# T. K. M. College of Arts & Science

(Affiliated to University of Kerala)

**Kollam-5**

## Department of Biochemistry


---

# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF *STROBILANTHES HEYNEANUS*

*Dissertation submitted to the University of Kerala in partial fulfilment of the requirement for the award of the Degree of Bachelor of Science in Biochemistry.*

*By*


| | | |
|---|---|---|
| **1.** | **Ganga. B** | **Reg. No. 23819142016** |
| **2.** | **J Saivinayak** | **Reg. No. 23819142017** |
| **3.** | **Muhsina A** | **Reg. No. 23819142018** |
| **4.** | **Nishana Irshad** | **Reg. No. 23819142019** |
| **5.** | **Parvathi Santhosh** | **Reg. No. 23819142020** |
| **6.** | **Sree Lekshmi. P** | **Reg. No. 23819142021** |
| **7.** | **Abeera. B. N** | **Reg. No. 23819142022** |


**May – 2022**

# PHYTOCHEMICALSCREENING AND ANTIOXIDANT ACTIVITIES OF *STROBILANTHES HEYNEANUS*

# DECLARATION

We hereby declare that the project titled '**Phytochemical Screening and antioxidant activities of *Strobilanthes heyneanus*** is based on the original work carried out by us under the supervision ofDr Ansil. P.N. Assistant Professor, Department of Biochemistry, T.K.M. College of Arts and Science, Kollam.

We also declare that the project report hasn't been submitted either partly or completely for the award of any other degree or diploma or other similar titles of any other University/Institution in India or abroad.

1. J Saivinayak
2. Abeera.B.N
3. Parvathi Santhosh
4. Ganga.B
5. Muhsina A
6. Sree Lekshmi P
7. Nishana Irshad

T. K. M. College of Arts & Science

(Affiliated to University of Kerala)

Kollam-5

Department of Biochemistry

# CERTIFICATE

This is to certify that the dissertation entitled '**Phytochemical Screening and antioxidant activities of *Strobilanthes heyneanus* s**ubmitted to the University of Keralain partial fulfilment of the requirements for the award of the degree of Bachelor of Science in Biochemistry, is a record of original research work carried out by the following candidates under my guidance and supervision.

1. Ganga.B Reg. No. 23819142016
2. J Saivinayak Reg. No. 23819142017
3. Muhsina A Reg. No. 23819142018
4. Nishana Irshad Reg. No. 23819142019
5. Parvathi Santhosh Reg. No. 23819142020
6. Sree Lekshmi.P Reg. No. 23819142021
7. Abeera.B.N Reg. No.23819142022

It is also certified that no part thereof has been presented for the award of any other degree or diploma or other similar titles of any other university.

**Dr. ANSIL. P.N**

Project Guide

# ACKNOWLEDGEMENT

We would like to perform our heartfelt gratitude to our guideDr Ansil. P.N., Assistant Professor, Department of Biochemistry, T.K.M. College of Arts and Science, Kollam for his guidance and encouragement that lead us to the successful completion of the work.

A special thanks to Dr. Latha. B., HOD of Biochemistry and other faculty members Dr. Sumayya, Dr. Hari Sankar, Ms. Soumya S and Ms. Ajinza A., for all their valuable help. We extend our sincere thanks to all the staff members of the college laboratory for their support and assistance.

Our heartfelt thanks to our dear friends for the moral support and help provided for the successful completion of this work. We wish to express our deepest gratitude to our parents for their support and encouragement throughout our work.

Above all, we are indebted to THE ALMIGHTY for moulding us in the present state and for his blessing throughout our life.

# CONTENTS

# 1. INTRODUCTION

Plants have played a unique holistic role inthe provision of food, drugs, clothing, shelter, etc. Natural compounds have been extensively exploredfor new drug discoveries. Indeed, plants have been used as medicines for more than 5000 years, as a source of antibiotics, antineoplastics, analgesics, cardioprotective, etc., (Chandraet al., 2017; Chen et al., 2013).

Medicinal plants are an important source of life-saving drugs for humans, especially in developing countries. More than 80% of the world's population in developing countries depend primarily on herbal medicine for basic health care. The issue is associated with the use of synthetic drugs, antibiotics, and the renewed interest in the use of plant-based drugs. Researchers on medicinal plants and their traditional medicinal plant use have increased in different regions of the world over the past few decades. It is important to document indigenous traditional knowledge through ethnobotanical studies to conserve and utilize biological resources. Plants can be used in their original or advanced form. Numerous biologically active substances are known to contain medicinal plants that have been isolated from plants and applied based on ethnobotanical expertise and approved drugs from medicinal plants (Carney et al., 1999).

Plant synthesizes a vast range of organic compounds that are tragically classified as primary and secondary metabolites. Primary metabolites are compounds like lipids, amino acids, phytosterols, and organic acids. They have an essential role associated with photosynthesis, respiration, growth, and development. Secondary metabolites are mainly flavonoids, phenolic compounds, terpenoids, nitrogen-containing alkaloids, and Sulphur containing compounds. They are of interest because of their use as dyes, fibres, ailments, flavouring agents, drugs, and perfumes and they are viewed as potential sources and new natural drugs, antibiotics, insecticides, and herbicides (Croteau et al., 2000; DeWitt 2002). The exploration of medicinal properties of plants throughout the ages was accomplished principally through careful observation, trial, and error, an accidental discovery, which is beneficial from nutritive and medicinal standpoints. Biologically active molecules isolated from the plants revolve around the research and developmentsector of the pharmaceutical industries as a source of new molecules leading to the development of novel drugs (Pye et al., 2017; Boucher et al., 2017).

The medicinal properties of these plants are mainly attributed to the phytochemicals in them, which are defined as bioactive non-nutrient compounds in fruits, vegetables, grains, and other plants. These are an amazing array of organic chemicals with an enormous diversity of

structural types. Many of these phytochemicals are essential for plant growth and development and are widely used by humans due to their health benefits. Phytochemicals are small molecules with diverse chemical profiles and are more "drug-like" than synthetic compounds, hence, they are considered good candidates for the development of drug leads. Phytochemicals, by acting individually or synergistically, help to reduce the risk for a variety of chronic and inflammatory conditions. These include atherosclerosis and stroke, myocardial infarction, certain types of cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, Alzheimer's disease, osteoporosis, psoriasis, septic shock, AIDS, menopausal symptoms, and neurodegeneration (Zhang et al., 2015).

India is the largest producer of medicinal plants and is rightly called the "Botanical Garden of the World". Medical information referred to in the old Indian literature includes several medicinal herbs, which have been in the use for thousands of years, in one form or the other, under the indigenous system of medicine. In India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. However, traditional communities use only about 7000-7500 plants for medicinal purposes. The Siddha system of medicine uses about 600, Ayurveda 700, Unani 700 and modern medicine about 30 medicinal plants for treating a variety of diseases in man and animal. Traditional medicines all over the world are nowadays being re-evaluated by extensive research on different plant species concerning their therapeutic principles (Madhuri and Pandey, 2008).

**Fig 1: Strobilanthes heyneanus**

**(Whole plant)**

*Strobilanthes heyneanus* Nees is a small aromatic shrub of the family Acanthaceae, commonly found on the western coast of India. The shrub has been used for years in folk medicine and various Ayurvedic medicinal preparations. In Kerala, the roots of *S. heyneanus* have been used as the plant source of the drug 'Sahachara'. 'Sahachara' is an important drug in Ayurveda, widely used against rheumatism and neurological disorders such as paraplegia, sciatica *etc*. This drug also is effective against a variety of human ailments such as ulcers, glandular swellings, poisonous affections, itching, leprosy and other skin diseases, cough, oedema, toothache and gum diseases and has the property of strengthening the nerves. The aqueous and ethanolic extracts of the stem possess marked aspirin type of analgesic, anti-inflammatory and immunosuppressant activities (Renjana et al.,2013).

The roots of *Strobilanthes heyneanus* are extremely used in the Ayurvedic drug preparation, one of the most efficient plant species available in India, and its pharmacological activity is not much revealed so far. S. heyneanus roots and leaves are used to treat leprosy, to control blood sugar, urinal problems, jaundice, inflammation, and excess menses. The members of the genus *Strobilanthes* are used as an anti-diabetic, diuretic, laxative, and potent antimicrobial agents (Vijayakumar et al.,2021).

**Fig 2: Flower & Root of Strobilanthes heyneanus**

# 2. OBJECTIVES

1. Collect the plant *Strobilanthes heyneanus*.
2. Prepare the water extract of the shade dried leaves and roots of the plant.
3. To carry out the phytochemical screening of the water extract of *Strobilanthes heyneanus*.
4. Determine the total antioxidant activity and phenolic content of the water extract of the leaf and root of *Strobilanthes heyneanus.*

# 3. REVIEW OF LITERATURE

The family Acanthaceae contains several species with a potential diversity of ethnobotanical uses. One of the important species in this family is *Strobilanthes heyneanus* (Nilgirianthusheyneanus) mostly found in the southwest regions of India, commonly called Karun kurinji. Ayurveda drugs prepared from this species are useful (Vijayakumar et al.,2021). The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plants. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in them known as phytochemicals.

Phytochemicals are biologically active, naturally occurring chemical compounds found in fruits, vegetables, grains, nuts, tea and seeds that promote human health and prevent diseases. The therapeutic effects of these medicinal plants can justifiably be attributed to, among others, the phytochemicals in them especially the flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins. The abundance of scientific evidence indicates that such bioactive compounds have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. This paper avails a review of medicinally important plant-derived compounds that can be used in the design of more efficacious therapeutic agents against many communicable and non-communicable diseases. (Miami, et al.,2016)

Phytochemicals or chemicals in plants play important roles in their growth and development. They protect plants from harmful agents such as insects and microbes as well as stressful events such as ultraviolet (UV) irradiation and extreme temperatures. They also attract beneficial birds and insects that promote pollination, germination, and seed dispersal. Phytochemicals provide colours to plants and an array flavour both pleasant and unpleasant when consumed. They are unique to specific plants and parts of plants, and they usually increase in abundance during stressful events. Phytochemicals also provide health benefits when consumed. They consist of nutrients essential for optimal health (e.g., proteins, carbohydrates, vitamins, and minerals) and other chemicals (e.g., phenolic acids, flavonoids, and other phenolics) with lesser-known roles in health promotion or disease prevention. A number of these phytochemicals are recognized as bioactive components in traditional herbal medicines (e.g., salicylates (aspirin) found in willow bark used to reduce inflammation, quinine

in cinchona bark used to treat malaria, and proanthocyanidins in cranberries used to treat urinary tract infections) (Kristina, et al., 2017). Polyphenols represent the largest category of phytochemicals and serve as powerful antioxidants due to their multiple hydroxyl groups (Pietta, et al.,2000).

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements plants synthesize low molecular weight antioxidants such as glutathione and ascorbate within the chloroplast stroma and cytosol using NADPH as the ultimate electron donor. These low molecular weight antioxidants function as redox buffers that interact with numerous cellular components and influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death In addition, these antioxidants may influence gene expression associated with biotic and abiotic stress responses to maximize defines. GSH acts as an antioxidantby quenching reactive oxygen species and is involved in the ascorbate-glutathione cycle, which eliminates damaging peroxides Plants also produce tocopherols (vitamin E) that act as important liposoluble redox buffer systems. Vitamin E, which is generally synthesized in chloroplasts and protoplastids, is located in the membranes of cells. This compound is a major singlet oxygen scavenger that protects against lipid peroxidation. (Deepak, e al.,2015).

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defences of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage some secondary antioxidant metabolites occur constitutively, while others are formed in response to biotic and abiotic stress conditions. In plants, phenolics can act as antioxidants by donating electrons to guaiacol-type peroxidases for the detoxification of H2O2 produced under stress conditions. Phenolics also protect against UV radiation through their potent radical scavenging ability. In addition, they function as enzyme inhibitors and feeding deterrents for herbivores while providing resistance against pathogens. Synthesis of flavonoids is known to be induced by UV stress, heavy metals toxicity, or low temperature and low nutrient conditions, whch might attributed to their UV-absorbing, radical scavenging and metal cheating ability. (Deepak, et al.,2015).

## 3.1 Scientific Classification

| | |
|---|---|
| **Kingdom** | **Plantae** |
| **Phylum** | Tracheophyta |
| **Division** | Tracheophyta |
| **Subdivision** | Spermatophyta |
| **Order** | Lamiales |
| **Family** | Acanthaceae |
| **Genus** | *Strobilanthes* |
| **Species** | *Strobilanthes heyneanus* |

## 3.2 Geographic Distribution

*Strobilanthes heyneanus*is found in India and Srilanka,In India, they are found abundantly in the western ghats, margins of shola forests and grasslands. they are also found in the state of Andhra Pradesh, Kerala and Odisha.In Andhra Pradesh, these are found in the EastGodavari district, and In Odisha, found in the district of Mayurbhanj.While in Kerala they are found in the districts of Kasaragod, Kannur, Wayanad, Kozhikode, Malappuram, Palakkad, Thrissur, Idukki, Kottayam, Pathanamthitta, Kollam and Thiruvananthapuram.

## 3.3 Botanical Description

Karinkurinji, is an undershrub, nearly a meter high, with a grooved stem, often covered with hairs. The leaves are oppositely arranged, unequal, ovate, and hairy and have a serrated margin. The flowers are blue urn shape of 1-1.2 cm long, which occurs in axillary spikes, and five sepals are combined at the base. The flower tube is swollen in the middle which is a white colour and has five rounded petals. In the flower, the stamens are 4, filaments are hairy at the base, and the capsule is 6–8mm long, oblong, and 4-seeded. (Vijayakumar et al.,2021). The swollen part is whitish.Like kurinji the flowering cycle is long, normally flowering from September to December.

## 3.4 Traditional Uses

*Strobilanthes heyneanus* (Nilgirianthusheyneanus), is mostly found in the southwest regions of India (Nair, et al.,1985). They are commonly used in rheumatic complaints, sprain of the angle, and hernia. Ayurvedic drugs prepared from this species are very useful. (Bhat, et al.,2014) *S. heyneanus* has been used for years in folk medicine and various Ayurvedic medicinal preparations (Pullaiah, et al.,2006).

In Kerala, the roots of *S. heyneanus* have been used as the plant source of the drug 'Sahachara'. 'Sahachara' is an important drug in Ayurveda, widely used against rheumatism and neurological disorders such as paraplegia, sciatica etc. Apart from that, this drug is effective against a variety of human ailments such as ulcers, glandular swellings, poisonous affections, itching, leprosy and other skin diseases, cough, oedema, toothache and gum diseases and has the property of strengthening the nerves (Sivarajan, et al.,1994).

It was found that the aqueous and ethanolic extracts of the stem possess marked aspirin type of analgesic, anti-inflammatory and immunosuppressant activities (Nair, et al.,1985). The petroleum ether extract showed anti-inflammatory and weak convalescent activities(Pullaiah, et al.,2006).Hypoglycemic and hypolipidemic effects of *S. heyneanus*have recently been reported in alloxan-induced diabetic rats (Kumar,et al.,2008).In addition, the members of the genus *Strobilanthes* are used as anti-diabetic, diuretic, laxative, and potent antimicrobial agents (Kumar,et al.,2008).The roots of *Strobilanthes heyneanus* are extremely used in the Ayurvedic drug preparation, one of the most efficient plant species available in India, and its pharmacological activity is not much revealed so far. The results of antimicrobial activity revealed that the species has potential antibiotics against infections caused by the pathogens (Sundaram, et al.,2021).

According to Agarwal and ranger, *S. heyneanus* is an aromatic herb found in South India which has been extensively studied for its anti-inflammatory property. The oil prepared from the plant is reported to be effective in various inflammatory conditions. The major constituents of the petroleum ether extract of these plants were isolated and identified as lupeol, its stereoisomer, and rare triterpenoid alcohol, 19 α-H lupeol. Preclinical studies have shown that lupeol possesses anti-inflammatory and antiarthritic properties and that this was due to its ability to prevent the production of some pro-inflammatory mediators, inhibit lipid peroxidation, and increase antioxidant effects (Arora, et al., 2013).

## 3.5 Pharmacological Properties

Several natural products are derived from plants and have been used traditionally to treat many diseases such as wound healing, injuries, and pain. Current synthetic medicine and combinatorial chemistry linked with the new technological tools such as proteomics, genomics, and metabolomics covered the broader use of natural products. Currently, most natural products are produced and industrialised as pharmacologically active agents with potential antioxidant, anti-angiogenic, anti-inflammatory, anti-infective, and anti-carcinogenic properties. Natural products have been engaged as the lead compounds to obtain extremely active pharmacological semi-synthetic derivatives for healing use with increased efficiency. The knowledge and practice from ancient times will significantly move to produce potential drugs for therapeutic strategy. There is considerable evidence that plant extracts, essential oils, and purified compounds have been used as a preventative agent or to treat various diseases. Hence, clinical trials of such natural products are essential for the safety and efficacy to offer therapeutic benefits before coming into the market. It should be helpful to use either alone or in combination with conventional therapies to reduce the overall burden of diseases worldwide. Sundaram, et al., (2021) reported that the root extract of *S. heyneanus* has good antioxidant and antibacterial properties.

**Table 1: Pharmacological properties of *Strobilanthes heyneanus***

| **Pharmacological properties** | **Findings** | **Parts and type of extraction** | **Author/year** |
|---|---|---|---|
| **Antibacterial properties** | The Gram-positive organisms such as Bacillus subtilis and Staphylococcus aureus as well as the Gram-negative organisms such as Salmonella typhi and Klebsiella pneumonia | Root | Sundaram et al. (2021) |

| | | | |
|---|---|---|---|
| | were chosen for the antibacterial activity study. | | |
| **Antifungal** | Antifungal potential of leaf extract of *S. heyneana* wasevaluated against two seed-borne fungi viz. Aspergillus niger, and Bipolarissp. by poisoned food technique | Leaf | Raghavendra et al. (2017) |
| **Antioxidant** | The phytochemical analysis of the root extract showed the presence of alkaloids, steroids, terpenoids, phenols, flavonoids, tannins, saponins, glycosides, and carbohydrates, which are responsible for theantibacterial root extract of *S. heyneanus* | Root | Sundaram et al. (2021) |

## 3.5.1 Antimicrobial activity

### 3.5.1.1Anti-bacterial activity

Evaluation of the antibacterial activity of methanol root extract of *S. heyneanus* was determined by the disc diffusion method against the Gram-positive bacteria such as Bacillus subtilis and Staphylococcus aureus as well as the Gram-negative bacteria such as Salmonella typhi and Klebsiella pneumonia. The effectiveness of the root extract on bacterial strains was determined by calculating the zone of inhibition. The root extract showed the highest zone of inhibition of 24 mm against Salmonella typhi at a 500 µg/mL concentration (Sundaram et al.,2021).

#### 3.5.1.2 Antifungal activity of leaf extract

The antifungal potential of leaf extract of *S. heyneana* was evaluated against two seed-borne fungi viz. *Aspergillus niger*, and *Bipolarissp*. by poisoned food technique. Control of phytopathogenic fungi commonly employs the use of synthetic fungicides which are reported to have certain drawbacks including the emergence of fungicide resistant strains, and adverse effects on humans. Higher plants seem to be potential alternatives for chemical agents as many studies revealed the inhibitory activity of crude solvent extracts and purified compounds from plants against a range of phytopathogenic fungi (Raghavendra, et al., 2017). The poisoned food technique, one of the widely usedantifungal assays, was used to evaluate the antifungal activity of *S. heyneana* (1mg extract/ml of potato dextrose agar medium) against two seed-borne fungi. Poisoning of the medium with the leaf extract caused a drastic reduction in the mycelial growth of test fungi when compared to the growth of fungi in control plates. The leaf extract was effective in causing a>50% reduction of the mycelial growth of test fungi. The extent of inhibition of A. niger and Bipolaris sp. was found to be 60.43% and 61.31%, respectively.

## 3.5.2 Anti-oxidant activity

The species are commonly used in rheumatic complaints, sprain of the ankle, and hernia. The objectives of the study were to evaluate the antioxidant activity, phytochemical analysis, and antibacterial activities of the root extract of *S. heyneanus*.The current study showed that the root extract of *S. heyneanus* has better antioxidant properties and potential anti-bacterial compounds. The phytochemical analysis of the root extract showed the presence of alkaloids, steroids, terpenoids, phenols, flavonoids, tannins, saponins, glycosides, and carbohydrates, which are responsible for the antibacterial root extract *of S. heyneanus* synergistically. (Vijayakumar Sundaram, et al. 2021)

# 4. MATERIALS AND METHODS

## 4.1 Collection of Plant

The *Strobilanthes heyneanus* plant (leaf and root) were collected from Kollam, Kerala.

## 4.2 Extraction

The collected plant was dried in shade and after a week dried material was made as fine. Powder by mechanical grinding. About 50gm of rice powder was mixed in 100ml of distilled water and subjected to 48 hours of occasional stirring at room temperature. After two days the extract was filtered with a cotton cloth and preserved in a refrigerator for future usage.

## 4.3 Reagents

1. **Mayer's reagent**: is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) in water (100.0 ml).
2. **Wagner's Reagent:** 2.5 gm iodine is dissolved in 12.5 gm of potassium iodide ($KI_2$); add 250 ml of water to produce a solution.
3. **Dragendroff reagent:** DR is a solution of potassium bismuth iodide composed of basic bismuth nitrate (Bi $(NO_3)_3$), tartaric acid, and potassium iodide (KI), and when contact with alkaloids DR produces an orange or orange-red precipitate
4. **Lead Acetate Solution:** It is prepared by dissolving 40g Pb (CH3COO)2·3H2O in water, adding 0.5 mL CH3COOH, and diluting to 100 mL.
5. **NaOH solution:** To make 1 M NaOH solution, you have to dissolve 40.00 g of sodium hydroxide pellets in 250 mL distilled water and then make up the solution to 1 litre.
6. Preparation of **Ferric Chloride Solution:**

   By dissolving iron ore in HCl (hydrochloric acid)

   $$Fe_3O_4 + 8HCl \rightarrow FeCl2 + 2FeCl3 + 4H2O$$

   By oxidizing iron (II) chloride with chlorine (Cl) $2FeCl2 + Cl2 \rightarrow 2FeCl3$ by oxidizing iron (II) chloride with oxygen.
7. **Potassium dichromate reagent:** It is produced industrially by reacting potassium chloride (KCl) with sodium dichromate (Na2Cr2O7).
8. **Sodium nitroprusside reagent:** Diluted preparation is stable for 24 hours at room temperature (must be protected from light).

## 4.4 Phytochemical Screening

### 4.4.1 Test for Alkaloids

a) **Mayer's test:** To 1ml of extract added 2ml of Mayer's reagent (Potassium Mercuric Iodide). The formation of a cream-coloured precipitate indicated the presence of alkaloids.
b) **Wagner's test**: To 1ml of extract added 2ml of Wagner's reagent (Iodine in potassium iodide). The formation of brown/ reddish precipitate indicates the presence of alkaloids.
c) **Dragendroff's test:** To 1ml of extract added 1 ml of Dragengroff's reagent (solution of potassium bismuth iodide) was added. The formation of an orange-red precipitate indicates the presence of alkaloids.

### 4.4.2 Test for Flavonoids

a) **Lead acetate test:** Extract was treated with a few drops of sodium hydroxide solution. The formation of intense yellow colour, which becomes colourless with the addition of dilute acid, indicated the presence of flavonoids.
b) **Alkaline reagent test**: The extract was treated with sodium hydroxide, the formation of yellow colour indicated the presence of flavonoids.
c) Few drops of 1% ammonia were added to 1 ml of sample, the formation of yellow colour indicated the presence of flavonoids.

### 4.4.3 Test for Phenolic Compounds and Tannins

a) 1 ml of the test solution was mixed with basic lead acetate solution and the formation of white precipitate indicated the presence of tannins and phenolic compounds.
b) To 1ml of the extract, ferric chloride solution was added. The formation of a dark blue or greenish-black colour product confirmed the presence of phenolic compounds and tannins.
c) Strong potassium dichromate solution was added to the test extract, yellow colour precipitate confirmed the presence of tannin and phenolic compounds.

### 4.4.4 Test for Glycosides

**a) Legal's test:** 1 ml of the extract was treated with sodium nitroprusside in pyridine and sodium hydroxide. The formation of pink to the blood-red colour indicated the presence of cardiac glycosides.

**b)** 5ml of aqueous extract was mixed with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution carefully added to 1 ml of concentrated Sulfuric acid (The concentrated sulphuric acid is seen underneath the mixture). The formation of the brown ring indicates the presence of glycosides.

### 4.4.5 Test for Saponins

**b) Froth test:**1ml of the extract was diluted to 20 ml with water and was shaken in a graduated cylinder for 15 min. Ring indicated the presence of cardiac glycosides.

**c) Foam test:** 0.5g of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

### 4.4.6 Test for Terpenoids

**a)** 5ml of the extract was added with 2ml of chloroform and 3ml of concentrated Sulfuric acid was added along the sides of all test tubes. The appearance of a brownish-redcolour indicates the presence of terpenoids.

### 4.5 Determination of Total Phenolic Content

The total phenolic content in plant extract was determined by using the colorimeteric method. Folin Cicalteau reagent and $Na_2CO_3$ are required for this method. Gallic acid was taken as standard. In an acid medium, the phenolic compounds react with sodium tungstate and sodium molybdate in the Folin's reagent to form blue colour, which was read at 650 nm.

The different volume of standard solution was taken in test tubes. All the tubes were made up of 1 ml of distilled water. Blank was prepared by taking 1ml of distilled water. 0.4 ml of Folin'sCiocalteau reagent was added to the test tubes and mixed well. All the tubes were kept at room temperature for 5 minutes. Then 4 ml of sodium carbonate was added. 100 mg sample is grinded with 10 ml distilled water it is then centrifuged at 2000rpm for 10 minutes. 1ml supernatant is made up to 5 ml with distilled water. 0.5 ml of this solution was taken as a test.

A standard graph was plotted by taking the concentration of the standard (gallic acid) on the X-axis and optical density along the Y-axis. From the graph, the total phenolic content was determined.

### 4.6 Estimation of Total Antioxidant Activity

The antioxidant activity of the extract was calculated by the phosphomolybdenum method. The assay is based on the reduction of Mo (IV) to Mo(V) by the extract and subsequent formation of a green colour phosphate Mo(V) complex at acidic $P^H$. The phosphomolybdenum reagent was prepared by mixing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate. Blank was prepared by 0.1 g of plant sample ground in 10ml distilled water. Centrifuge at 2000rpm for 10 minutes. Then 1 ml stock diluted to 5ml distilled water. From this, 0.5 ml was taken as a test. The sample was incubated at 95ºC for 90 minutes. The absorbance of the solution was measured at 690 nm against blank after cooling.

A standard graph was plotted by taking the concentration of ascorbic acid on the X-axis, and optical density on the Y-axis. From the graph total antioxidant activity was determined.

# 5. RESULTS AND DISCUSSION

The powdered leaves and roots of *Strobilanthes heyneanus* were extracted with water. The resultant cold extracted aqueous solution was subjected to screening of various phytochemical constituents. Table 1&2 shown below indicates the presence and absence of various phytoconstituents in the Aqueous extracts of *Strobilanthes heyneanus* leaf and root extract. It revealed the presence of alkaloids, flavonoids, phenols, glycosides, saponins, terpenoids and steroids.

## 5.1 Phytochemical analysis of the aqueous extract of *Strobilanthes heyneanus* (Leaf)

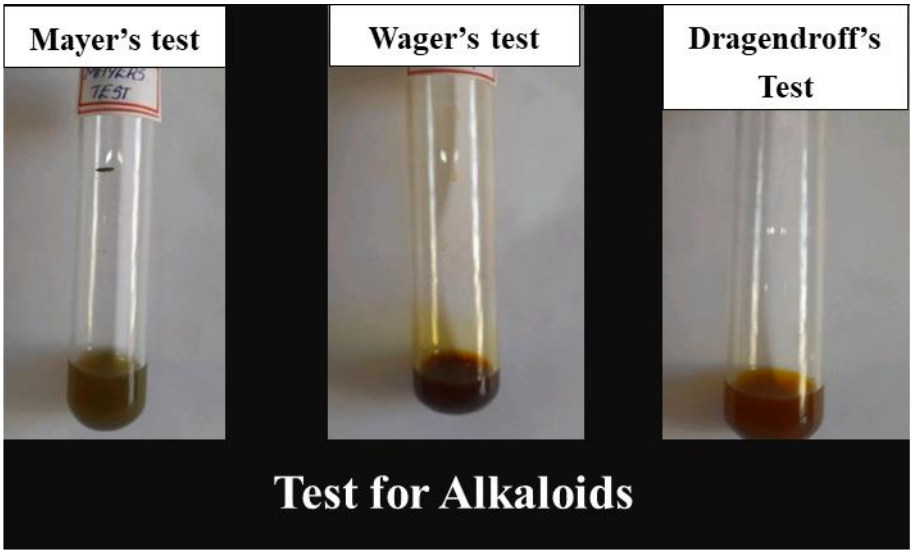
The aqueous extract of Strobilanthes heyneanus leaf showed the presence of alkaloids, phenolics, tannins and saponins.

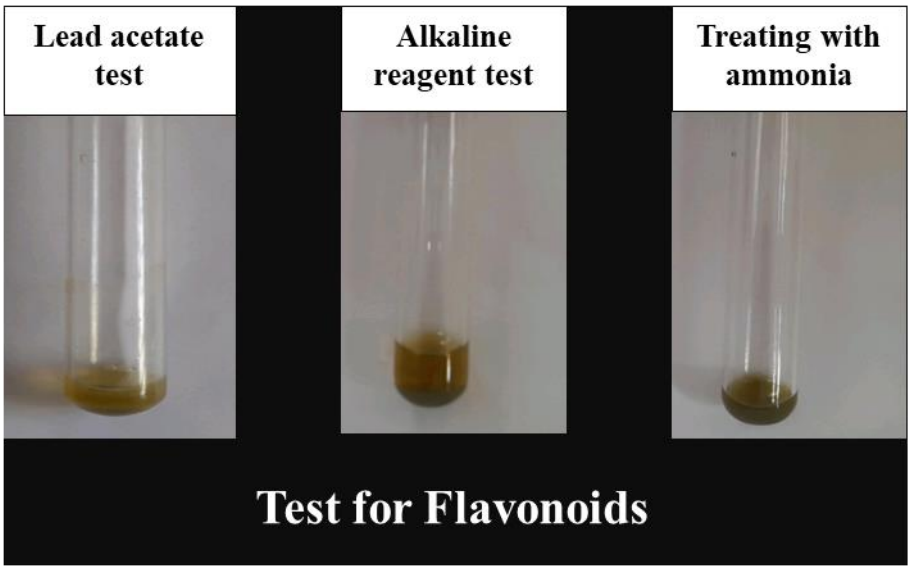
**Table 2: Phytoconstituents of the aqueous extract of *Strobilanthes heyneanus* (Leaf)**

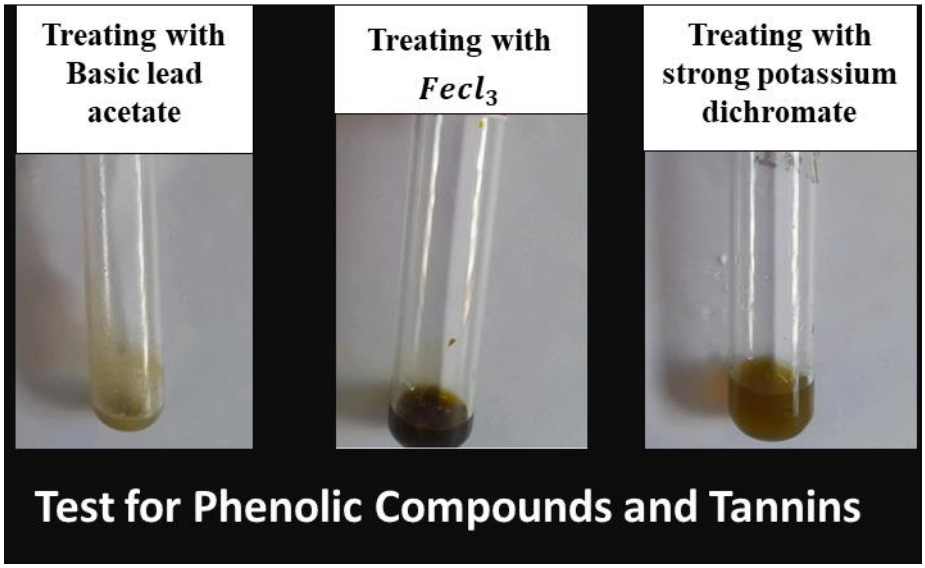
| SL.NO | NAME OF TEST | RESULT |
|---|---|---|
| 1. | Test for alkaloids | + |
| 2. | Test for flavonoids | - |
| 3. | Test for phenolic compounds and tannins | + |
| 4. | Test for glycosides | - |
| 5. | Test for saponins | + |
| 6. | Test for terpenoids | - |

**+ Indicates the Presence of Constituents;**

**- Indicates the Absence of Constituents**


Mayer's test
Wager's test
Dragendroff's Test
Test for Alkaloids


Lead acetate test
Alkaline reagent test
Treating with ammonia
Test for Flavonoids


Treating with Basic lead acetate
Treating with $Fecl_3$
Treating with strong potassium dichromate
Test for Phenolic Compounds and Tannins

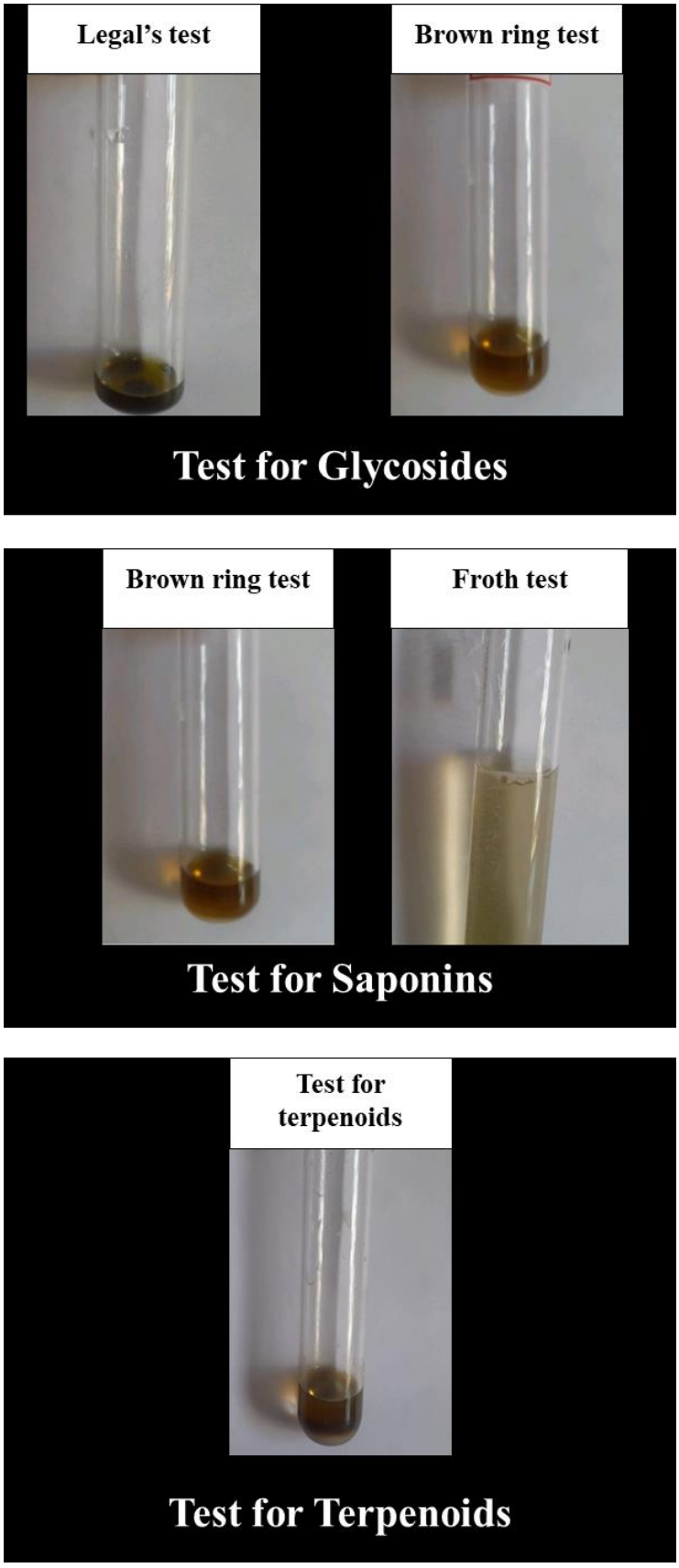



**Fig 3. Result of the Phytochemical analysis of the aqueous extract of *Strobilanthes heyneanus* (Leaf)**

**5.2 Phytochemical analysis of the aqueous extract of *Strobilanthes heyneanus* (Root)**

The aqueous extract of Strobilanthes heyneanus root showed the presence of alkaloids, flavonoids, phenolics, glycosides, tannins, terpenoids and saponins.

**Table 3: Phytoconstituents of the aqueous extract of *Strobilanthes heyneanus* (Root)**

| SL.NO | NAME OF TEST | RESULT |
|---|---|---|
| 1. | Test for alkaloids | + |
| 2. | Test for flavonoids | + |
| 3. | Test for phenolic compounds and tannins | + |
| 4. | Test for glycosides | + |
| 5. | Test for saponins | + |
| 6. | Test for terpenoids | + |

**+ Indicates the Presence of Constituents;**

**-Indicates the Absence of Constituents**

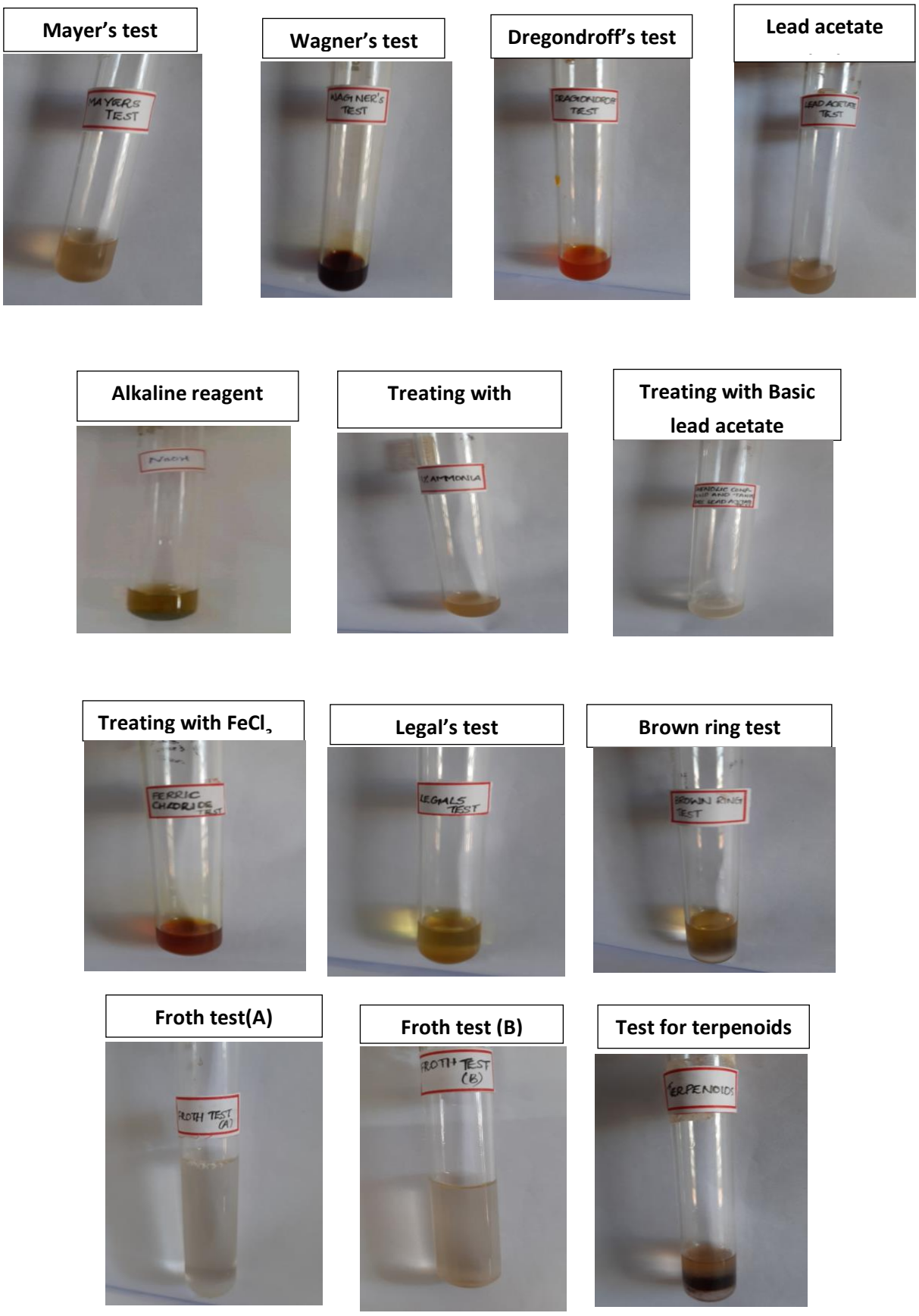



**Fig 4. Result of the Phytochemical analysis of the aqueous extract of *Strobilanthes heyneanus* (Root)**

## 5.3 Estimation of Total phenolic content of root and leaf extract of *Strobilanthes heyneanus*

**Table 4. Standard valves of The Phenolic Content**

| Sl. No. | The concentration of the standard (µg) | Optical density at 650nm |
|---|---|---|
| **B** | 0 | 0 |
| $S_1$ | 8 | 0.13 |
| $S_2$ | 16 | 0.25 |
| $S_3$ | 24 | 0.37 |
| $S_4$ | 32 | 0.50 |
| $S_5$ | 40 | 0.63 |

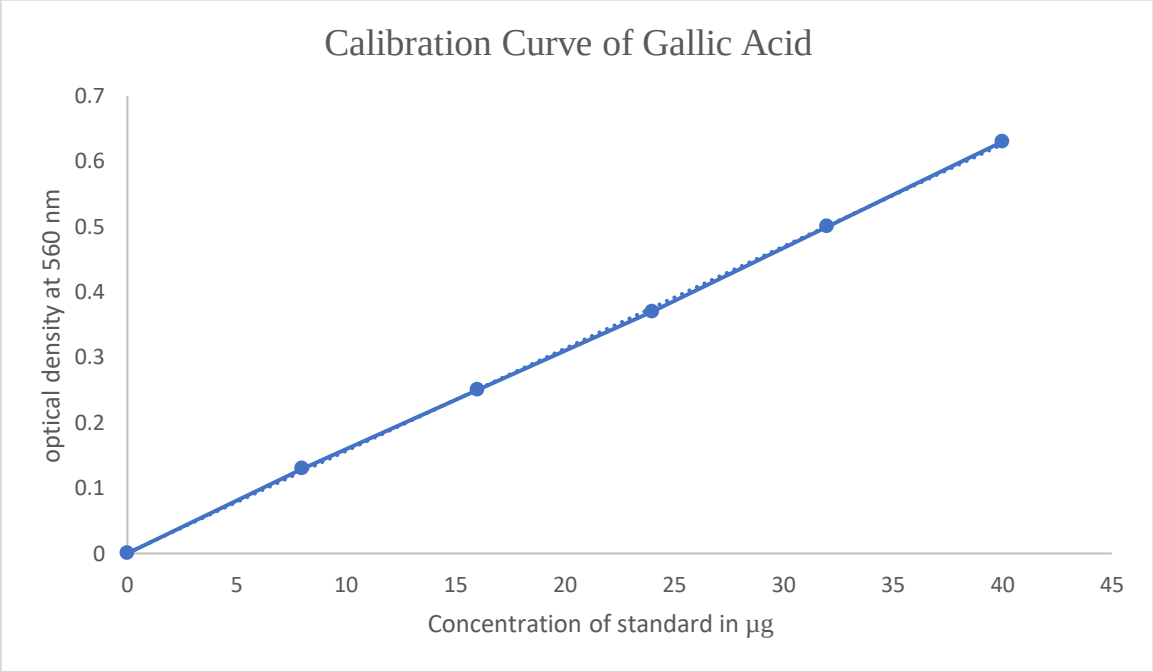



**Fig 5: Calibration Curve of Gallic Acid**

**Table 6: Total phenolic content of root and leaf extract of *Strobilanthes heyneanus***

| **Leaf extract (ml)** | **OD valves** | **Gallic acid equivalence per ml of extract** |
|---|---|---|
| 0.5 | 0.20 | 25.6 |
| 0.5 | 0.21 | 27.2 |
| 0.5 | 0.19 | 24.60 |
| **Root extract (ml)** | **OD valves** | **Gallic acid equivalence per ml of extract** |
| 0.5 | 0.05 | 3.2 |
| 0.5 | 0.07 | 4.4 |
| 0.5 | 0.06 | 3.8 |

The total phenolic content in the leaf extract of Strobilanthes heyneanus = **25.8±1.07µg/ml**

The total phenolic content in the root extract of Strobilanthes heyneanus = **3.8±0.48µg/ml**

**5.4 Estimation of Total antioxidant activity of root and leaf extract of *Strobilanthes heyneanus***

**Table 5: Estimation of Total Antioxidant Activity**

| **Sl. No.** | **The concentration of the standard (µg)** | **Optical density at 690nm** |
|---|---|---|
| **B** | 0 | 0 |
| $S_1$ | 8 | 0.11 |
| $S_2$ | 16 | 0.20 |
| $S_3$ | 24 | 0.31 |
| $S_4$ | 32 | 0.44 |
| $S_5$ | 40 | 0.55 |

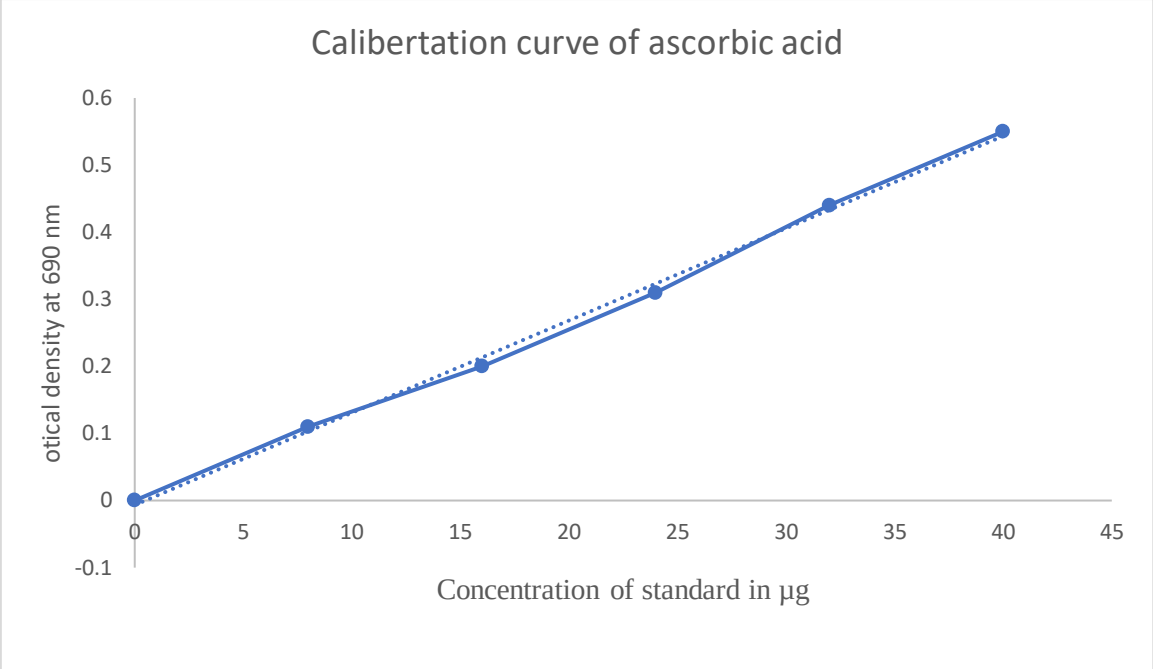



**Figure 6: Calibration Curve of Ascorbic Acid**

**Table 6: Total antioxidant content in the leaf and root extract of *Strobilanthes heyneanus***

| **Leaf extract (ml)** | **OD valves** | **Ascorbic acid equivalence per ml of extract** |
|---|---|---|
| 0.5 | 0.18 | 26.4 |
| 0.5 | 0.17 | 24.9 |
| 0.5 | 0.19 | 27.8 |
| **Root extract (ml)** | **OD valves** | **Ascorbic acid equivalence per ml of extract** |
| 0.5 | 0.05 | 7.2 |
| 0.5 | 0.04 | 5.7 |
| 0.5 | 0.06 | 8.5 |

The total antioxidant content in the leaf extract of *Strobilanthus heyneanus* = 26.3±1.18µg/ml

The total antioxidant content in the root extract of *Strobilanthus heyneanus* = 7.13±1.14µg/ml

The secondary metabolites from plants, which are distinguished from primary metabolites such as nucleic acids, amino acids, carbohydrates, fats, etc., are extremely diverse. The plant chemical used for drug purposes are largely the secondary metabolites and are not directly involved in growth, development and reproduction of plant. These secondary metabolites can be classified into several groups according to their chemical classes as terpenoids, alkaloids, phenolics, etc. (Harborne, 1998).

Plants synthesize secondary metabolites (small organic molecules) that are not required for their normal growth or development but are essentially required for reproduction and defence mechanism against bacteria, fungus, virus, vertebrates, etc. These products have a great potential to act as drugs. Many secondary metabolites are involved in the antagonistic relationship between plants and other organisms, but also in mutualistic ones (i.e., plants/pollinators, plants/disseminators, nitrogen-fixing plants/microorganisms, etc.). Secondary metabolites are the heterogeneous group of naturally occurring compounds, which have been used to treat various diseases. The biochemistry of medicines based on traditional natural products have made a tremendous contribution to public healthcare and has boosted the development of affordable medicines globally. Secondary metabolites have been investigated extensively since the 1850s. Their classification can be based on the chemical composition (containing nitrogen or not), chemical structure (e.g., having rings, containing a sugar), the biosynthetic pathway (e.g., phenylpropanoid, which produces tannins) or their solubility. They are divided into three large categories, namely alkaloids, terpenes, and phenolics. The greater part of plant derived compounds are phytochemicals, and secondary metabolites, which play a dominant role as antimicrobials and antivirals and are classified in many groups such as, alkaloids, phenolics, polyphenols, flavonoids, quinones, tannins, coumarins, terpenes, lectins and polypeptides, saponins, etc. (Anand et al., 2019).

Metabolites such as alkaloids, polyphenolic compounds and terpenes are known to exhibit a range of bioactivities including antimicrobial, antioxidant and anticancer activity and are shown to be responsible for therapeutic potential of plants. Hence, it is important to screen the plants for the presence of secondary metabolites. The phytochemical analysis of the root extract of *S. heyneanus* showed the presence of compounds such as alkaloids, steroids, terpenoids, phenols, flavonoids, tannins, saponins, glycosides.

Many phenolic acids and flavonoids possess antimicrobial, antiviral, and cytotoxic activity and have been reported to stimulate the secretion of bile acids and to prevent enzymatic

reactions The phytochemical analysis of the root extract showed the presence of terpenoids, phenolic compounds, flavonoids, and tannins which are responsible for antioxidant and antibacterial activity. Antioxidants are substances that may protect cells from ROS (reactive oxygen species) and free radicals which cause damage to cells. The phenolic acids and flavonoids, as effective anti- oxidants, play an important role in the control of different oxidative human diseases such as cancer inflammation, tissue damage, and DNA injury.

Antioxidant substances from natural products are good free radical scavengers leading to reduced risk of cancer development and protecting cells against the harmful effects of ROS on macromolecules, such as proteins, lipids, and DNA. Flavonoids and phenolic compounds as potential antioxidants may assist in health promotion by preventing oxidative damage responsible for many diseases. Phenolic compounds increase the antioxidant enzyme activity and indirectly affecting the concentration of harmful oxygen radicals in the living cells and showed anti-inflammatory and anticancer activity. Flavonoids eliminate pathological changes on capillaries and are used for the treatment of hypertension, diabetes, and atherosclerosis.

*Strobilanthes heyneanus* roots and leaves are used to treat leprosy, to control blood sugar, urinal problems, jaundice, inflammation, and excess menses. The members of the genus Strobilanthes are used as anti-diabetic, diuretic, laxative, and potent antimicrobial agents.

The anti-inflammatory and antimicrobial activities of the 95% ethanol extract, benzene fraction and isolated triterpenoids of *Strobilanthes callosus* were investigated. In the carrageenan-induced paw oedema inflammation model, the taraxerol showed a high reduction of oedema, but the antimicrobial effect observed was lower at the two doses employed. These results confirm the use of this plant in folk medicine as an anti-inflammatory and antimicrobial herbal drug.

*Strobilanthes heyneanus* (Nilgirianthusheyneanus) belongs to the family Acanthaceaewhich contains many species with potential for diverse medicinal uses. It is also called ‘Karun kurinji’ and is commonly found in the South-West regions of India. The species are commonly used in rheumatic complaints, sprain of the ankle, and hernia.

Several natural products are derived from plants and have been used traditionally to treat many diseases such as wound healing, injuries, and pain. The present study showed that the root extract of S. heyneanus has good antioxidant properties and potential antibacterial agents. Further studies are necessary to isolate active compounds responsible for antibacterial activity in order to overcome the demand for natural antibiotics for human welfare The mechanism of action of the plant extracts revealed that plant polyphenols and terpenoids play

important roles against bacteria, fungi, and viruses. Phenolic compounds increase the antioxidant enzyme activity and indirectly affecting the concentration of harmful oxygen radicals in the living cells and showed anti-inflammatory and anticancer activity

# 6. SUMMARY AND CONCLUSION

*Strobilanthes heyneanus* possess potential therapeutic effects on several diseases including rheumatism and neurological disorders such as paraplegia, sciatica etc. Apart from that it has been to be effective against a variety of human ailments such as ulcers, glandular swellings, poisonous affections, itching, leprosy and other skin diseases, cough, oedema, toothache and gum diseases and has the property of strengthening nerves etc. It is used as a pharmacologically active agents with potential antioxidant, anti-infective, anticarcinogenic properties etc. Many phytochemicals like alkaloids, phenolic compounds, tannins, saponins, flavanoids, glycosides and terpenoids have been detected from the leaves and root of *Strobilanthes heyneanus*. The traditional claim of medicinal properties of the plant may be due to the presence of the identified secondary metabolites in the leaf and root of *Strobilanthes heyneanus*. The present investigation further revealed the potential pharmacological role of the plant as a natural antioxidant with significant amount of phenolic compound. However, further research works are needed to support the complete traditional claims and health benefits of *S. heyneanus.* Studies also needs to be conducted on the toxicological effects of various parts of *Strobilanthes heyneanus.*

# 7. REFERENCES

Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 2019 Nov 1;9(11):258.

Arora R, Malhotra P, Sharma A, Haniadika R, Yashwanth H S, BaligaM S. Bioactive food as dietary interventions for arthritis and related inflammatory diseases.2013;14:601-617.

Bhat GK (2014) Flora of South Kanara. Akriti Prints Mangalore India 638.

Bouchar,H.W. Ambrose, P .G. Chambers H.F.; Ebright R.H; Jezek, A.; Murray, B.E.; Newland,J.G.; Ostrowsky, B.; Rex, J.H. White paper: Developing antimicrobial drugs for resistant pathogens,narrow-spectrum Indication, and unmet needs. J.Infect.Dis.2017,216,228-236.

Carney, CalcipaiG.Barvort .D (1999). European legislation of herbal medicine a look into the future drug safe.31428-431.

Chandra,H.; Bishnoi, P.; Yadav, A.; Patni,B.; Mishra,A.P.; Nautiyal,A.R. Antimicrobial Resistance and the Alternative Resources With special Emphasis on Plant- Based-Antimicrobials - A Review. Plants 2017,6,16.

Chen,S.; Song, J. ; Sun, C. ; Xu,J .; Zn, Y.; Verpoorte ,R .; Fan,T.P. Herbal genomics: Examining the biology of traditional medicines. Science 2015,347, S27-S29.

Corteau R, Kutchan TM, Lewis NG (2000) Natural products, Biochemistry and Molecular Biology of plant, American Society of Plant Physiologist, Rockville .1250-1318.

Dewick P M, (2002) Medicinal natural products: A Biosynthetic Approach, 2nd edition, Tom Wiley and Sons,Chinchester.

Dorothy wavinyanyamia, Wycliffe arika, Philip Einstein ogola, Eluid N M Njagi (2016). Medicinally Important phytochemicals: An Untapped Research Avenue.

Harborne . J . B , 1998 , Phytochemical methods : A guide to modern techniques of plant analysis , 3rd edition . Chapman and Hall , London

Honda G, Tabata M. Isolation of Antifungal principle Tryptanthrin, from*Strobilanthes*O.kuntze. Planta Med 1979 ;36(5):85-86.

Kristina B. Martinez, Michael K.McIntosch, in Nutrition and Functional Foods for Healthy Aging, 2017.

Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, Krishan MRV (2008) Hypoglycemic and hypolipidemic effect of *Strobilanthesheyneanus*in alloxan-induced diabetic rats. J Med plants Res 9:246-249.

Madhuri S, Pandey G. Some dietary agricultural plants with Anticancer properties. Archives 2008; 8(1):1 3-6.

MangangHC,Chhetry GKN . Antifungal properties of Certain plant extracts against *Rhizoctonia solani*causing *root* rot in French bean in organic soil Manipur. International Journal of Scientific and Research Publications 2012;2(5): 1-4.

Nair KV, Yoganarasimhan SN, GopaKumar K, shantha TR,Keshavamurthi KR (1985), Studies on some South Indian market samples of Ayurvedic drugs IV.Anc Sci Life 4(4) :211-216 PMID: 22557481; PMCID: PMC331526.

Nair RB,RavishankaranB,VijayanNP,SasikalaCK,Saraswathy VN(1988). Anti-inflammatory effect Of *Strobilanthesheyneanus*leaves. Biochemical Study. J Res Ay Sid 9(1-2).

Pullailah T. Encyclopedia of World Medicinal plants.Vol. 4, Regency publications, New Delhi, 2006.

Pye, CR.; Bertin, M J.; Lokey, R S.; Gerwick, W. H.; Linington, R G. Retrospective analysis of natural products provides Insight for future discovery trends. Proc. Natl. Acad. Sci. USA 2017,114,5601-5606.

Raghavendra HL, Kekuda PTR, Akarsh S, Renjitha MC, Ashwini HS. Phytochemical analysis, Antimicrobial and Antioxidant activities of different parts of *Pleocaulus*sessilis (Nees) Bermek (Acanthaceae). Int J Green Pharm 2017;11(2):98-107.

Renjana P.K, John E Thoppil, Toxicological evolution of Root Methanolic Extraction of *Strobilanthesheyneanus*Nees Using Allium Test, International Journal of Pharmaceutical Science and Drug Research 2013,5(3):125-128.

Sundaram, V., Sadhasivam, S., Chandrasekaran, S. et al. Strobilanthesheyneanus root extract as a potential source for antioxidant and antimicrobial activity. Future J Pharm Sci 7, 91 (2021).

Vijay Kumar Sudaram,SelvarajuSadhasivam ,Sivaraj Chandrasekaran , RaamanNanjian and Arjun Pandian. Future Journal of Pharmaceutical Sciences 7, Article number: 91 (2021).

Vijay Kumar Sudaram,SelvarajuSadhasivam, Sivaraj Chandrasekaran, RaamanNanjian and Arjun Pandian. *Strobilanthes*heyneanus root extract as a potential source for antimicrobial and Antioxidant activity 2021 7:91.