Photochemical Analysis of Catharanthus Roseus (Periwinkle)

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Abstract

The present study was made to investigate the phytochemical analysis of Catharanthus Roseus (Periwinkle) plant extract. The physical parameters determined were pH, total ash, acid soluble ash, acid insoluble ash value, water extract, chloroform extract. The phytochemical analysis were also performed & the results of phytochemical screening of the aqueous leaves extract revealed the presence of alkaloids, flavonoids, proteins, tannin & Phenolic compound. The FTIR Spectra of total water Extractives were performed. The present study clearly indicates that the compounds like alkaloids, flavones, terpenoids and saponins are the active principles present in the plant samples. The FTIR spectra analysis in Catharanthus Roseus (Periwinkle)Plant Extract gives an idea about the different functional groups were present in this plants and it can be isolate and can be use the active components of this natural plant for further drug preparation.

Key words: total ash, water extracts, chloroform extract, UV fluorescence, alkaloids, flavonoids, Proteins, tannin.

Introduction:

The recent trend shows increasing interest in traditional medicines and there has been an increasing demand for more drugs from plant sources. Biologically active compound from natural source has always been of great interest to scientist working on infectious disease[1,2,3] Efficiency of herbal medicines has been proved in Ayurveda. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious disease.[4-8]The most important of these are flavonoids, tannins and Phenolic compound.[10]It is a well known fact that plant produces various chemicals for self defense.

fig no 1 : Catharanthus roseus (L.) .Don.

Catharanthus roseus (L.) .Don. : Common name is Vinca rosea, Periwinkle, Sadabahar, Sadaphul, Nayantra, Nityakalyani, Old maid. It belongs to the Kingdom Plantae ,Phylum Magnoliophyta, Class Magnoliopsida, Family Apocynaceae. Catharanthus roseus is a perennial, evergreen herb and is best grown as an annual bedding plant in well drained sandy loam in full sun to part shade. It is flowering plant, white,pink,red,yellow are commonly found in garden area.[11-12]

Medicinal uses:

1) Used against malaria [16]
2) The infusion of the leaves is given in the treatment of menorrhagia
3) The alkaloids like ajmalicine, serpentine and reserpine have hypotensive, sedative and ranquillizing properties. Therefore, the plant is used in the treatment of hypertension and mental disorders like depression and insanity.[17-19]
4) Herbal remedies made with periwinkle help in toothache, memory loss and blood circulation.
5) The alkaloids also inhibit the growth of Vibrio cholerae (Cholera causing bacterium).[20]

Materials and Methods:

Sampling and Collection of leaves: The plant samples was collected from the Himayat Bagh of Aurangabad M.S India . It was washed 2-3 times with d/w and air dried in open air. It was kept in plastic bag in refrigerator at 4^{o}c till it was analyzed. All the chemicals used in analysis was AR Grade. All the Apparatus used was first washed with d/w. The physical parameters includes pH, total ash ,acid soluble ash ,acid insoluble ash, water extraction, methanol extraction, Fluorescent test was done with the crude plant powder by the procedures given in the Ayurvedic pharmacopoeia of India . FTIR screening was carried out by using aqueous extract. The preliminary phytochemical analyses was done by using water extraction and Chloroform extract followed by the standard procedures [20,21].

Determination of pH range : 5gm of the sample weighted and immersed in 100ml of water in a beaker and the pH of the formulation was determined using a calibrated pH meter. [18] as shown in table no 1

Determination of Total ash: Accurately 5 gm of sample was taken in finely clean & previously weighed silica crucible and ignited for 3-4 hrs with gradually increasing in temperature up to 500^{o}C. After ignition of leaves crucible was cooled in a desiccator and weighed as total ash. The ash was used to determine the acid soluble ash and acid insoluble ash.

Determination of Acid insoluble ash: The ash was dissolved in 2N hydrochloric acid in the beaker. Stirred well for the digestion of ash and filtered through whatmann filter paper number 41. The residue remains after filtration is ignited in clean silica crucible by gradually increasing temperature up to 500^{o}C. The crucible with the residue was cooled in desiccators and weighed. The residue remains after ignition was calculated as acid insoluble ash in percentage. From this calculation the acid insoluble ash was calculated by taking the difference between the Total ash & acid insoluble ash in percentage as shown in table no 2

Fluorescence Analysis: The Fluorescence property of the sample was observed both in visible and ultra-violet light for their fluorescence characters (short wave length 254nm and long wave length

365nm) powered leaf sample was taken with different solvents and florescence property was noted . [19]as shown in table no 3

Water extraction: Accurately weight 10gm of sample was introduced into the 500ml round bottom flask with 100ml of double distilled water. The sample was refluxed on flame for six hrs. The sample was cooled and filtered by the suction pump. The excessive water was evaporated for the preservation of the sample and it was kept at 40C for 12 hrs. The percentage of the extracts was calculated as shown in table no 4

Microwave Extraction (Methanol):10 gm of the sample was kept in the clean round bottom flask. Approximately 100ml methanol was used as a solvent for extraction0. The sample was refluxed by microwave radiations using microwave oven for 20-30 min. at 50% power, 420 watt and 150^{o}C. The sample was cooled and filtered by the suction pump. The excessive solvent was evaporated & it was kept for 12hrs.The extract was weighed and calculated in percentage as shown in table no 4

Chloroform and petroleum ether extracts: 10 gm of the sample was kept in the two clean beaker 100ml each solvent was added in each beaker respectively. The sample was soaked in solvent for 12hr at room temperature. It is than filtered .The excessive solvent was evaporated. The extract was weighed and calculated in percentage as shown in table no 4

Preliminary phytochemical screening : The aqueous, Chloroform, methanol and petroleum ether extracts were used for preliminary phytochemical analyses using standard procedures [20,21].

a) Test for alkaloids:

Wagner's test: About 10 mg of extract was taken and few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

b) Test for Flavonoids: Shinoda Test:10mg of extract was added to pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. Formation of pink colour indicates the presence of Flavonoids.

c) Test for Phenols & Tannins:

Lead acetate test: 10mg of extract was taken and 0.5 ml of 1%leadacetate solution was added & the formation of precipitate indicates the presence of tannin and Phenolic compounds.

Ferric chloride test: 5mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Sodium hydroxide test: 5mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

d) Test for steroids and sterols:

Salkowski's test: 5mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extract.

e) Test for carbohydrate:

Fehling's test: 5ml of Fehling's solution is a water bath. The formation of yellow or rest for precipitate indicates the presence of reducing power.

f) Test for Saponins: **Foam test:** 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for15 min. The formation of foam to a length of 1cm indicated the presence of saponins

g) Test for Glycosides: Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

h)Test for Protein & amino acids: Biuret test: To 0.5 mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

Ninhydrin test: About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for Anthraquinone: Borntragers test :About 0.5 gm of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10%ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Mayer's Test: 5ml of stock solution and add few drops of Mayer's reagent then formed white and creamy precipitate in the tube.

Result & Discussion:

Table no 1: Determination of pH of Catharanthus Roseus plant

Sr. No.	Parameter	Sample
1	pH meter value	6.08

Table no 2: Determination of different ash values of Catharanthus Roseus

Sr. No.	Parameter	Sample
1	Total Ash value	3.4%
2	Acid Insoluble Ash value	1.2%
3	Water Soluble Ash value	1.4%

Table no :3 Quantitative determination of water extract and chloroform extract.

Sr. No.	Parameter	Sample
1	Water Extraction	26.4%

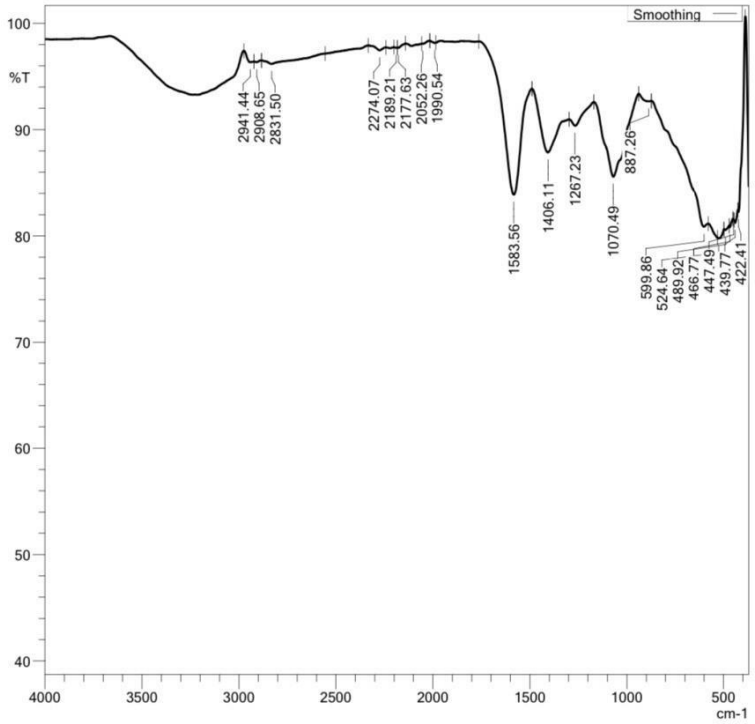
Table no 4 : Phytochemical test:

Plant constituent	Sample	Name of test
1)Alkoides	+	Wagner's test

2)Flavonoides	+	Lead acetate test
3)Phenols & Tannins	+	Lead acetate test
	+	Ferric Chloride Test
4)Steroids & Sterols	_	Salkoswi's test
5)Carbohydrates	+	Fehling's test
6)Saponins	_	Honey comb test
	+	Foam test
7)Glococides	+	Glococides
8)Protien and amino acid	+	Biuret test
	_	Ninhydrin test
9)Anthraquoine test	_	Borntrager's test
10)Phlobatannins	_	-
11)Mayer's test	_	-

FTIR analysis :All the extracts were examined for Fourier transforms infrared spectroscopy. All theextract are having near about FTIR spectra. The peaks at 3829, 3775, 3664, 3644, 3444,

3367, 3262, 3079 Cm are observed are responsible for different functional groups. Such as NH2 stretching, OH stretching, C-H stretching, Etc.



Water Extract of C. Roseus				
Sr. No.	**Absorption**	**Appearance**	**Group**	**Comp Class**
1	3291.23	Strong, Broad	O-H Stretching	Carboxylic acid
2	2971.90	Medium	C-H Stretching	Alkane
3	2962.58	Medium	C-H Stretching	Alkane
4	2882.16	Medium	C-H Stretching	Alkane
5	1559.40	Medium	C=C Stretching	Cyclic alkane
6	1385.21	Medium	C-H Bending	Alkane
7	1192.82	Strong	C-O Stretching	Ester
8	1077.92	Strong	C-O Stretching	Primary Alcohol
9	878.15	Strong	C-H Bending	1,2,3 - tri substituted
10	780.91	Strong	C-H Bending	1,2,3 - tri substituted
11	650.34	Strong	C-Br Stretching	Halo compound
12	534.78	Strong	C-Cl Stretching	Halo compound

Conclusion : FTIR spectra Analysis shown different peaks of chemical constituents present in it as shown in table no 6, 7 this spectra analysis gives an idea about the different functional groups were present in this sample and it can be isolate the active components of this natural plant for further drug preparation.

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