Chapter 14

Comparative study on bioactive compounds of leaves of *Carica papaya, Catharanthus roseus,* and *Callistemon viminalis* with current prospects

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ABSTRACT:- Plants are considered a good source for the exploration and discovery of new pharmaceutical compounds as well as medicines. It can be a potential drug for humans as it acts as an intermediate for the synthesis of useful medicines. Plants possess various Phytochemicals with several bioactivities such as anti-inflammatory, antioxidant, and anticancer. Therefore, the phytochemical characteristics of medicinal plants need to be studied. Some such medicinal plants are *Carica papaya*, *Catharanthus roseus*, and *Callistemon viminalis*. The Study revealed the presence of major bioactive compounds like Alkaloids, Carboxylic acids, Carbohydrates, Phenols, etc. in the three plants. Flavonoids are absent from the leaves of *Catharanthus roseus*. Steroids were absent only from the *Catharanthus roseus*. Tannin was absent from the *Callistemon viminalis* leaves. Saponins were absent from *Carica papaya* and *Callistemon viminalis*. Quinones were absent from the *Carica papaya* and *Catharanthus roseus*. The FTIR technique has been used to monitor and understand the chemical and surface chemistry of leaf extracts of these three plants.

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The present study can be used for large-scale pharmaceutical and commercial production of drugs from these important medicinal plants.

Keywords:- Bioactive Compounds, Ftir, Tlc, Medicinal Plants

INTRODUCTION

Plants are considered a unique source of structure of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. In the context of the worldwide spread of different diseases such as AIDS, chronic diseases, and a variety of cancers, an intensive search for new lead compounds for the development of novel pharmacological therapeutics is extremely important. With this view in mind, extensive studies are being carried out to know the bioactive compounds of the plants present in nature. *Carica papaya* L. also known as papaya belongs to the family Caricaceae and has nutritional and medicinal value worldwide. It is a herbaceous plant that grows well in the tropics and can reach up to 10m high[1]. Papaya fruits are consumed either raw or in processed forms such as jelly, candy, jam, and pickles[2]. In the tropics and subtropics, where it is frequently grown, the plant has long been regarded as an ethnomedicine[3,4]. Papaya seeds and leaves were linked to the improvement of diabetic mellitus, hepatic and renal problems, fertility, hyperglycemias, amoebic dysentery, and current anticancer activity[5-8]. Phytochemicals found in papaya seeds and leaves include phytosterols, tocopherols, flavonoids, alkaloids, and carotenoids, all of which have medicinal value for a variety of illnesses[2,7-12]. *Catharanthus roseus* (L) is a perennial plant that belongs to the Apocynaceae family. It is commonly seen in tropical countries and is native to Madagascar and Southern Asia[13]. The plant has spread all over tropical and subtropical parts of India and grows wild all over the plains and lower foothills in the northern and southern hills of India[14]. Also 'the Periwinkle' is a logo/ symbol of hope for cancer patients used by the National Cancer Council of Malaysia[15]. *Catharanthus roseus* (L) is an important medicinal plant cultivated by common names, which is named based on its flower colors, Pink: Rosea, White: Alba. It is an herbaceous plant or an evergreen sub-shrub growing to 32 to 80 cm in height. It has glistening, dark green leaves and flowers all summer long. The flowers appear pale pink with a purple "eye" in their centers. Erect/accumbent suffrutex, to 1m, usually with white latex. Stems are green, often permeated with purple or red. Traditionally, leaves of *Catharanthus roseus* (L) are used as medicine for the treatment of diseases[16,17]. Alkaloid-like vinblastine produced from *Catharanthus roseus* (L) is used in anti-tumor activity and for other wide pharmaceutical use[18]. For its capacity to relieve pain, *C. roseus* (L) can develop contemporary chemotherapeutic agents[19]. *Callistemon viminalis* belongs to the family Myrtaceae, consists of 34 species, and is known for its brush-like flower also called a bottlebrush. *Callistemon viminalis*, also known as weeping bottlebrush, is a small tree or shrub that is native to Australia. It typically reaches a height of around 4 meters in temperate areas[20-22]. *C. viminalis*, also known as the weeping willow or the crack willow, is commonly planted as a farm tree for forestry plantations or for ornamental purposes. It is also often used for weed control due to its fast growth and dense foliage [20,23]. *C. viminalis*, also known as the weeping willow, is indeed used in traditional Chinese medicine (TCM) for treating haemorrhoids[24,25]. In Jamaica, the hot drink locally known as 'tea' is made from the plant *C. viminalis* and has been traditionally used for treating gastroenteritis, diarrhea, and skin infections[26,27]. These are the Phytochemicals, a chemical derived from plants that helps in treating several diseases and the term indicates the number of secondary metabolites found in plants. Overall, phytochemical screening assays play a crucial role in the initial evaluation of plant extracts for their potential medicinal properties. They provide valuable information about the presence of bioactive compounds, allowing researchers to prioritize extracts for further investigation and potential development of new drugs or therapeutic agents. After obtaining the crude extract or active fraction from plant material, phytochemical screening is performed with the appropriate test protocol as shown in Tables 1 and 2 to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction. FTIR has also proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extract[28,29]. In addition, FTIR spectra of pure compounds can produce molecular information called molecular "fingerprints". For most usual plant compounds, the spectrum of an unknown compound may be diagnosed with the aid of using the evaluation of a library of recognized compounds. FTIR spectroscopy is a powerful analytical technique that uses infrared radiation to identify and characterize chemical compounds. The technique is based on the principle that different chemical bonds absorb specific frequencies of infrared radiation, resulting in characteristic absorption spectra. The development of the imaging of tissues using infrared spectroscopy is a recent advancement in the field. Infrared spectroscopy is a technique that uses the absorption of infrared light by molecules to identify and analyze their chemical composition. By applying this technique to biological tissues, researchers have been able to create images that provide valuable information about the molecular structure and composition of the tissues. Another test method, Thin-layer chromatography (TLC) can also be employed for bioactive compound analysis. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. Another test that can be done is the thin-layer chromatography (TLC) test. In this test, the plant extract is applied as a spot on a TLC plate and then developed using a suitable solvent system. The different phytochemicals present in the extract will separate and form distinct spots on the plate. These spots can be visualized by spraying with a suitable reagent or by exposing the plate to UV light. This method has also been utilized to confirm the purity and identity of isolated compounds.

MATERIALS AND METHOD

Collection and preparation of methanolic extract from plant leaves

The plant material was collected from the local areas of Ranchi, Dangratoli, and Morabadi. The plant was identified as *Carica papaya*, *Catharanthus roseus* and *Callistemon viminalis* at the University Department of Botany, Ranchi University, Ranchi. The fresh plant leaves were washed 2-3 times under running tap water, then once with distilled water and was air dried. It was kept in a hot air oven for 3 days. It was homogenized to fine powder by using an electric grinder and was kept in air-tight bottles for further use. A 5g powder sample was added in 50ml acetone and distilled water, it was kept for shaking in an orbital shaker for 72hrs at room temperature. After incubation, the extract was filtered with Whatman filter paper into a clean petri plate for the solvent to evaporate. After evaporation, the plates were weighted. Residual concentrates were dissolved in 5 ml of DMSO. The extracts were collected in screw-capped bottles. The extracts were used for antifungal activity, antibacterial activity, or phytochemical test.

Table 1: Method of extract preparation from plant materials.

	Maceration
Solvents	Methanol, Ethanol or a mixture of alcohol and water
Temperature (ºC)	Room temperature
Pressure applied	Not applicable
Time required	3-4 days
Volume of solvent required (ml)	Depending on the sample size
Reference	[30,31,32,33]

Fourier transforms infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectroscopy a popular technique today, due to its unique combination of sensitivity, flexibility, specificity and robustness. It is a widely practiced analytical instrumental technique in science that can handle solid, liquid, and gaseous samples. To organize FTIR samples, liquid samples can be placed between two plates of sodium chloride. One drop of the sample is sufficient to create a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) and then compressed into the thinnest pellet, which can be analyzed. Otherwise, solid samples may be dissolved in a solvent that include methylene chloride, and the solution is then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

Qualitative test

Table 2: Phytochemical screening assay of secondary metabolites

Secondary metabolite	Name of test	Methodology	Result(s)	Ref.
Alkaloid	Dragendorff's test	Spot a drop of extract on a small piece of percolated TLC plate. Spray the plate with Dragendorff's reagent.	Orange spot	34
	Wagner test	To the 2ml filtrate, add 1% HCl + steam. Then, add 1ml of the solution with 6 drops of Wagner's reagent.	Brownish-red precipitate	35
	TLC method 1	The solvent system used in this case is a mixture of chloroform, methanol, and 25% ammonia in a ratio of 8:2:0.5. After the TLC plate is developed, spots can be visualized by spraying it with Dragendorff's reagent.	Orange spot	36
	TLC method 2	Wet the powdered test samples with diluted NH4OH and extract with EtOAc for 24hrs at room temperature. Separate the organic phase from the acidified filtrate and basify with NH4OH (pH 11-12). Extract with chloroform (3X), evaporate, and use for chromatography. Separate alkaloid spots using chloroform and methanol (15:1) solvent mixture. Spray spots with Dragendorff's reagent.	Orange spot	37

Flavonoid	Shinoda test	Add 2-3ml of methanolic extract, along with a piece of magnesium ribbon and 1ml of concentrated hydrochloric acid.	Pink-red or red colorationof the solution	34
	TLC method	Extract 1g of powdered test samples with 10ml of methanol in a water bath at 60°C for 5 minutes. Condense the filtrate by evaporation, then add a mixture of water and EtOAc in a 10:1 ratio and mix well. Keep the EtOAc phase for chromatography. Separate the flavonoid spots using a solvent mixture of chloroform and methanol in a 19:1 ratio.	The color and hRf values of these spots can be recorded under ultraviolet (UV254nm) light	37
	NaOH test	The extract should be treated with dilute NaOH, followed by the addition of dilute HCl	A yellow solution with NaOH turns colorless with dilute HCl	38
Phenol	Phenol test	The extract will appear as a spot on the filter paper. Add a drop of phosphomolybdic acid reagent and expose it to ammonia vapors.	The blue coloration of the spot	34
Pyrrolizidine alkaloid		Prepare a 1ml oxidizing agent by combining 0.01ml of 30% w/v hydrogen peroxide stabilized with tetrasodium pyrophosphate (20mg/ml) and diluting it to 20 ml with isoamylacetate. Add this oxidizing agent to 1ml of plant extract and mix well using a vortex. Then, add 0.25ml of acetic anhydride to the mixture. Heat the sample at 60°C for 50-70 minutes and allow it to cool to room temperature. Next, add 1ml of Ehrlich reagent to the cooled sample and place the test tubes in a water bath at 60°C for 5 minutes. Measure the absorbance at 562nm. To confirm the results of the screening method, use the method of Holstege *et al.* (1995).	Peaks were compared withthe GC–MS library	39-41
Reducing sugar	Fehling test	Mix 25ml of diluted sulphuric acid (H_2SO_4) with 5ml of water extract in a test tube. Boil the mixture for 15 minutes, then let it cool. Neutralize the solution with 10% sodium hydroxide until it reaches pH 7. Finally, add 5ml of Fehling solution.	Brick red precipitate	42
	Molisch test	To 2ml of extract 2ml of Molisch reagent was added. 1ml of conc. sulphuric acid was added along the side wall of the test tube in a slanting position.	The violet color ring at the junction	
	Starch test	To 1ml of extract 1ml of iodine was added dropwise.	Blue- Black color form	
	Cellulose test	To 1ml of extract 1ml of iodine was added dropwise. Then dropwise 1ml of sulphuric acid was added.	Dark brown-red color form	
Saponin	Frothing test /Foam test	Mix 0.5ml of filtrate with 5ml of distilled water, shake thoroughly.	Persistence of frothing	43
	TLC method	To extract the test samples, reflux 2 grams of powdered samples with 10 ml of 70% EtOH for 10 minutes. Condense the filtrate and enrich it with saturated n-BuOH. Mix thoroughly and retain the butanol. Condense the butanol and use it for chromatography. Separate the saponins using a solvent mixture of chloroform, glacial acetic acid, methanol, and water (in a ratio of 64:34:12:8). Expose the chromatogram to iodine vapors.	The colour (yellow) and hRf values of these spots were recorded by exposing the chromatogram tothe iodine vapours	37

Steroid	Liebermann-Burckhardt test	Added 1 ml of methanolic extract, then 1 ml of chloroform, 2-3 ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid stepwise in a tube.	The dark green color appears	34
		To 1ml of extract, add 2ml acetic anhydride and 2ml concentrated H_2SO_4.	Blue or Green color	44
	TLC method	Extract 2 grams of powdered test samples with 10ml methanol in a water bath at 80°C for 15 minutes. Use the condensed filtrate for chromatography. Separate the sterols using a solvent mixture of chloroform, glacial acetic acid, methanol, and water in a ratio of 64:34:12:8. Record the color and hRf values of the spots under visible light after spraying the plates with an isoaldehyde-sulphuric acid reagent and heating at 100°C for 6 min.	The color (Greenish blackto Pinkish black) and hRf values of these spots can be recorded under visible light	37
Tannin	Braemer's test	Add 10% alcoholic ferric chloride to 2-3ml of methanolic extract (1:1) for clarification.	Dark blue or greenish-greycolor	34,35, 43
Terpenoid	Liebermann-Burckhardt test	Add 1ml of chloroform and 2-3ml of acetic anhydride to 1ml of methanolic extract. Then, add 1 to 2 drops of concentrated sulphuric acid.	The Pink or red coloration	34
	Salkowski test	5ml extract was added with 2ml of chloroform and 3ml of concentrated sulphuric acid H_2SO_4.	The Reddish-brown color of the interface	44
Quinones	Chloroform-Ammonia Test	Added 1ml of Concentrated sulphuric acid and 5ml of chloroform in a few ml of hot extract and kept in a boiling water bath. From that 2ml was taken and 1ml of 10% ammonia was added and shaken well.	pink-red layer indicates, anthracene derivative.	
Anthraqui-nones	Borntrager's test	In 5ml of extract, 10% ferric chloride was added and 1ml of Concentrated HCl was added. Cool and filter it. Then filtrate was shaken with diethyl ether and strong ammonia was added.	Formation of pink and deep red color	

Quantitative test

1. Total Phenolic Content

In order to determine the total phenolic content (TPC), the method described by Hinneburg et al. in 2006 was followed, with some modifications. 1 ml of diluted sample was added to 0.5 ml of Folin-Ciocalteau reagent and kept aside for 5 minutes. 2 ml of 20% Na2CO3 was then added to the reaction solution. The above solution was then kept for incubation in a boiling water bath for a few hrs. Absorbance was measured at 760nm using a 1cm cuvette UV-1800 spectrometer [Shimadzu, Japan]. Gallic acid [0-800mg/L] was used to produce a standard calibration curve. The total phenolic content was expressed in mg of gallic acid equivalent [GAE]/100ml of extract.

Plant name	Test 1	Test 2
Carica papaya	1.426	1.61
Catharanthus roseus	1.612	1.711
Callistemon viminalis	1.745	1.839

2. Determination Of Total Flavonoid Content

The total flavonoid content in the extracts was determined using the aluminum chloride colorimetric assay. A stock solution of quercetin was prepared by dissolving 4 mg of quercetin in 1 ml of methanol, resulting in a concentration of 4 mg/L.

To create various concentrations for the calibration curve, the stock solution was diluted serially. The concentrations used were 0.25 mg/ml, 0.50 mg/ml, 0.75 mg/ml, and 1 mg/ml.

In each test tube, 1 ml of quercetin solution at each concentration was added. After 5 minutes, 0.3 ml of 10% AlCl3 was added to the test tube. After 6 minutes, 2 ml of 1N NaOH was added to the mixture. The volume of the mixture was then made up to 10 ml by immediately adding 4.4 ml of distilled water. The total flavonoid content was expressed as quercetin equivalents using a linear equation based on the calibration curve. The absorbance of the mixture was measured at 510 nm using a spectrophotometer.

Plant name	Test 1	Test 2
Carica papaya	-	-
Catharanthus roseus	-	-
Callistemon viminalis	0.941	1.155

OBSERVATIONS

Table 3. Phytochemical Screening assay of Secondary metabolites of *Carica papaya, Catharanthus roseus, Callistemon viminalis*

Test	*C.papaya*	*C.roseus*	*C.viminalis*
Phenol	+	+	+
Flavonoid	-	-	+
Steroid	+	-	+
Bayer's	+	+	+
Tannin	+	+	-
Carbohydrate	+	+	+
Saponins	-	+	-
Alkaloids	+	+	+
Quinones	-	+	+
Coumarin	-	-	+
Carboxylic group	+	+	+

Fig 1: ***Carica papaya***

Fig 2: ***Catharanthus roseus***

Fig 3: ***Callistemon viminalis***

Fig 4: Showing the homogenized fine plant leaves powder

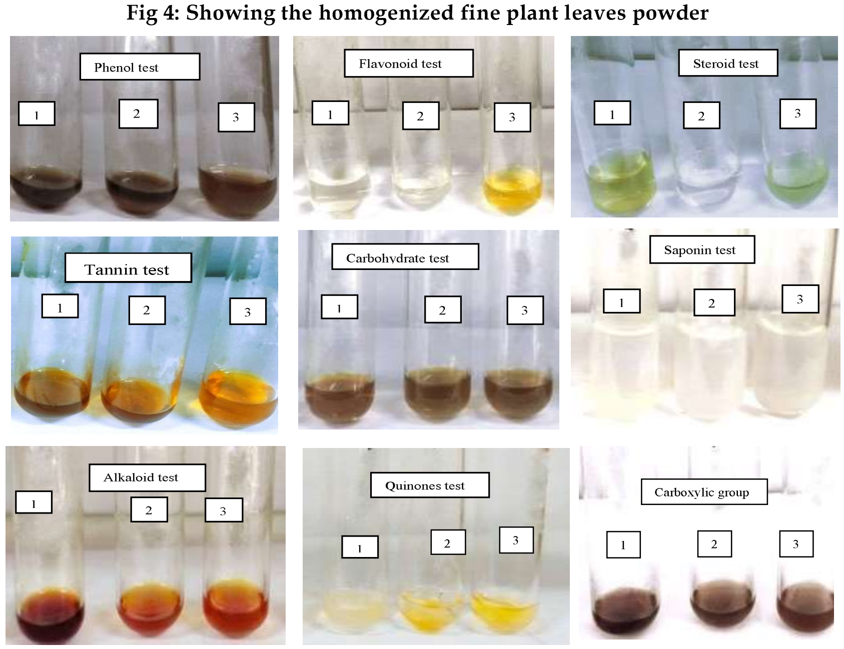

Fig 5: Above plates show different phytochemical screening assay test, as in Table 3. The number (1, 2, 3) represents the three plant extracts test i.e. ***Carica papaya, Catharanthus roseus,*** **and** ***Callistemon viminalis*** **respectively**

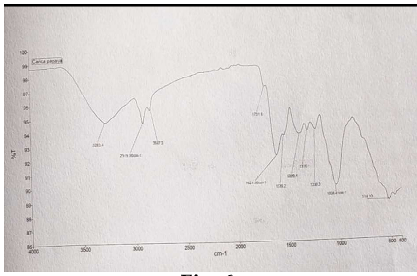

Fig. 6

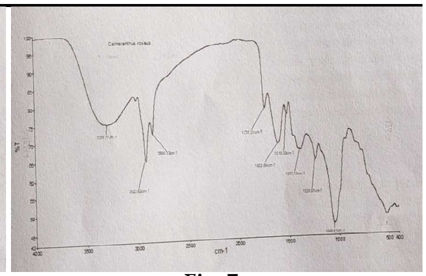

Fig. 7

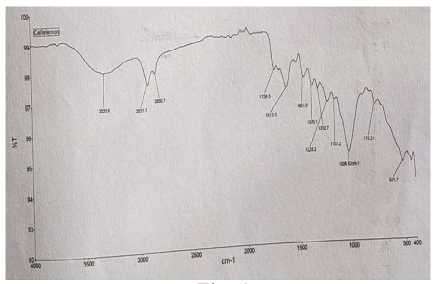

Fig. 8

Fig. 6-8: Showing FTIR test on *C. papaya, C. roseus,* and *C. viminalis* plants.

RESULT AND DISCUSSION

This study aims to present evidence-based information on *Carica papaya*, *Catharanthus roseus* and *Callistemon viminalis* leaf functional bioactivities. *Carica papaya, Catharanthus roseus,* and *Callistemon viminalis* is a plant of diverse ethnomedicinal value. *C. papaya* shows the presence of alkaloids, phenol, tannins, carbohydrates, steroids, etc and *C. roseus* shows the presence of alkaloids, Quinones, carbohydrates, tannins, phenol, saponins and carboxylic groups. Similarly, *C. viminalis* showed the presence of bioactive compounds like alkaloids, quinines, carbohydrates, steroids, flavonoids, phenols, and carboxylic groups. These leaves of *C. papaya* extracts showed the absence of Flavonoids, saponins, and quinones. *C. roseus* showed the absence of Flavonoid and steroids whereas *Callistemon viminalis* leaf extract showed the absence of tannin and saponins (Table 3). Pharmaceutical companies in the production of valuable drugs can use these bioactive compounds. These drugs from *C. papaya* can be used for the treatment of some diseases like hyperglycemia, fertility-related complications, inflammation, hypertension, and anticarcinogenic activities. *C. roseus* leaves are antitumors, pain-relieving, antioxidant, and anticancerous. The phytochemical screening of *C. viminalis* leaf extracts showed the presence of glycosides, flavonoids, alkaloids, proteins, carbohydrates, phenol, etc. has the potential biological activities. The bioactive compounds show antibacterial, antifungal, antiviral, anti-platelet aggregation, allelopathic, anti-quarum sensing, hemolytic, anthelmintic, insecticidal, and antioxidant activities (Figure 5 and Table 3). Medicinal plants play a vital role in human health care, about 80% of the world population relies on the use of traditional medicine, concomitantly based on plant materials. The antimicrobial activity was found in this present study. The Secondary preliminary phytochemical screening revealed the presence of Alkaloids, Saponins, Steroids, Tannins, Flavonoids, Proteins, and Carbohydrates in Leaves. Several studies provide support for the plant's traditional and alternative use against various diseases and infections of these three plants[3,4,14,16,17,22]. *Carica papaya* has been studied and its bioactive compounds have been found to have various beneficial effects including anticancer, gastroprotective, antioxidant, antifungal, antiparasitic, hypoglycemic, antimicrobial, contraceptive, and hepatorenal-protective activities[5,6]. *C. papaya* leaves are rich in macro and micronutrients for eg. Proteins, carbohydrates, and alkaloids making them good alternative energy sources that may complement the undernourished populations. Similarly, *C. roseus* has been discovered to be an important medicinal plant for the creation of novel pharmaceuticals as most of the bacterial pathogens were improving resistance against many of the available anti-microbial drugs. Major alkaloids like vincristine, vinblastine, catharanthamine, and vincoline were found. Other alkaloids i.e. deoxyvinblastine, leurosine, pleurosin, leurocristine, leurosidine, vincolinine, vinacardine, and roseadine were also isolated. Plants have been justified to be valuable natural resources for active chemotherapeutic agents and suggest a broad spectrum of action with a greater emphasis on preventive action[45]. FTIR (Fourier Transform Infrared) techniques are widely used in the field of membrane application to analyze and evaluate the properties and performance of membranes in various applications. This paper provides an overview of FTIR spectroscopy and its application in the biological field. It discusses the fundamental concepts related to FTIR and highlights the latest research in this area. The technique distinguishes the three selected plant samples at the molecular level (Figure 6 – 8). In recent studies, *C.*

papaya, *C. roseus,* and *C. viminalis* leaves bioactive compounds, molecular information, and confirmed safety profiles as nutraceutical food are being represented.

CONCLUSION

The medicinal plant is the most exclusive source of life-saving drugs for the majority of the world's population. They continue to be an important therapeutic aid for alleviating the ailments of humankind. In the present study, the phytochemical characteristics of medicinal plants tested were summarized. The result revealed the presence of medicinally active compounds in plants which is responsible for many pharmacological activities. It was seen alkaloids, carboxylic groups, quinones, and carbohydrates were present in the plants. Steroids and coumarins were absent from *Catharanthus roseus.* The plant studied here can be seen as a source of useful drugs. It also justifies the folklore medicinal uses and claims about the therapeutic values of these plants as curative agents. A positive result in phytochemical analysis supports the use of plants for therapeutic purposes. The identified classes from the analysis can be modified to enhance their biological activities. Bioactive compounds found in plant material are complex mixtures, making their separation and determination challenging. To isolate these compounds, a combination of chromatographic techniques and other purification methods is often required.

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