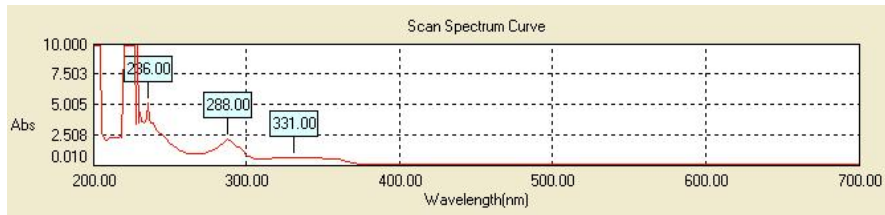


Glycosmis pentaphylla

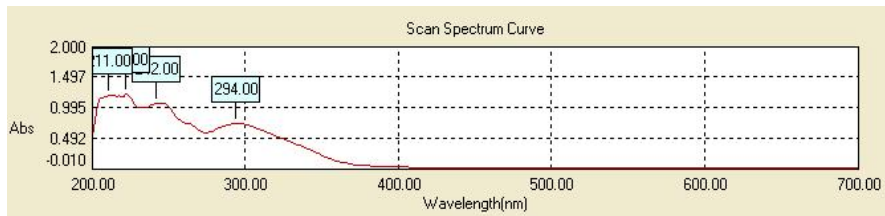


C. Comparison of methanol extracts

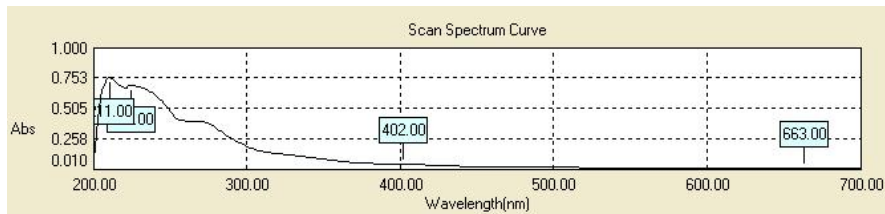
Murraya koenigii



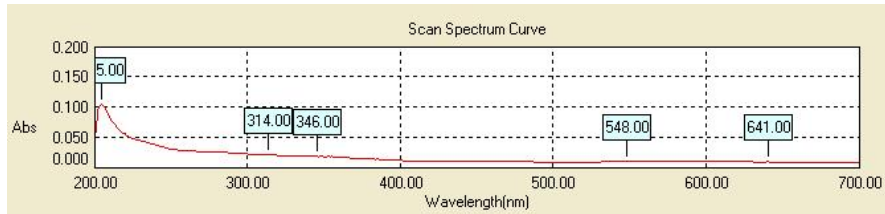
Aegle marmelos



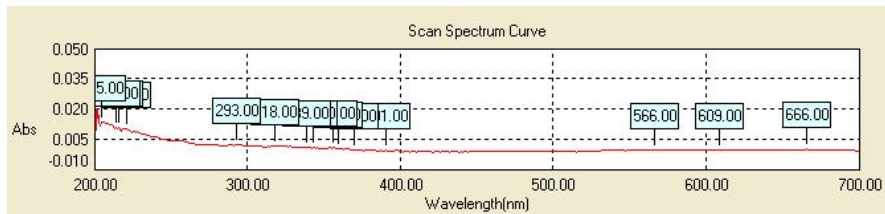
Clausena indica



Paramignya monophylla

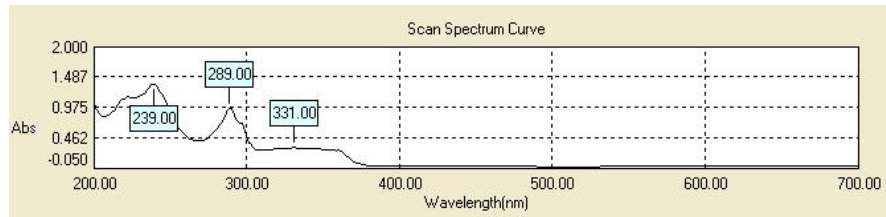


Glycosmis pentaphylla

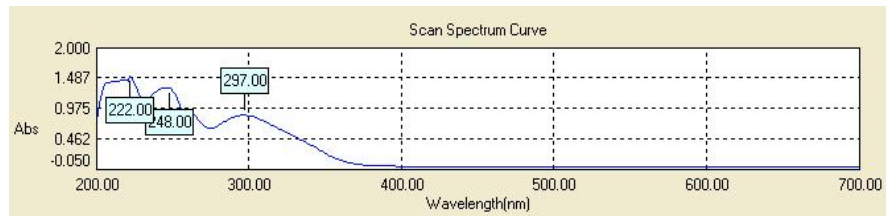


D. Comparison of chloroform extracts

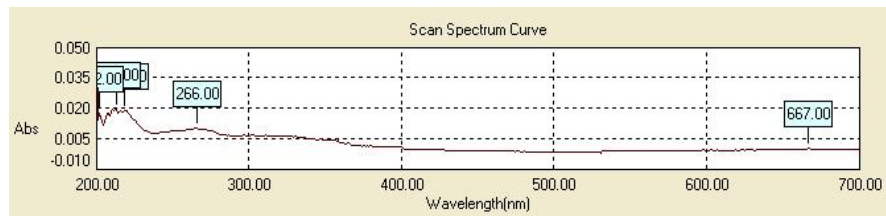
Murraya koenigii



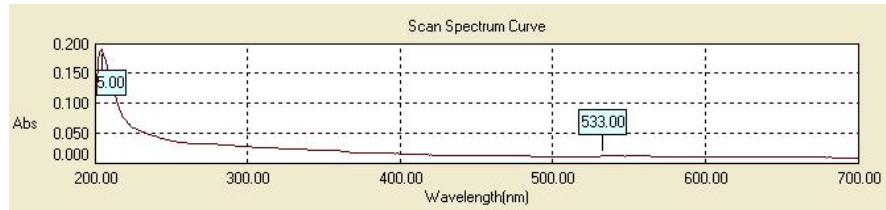
Aegle marmelos



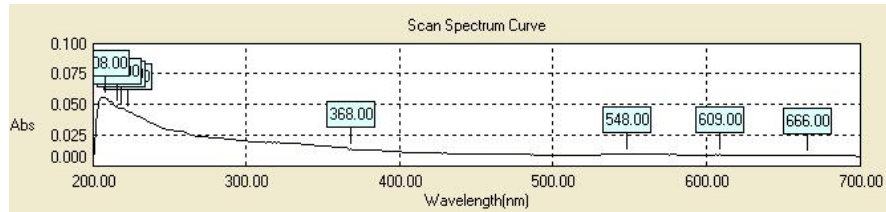
Clausena indica



Paramignya monophylla



Glycosmis pentaphylla



When the absorbance profiles of different extracts (Figure 17) were compared, *Aegle marmelos* and *Murraya koenigii* showed more or less similar optical density pattern in methanolic, ethanolic, chloroform and acetone extracts to the standard. In methanolic extract, *Murraya koenigii* gave better results than *Aegle marmelos*. Chloroform and acetone extracts did not give much yield when compared to the other two species. But *Aegle marmelos* and *Murraya koenigii* showed better profile. *Paramignya monophylla* and *Glycosmis pentaphylla* gave similar results with each other but deviated from the absorbance value of the standard. *Clausena indica* produced a comparatively different absorption profile.

The presence of psoralen in *Aegle marmelos* and *Murraya koenigii* detected by medium pressure liquid chromatography was confirmed by HPLC.

The chloroform and acetone extracts of both the plants were analysed in HPLC (Shimadzu, Japan). The separation was performed using reverse-phase C18 column at 25 °C with a flow rate of 1 ml/min of the solvent acetonitril. The absorbance for the psoralen was monitored at 288 nm with a UV detector SPD-10A and CLASS VP software. The retention time of standard psoralen (Sigma) was 4.7 min. Peaks in the same retention time (4.7 min) were also detected in chloroform as well as acetone extracts of *Aegle marmelos* and *Murraya koenigii* (Figures 18 and 19).

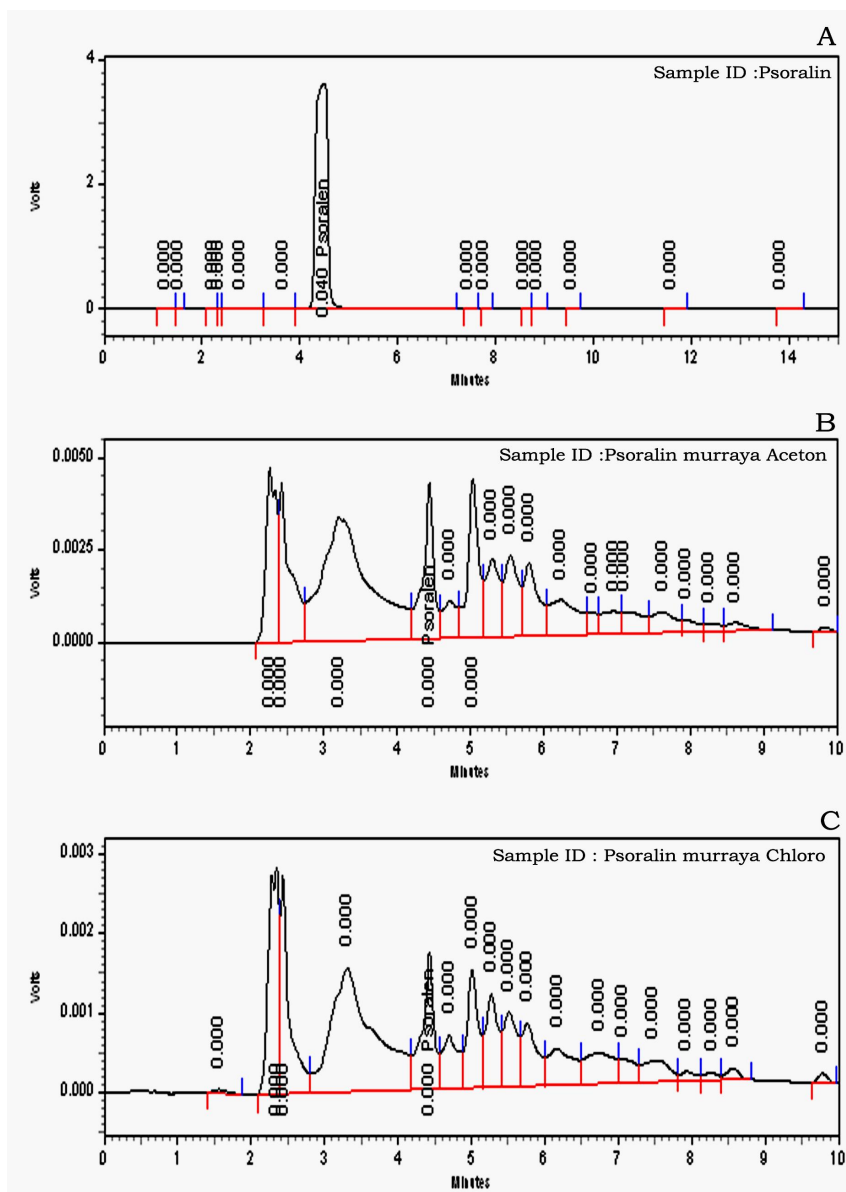


Figure 18. HPLC analysis profiles of standard psoralen (Sigma) (A) and extracts of *Murraya koenigii* through C18 column extracted in acetone (B) and chloroform (C).

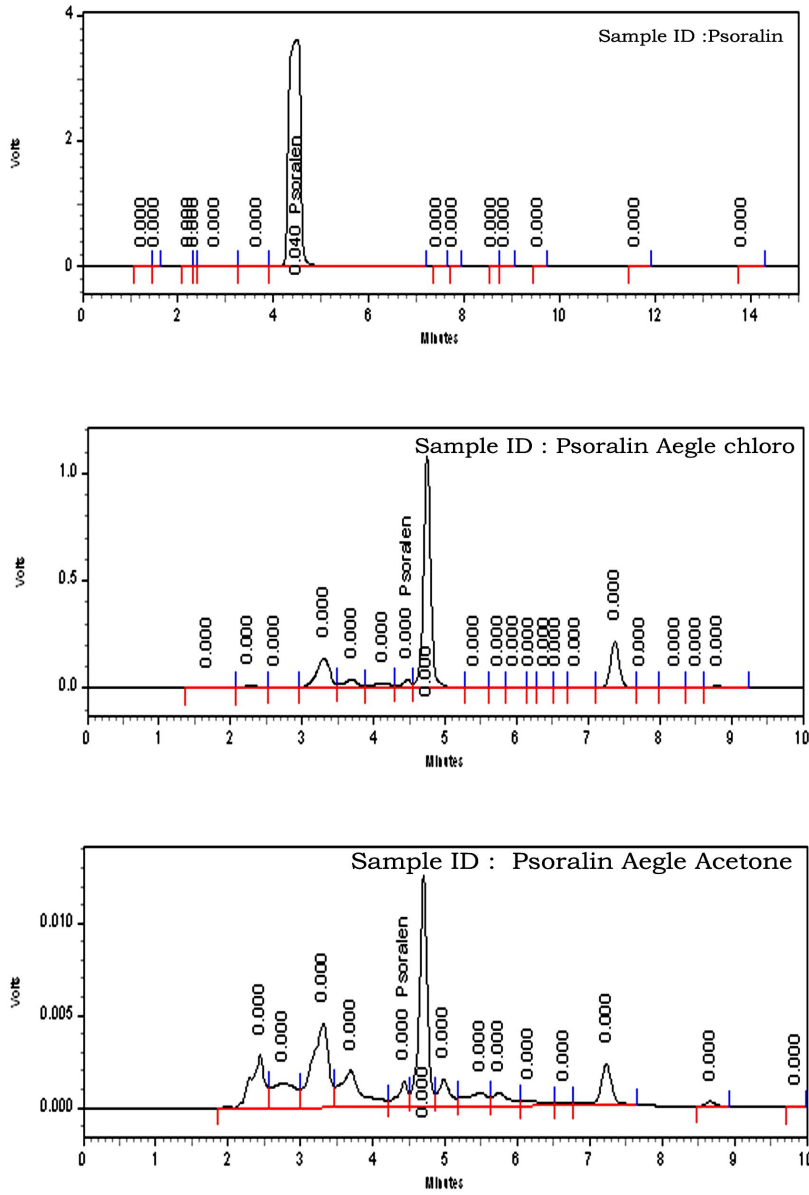


Figure 19. HPLC analysis profiles of standard psoralen (Sigma) (A) and extracts of *Aegle marmelose* through C18 column extracted in acetone (B) and chloroform (C).

Among the five species studied for the detection of psoralen, *Aegle marmelos* and *Murraya koenigii* showed the presence of psoralen in both thin layer chromatography and spectrophotometric analyses. *Aegle marmelos*, one of the *Dasamoola* of Ayurveda is used in several medicinal preparations including *Dasamoolarishtam*, a health rejuvenator. The three other species studied, *Clausena indica*, *Paramignya monophylla* and *Glycosmis pentaphylla* did not show positive results for psoralen content. However, the absorbance values obtained through spectrophotometric analyses were different. *Glycosmis pentaphylla* is mostly used in folklore medicines.

C. Luteolin content

The compound luteolin is not detected in *Premna serratifolia* as per the procedure used.

3.2. Biological Properties

Scutellaria colebrookiana* and *S. violacea

Superoxide radical scavenging activity

Among the four extracts studied, chloroform and acetone extracts were found to possess significant superoxide radicals scavenging activity. The chloroform extracts needed for 50% inhibition were 86 and 93 μg and that of acetone extracts were 76 and 83 μg for *Scutellaria colebrookiana* and *S. violacea*, respectively. Methanol extract was also less effective in scavenging free radicals. The amount of extract needed for 50% inhibition was 610 μg for *Scutellaria colebrookiana* and 640 μg for *S. violacea* (Figure 20(I)).

Hydroxyl radical scavenging activity

The degradation of deoxyribose to TBARS by hydroxyl radicals generated from Fe^{3+} /ascorbate/EDTA/ H_2O_2 system markedly decreased by plant extracts. The concentration of the extracts needed for 50% (IC_{50}) inhibition of hydroxyl radical are given in Figure 20. The chloroform extracts needed for 50% inhibition were 240 and 275 μg and that of acetone extracts were 270 and 296 μg for *Scutellaria colebrookiana* and *S. violacea* respectively. The scavenging activity of methanol extract on hydroxyl radical was less. The amount of extract needed for 50% inhibition was 800 and 887 μg for *Scutellaria colebrookiana* and *S. violacea* respectively (Figure 20(II)).

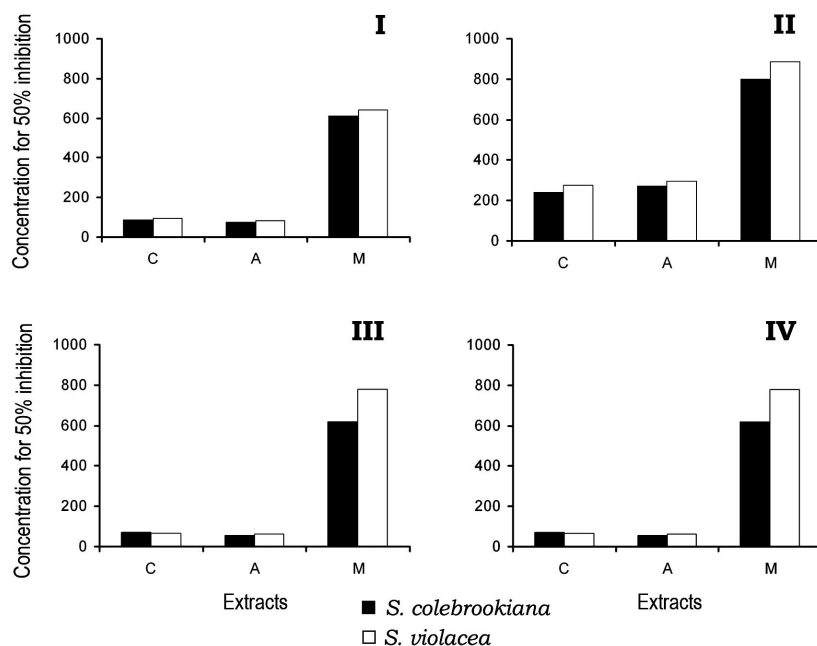


Figure 20. The effect of extracts of *Scutellaria* with chloroform (C), acetone (A) and methanolic (M) on superoxide (I), hydroxyl (II), lipid peroxidation (III) and DPPH radicals (IV)

Inhibition of lipid peroxidation

Chloroform and acetone extracts of *Scutellaria* were effective in inhibiting lipid peroxidation induced by Fe²⁺-ascorbate system in rat liver homogenate. The generation of malondialdehyde (MDA) and related substances that react with thiobarbituric acid (TBA) were found to be inhibited by these extracts. The IC₅₀ value for chloroform extracts were 160 µg and 205 µg for *Scutellaria colebrookiana* and *S. violacea*, respectively. The amount of acetone extract needed for 50% inhibition was found to be 210 and 196 µg for *Scutellaria colebrookiana* and *S. violacea* respectively. In the case of methanol extract, the values were found to be 700 µg for *Scutellaria colebrookiana* and 790 µg for *S. violacea*, respectively (Figure 20 (III)).

DPPH radical scavenging activity

The stable free radical, DPPH was effectively scavenged by chloroform and acetone extracts of *Scutellaria colebrookiana* and *S. violacea*. The IC₅₀ values were 72.5 and 67.5 µg for chloroform extracts of *Scutellaria colebrookiana* and *S. violacea*, respectively. The amount of acetone extract needed for 50% inhibition was 56 and 62 µg for *Scutellaria colebrookiana* and *S. violacea* respectively. In the case of methanol extract, a higher quantity was needed for obtaining 50 % inhibition. The values were 620 and 778 µg for *Scutellaria colebrookiana* and *S. violacea*, respectively (Figure 20(IV)).

The compounds from *Scutellaria baicalensis* have implications in tumor prevention due to their antioxidant activities. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of *Scutellaria baicalensis* has been reported (Huang *et al.*, 2000; Shieh *et al.*, 2006). The data obtained with the *in vitro* antioxidant studies showed that chloroform and acetone extracts of *Scutellaria*

colebrookiana and *S. violacea* possess significant antioxidant activity. A high radical scavenging activity was observed in scavenging super oxide radical, hydroxyl radical, 1,1-Diphenyl-2 Picrylhydrazyl (DPPH) radical and also showed inhibition against lipid peroxidation.

More than 10 flavonoids have been identified from different sources of *Scutellaria*. Besides wogonin, baicalein and baicalin, a number of minor flavonoids (eg. chrysin, luteolin and apigenin) have been also reported to possess potent antitumor activities. The data obtained from chromatographic as well as spectrophotometric analysis suggest the presence of baicalin in *Scutellaria colebrookiana* and *S. violacea*. Results of the present study clearly demonstrate that the chloroform and acetone extracts of root of *Scutellaria* species offers protection against the adverse effects of free radicals such as superoxide, hydroxyl, DPPH and inhibited tissue lipid peroxidation. This preliminary data also suggests their wide spectrum biological potentials.

Premna serratifolia

Superoxide scavenging activity

Extract of *Premna serratifolia* roots were found to scavenge the superoxides generated by photoreduction of riboflavin. Concentration of methanolic extract needed for 50% of inhibition of superoxide was 180 $\mu\text{g}/\text{ml}$ (Figure 21).

Hydroxyl radical scavenging activity

Degradation of deoxyribose by hydroxyl radical generated by Fe^{3+} /ascorbate/EDTA/ H_2O_2 system was found to be inhibited by *Premna serratifolia* root extract. Concentration of methanolic extract needed for 50% inhibition of hydroxyl radical was 156 mg/ml (Figure 21).

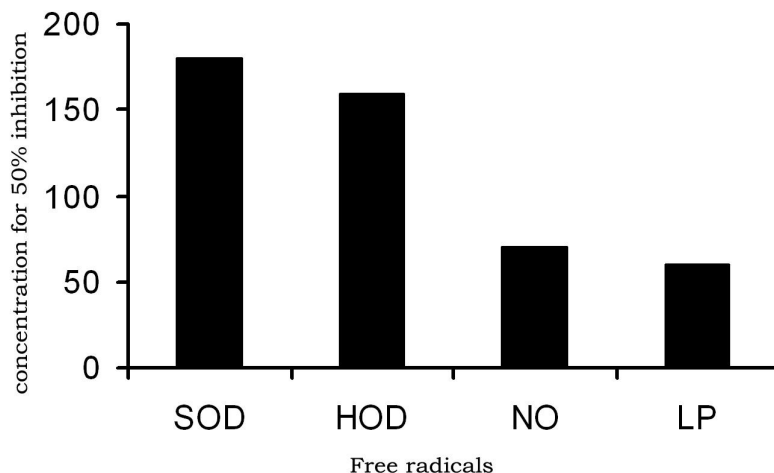


Figure 21. *In vitro* antioxidant analysis of *Premna serratifolia*. SOD - Super oxide, HOD - Hydroxyl radical, NO-Nitric oxide, LP - Lipid peroxidation

Nitric oxide radical scavenging activity

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by the *Premna serratifolia* root extract. Concentration of 70% methanolic extract needed for 50% of inhibition of nitric oxide radical was 79 $\mu\text{g}/\text{ml}$ (Figure 21).

Lipid peroxidation activity

The root extract was found to inhibit lipid peroxides generated by induction of Fe^{2+} /ascorbate and Fe^{3+} /ADD/ascorbate in rat liver homogenate. Concentration of methanolic extract needed for 50% inhibition of lipid peroxides was 60 $\mu\text{g}/\text{ml}$ (Figure 21).

Anti-inflammatory activity

The administration of the root extract inhibited the carragenan induced acute paw oedema and formalin induced chronic paw oedema in a dose dependent manner (Table 6). The animals treated with 70 per cent methanolic extract @ 250 mg/kg and 500 mg/kg showed 47.05 and 70.58% inhibition of carragenan induced acute inflammation. In the case of formalin induced chronic inflammation, 250 mg/kg and 500 mg/kg showed inhibition of 50.00 and 72.00%, respectively (Table 7).

Table 6. Effect of *Premna serratifolia* (70% methanolic extract) on carrageenan induced paw odema

Groups	Initial paw thickness (mm)	Paw thickness on 3 hrs (mm)	Increase in paw thickness	% of inhibition
Control	0.21 ± 0.01	0.38 ± 0.02	0.17
Dichlofenac	0.22 ± 0.02	0.29 ± 0.008 ^a	0.07	58.82
<i>Premna serratifolia</i> 250 mg/kg body wt	0.21 ± 0.02	0.30 ± 0.008 ^a	0.09	47.05
<i>Premna serratifolia</i> 500 mg/kg body wt	0.20 ± 0.01	0.25 ± 0.027 ^a	0.05	70.58

Values are Mean ± SD; for six animals in each group; (a) p, 0.01, (b) p<0.05 as compared to control.

Table 7. Effect of 70% methanolic extract of *Premna serratifolia* on formalin induced paw odema

Groups	Initial paw thickness (mm)	Paw thickness on 6 th day(mm)	Increase in paw thickness (mm)	% of inhibition
Control	0.21 ± 0.012	0.39 ± 0.015	0.18
Dichlofenac	0.23 ± 0.013 ^b	0.30 ± 0.070 ^a	0.07	61.11
<i>Premna serratifolia</i> 250mg/kg body wt	0.22 ± 0.013	0.31 ± 0.018 ^a	0.09	50.00
<i>Premna serratifolia</i> 500mg/kg body wt	0.23 ± 0.014 ^b	0.28 ± 0.011 ^a	0.05	72.22

Values are Mean ± SD; for six animals in each group; (a) p, 0.01, (b) p<0.05 as compared to control.

6. Discussion

Based on the biosynthetic origins, plant secondary metabolites can be divided in to 3 major groups. First group is flavonoides and allied phenolic compounds second are terpenoids and third are nitrogen containing alkaloids and sulphur containing compounds. Secondary metabolites and their effects are currently being intensively researched. These include carotenoids, phytosterols, saponins, glucosinolates, flavonoids, protease-inhibitors, alkaloids, terpenes etc. Among these metabolites, the flavonoid groups include organic pigments found throughout the plant kingdom which have a particularly broad spectrum of efficacy. Flavonoids can prevent injury caused by free

radicals by directly scavenging them. By scavenging free radicals, flavonoids can inhibit LDL oxidation *in vitro*. This action protects the LDL particles and, theoretically, flavonoids have preventive action against atherosclerosis also.

Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. Thus flavonoids have received much attention over the past 10 years and a variety of potential beneficial effects have been elucidated. Baicalein, a bioactive flavonoid extracted from root of *Scutellaria baicalensis* and *S. radix*, exerts antitumor activity (Bonham *et al.*, 2005; Miocinovic *et al.*, 2005; Ma *et al.*, 2005). It leads to cell cycle arrest and suppression of proliferation in cancer cells. Baicalein induces apoptosis of a variety of human cancer cell lines (Kuntz *et al.*, 1999; Chen *et al.*, 2000; Pidgeon *et al.*, 2002). Baicalein and Baicalin were shown to cause depletion of GSH content in human hepatoma cell lines and therefore it is believed that the anticancer activity of these compounds may also involve a pro-oxidant mechanism (Yu *et al.*, 2007; Chang *et al.*, 2002).

Considering the wide attention on potential biological activities of baicalein, phytochemical as well as biological analyses of baicalein content in *Scutellaria colebrookiana* and *S. violacea* occurring in Western Ghats of Kerala were carried out. In view of the findings of baicalein in *Oroxylum indicum*, analyses for the baicalein content in *Stereospermum colais*, *S. suaveolens*, *Dolichandrone arcuata*, *Radermachera xylocarpa* and *Millingtonia hortensis* belonging to the family Bignoniaceae were carried out. Baicalein content in *Scutellaria* spp. was confirmed through column chromatography, spectrophotometry, TLC and HPLC analyses. The antioxidant analysis shows its very good potency, especially to scavenge free radicals such as superoxide, hydroxyl radicals and inhibit lipid peroxidation which is generated in various *in vitro* experimental models. Among the various solvent extracts, the chloroform extracts

show most potent activity. Maximum quantity of baicalein content was observed in chloroform extracts in HPLC analysis. It suggests that the free radical generation may be due to the presence of baicalein content. The earlier reports (Middleton *et al.*, 2000; Havsteen *et al.*, 2002) on the bioactivity and structure relationships of different flavonoids has revealed that the antioxidant properties of flavonoids are due to the presence of 2, 3-unsaturation in conjugation with a 4-oxo group in the C-ring, the hydroxyl groups in the B-ring and the 5-hydroxy group in the A-ring. (Brown *et al.*, 1994; Williams, 2004). Baicalein and baicalin possess the 2, 3-unsaturation and the 4-oxo in the C-ring and 5-hydroxyl group in the A-ring (Figure 1).

As in the case of baicalin, luteolin is the most common flavonoid widely distributed in the plant kingdom. Dietary sources of luteolin include carrot, pepper, celery, olive oil, peppermint, thyme, rosemary and oregano. Preclinical studies have shown that this flavone possesses a variety of pharmacological activities, including antioxidant (Katarzyna *et al.*, 2007) anti-inflammatory, antimicrobial (Sousa *et al.*, 2006) and anticancer (Zhou *et al.*, 2009). Considering the activities of luteolin and its presence in various plants, analyses were carried out for the luteolin content in *Premna seratifolia*. But luteolin content was not detected in *Premna seratifolia* with the procedure followed. However, the biological property analysis shows very potent antioxidant and anti-inflammatory activities.

The phototoxic phytoalexin, psoralen was originally extracted from *Psoralea corylifolia*, generally used to increase melanin in the skin. It is used in the treatment of vitiligo, alopecia and psoriasis. The psoralen is most prevalent in the genus *Psoralea*. Plants produce psoralen as natural pest defence, since it can stop some infections in their tracks and is also deadly to insects. Recent studies have

reported that psoralen induces interstrand cross links in DNA when activated by UV light (Ashwood-Smith *et al.*, 1980).

The presence of psoralen content in *Aegle marmelos*, one of the *Dasamoola* has been reported recently (Dhalwal *et al.*, 2007; 2008). Psoralen content usually accumulates in the fruits and seeds. Therefore, the fruits and seeds of *Aegle marmelos*, *Murraya koenigii*, *Glycosmis pentaphylla*, *Clausena indica* and *Paramygnia monophylla* were analysed for psoralen content. Among the plants studied, the fruits of *Aegle* and *Murraya* showed positive results for the psoralen. The psoralen content usually varies with plants. The techniques used for the detection also vary according to the nature and the occurrence of psoralen. The difference in the results obtained in plants may be due to the presence of glycosidic bonds in different levels. Glycosidic bonds and shifts in absorbance interfere in the identification procedure.

The finding of baicalein in *Scutellaria colebrookiana* and *S. violacea* and the significant biological properties are very promising. This preliminary data also suggests its wide spectrum biological potentials. Because of the broad toxicities to various types of tumour cell lines and low toxicities to normal tissues, the *Scutellaria* species of the Western Ghats are attractive candidates for the development of new anticancer drugs.

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