# *Lepidagathis keralensis*: Pharmacognostical, Phytochemical, and Pharmacological Investigations for Therapeutic Applications

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**Abstract: This paper describes the Pharmacognostical and Phytochemical screening of selected plants are *Lepidagathis keralensis. Lepidagathis keralensis* is a plant belonging to the family *Acanthaceae* commonly called as 'Paramullu'.This review paper discusses in detail study of Pharmacognostical, Phytochemical and In vitro cytotoxicity screening of *Lepidagathis keralensis* . In Pharmacognostical study discusses the Macroscopy and Microscopy of selected plant. Here the Macroscopical study describe the characters like colour, odour, taste, size, shape. The Microscopical study describe the fresh leaves, Stem and Root were subjected to various anatomical studies. The sections of leaf,stem and root specimens were taken using sharp blade and stained with various staining reagents like phloroglucinol-HCL, iodine, safranin and observed under microscope. Phytochemical screening of *Lepidagathis keralensis* shows the presence of alkaloid, flavonoid and Phenol. Rf value of *Lepidagathis keralensis* crude extract was determined by thin layer chromatography and it was 0.40. Cytotoxicity screening of *Lepidagathis keralensis* was done by MTT assay method.The sample was found to cause significant reduction in cell viability of normal fibroblast cells at highest concentration. Since no significant reduction was observed in lower concentrations, test samples can be considered as non-cytotoxic to normal cells at lower concentrations.**

**Keywords: *Lepidagathis keralensis*, Microscopy, Macroscopy, Phytochemical screening, Alkaloid, Flavonoid, Phenol, Cytotoxicity.**

## INTRODUCTION

Nature has provided a wealth of medicinal substances from plants for thousands of years, and an astounding number of modern medications have been identified from natural sources. Many of these isolations were based on how the agents were used in traditional medicine. Plant-based, traditional medicine systems continue to play an important role in health care, with around 80% of the world's population relying on traditional medicines for primary care. Medicinal plants are plants that have active compounds that can be used to treat sickness or relieve suffering. In most developing nations, traditional medicines and medicinal plants are widely used as therapeutic agents for the preservation of health.[1]

*Lepidagathis keralensis* family *Acanthaceae* is a plant endemic to Kerala. The genus comprises about 110 species, mainly distributed in tropical and subtropical countries with 33 species in India. It is a hard prostrate shrub with woody root stalk. It is found in lateritic hills near sea coast mainly in exposed lateritic rocks. Commonly it is known as Paramullu. Previous studies showed that, plant is a rich source of many bioactive constituents and it possesses medicinal properties. Here the review designed to point out the pharmacological effect of *Lepidagathis keralensis.*Medicinal plants also called medicinal herbs.

An herb can be any form of a plant or plant product, including leaves, stems, flowers, root, and seeds. An herbal medicine may be defined as a plant derived products for medicinal and health purpose. Natural products, mainly plant have been used for the treatment of various disease for thousands of years. *Lepidagathis* genus represented by 100 of species widely spread in tropical and subtropical regions of Asia and Africa.*Lepidagathis keralensis* Madhus. & NP Singh. is a species belonging to Acanthaceae family It is a perennial, prostrate, woody herb tenaciously attached to hard laterite soil. It is used by traditional practitioners of North Malabar for various ailments. Safety is of primary importance as far as any drug is concernedThe plant had many medicinal properties. The spines of the plant used by Paniya tribes for digestive disorders. The plant is also used for kidney stone, asthma, chest pain, blood purifieretc.The major isolated chemical constituents of the genus include essential oils, sesquiterpenes, flavanoids, phenolics, alkaloids, inorganic minerals and saponins.[2,3]

**Common Name**

*Kerala Lepidagathis*

**Vernacular Name**

Nonganampullu

Paramullu

Venappacha

Fig 1: *Lepidagathis keralensis*

**SCIENTIFIC CLASSIFICATION**

| | |
|---|---|
| Kingdom | Plantae |
| Phylum | Tracheophyta |
| Class | Magnolisidae |
| Order | Laminale |
| Family | Acantaceae |
| Genus | *Lepidagathis* |
| Species | *Lepidagathis keralensis* |

Table 1: Scientific classification of *Lepidagathis keralensis*

Fig 2 : Leaves of *Lepidagathis keralensis*

Fig 3 : Stem of *Lepidagathis keralensis*

Fig 4 : flowers of *Lepidagathis keralensis*

Fig 5 : Root of of *Lepidagathis keralensis*

**Traditional claims of *Lepidagathis keralensis*[4]**

- Used for treating kidney stones,
- Prevention of malnutrition, malabsorption
- Digestive issues
- Blood purifier

**Chemical constituents of *Lepidagathis keralensis***

Glycosides, saponins, phlobatannins, flavanoids, resins, sterols, amino acids protein, phytosterols, phenols, Terpenes. Alkaloids, Tannins are found in *Lepidagathis keralensis.*

**Distribution**

- Global Distribution: South India (Kerala)
- Indian Distribution: Kerala (Kannur- Ezhimala, Madayipara, Pazhayangadi)
- Endemic Distribution: Southern Western Ghats

## Materials and Methods

### Pharmacognostical studies[5,6]

### Macroscopical characters

The Macroscopical characters like colour, odour, taste, size, shape, extra features of the leaves were studied, and the results were tabulated.

### Microscopical studies

The fresh leaves were subjected to various anatomical studies. The sections of leaf, stem and root specimen were taken using sharp blade and stained with various staining reagents like phloroglucinol-HCL, iodine, safranin and observed under microscope.

### Preparation of powder

The collected leaves of *Lepidagathis keralensis* were washed with running tap water to remove adhering materials. Then the leaves were dried under shade. The dried leaves were pulverized mechanically into coarse powder. The coarse powder (passed through sieve no. 18 and retained on sieve no. 60) was collected and used for Pharmacognostical and Phytochemical studies. The fine powder was used for Powder Microscopy.

### Powder Microscopy

Majority of the crude drugs for commercial purpose are available in powder form. So, the powder microscopic studies give the anatomical characters of fragmented crude drugs. Glycerine mounted temporary preparations were made for powdered leaves.

### Phytochemical studies[7,8]

### Preparation of Extracts

The shade dried, coarsely powdered whole plant of *Lepidagathis keralensis* (500gm) was successively extracted with Methanol in a soxhlet apparatus separately. Each extract was concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and used for further detailed phytochemical studies. The crude samples were subjected to screening and estimation of phytochemical parameters *viz*. total alkaloids, tannins, flavonoids and phenol as well as LCMS analysis.[5]

### Phytochemical analysis

In the present study qualitative and quantitative analysis were carried out on the alcoholic extract of *Lepidagathis keralensis* using standard procedures to identify the constituents(Senguttuvan, Paulsamy et al. 2014). A preliminary screening was carried out as per the standard methods described by Treese and Evans (Trease and Evans 1989)

### Quantitative analysis

#### a. Estimation of Total Alkaloid content

**Materials required**

Dimethyl sulphoxide (DMSO), 2N Hydrochloric acid, Bromocresol green, 2N Sodium hydroxide, Phosphate buffer (PH-4.7), Water, Chloroform, Standard atropine.

**Procedure**

**Preparation of solutions**

**Bromocresol green solution:** Dissolve 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water and make up to 100 ml with distilled water.

**Atropine standard solution:** Dissolve 1mg pure atropine in 10 ml distilled water.

**Preparation of standard curve of Atropine**

A set of standard solutions of atropine (20, 40, 60, 80 and 100 µg) were prepared in separate test tubes. 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution were added to each test tube and shake mixture. Extract them with chloroform and make up to 10 ml with chloroform. The absorbance of the complex was measured at 470 nm against blank prepared as above but without atropine.

**Estimation of total alkaloids**

The plant extract (1mg) was dissolved in 1 ml dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3, 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. The absorbance was determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer.

**Estimation of Total Flavonoid content**

**Materials required**

Standard Quercetin, Aluminium chloride solution (10 %), Sodium nitrite solution (5 %), Sodium hydroxide solution (1 M), Methanol, Distilled water, Extracts.

**Procedure**

**Preparation of standard solution**

10 mg of quercetin was dissolved in methanol and make up to 10 ml. From the above solution (1 mg/ml), 1 ml was pipetted out and made up to 10 ml with methanol to get 100 mcg/ml of quercetin stock solution. From the stock solution, solutions of concentration 20, 40, 60, 80 and 100 µg/ml were prepared. To each of these 4 ml water was added followed by 0.3 ml of 5 % sodium nitrite. After 5 minutes, 0.3 ml of 10 % aluminium chloride solution and at the 6th minute, 2 ml of 1 M sodium hydroxide was added and made up to 10 ml with distilled water. The solutions were mixed and absorbance was measured against the blank without addition of aluminium chloride at 510 nm using UV-Visible spectrometer. A standard graph was plotted using various concentration and their corresponding absorbance.

**Estimation of flavanoid content**

Each extract (1 ml) was mixed with 4 ml of distilled water and subsequently with 0.3 ml of sodium nitrite (5 %). After 5 minutes, 0.3 ml aluminium chloride solution (10 %) was added followed by 2 ml of I M sodium hydroxide. The mixture was thoroughly mixed and absorbance was determined at 510 nm versus blank. Results were expressed as quercetin equivalents (mg quercetin/g of dried material). The estimation of flavonoid content was carried out in triplicate.

**c. Estimation of Total Phenolic content**

**Materials required**

Folin-Ciocalteu- phenol reagent, 7 % sodium carbonate solution, Extracts, Standard gallic acid, Deionized water.

**Procedure**

The total phenolic content (TPC) of dry methanolic extract was estimated by Folin – Ciocalteu assay. It is also called Gallic Acid Equivalent method. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin – Ciocalteu phenol reagent. After 5 minutes, 10 ml of 7 % sodium carbonate solution was added to the mixture followed by the addition of 13 ml of deionized water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 230 C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE)/g of dried sample.

**Determination of total chlorophyll content[9]**

Chlorophyll is a green photosynthetic pigment found in plants. It absorbs sunlight and uses its energy to synthesize carbohydrates from carbon dioxide and water. There are two types of chlorophyll in plants, chlorophyll a and b which both of them works as photoreceptor in photosynthesis.

**Materials required:** Acetone, Fresh leaves

**Procedure**

One gram of finely cut fresh leaves of *Lepidagathis keralensis* were taken and ground with 20-40 ml of 80% acetone. It was then centrifuged at 5000-10000 rpm for 5 min. The supernatant was transferred and the procedure was repeated till the residue becomes colourless. The absorbance was read at 645 and 663nm against the blank. The concentration of chlorophyll a and b are calculated by following equation.

Total chlorophyll=20.2 (Absorbance at 645) + 8.02 (absorbance at 663)

Chlorophyll a=12.7 (Absorbance at 663)–2.69 (absorbance at 645)

Chlorophyll b=22.9 (Absorbance at 645)–4.68 (absorbance at 663)

**Thin layer chromatography (TLC)[10,11]**

Thin layer chromatography is a solid- liquid technique which works on the principle that different compounds have different affinities and the compounds will separate based on their affinities towards stationary phase and mobile phase. Plant extracts contains various types of bioactive compounds having different polarities. Their separation can be easily done using Thin Layer Chromatography (TLC). In the present investigation, the studies were aimed at designing qualitative TLC parameters for extracts of *Lepidagathis keralensis* and to find out suitable eluents for it.

**Selection of mobile phase**

Solvent mixture was selected on the basis of the phytoconstituents present in each extracts from the vast analysis, best solvent was selected which showed good separation with maximum number of components.

**Procedure**

TLC plates were prepared by using silica gel G and the coated plates were allowed to dry in air and activated by heating in hot air oven at 1050C for 1 hour. The extracts were dissolved in respective solvents and applied in TLC plates by means of capillary tube, a few centimeters above from the edge of plate. The plates were then developed in TLC chamber previously saturated with different solvent systems. By trial and error method appropriate solvent system were developed and different spots developed were. The colored substances were visual on the chromatogram. Colorless components were detected using visualizing agent, UV light and spray reagents and Rf values were calculated.[12]

**In-vitro pharmacological studies[13,14,15]**

**Cytotoxicity study**

**MTT assay**

**Cell lines and maintenance**

The cell line / cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune,India.

**Cell line used in this study:**

Fibroblast cell line

**Procedure**

The cells (10000 cells/well) were seeded on 96 well plates and allowed to acclimatize to theculture conditions such as 37°C and 5% $CO_2$ environment in the incubator for 24 hours. The test samples were filter sterilized using 0.2 µm Millipore syringe filter. The test samples wereprepared in DMEM media (10 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter.The samples were added to the cells at final concentrations of 12.5, 25, 50, 100, 500 µg/mLrespectively. Untreated wells were kept as control. All the experiments were done in triplicateand average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 hours for the development of formazan crystals.The supernatant was removed and 100 µL DMSO (100%) were added per well.The absorbance at 570 nm was measured with micro plate reader. Three wells per plate without cells served as blank.All the experiments were done in triplicates.The cell viability was expressed using the following formula:

$$Percentage of cell viability = \frac{Average absorbance of treated}{Average absorbance of control} \times 100$$

**Cell Morphology**

Representative photomicrographs of cells belonging to each experimental group were taken using an inverted phase contrast microscope (LABOMED, TCM 400, USA). Magnification- 10x.Total magnification- 100x (10x Objective lens × 10x Ocular lens).

## Result and Discussion

### Pharmacognostical studies

### Macroscopic character of *Lepidagathis keralensis*

Table No. 2: Macroscopic characters of *Lepidagathis keralensis*

| Organoleptic characters | Leaves | Stem | Root |
|---|---|---|---|
| Colour | Dark green | Purple | Brown |
| Odour | Pleasant odour | Pleasant odour | No odour |
| Taste | Tasteless | Tasteless | Tasteless |
| Shape | Lanceolate | Quadrangular | Fibrous |
| Size | Length 0.5 - 1cm<br>Width 3 - 10mm | Length 10 - 15cm Width 5 - 10mm | Length 10 - 20cm Width 0.1 - 5 mm |
| Texture | Soft and spikes | Woody | Woody |

### Microscopical evaluation of Leaf of *Lepidagathis keralensis*

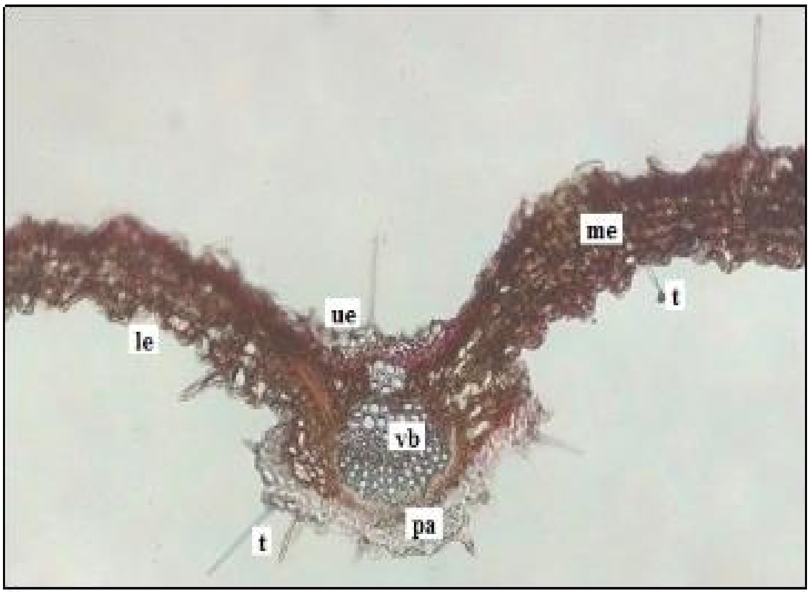



Fig.6: T.S of Leaf of *Lepidagathis keralensis*

Stain: Iodine & Magnification: 10 x

Ground plan; **le**.: lower epidermis; **me**.: mesophyll cells; **pa.:** parenchyma cells; **t**.:trichome; **ue**.: upper epidermis.

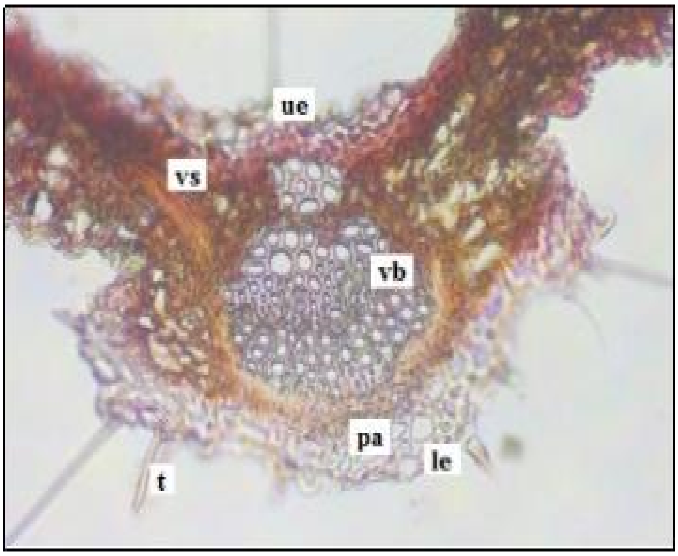



Fig.7: T.S of Leaf of *Lepidagathis keralensis*

Stain: Iodine & Magnification: 45 x

Transverse section of the Lepidagathis (Leaf) through midrib: **le**.: lower epidermis; **pa.**:parenchyma cells; **t.**:trichome; **ue.**: upper epidermis; **vb**.: vascular bundle**; vs**.: vascular strand.

**Microscopical evaluation of Stem of *Lepidagathis keralensis***

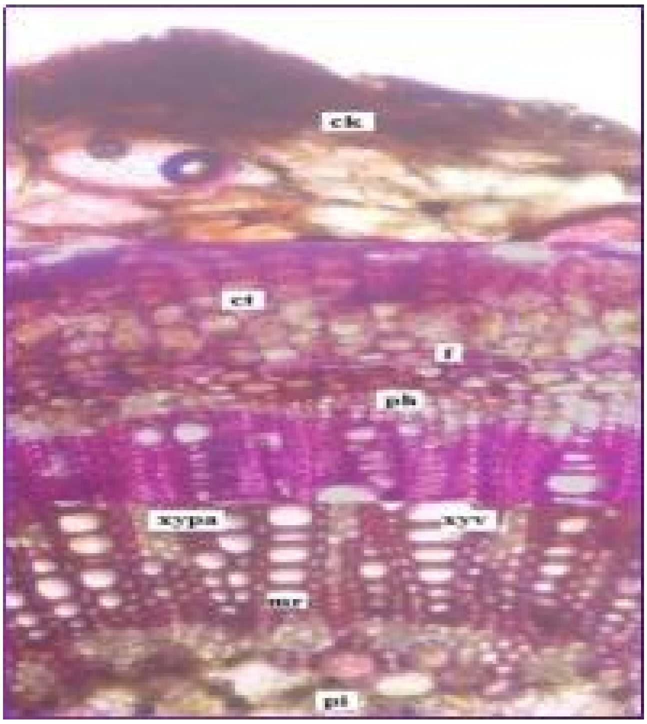


Fig.8. T.S of stem of *Lepidagathis keralensis*

Stain: Phloroglucinol + HCL & Magnification: 45 x

Fig.16: Portion enlarged *Lepidagathis* (Stem): ck.: cork; ct.: cortex; f.: fiber; mr.: medullaryrays; ph.: phloem; pi.: pith; xypa.: xylem parenchyma; xyv.: xylem vessel

**Microscopical evaluation of Root of *Lepidagathis keralensis***

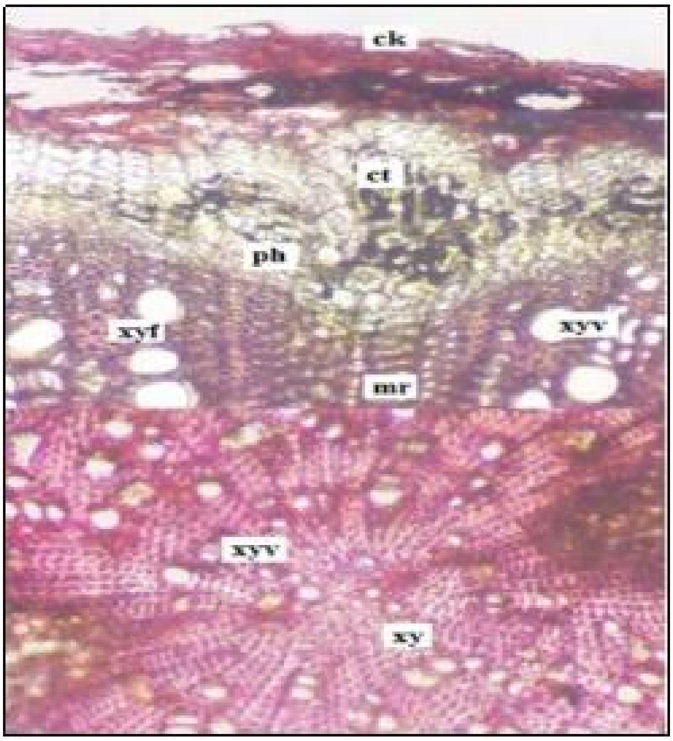



Fig.9.T.S of root of *Lepidagathis keralensis*

Stain: Phloroglucinol + HCL & Magnification: 45 x

Fig.23: Transverse section of *Lepidagathis keralensis* (Root) a portion enlarged: **ck**.: cork; **c**t.: cortex**;mr.**: medullary ray; **ph.**: phloem; **xy** .: xylem; **xyv** .: xylem vessel**; xyf** .: xylem fiber.

**Powder microscopy of *Lepidagathis keralensis***

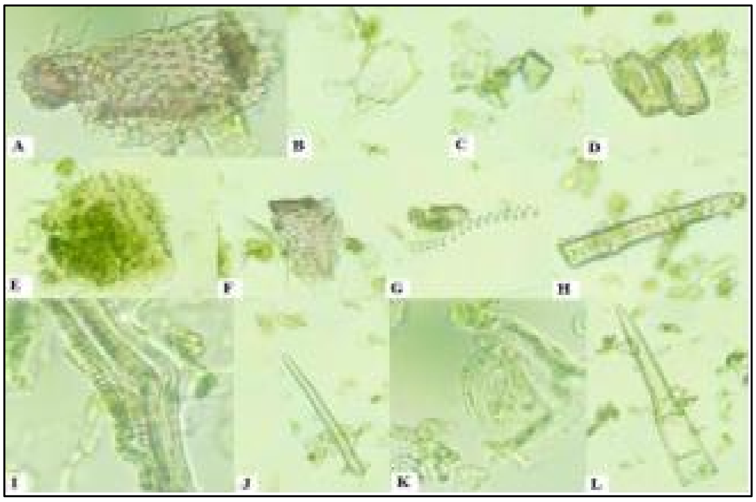



Figure 10*: Powder microscopy of Lepidagathis keralensis*

bordered pitted vessel; B.: parenchyma cells; C.: crystal of calcium oxalate; D.: sclereids; E.: fragment of mesophyll cells; F.: pitted vessel; G.: spiral vessel; H.: sclereids; I.: non-lignified fiber with annular vessel; J.: trichomes; K.: stomata; L.:multicellular hair.

**Phytochemical studies**

**Estimation of total alkaloids**

**Alkaloid present in *Lepidagathis keralensis***

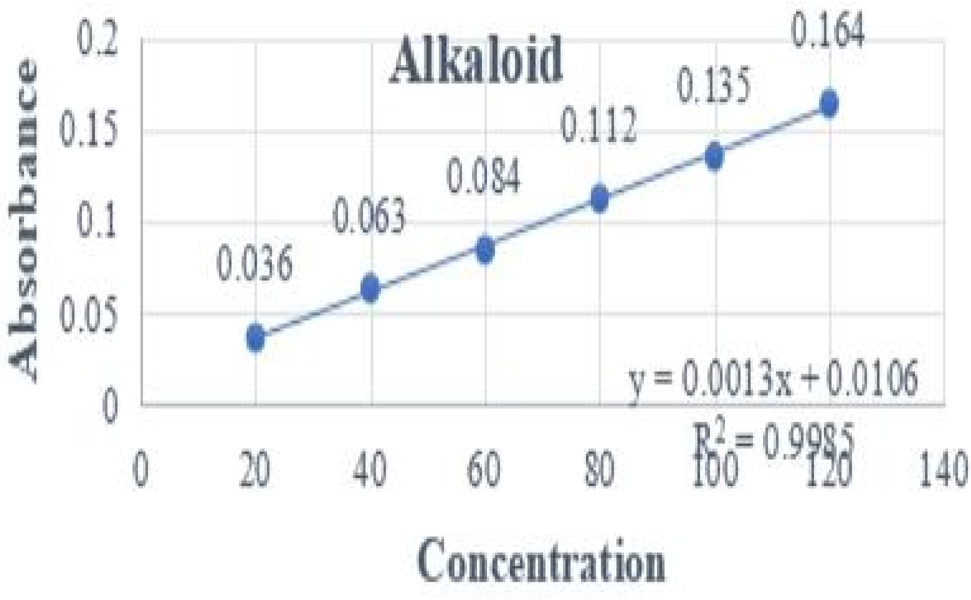
Table no.3: Absorbance of standard atropine and extracts of *Lepidagathis keralensis*

| Standard | Concentration of Atropine (µg/ml) | Absorbance (470 nm) |
|---|---|---|
| S1 | 20 | 0.036 |
| S2 | 40 | 0.063 |
| S3 | 60 | 0.084 |
| S4 | 80 | 0.112 |
| S5 | 100 | 0.135 |
| S6 | 120 | 0.167 |

| Sl. No: | Absorbance (470 nm) | Concentration of alka loid (µg/ml) |
|---|---|---|
| 1 | 0.146 | 104.15 |

Values were expressed as Mean ± SEM, n=3

**Graph no.1: Calibration curve of standard atropine**



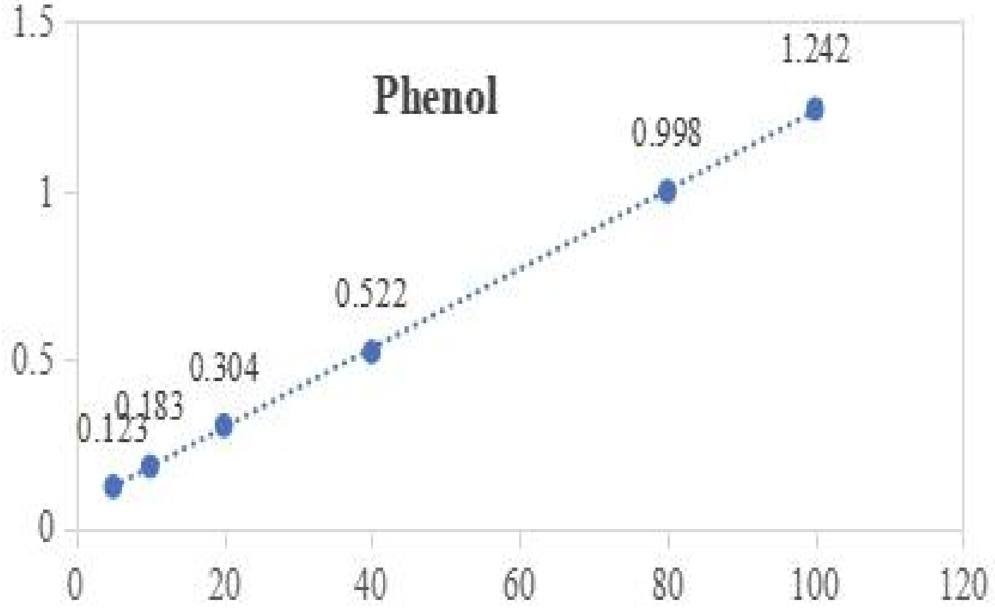
**Estimation of total phenols in *Lepidagathis keralensis* extract using Folin-Ciocalteu method**

Table no.4: Absorbance of standard gallic acid and extracts of *Lepidagathis keralensis*

| Standards | Concentration of gallic acid (µg/ml) | Absorbance(750 nm) |
|---|---|---|
| S1 | 5 | 0.123 |
| S2 | 10 | 0.183 |
| S3 | 20 | 0.304 |
| S4 | 40 | 0.522 |
| S5 | 80 | 0.998 |
| S6 | 100 | 1.242 |
| Sl. No. | Absorbance (750 nm) | Concentration of phen ol (µg/ml) |
| 1 | 1.215 | 98.40 |

Values were expressed as Mean ± SEM, n=3

**Graph no.2 Caliberation curve of standard gallic acid**



### Estimation of total Flavonoids in *Lepidagathis keralensis*- Aluminium chloride colorimetric method

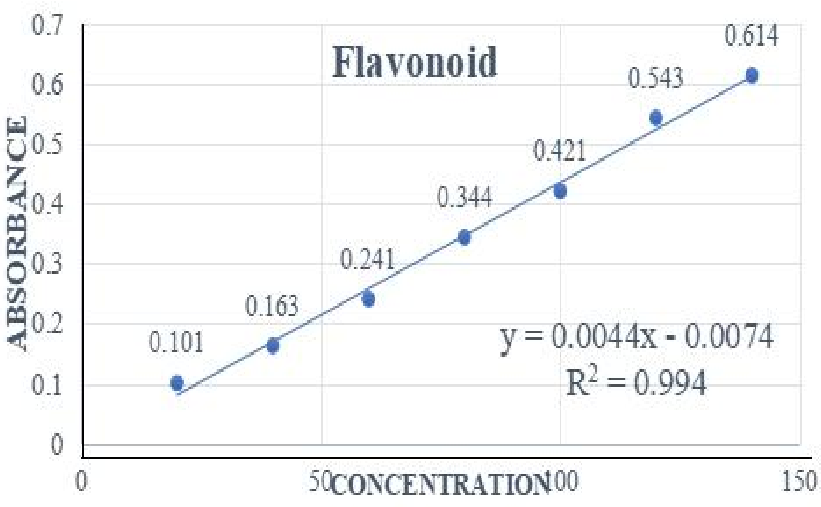
**Table no.5: Absorbance of standard quercetin and extracts of *Lepidagathis keralensis***

| Standards | Concentration of Quercetin (µg/ml) | Absorbance (510 nm) |
|---|---|---|
| S1 | 20 | 0.101 |
| S2 | 40 | 0.163 |
| S3 | 60 | 0.241 |
| S4 | 80 | 0.344 |
| S5 | 100 | 0.421 |
| S6 | 120 | 0.543 |

| Sl. No: | Absorbance( 510nm) | Concentration of Flavonoid (µg/ml) |
|---|---|---|
| 1 | 0.406 | 93.95 |

Values were expressed as Mean ± SEM, n=3

**Graph no. 3 Caliberation curve of standard Quercetin**



### Determination of total chlorophyll content

Table 6: Absorbance of chlorophyll a and b in *Lepidagathis keralensis*

| Solvent | Wave length (nm) | Mean absorbance |
|---|---|---|
| Acetone | 645 | 0.8437 |
| | 663 | 1.7690 |

Table 7: Amount of chlorophyll a and b in the leaves of *Lepidagathis keralensis*

| Solvent | Total chlorophyll | Chlorophyll a | Chlorophyll b |
|---|---|---|---|
| Acetone | 31.2005 | 20.173 | 11.0458 |

### Thin layer chromatography

| Sl. No. | Mobile Phase – (Solvent System Used) | Proportionof Solvent system | Spraying Reagent | Observation (Visible light) | Observation (UV light) |
|---|---|---|---|---|---|
| 1 | Toluene:Acetone: Formic acid | 4.5:4.5:1 | 1% Ferric chloride solution | Blue band | Orange fluorescence in UV |

| Solvent front (cm) | Solute front (cm) | Rf value |
|---|---|---|
| 7 | 2.8 | 0.40 |

Table no.8 TLC of *Lepidagathis keralensis*

Figure 11: TLC Plate

**Pharmacological studies**

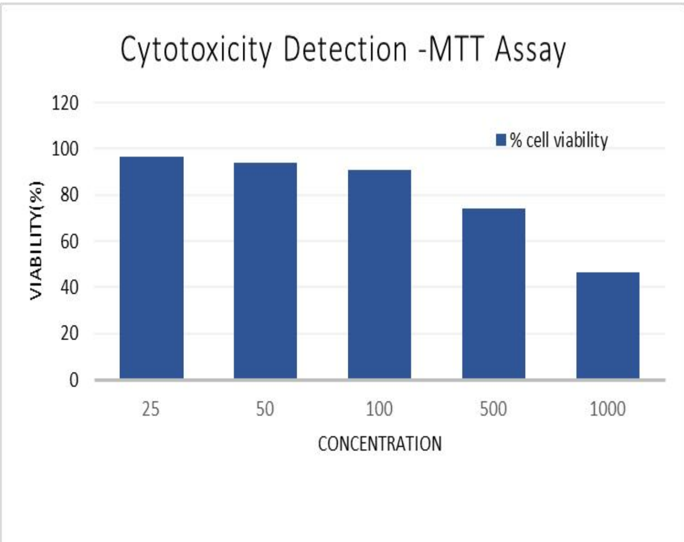
Cytotoxicity screening of *Lepidagathis keralensis*

Table 9: MTT assay results for varying concentration of test sample

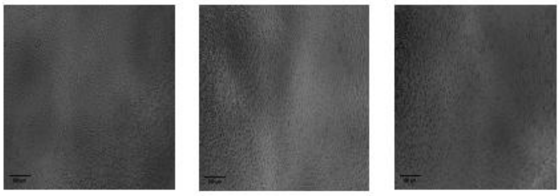
| Concentration (µg/mL) | Triplicate values | | | Average OD |
|---|---|---|---|---|
| | OD1 | OD2 | OD3 | |
| Control | 0.972 | 0.968 | 0.978 | 0.973 |
| 25 | 0.932 | 0.942 | 0.936 | 0.937 |
| 50 | 0.917 | 0.915 | 0.912 | 0.915 |
| 100 | 0.883 | 0.881 | 0.885 | 0.883 |
| 500 | 0.721 | 0.720 | 0.719 | 0.720 |
| 1000 | 0.445 | 0.459 | 0.453 | 0.452 |

Table 10: Percentage of viability for varying concentration of test sample

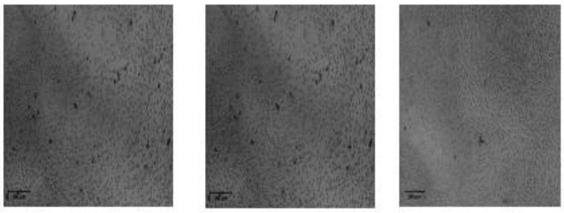
| Concentration (µg/mL) | Percentage of viability |
|---|---|
| 25 | 96.30 |
| 50 | 94.03 |
| 100 | 90.75 |
| 500 | 73.92 |
| 1000 | 46.45 |
| IC 50* | 944.46 µg/mL |




Graph no.4, Cytotoxicity of *Lepidagathis keralensis*



CONTROL 25µg/ml 50 µg/ml



100 µg/ml 500 µg/ml 1000 µg/ml

Fig.12.Normal cell line after treatment

**Conclusion**

In the present investigation, Selected *Lepidagathis keralensis* plant belonging to terrestrial source**.** This review gives information about the Pharmacognostical and Phytochemical and Pharmacological Characteristics of *Lepidagathis keralensis*. The *plant Lepidagathis keralensis* used in folk treatments the plant parts are widely used for treating kidney stone, Asthma and as a blood purifier..Pharmacognostical investigation includes the Macroscopy, Microscopy and Powder Microscopy. Here in Macroscopy determine the color, odour, taste, shape, size and texture. Microscopy identify the parts glandular trichome, xylem, phloem, sclerenchyma cells, vascular bundle, upper epidermis, lower epidermis, parenchyma cells, and collenchyma cells. Quantitative screening of *Lepidagathis keralensis* shows the presence of alkaloid,, flavonoid and Phenol.Rf value of *Lepidagathis keralensis* crude extract was determined by thin layer chromatography and it was 0.40. The *Lepidagathis keralensis* methanol extrac*t* was found to cause significant reduction in cell viability of Normal cells at highest concentration. Since no significant reduction was observed in lower concentrations, test samples can be considered as non-cytotoxic to normal cells at lower concentrations. The Pharmacognostical, Phytochemical and Pharmacological evaluation of *Lepidagathis keralensis* reveals its potential as a valuable botanical resource for traditional medicine.

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