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Phytochemical Screening and TLC Studies on Ethanolic Extract of Catharanthus Roseus Leaves

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Abstract: ***Medicinal plants are of great importance to the health of individuals. Its various extract posses reported number of pharmacological activities such as nootropic, anti-oxidative, anti-diabetic, anti-bacterial, anticonvulsant, sedative, anti-pyretic, anti-inflammatory, anti-stress. Catharanthus roseus commonly known as evergreen herb is one of the famous medicinal herb in the field of Diabetic and Cancer Treatment. The ethanolic extract of Catharanthus roseus showed the presence of Flavanoids, Protein, Cardio glycosides, Phenol. For qualitative and quatitative analysis of the ethanolic extract of the Leaves of Catharanthus roseus acts as a source of therapeutic agents. Many famous Phytochemicals such as Vincristine and Vinblastin were isolated from their Medicinal Plant. It has many Pharmacological properties such as Anti-diabetic, Anti-oxidant, Anti-microbial, Wound Healing, Anti-ulcer, Hypotensive, Antidiarrhoeal, Hypolipidemic and Memory enhancement. Catharanthus roseus are used in folklore herbal medicine for treatment of many types of Cancer, Diabetes, Stomach disorders, Kidney, Liver and Cardiovascular disease. Catharanthus roseus leaf exhibited in vitro Anti-diabetic property. Vincristine and Vinblastine are used for the treatment of various types of cancer such as Hodgkin's disease, Breast cancer, Skin cancer and Lymphoblastic leukemia. It produce nearly 130 alkaloids mainly Ajmalcine, Vinceine, Resperine, Vincristine, Vinblastine and Raubasin. It is an Endangered Species and need to be conserved using techniques like Micropropagation.***

Keywords: Catharanthus roseus, Flavanoids, Protein, Cardioglycosides, Phenol

1. Introduction

Medicinal Plant

Figure: Catharanthus Roseus

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistorictimes. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, and herbivorous mammals (BernhoftA.2010).Medicinal plants are those plants rich in secondary metabolites and are potential sources of drugs. These secondary metabolites include alkaloids, glycosides, coumarins, flavonoids, steroids etc. These plants form the main base for the manufacture of drugs of Indian system of medicine (ayurveda, Unani, Siddha) and Homeopathy (BernhoftA.2010).

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. In many countries, there is little regulation of traditional medicine, but the World Health Organization coordinate network to encourage safe and rational usage. The botanical herbal market has been criticized for being poorly regulated and containing place pseudoscience products with no scientific research support their medical claims. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand (Sahana, S., Kumar, R., et al., 2020). The compounds found in plants are of many kinds, but most are in four major

biochemical classes alkaloids, glycosides, polyphenols, and terpenes (Saha, P., Kumar, R., et al., 2021)

Figure: Catharanthus Roseus

Alkaloids produced by Catharanthus roseus

1) Vindolicine
2) Anhydrovinblastine
3) Vincristine
4) Ajmalicine
5) Tabersonine
6) Catharanthine
7) Vindoline
8) Vinblastine
9) Ajmalicine (Hirata, K. et al., 1994).

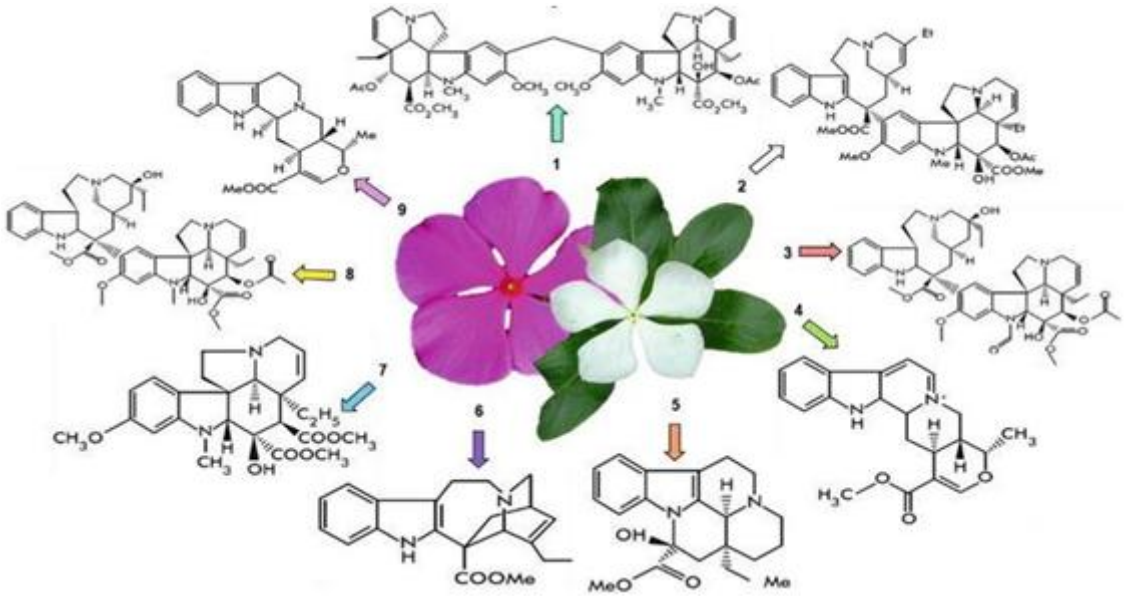

Figure: Chemical Structure of Catharanthus Roseus

Taxonomic Classification
Kingdom: Plantae
Sub Kingdom: Tracheobionta (Vascular plants)
Division: Magnoliophyta
Class: Magnoliopsida
Sub Class: Asteridae
Order: Gentianales
Family: Apocynaeean(Dog-bane family)
Subfamily: Plumeroideae

Catharanthus roseus, commonly known as bright eyes, Cape periwinkle, graveyard plant, Catharanthus roseus, old maid, pink periwinkle, rose periwinkle, is a perennial species of flowering plant in the family Apocynaceae. It is native and endemic to Catharanthus roseus,but grown elsewhere as an ornamental and medicinal plant.(Heijden,Robert;et al.,2004).
Catharanthus roseus is an evergreen, erect-to-spreading, herbaceous, tender perennial that grows to 4 feet tall. It is frequently planted as an annual, as it is not cold-hardy. Plantitin full sun to partial or dappled shade in moist, well-drained soil. While it prefers acidic soil, it tolerates a range of soil types, so as long as it has good drainage. Seeds can be sown in March, in warm temperatures, and planted outside in early June. It does not need pinching or dead heading. (Fraser, Valerie N et al., 2022).

Leaves
Leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm broad, glossy green, hairless, with a pale mid rib and a short petiole1–1.8cmlong they are arranged in opposite pairs. Herbaceous to thinly leathery, glossy green above and pale green below, sparsely shortly hairy to glabrous on both sides.

Flower
Flowers are bisexual, 5-merous, regular, almost sessile; sepals slightly fused at base, (2–)3–5mm long, erect, green; corolla tube cylindrical, 2–3 cm long, widening near the top

at the insertion of the stamens, laxly shortly hairy to glabrous outside, with a ring of hairs in the throa tand another lower down the tube, greenish, lobes broadly obovate, 1–2(–3) cm long, apex mucronate, glabrous, spreading, pink, rose-purple or white with a purple,red,pink,pale yellow or white center stamen sinserted just below the corolla throat.

Fruit
Fruit is composed of 2 free cylindrical follicles 2–4.5 cm long, striate, laxly shortly hairy to glabrous, green, dehiscent, 10–20-seeded.Seeds are oblong,2–3mmlong, grooved at one side and are black colored (Moudi, Maryam et al., 2013).

Chemical Consituents of Catharanthus Roseus

From the Leaf
- Limonene (37.2%) and
- Dotriacontane (16.1%)

Morphology of Catharanthus Roseus
Catharanthus roseus is an evergreen plant with smooth, slightly hairy, simple, or slightly branched, about 1-2 m tall. The stems of the Catharanthus roseus plant are up to 60 cm long and are narrowly winged, and greenish-red in color. The leaves are oval, 2.5-9cm long, and 1- 3.5 cm wide glossy green. It is known for its antitumour, anti-diabetic, anti-microbial, antioxidant and antimutagenic effects. It produces nearly 130 alkaloids mainly ajmalcine, vinceine, resperine, vincristine, vinblastine and raubasin. Vincristine and vinblastine are used for the treatment of various types of cancer such as Hodgkin's disease, breast cancer, skin cancer and lymphoblastic leukemia. It is an endangered species and need to be conserved using techniques like micropropagation. It has high medicinal values which need to be explored extensively (RoshanKumar2022).

2. Materials and Methods

Sample Collection:
The Sample was collected from Chennai, Tamil Nadu, India.

Figure: Leaves of Catharanthus Roseus and Powdered form of Catharanthus Roseus

The leaves of Catharanthus roseus were washed thoroughly in tap water to remove dustparticles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder.

Phytochemical Analysis

Qualitative Phytochemical Screening Method:
Preliminary phytochemical screening was carried out by the following method

Test for Tannins:
To the 1ml of extract, 1ml of Ferric chloride solution was added. 2ml of distilled water was added.Formation of green precipitate indicates the presences of tannin.

Test for Saponins:
To 1ml of extract, 5ml of distilled water was added and shaken vigorously. Formation of forth indicates the presence of saponin.

Test for Flavonoids:
To the 1ml of extract, 2ml of distilled water was added and few drops of 10% Ferric Chloride were added. A Green ,blue or violet colouration indicates the presence of flavonoids.

Test for Alkaloids:
To the 1ml of extract,1ml of distilled water were added and 25µl of concentrated hydrochloricacid was added and few drops of Dragendroff's reagent was added. Formation of Orange red colour indicates the presence of Alkaloids.

Test for Protein:
To the 100ml of extract, 3ml of Coomassie brilliant blue(CBB)was added.

Test for Steroids:
To 1ml of extract, 2ml of distilled water is added and 2ml of Chloroform and 2ml of Sulphuric acid was added.A Red colour indicates the presence of Steriod.

Test for Anthro Quionone:
To 1ml of extract, add 1 or 2ml of Chloroform was added and 2 or 4ml of 10% Ammonium Solution (3.3ml ammonium solution make up to 10ml distilled water) shake well. An Upper aqueous layer Pink colour indicates presence of Anthoquionone.

Test for Terpenoids:
To 1ml of extract, 2ml of sulphuric acid was added. Formation of Greyish colour indicates the presence of Terpenoids.

Test for Cardioglycosides
To 1ml of extract, add 1ml of Baljet's reagent ,tube kept for incubation for 1 hour. After incubation add 2ml of Distilled water. Test tube were read at 495nm.

Test for Phenols
To the 1ml of extract, 2ml of Distilled water was added. 20µl stock solution with 180µl of distilled water and 0.5ml of follin phenol reagent with 1ml of 7.5% Sodium Carbonate (0.37gmake upto 5ml) add 0.5ml of Distilled Water. After 2 hours of incubation formation of blue Colour indicates the presence of phenols was read at 728 nm.

3. Results

Phytochemical Analysis
The phytochemical components were analysed for phenolic compounds, Flavanoids, Alkaloids, Saponins, Terpenoids, Tannins, Cardioglycosides, Proteins, phenol, Steroids, Anthraquinone.

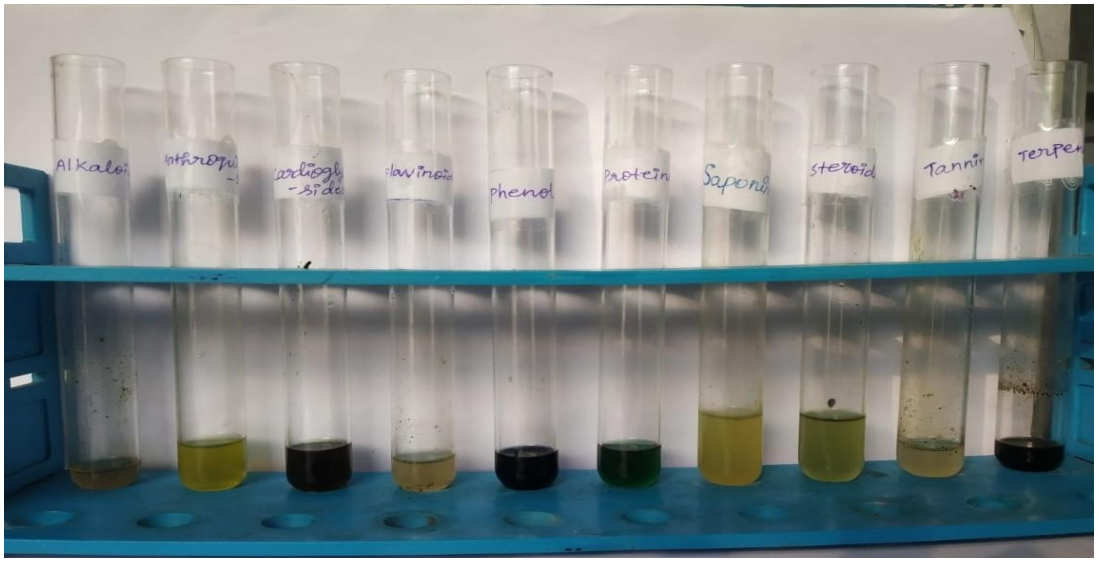

Figure: Phytochemical Qualitative Test

1	FLAVANOIDS	Present
2	TERPENOIDS	Absent
3	TANNIN	Absent
4	SAPONIN	Absent
5	ALKALOIDS	Absent
6	PROTEIN	Present
7	STEROIDS	Absent
8	ANTHROQUIONONE	Absent
9	CARDIOGLYCOSIDES	Present
10	PHENOL	Present

Thin Layer Chromatography (TLC)

SAMPLE NAME: CATHARANTHUS ROSEUS (ethanolextract)
Mobile Phase: Chloroform: Ethanol
Hexane: Ethylacetate
Ratio: 9:1
Peak Value: 0.763

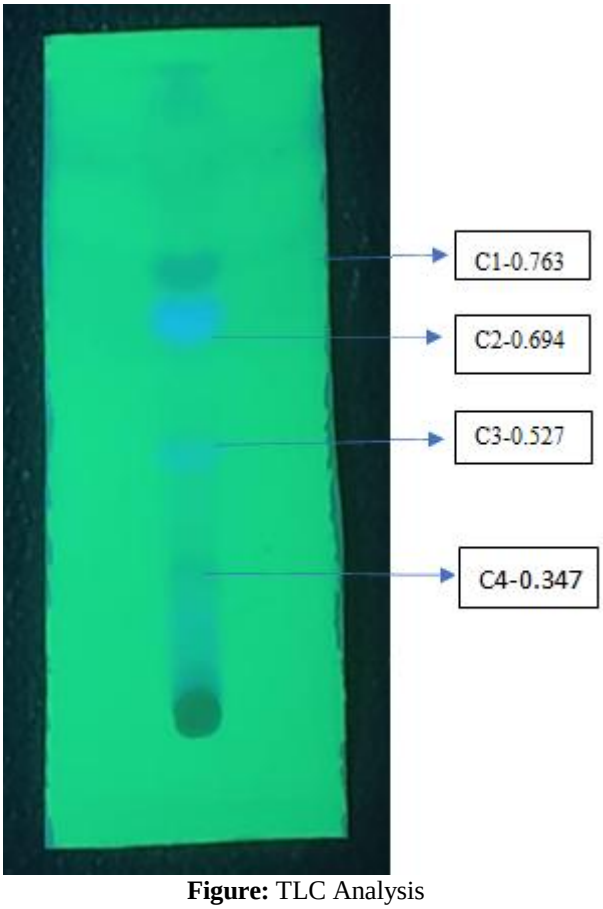

Figure: TLC Analysis

4. Calculation

$$Rf = \frac{\text{Distancetravelledbysolute}}{\text{Distance travelledbysolvent}}$$

$$C1 = \frac{5.5}{7.2} = 0.763$$

$$C2 = \frac{5.0}{7.2} = 0.694$$

$$C3 = \frac{3.8}{7.2} = 0.527$$

$$C4 = \frac{2.5}{7.2} = 0.347$$

S. No	Rf	Colour
1	0.763	Green
2	0.694	Green
3	0.527	Green
4	0.347	Green

5. Discussion

Plants have been used for medicinal purposes long before prehistoric period these medicinal plants are also used as foods, flavonoids, medicines,side effects of several synthethic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant material as a source of medicines for a wide variety of humans ailments.

The qualitative phytochemical analysis of the leaves of Catharanthus roseus is summarized in the quantifications of important phytocompounds of the leaves of Catharanthus roseus. The ethanolic extract of leaves shows the presence of high number of phytocompounds. It shows the presence of Alkaloids, Steriods, Terpenoids, Flavonoids, Phenol, Carbohydrate, Saponins, and Reducing Sugars. Phytochemicals such as Saponins, Terpenoids, and Alkaloids have hypoglycemic activites. The leaves show the presence of terpenoids and they play a major role in the treatment of intestinal disorders like of Anti-inflammatory activity. The leaves show positive result for phenols which can be act as antioxidants. The leaves also have Flavanoids which can act as antioxidants. Phytochemicals have highest therapeutic efficiency in pharmaceutical field. It helps to undertake further studies on isolation and identification of specific phytocomponents for pharmacological studies.TLC analysis were carried to study separation based on molecular weight of Catharanthus roseus.

Catharanthus roseus has a wide range of applications in the traditional system of medicine especially in cancer and diabetes. During phytochemical investigation, total of 344 compounds including monoterpene indole alkaloids, bisindole alkaloids, flavonoids, phenolic acids and volatile constituent have been reported in the various extracts and fractions of different plant parts of C. roseus. The extracts and isolated compounds of C. Roseus have to exhibit many pharmacological activities such as anticancer, cytotoxic, antidiabetic, antimicrobial, and antioxidant, larvicidal and pupicidal.

6. Conclusion

The present research work was aimed to phytochemical screening and TLC analysis on ethanolic extract of Catharanthus roseus leaves. The qualitative analysis showed that Flavonoids, Cardiacglycosides, Phenol, and Proteins. TLC study revealed the presence of various compounds in the plant extract. The study provides support to the plant traditional and alternative use against various diseases. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance. Catharanthus roseus can be considered as a rich source of alkaloids, phenolics, which possess diverse biological properties. Consequently, the identification and isolation of new phytochemicals with in the different structural components of Catharanthus roseus have been identified. Catharanthus roseus has its unique therapeutic efficacy the plant could be utilized as an alternative source of useful antidiabetic drugs. In addition ,potential uses of bioactive compounds derived from this material need to be further investigated for applications in the nutraceutical and pharmaceutical industries. Since many of the existing synthetic drugs cause various side effects,however,extensive research still needs to be done on photochemical of this plant for the development of cost effective drugs for the future.

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