IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 6, Issue 5 (Sep. – Oct. 2020), PP 13-26
www.iosrjournals.org

Biochemical profile of *Vinca rosea* Linn (*Catharanthus roseus* G. Don)

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Abstract: *Vinca rosea Linn. (Syn Catharanthus roseus G. Don) is commonly known as periwinkle, Nayantara and Sadavahar plant. It is native to the Indian Ocean Island of Madagascar. It is an evergreen herbaceous plant growing to about 1 miter in height. Catharanthus roseus is an important medicinal which synthesizes two terpene indole alkaloids viz. vinblastine and vincristine. These alkaloids are used to treat cancer. The phytochemicals of Catharanthus roseus has been also used to treat diabetes, microbial infection, high blood pressure, hyperlipidemia, wound healing, CNS disorders etc.*

Phytochemical analysis conducted on the leaves and flowers extracts of Catharanthus roseus revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical screening of the leaves and flowers of Catharanthus roseus was done with chloroform, petroleum ether, ethanol, methanol, hexane and distilled water. Among the six solvent extracts studied the carbohydrate was present in aqueous extract of flowers as evidenced by positive Fehling's test. All the six solvent extracts of flowers showed positive Benedict's test for carbohydrate. Only the chloroform and hexane extracts of flowers showed positive Iodine test for carbohydrate. The phenols and tannins were detected only in chloroform, ethanol, methanol and hexane extracts but not in petroleum ether and aqueous extracts. All the six solvent extracts of flowers showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in four solvent extracts of flowers viz. ethanol, methanol, hexane and distilled water as evidenced by positive froth foam test. Glycosides were not detected in any of the six solvent extracts of flowers in Libermann's test, but in Salkowski and Killer- Kilani tests all the six solvent extracts showed the presence of glycosides. Phenolic compounds were detected in all extracts except petroleum ether. The steroids were recorded in all the six solvent extracts of flowers. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of flowers. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of flowers of Catharanthus roseus.

In the solvent extracts of leaves of Catharanthus roseus carbohydrates were detected in all the six solvent extracts. Fehling's test was positive in chloroform, petroleum ether, ethanol and methanol extract but negative in hexane and aqueous extracts. Benedict's test was positive in all except petroleum ether extract. Similarly, Iodine test for carbohydrates was positive in all except chloroform extract. Phenols and tannins were detected in all except chloroform and aqueous extracts. Flavonoid was detected in all except chloroform, and saponins in all except chloroform and petroleum ether extracts. Petroleum ether extract showed negative results for the presence of glycosides with all the three tests viz. Libermann's, Salkowski and Killer- Kilani. In petroleum ether and hexane extract of leaves glycosides were not detected. Similarly, glycosides were also not detected in chloroform and petroleum ether extracts as Salkowski test was found to be negative. Killer- Kilani test for glycosides was positive with chloroform, ethanol and methanol extracts but negative with petroleum ether, hexane and distilled water extracts. Phenolic compounds and steroids were detected in all the solvent extracts of leaves except hexane and distilled water extracts. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of leaves. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of leaves of Catharanthus roseus.

The leaves and flowers of Catharanthus roseus contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The leaves of Catharanthus roseus contained relativelely higher amounts of alkaloids, flavonoids and phenols than flowers. The amount of flavonoids in leaves was maximum

(47.65QE) followed by phenols (26.85GAE), alkaloids (23.75mg/gm), saponins (19.65 mg/gm) and tannins (17.35mg/gm). The amount of phytochemicals in flowers also showed a similar trend i.e. the amount of flavonoids was maximum (37.75QE), followed by phenol (22.67GAE), alkaloids (21.35mg/gm), saponins (19.75mg/gm), and tannins (17.45mg/gm).

As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from Catharanthus roseus should be emphasized for the control of various diseases. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study.

Key Words: *Catharanthus roseus, polar solvents, non-polar solvents, leaves, flowers, phytochemicals*

Date of Submission: 05-10-2020 Date of Acceptance: 19-10-2020

I. Introduction

Vinca rosea Linn. (Syn *Catharanthus roseus* G. Don) is commonly known as periwinkle, Nayantara and Sadavahar plant. It is native to the Indian Ocean Island of Madagascar. It is an evergreen herbaceous plant growing to about 1 miter in height. The leaves are oval to oblong, 2.5- 9.5 cm. long and 1-3.5 cm. broad glossy green hairless with a pale midrib and a short petiole about 1- 1.8 cm. long and they are arranged in the opposite pairs. The flowers are white to dark pink with a dark red center, with a basal tube about 2.5-3 cm. long and a corolla about 2-5 cm. diameter with 5 petal like lobes. The fruit is a pair of follicles about 2-4 cm. long and 3 mm broad (Monika Sain and Vandana Sharma, 2013) [1].

Systematic Position

Kingdom :	Plantae
Division :	Magnoliophyta (Flowering plants)
Class :	Magnoliopsida (Dicotyledons)
Order :	Gentianales
Family :	Apocynaceae
Genus :	*Catharanthus*
Species :	*C. roseus* (*Vinca rosea*)

Medicinal importance of *Catharanthus roseus*:

Catharanthus roseus is an important medicinal which synthesizes two terpene indole alkaloids viz. vinblastine and vincristine. These alkaloids are used to treat cancer (Ajaib *et al*., 2010) [2]. This plant has historically been used to treat a wide range of diseases. It is used as folk remedy for treatment of diabetes. The extract of leaves is used to treat wasp stings. The extract of dried leaves is also used to treat Hodgkin's disease. The root extract is used orally to relieve menorrhagia.

Catharanthus roseus is known to exhibit a wide range of pharmacological activities viz.

Anti-cancer activity: The Vinblastine and Vincristine are anticancer alkaloids derived from stem and leaf of *Catharanthus roseus*. These alkaloids exhibit growth inhibition effect to some human tumors. Vinblastine is used experimentally for treatment of neoplasmas and is recommended for Hodgkins disease, chorio carcinoma. Vincristine alkaloid is used for leukemia in children. Different percentage of the methanolic crude extracts of *Catharanthus* was found to show the significant anticancer activity against numerous cell types in the *in vitro* condition and especially greatest activity was found against the multidrug resistant tumor types. Vinblastine is sold as Velban or Vincristine as oncovin (Banskota, 2002; Wang et al., 2004) [3, 4].

Anti-diabetic activity: The ethanolic extracts of the leaves and flower of *C. roseus* shows a dose dependent hypoglycemic activity. The hypoglycemic activities of alkaloids have been studied pharmacologically and have been marketed under the proprietary name Vinculin as a treatment for diabetes (Chattopadhyaya, 1991; Singh *et al*., 2001; Chattopadhyaya, 1994) [5, 6, 7].

Anti-microbial activity: Crude extracts from different parts of the plant show anti-bacterial activity against microorganism like *Pseudomonas aeruginosa* NCIM2036, *Salmonella typhimuruim* NCIM2501, *Staphylococcus aureus* NCIM5021. The extracts could be used as the prophylactic agent in the treatment of many bacterial diseases (Prajakta *et al*., 2010) [8].

Anti-oxidant property: The anti-oxidant potential of the ethanolic extract of the roots of *C. roseus* has been assayed as Hydroxyl radical-scavenging activity, Superoxide radical-scavenging activity, DPPH radical-scavenging activity and nitric oxide radical inhibition method. The results exhibited the satisfactory scavenging effect in the entire assay in a concentration dependent manner (Alba Bhutkar and Bhise, 2011) [9].

Anti-helminthic activity: The anti-helminthic property of *C. roseus* has been evaluated by using *Pheretima posthuma* as an experimental model and with Piperazine citrate as the standard reference. The ethanolic extract of the concentration of 250 mg/ml was found to show significant anti helminthic activity (Swati Agarwal et al., 2011) [10].

Anti-ulcer property: Vincamine and Vindoline alkaloids shows anti-ulcer property. The alkaloid vincamine, present in the leaves shows cerebrovasodilatory and neuroprotective activity (Babulova *et al*., 2003) [11].

Hypotensive property: Extract of leaves shows significant hypotensive activity. Significant antihyperglycemic and hypotensive activity of the leaf extracts (hydroalcoholic or dichloromethane-methanol) have been reported in laboratory animals (Pillay *et al*., 1959) [12].

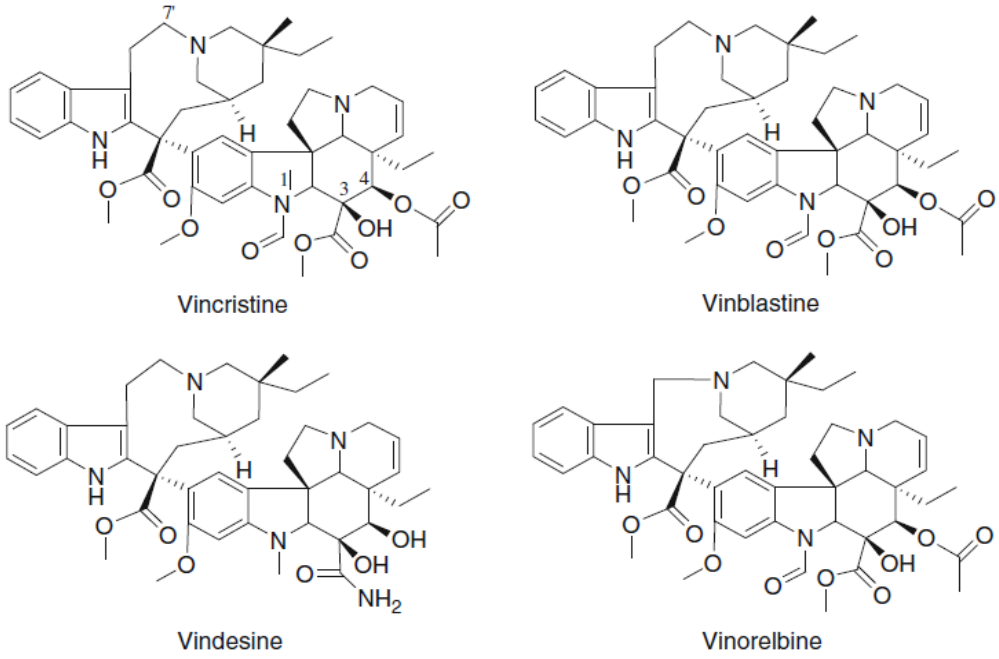
Anti-diarrheal property: The anti-diarrheal activity of the ethanolic leaf extracts has been assayed in the wistar rats and the results showed the dose dependant inhibition of the castor oil induced diarrhea (Mithun Singh Rajput *et al*., 2011) [13].

Wound healing property: Rats treated with 100 mg /kg/day of the *Catharanthus roseus* ethanol extract had high rate of wound contraction significantly decreased epithelization period, significant increase in dry weight and hydroxyproline content of the granulation tissue when compared with the controls. Wound contraction together with increased tensile strength and hydroxyproline content support the use of *C. roseus* in the management of wound healing (Nayak *et al*., 2007) [14].

Hypolipidimic effect: In a study, significant anti atherosclerotic activity as suggested by reduction in the serum levels of total cholesterol, triglycerides, LDL- cholesterol, VLDL- cholesterol and histology of aorta, liver and kidney was observed with the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. (Yogesh Patel *et al*., 2011) [15].

Memory enhancement activity: Vinpocetine has been reported to have a variety of actions that would hypothetically be beneficial in Alzheimer's disease (AD). Vinpocetine has been well tolerated at doses up to 60 mg/d in clinical trials of dementia and stroke, and no significant adverse events (Sekar, 1996) [16].

Active Phytochemicals: *Catharanthus roseus* contains a group of alkaloids that are extremely toxic, and has the ability to treat cancer. Plants synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack of insects, herbivorous mammals, fungi and bacteria. Carbohydrates, flavonoids and saponins are also present in appropriate quantities. Alkaloids are the most potentially active phytochemicals of *Catharanthus roseus.* More than 400 alkaloids are present in the plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance, food additives and pesticides. The alkaloids like actineo plastidemeric, Vinblastine, Vincristine, Vindesine, Vindeline Tabersonine etc. are mainly present in aerial parts whereas ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine etc. are present in roots and basal stem. Rosindin is an anthocyanin pigment found in the flower of *C. roseus* (Bennouna *et al*., 2008) [17]. Of the 400 alkaloids, only two viz. vincristine and vinblastin have been isolated, characterized and are widely used as chemotherapeutic agents (Cutts *et al*., 1960; Johnson *et al*., 1963) [18, 19]. Numerous other natural alkaloids were also investigated but not pursued due to severe toxicity (Creasey, 1975) [20]. Now the vinca alkaloids have become part of the standard of care for more than 30 years. A number of semisynthetic derivates have since been identified and tested. Two of these, vindesine and vinorelbine, are currently used in clinical practice. A third, vinflunine, is presently in phase III clinical trials (Bennouna *et al*., 2006; Campone *et al*., 2006) [21, 22]. Structure of some important vinca alkaloids is illustrated by molecular structures:



The molecular structures of the four vinca alkaloids viz. Vincristine, Vinblastine, Vindesine and Vinorelbine

These compounds are commonly administered as sulfate salts to enhance solubility and increase stability. All members of this family of molecules enact their cytotoxic activity primarily by binding to tubulin and inhibiting polymerization or extension of microtubules. Microtubules are crucial for a wide range of cellular activities, including mitotic spindles formation necessary for cell division. The naturally occurring vinca alkaloids have been used in the treatment of a wide range of malignancies, most prominently hematological cancers such as leukemia and lymphoma, but also testicular cancer. The semi-synthetics have exhibited clinical activity against lung, ovarian, and breast malignancies.

Chemically the vinca alkaloids contain both an indole nucleus (catharanthine portion) and a dihydroindole nucleus (vindoline portion) connected by a carbon-carbon ring with variable substituents attached to the rings. Vincristine differs from vinblastine, vindesine, and vinorelbine in having an acetaldehyde group at the nitrogen atom at position one in the vindoline nucleus instead of a methyl group. Vincristine, vinblastine, and vinorelbine all have a methyl ester moiety attached to carbon 3 in the vindoline nucleus while vindesine has an amide attached at this same site. Vincristine, vinblastine, and vinorelbine are all acetylated at carbon 4 while vindesine has a hydroxyl group. Vinorelbine also has a different structure in the catharanthine portion of the molecule with the 11-membered ring being replaced with a 10-membered ring by the elimination of carbon 7'.

Mechanism of action of Vinca alkaloids: The vinca alkaloids interact with tubulin thereby disrupting the mitotic spindle apparatus (Na GC and Timasheff, 1982; Lobert *et al*., 1996; Himes, 1991) [23, 24, 25]. Tubulin is a heterodimer of α-tubulin and β-tubulin each with a molecular weight of 55 kDa. The heterodimers polymerize to form microtubules which are involved in mitosis and meiosis through the formation of the spindle apparatus which separates the chromosomes. In addition microtubules are involved in cell shape, axonal transport, and secretion (Luduena *et al*., 1977) [26]. The biological function of microtubules is determined largely by their polymerization dynamics (Waterman-Storer and Salmon, 1997) [27]. The two main types of dynamic behavior are "dynamic instability" and "tread milling." The assembly and disassembly of the microtubule polymers are regulated by the binding of tubulin and guanosine 5-triphoshpate (GTP) (Mitchison, 1993) [28]. All microtubules have a plus end of the microtubule that polymerizes faster and thereby grows faster than the opposing minus end. Dynamic instability is characterized by changes in the length of the microtubule structure, primarily at the plus end whereas tread milling is characterized by the movement of cellular components along a tubule that is maintained at constant length, with equal addition at the minus end and subtraction at the plus end. It has been suggested that tread milling might be particularly important in mitosis (Chen and Zheng, 2004) [29]. In mitosis the microtubules form the spindle apparatus which aligns the chromosomes along the metaphase plate and then pulls the chromosomes apart during the mitotic process.

All the vinca alkaloids bind to tubulin with high affinity and inhibit further polymerization. Since microtubules are in a constant dynamic state of polymerization and depolymerization the inhibition of polymerization by the vinca alkaloids functions to create a state of net depolymerization. The interaction of the vinca alkaloids with the microtubules of the spindle apparatus disrupts the spindle apparatus and leads to metaphase arrest. Vinorelbine, vincristine, and vinblastine have all been shown to possess roughly equal tubulin binding constants (Lobert *et al*., 1996) [30] and cause metaphase block at roughly the same concentrations. It has been suggested that the differences in the relative potencies of the vinca alkaloids may not be due to their binding efficiencies but rather to differences in their intracellular retention times or the stability of the drug-tubulin complexes (Singer and Himes, 1992) [31]. In addition, vincristine is a much more potent inhibitor of axonal microtubule formation (Binet et al., 1990) [32]. While the disruption of the mitotic process is the key feature of the vinca alkaloids the final effect of this metaphase arrest is the death of the cell through activation of apoptotic pathways (Tsukidate et al., 1993; Harmon et al., 1992) [33, 34]. In vitro experiments with these agents have shown that exposure can lead to apoptosis through both p53-dependent and p53-independent pathways (Li et al., 1998; Yu et al., 1997; Fan et al., 1998) [35, 36, 37]. Tumor cells that have been exposed to the agents show characteristic morphological and molecular changes that are associated with the induction of apoptosis in a dose and time dependent fashion. Since the drugs attempt to induce apoptosis by halting the cell in mitosis, cytotoxicity is strongly dependent on the duration of exposure. A number of other cellular effects beyond microtubule inhibition have also been reported for the vinca alkaloids. These include inhibition of amino acid metabolism (Cline, 1968) [38], calmodulin-dependent Ca^{2+} ATPase activity (Watanable and West, 1982) [39], nucleic acid synthesis (Creasey, 1975) [40].

The aim of the present study is to analyse the important phytochemicals in the six different solvent extracts of and leaves of *Catharanthus roseus* qualitatively as well as quantitatively.

II. Materials and Methods

Fresh leaves and flowers of *Catharanthus roseus* were collected in the month of February 2020 from the campus of College of Commerce (Patna). The collected plant materials were brought to the laboratory on the same day. Plant samples were washed with water and air–dried at room temperature for 7 days, oven – dried at 40 °C to remove the residual moisture. The dried leaves and flowers were powdered using a mixer grinder and stored in air-tight container for future use. Six different solvents such as Chloroform, Petroleum ether, Ethanol, Methanol, Hexane and Distilled water were used for extraction. About 1 gm of the plant samples were added respectively into the test tubes containing 5 ml solvents, and were extracted at room temperature. The extracts in all the six solvents of leaves were tested for the presence of biological compounds following standard methods.

Qualitative estimation of Phytochemicals: Qualitative analysis of phytochemicals was done for carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, cardiac glycosides and alkaloids

Test for Carbohydrates

Fehling's test: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Iodine test: Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for Phenols and Tannins: Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue–green or black coloration indicated the presence of phenols and tannins.

Test for Flavonoid

Alkaline reagent test: Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Saponins (Frothing test): Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for Glycosides

Liebermann's test: Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test (Cardiac Glycosides): Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the inter phase indicated the presence of cardiac glycoside.

Test for Alkaloids: The crude extract of all the six solvents was boiled in 10 ml methanol and filtered separately. 1% HCl was added followed by 6 drops of Dragendroff reagent, and the brownish-red precipitate was taken as evidence for the presence of alkaloids.

Phlobatannins: The deposition of a red precipitate denoted the presence of phlobatannins when crude extract of all the six solvent of plant material was dissolved in 10 ml of aqueous extract and few drops of 1% HCl were added in the boiling tube.

Anthraquinones: All the six solvent extracts of leaves were boiled in 10% HCl for 5 minutes separately and the filtrate was allowed to cool. An equal volume of $CHCl_3$ with few drops of 10% NH_3 was added to the 2ml filtrate. The formation of rose-pink colour implies the presence of anthraquinones.

Quantitative estimation of phytochemicals: Phytochemicals were determined quantitalively for alkaloids, flavonoids, tannins, saponins and total phenols.

Determination of Alkaloids: Alkaloids content was measured by method suggested by Harborne (Harborne, 1973) [41]. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

Determination of Flavonoids: The flavonoids content was also determined by Harborne (Harborne, 1973) [41] method. 5 gm of leaves and flowers were boiled in 2M HCl separately for 30 min under reflux condition and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as Quercetin equivalents (QE) in mg/100 gm.

Determination of Tannins: The finely powdered leaves and flowers of *Catharanthus roseus* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80oC in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins: 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

Determination of total phenols

Five gms of the powdered leaves and flowers were boiled separately with 50 ml of ether for 15 minutes and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

For measuring alkaloids a suspension was prepared by dispersing 5 gm of the dried leaves and flowers separately in 10% acetic acid solution in ethanol and kept at 28^0C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous NH_4OH were added. Finally, the precipitate was washed with 1% ammonia solution and dried at 80^0C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

For determining flavonoids 5 gm of leaves were boiled separately in 2M HCl for 30 min under reflux and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g.

For measuring tannin the finely powdered leaves and flowers of *Catharanthus roseus* were kept separately in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80^0C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

For determining saponin content 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered leaf samples separately and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 minutes for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

For determining total phenolic content five gms of the powdered leaves of *Catharanthus roseus* were boiled with 50 ml of ether for 15 minutes and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

Quantitative analysis of phytochemical constituents in six different solvent extracts

Six solvent extract of leaves and flowers of *Catharanthus roseus* viz. chloroform, petroleum ether, ethanol, methanol, hexane and distilled water were prepared by soaking 10gm of the powdered sample in 200 ml of each of the solvent separately for 12 hrs. The extracts were then filtered using filter paper. The extracts were then concentrated to ¼ of the original extracts i.e. 50 ml.

The amount of total phenolics in both leaves and flower extracts was determined by the Folin–Ciocalteu method. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate and the mean values with ± SD are presented.

The aluminium chloride colorimetric method was used for flavonoids determination. Each solvent extract (0.5 ml of 1:10 gm ml^{-1}) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quercetine equivalent (QE) (200-1000µg) plotted by using the same procedure and total flavonoids was expressed as Qeurcetin Equivalent (QE) equivalents in mg per gm sample.
The results obtained have been presented in Table- 1, 2, 3and 4; Fig- 1, 2 and 3.

Table- 1: Qualitative phytochemical analysis of flowers of *Catharanthus roseus*

Tests		Extracts used					
		Chloroform	Petroleum ether	Ethanol	Methanol	Hexane	Distilled water
Carbohydrates	Fehling's test	-	-	-	-	-	+
	Benedict's test	+	+	+	+	+	+
	Iodine test	+	-	-	-	+	-
Phenols and Tannins	Ferric chloride test	+	-	+	+	+	-
Falavonoids test	Alkaline reagent test	-	-	-	-	-	-
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	-	-	-	-	-	-
	Salkowski test	+	+	+	+	+	+
	Killer- Killani test	+	+	+	+	+	+
Phenolic compounds		+	-	+	+	+	+
Steroids		+	+	+	+	+	+
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+
	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table- 2: Qualitative phytochemical analysis of leaves of *Catharanthus roseus*

Tests		Extracts used					
		Chloroform	Petroleum ether	Ethanol	Methanol	Hexane	Distilled water
Carbohydrates	Fehling's test	+	+	+	+	-	-
	Benedict's test	+	-	+	+	+	+
	Iodine test	-	+	+	+	+	+
Phenols and Tannins	Ferric chloride test	-	+	+	+	+	-
Falavonoids test	Alkaline reagent test	-	+	+	+	+	+
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	+	-	+	+	-	+
	Salkowski test	-	-	+	+	+	+
	Killer- Killani test	+	-	+	+	-	-
Phenolic compounds		+	+	+	+	-	-
Steroids		+	+	+	+	-	-
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+
	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table- 3: Quantitative estimation of phytochemicals in leaves and flowers of *Catharanthus roseus* (amount in mg/gm)

Phytochemicals	Leaves	Flowers
Alkaloids	**23.75±0.27**	**21.35±0.43**
Flavonoids	**47.65±0.45**	**37.75±0.32**
Phenols	**26.85±0.35**	**22.67±0.21**
Saponins	**19.65±0.31**	**19.75±0.32**
Tannins	**17.35±0.21**	**17.45±0.31**

Mean ± SD of five measurements

Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm

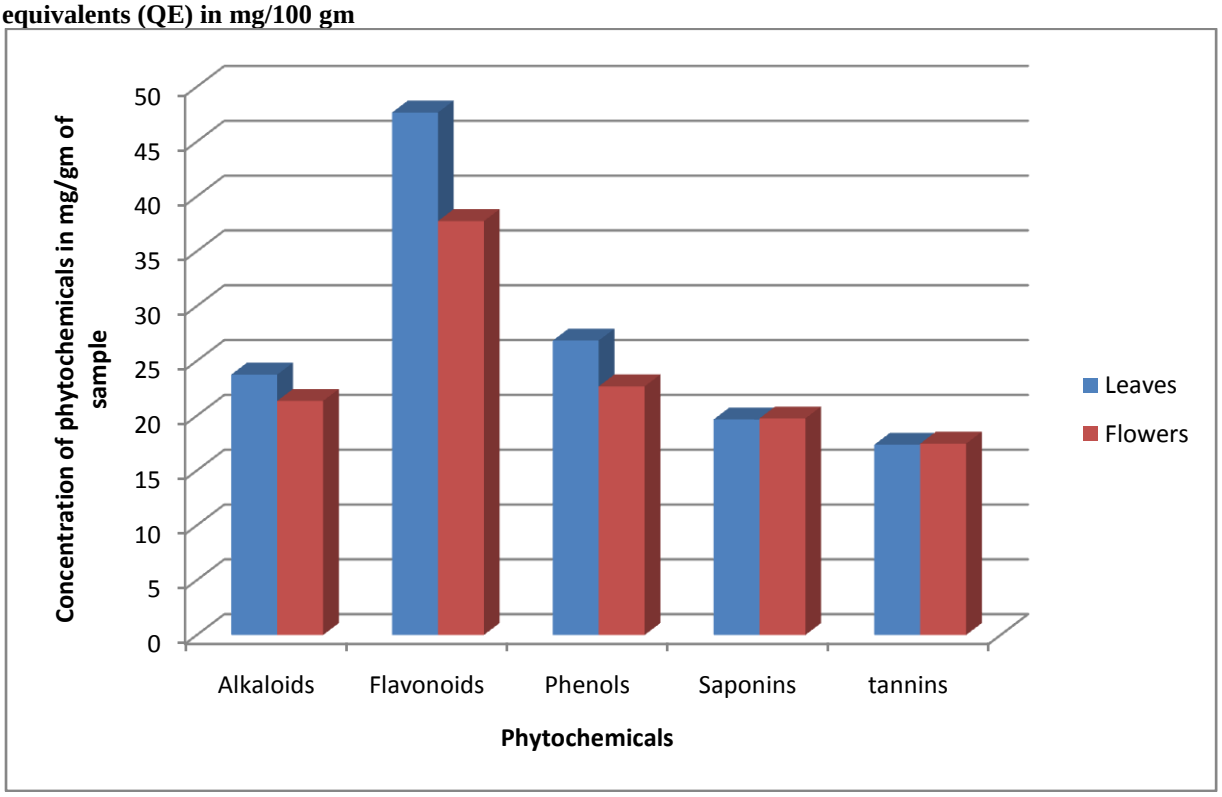


Figure-1: Amount of phytochemicals in leaves and flowers of *Catharanthus roseus*

Table- 4: Comparative analysis of total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts of bark and leaves of *Saraca indica* (amount in mg/gm)

Solvents	Extract type	Total alkaloids	Total flavonoids	Total phenols	Total saponins	Total tannins
Chloroform	Leaf	16.35±0.21	4.35±0.21	41.36±0.51	10.15±0.31	15.45±0.18
	Flower	13.66±0.32	6.75±0.25	38.67±0.54	8.45±0.21	12.65±0.21
Petroleum ether	Leaf	13.71±0.71	5.15±0.17	45.31±0.61	11.43±0.31	11.55±0.18
	Flower	11.25±0.22	5.75±0.18	35.35±0.71	10.45±0.24	12.45±0.12
Ethanol	Leaf	38.35±0.16	7.35±0.32	120.75±0.70	75.25±0.19	65.75±0.15
	flower	35.65±0.15	7.58±0.31	145.35±0.63	64.37±0.17	55.67±0.17
Methanol	Leaf	40.45±0.17	6.75±0.71	117.98±0.71	75.45±0.16	51.35±0.16
	Flower	35.35±0.21	6.86±0.61	145.37±0.63	65.35±0.13	57.65±0.31
Hexane	Leaf	31.65±0.31	7.15±0.17	42.28±0.60	63.65±0.15	14.75±0.15
	Flower	25.36±0.23	4.85±0.18	35.35±0.62	13.37±0.21	11.70±0.14
Distilled water	Leaf	45.65±0.31	7.45±0.31	123.76±0.51	75.35±0.19	67.45±0.19
	Flower	43.45±0.17	7.75±0.24	148.37±0.42	65.75±0.17	65.27±0.22

Mean ± SD of five measurements

Phenols are expressed as Gallic acid equivalent (GAE) and Flavonoids are expressed as Quercetin equivalents (QE) in mg/100 gm

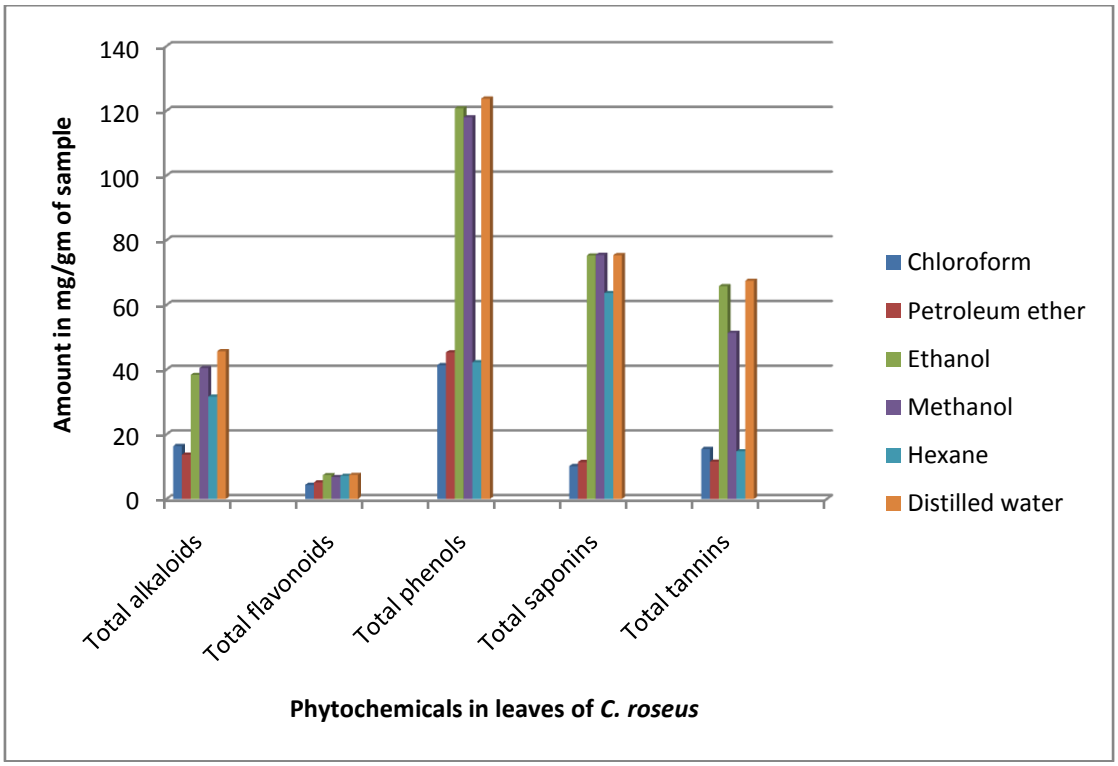


Figure-2: Phytochemicals in six different solvent extracts of leaves of *Catharanthus roseus*

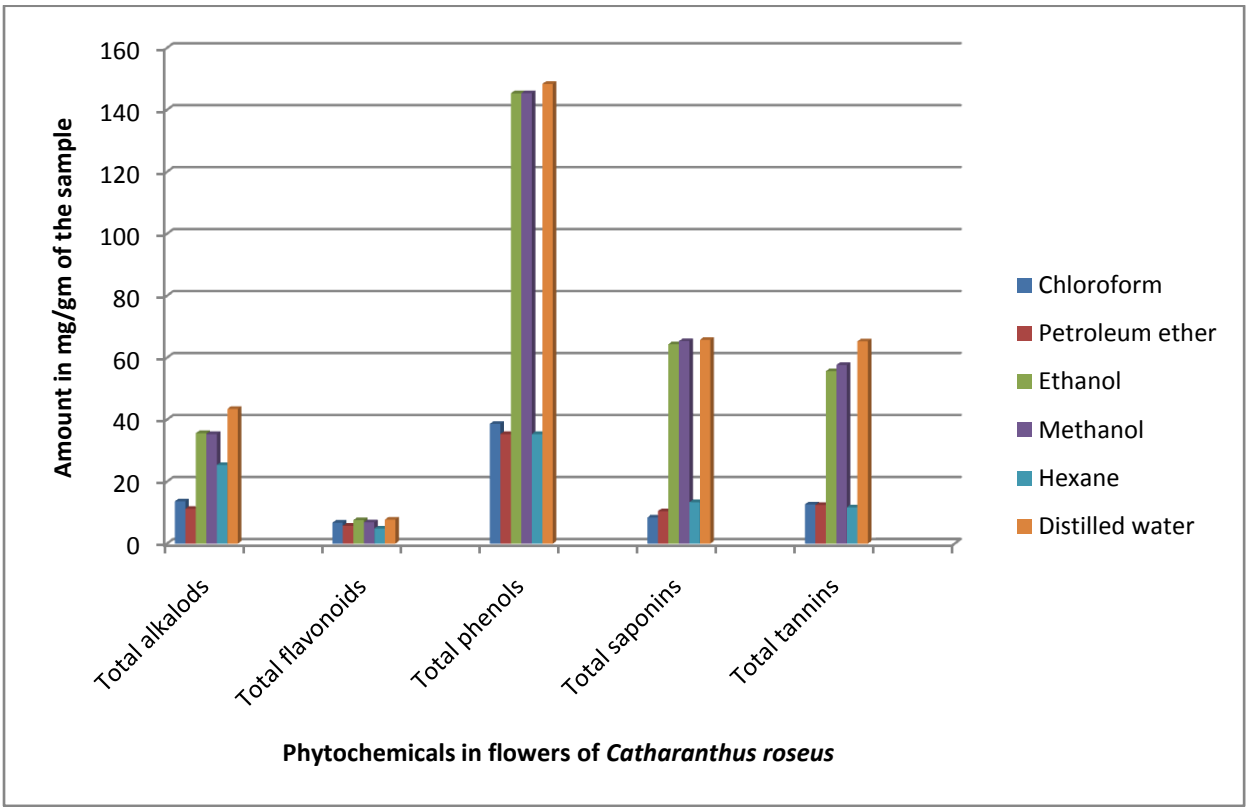


Figure-3: Phytochemicals in six different solvent extracts of flowers of *Catharanthus roseus*

III. Results

Phytochemical analysis conducted on the leaves and flower extracts of *Catharanthus roseus* revealed the presence of phytochemicalzs which are known to exhibit medicinal as well as physiological activities. The phytochemical screening of the and leaves and flowers of *Catharanthus roseus* was done with chloroform, petroleum ether, ethanol, methanol, hexane and distilled water. Among the six solvent extracts studied the

carbohydrate was present in aqueous extract of flowers as evidenced by positive Fehling's test. All the six solvent extracts of flowers showed positive Benedict's test for carbohydrate. Only the chloroform and hexane extracts of flowers showed positive Iodine test for carbohydrate (Table- 1). The phenols and tannins were detected only in chloroform, ethanol, methanol and hexane extracts but not in petroleum ether and aqueous extracts. All the six solvent extracts of flowers showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in four solvent extracts of flowers viz. ethanol, methanol, hexane and distilled water as evidenced by positive froth foam test. Glycosides were not detected in any of the six solvent extracts of flowers in Libermann's test, but in Salkowski and Killer- Kilani tests all the six solvent extracts showed the presence of glycosides. Phenolic compounds were detected in all extracts except petroleum ether. The steroids were recorded in all the six solvent extracts of flowers. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of flowers. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of flowers of *Catharanthus roseus* (Table- 1).

In the solvent extracts of leaves of *Catharanthus roseus* carbohydrates were detected in all the six solvent extracts. Fehling's test was positive in chloroform, petroleum ether, ethanol and methanol extract but negative in hexane and aqueous extracts. Benedict's test was positive in all except petroleum ether extract. Similarly, Iodine test for carbohydrates was positive in all except chloroform extract (Table- 2). Phenols and tannins were detected in all except chloroform and aqueous extracts. Flavonoid was detected in all except chloroform, and saponins in all except chloroform and petroleum ether extracts. Petroleum ether extract showed negative results for the presence of glycosides with all the three tests viz. Libermann's, Salkowski and Killer-Kilani. In petroleum ether and hexane extract of leaves glycosides were not detected. Similarly, glycosides were also not detected in chloroform and petroleum ether extracts as Salkowski test was found to be negative. Killer-Kilani test for glycosides was positive with chloroform, ethanol and methanol extracts but negative with petroleum ether, hexane and distilled water extracts. Phenolic compounds and steroids were detected in all the solvent extracts of leaves except hexane and distilled water extracts. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of leaves. Similarly the phytosterols were present in all extracts except ethanol and methanol extracts of leaves of *Catharanthus roseus* (Table- 2).

From the results (Table- 3; Fig- 1) it is evident that the leaves and flowers of *Catharanthus roseus* contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The leaves of *Catharanthus roseus* contained relativelely higher amounts of alkaloids, flavonoids and phenols than flowers. The amount of flavonoids in leaves was maximum (47.65QE) followed by phenols (26.85GAE), alkaloids (23.75mg/gm), saponins (19.65 mg/gm) and tannins (17.35mg/gm). The amount of phytochemicals in flowers also showed a similar trend i.e. the amount of flavonoids was maximum (37.75QE), followed by phenol (22.67GAE), alkaloids (21.35mg/gm), saponins (19.75mg/gm), and tannins (17.45mg/gm) (Table- 3; Fig- 1).

The phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts from leaves and flowers of *Catharanthus roseus* has been presented in Table- 4 and Fig- 2 and 3. In all the six solvent extracts it was found that the leaves of *Catharanthus roseus* contained higher amount of phytochemicals in comparison to flowers. The concentration of total alkaloids in leaves was maximum in distilled water extract (45.65mg/gm), followed by methanol extract (40.45mg/gm), ethanol extract (38.35mg/gm), hexane extract (31.65mg/gm), chloroform extract (16.35mg/gm), petroleum ether extract (13.71mg/gm). Similarly, the concentration of total alkaloids in flowers also showed a more or less similar trend, being maximum in distilled water extract (43.45mg/gm), followed by methanol extract (35.35mg/gm), ethanol extract (35.65mg/gm), hexane extract (25.36mg/gm), chloroform extract (13.66mg/gm) and petroleum ether extract (11.25mg/gm). In all the six solvent extracts it was found that the flowers contained relatively less amount of total alkaloids in comparison to leaves. The amount flavonoids was comparatively less in all the six solvent extracts of both leaves and flowers. The leaves contained 4.38 to 7.45 QE of flavonoids. Similarly, the flowers contained 5.75 to 7.45 QE of flavonoids. The amount of total phenols was highest in ethanol, methanol and distilled water extracts of both leaves and flowers. The distilled water extracts of leaves and flowers contained 123.76GAE and 148.37GAE respectively; of ethanol extracts contained 120.75GAE and 145.35GAE respectively and of methanol extracts 117.98GAE and 145.37GAE respectively of total phenols. Other extracts contained lesser amount of total phenols in leaves and flowers. The concentration total saponin and total tannins was maximum in ethanol, methanol and distilled water extracts of both leaves and flowers in comparison to non polar solvents viz. chloroform, petroleum ether and hexane which contained less amount of total saponins and tannins in both leaves and flowers. The total saponin concentration in leaves and flowers was maximum in distilled water extract (75.35mg/gm and 65mg/gm respectively), followed by methanol extract (75.45mg/gm and 65.35mg/gm respectively) and ethanol extract (75.25mg/gm and 64.37mg/gm respectively). Other extracts

contained relatively very low amount of total saponins in both leaves and flowers. A more or less similar pattern for total tannins was recorded in all the six solvent extracts, being maximum in polar solvents viz. distilled water, ethanol and methanol extracts and lesser in non polar solvents i.e. chloroform, petroleum ether and hexane extracts (Table- 4; Fig- 2 and 3).

IV. Discussion

The present investigation revealed that the non polar solvents viz. chloroform, petroleum ether, ethanol, methanol and hexane dissolve many hydrophilic and lipophilic components of plants. Distilled water is universal solvents for a large number of phytochemicals and is largely used for bioassay because of its low toxicity. It is a very useful extractant, especially for antimicrobial studies where more phenolic compounds are required to be extracted. A study reported by Das *et al*., 2010 [42] revealed that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol. The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. It means that they are more efficient in cell walls and seed degradation which have nonpolar character and cause polyphenols to be released from cells. More useful explanation for the decrease in activity of aqueous extract can be ascribed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts, whereas in methanol and ethanol they are inactive. The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing 70% ethanol the polarity of solvent was increased (Bimakr, 2010) [43]. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang 2010) [44]. In the leaves and flowers of *Catharanthus roseus* maximum results such as carbohydrates, phenols, flavanoids, saponins, glycosides and steroids were seen in ethanol in the present study (Table- 1 and 2). The present observations are supported by Kabesh et al., (2015) [45] who have screened a more or less similar phytochemicals from leaves and flowers of *Catharanthus roseus*. The present findings also gain support from the work of Gomathi and Anuradha (2012) [46] who analyzed qualitatively and qualitatively a more or less similar phytochemicals from the leaves of *Vinca rosea using* thin layer chromatography (TLC) technique.

Alkaloids are major chemical compound present in leaves of *Vinca rosea*. Alkaloids are important defence of the plant against pathogenic organism and herbivores. It is also toxic for insects which further modify the alkaloids and incorporate them into their own defence secretion (Khanuja *et al*., 2002) [47]. Flavonoids have been reported to expert multiple biological effects such as, anti-inflammatory, anti-allergies, anti-viral and anti-cancer activities. Tannins are an important ingredient in the process of tanning leather. Oak bark, Momosa and some other plants are the primary source of tannery tannin, though inorganic tanning agents are also in use today and account for 90% of the world's leather production (Marion Kite and Roy Thomson, 2006) [48]. Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources. Specifically, they are amphipathic glycosides and produce when shaken in aqueous solutions.

V. Conclusions

The present study revealed the presence of various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids in the leaves and flowers of *Catharanthus roseus*. *Catharanthus roseus* has many medicinal uses and is a nontoxic traditional medicinal plant. Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. In the Ayurvedic system of medicine, herbal extracts instead of purified compounds have been used since centuries because many constituents with more than one mechanism of action are considered essential for the required holistic therapeutic action. *Catharanthus roseus* has many known medicinal uses and is a nontoxic traditional medicinal plant. The use of phytochemicals of *Vinca rosea* against diseases is a challenge in the development of modern drug discovery. This versatile plant is the source of various types of compounds. In the present scenario many plant are used to treat many diseases. But catharanthus roseus is ancient and reliable source of medicine so this plant is used in many pharmacological activities. It has many uses like to treat cancer, diabetes, microbial infection, wound healing, CNS function, genitor-urinary functions etc. As the global scenario is now changing towards the use of nontoxic plant product having traditional medicine use, development of modern drug from *Catharanthus roseus* should be emphasized for the control of various diseases. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study. The bioactive phytochemicals of *Catharanthus roseus* responsible for various pharmacological purposes require further investigation at scientific level.

Acknowledgement: Authors are thankful to Dr Baidyanath Kumar, Visiting Professor, Department of Biotechnology, Patna Science College, Patna University for providing necessary suggestions and support for the preparation of this manuscript.

Conflict of interest: Authors declare no conflict of interest directly or indirectly.

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Dr. Baidyanath Kumar, et. al. "Biochemical profile of Vinca rosea Linn (*Catharanthus roseus* G. Don)." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 6(5), (2020): pp. 13-26.