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Screening of Bioactive Compounds from Madagascar Periwinkle and Their Importance in Drug Discovery

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Abstract

The phytochemicals present in medicinal plants are effective and safe alternative to the chemically synthesized drugs for various diseases. Madagascar periwinkle grown as ornamental plant for its flowers. It is an evergreen that was native to the Madagascar Island. It is so special due to the variety of bioactive compounds present which have medicinal values, including anti-inflammatory, anti-cancer and anti-diabetic quality. This study aims to investigate phytochemical analysis of crude aqueous, methanol, chloroform and acetone extracts from leaves and flower of *C. roseus*. Qualitative preliminary phytochemical analysis revealed that the presence of alkaloid, flavonoids, tannin, phenol, and carbohydrate in all extracts of *C. roseus*. Both the aqueous and acetone extract of leaf and flower lacks terpenoid, protein, and resin. The most important alkaloids vinblastine and vincristine are derived from leaves and they exhibits anti-cancer and anti-diabetic property. These findings imply that the plant still has the potential to be a valuable source of bioactive substances. Further pharmacological and toxicitystudies are necessary to be explored which can be used for the development of medicines.

Keywords: Phytochemicals, *C. roseus*, Alkaloid, Flavonoid, Tannin, Anti-Cancer, Anti-Diabetic.

1. Introduction:

Plants which have medicinal values are effective for treating many human diseases because they contain several bioactive compounds. Herbal plants are considered as natural chemical factories with therapeutic sources of biomolecules that can produce an effect physiologically on human system properties [1]. These compounds are present in all parts like stem, bark, leaves, roots, flowers and fruits [2]. Many studies proved that the metabolites like alkaloids, flavonoids, coumarin, terpenoids, steroids, tannins which exhibit certain important pharmacological activities like anti-microbial, anti-cancer, anti-diabetics and anti-inflammatory [3]. The natural plant products minimize the harmful effects of various pharmaceutical agents and attaining positive general health. India is the richest, oldest and diverse cultural associations with the use of medicinal plants.

Catharanthus roseus which belong to the family *Apocynaceae* is an ornamental shrub and it is commonly known as Madagascar periwinkle [4]. Madagascar Island is the origin of this plant but it can be found around the world because it has the ability to survive varieties of habitats [5]. Leaves of *C. roseus*, which are present in pairs on stem, used extensively in folk medicine for decreasing sugar level

of blood [6]. *C roseus* widely grows in the Indian subcontinent having two common flower colors as pink and white.

The plant synthesize more than 400 alkaloids, which are the chemical compounds of the plant that are utilized in pharmaceutical processes, flavour and fragrance, food additives, insecticides, and agrochemicals. Vinesine, vindeline tabersonine, vinblastine, vincristine are just a few of the alkaloids are found. This plant produces more than 140 mono terpenoid indole alkaloids (TIA) in different parts. The leaves and stems are source of alkaloids, flavonoids, phenols and roots have ajmalicine, reserpine, catharanthine, vinceine, vineamine, serpentine which can be used to treat hypertension [7].

The significant alkaloid vinpocetine has vasodilating and memory enhancing actions which can be used to cure Alzheimer's disease [8]. Many pharmacological researchers have found that terpenes or terpenoids indole alkaloids have anti-cancer, anti-inflammatory, anti-malarial properties and can be used as medicines [9]. Vinblastin is mainly used for treatment of Hodgkin's disease, advanced testicular cancer, advanced breast cancer, Kaposi's sarcoma. The present investigation was carried out for evaluation of crude leaf and flower extracts of *Catharanthus roseus* in aqueous, methanol, chloroform and acetone for phytochemical analysis.

Table.1: Presence of Alkaloids in different part of madagascar periwinkle

PLANT PART	ALKALOIDS
FLOWER	Catharanthine, Vindoline, Leurosine, Lochnerine, Tricin
STEM	Leurosine, Lochnerine, Catharanthine, Vindoline
LEAF	Catharanthine, Vindoline, Vindolidine, Vindolicine, Vindolinine, Ibogaine, Yohimbine, Raubasine, Vinblastine, Vincristine
ROOT	Ajmalacine, Serpentine, Catharanthine, Vindoline, Leurosine, Lochnerine, Reserpine, Alstonine, Tabersonine, Horhammericine, Lochnericine, Echitovenine

2. Materials and Methods:

2.1. Sample Collection: Plants leaves and flowers were collected from the horticulture of Bhubaneswar, Odisha. Fresh leaves and flowers of selected plants were used for phytochemical analysis.

2.2. Preparation of Plant Extract: The leaves and flowers of the chosen plants were collected**.** Then dust and soil particles were removed by washing under running tap water followed by distilled water. The samples were then dried under shade at room temperature for about 15 days and crushed to fine powder and kept in air tight container for future uses.

2.3. Preparation of Solvent Extract of Sample: The extracts of sample were prepared by soaking 10 g of powdered sample in distilled water, methanol, chloroform, acetone each of 100 ml and shaken well. The solution was left at room temperature for 36 hours and then filtered with the help of Whatsman filter paper. The filtrate of the samples were taken and used for further phytochemical analysis.

2.4. Qualitative phytochemical Screening:

Selected plant extracts were tested for phytochemical through various test methods.

2.4.1. Test for Alkaloids:

2.4.1.1. Wagner's Test: To the 1 ml extract, few drops of Mayer's reagent were added and observed the formation of reddish-brown precipitate (or colouration) [10].

2.4.1.2. Dragendroff's Test: Selected extracts of 2 ml were treated with 1 ml of Dragendroff's reagent which forms orange-red precipitation.

2.4.1.3. Hager's Test: 2 ml of extract were treated with 5-10 drops of Hager's reagent. A yellow precipitate was formed.

2.4.1.4. Mayer's Test: To the 1 ml of extract, 3-5 drops of Mayer's reagent was added. Cream coloured precipitate will indicate the presence of alkaloids.

2.4.2. Test for flavonoids:

2.4.2.1. Alkaline Test: 1 ml of extract was treated with few drops of 20% sodium hydroxide solution shows yellow colouration which disappeared on addition of dilute hydrochloric acid [11].

2.4.2.2. Shinod's test: 10 drops of dilute hydrochloric acid and a piece of magnesium were added to 1 ml of extract which gives red or orange colouration.

2.4.2.3. Pew's Test: 5 ml of the extract was mixed with 0.1 g of metallic zinc and 8 ml of concentrated sulphuric acid. The mixture was observed for red colouration.

2.4.2.4. Ferric Chloride Test: 2 ml of the filtrate and few drops of 10% ferric chloride solution were mixed to observe a greenish-blue or blackish-red coloration.

2.4.3. Test for Tannins:

2.4.3.1. Lead Acetate Test: To 5 ml of extract, few drops of 1% lead acetate solution were added. The formation of orange or red precipitate indicates the presence of tannins.

2.4.3.2. Braymer's Test: 2 ml extract and 2 ml of 10% ferric chloride solution was mixed, which gives bluish-green or black precipitation.

2.4.3.3. Gelatin Test: The extracts were mixed with basic 1% solution of gelatin containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

2.4.4. Test for Saponins:

2.4.4.1. Froth Test: To 5 ml of aqueous extract add a drop of sodium bicarbonate solution and left to rest for 5 minutes after shaking vigorously. Formation of a honey comb like froth indicates the presence of saponins.

2.4.4.2. Foam Test: Distilled water of 6 ml was added in 2 ml of extract. The mixture was shaken thoroughly and observed for the formation of constant foam [12].

2.4.5. Test for Terpenoids:

2.4.5.1. Salkowki's Test: 2 ml of chloroform was added to 2 ml of extract and few drops of concentrated sulphuric acid were added. A reddish brown precipitate produced immediately after the mixture was shaken well [13].

2.4.5.2. Horizon Test: Red colour precipitate was observed after 2 ml of trichloroacetic acid was added to 1 ml of extract.

2.4.6. Test for Cardiac Glycosides:

2.4.6.1. Keller Kelliani Test: 1 ml of extract was treated with glacial acetic acid and 2-3 drops of 5% aqueous ferric chloride solution. To this mixture 0.5 ml of concentrated sulphuric acid was added and observed for a reddish-brown ring at the interface [14].

2.4.6.2. Legal Test: To 2 ml of the extract, 1 ml of pyridine and 1 ml of sodium nitropruside were added. The formation of pink or red indicates the presence of cardiac glycosides.

2.4.7. Test for Phenols:

2.4.7.1. Ferric Chloride Test: 2 ml of extract were treated with 0.5 ml of aqueous 5% ferric chloride and observed for the formation of deep blue or black colouration [15].

2.4.7.2. Lead Acetate Test: 5 ml of extract and 3 ml of 10% lead acetate solution were mixed. A bulky white precipitate indicates the presence of phenols.

2.4.8. Test for carbohydrate:

2.4.8.1. Molish's Test: The extracts of 1 ml added to 6 drops of Molisch's reagent, along with 1 ml of concentrated sulphuric acid down the side of the test tube. Then allow the mixture to stand for 2-3 minutes. Then the formation of red or dull violet colour at the interface of the two layers is a positive result.

2.4.8.2. Fehling's Test: 5 ml of Fehling's solution A and B were mixed with 2 ml of extract and boiled in water bath for 5 minutes. The brick-red precipitate formation shows positive result.

2.4.9. Test for Proteins:

2.4.9.1. Ninhydrin Test: 1 ml of extract and 2-5 drops of 1% ninhydrin solution were added and placed in a boiling water bath for 1-2 minutes and observe for the formation of purple colour [16].

2.4.10. Test for Resin:

2.4.10.1. Sulphuric Acid Test: To 2 ml of extract 5 to 10 ml of acetic anhydride added and dissolves gently by heating. After cooling add 0.5 ml of sulphuric acid which gives bright purple colour.

3. Results and Discussion:

The current investigation was carried out for the qualitative phytochemical analysis of *C. roseus* pink flower type leaf and flower extracts in different solvents is shown in Tables 2 and 3 respectively.

Table.2: Qualitative phytochemical analysis of different solvent extract of leaf of *C. roseus*

PHYTOCHEMICALS	TEST NAME	SOLVENT EXTRACT OF LEAF			
		AQUEOUS	METHANOL	CHLOROFORM	ACETONE
ALKALOID	Wagner's Test	positive	positive	positive	positive
	Dragendroff's Test	positive	positive	positive	positive
	Hager's Test	positive	positive	positive	positive
	Mayer's Test	positive	positive	positive	positive
FLAVONOID	Alkaline Test	positive	positive	positive	positive
	Shinod's test	positive	positive	positive	positive
	Pew's Test	positive	positive	positive	positive
	Ferric Chloride Test	positive	positive	positive	positive
TANNIN	Lead Acetate Test	positive	positive	positive	positive
	Braymer's	positive	positive	positive	positive

	Test				
	Gelatin Test	positive	positive	positive	positive
SAPONIN	Froth Test	positive	positive	negative	negative
	Foam Test	positive	positive	negative	negative
TERPENOID	Salkowki's Test	negative	positive	positive	negative
	Horizon Test	negative	positive	positive	negative
CARDIAC GLYCOSIDE	Keller Kelliani Test	positive	positive	positive	negative
	Legal Test	positive	positive	positive	negative
PHENOL	Ferric Chloride Test	positive	positive	positive	positive
	Lead Acetate Test	positive	positive	positive	positive
CARBOHYDRATE	Molish's Test	positive	positive	positive	positive
	Fehling's Test	positive	positive	positive	positive
PROTEIN	Ninhydrin Test	negative	positive	negative	negative
RESIN	Sulphuric Acid Test	negative	negative	negative	negative

Table.3: Qualitative phytochemical analysis of different solvent extract of flower of *C. roseus*

PHYTOCHEMICALS	TEST NAME	SOLVENT EXTRACT OF FLOWER			
		AQUEOUS	METHANOL	CHLOROFORM	ACETONE
ALKALOID	Wagner's Test	positive	positive	positive	positive
	Dragendroff's Test	positive	positive	positive	positive
	Hager's Test	positive	positive	positive	positive
	Mayer's Test	positive	positive	positive	positive

FLAVONOID	Alkaline Test	positive	positive	positive	positive
	Shinod's test	positive	positive	positive	positive
	Pew's Test	positive	positive	positive	positive
	Ferric Chloride Test	positive	positive	positive	positive
TANNIN	Lead Acetate Test	positive	positive	positive	positive
	Braymer's Test	positive	positive	positive	positive
	Gelatin Test	positive	positive	positive	positive
SAPONIN	Froth Test	negative	negative	negative	negative
	Foam Test	negative	negative	negative	negative
TERPENOID	Salkowki's Test	negative	positive	positive	negative
	Horizon Test	negative	positive	positive	negative
CARDIAC GLYCOSIDE	Keller Kelliani Test	negative	positive	positive	negative
	Legal Test	negative	positive	positive	negative
PHENOL	Ferric Chloride Test	positive	positive	positive	positive
	Lead Acetate Test	positive	positive	positive	positive
CARBOHYDRATE	Molish's Test	positive	positive	positive	positive
	Fehling's Test	positive	positive	positive	positive
PROTEIN	Ninhydrin Test	negative	positive	negative	negative
RESIN	Sulphuric Acid Test	negative	negative	negative	negative

Medicinal plants are important, cheap, and widely available resource to cure human diseases. Out Of the 10 phytochemicals analyzed, only resin was absent in all leaf and flower extracts. Five phytochemicals which are alkaloid, flavonoid, tannin, phenol, and carbohydrate were present in each extract.

Phenols are used as antiseptic for surgical instruments, in the production of drugs, in cosmetics, etc. Tannins bind to proline rich protein to interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances have been found to be antimicrobial substances against wide array of microorganisms in vitro [17].

Alkaloids have been associated with medicine and common biological property is the cytotoxicity [18]. Glycosides are known to lower the blood pressure [19]. Saponins were detected in aqueous and methanolic extract of leaf. These saponins are used medically for the treatment of high cholesterol, hypertension, and in the control of post menopausal syndrome [20].

Previous research shows that, *C. roseus* has mostly been studied with respect of anti-hypertension, anti-diabetic and anti-cancer properties [21]. Moreover, many species of *Apocynaceae* family has been commonly used in traditional and folk medicine. Hence the present investigation on *C. roseus* plant extracts could be significant for the progress of new life saving drugs and an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Further more advanced research is also required to isolate the new bioactive compounds.

4. Conclusion:

The modern chemotherapeutic agents for different diseases are also produced by the *C. roseus*. So, various parts of the plant can be considered for the development of new therapeutic drugs which are effective against various chronic diseases. The screening of phytochemical constituents of plant *Catharanthus roseus* indicates the presence of alkaloid, flavonoids, tannin, phenol, and carbohydrate in all the eight different extracts viz., aqueous, methanol, chloroform, and acetone extract of both leaf and flower. Both the aqueous and acetone extract of leaf and flower lacks terpenoid, protein, and resin. It can be concluded from the above study that among the four solvents used for extraction, methanol and chloroform extracts were found more affective, followed by aqueous and acetone. The result indicated that leaf extract possesses the highest phytochemical activity than the flower extract of the plant. However, further pharmacological and toxicity studies are necessary to be explored so that the new bioactive compounds of this plant can be used for the development of medicines.

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6. References

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