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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *CATHARANTHUS ROSEUS*

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ABSTRACT

***Catharanthus roseus* is important medicinal plants which are used for cancer treatment. In the present study an attempt was made to investigate the phytochemical analysis and antimicrobial activity of different solvent extract of *catharanthus roseus* was conducted using agar disc diffusion method. The maximum activity observed against some microorganism. The qualitative analysis of phytochemical screening reveals the presence of some bioactive compounds.**

KEYWORDS: *Catharanthus roseus*, Antimicrobial Activity, Phytochemical Analysis.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body as well as their physiological activities due to the presence of bioactive substance include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Paikara *et. al.,,* 2015). Above activity of those compounds should depend on the methods and solvent used for extraction (Kabesh *et. al.,* 2015). *Catharanthus roseus* is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et. al*., 2009). The leaves and flowers of this plant are effective for diabetic patients. *Catharanthus roseus* are cultivated two common names, which is named on the basis of their flower colours, Pink: Rosea, White: Alba. (Sain *et. al.,* 2013).

ANTIMICROBIAL ACTIVITY

The different parts of plants were used and extracts were subjected to antimicrobial assays (Patil *et al*., 2010). *C. roseus* against clinically significant bacterial strains. Emerging and remerging infections and spread of deadly, drug-resistant strains of organisms pose a challenge to the global public health for their treatment. Bacterial resistance to antibiotics is a major therapeutic problem and the rate at which new antibiotics are being produced is slowing, (Russell *et al*., 2002). The studies of antimicrobial antifungal property of periwinkle leaves extract have been checked against microorganisms like *Escherichia coli, Staphylococcus aureus (*Balaabirami *et al.,* 2012). The antifungal activity have been checked against *Aspergillus niger, Aspergillus flavus* (Patharajan *et al*., 2012)

PHYTOCHEMICAL TEST

Phytochemicals are basically divided in two groups that are primary and secondary metabolites according to their functions in plant metabolism. Primary metabolites comprise of common sugar, amino acids, proteins, whereas Secondary metabolites alkaloids, flavonoids, and tannins. The phytochemical screening of different solvent the crude plant extracts revealed the presence of various secondary metabolites.

MATERIALS AND METHODS

Collection of the Plant Samples

Fresh plant parts were collected randomly from Durg district of Chhattisgarh. The plants were identified and studied according to their families Fresh plant materials were collected and washed under tap water, shade dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of Plant Extract

Ten grams of air dried powder was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 hours. After 24h, the supernatant was discarded and petroleum ether was evaporated from the powder. This dry powder was then taken in 100 ml of solvent (methanol or acetone) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, the extracts were centrifuged at 5000 rpm for 10 min, the supernatant was collected, solvents were evaporated and the dry extract was weighed and stored at 4^0C in airtight bottles. The extraction was done at least three times for each plant. The preliminary qualitative phytochemical analysis was carried out in crude dry powder of selected plants.

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Soxhlet Extraction Method

Leaves of selected plants were collected locally. Leaves were washed; air dried under shade and powdered with the help of Grinder. Powdered leaves were weighed and packed in soxhlet. Solvent used for soxhletion was petroleum ether and ethanol. Extraction was continued at the temperature of 35°C till clear solvent was observed in thimble. Extract was concentrated in water bath at 40°C. Concentrated extract was concentrated at 40°C in hot air oven. Concentrated extract was packed in an air tight container.

Qualitative Phytochemical screening

Catharanthus roseus with petroleum ether extract were subjected to various qualitative tests for the identification of plant constituents present in this species (Nandkarni & Khare, 2007 and Bakshi,1999).

Table 1: Qualitative Analysis of Phytochemicals

S No.	Test	Observation
1	**Test for Alkaloid**	
	1.0ml of plant extract was taken and then adds 1.0 ml of saturated solution of picric acid was added.	Yellow colour appears
2	**Test for Tannins**	
	About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 $fecl_3$ was added	Brownish green or blue- black coloration.
3	**Test for Saponins**	
	0.5g of extract was added in 5ml of distilled water in a test tube. The solution was shaken vigorously. The frothing was mixed with 3 drops of olive oil and shaken vigorously.	Stable persistent froth appears. Formation of an emulsion
4	**Test for Cardiac Glycosides**	
	0.5g of extract was diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of $feCl_3$. This was under laid with 1 ml of conc. Sulphuric acid.	A brown ring at the interface. A violet ring was appeared below the brown ring. Greenish ring may form just above the brown ring.
5	**Test for Tarpenoids**	
	5 ml of extract was mixed with 2 ml of chloroform and 3 ml of conc. H_2SO_4 was carefully added to form a layer.	A reddish brown coloration of the interface was formed.
6	**Test for Phenol**	
	2 ml of extract was taken and add 2 ml of Folin's reagent.	Appearance of violet or brown colour.
7	**Test for Flavonoids**	
	5 ml of dil. Ammonia solution were added to a portion of the crude extract followed by addition of conc. H_2SO_4.	Yellow coloration occurs.
8.	**Test for Carbohydrates**	
	10 ml H_2O was added in 2 ml of extract and 2 drops of ethanolic α-naphthol were added which was followed by addition of 2 ml of conc. H_2SO_4.	Reddish violet ring at the junction appears.

Antimicrobial Activity

The antimicrobial activities of different plants were evaluated by Agar well diffusion test technique.

Antimicrobial Screening Test Microorganisms

Antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive bacteria- *Staphylococcus aureus*, Gram negative- *Escherichia coli* and fungal strains *Aspergillus niger* and *Aspergillus flavus*. The

bacterial cultures were grown and maintained on Nutrient Broth medium at 37ºC for 24h while the fungal culture were maintained on Potato Dextrose Agar slants and incubated at 27ºC for 48h.

Antibacterial Assay

Fresh microbial culture of 0.1ml was spread on nutrient agar plate with glass spreader. A well of 6 mm diameter was punched off into agar medium with sterile cork borer and filled with 50 µg of ethanol, acetone, chloroform and petroleum ether extracts by using micropipette in each well in aseptic condition. The petriplates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes and further incubated in an incubator at 37 ℃ for 24 h. The antibacterial screening was evaluated by measuring the zone of inhibition.

Antifungal Assay

The antifungal activity of the leaf extracts was determined using agar well diffusion method. Small amount of diluted fungal suspension were poured over the media to spread uniformly on the surface. Later when the surface was little dried wells of 8mm were punched in the agar with stainless steel borer and filled with 300µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 28°C for 72 hours and the antifungal activity was assessed by measuring the diameter of the zone of inhibition at the interval of every 24hrs.

RESULTS AND DISCUSSION

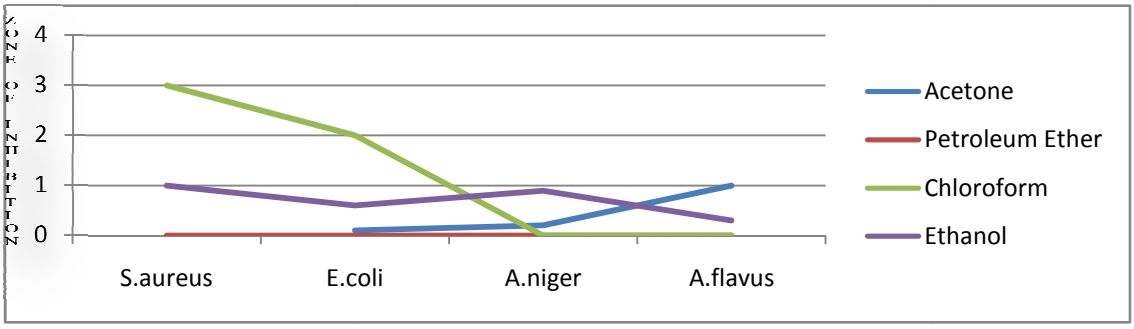
In the present study plants were collected and were authenticated. Then they were shade dried and powdered and were subjected to phytochemical screening antimicrobial activity. The dried powdered leaves of *Catharanthus roseus* were subjected to soxhlet extraction with petroleum ether. The qualitative chemical tests for the extracts were performed. The investigation showed that *Catharanthus roseus* contains tannins, alkaloid, flavonoid, terpenoid and Cardiac glycosides were present in *Catharanthus roseus*. Carbohydrate proteins were absent. Antimicrobial efficacy of the crude extract of *Catharanthus roseus* was quantitatively assessed on the basis of inhibition zone. The ethanol, chloform, petroleum ether and acetone of *Catharanthus roseus* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table).

Table 2: Phytochemical Evaluation of *Catharanthus roseus*

S.No.	Phytochemical	Result
1	Tannins	+
2	Cardiac glycosides	+
3	Alkaloid	+
4	Flavonoid	+
5	Carbohydrate	-
6	Tarpenoids	+
7	Proteins	-

Table 3: Antimicrobial activity of leaf of *Catharanthus roseus* (inhibition zone)

S No.	Solvent	Zone of inhibition (mm)			
		Bacteria		Fungus	
		S. aureus	*E.coli*	*A.niger*	*A. flavus*
1.	Acetone	-	.1	0.2	1
2.	Petroleum ether	0.5	-	-	-
3.	Chloroform	3	2	0.1	0.1
4.	Ethanol	1	0.6	0.9	0.3



Graph: Antimicrobial activity of *Catharathnus roseus* using different solvents

The most susceptible bacterium and fungi are *S. Aureus* and *A. niger*, respectively. The inhibition zones were in the range of 0.1 to 3 mm for most of the tested strains. Chloroform extract of *Catharanthus roseus* shows highest antimicrobial activity and petroleum ether least antimicrobial activity. According to table and graph shows that the Chloroform leaves extract exhibited highest zone of inhibition against S. aureus and ethanol leaves extracted exhibited highest zone of inhibition A. niger (0.9 mm respectively).

CONCLUSION

Many plants were being investigated for their antimicrobial activity by many scientists of different parts world. They found thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. From the above result it can be concluded that the leaf extract of selected plants have potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by many microorganisms. Phytochemical analysis of *Catharanthus roseus* leaves extracts was done by using the extracts which were obtained by cold extraction method and soxhlet method. The screening of phytochemical constituents of plants *Catharanthus roseus* indicated the presence of tannins, alkaloid, flavonoid, terpenoid and Cardiac glycosides were present in common. The results of the maximum antibacterial activity was identified with different solvents leaf extract of *C. roseus* against *S. aureus* and *A.niger* might be due do the presence of the unique phytochemical constituents. Chloroform extract of *Catharathnus roseus* shows highest antimicrobial activity and petroleum ether extract shows least antimicrobial activity.

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