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PHYTOCHEMICAL PROFILE, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITYOF *CATHARANTHUS PUSSILUS*

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ABSTRACT: Catharanthus pussilus is well known for its medicinal properties. In this study DPPH Scavenging activity and amylase inhibition assay is described. In DPPH scavenging activity alcohol shows zone of inhibition and the values are compared with butylated hydroxy anisole (BHA). BHA used as a reference standard. More phytochemicals are present in present in Catharanthus pussilus. It is responsible for perspective activity.

KEYWORDS: Catharanthus pussilus, DPPH scavenging, phytochemicals

1. INTRODUCTION:

Many plants are used as a medicine because of the presence of secondary metabolise such as such as alkaloids, glycosides, flavonoids, volatile oils, tannins, resins, quinines etc are produced from the plant and it has been widely used for medicinal, industrial and commercial purpose. *Catharanthus pussilus* is a small erect annual herb, native to India. It has many branches, spreading from the base with narrow leaf. White color flowers appear, in the upper leaf axils [1-5]. Flowers with 5 petals the length about 6-8mm tiny and flat. Seed pod is very slender. Flowering during the month between July-August.

2. EXPERIMENTAL SECTION

2.1. MATERIALS:

2.1.1. PREPARATION OF CP-AL EXTRACT:

The freshly collected whole plant of *Catharanthus pussilus* were shaded dried and then crushed using mechanical grinder. A weighed quantity of powder was subjected to continuous hot extraction with alcohol in soxhlet apparatus for 3 days. Then the extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample.

2.2. METHODS

2.2.1. PHYTOCHEMICAL STUDIES:

The extract obtained from successive solvent extraction of *Catharanthus pussilus* were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, carbohydrates, proteins/amino acids, glycosides, phenol, tannins, phytosterols, flavonoids, Saponins, lactones, coumarins, terpenes [6].

2.2.2 ANTI-BACTERIAL ACTIVITY:

The Anti microbial activities of the extract were performed with Escherichia coli, Staphylococcus aureus were used as test organism by Agar diffusion method. The stock cultures of bacteria were revived by inoculating in broth media and grown at 37 degree Celsius for 18 hrs. The agar plates of the media were prepared and wells were made in the plate. Each plate was inoculated with 18 hold cultures (100µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. All the plates were incubated at 37 degree Celsius for 24 hours and the diameter of inhibition zone were noted and compared with standard Ciprofloxacin. [7].

2.2.3 ANTI-FUNGAL ACTIVITY:

The Anti fungal activity of the extract were performed with Aspergillus Niger, Aspergillus flavus were used as test organism by Agar diffusion method. The stock cultures of bacteria were revived by inoculating in broth media and grown at 37 degree Celsius for 18 hrs. The agar plates of the media were prepared and wells were made in the plate. Each plate was inoculated with 18 hold cultures (100µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. All the plates were incubated at 37 degree Celsius for 24 hours and the diameter of inhibition zone were noted and compared with standard Amphoterician [8].

2.2.4 ANTI-OXIDANT ACTIVITY:

The antioxidant activity of the sample was determined in different concentrations of samples in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500µl by adding Methanol. Five millilitres of a

0.1 mM methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH; from Sigma –Aldrich, Bangalore) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the samples was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as reference standard. [9]
Free Radical scavenging activity was calculated using the following formula:
Percentage radical scavenging activity = (control OD - sample OD)/ control OD × 100

2.2.5 AMYLASE INHIBITION ASSAY:
The inhibition assay was performed using the chromogenic DNSA method [Miller, 1959]. At 37°C the total assay mixture is incubated with 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase and samples at concentration 20, 60, 80 µl for 10 min. Then 500 µl of 1% (w/v) starch solution is added after pre-incubation, in the above buffer, that will be incubated at 37°C for 15 min. The solution is placed in a boiling water for 5 minutes ,after the reaction was terminated with 1.0 ml DNSA reagent, and then allowed to cooled at room temperature and the absorbance measured at 540 nm. After adding DNS solution the extract are added with the reacting mixture. The liberated sugar was determined by the help of standard maltose curve and activities that were calculated according to the following formula. Concentrated of maltose liberated is multiplied with ml of enzyme used ,that value will be divided by molecular weight of maltose multiplied with incubation time the whole value is calculated with dilution factor .

3. RESULTS AND DISCUSSION:

3.1 PHYTOCHEMICAL ANALYSIS:

Preliminary phytochemical screening of (CP AL) mainly revealed the presence of alkaloids, carbohydrates, proteins, lactones, steroids, and negative results were obtained for tannins, terpenes, Flavanoids, phenol and glycosides in ethyl acetate extract.

3.2 ANTIBACTERIAL ACTIVITY:
The whole plant of *Catharanthus pussilus* in ethyl acetate (CP AL) was acted for their anti-bacterial and anti-fungal activities using agar diffusion method. The extract of the inhibitors (mm) are mentioned in the table.2 shows the antibacterial and anti-fungal activities of CP in AL .At higher concentrations DTZ against Catharanthus pussilus was 4mm at E-coli. Sample has not shown any zone of inhibition for S.aureus

TABLE: 1 ANTIBACTERIAL ACTIVITY OF CP IN AL

Sample	20 µl	60 µl	80 µl
E.coli	0	4	3
S.aureus	0	0	0

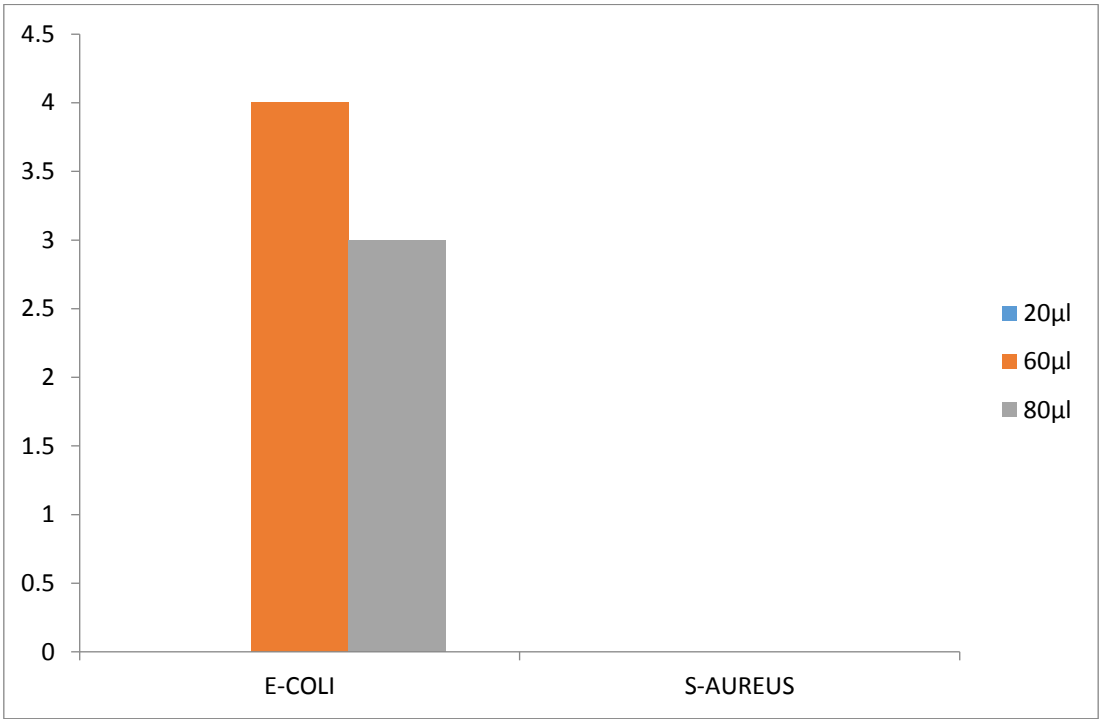


FIG: 1 ANTIBACTERIAL SUSCEPTIBILITY ASSAY OF ALCOHOL EXTRACTS WHOLE PLANT OFCATHARANTHUS PUSILLUS AT HIGHER CONCENTRATION

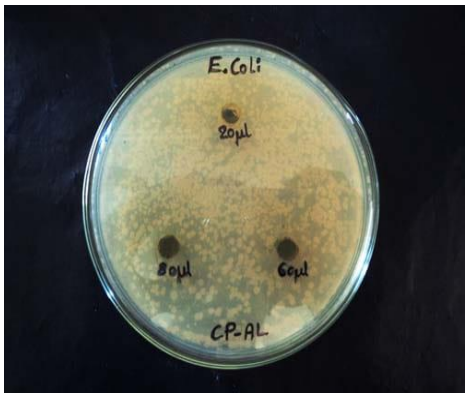
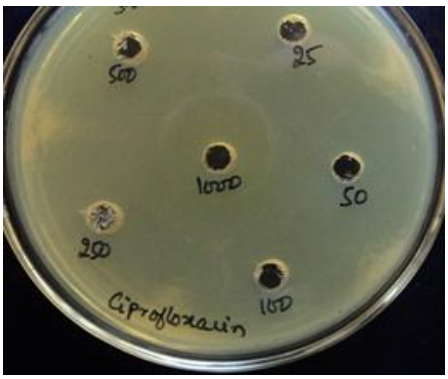


FIG: 2 CP AL E.coli FIG: 3 CP AL S.aureus

TABLE: 2ANTIBACTERIAL ACTIVITY OF STD CIPROFLAXIN

Organism	25	50	100	250	500	1000	MIC
E-COLI	26	29	32	34	38	*	25
S.aureus	25	28	31	34	36	*	25

*Zones could not be measured due to merging



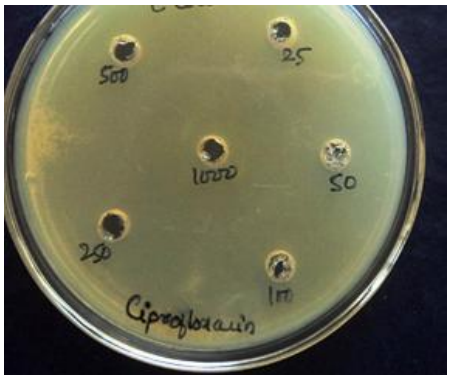


FIG: 4 E.COLI WITH STD CIPROFLAXIN FIG: 5 S.AUREUS WITH STD CIPROFLAXIN

3.3 ANTI FUNGAL ACTIVITY:

The alcoholic extract of Catharanthus pussilus is totally inactive against human pathogens .Sample has not shown any zone of inhibition for A.niger and A.Flavus.

3.4 ANTI-OXIDANT ACTIVITY

Accordingly to DPPH antioxidant assay of CP in AL showed the activity of 50.53 % 44.06% and 42.44% at three different concentration (20µl, 60µl, 80µl) respectively. The whole plant extract of *Catharanthus pussilus* was compared with reference standard burylated hydroxyl anisole with the same concentration are shown in the table 2.

TABLE: 3 DPPH SCAVENGING ASSAY CP IN AL:

CONCENTRATION	%FREE RADICAL SCAVENGING	
	CP-AL	BHA
20µl	50.5396	54.3
60µl	44.0647	70.1
80µl	42.4460	91.7

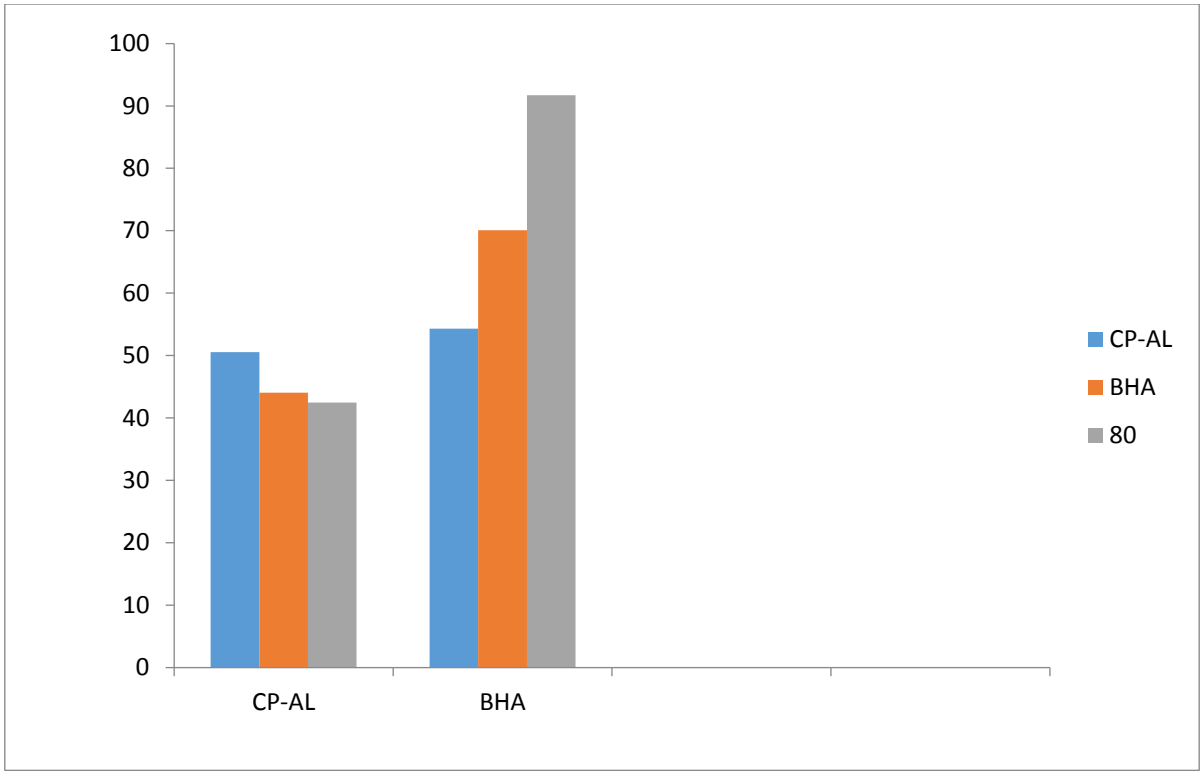


FIG: 6 PERCENTAGE FREE RADICAL SCAVENGING ACTIVITY OF SAMPLE IN COMPARISON (BHA)

3.5 ANALYSIS OF ACARBOSE AS STANDARD INHIBITOR IN CATHARANTHUS PUSSILUS:

Acarbose was used as a standard inhibitor and it was assayed at above mentioned test sample concentrations. The test sample was compared to that of results.

TABLE: 4PERCENTAGE INHIBITION OF ALPHA AMYLASE ENZYME BY THE SAMPLES:

	CP-AL
20µl	0.05634037
60µl	0.05777432
80µl	0.05499894

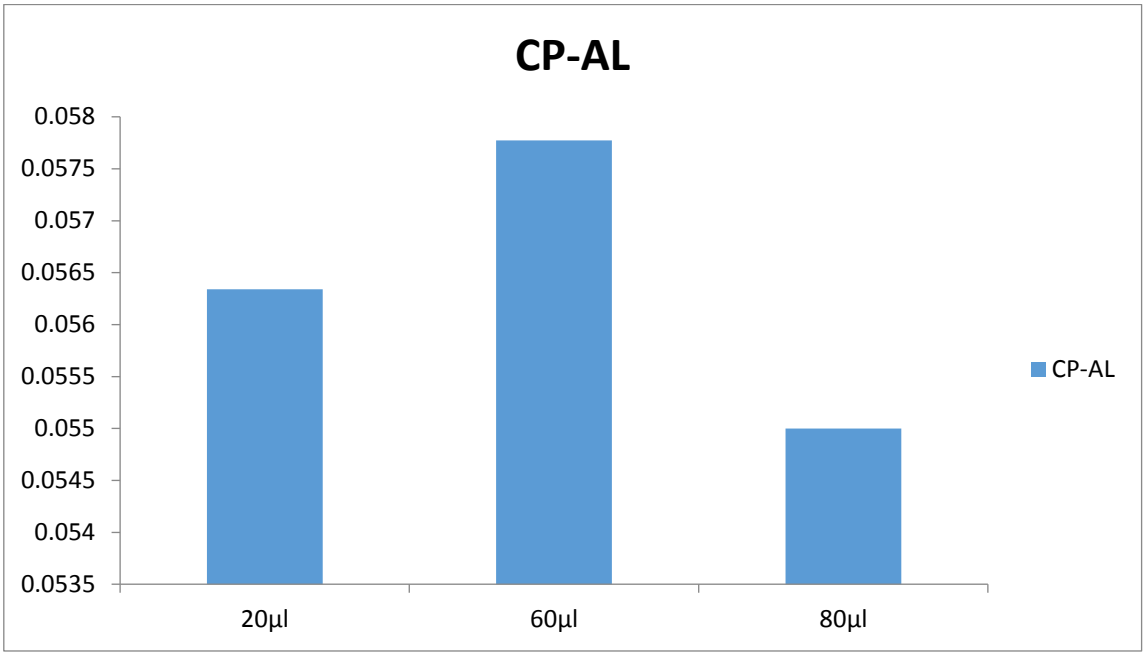


FIG: 7 AMYLASE INHIBITION ASSAY

4. CONCLUSION:

The current study describes many phytochemicals present in Catharanthus pussilus. Due to the presence of phytochemicals, it is used as a medicine .Because of its much medicinal usage there is numerous scope of future research on Catharanthus pussilus.

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