Spectrophotometric Quantification of Triterpenoids in Selected Medicinal Plants

Sowmiya, K

(12PCH011)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, University

Coimbatore-641043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Chemistry

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K.SOWMIYA

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List of abbreviations

TLC	-	Thin Layer Chromatography
OD	-	Optical Density
mg	-	milligram
g	-	gram
%	-	percentage
NaOH	-	Sodium hydroxide
nm	-	nano meter
$Fecl_3$	-	Ferric chloride

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1.INTRODUCTION

Medicinal plants have played an essential role in the development of human culture. India has a rich culture of medicinal herbs and spices, which includes about more than 2000 diverse ailments species and has a huge geographical area with high suppressed abilities for Ayurvedic, Unani, Siddha habitual medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value.

Medicinal plants have been identified and used throughout human history. 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. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants intervene their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

Phytochemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. These concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with these chemical structure of these substance, their biosynthesis turnover and metabolism their biological function.

Throughout human history natural products have been used as remedies to cure or treat various diseases. Human continues to be affected by various diseases, mainly due to natural forces such as drug resistant microbes and insects. Consequently an imperative need exist to connect the etho pharmacological information with the newest drug discovery new active natural metabolites. Hence

it an urgent needs to screen and isolate new natural products from plant sources.(**Ekta menghani *et al*., 2013).**

Triterpenoids are a class of naturally occurring compounds found in plants, and their beneficial effects on the immune system and in the treatment of cancer are currently being investigated. Some triterpenoids of interest include glycyrrhetinic, betulinic, oleanolic and ursolic acids, where ursolic has been recently reported to exist at high concentration in apple skin**(*Marc Plante etal.,2012).***

Lupeol a triterpene (also known as Fagarsterol) found in white cabbage, green pepper, strawberry, olive, mangoes and grapes was reported to possess beneficial effects as a therapeutic and preventive agent for a range of disorders. These studies also provide insight into the mechanism of action of Lupeol and suggest that it is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways which involve nuclear factor kappa B (NFjB), cFLIP, Fas, Kras, and Wnt/b-catenin in a variety of cells. It is notable that Lupeol at its effective therapeutic doses exhibit no toxicity to normal cells and tissues. These also determine the utility of Lupeol as a therapeutic and chemopreventive agent for the treatment inflammation and cancer (**Mohammad Saleem., 2009).** The 3-O-acyl-derivatives of lupeol have anti-inflammatory properties and many of them are present in different medicinal plants, as are lupeol acetate and lupeol docosanoylate .

Lupeol figure.1 is a pharmacologically active triterpenoid, commonly found in plant species of the *Asteraceae* family.

It has several medicinal properties such as antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemo preventive properties. It is an effective inhibitor in laboratory models of prostate and skin cancers.

Quantification of Lupeol showed high resolution and separation from other constituents of the extracts **(V Leela *et al*., 2013).**Its advantages are its simplicity,

accuracy and selectivity. These can be used for the estimation of these compounds in other herbal preparations and may be useful for standardisation purposes.

The main aim of the present work is to quantify in chosen indigenous plant materials by Colorimetric method and UV-Spectrometric method.

Fourteen indigenous plants were selected for the study. Table 1 gives the details of plants chosen.

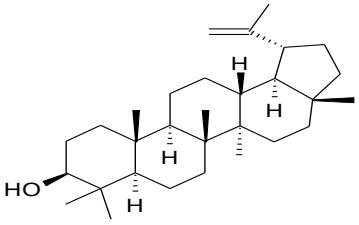


Figure.1 Structure of Lupeol

Properties of Lupeol

chemical formula	$C_{30}H_{50}O$
Melting point	215-216°c
Molecular weight	426.7174g/mol
H-Bond donor	1
H-Bond acceptor	1
Rotatable bond	1
Heavy atom count	31
Complexity	766
Isotope atom count	0

Details of plant selected for the study

Plant Name	Family Name	English Name	Tamil Name	Malayalam Name	Hindi Name
Aloe vera(Linn)	*Xanthorrhoea c-eae*	*Aloe vera*	*Sotrukatraz --hai*	*Kattuvala*	*Ghritkumari*
Aegle marmelos(Linn)	*Rutaceae*	*Bael tree*	*Vilvam*	*Kulakam*	*Sirphal*
Cajannus cajan(Linn0	*Fabaceae*	*Angolan pea*	*Thovaray*	*Kacang Bali*	*Arhar*
Calendula Officinalis(Linn)	*Asteraceae*	*Garden Marigold*	*Sendigai*	*Zendu*	*Zergul*
Capsicum annuum(Linn)	*Solanaceae*	*Red chillies*	*Milagay*	*Vattalmulaku*	*Degimirch*
Cassia fistula(Linn)	*Fabaceae*	*Golden shower*	*Konnai*	*Bereksa*	*Amaltas*
Limonia aurantifolia (Christm)	*Rutaceae*	*Limes*	*Elumichai*	*Limaunipis*	*Nibu*
Helianthus annuus(Linn)	*Asteraceae*	*Sun flower*	*Suryakanthi*	*Sooryakanthi*	*Surajmukhi*
Jatropha curcas(Lin)	*Euphorbiacee*	*Barbados nut*	*Kadalamanakku*	*Kattamanak*	*Bagbherenda*
Lawsonia liermis(Linn)	*Lythraceae*	*Camphire*	*Maruthani*	*Milanchi*	*Mehanti*
Lycopersicon esulentum(Linn)	*Acanthaceae*	*Strobilant -hus*	*Karimkurujji*	*Karimkuruji*	*Karvi*
Mangifera indica(Linn)	*Anacardiacee*	*Mango*	*Mamaram*	*Mavu*	*Amra*
Psidium guajava(Linn)	*Myrtaceae*	*Guava*	*Koorayaa*	*Jambubatu*	*Amaruud*
Strobilanthus cilatus	*Aanthaceae*	*Strobilath -us*	*Karimkurunji*	*Karimkurunji*	*Karvi*

Description of Plants

***Aloe vera* (Linn)**

Aloe vera is an herbaceous continuing in the family *Liliaceae* grown for its luscious leaves which have a variety of gastronomic and medicinal uses. The plant has a short, plump stem and a rosette of fleshy leaves which have a ragged margin of small white teeth. The leaves are pale green or gray-green in colour. Aloe vera plants produce a clear inflorescence composed of densely packed pendulous yellow flowers on a spike which can be up to 90 cm (35 in) in height. The plant can grow to be 1 m (3 ft) in height. The plant may be called as Aloebarbadensis or Aloeperfoliata .

Aegle marmelos(Linn)

Aegle marmelos is a medium size tree. It grows to 12-15 m tall with short trunk, thick and soft. It is sweet at first taste and then irritating to the throat. The deciduous, alternate leaves are 4-10 cm long, 2-5 cm wide, and the terminal one with a long petiole. New foliage is glossy and pinkish-maroon. Mature leaves emit a disagreeable odour when bruised. Fragrant flowers, in clusters of 4 to 7 along the young branchlets, have 4 recurved, fleshy petals. The fruit, round, oval, or oblong, 5-20 cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, gray-green until the fruit is fully ripe. It is dotted with aromatic, minute oil glands. Embedded in the pulp are 10 to 15 seeds, flattened-oblong, about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying.

Cajanus cajan(Linn)

Cajanus cajan is a glandular-pubescent, short-lived persistent (1-5 years) shrub, usually grown as an annual, 0.5-4 m high, with thin roots up to 2 m deep. Stems up to 15 cm in diameter. Leafs are 1-2 cm long; stipules acuminate, 2.5-5 mm long, persistent; leaflets lanceolate or narrowly elliptic, puberulent above and underside, the largest to 7.5-8 x 2.8-3.5 cm ,acute apically, bases similar, venation strongly reticulate, prominent underneath. Inflorescence in terminal or axillary racemes in the upper branches of the bush. Flowers multi-colored with yellow

predominant, red, purple, orange occurs in streaks or fully covers the dorsal side of the flag. Flowers are 2cm long.

Calendula officinalis(Linn)

Marigold is an annual plant, growing to 80 centimeters in height. It has diamond leaves. They are pale-green in color. The inflorescences are usually golden-yellow or orange, which flower for a long growing period up to 4-7cm long .The fruit is a spiky curved achene. The petals have a spicy flavour and the leaves a bitter aftertaste. All parts are highly scented and therefore attractive to bees and drift flies.

Cassia fistula(Linn)

Cassia fistula is a medium sized deciduous tree, 10 m tall with a straight trunk to 5 m, 1 m diameter and spreading branches. Stem bark are pale grey, smooth and trim. Leaves alternate, pinnate, 30-40 cm long, with 4-8 pairs of ovate leaflets, 7.5-15 cm long, 2-5 cm broad, entire, the petiolules 2-6 mm long. Flowers bright yellow in terminal, drooping racemes, 30-60 cm long; calyx oblong, obtuse, pubescent; corolla with five subequal, obovate, shortly clawed petals, to 3.5 cm across; stamens 10, upper three with erect filaments to 0.7 cm long an. Fruit an indehiscent pod, 40-60 cm long by 1-2 cm diameter, cylindrical, pendulous and terete, containing 25-100 seeds. The pod develops numerous transverse septa between the seeds. When fresh the pods contain a black pulp which on drying adheres to the septa. Seeds lenticular, light brown and lustrous.

Capsicum annuum(Linn)

Capsicum annuum is belongs to the solanaceae family.It grows up to 1m (3.3 ft)to 3m(9.8 ft).Its commonly known as pepper, spreads over 1to 2 feet. Some strains of tepin peppers are much closer to perfectly round when flesh. If a tepin pepper is dried, it appears quite round even if it was slightly ellipsoidal when fresh.

Limonia aurantifolia (Christm)

It is a shrubby tree, to 5m (16ft.), with many thorns. Dwarf varieties are popular with home growers and can be grown indoors during winter months and in colder climates.

Helianthus annus(Linn)

Common sunflower is a widely branching, stout annual, 1 1/2-8 ft. tall, with coarsely hairy leaves and stems. The terminal flowers heads are large and showy, up to 5 in. across. A tall, coarse leafy plant with a hairy stem commonly branched in the upper half and bearing several or many flower heads, the central maroon disk surrounded by many bright yellow rays. Yellow ray flowers surround brown disk flowers. Flowering head terminal on main stem, 10–40 cm in diameter, rotating to face the sun, sometimes drooping, heads on lateral branches smaller; outer ray flowers neuter with yellow ligulate corolla. Taproot strong, penetrating to depth of 3 m and with large lateral spread of surface roots. Sunflower seeds are popular in breads, cereals, salads and many other dishes.

Jatropha curcas(Linn)

Jatropha curcas tree to 6m long, with spreading branches and stubby twigs, with a milky or yellowish rufescent exudates. Leaves are alternate 3 to 5-lobed in outline, 6–40 cm long, 6–35 cm broad, the petioles 2.5-7.5 cm long. Male and female flowers are produced on the same inflorescence, averaging 20 male flowers to each female flower or 10 male flowers to each female flower. The petiole length ranges from 0.24 to 0.90 inches (6.1-23.1mm).

Lycopersicon esculentum(Linn)

These leaves are 10-25 centimetres (4-10)in long, odd pinnate, with five to nine leaflets on petioles, each leaflet up to 8 centimetres (3 in) long, with a serrated margin; both the stem and leaves are densely glandular-hairly. Their flowers, appearing on the apical meristem, have the another fused along the edges, forming a column surrounding the pistil's style.Flowers in domestic cultivars tend to be self- fertilizing.

Lawsonia inermis (Linn)

Lawsonia inermis is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. These leaves are bark greyish-brown colour. Leaves are opposite, entire, sub-sessile, elliptic and broadly lanceolate, 1.5-5 x 0.5-2 cm, glabrous, acuminate; veins on the upper surface depressed. Flowers are small, white, numerous. Calyx with 2-mm tube and 3-mm spread lobes. Petals are obovate, white or red; stamens inserted in pairs on the rim of the calyx tube; ovary 4 celled style up to 5 mm long. Fruits are small, brown, globose capsules 4-8 mm in diameter. These seeds are angular and 3mm across with thick seed coat.

Mangifera indica(Linn)

It is a large evergreen tree to 20 m tall with a dark green, umbrella-shaped crown. These trees are 90 cm in diameter; bark brown colour thick, becoming darker, rough and scaly or furrowed; branchlets rather stout, pale green and hairless. Inner bark light brown and bitter. Whitish latex exudes from cut twigs and a resin from cuts in the trunk. Leaves are simple, leathery, oblong-lanceolate, 16-30 x 3-7 cm long .Flowers radially symmetrical, usually have 5 spreading petals, 3-5 mm long, 1-1.5 mm broad. The flower has a noticeable 5-lobed disc between the petals and stamens. Fruit an irregularly egg-shaped and slightly compressed fleshy drupe, 8-12 (max. 30) cm long, attached at the broadest end on a pendulous stalk. The skins were smooth and greenish-yellow colour. The basic yellow-orange flesh varies in quality from soft, sweet, juicy. The single, compressed-ovoid seed is encased in the white fibrous inner layer of the fruit.

Psidium guajava(Linn)

A small evergreen tree to 3-10 m high, many branches. Stems are warped, bark light to reddish brown, thin, smooth, continuously flaking. Leaves are opposite, simple and 3-10 mm long. Inflorescence, axillary, 1- to 3-flowered, pedicles about 2 cm long. Calyx splitting irregularly into 2-4 lobes. Petals 4-5, white, linear-ovate c. 2 cm long. Fruit an ovoid or pear-shaped berry, 4-12 cm long, weighing up to 500 g. The flesh is red; pulp juicy, creamy-white to pink or red in colour .The exterior of the fruit is fleshy, and the centre consists of a seedy pulp.

Strobilanthus cilates (Nees)

An aromatic, much branched shrub, with broad elliptic-lanceolate leaves, small purplish flowers, in slender axillary spikes and 2-4 seeded capsule containing compressed, smooth seeds.

Objectives of the study

1. To estimate the lupeol content of chosen the medicinal plants by spectrocolorimetric method.
2. To estimate the triterpenoid and steroid content of the chosen the medicinal plants by spectrocolorimetric method and UV-spectrometric method.

2.REVIEW OF LITERATURE

A review of past literature helps to proceed with new investigation .In the present investigation past literature has been reviewed on the following aspects and it covers the period from 2008 till date.

- Reports on quantification of lupeol from medicinal plants
- Reports on quantification of natural products in plant materials
- Reports on quantification of synthetic compounds in drug formulation
- Reports on the medicinal and phytochemical aspects of the chosen plants

Quantitative Estimation of Lupeol

The function of lupeol as a therapeutic and chemopreventive agent for the treatment of inflammation and cancer was studied .White cabbage, green pepper, strawberry, olive, mangoes and grapes possess beneficial effects as a therapeutic and preventive agent for a range of disorders (**Mohammed *et al*., 2009).**

Quantification of triterpenic glycosides *Centella asiatica* in was done by HPLC-UV method *(***M.H. Rafamantanana *et a*l.,2009**).

HPLC method was used for a custom quality control analysis and simultaneous quantitation of β-sitosterol and Lupeol in*Vernonia cinerea*Linn**(Willy shah *et al*.,2010).**

A sensitive, simple and accurate high-performance thin layer chromatographic method has been established for the simultaneous determination of β-sitosterol and Lupeol both in *Artocarpus lakoocha* Roxb. Leaf powder. The chromatographic separation was performed on silica gel 60 F254 HPTLC plate, with toluene: methanol: formic acid, 7.0:2.0:0.3 (*v/v/v*), as mobile phase. After development, plates were treated with methanolic sulphuric acid reagent. Detection and quantification were performed by

densitometry at 366 nm in fluorescence mode. The developed method was then validated using statistical analysis. **(Vikas *et al.*, 2011).**

Lupeol content in *Rhizophora mucronata*barkhas been quantified by HPTLC method. The amount of lupeol was found to be 3.34 μg/ mg of ethyl acetate extract of *R mucronata*bark(**Rohini rm.,2011).**

Two marker compounds lupeol and stigmasterol from methanolic extract of *Hygrophila auriculata*(K. Schum) Heine were quantified by HPTLC-UV530 nm analysis(**Md Sarfaraj Hussain.,2012**).

Lupeol has been isolated from the aerial part of *Strobilanthus callosous* Nees (**Venkatachapathi *et al.*, 2012**), *Curculigo orchlorides* (**Leela *et al.*, 2013**) and *Costus igneus* Stems **(K. Manjula *et al.*, 2013**) and quantified by HPTLC.

A HPLC method has been developed for determination of lupeol in the medicinal plants-*Vernonanthura Ferruginea* (**Ansamathew *et al.*,2012**), *AcaciaLeucophloea* Willd Flowers **(V.Leela *et al.*, 2013**).

Quantification of Natural Product in Plant Materials

A UV/Visible spectrophotometric method for the quantification of chlorophyll derivatives in complex mixtures was developed (**Hendrik Kupper., 2000).**

Triterpenoid acids were isolated and quantified from *Ganoderma applanatum*. An optimised extraction procedure was developed.IR, UV and NMR spectra were used for the identification of ganoderic and ganoderenic

acids. The highest fraction of triterpenoid acids was found in the tubes (6.4 mg/g of air-dry weight), followed by the dark context layer, which is the young part of the pileus (2.5 mg/g). The white context layer of the older pileus and the upper surface of the fruiting body contained only about 0.6 mg/g of triterpenoid acids(***Bojana Boh et al.,2000)***.

The determination of tryptophan content in protein hydrolysates has been developed by spectrophotometric method **(Mouming *et al*., 2006).**

The determination of two groups of flavonoids from *Romanian propolis* by sonication and maceration methods has been reported**(Laura *et al*., 2007).**

Parthenolide was estimated *Tanacetum parthenium (L*bythree methods colorimetric , planar chromatographic and high performance liquid chromatographic method. Parthenolide was estimated for the first time in the callus culture of the plant **(El *et al*., 2007).**

Hydroxycinnamate derivatives of *Stevia rebaudiana* have been investigated qualitatively and quantitatively by LC-MS. Twenty-four hydroxyl cinnamic acid derivatives of quinic and shikimic acid were detected and 19 of them were successfully characterized .The identification of these phenolic compounds might affect the organoleptic properties and add additional beneficial health effects to *stevia*-based products**(Hande Karakose *et al*., 2011**).

Triterpenes of roots of Beta vulgaris L .were quantified by HPLC-ESI method(**Agnieszka Mroczek *et al*., 2012**).

Identification and quantification of triterpenoids from fruits and leaves extracts of bilberry *Vaccinium myrtillus L* was done by GC-MS/FID method.(**Anna Szakiel *et al*.,2012).**

Quantification of Synthetic Compound in Drug Formulation

A new, simple and sensitive spectrophotometric method has been developed for the determination of rosiglitazone maleate in bulk and in tablets. Statistical analysis and recovery studies validated the methods. **(Kashaw *et al*.,2008).**

Two simple, sensitive, accurate and economic methods have been developed for the quantitative estimation of pramipexole dihydrochloride drug and its formulations**(Gurupadayya *et al.*, 2009**).

For the quantitative estimation of ritonavir in bulk drug in pharmaceutical dosage forms two simple, precise and sensitive visible spectrophotometric methods were developed**(Priyadarsini *et al*., 2010).**

The amount of Meloxicam in bulk drug and its tablets has been determined by a UV Spectrophotometric method (**Dhandapani *et al*., 2010).**

A rapid, accurate, precise and specific colorimetric method of estimation of rifabutin in pharmaceutical formulations and in bulk drugs was developed (**Medikondu *et al*., 2010**).

Estimation of nitrazepam by colorimetric method has been reported. The method is based on the formation of a yellow coloured complex by nitrazepam in presence of $FeCl_3$ and NaOH. This colored complex showed absorbance of 490 nm in colorimeter and obeyed Lambert- Beer's law in the concentration range of 4-24 µg/ml.(**Rakesh *et al*., 2010**).

Quantification of tobramycinin bulk and pharmaceutical formulations was done by a simple and selective colorimetric method . The method was

based on the coupling of tobramycin with ascorbic acid to give a coloured product having analytically useful maxima at 390 and 532 nm (***Shaza et al., 2011***).

A colorimetric estimation of prasugrel in bulk and pharmaceutical formulations has been reported **(Paramdeep *et al.*, 2011)**

Estimation of amoxicillin trihydrate in bulk drug samples was done by LC method. The method was validated for system suitability, linearity, precision and accuracy , specificity and sensitivity **(Beg *et al.*, 2011).**

A simultaneous estimation method of cetirizine hydrochloride and phenylephrine hydrochloridewas developed by spectrophotometry .The proposed methods are simple, rapid, economical and accurate **(Wankhede *et al.*, 2012).**

A colorimetric method for estimation of tranexamic acid in bulk and pharmaceutical dosage form has been developed and relevant statistical parameters determined. In this method a orange-yellow coloured complex is developed by tranexamic acid on reacting with a dye solution of 1,2-naphthoquinone-4-sodium-sulphonate in presence of borate buffer pH 9.5. The absorbance of this derivative was measured at 460 nm against a reagent blank and calibration curve was plotted (**Shweta *et al.*, 2012**).

Spectrophotometric method of estimation of hesperidin and diosmin in tablet dosage form was developed.**(Doddi *et al.*, 2013).**

Chondroitin sulphate (CHS) belongs to a family of heteropolysaccharides called glycosaminoglycans or GAGs. It is found in humans in cartilage, bone, cornea, skin and the arterial wall. A simple and sensitive spectrophotometric method for the determination of chondroitin sulphate in bulk drug and pharmaceuticals was developed CHS mainly used in promotion and maintenance of the structure and function of cartilage in

pain relief of osteoarthritic joints andexhibits anti-inflammatory activity **(Somashekar *et al.*, 2013).**

The quantification of moclobemide by spectrophotometric method using Folin Ciocalteu's reagent has been carried out andthe method validated **(Shital *et al.*, 2013).**

Medicinal and Phytochemical Aspects of the Chosen Plants

Aloe vera (Linn)

Aloe vera belongs to the family *xanthorrhoaeacae*.The natural range of Aloe vera is unclear , as the species has been widely cultivated throughout the world. Aloe vera has been used in herbal medicines and cosmetics.

- Tannin, Saponin, Terpenoids and Flavonoids of *Aloe vera* have been identified by GC-MS method **(Rubina *et al*.,2009).**

- Methanolic extract of *Aloe barbandensis Miller* inner leaf gel has been fractionated by RP-HPLC method and antibacterial activity was analysed **(Bushra *et al*.,2009).**

- A GC –MS method of identification of lupeol from *Aloe vera* has been reported **(Narish *et al*.,2012) .**The amount of lupeol was found to be 3.45%

Aegle marmelos (Linn)

Aegle marmelos is traditional plant of Tamilnadu, India. It belongs to *Rutaceae* family. It is useful for several medicinal properties

- The methanolic extract of the leaves, root, stem bark of *Aegle marmelos* has been quantitatively estimated and the presence of phenols and flavonoids has been reported **(Nadeem *et al.*, 2009).**

- A phytochemical and biological evaluation of different parts of *Aegle marmelos* plants has been reported recently**(Sandeep Dhankhar *et al.*, 2010).**

- A HPLC and HPTLC quantification of lupeol in *Aegle marmelos* plant has been reported **(Gulshan *et al.*, 2011).**

- The antibacterial activity of *Aegle marmelos* extract of leaves, fruits and peels was analysed by agar-agar method **(Amit Pandey *et al.*, 2011).**

- Phytochemical and pharmacological studies on *Aegle marmelos* plant.**(Shahedur Rahman *et al.*, 2014).**revealed that the plant possesses antimicrobial, antiviral, anticancer, antipyretic, ulcer healing, anti-inflammatory properties .

Cajanus cajan (Linn)

Cajanus cajan belongs to the *Fabaceae* family. It is cultivated in central India. It has antiulcer, wound healing, hepatoprotective, anti asthamatic ailments properties.

- The antioxidant activity of the aqueous and ethanol extracts of *Cajanus cajan* has been estimated by DPPH and BHT methods **(Nan Wu *et al.*, 2009).**

- The ethanolic extract of the leaves and roots of *Cajanus cajan* has been estimated for it tannin, flavonoid and alkaloid content **(Samara *et al*., 2012).**

Calendula officinalis (Linn)

Calendula officinalis is also known as pot marigold. It belongs to the *Asteraceae* family. Extract of the plant has antiviral and anti-inflammatory properties.

- Carotenoids of *Calendula officinalis* have been separated by chromatographic methods **(Adela *et al*., 2008)**and the plant is found to contain a higher percentage of carotenoids, phenols, and terpenoids **(Monica *et al*., 2009).**

- The phytochemical screening of petroleum ether, chloroform, methanol and water extracts of *Calendula officinalis*leaf has been reported**(Chakra ., 2010) .**

- The antimicrobial activity of aqueous, ethanolic, chloroform, n-butanol and petroleum ether extracts of C.officinalis was assessed by agar gel diffusion method. All the plant parts showed significant anti microbial activity but the highest antimicrobial activity was observed in petroleum ether extract of stem part of *Calendula officinalis* against Escherichia coli (**Mathur et al., 2011**).

Capsicum annuum (Linn)

Capsicum annuum is belongs to the *Solanaceae* family. It's used for aurveda medicines. The plant contains a number of bioactive compounds.The alkaloid capsaicin has been identified by GC-MS method **(Aneta *et al*., 2011).**

- Antioxidant , anticoagulant and free radical scavenging activity of this *Capsicum annuum* has been reported recently**(Narayan *et al.*, 2012) , (Muhammad *et al.*, 2012).**

Cassia fistula (Linn)

Cassia fistula belongs to the family *Leguminosae* and is very common Indian plant known for its medicinal properties. It is also used in folk medicines for liver and throat cancer.

- Primary and secondary metabolite composition of vegetative and reproductive plant parts of this plant has been reported **(Theeshan *et al* ., 2005).**

- Pharmacognostic, pharmacological properties and phytochemical constituents of *Cassia fistula*(**Thirumal.M *et al*.,2012)** andantioxidant activities of the extracts (**Hermien *et al.*, 2012) (Md. Irshad *et al*., 2012)** has been reported.

Limonia aurantifolia (christm)

The *Limonia aurantifolia* is a citrus species with a globose fruit usually yellow colour. It belongs to the *Rutaceae* family.

- Flavonoids, terpenoids and higher concentration of an aldehyde were identified from this plant extract (**Maria *et al*., 2010), (Nagwa *et al*.,2010)** . Various monoterpenes have been identified by GC-MS method.

- The *Citrus* extracts have the potential to develop a clinically useful antiosteoporotic agent **(Nagwa *et al.*, 2010).**

Helianthus annuus (Linn)

Helianthus annuus is an annual plant. Commonly known as sunflower. It belongs to *Asteraceae* family.

- The sunflower seed is a good source of protein with high nutritional value (**Satish et al ., 2011**).

- It is reported that this plant produces a complex array of secondary compounds that are secreted into glandular trichomes - specialized structures found on leaf surfaces and appendages of flowers **(Heather et al., 2012).**

Jatropha curcas (Linn)

Jatropha curcas is a genus of flowering plants of *Euphorbiacea* family. The oil from this plant find main use as biodiesel for energy.

- The chemical composition and insecticidal activity of *Jatropha curcas* seed was evaluated by standard techniques. The oil content of the seed was 66.4% **(Adebowale *et al.*, 2006).**

- The methanolic extract of *Jatropha curcas*has been analysed by GC-MS method and the antifungal activity of this plant determined by using paper disc diffusion method **(Isamil *et al.*,2011).**

- The phytochemical composition and antimicrobial activity of *Jatropha curcas* was analysed . The phytochemicals present in the samples were found to be phenolics and terpenoids **(Tadeu *et al*., 2012).**

Lawsonia inermis (Linn)

Lawsonia inermis also called henna tree is a flowering plant used to colour hair, fingernails and leather. Henna belongs to the family *Lytheraceae*. Henna also acts as an anti-fungal and a preservative for leather and cloth.

- Antibacterial and antifungal activity of henna leaves has been reported **(M.A. Abdulmoneim Saadabi ., 2007).**

- The effect Water and chloroform extracts of the leaves of *Lawsonia inermis* against the primary invaders of burnt wounds was investigated **(Muhammad *et al*., 2005).**

- The plant contains carbohydrates, proteins, flavonoids, tannins and phenolic compounds.It also reported to have antibacterial, antimicrobial, antifungal, antiviral and anticancer properties **(Gagandeep *et al*., 2010).**

- The leaves contain tannin and saponin. Flowers contain essentialoil and bark contains triterpenoids. The whole plant is found to elaborate coumarin and xanthones **(Sonam *et al*., 2012).**

Lycopersicon esculentum (Linn)

The tomato *(Lycopersicon esculentum)* is most widely consumed fresh vegetables in the world. It is also used by food industries as a raw material for the production of derived products. It is also the most common vegetable

in the diet, beneficial for health and development of chronic degenerative disease.

- Chemical composition (moisture, ash, total fibre, protein, glucose and fructose), the taste and maturity were determined in five tomato cultivars. These were cultivated using intensive, organic and hydroponic methods **(Diaz *et al*., 2007).**

Mangifera indica(Linn)

Mangifera indica Linn, locally known as mango tree, is a medicinal plant tradicinally used in tropical regions. It has been claimed to possess antidiabetic potential.

- Reports on antioxidant, radioprotective,antitumor, immunomodulatory, anti-allergic, anti- inflammatory, antidiabetic, lipolytic, antibone resorption, monoamine oxidase inhibiting, antiviral, antifungal ,antibacterial and antiparasitic properties of *Mangifera indica* support the numerous traditional uses of the plants **(Nathalie *et al.*,2006).**

- Lupeol ,a triterpene was found to have *in vivo* and *in vitro* activity in mango extracts(**Sahdeo *et al.*, 2008).**

- Antioxidant and antidiabetic activity **(Nathalie *et al*., 2009)**anti inflammatory effects**(Lucia *et al*., 2010)**and anti-inflammatory activity **(Latha *et al*., 2012)**of leaf extracts of *M.indica* leaves has been reported.

Psidium guajava (Linn)

Psidium guajava L, commonly known as guava, of the family *Myrtaceae,* is a native plant of tropical America. Different parts of the plant are used in medicine for the treatment of various human diseases.

- Phytochemical and pharmacological studies on *Psidium guajava* leaves has been reported**(Gutiérrez *et al*., 2008).**

- The composition of the volatile oil obtained from the hydro distillation of the leaves of *Psidium guajava* was studied by GC-MS method. 40 compounds representing more than 90% of the volatile mixture were identified **(Nuerni *et al.*, 2008**).

- *Psidium guajava* has been widely used in traditional medicine for the treatment of diarrhoea, dysentery, gastroenteritis and indigestion. The aqueous extract of leaves of *Psidium guajava* was studied for its pathogenicity of infectious diarrhea **(Tannaz *et al.*, 2010).**

- The ethanolic extracts of *Psidium guajava* leaves has been investigated in rats infected with *Trypanosaoma bruceli*. Results showed that the extract contained a higher amount of flavonoid, steroids and terpenoids **(Adeyemi *et al*., 2010) .**

Strobilanthus ciliatus(Nees)

Strobilanthus ciliatus is found mainly in the low hills of the Western Ghats all along the west coast of India. It is used as a tradicinal medicinal herb for the treatment of inflammatory disorders. It also finds use in folk medicines.

- Phytochemical investigation of *Strobilanthus ciliatus Nees* was carried out and lupeol, stigmasterol and betulin were isolated and characterized **(Reneela *et al.*, 2010).**

- Lupeol was isolated from the petroleum ether extract of aerial parts of *Strobilanthes ciliatus Nees* by column chromatography and identified by IR, NMR, and MS spectral data. It was quantified by HPTLC method **(Suppan *et al*., 2012)**

3.MATERIALS AND METHODS

The present work titled "Spectrophotometric Quantification Of Triterpenoid In Selected Medicinal Plants" comprises of the following stages.

- Extraction of plant material
- Identification of terpenoids in extracts by colour tests
- Colorimetric estimation of triterpenoids in the extracts
- UV-spectrophotometric method of quantification of triterpenoids in the extracts.
- Quantification of the triterpenoid Lupeol by preparative TLC method.

Instruments used

Quantification of triterpenoides and steroids content by using Photocolorimeter (model 1311) and UV-spectrometer (model Shimadezu).

Chemicals used

Solvent and reagent used for the study were of laborating grade. Silica gel G was used for TLC analysis.

Methodology

The methodology adopted is presented below.

Collection of Plant Materials

The leaves of *Cassia fistula* was collected from local areas of Coimbatore. The stem and root of Strobilanthus cilatus were collected from Kerala.The leaves of *Aloe vera ,Aegle marmelos, Cajanus cajan, Calendula officinalis, Capsicum annuum, Limonia aurantifolia, Helianthus annuus, Jatropha curcas, Lawsonia inermis, Lycopersicon esculentum, Mangifera indica* and *Psidium guajava* were collected from *Che*nnimalai, Erode(Dt).The plant parts were pulverized and air dried..In present work selected parts of each plants were analysed. Totally seventeen samples were analysed. The details of plant materials used is presented in the following table2

Extraction of Plant Materials

The dried plant material (50g) was extracted with petroleum ether (150 ml) by heating over a water bath for 1 hour. The extract was filtered and concentrated. The extraction procedure was repeated again with fresh alcohol (130 ml).The extract was filtered and concentrated to give a residue which was weighed. The above extraction procedure was carried out for each of the 17 plant materials taken up for the present study. The pet-ether extract was taken up for the quantification study.

The percentage yield of residue was calculated using the formula

Weight percentage = Weight of substance extracted/Weight of the original material×100

Details of Plant Materials Selected and their Designated Code Names

S.NO	Chosen plant material	Sample code
1	*Aloe vera* leaf	AVL
2	*Aegle marmelos* leaf	AML
3	*Cajanus cajan* leaf	CCL
4	*Calendula officinalis* leaf	COL
5	*Calendula officinalis* flower	COF
6	*Capsicum annuum* leaf	CAL
7	*Cassia fistula* leaf	CFL
8	*Limonia aurantifolia* leaf	CASL
9	*Helianthus annuus* leaf	HAL
10	*Helianthus annuus* flower	HAF
11	*Jatropha curcas* leaf	JL
12	*Lawsonia intermis* leaf	LAL
13	*Lycopersicon esulentum* leaf	LEL
14	*Mangifera indica* leaf	MIL
15	*Psidium guajava* leaf	PGL
16	*Strobilanthus ciliatus* stem	SCS
17	*Strobilanthus ciliatus* root	SCR

Tests for Terpenoids

Salkowski test

The sample was taken in a test tube and chloroform (1ml) was added. Then concentrated sulphuric acid was added carefully along the sides of the test tub.Formation of reddish brown coloured solution indicates presence of terpenoids.

Liebermann-Burchard test

The sample was treated with chloroform (1 ml), acetic anhydride (1ml) and add few drops of concentrated sulphuric acid. The dark green coloured solution indicates the presence of terpenoids.

Spectrocolorimetric Method of Quantification of Lupeol

A colorimeter is a light-sensitive instrument that measures how much colour is absorbed by an substance. It determines colour based on the red, blue, and green components of light absorbed by the sample. When light passes through a medium, part of the light is absorbed, and as a result, there is a decrease in how much of the light reflected by the medium. A colorimeter measures that change so users can analyze the concentration of a particular substance in that medium. The device works on the basis of Beer-Lambert's law, which states that the absorption of light transmitted through a medium is directly proportional to the concentration of the medium.

Procedure 1

- Extract (5mg) was taken in a beaker. Chloroform (1.5ml) was added to dissolve the extract and transferred to a clean test tube.
- Concentrated sulphuric acid (0.1ml) was added to it. The test tube solution was allowed to stand for 3hrs at room temperature.
- A reddish brown precipitate was formed on standing. The supernatant liquid was removed.

- The precipitate was dissolved in 95% methanol and made up to 5ml.The solution was transferred to a colorimetric cuvette and the absorbance was measured at 540nm.Methanol (95%) was used as a blank.

Procedure 2

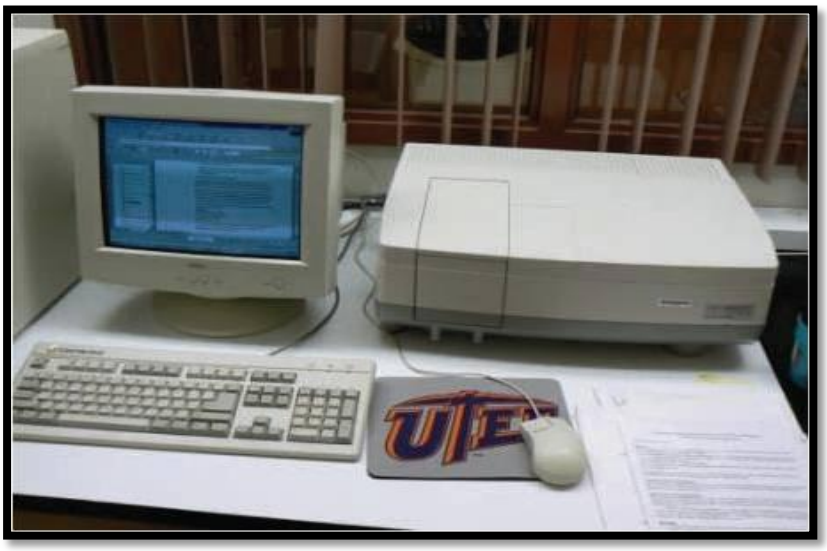
- The following procedure was adopted for isolating the terpenoid rich fraction of the plant material.
- The plant material (5g) was taken in a round bottomed flask ,Hydromethanol (150ml , 1:4 ratio) was added to it and refluxed for 12 hours.
- The extract was then filtered. Filtrate was acidified with 2M H_2SO_4 and extracted with chloroform(75ml).
- The extraction was repeated until the chloroform layer was colourless. The chloroform layer was concentrated and the concentrate was placed in a desiccator for removing its moisture content.
- This fraction consists of phenolics and terpenoids.
- Procedure 1 was repeated for the Colorimetric estimation of this fraction. For comparison only plant material (HAF) was analysed by this method.
- The OD was measured for the various samples of pet-ether extracts of the chosen plant materials at different concentrations.

UV-Spectrometeric Quantification of Triterpenoids and Steroids

A UV visible spectrum represents the absorption of UV light by a molecule promotion of an electron from a ground electronic state to an excited electronic state.

The UV Visible spectrum of the pet-ether extracts of the plant materials was recorded at room temperature using in hydromethanol (95% MeoH) Systronics-PC based double beam spectrometer 2202 in 200-600 nm range.

The complex formed by each plant extract with conc.H_2SO_4. In Salkowski test diluted with 95% methanol of UV solution was recorded. Peak absorbance value are noted.



Quantification of Lupeol by Preparative TLC Method

In this method concentrated petroleum ether extract of four samples (SCS, SCR, HAF and CFL) was subjected to TLC examination by a preparative method using petroleum ether as developing solvent system. The samples were compared with standard lupeol. The plate was observed under short UV light . The R_f of spot corresponding to standard lupeol was noted.

A preparative thin layer chromatogram of the sample was run. The silica gel band corresponding to the R_f of standard lupeol was cut and extracted with chloroform under warm condition. The chloroform extract was concentrated. The residue was treated with Salkowski reagent and the optical density (OD) observed. The amount of lupeol was calculated by correlating the observed OD in the standard calibration curve.

4.RESULTS AND DISCUSSION

The present study was undertaken with the aim of analyzing extracts of 17 chosen plant materials from 13 anti-inflammatory plants for the presence of pharmacologically active triterpenoid molecule lupeol in them by colorimeteric method and UV spectrometric method and to quantify the extracts for their triterpenoid and steroid content by the same methods. The results of the study are presented below.

EXTRACTION

Each of the 17 plant materials (50 g each) was first extracted with petroleum ether for one hour followed by a second extraction of the residual plant material with ethanol for one hour (heating over a water bath for petroleum ether and ethanol extraction).The extraction strategy revealed that most of the plants gave a higher yield of residue in the second ethanol extraction . The percentage yield of residue obtained is given in Table 3.

Under the above conditions seven plant samples (leaves of *Aloe vera*, *Capsicum annuum* leaves, *Mangifera indica* leaves , *Cassia fistula* leaves, *Cajanus cajan* leaves, *Calendula officinalis* leaves, *Psidium guajava* leaves) gave maximum yield of residue in the range 3-5% from only 50g of plant material.This is a notable aspect of the extraction analysis. However eight plants (*Helianthus annuus* flower, *Jatropha curcas* leaves, *Limonia aurantifolia* leaves, *Lycopersicon esculentum* leaves, *Helianthus annuus* leaves, *Aegle marmelos* leaves, *Calendula officinalis* leaves, *Lawsonia inermis* leaves) gave a minimum yield of residue in the range 1-3%.

Table3 - Yield of Residue

S.NO	Sample	Residue weight(g) in petroleum ether extraction	Yield(%) in petroleum ether extraction	Residue weight(g) in ethanol extraction	Yield (%) in ethanol extraction
1	AVL	0.7	1.4	1.8	3.6
2	AML	0.5	1.0	1.0	2.0
3	CCL	0.8	1.6	2.4	4.8
4	COL	0.4	0.8	0.8	1.6
5	COF	1.0	2.0	2.2	4.4
6	CFL	1.1	2.2	2.0	4.0
7	CAL	0.8	1.6	1.9	3.8
8	CASL	0.6	1.2	1.2	2.4
9	HAL	0.5	1.0	0.9	1.8
10	JL	0.7	1.4	1.4	2.8
11	LAL	0.6	1.2	0.8	1.6
12	LEL	0.8	1.6	1.4	2.8
13	MIL	0.9	1.8	2.2	4.4
14	PGL	1.0	2.0	2.2	4.4
15	HAF	0.5	1.0	1.2	2.4
16	SCS	1.0	2.0	1.2	2.4
17	SCR	1.4	2.8	2.0	4.0

Quantification of Triterpenoids and Steroids by Colorimetric analysis

According to methodology already discussed, the optical density was recorded for the complex formed by the 17 samples of the plant extracts with Salkowski reagent. Optical Density (OD) was recorded at low, medium and high concentration of samples. Table 4 gives the OD value for standard compound lupeol. Table 5, 6,7 give the OD values for samples at three concentrations. The results indicate the relative quantity of triterpenoids and steroid content in these extracts.

TABLE 4

S.NO	Concentration of standard (mg)	Optical Density
1	0.2	0.29
2	0.4	0.35
3	0.6	0.42
4	0.8	0.48
5	1	0.55
6	1.2	0.62
7	1.4	0.68
8	1.6	0.77
9	1.8	0.86
10	2	0.94

TABLE 5

S.NO	Sample code	Optical density
1	AVL	0.38
2	JL	0.37
3	CFL	0.34
4	LEL	0.27
5	COF	0.32
6	MIL	0.45
7	PGL	0.35
8	LAL	0.27
9	HAL	0.41
10	CASL	0.24
11	CAL	0.51
12	CCL	0.29
13	SCR	0.33
14	SCS	0.40
15	HAF	0.47
16	COL	0.40
17	AML	0.22

TABLE 6

S.NO	Sample code	Optical density
1	AVL	0.51
2	JL	0.74
3	CFL	0.57
4	LEL	0.46
5	COF	0.44
6	MIL	0.53
7	PGL	0.45
8	LAL	0.59
9	HAL	0.43
10	CASL	0.48
11	CAL	0.64
12	CCL	0.64
13	SCR	0.37
14	SCS	0.48
15	HAF	0.58
16	COL	0.65
17	AML	0.47

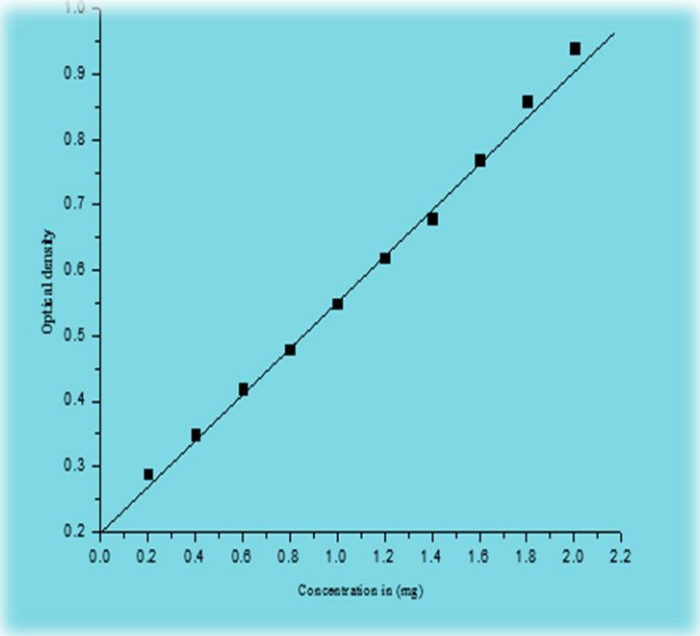
TABLE 7

S.NO	Sample code	Optical Density
1	AVL	0.68
2	JL	0.87
3	CFL	0.63
4	LEL	0.72
5	COF	0.49
6	MIL	0.93
7	PGL	0.59
8	LAL	0.62
9	HAL	0.73
10	CASL	0.78
11	CAL	0.71
12	CCL	0.82
13	SCR	0.47
14	SCS	0.51
15	HAF	0.72
16	COL	0.70
17	AML	0.49

Standard Calibration Curve

Standard calibration curve was plotted using origin 8 software. In this graph linearity, slope ,intercept and correlation co-efficient were calculated.

Figure 2



Precision of the method

Parameters	**Lupeol**
Linearity range(mg)	0.2-1.6 (mg)
Slope (m)	0.358788
Intercept (c)	0.201333
Correlation coefficient(R)	0.998

Figure 3

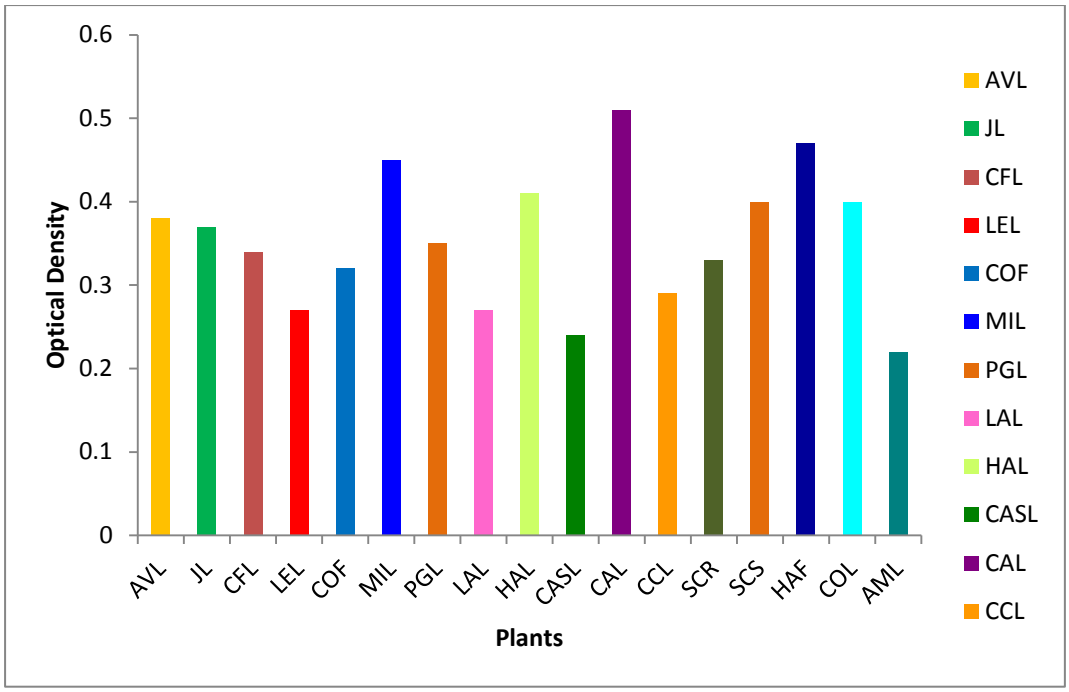


Figure 4

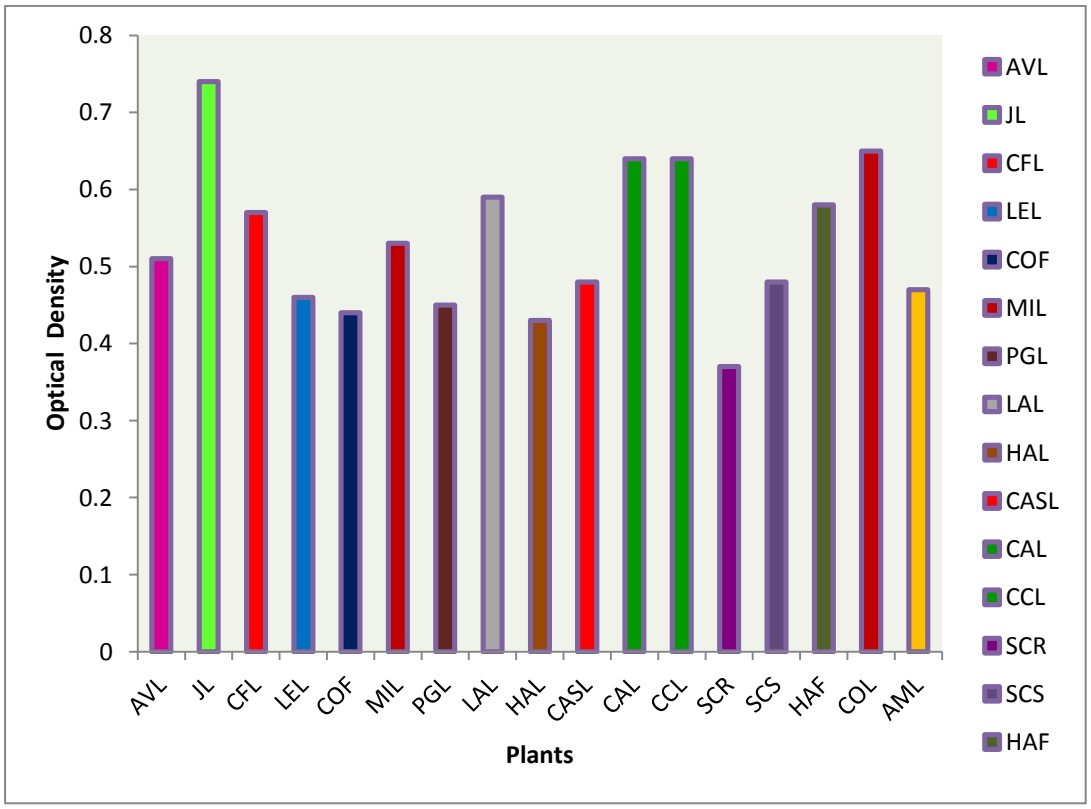
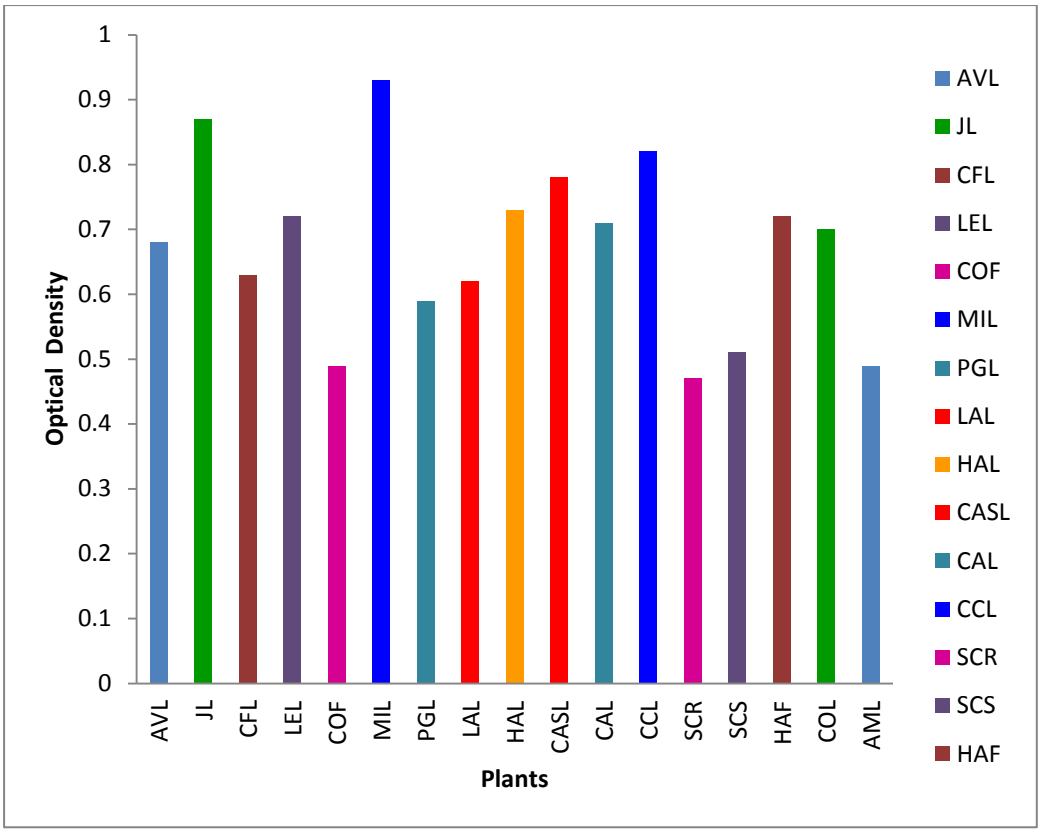


Figure 5



The following figures represent the histogram of optical density of samples.

Figure 3 represents the histogram for low concentration of the plant extracts. It reveals that *Capsicum annuum* (CAL) contains high terpenoid and sterol content.

Figure 4 represents the histogram for medium concentration of the plant extracts. In this histogram reveals that *Jatropha curcas* (JL) extract contains higher terpenoid and sterol content.

Figure 5 represents the histogram for high concentration of the plant extracts. According to this histogram *Mangifera indica (*MIL) extract is found to contain high terpenoid and sterol content.

Isolation of Lupeol by Preparative TLC Method

Figures 6 and 7 represents the preparative TLC chromatogram of plant samples SCS and HAF respectively. The rounded rectangle represents the TLC spot corresponding to lupeol.

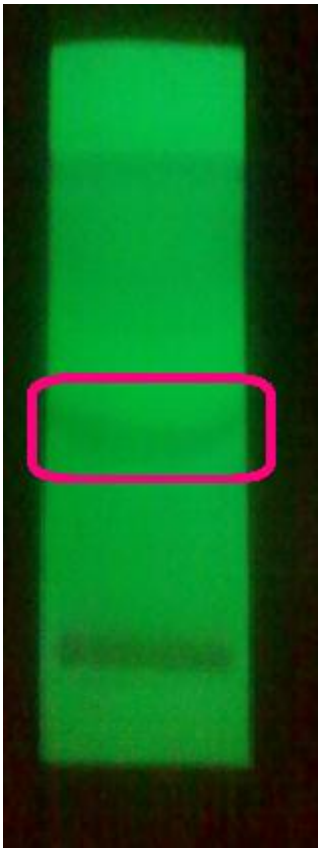


Fig 6

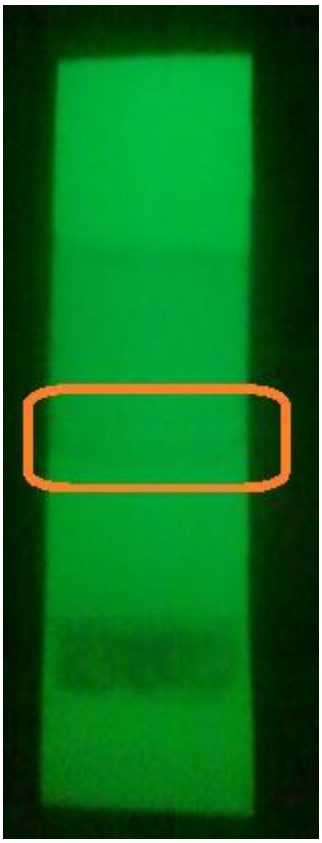


Fig 7

Quantification of Lupeol Extracted from the Plant Sample by Spectrophotometric Method

The lupeol isolated for each chosen plant extract (SCS, SCR, HAF and CFL) after preparative TLC, was treated with Salkowski reagent. The optical density of the complex solution was noted and the quantity of lupeol of extracts corresponding to this OD value was obtained from the calibration curve (Figure.2) of standard lupeol. Table 8 gives details of plant sample code, OD values and the amount of lupeol quantified.

Table 8

Spectrophotometric data for various extracts

Sample code	OD	Quantity of Lupeol in mg/mg of Plant extracts
SCS	0.16	0.25
SCR	0.20	0.28
HAF	0.04	0.1
CFL	0.02	0.06

UV Spectrum of solution of 5mg of lupeol reaction with Salkowski reagent (concentrated sulphuric acid)

Fig 8

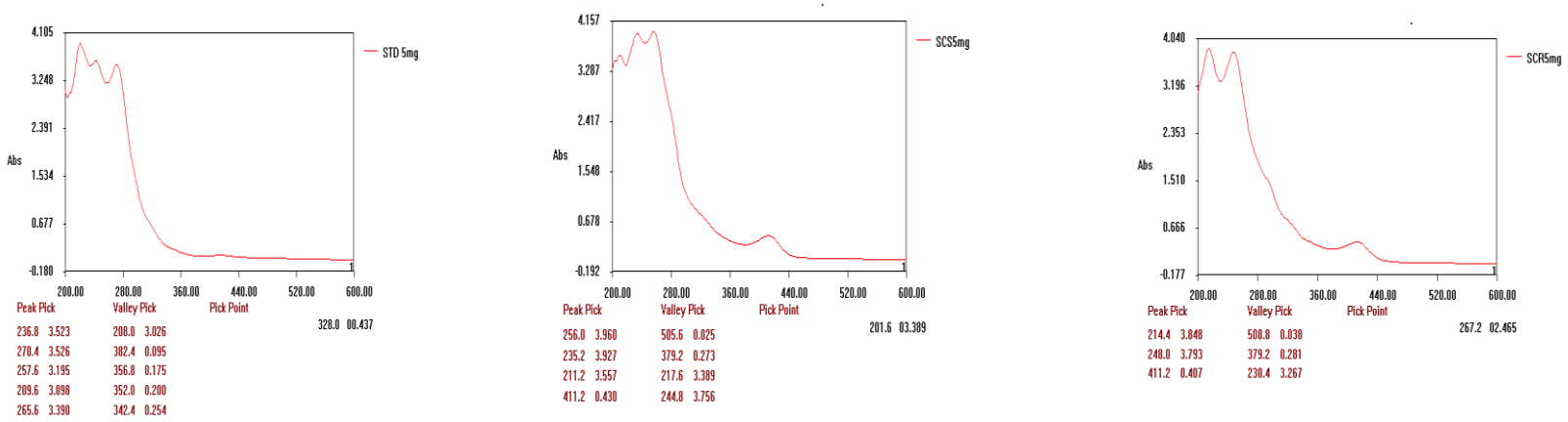
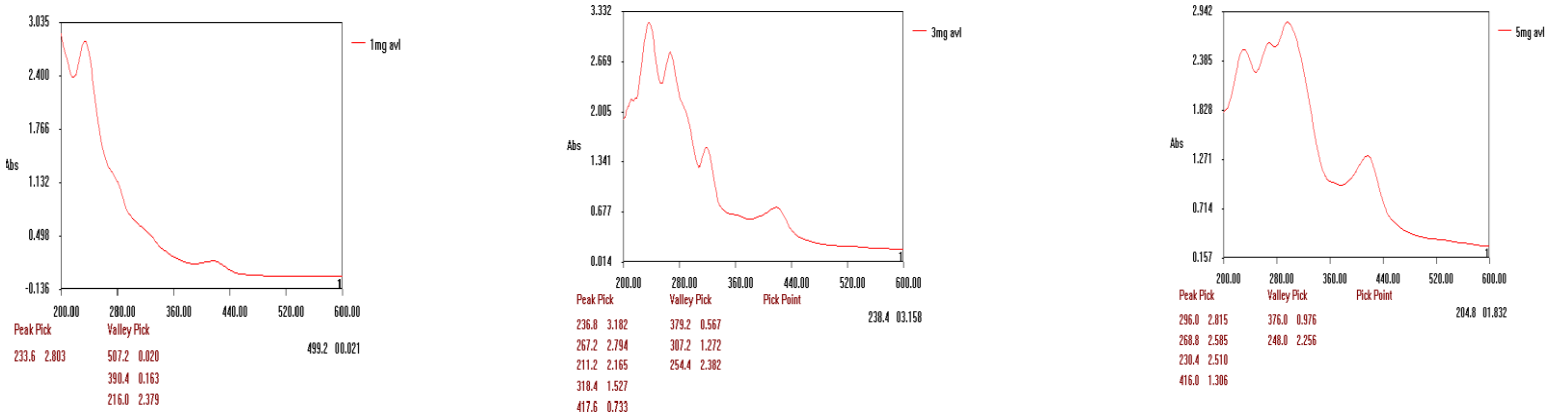


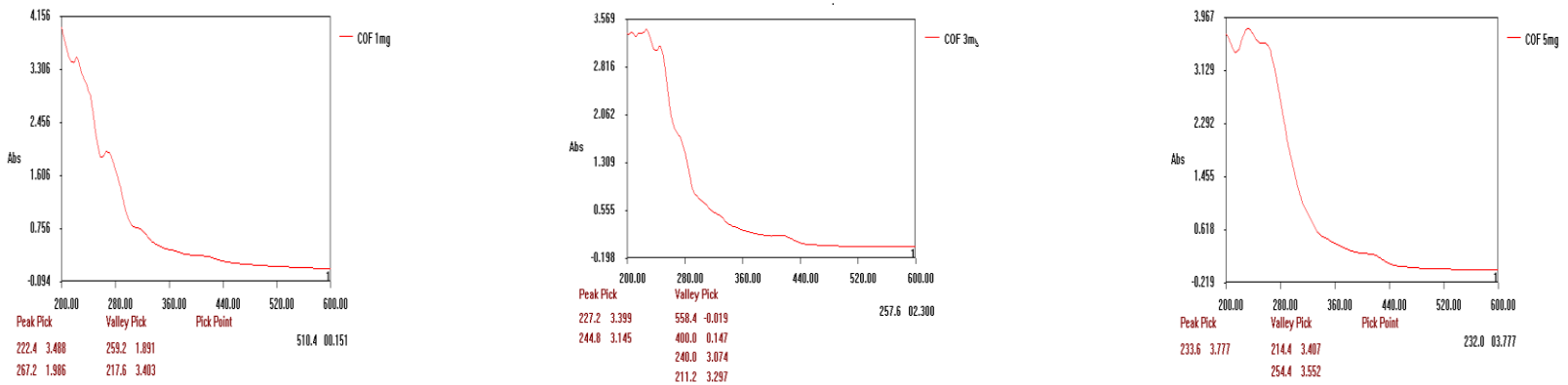
Fig 8 show the comparative UV Spectrum of standard lupeol with plant extract SCR and SCS

UV Spectrum of the complex formed by the plant extracts (AVL) extract with Salkowski reagent at various concentrations

Fig 9

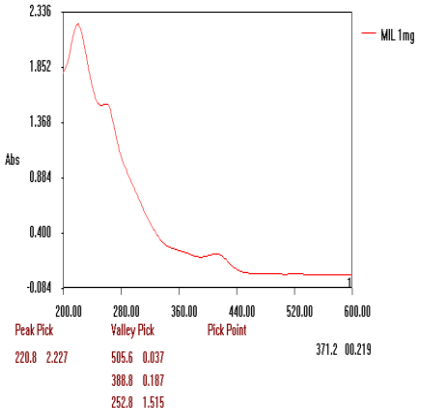


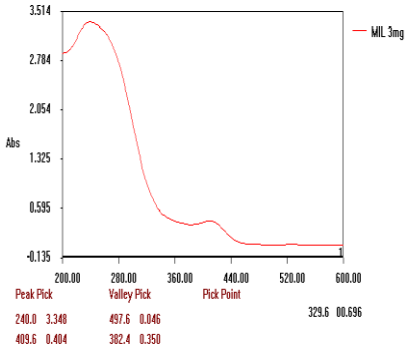
UV Spectrum of the complex formed by the plant extracts (COF) extract with Salkowski reagent at various concentrations

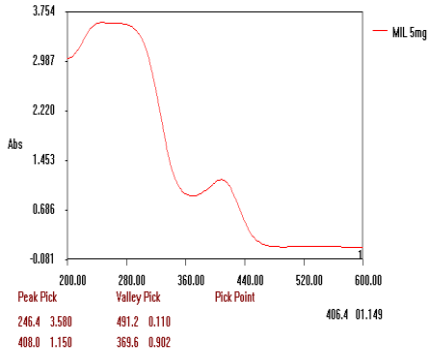


UV Spectrum of the complex formed by the plant extracts (MIL) with Salkowski reagent at various concentrations

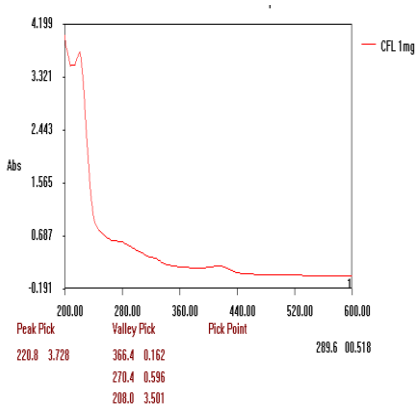
Fig 10

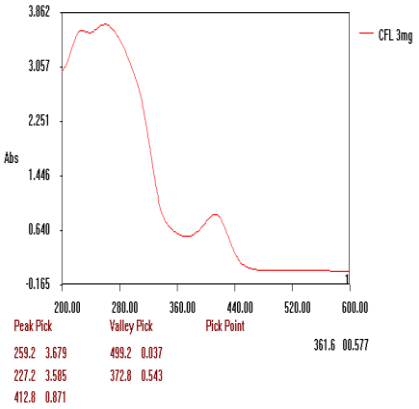


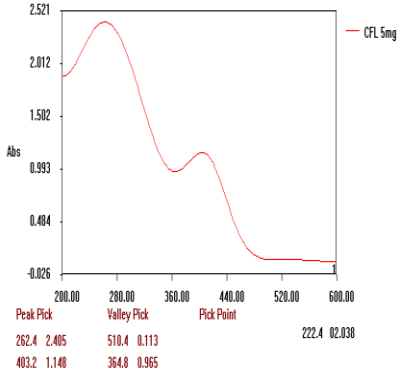




UV Spectrum of the complex formed by the plant extracts (CFL) with Salkowski reagent at various concentrations

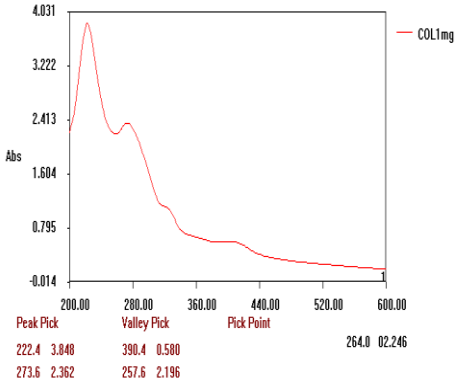


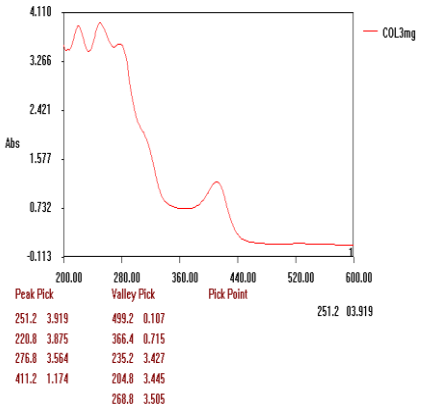


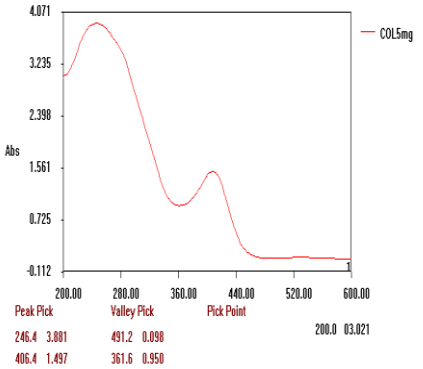


UV Spectrum of the complex formed by the plant extracts (COL) with Salkowski reagent at various concentrations

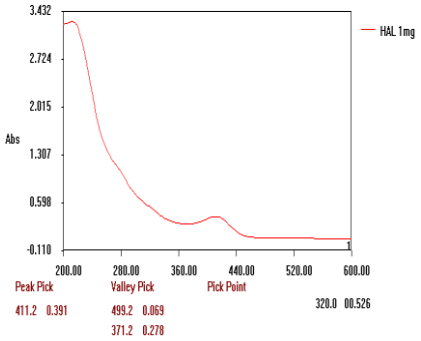
Fig 11

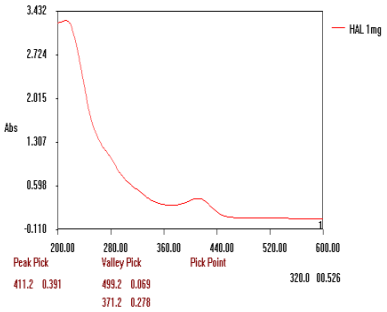


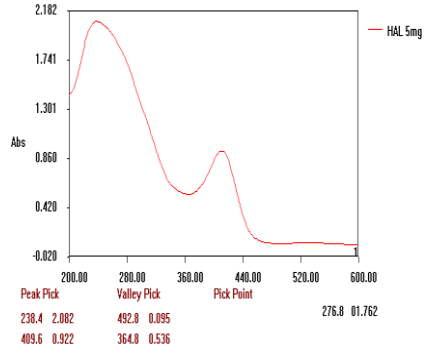




UV Spectrum of the complex formed by the plant extracts (HAL) with Salkowski reagent at various concentrations

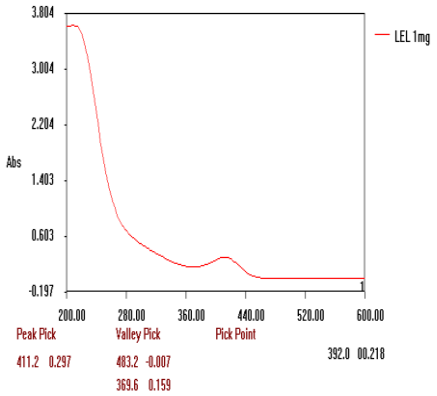


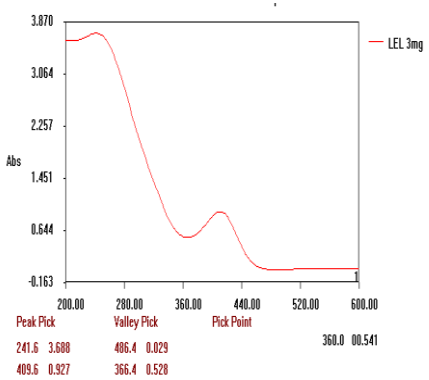


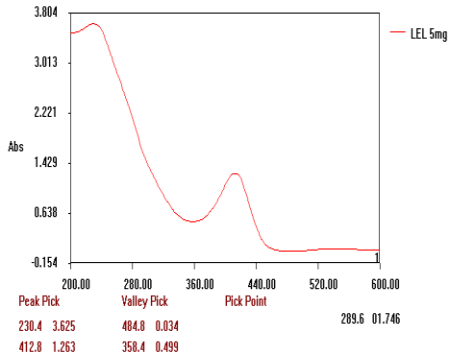


UV Spectrum of the complex formed by the plant extracts (LEL) with Salkowski reagent at various concentrations

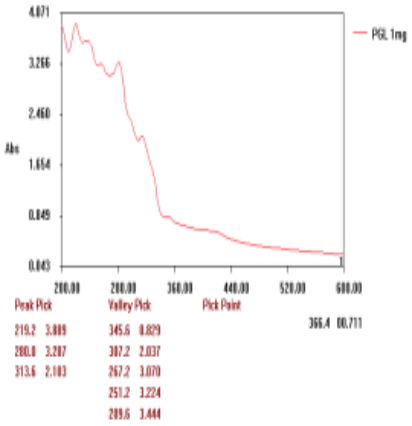
Fig 12

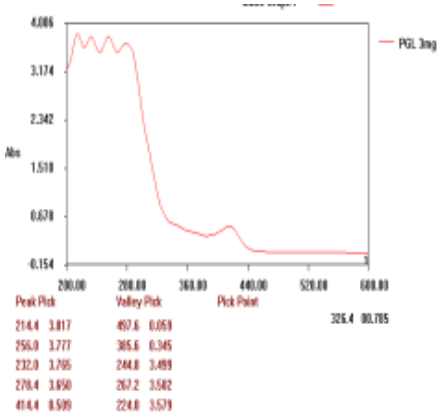


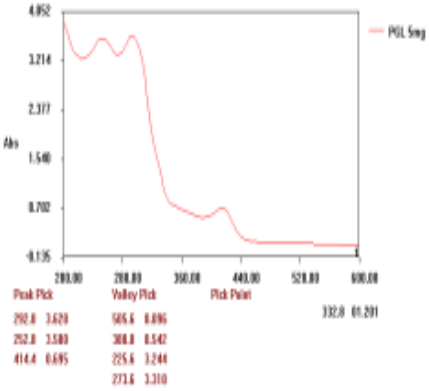




UV Spectrum of the complex formed by the plant extracts (PGL) with Salkowski reagent at various concentrations

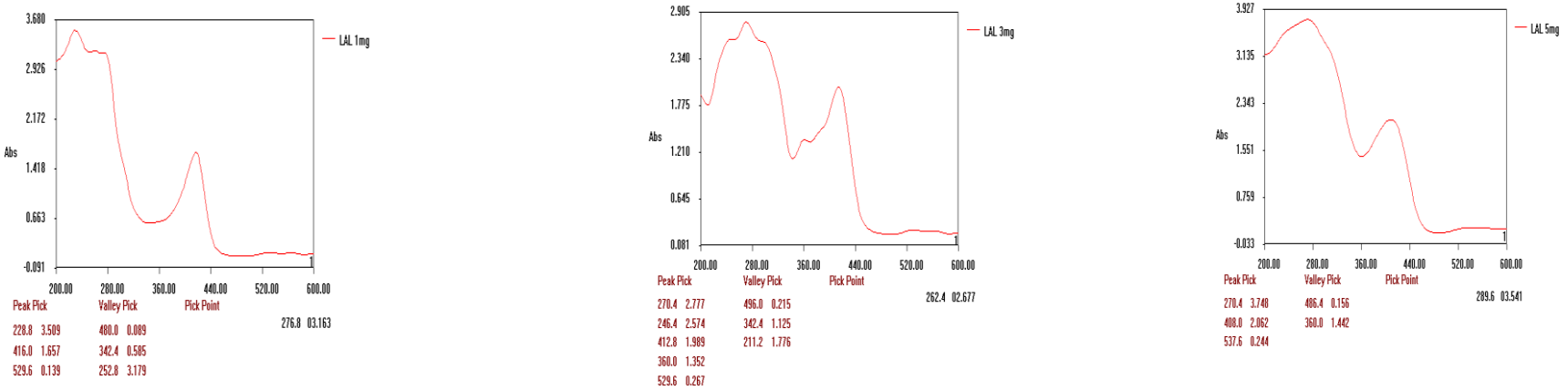




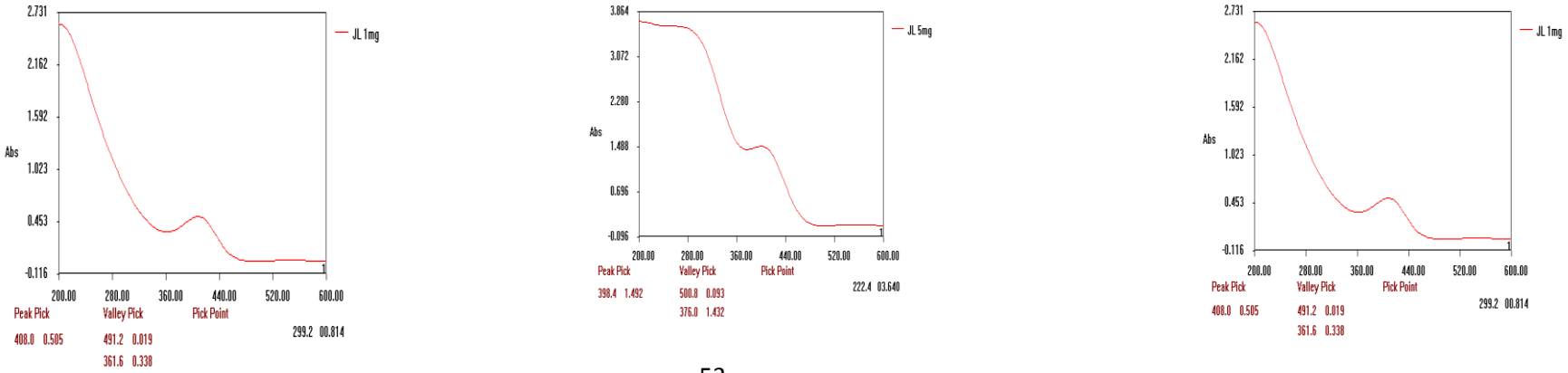


UV Spectrum of the complex formed by the plant extracts (LAL) with Salkowski reagent at various concentrations

Fig 13



UV Spectrum of the complex formed by the plant extracts (JL) with Salkowski reagent at various concentrations



The present study was undertaken to establish a simple method of estimation of triterpenoids in plant extract. Also, the medicinally valuable molecule Lupeol was quantified in chosen plant extracts. The method is simple and cost effective.

5.SUMMARY AND CONCLUSION

The present study titled "Spectrophotometric Quantification of Triterpenoids in Selected Medicinal Plants "was undertaken to quantify triterpenoids in the extracts of chosen plants.

Chapter 1 gives a brief introduction to the study. A recent review is presented in the Chapter 2 under the following headings.

- Quantification of lupeol from medicinal plants
- Quantification of natural products in plant materials
- Quantification of synthetic compounds in drug formulation
- Medicinal and phytochemical aspects of the chosen plants

Chapter 3 deals with the methodology adopted for the study. The results of the study and the relevant discussions are presented in Chapter 4.

- The present work involves the spectrophotometric quantification of lupeol in four selected plant extracts and quantification of triterpenoids and steroids content of 17 chosen plant extracts .

- The present study reveals that the method adopted for lupeol content in selected plant extracts is simple and feasible.

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