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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF CATHARANTHUS ROSEUS

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ABSTRACT:
Catharanthus roseus, a Subtropical evergreen shrub serves as a high valued medicinal plant . The extracts of the plant are used for treating various diseases like Cancer and Diabetics Catharanthus roseus also exhibits enormous biological properties such as Antioxidant, Antibacterial, Antidiabetic ,Antiulcer , Hypotensive etc. In the present study we attempted to study the Phytochemical Screening , Antioxidant activity by Free radical scavenging against DPPH, O_2^-, H_2O_2 and Antibacterial activity by Disc Diffusion method . The extracts prepared by Batch method from the leaves of Catharathus roseus are used for the study.

KEY WORDS: Antibacterial**,** Antioxidant, Cathranthus Roseus, Phytochemical Studies

INTRODUCTION:

The plant kingdom is a treasure trove of potential drugs and in the past few years there has been a growing awareness about the importance of herbs. The plant that was selected for medicinal use over 1000 years is the most evident choice of examine current research into new effective therapeutic drugs such as anticancer drug.[1] antimicrobial drug [2], antihepatotoxic compounds. According to the World Health Organization (WHO), medicinal plants would be the best source for various medications. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, these plants should be studied for a better understanding of their properties, safety and effectiveness[3]. Medicinal plants contain certain organic compounds that provide a definite physiological effect on the human body and these bioactive substances includes tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [4.]These compounds are synthesised by primary or rather secondary metabolism of the living body. Secondary metabolites are compounds of extreme chemical and taxonomic diversity with obscure function. They are widely used in human, veterinary, agricultural, scientific research and countless other areas [5]. Phytochemicals (from the Greek term phyto meaning plant) are biologically active, natural chemical compounds present in plants, which has predicted plant chemicals, which protect plant cells from environmental hazards such as pollution, stress, dryness, UV exposure and .Pathogenic attacks are referred to as phytochemics [7,8] Human health benefits greater than those attributed to macro- and micro-nutrients. [6] In general, plant chemicals that protect plant cells from environmental hazards like pollution, stress, and drought, exposure to ultraviolet and pathogenic attack are called as phytochemicals. Phytochemical compounds build up in different parts of plants, for example in roots, stems, leaves, flowers, fruits or seeds.[9] Plant chemicals are also available in additional form, but there is no evidence that they provide the same health benefits as food plant chemicals. These compounds are referred to as secondary metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxifying enzymes, stimulation of the immune system, reduction of aggregation and modulation of platelets of hormone metabolism and anticancer property. Plants

produce a variety of bioactive phytochemical substances that can be grouped into two categories: primary and secondary metabolites. Primary metabolites include proteins, carbohydrates, amino acids and chlorophyll, while polyphenols, alkaloids and terpenenoids are a few example of secondary metabolites[10]. Secondary metabolites are chemicals that are not necessary for the immediate survival of the plant, but are synthesized to improve the survival of the plant allowing them to interact with pathogens, herbivorous insects and the environment.[11]

Secondary metabolites like alkaloids, glycosides, flavonoids, steroids, saponins and terpenenoids play an important role in protection of the plant from environmental stress, attack of pathogens and insects pests [12].Due to the presence of these bioactive phytochemical compounds, plants provide a source of medicines from historical times and now they are an important part of all the world's pharmaceuticals and serve as starting material for drug development [13].Peckolt and all have reported that it controls bleeding and scurvy, as a mouthwash for toothaches, and for curing and cleaning chronic wounds as the applications of catharanthus roses. In the British West Indies it was used to treat diabetic ulcers and in the Philippines, it was considered an effective Oral Hypoglycemia Agent. More recently, Chopra et al. reported that total alkaloids have limited antibacterial activity and extensive and sustained activity hypotensive action. The physiologically important alkaloids are antineoplastic dimeric alkaloids, vinblastine, vincristine in the aerial parts and ajmalicine ,serpentine in the roots. Another alkaloid, vinflunine, not universally accepted except in Europe and said to possess anti-tumour activity. Vinblastine and vincristine are chemotherapy medications used to deal with several types of cancers and are biosynthesised from the linking of the alkaloids catharanthine and vindoline .The newer semisynthetic chemotherapeutic agent vinorelbine is used to deal with non small-cell lung cancer, can be prepared either from vindoline and catharanthine or from the vinca alkaloid leurosine in both cases via anhydrovinblastine. Rosinidin is an anthocyanidin pigment found in the flowers of Catharanthus roseus. In traditional way of medications , the periwinkle has been used for relieving muscle pain, depression of the central nervous system, also used for applying to wasp stings and to heal wounds[14].

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials:

The plant species namely pink vinca rosea plant were collected by in and around Villupuram District, Tamil Nadu, India.

Preparation of Plant powder

The plant leaves was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electrical grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the Ethanolic extract

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The leaves powder material was subjected to Batch extraction separately and successively with 140ml ethanol and 60ml distilled water. These extract was filtered by Whatmann filter paper. Then the extract was put in an air tight container stored.

PRELIMINARY QUALITATIVE ANALYSIS:[15 - 26]

The preliminary phytochemical investigation of the whole plant of Catharanthus roseus were carried out with the standard protocol. The extracts are subjected to preliminary phytochemical analysis

Qualitative analysis of phytochemical:

The leaf extract were tested for the presence of bioactive compounds by using the following standards method.

1.Test for alkaloids:

The plant extracts is mixed in 1% of Conc. HCl. Warmed and filtered. Now the filtered is used for following test.

a. Mayer's test:

The filtrate is treated with Mayer's reagent (Mercuric chloride +Potassium iodide in water).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b.Wanger 's test:

The plant extract is added to 1.5% HCl & a few frops of Wagner's reagent. Yellowish Brown appearance indicates the presence of Alkaloid.

.2.Test for carbohydrate:

The plant extract is Dissolved in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Molisch'test:

Filtrate is treated with two drops of Alcoholic ά- naphthol solution in a test tube. Carefully, Incline tubes and pour drop wise conc. Sulphuric acid Using a dropper, along the sides of test tube. Formation Of violet colour at the junction or interface of two Liquids indicates the presences of carbohydrates.

b.Benedict'test:

Filtrate is treated with Benedict's Reagent then the mixture was heated on a Boiling water bath for 5 minutes and cooled. Orange red Precipitate indicates the presence of carbohydrates.

3.Test for steroids:

To the 1 ml of the plant extract is mixed with 1 ml of chloroform and concentrated sulfuric acid sidewise added . A red colour presence at the lower chloroform layer indicates presence of steroids.

4.Test for diterpenes:(Copper acetate test)

The plant extract is dissolved In distilled water and treated with copper acetate Solution. Formation of emerald green colour indicate . The presence of diterpenes.

5.Test for terpenoids:

To 5 ml of the plant extract was mixed with 2 ml of chloroform and 3 ml of sulfuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed which indicates the presence of terpenoids.

6.Test for phenol :

To 1 ml of the plant extract was treated with distilled water and a few drop of aqueous FeCl3 solution is added. Formation of a blue or green precipitate indicated the presence of phenol.

7.Test for saponins:

To 1 ml of the plant extract added 2 ml of distilled water and shaken vigorously and formation of 1 cm layer of foam indicates presence of saponins.

8.Test for flavonoids :

The plant extract is treated with 2-3 drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of few drops of sulfuric acid which indicates the presence of flavonoids.

9.Test for protein:

To 1 mL of the plant extract to added a few drop of mercuric chloride. Formation of yellow colour indicates the presence of protein.

10.Test for phytosterol:(Salkowski's test)

The plant extracts was treated with chloroform and filtered. The filtered was treated with 5-10 drop of Concentrated sulphuric acid and shaken gently. The golden yellow colour indicates the presence of phytosterol.

11.Test for Glycosides:

The Plant Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides.To 1ml of plant extract, 1ml of acetic acid and few drops of FeCl2 and concentrated H2SO4 were added. Formation of brown ring indicates presence of glycosides.

12.Test for Quinines:

To 1 ml of the plant extract is added 1 ml of NaoH and mixed well . Formation of blue green or red indicates the presence of Quinines.

13.Test for Tannins:

The2ml of the plant extract is added to 10% ferric chloride .The appearance of greenish black indicates the presence of catechol tannins .

IN VITRO ANTIOXIDANT ACTIVITY

DPPH, H_2O_2, and O_2- radical scavenging assays were used to assess the extract Catharanthus roseus antioxidant activity in vitro. The IC50 values were calculated and compared to ascorbic acid, a standard antioxidant (**Table 2**). The ABTS assay is a relatively new one that uses a more powerful, chemically created radical to screen

complex antioxidant mixtures including plant extracts, drinks, and biological fluids. The solubility of $ABTS^{\bullet+}$ in both organic and aqueous environments, as well as its stability over a wide pH range, piqued researchers' interest in using it to estimate antioxidant activity.[14] When the DPPH-free radical combines with hydrogen donors, it forms a matching hydrazine. The DPPH radical is purple in appearance and turns yellow when it reacts with hydrogen donors. It's a discoloration test that involves adding the antioxidant to a DPPH solution in ethanol or methanol and measuring the decrease in absorbance at 490 nm. Free radical participation, particularly increased generation, appears to be a characteristic of most human diseases, including cardiovascular disease and cancer. The addition of sodium hydroxide to air-saturated dimethyl sulfoxide produces superoxide radicals in the alkaline DMSO method (DMSO). At normal temperature, the produced superoxide remains stable in solution, reducing nitro blue tetrazolium to Formosan dye, which can be detected at 560 nm. The creation of a red dye formazan is inhibited by a superoxide scavenger capable of reacting. [39] Several oxidase enzymes produce hydrogen peroxide in the body. There is mounting evidence that hydrogen peroxide causes serious harm to biological systems, either directly or indirectly through its reduction product, the hydroxyl radical (OH•). The decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm when a scavenger is incubated with hydrogen peroxide in this method. [14]

The inclusion of phytochemicals such as alkaloids, carbohydrates, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids in the Catharanthus roseusLinn is extract resulted in a higher antioxidant activity. By chemical technique, the Catharanthus roseusLinn extract has the least antioxidant activity. All of the Catharanthus roseusLinn show strong antioxidant activity when compared to typical antioxidants such as ascorbic acid, based on the above antioxidant findings. All of the evaluated methods had the same antioxidant activity order. Because it includes a high amount of phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, the Catharanthus roseus has remarkable antioxidant action when compared to conventional ascorbic acid.

The inhibition-based in vitro approaches are used. The inhibition of free radical action is evaluated after samples are added to a free radical producing system, and this inhibition is connected to the sample's antioxidant activity. The generated radical, the reproducibility of the creation procedure, and the endpoint employed for the determination all differ significantly. Despite the fact that in vitro methods provide a valuable indication of antioxidant activity, data derived from in vitro methods are challenging to adapt to biological systems and do not always predict similar in vivo antioxidant activity. It's important to remember that all of the methodologies established have advantages and disadvantages, and that a single measurement of antioxidant capacity is rarely enough. It's possible that a variety of approaches will be required.

The Catharanthus roseus and standard solutions had concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 g mL^{1}. To avoid extract agglomeration, the dilute solution of Catharanthus roseus was sonicated for 30 minutes at room temperature in a sonicator bath. The absorbance was compared to the equivalent blank solutions using spectrophotometry. Using the following formula, the % inhibition was calculated:

IN VITRO ANTIBACTERIAL ACTIVITY:

Infectious diseases caused by bacterial and fungal organisms pose a severe hazard to public health around the world. Antibiotics are a common therapeutic option for bacterial and fungal illnesses. The emergence of antimicrobial resistance and toxicity concerns, on the other hand, has reduced the usage of antibacterial agents. Antibiotics' safety and efficacy limits complement biological research on the antibacterial role of plants, which have similar toxicity and efficacy. The antibacterial properties of Catharanthus roseus ethanolic extract against pathogenic bacterial strains Pseudomonas aeruginosa and Escherichia coli were investigated. The antibacterial and antifungal potential of ethanolic extracts was measured in terms of bacterial and fungal growth inhibition zones. The antibacterial outcome has sparked interest in the development of alternative antimicrobial medications for the treatment of infectious disorders. without any negative side effects The consequences of such colonisation in vital human organs as the lungs, urinary tract, and kidneys might be lethal. This bacterium is found on and in medical equipment, especially catheters, because it thrives on damp surfaces, causing cross-infections in hospitals and clinics. It can also breakdown hydrocarbons, therefore it's been used to break down tar balls and oil spil.

RESULT AND DISSCUSION:

PHYTOCHEMICAL TEST : The phytochemical characters of the Catharanthus roseus leaves investigated and summarized in the table.1. The phytochemical screening of Catharanthus roseus leaves showed that the presence of Glycosides, Tannins, Steroids, Flavonoids, and Proteins, Alkaloids, Phenols, Diterpenes, Saponins.

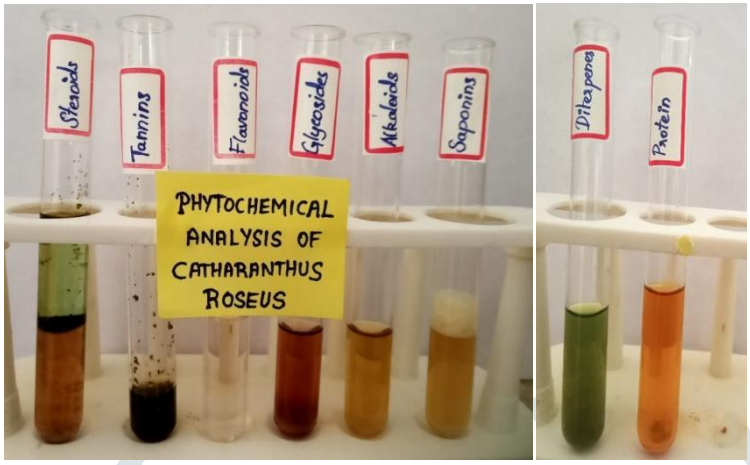

Figure.3 The Phytochemical analysis test tubes of Catharanthus roseus.

Table: 1 QUALITATIVE ANALYSIS OF CATHARANTHUS ROSEUS:

S.NO	TEST	AQUEOUS EXTRACT	S.NO	TEST	AQUEOUS EXTRACT
1	Alkaloid	+	7	Saponins	+
2	Carbohydrate	-	8	Flavonoids	+
3	Steroids	+	9	Proteins	+
4	Diterpenes	+	10	Phytosterol	-
5	Terpenoids	-	11	Glycosides , Tannins	+
6	Phenols	+	12	Quinones	-

(+)Presence (-) Absence

ANTIOXIDANT ACTIVITY

1. Assay for DPPH

The experiment was done in a 96-well micro titer plate. In each well of the micro titer plate, 10 l of each sample or standard solution was added separately to 200 l of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) solution. The absorbance of each solution was measured at 490 nm after 30 minutes of incubation at 37 °C.

The DPPH radical scavenging assay method was used to assess the antioxidant activity of the extract Catharanthus roseusLinn. The anti-oxidant activity of Catharanthus roseusLinn equal doses of 100 nm measured at 230 nm is shown in **Table 2.**

2. Assay Hydroxyl Radical Scavenging

Various amounts of samples or standard (0.5 mL) were added to a reaction mixture including ferric chloride (0.5 mL,0.1 mM); EDTA (0.5 mL, 0.1 mM); ascorbic acid (0.5 mL,0.1 mM); hydrogen peroxide (0.5 mL, 2 mM); and p-nitrosodimethyl aniline (p-NDA; 0.5 mL,0.01 mM) By combining 0.5 mL sample with 2.5 mL phosphate buffer, a sample blank was created. These solutions' absorbance was measured at 440nm.

H_2O_2 radical scavenging assay technique was used to study the antioxidant activity of extract Catharanthus roseus. The anti-oxidant activity of Catharanthus roseus equal doses of 100 nm measured at 230 nm is shown in **Table 2.**

3. Assay for Superoxide Radical Scavenging (Alkaline DMSO Method)

0.1 mL of nitro blue tetrazolium (NBT;1 mg mL1) was added to a reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) and 0.3 mL of the sample in freshly distilled DMSO at varied concentrations. At 560nm, the absorbance was measured.

The superoxide scavenging assay method was used to assess the antioxidant activity of Catharanthus roseusLinn once more. The superoxide scavenging activity of Catharanthus roseusLinn at a concentration of 100 nm was measured at 560 nm in this work. **Table. 2**

Table:2 Antioxidant scavenging percentages against DPPH, Hydrogen peroxide, and Superoxide radicals in Catharanthus roseus compared to normal

Compound	Free radical Scavenging Activity (%)		
	DPPH	H_2O_2	O_2^-
Catharanthus roseus	**70.3**	**75.2**	**74.2**
Ascorbic Acid	**78.2**	**80.0**	**78.2**

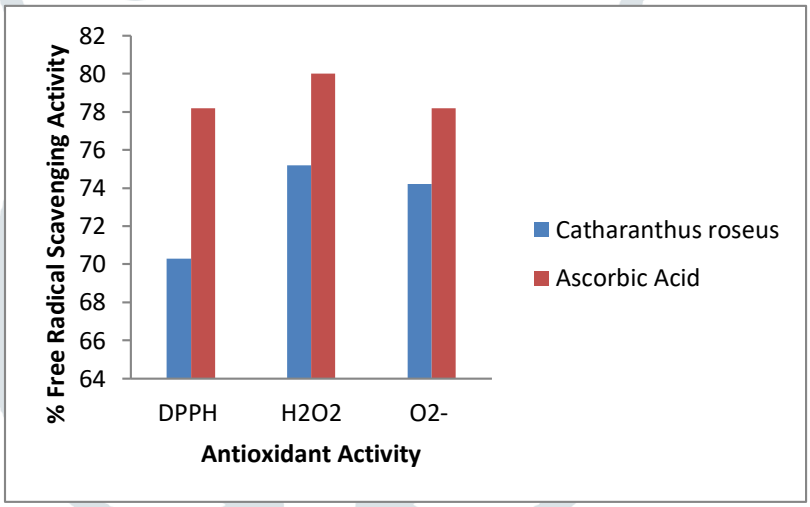

Fig. 4: In vitro antioxidant activity various free radical assay method compared with Standard Ascorbic Acid

DPPH, H_2O_2, and superoxide radical scavenging assays were used to study the antioxidant activity of the extract Catharanthus roseus. **Figure 4** compares Catharanthus roseus anti-oxidant activity to that of normal ascorbic acid. Superoxide radical scavenging has stronger antioxidant activity than hydrogen peroxide radical scavenging, according to free radical scavenging.

In comparison to other free radical scavenging methods, DPPH free radical scavenging activity reveals superior antioxidant activity. It has 70 % activity compared to 78 % for normal ascorbic acid.

H_2O_2 free radical scavenging activity is superior to other free radical scavenging methods in terms of antioxidant activity. It has a 75 % activity compared to 80 % for normal ascorbic acid.

In comparison to other free radical scavenging methods, superoxide free radical scavenging activity demonstrates the best antioxidant activity. It shows a 74% increase in activity when compared to standard ascorbic acid 78%.

ANTIBACTERIAL ACTIVITY

By disc diffusion method, Catharanthus roseus leaf extract has strong activity against Staphylococcus aureus with a zone of inhibition of 17 mm and Pseudomonas aeruginosa with a zone of inhibition of 18 mm.

Table: 3 Zone of inhibition of the synthesized Catharanthus roseus

S. No	Positive and negative Pathogen	Zone of inhibition (diameter in mm)				Standard (Gentamicin)
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	Staphylococcus aureus	9	12	15	17	16
2	Pseudomonas aeruginosa	12	13	11	18	18
3	Control (DMSO)	NI	NI	NI	NI	NI

NI: No Inhibition

The antibacterial properties of Catharanthus roseus Linn ethanolic extract were investigated against the pathogenic microorganisms Staphylococcus aureus and Pseudomonas aeruginosa. The antibacterial activity of ethanolic extracts was measured in terms of bacterial growth inhibition zone. The antibacterial outcome has sparked interest in developing new antimicrobial medications that are free of adverse effects for the treatment of infectious disorders. The consequences of such colonisation in vital human organs as the lungs, urinary tract, and kidneys might be lethal. This bacterium is found on and in medical equipment, especially catheters, because it thrives on damp surfaces, causing cross-infections in hospitals and clinics. It can also degrade hydrocarbons and has been used to oil spills produce tar balls and oil. By disc diffusion technique, Catharanthus roseus leaf extract has high activity against Staphylococcus aureus with a zone of inhibition of 17 mm and Pseudomonas aeruginosa with a zone of inhibition of 18 mm..

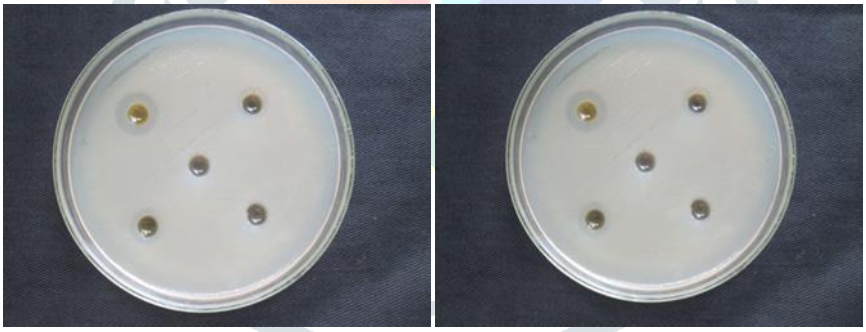

Figure.5 Catharanthus roseus- Antibacterial Activity

CONCULSION

The antioxidant activity of Catharanthus roseus extract was investigated using the DPPH, H_2O_2, and O_2-free radical assay methods. In vitro antioxidant studies using various methodologies reveal that Catharanthus roseus has significant antioxidant activity when compared to Ascorbic acid and its give antibacterial activity with various concentrations, when concentration increase antibacterial activity also increases Because it contains various phytochemicals such as alkaloids, flavonoids, phenolic compounds, and terpenoids, Saponins, Tannins, Diterepenes, Steroids and Glycosides . Catharanthus roseus displays exceptional good activities in all biological research.

REFERENCES:

1. Dewick, P.M. Tumor inhibition from plants: Tease and Evans 1996.
2. Phillipson, J.D., Wright, C.W. Plants With Antiprotozoal Activity : Tease and Evans, Pharmacognosy, 14th edn., *WB Saunders Company, London,* **1996**, 612.
3. Arunkumar, S., Muthuselvam. Analysis of phytochemical constituents and antimicrobial activities of aloevera L. against clinical pathogens. *World J.Agril. Sc.*,**2009**, 5, 572-576.

4. Mann, J. Secondary Metabolism. *Oxford University press, London,***1978**, 154.
5. Vasu, K., Goud, J.V., Suryam, A., Singara, Chary, M.A. Biomolecular and phytochemical analyses of three aquatic angiosperms. *Afr. J. Microbiol. Res.*, 2009, 3, 418-421.
6. Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition***1999**, 129, 756-757.
7. Gibson EL, Wardel J, Watts CJ. *Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children.* Appetite **1998**, 31, 205-228.
8. Mathai K. *Nutrition in the Adult Years. In Krause's Food, Nutrition*, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, **2000**, 271, 274-275.
9. Costa MA, Zia ZQ, Davin LB, Lewis NG. Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. *In Recent Advances in Phytochemistry, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense,* ed. JT Romeo, New York, **1999**, 33, 67-87.
10. Wadood, A., Ghufran, M., Jamal S.B., Naeem, M., Khan, A., Gaffar, R. And Asnad. *"Phytochemical analysis of medicinal plants occurring in local area of Mardan", Biochemistry and analytical biochemistry*, **2013**, 2, 1-4.
11. Kennedy, D.O. and Wightman, E.L., "Herbal extracts and phytochemicals: plant secondary metabolites and enhancement of human brain function", *Advances in nutrition*, **2015**, 2, 32-50.
12. Shalini, S. and Sampathkumar, P., "phytochemical screening and antimicrobial activity of plant extracts for disease management", *International Journal of current science*.**2012**, 209-218.
13. Ajai, I.A., Ajibade, O. and OderindeR.A.,"Preliminary phytochemical analysis of some plant seeds", *Research Journal of chemical sciences*, **2011**, 1, 58-62.
14. Jai Narayan Mishra, Navneet Kumar Verma "A brief study on Catharanthus Roseus: A review",*International Journal of Research in Pharmacy and Pharmaceutical Sciences,***2017**, 2,20-23.
15. Asheesh Kumar, K.C. Singhal, R.A.Sharma, Govind K. Vyas, Vinod Kumar. *Analysis of Antioxidant Activity of Catharanthus Roseus L. and it's Association with Habitat Temperature*. Asian J. Exp. Biol. Sci. 3(4): 706-713 (2012)
16. Chinnavenkataraman Govindasamy and Rajendran Srinivasan. *In vitro antibacterial activity and phytochemical analysis of Catharanthus roseus (Linn.) G. Don. Asian Pacific Journal of Ttopical Biomedicine*, S155-158 (2012)
17. Dash, B.K. Sultena, S. Sultana, N., *Anti bacterial activities of methonal and acetone extracts of Fenugreek and coriander*. Lif Sci Med Res, 27: pp:1-8 (2011)
18. G.M. Cragg and D.J. Newman. *Plants as a source of anti-cancer agents. Ethnopharmacology*, 100: 72-79 (2005)
19. Kratika Kumari and Sharmita Gupta. *Phytopotential of Cathanthus Roseus L.(G.) Don. Var. "Rosea" And " Alba" Against Various Pathogenic Microbes In vitro. International Journal of Research in Pure and Applied Microbiology*, 3(3): 77-82 (2013)
20. Monika Sain and Vandana Sharma. Catharanthus roseus- *A Review of Potential Therapeutics Properties*. Int. J. Pure App. Biosci. 1(6): 139-142 (2013)
21. Rischer, H. Oresic, M. Seppanen-Laakso, T. Katajamaa, M. Lammertyn, M. and Ardiles- Diaz, W., *Gene-tometabolite networks for terpenoid indole alkaloid biosynthesis in cathanthus roseus cells*. Proceedings of the national Academy of sciences of the United States USA. 103:5614-5619 (2006)
22. Salah, N. Miller, N.J. paganga, G. Tijburg, L. Bolwell, G.P. and Rice-Evans, C. *Polyphenolc flavanoids as scavengers of aqueous phase radicals and as chain-breaking antioxidants.* Archives of Biochemistry and Biophysics, 322: 339-346 (1996).
23. Sarabjot Kaur, Poonam Mondal. *Study of Total Phenolic and Flavonoids Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. Journal of microbiology and Experimentation*. 1(1): 1-6 (2014)
24. Shalini, S. and Prema Sampathkumar. *Phytochemical screening and antimicrobial activity of plant extracts for disease management*. Int. J. Curr. Sci. 209-218 (2012)
25. Sheeraz Ahamad Wagay, Sudhansud Dhar Dwivedi, Manik Sharma Jagrati tripathi and Mushtaq ahmad. *Antimicrobial Activity of Catharanthus rosrus*. Chemistry and Materials Research 3(9): 61-63 (2013)

26. V. Govindaraji, *PGR mediated in vitro metabolic engineering of alkaloid production in somatic explants of Catharanthus roseus (L.) G. Don.*, M.Phil. Dissertation, PRIDE,PU, Salem, (2007).