Research Article

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PHYTOCHEMICAL AND PHARMACOGNOSTIC STUDY OF *CATHARANTHUS ROSEUS* USING DEEP EUTECTIC SOLVENTS

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ABSTRACT

Medicinal plant derived compound have an important role in the development of many clinically useful agents. *Catharanthus roseus* is a significant medicinal plant of the Apocynaceae family. *Catharanthus roseus* plant is used for different disease treatment such as skin cancer, Hodgkin's disease, breast cancer, and lymphoblastic leukemia. The aim of this study is conferring the comparative abundances of different phytochemicals, physiochemical and fluorescence analysis from *Catharanthus roseus*. Some of the chemical constituent's tests have been performed for phytochemical analysis. Fluorescence analysis was done by using dried powder mixed with different chemicals and have been observed under UV light (365 nm) and ordinary light. Total ash, acid insoluble ash, and moisture have been selected for physiochemical analysis. The precipitate formation or colour change was used as analytical answers for phytochemical analysis. The result of fluorescence analysis observed under UV light (365nm) and ordinary light by observing colour changes in sample. The percentage of loss of weight on drying, total ash and acid insoluble ash were obtained by employing standard methods of analysis for the physiochemical test. Extraction of *Catharanthus roseus* plant using deep eutectic solvent gave positive results.

Keywords: *Catharanthus roseus*, deep eutectic solvent, phytochemical, fluorescence, physiochemical.

INTRODUCTION

Naturally grown plants around us are used for medication purpose. Over decades, cultures around the globe have learned about so many plants how to use them against illness and maintain human health. Plants are readily available and culturally significant medicines from the basis of an accessible and economical health care system and are a very significant basic source of livelihood for indigenous and rural population¹. The plant showed to be a source of many alkaloids which *Vinca rosea* is an ever-blooming Apocynaceae, pubescent herb or subshrub. Which has been enjoyed a very good reputation in indigenous medicine in different areas of the globe. The plants alkaloids are hypotensive, sedative and have tranquilizing properties and are also anti-cancerous, that helps in relieving the pain of muscle, wasps stings and also depression of central nervous system. It has been used in different cases like a nose bleed, sore throats, bleeding gums, and also mouth ulcers. Furthermore, It also has been used internally for hypertension, loss of memory, cystitis, enteritis and gastritis, raised blood sugar levels, and diarrhea². This plant produces a diverse array of secondary metabolites that are pharmaceutically important and used as chemotherapeutic agents in the treatment of several types of cancers³.

The species of *Catharanthus roseus* (Madagascar periwinkle) is *Catharanthus* native and endemic to Madagascar. Synonyms include *Vinca rosea* (the basionym), *Ammocallis rosea*, and *Lochnera rosea*; it also has other different English names occasionally used include Rosy Periwinkle, Cape Periwinkle, Rose Periwinkle, and "Old-maid". The common name of *Catharanthus roseus* in India is known as "Nithyakalyani" and in Pakistan "Sadabaharphol." Scholars Studies in the 1950s find out that *Catharanthus roseus* has 70 alkaloids, many of the are biologically active. In the same time studies for its use in diabetes mellitus was disappointing, the findings that it is caused myelo suppression led to its study in mice with leukemia, because of *Vinca* preparation the lifespan was a bit prolonged. The other scientific name for "*Vinca rosea*" is *Catharanthus roseus*. Vincristine is *Vinca* Alkaloids from *Catharanthus roseus*. The plant is a mitotic inhibitor and used in cancer chemotherapy. The leaf juice of *Catharanthus roseus* makes a dose-dependent reduction in blood-glucose of both diabetic and normal rabbits and comparable with that of the standard drug, glibenclamide. The findings indicate a prolonged action in reduction of blood glucose by *Catharanthus roseus* and the mode of action of the compound(s) of *Catharanthus roseus* is probably mediated through enhancing secretion of insulin through extra pancreatic mechanism or from the β -cells of Langerhans. The plant has been cultivated as an ornamental

plant and herbal medicine. The substances vincristine and vinblastine extracted from the *Catharanthus roseus* are used in the treatment of leukemia. This conflict between historical indigenous use, and recently patents on *Catharanthus roseus* derived drugs by European pharmaceutical companies, without compensation, has led to accusations of bio piracy. If consumed orally it can be dangerous. It can be hallucinogenic, and is cited (under its synonym *Vinca rosea*) in Louisiana State Act 159⁴.

In Chinese medicine, extracts from *Catharanthus roseus* have been used to treat many diseases, including diabetes, malaria, and Hodgkin's disease. It is also used as an astringent, diuretic and expectorant⁵. The alkaloids like Vinblastin, actinoplastidemic, Vincristine, Vindesine, Vindoline Tabersonine etc. are mainly present in aerial parts whereas Raubasine, Vincamine, Ajmalicine, Catharanthine, Reserpine, etc. are present in basal stem and roots. These are used as indispensable antihypertensives and cancer drug. The tropical plant produces more than 100 monoterpenoids indole alkaloids in different organs⁶.

The research for new therapeutic treatments for various disease conditions is expanding. In different developing countries, *Catharanthus roseus* have been looked at as a very promising source of new lead compounds for drug development and discovery.

MATERIALS AND METHODS

Investigated Plant: *Catharanthus roseus*

Family: Apocynaceae

Habit: 20-40 cm, perennial under shrubs

Botanical Characters

Catharanthus roseus is an evergreen sub herb plant having a height of 1 m tall. The shape of leaves is oval to oblong with 2.5cm - 9.5cm long and 1cm - 3.5cm broad having colour glossy green without hairs with a pale colour midrib and a short petiole about 1cm - 1.8cm long and these are arranged against each other. The flowers having two colour means from the back is white, and top dark pink with a dark red center, with a basal tube about 2.5cm - 3cm long and a corolla is between 2cm to 5cm diameter having lobes of 5 petals. The fruit is a pair of follicles about 2cm - 4cm long and 3 mm broad⁷.

Part Used

Root, Stem, Leaves, and Flower Petal

Vernacular Names

Table 1: Vernacular Names of *Catharanthus roseus*⁸

Country	Vernacular Names
English	Cayenne jasmine, old maid, periwinkle
Hindi	Sadabahar, Sada bahar
Kannada	Batlahoo, bilikaasikanigalu, ganeshanahoo, kempukaasikanigalu
Malayalam	Banappuvu, nityakalyani, savanari, usamalari
Marathi	Sadaphool, sadaphul, sadaphuli
Sanskrit	Nityakalyani, rasna, sadampuspa, sadapushpi
Tamil	Cutkattumalli, cutukattumalli, cutukattuppu
Telugu	Billaganneru
Gujarati	Barmasi
Bengali	Noyontara

Taxonomy

Table 2: Classification of *Catharanthus roseus*⁹

Scientific Classification	
Domain	Eukarya: eukaryotes.
Kingdom	Plantae: plants.
Subkingdom	Tracheobionta: vascular plants.
Superdivision	Spermatophyta: seed plants.
Division	Magnoliophyta: flowering plants.
Class	Magnoliopsida: dicotyledons.
Subclass	Asteridae.
Superorder	Gentiananae.
Order	Gentianales.
Family	Apocynaceae: dogbane.
Subfamily	Rauvolfioideae.
Tribe	Vinceae.
Genus	<i>Catharanthus</i> G. Don
Specific epithet	Roseus (Linnaeus) G. Don.
Botanical name	<i>Catharanthus roseus</i> (Linnaeus) G. Don.

Collection of plant

Healthy plant *Catharanthus roseus* was collected from Institute of Sustainable Agro technology, Sg. Chuchuh, University Malaysia Perlis (UniMAP). The plant parts like roots, stem, leaves, and the flower was washed properly with tap water and then with distilled water for the removal of dust and sand particles. The plant was dried using the dry oven at 40 °C for two days and powdered by the electrical blender. The powdered samples were hermetically preserved in separate Polythene bags until the time of the extraction. This was used as the raw material for the extraction and phytochemical screening.

Chemicals

Ethyl acetate, ethanol, methanol, and chloroform (Fisher Scientific, UK), sulphuric acid (HmbG), ferric chloride, choline chloride, glycerol, benedict solution, ninhydrin, acetone, potassium hydroxide, and sodium hydroxide (Sigma-Aldrich, USA)

Deep eutectic solvents preparation

Deep Eutectic Solvents can be easily prepared by the heating method. In the deep eutectic solvent, choline chloride mixed with glycerol in the reaction flask at 80°C under vigorous agitation for 60 min. After that homogeneous liquid can be formed¹⁰.

Extraction of plant

The dried plant parts were used for the solvent extraction procedure. Took 10g dried plant sample and added into a flask with 150ml of a deep eutectic solvent solution. The flask was placed onto a magnetic stirrer at 50 °C for 40 minutes. The solution then filtered with the filter paper and filtered extract of the selected plant sample were taken and this used for further phytochemical analysis.

Catharanthus roseus

Flower

Leaves

Stem

Roots

Flower powder

Leaves powder

Stem powder

Roots powder

Figure 1: Stages of *Catharanthus roseus* plant extraction (flower, leaves, stem, roots)

Phytochemical Test

The standard procedure methods of Phytochemical analysis were carried out for each sample's crude extract of the plant^{11,12}.

Test for phenolic compounds and tannin

2 ml of crude extract in a test tube and add 3% ferric chloride drop by drop. The appearance of bluish black precipitate indicates the presence of phenolic compounds and tannin.

Test for saponin

First of all, 0.5ml of plant extract was diluted with 5ml of distilled water. The suspension was shaken vigorously for a few minutes. And then check if the foam developed and persisted for about 10 minutes it showed the presence of saponin.

Test for amino acids

Few drops of ninhydrin were added to 1ml of the extract. The appearance of purple colour showed the presence of amino acids.

Test for flavonoids

For flavonoids, the test took few drops of dilute sodium hydroxide added in 1 ml of extract. The intense yellow colour was produced in the plant extract, and this colour became colourless on the addition of a few drops of diluted acid that indicating the presence of flavonoids.

Test for steroids

One ml of extracts of the plant was dissolved in 10ml of chloroform and added an equal volume of concentrated sulphuric acid by sides of the test tubes. The upper layer of the solution turns red and sulphuric acid layer shows yellow colour with green fluorescence. This change in colour shows the presence of steroids.

Test for reducing sugar

Add 2ml to 3ml of the sample solution to a test tube. Add an equal volume of Benedict's solution to the test tube and swirl or vortex the mixture. Leave the test tube in boiling water for about 5 minutes and yellowish green color observed.

Test for terpenoids

1 ml of concentrated sulphuric acid added to 1 ml crude extract and heated for 2 minutes. And grayish colour would show the presence of terpenoids.

Test for quinines

1 ml of extract added to 1 ml of 1% NaOH and properly mixed. Appearance of bluish green or red indicates presence of Quinines

Fluorescence Analysis

Fluorescence analysis is an important phenomenon exhibited by various chemical constituents present in plant substances. If the material themselves are not fluorescent they may often be changed into fluorescent derivatives by reagents. Thus some crude drugs are often assessed qualitatively in this way, and it is a significant parameter of pharmacognostic evaluation¹³.

Physicochemical Study

The ash values generally represent the inorganic residue present in the medicinal plant. The ash values are important to illustrate the quality as well as purity of herbal medicine. Physicochemical constants such as the percentage of total ash content, acid insoluble ash and loss of weight on drying were calculated based on standard procedures¹⁴.

RESULTS

Phytochemical analysis of *Catharanthus roseus* roots, stem, leaves, and flower petal were carried out in an aqueous solvent using deep eutectic solvent. This study is showing the presence of phytochemicals considered as active chemical constituents. The selected plant showed the phytochemicals activity. Table 3 shows the relative frequencies of phytochemicals of selected plant.

Table 3: Phytochemical Analysis of *Catharanthus roseus* Root, Stem, Leaves and Flower

Name of phytochemicals	Root	Stem	Leaves	Flower (Petal)
Alkaloids	+	+	+	+
Amino acids	-	+	+	-
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Reducing sugar	+	+	+	+
Terpenoids	+	+	+	+
Quinines	-	-	-	-
Phenol	+	+	+	+

Fluorescence Analysis

The plant powder treated with different chemical reagents which produces characteristic colour when seen under UV light (365 nm), and it is compared with colour observed under visible light. The chemicals were used for fluorescence analysis water, methanol, ethanol, chloroform, sulphuric acid, potassium hydroxide, Acetone, and ethyl acetate. Table 4 shows the fluorescence analysis of *Catharanthus roseus*.

Table 4: Fluorescence Analysis of *Catharanthus roseus*

Treatment With Reagent	Root		Stem		Leaves		Flower (PETAL)	
	Ordinary Light	UV Light (365nm)	Ordinary Light	UV Light (365nm)	Ordinary Light	UV Light (365nm)	Ordinary Light	UV Light (365nm)
powder with methanol	light yellow	bluish green	very light green	bluish green	dark green	reddish brown	yellowish white	bluish white
powder with ethanol	light yellow	bluish green	very light green	bluish green	dark green	reddish brown	yellowish white	whitish purple
powder with chloroform	light brown	yellow	light green	bluish green	brownish green	reddish grey	off white	bluish white
powder with sulphuric acid	dark brown	blackish brown	dark brown	blackish brown	dark blackish brown	blackish brown	dark brown	blackish brown
powder with potassium hydroxide	dark yellowish	greenish brown	yellow	reddish brown	green	greenish gray	yellow	brownish yellow
powder with acetone	light yellow	light greenish brown	very light green	bluish green	green	reddish brown	light yellow	bluish white
powder with ethyl acetate	light yellow	off white	light green	reddish yellow	light green	reddish brown	off white	bluish white
powder with water	greyish yellow	turbid	turbid green	bluish green	green	green	turbid	bluish turbid

Physicochemical Study

Physicochemical parameters were evaluated, and the results are presented in Table5.

Table 5: Summary of Physicochemical Analysis

Physicochemical Constants	Root (% w/w)	Stem (% w/w)	Leaves (% w/w)	Flower (% w/w)
Total ash	0.450	0.323	0.072	0.158
Acid insoluble ash	0.161	0.219	0.033	0.101
Moisture content	3.5	3.7	2.3	2.0

DISCUSSION

Catharanthus roseus is one plant recognized well in Ayurveda. It is known for its anti-tumour, anti-diabetic, anti-microbial, anti-oxidant and anti-mutagenic effects. It is an evergreen plant first originated from islands of Madagascar. The flowers may vary in colour from pink to purple and leaves are arranged in opposite pairs. It produces nearly 130 alkaloids mainly ajmalicine, risperidone, vincristine, vinblastine, and raubasine. Vincristine and vinblastine are used for the treatment of various types of cancer such as Hodgkin's disease, breast cancer, skin cancer and lymphoblastic leukemia¹⁵. Phytochemical analysis of the medicinal plants is also important and has commercial attention in both pharmaceutical companies and research institutes for new drugs manufacturing. Thus we hope that the important phytochemicals properties acknowledged by this study in the local plant of *Catharanthus roseus* will be supportive in managing different diseases. Fluorescence analysis is a very important parameter of pharmacognostic evaluation. The selected medicinal plants are the source of the phytochemicals play a very important role in preventing a variety of diseases. The value of ash is important indices to illustrate the purity as well as the quality of the medicinal plant. Acid insoluble ash value is normally necessary to evaluate the crude drugs. Ash value indicates contamination with siliceous material e.g. sand and earth. The comparison of this with the total ash value of the sample will differentiate between variations of the natural ash and contaminating minerals of the drug¹⁶. So, to discover and develop *Catharanthus roseus* plant is crucial, especially in the view of the quickly growing need for improvement in the medicine. More so, the plant, if developed

can be of immense use to cosmetic, pharmaceutical, and industries since it contains bioactive compounds.

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