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PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES ON THE AQUEOUS EXTRACT OF THE WHOLE PLANT OF CATHARANTHUS ROSEUS

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ABSTRACT

India is one of the richest countries in the world with regard to diversity of medicinal plant. There are a wide variety of medicinal plants having various pharmacological activity suchas Antispasmodic, Antidiabetes, Antirheumatics, Anthelmintics, Antihypertensives, Antimalarials, Antifungal, Antidiuretics, Antidepressants, etc., *Catharanthus roseus* is an evergreen shrub herb belong to the family Apocynaceae which is also known as Vinca rosea. The extracts of Vinca have demonstrated significant anticancer activity against numerous cell types. Many scientists investigated and reported that *Catharanthus roseus* widely employed for medicinal purposes. In our project by using aqueous extract of the whole plant of *Catharanthus roseus* were studied by using phytochemical investigation and determine their antimicrobial activity against selective pathogens by using Kirby-Bauer agar well diffusion assay method. Fractions showed higher inhibitory effect against the pathogenic such as Staphylococcus, Enterobacter, Streptococcus, Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella Para thyphi, Candida albicans and A.niger. Since this organism is reported to the major pathogen and resistance towards the most antibiotic and hence receives special attention from the researchers. Gentamycin is used as a standard.

KEYWARDS: *Catharanthus roseus*, phytochemical investigations, antimicrobial and antifungal activity, Kirby-Bauer agar well diffusion assay method.

INTRODUCTION

Herbal plant used in traditional medicine consist of a wide range of bioactive compounds that can be used as alternative therapeutic tools for the prevention or treatment of many contagious diseases. Plants produce several secondary metabolites including alkaloids, cyanogenic glycosides flavonoids, saponins, steroids & terpenoids to protect themselves from the continuous attach of environmental stresses. There are a wide variety of medicinal plants having Antimicrobial, Antispasmodic, Antidiabetes, Antirheumatics, Anthelmintics, Antihypertensives, Antimalarials, Antifungal, Antidiuretics, Antidepressants, etc., Plants are considered as clinically effective and safer alternative to synthetic antibiotics.[1] Catharanthus roseus is native to the Indian Ocean Island of Madagascar. Catharanthus roseus Linn., belong to the family Apocynaceae which also known as Vinca rosea. It has more than 400 known alkaloids, some of which are approved as Antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, and other cancer. Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer's disease.

The two classes of active compounds in vinca named as alkaloids and tannins. The major alkaloid is vincamine and ethyl-apovincaminate or vinpocetine(a semisynthetic derivative) has vasodilating, blood thinning, hypoglycemic and memory-enhancing actions. The extracts of Vinca have demonstrated significant anticancer activity against numerous cell types. *Catharanthus roseus* commonly known as the Madagascar periwinkle, rosy periwinkle or teresita a source of drugs vincristine and vinblastine, used to treat the cancer.[2,3] Several studies of vinpocetile suggest that it may improve brain function and memory, particularly in people affected by diseases that decrease mental capacity such as Alzheimer’s disease or dementia.[4,5] The world antibiotics is derived from the term antibios, which literally means against like(anti-against/bios-life)antibiotics as a chemical substance produced by a micro organism that has the capacity, in low concentration, to inhibit selectively (or) even to destroy bacterial and other micro organisms through an Anti metabolic mechanism.[6]

PLANT SELECTION

The selection of a particular plant, to study about the phytochemical nature and antimicrobial activity are becoming very important to localize the plant first and it is most critical aspect in formulating a project, first of all, to select the plant. Searching for the ethnobotonical data’s will makes the way for obtaining information about the yield of medicinal plants and its

locality from where it is easily obtained. It should be known that whether the plant has undergone any previous research work and should specify the type and nature of the work and the part of the plant in which in the research is made should be noted up.

AIM OF THE WORK

The aim of the present work is to investigate "Phytochemical Screening, Antibacterial And Antifungal Activities On The Aqueous Extract Of The Whole Plant Of Catharanthus Roseus'**.** Preliminary Phytochemical screening was performed by using chemical test and antimicrobial action screened by using Kirby-Bauer agar well diffusion assay method.

CHEMICAL CONSTITUTENTS

Vinblastine contain (A) Indole alkaloid - Catharanthine B) Dihydro indole alkaloid - Vindoline.

Other alkaloids : Ajmalicine : Leurosidine
Lochnerine : Leurosine
Serpentine : Isoleurosine
Tetrahydrolstonine : Deacetyl-vindoline hydrazide
PIGMENT: Rosinidin is an anthocyanidin pigment found in the flowers of *Catharanthus roseus.* Table.1 shows various pharmacological activity of Cathranthus roseus.[8,9]

Traditional uses of the *Vinca rosea* world-wide[10]

Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and could thus serves as a source of traditional drugs(Kim et.al., 1995).

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT AND PHYTOCHEMICAL SCREENING

The whole plant of the selected plants were collected from various places of Perambalur District, Tamilnadu and then washed off under running tap water to eradicate dust. The plant samples were then air dried for few days and the leaves were grinded off into powder and kept in polythene bags for further uses.

EXTRACTION- PRINCIPLE

The active constituents were removed or separated or extracted from the crude drugs by using suitable solvent (water, alcohol, solvent ether, light petroleum and chloroform). This is basic

principle involved in extraction processes. Percolation, maceration process is most commonly used for extracting active constituents. The active constituents that have been extracted from crude drugs called extractives and the preparation so obtained called extracts.

PREPARATION PROCEDURE-AQUEOUS EXTRACTION

10 grams of dried powder was taken into distilled water and extracted for 6 hours at low heat. Filtration was done for every 2 hours, through 8 layers of muslin cloth and centrifuged at 5000rpm for 15min. The supernatant was collected. The process was repeated twice and after 6 hours, the supernatant was concentrated to make the final volume to one-fourth of the original volume. Then the final supernatant was autoclaved 121°c Ibs pressure for 15 minutes and then stored at 4°c.[11]

PHYTOCHEMICALSCREENING

The whole plant of aqueous extract of the catharanthus roseus were subjected to qualitative examination for an identification of various phytoconstituents and the given table-3 shows the investigation of phytochemicals.[12]

ANTIMICROBIAL ACTIVITY

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The plant samples were collected from Perambalur, south India. The collected plant materials were transported to the laboratory.

MICRO ORGANISMS AND CULTURE MEDIA

Bacterial cultures such as *Staphylococcus, Enterobacter, Streptococcus, Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella Para thyphi, Candida albicans* and *A.niger* were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4˚C.

INOCULUM PREPARATION

Bacterial cultures were sub cultured in liquid medium (Nutrient broth) at37˚C for 8h and further used for the test (10^5-10^6CFU /ml). These suspensions were prepared immediately before the test was carried out.

PREPARATION OF CULTURE MEDIA

NUTRIENT AGAR MEDIUM

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients Grams/Liter

Peptone	:	5gm
Beef extract	:	3gm
Agar	:	15gm
Sodium chloride	:	5gm
Yeast extract	:	1.5gm
p^H	:	7.0

After adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ibs psi pressure (121°C) for 15 minutes.

NUTRIENT BROTH

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ibs psi pressure (121°C) for 15 minutes.

PREPARATION OF PLANT MATERIAL

Whole plant of *Catharanthus roseus*, plant materials taken for this study were shade dried individually at room temperature and then powdered by using electric, blender. About 10gm of fresh plant materials (whole plant).Were extracted with 100ml of distilled water 91:10. They were kept for seven days at room temperature (31˚C) for complete extraction. After seven days. The extracts were filtered through what man no.1 filter paper. This extract was collected in both and kept in refrigerator.[13,14]

ASSAY OF ANTIMICROBIAL ACTIVITY

Microbial Inoculum Preparation

The nutrient broth were prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

KIRBY BAUER AGAR WELL DIFFUSION ASSAY

The nutrient agar medium was prepared and sterilized by autoclaving at 121˚C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile Petri plates and allowed to solidify the Bacterial broth culture was swabbed on each Petri plates using a sterile buds. Then wells were made by well cutter. The organic solvent extracts of leaves were added to each well aseptically. This procedure was repeated for each Petri plates then the Petri plates were incubated at 37˚C for 24 hrs. After incubation the plates were observed for the zone of inhibition.[15]

USES OF VINCA PLANT

Extracts from dried or wet flower and leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the flowers of *Catharanthus roseus* made into a tea has been used by Ayurvedic physician in India for external use to treat skin problems, dermatitis, eczema and acne.

Vinorelbine used to treat breast cancer and bee effective against bone marrow and osteosarcoma. Vincristine used to treat acute leukemia, neuroblastoma, wilm's tumor, Hodgkin's disease. Vindesine also possess antineoplastic activity and is seen in acute lymphocytic leukemia, chronic myeloid leukemia, malignant melanoma and breast carcinoma. Lesser periwinkle is generally recommended for improving circulation, particular in brain.

Precautions

Catharanthus roseus is not known to be harmful when taken in recommended dosage, though it is important to remember that long-term effects of tacking the herb have not been investigated.

Catharanthus roseus should not be used by people with low blood pressure or constipation. Due to look of sufficient medical study, periwinkle should be used with caution in children women who are pregnant or breast feeding and people with liver or kindly disease.

SIDE EFFECTS

Vinblastine- Nausea, vomiting, constipation, dyspnea, toxicity to WBC, chest pain, wheezing and fever. Vinorelbine-Decreased resistance to infection, bleeding, anaemia, constipation, diarrhoea, nausea, vomiting, numbness(or) tingling in hands and feet, fatigue Vincristine-

Peripheral neuropathy, bone marrow suppression, nausea, vomiting, constipation, toxicity of nervous system. The only effective way to reduce neurotoxicity caused by Vinca alkaloid is by discontinuity treatment (or) by decreasing dose and dosing frequency. Thrombocytopenia and anemia rarely reported.

CONTRA INDICATION

Vinca alkaloids are contraindicated during pregnancy, have been planning for pregnancy or during breast feeding as it may cause birth abnormalities. Patients should not receive any vaccination while taking this medication. Vincristine may also cause weakness of immunity system and can lead to an illness. The drug concentration and treatment duration are important factor for determining drug accumulation and cytotoxicity.[16,17,18]

RESULT AND DISCUSSION

Phytochemical screening of the plant is very important and has great interest in Pharmaceutical companies for the production of new drugs for curing varies diseases Uses of these plant extracts also have proven benefits in the treatment of various diseases. This study has exposed the presence of phytochemicals considered as active medicinal chemical constituents such as tannins, flavonoids, alkaloids, glycosides, steroids and triterpenoids. The inhibitory effect of the aqueous extract of the whole plant of catharanthus roseus against pathogenic species in nutrient agar were studied.

Fractions showed higher inhibitory effect against the pathogenic such as *Staphylococcus, Enterobacter, Streptococcus, Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella Para thyphi, Candida albicans* and *A.niger. Since this organism is reported to the major pathogen and resistance towards the most antibiotic and hence receives special attention from the researchers.*

Finally from the obtained results we get a conclusion that indicate the Antibacterial and Antifungal Activity of Catharanthus roseus increases in concentration of extracts increases the zone of inhibition, these extracts may be of great interest for future studies about treatment of many diseases. Further studies are needed for confirmation of antimicrobial action by isolating pure chemical constituents and also identify which compound is responsible for antibacterial and antifungal action of *Catharanthus roseus.*

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Table 1: Pharmacological activities of *Vinca rosea.*

S. No.	Source	Pharmacological activity
1	Ethanol (70%) extract of leaf and stem	Cardio tonic Activity
2	Water extract of callus tissue	Antiviral Activity
3	Benzene extract of dried flowers	Antibacterial Activity
4	Hot water extract of dried aerial parts	Antihyperglycemic Activity
5	Alkaloid fraction of dried leaves	Cytotoxic Activity
6	Chloroform extract of root	Antimalarial Activity
7	Alkaloid fraction of the entire plant	Antidiuretic Activity
8	Ethanol (70%) extract of leaves	Antimitotic Activity
9	Hot water extract of dried leaves	Animutagenic Effect
10	Methanol/water (1:1) extract of dried leaf and stem	Antifertility Activity
11	Total alkaloids of root	Antihypertensive Activity
12	Acetone and water extracts of dried aerial parts	Antifungal Activity

Traditional uses of Vinca rosea in world wide as represented below in table-2

Table 2:

Country	Use
Australia	Hot water extract of dried leaves is taken orally for menorrhagia, diabetes and extract of root bark is taken orally as febrifuge.
Brazil	The hot water extract of dried entire plant is taken orally by human for diabetes mellitus.
China	Hot water extract of the aerial parts is taken orally as a menstrual regulator.
Cook Island	Decoction of dried leaves used orally to treat diabetes, hypertension and Cancer.
Dominica	Extract of leaves is taken orally by pregnant woman to combat primary inertia in child birth and the boiled leaves are drink to treat diabetes.
England	Hot water extract of dried entire plant is taken orally for the curing of diabetes.
Europe	Decoction of dried leaves is taken orally for diabetes mellitus
France	Hot water extract of entire plant is taken as an anti galactagogue.
French Guinea	Hot water extract of entire plant is taken orally as a cholagogue
India	The hot water extract of dried entire plant is taken orally by human for cancer. Hot water extract of dried leaves is takenorally to Hodgkin's disease. The root extract is taken orally for menorrhagia.
Jamaica	Hot water extract of dried leaves is taken orally for diabetes.

Table 3: Data showing the preliminary phytochemical screening of the whole plant of aqueous extract of the catharanthus roseus.

S. No.	Name Of The Test	Aqueous Extract Catharanthus Roseus
1.	Test for phenol	Absence
2.	Test for tannins	Presence
4.	Test for alkaloids	Absence
5.	Test for glycosides	Presence
6.	Test for triterpenes	Presence
7.	Test for steroids	Absence

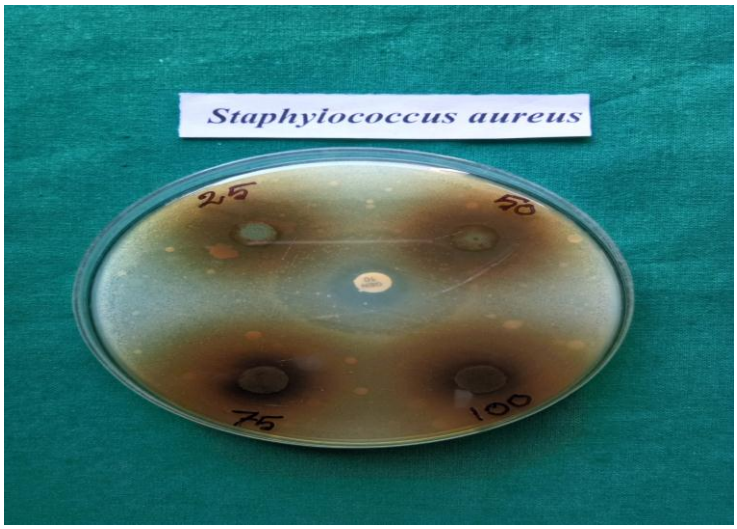

Fig. 1:

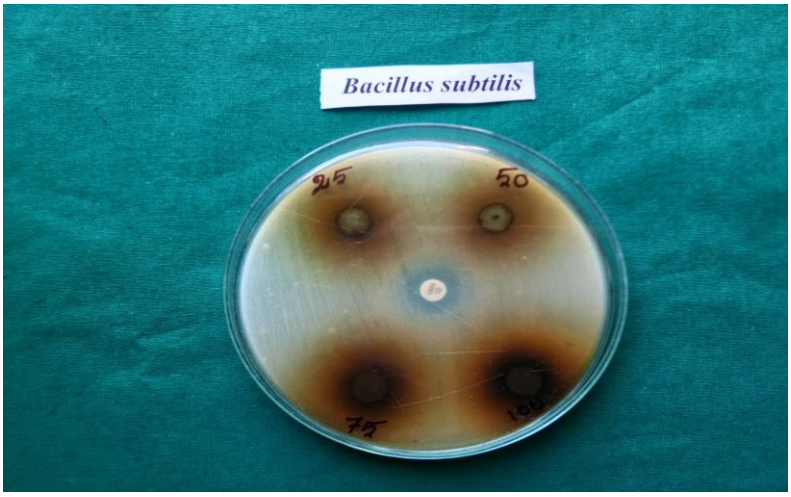

Fig. 2:

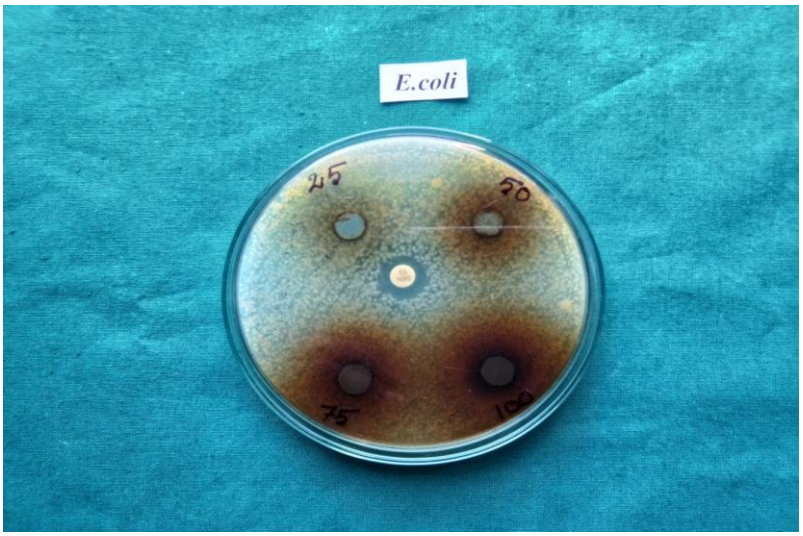

Fig. 3:

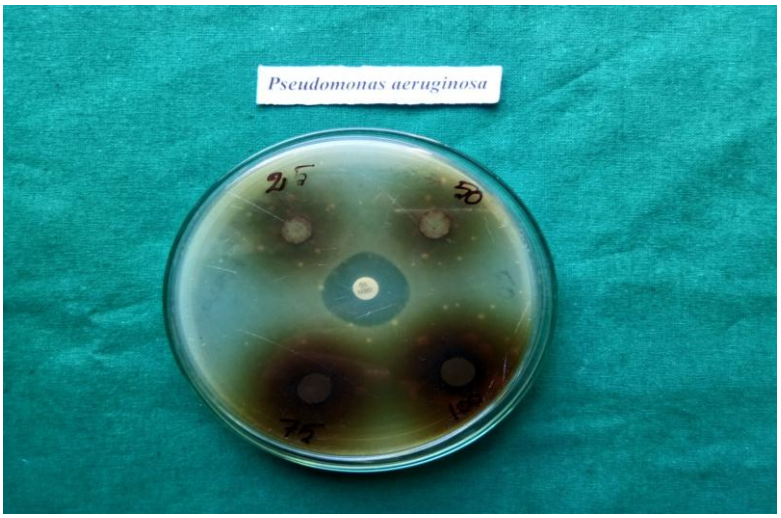

Fig. 4:

Fig. 5:

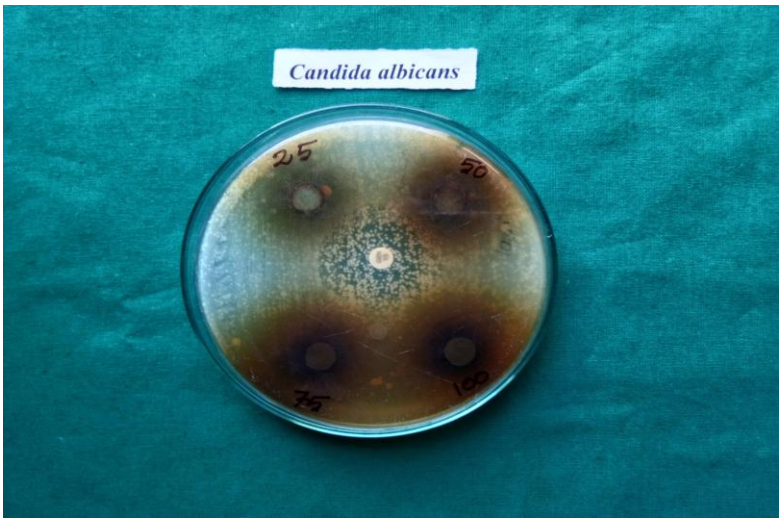

Fig. 6:

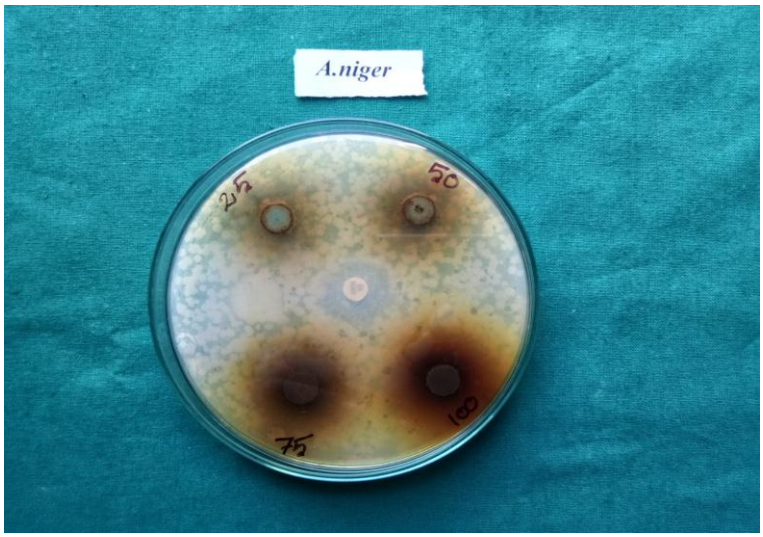

Fig. 7:

Zone of inhibition of plant of vinca

Name of the micro organism

Control: **gentamycin**

Micro organism	**M1 Plant Extract 100 µl added and Zone of inhibition(mm/ml)**				
	25 µl	50 µl	75 µl	100 µl	Control
Staphylococcus aureus	20	24	30	35	35
Enterobacter	20	22	26	30	30
Bacillus subtilis	18	20	25	30	30
E.coli	15	20	25	30	18
Pseudomonas aeruginosa	16	18	21	25	25
Klebsiella pneumoniae	18	21	25	28	22
Candida albicans	18	20	25	28	30
A.niger	18	21	23	26	25

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