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Research Article

ANTIBACTERIAL AND ANTIDIABETIC EVALUATION OF *CATHARANTHUS ROSEUS* PLANT

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ABSTRACT

The present work is aimed at evaluating antibacterial and antidiabetic activities of whole plant extract of *Catharanthus roseus.* Dichloromethane: methanol (1:1) extract was used for carrying out *in-vitro* antibacterial and *in-vivo* antidiabetic activity. Antibacterial activity was carried out using seven different gram positive and gram negative bacterial strains. The extract was found to have considerable antibacterial action against all bacterial strains. The study on antidiabetic activity involves induction of diabetes to all male Wister albino rats using alloxan monohydrate (80mg/kg body/weight) except control group followed by treatment of diabetic rats with extract (500mg/kg body weight) daily for 14 days. Glucose level was found to decrease and body weight was increased in extract treated and standard treated groups when compared to diabetic group. The influence of extract on biochemical parameters like total cholesterol, urea, creatinine and alkaline phosphatase were also measu red. The results obtained confirms antibacterial and antidiabetic activity of extract and therefore can be used not only against treating infections but also be used in treating diabetes.

Keywords: Antibacterial, Antidiabetic, *Catharanthus roseus,* Alloxan monohydrate, Glibenclamide.

INTRODUCTION

Catharanthus roseus which is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases. *C. roseus* also possess good antioxidant potential. There are about two common cultivars of *C. roseus* which is named on the basis of their flower color that is the pink flowered 'Rosea' and the white flowered 'Alba'. *C. roseus* is extensively cultivated in northern India in order to meet their commercial and the ever increasing demand in the indigenous systems of the medicine also their need to the pharmaceutical industry. Medicinally important plants are also rich source of antibacterial agents. There are number of plants investigated for their antibacterial activity, to cite few examples are: St. John's wort against *S. aureus ,S. mutans, P. vulgaris, E. coli, P. aerugino* Liquorice root against *S. aureus, S. mutans,* Uva ursi against *E. coli, S. aureus,* Garlic against *S. typhimurium, B. cereus, E. coli, S. aureus* and Sage against *B. cereus, S. aureus*[1-5]. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia (high blood glucose concentration) caused by insulin deficiency, often combined with insulin resistance[6]. Insulin is hormone released from β cells of pancreas and convert glucose (source of energy in body) to glycogen thus maintaining the glucose levels in the body. The deficiency of insulin leads to increase of glucose levels in blood and urine. Diabetes can also gives rise to other diseases like cataracts, cardiac problems, etc., There also occurs changes in biochemical parameters like cholesterol, urea, creatinine etc. The present study involves *Catharanthus roseus* (Apocynaceae) also known as medagascar periwinkle, is a perennial subshrub with green color simple, entire, petiolate leaves and violet pink-white or carmine red color flowers.[7] It contains 150 alkaloids including vincristine vinblastine, ajmalicine, etc[8]. The plant has been considered due to its wide range of pharmacological activity like anti-inflammatory, antimalarial, antimitotic, antihypertensive, antifertility, antihypercholesterolemic, antimutagenic, antidiuretic, antifungal, antispasmodic, antiviral, cardio tonic, CNS depressant, antitumor, cytotoxic, antispermatogenic, anticancer activities.The study involves whole plant of *Catharanthus roseus* for evaluating both antibacterial and antidiabetic activity[9] (Figure 1).

Figure 1: Represents *Catharanthusroseus* plant.

MATERIALS AND METHODS

Plant Collection and Identification: The whole plant material of *Catharanthus roseus* were collected from Medicinal Garden.

Preparation of Extract: The whole plants of *Catharanthus roseus* were collected, washed with tap water and then with distilled water and kept for shadow drying in a clean space. The dried material was then subjected to chopping and grinding for obtaining coarse powder. About 100gms of powder was weighed and subjected for extraction with dichloromethane: methanol (1:1).[10] using soxhlet apparatus for 11 hr. The extract so obtained was concentrated to obtain the dried form. The extract yield obtained was about 10 gm. The dried extract obtained was subjected to phytochemical screening to know the presence of alkaloids and further for carrying out *in-vitro* antibacterial and *in-vivo* antidiabetic studies.

Phytochemical screening: The extracted was subjected to chemical analysis for the presence of Carbohydrates, Flavonoids, Phenols, Tannins Alkaloids, Glycosides, Carbohydrates, Proteins and amino acid, Fats and Oils[11]. TLC was also performed for qualitative analysis to know the presence of alkaloids. TLC was performed using precoated plates on which the standard vinblastine sulphate solution and extract were spotted and then placed in chamber equilibrated with the mobile phase choloroform**:**methanol (19:1). After running ¾th of chromatogram Rf values were calculated by observing under UV light.[12]

IN-VITRO ANTIBACTERIAL STUDY

Test organisms: Pure cultures of seven bacterial isolates (B.*cereus, B. subtilis, S. aureus, K.pneumoniae, E. coli, P. aeruginosa, and P. vulgaris)* were collected from the Medicinal Garden.

Preparation of extract solutions: 10, 25, 50, 75 and 100 mg of the dried plant extract were dissolved separately in each 1 ml DMSO (dimethylsulphoxide).

Procedure: The method used for the determination of antibacterial activity is, agar diffusion method[13,14] In this method, nutrient agar medium was prepared and sterilized along with washed and dried petriplates. After sterilization the nutrient agar medium approximately 20 ml was poured in all the petriplates under aseptic conditions and allowed to solidify. After solidification, all the petriplates were marked and were inoculated with 100µl of respective bacterial culture from the nutrient broth medium and incubated for 5 min in inverted position. Then bores were made using cork borer and 100 µl of extract of different concentrations were poured in the wells. Similarly standard Streptomycin was also placed in seven different bacterial strains. All the plates were incubated at 37ºC for 24 hrs and zone of inhibitions were measured in mm.

IN-VIVO *A*NTIDIABETIC ACTIVITY:

Animals: Healthy, adult female albino rats of Wistar strain, weighing 180-220 g and having normal glucose levels were obtained from the animal house. The animal house was well ventilated and the animals were exposed to 12 h day and night cycles at a temperature of 20 ± 20C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period.

Animal Grouping: Group I: Control (Normal rats)

Group II: Alloxan induced Diabetic rats (80mg/kg body weight)

Group III: Diabetes induced rats+ whole plant extract (500mg/kg body weight)

Group IV: Diabetes induced rats+ Glibenclamide (5mg/kg body weight)

Diabetes induction: Alloxan monohydrate was used for the induction of diabetes and the solution (80 mg/kg body wt) was prepared in normal saline and was given intraperitoneally.

Preparation of standard solution: The weighed amount of powered standard glibenclamide (5mg/ kg body wt.) was dissolved in normal saline and was given p.o.

Preparation of extract solution: The weighed amount of powdered extract was suspended in normal saline solution using few drops of tween 80 and was given the dose 500 mg/ kg body wt., p.o.[15]

Diagnostic kits: Diagnostic kits used for the estimation of Urea, Cholesterol, Creatinine, Alkaline Phosphatase were collected.

Procedure: Animals having normal glucose level wereconsidered and diabetes was induced, using 0.5ml of Alloxan monohydrate (80mg/kg body weight) i.p togroup II, III and IV keeping group I as normal control. After 24 hrs, the body weights and glucose levels were measured. Daily dose of whole plant extract (500mg/kg body weight) and standard (glibenclamide, 5mg/kg body weight) was given orally (0.5ml p.o) to group III and IV for 14 days respectively. Glucose levels and body weights were measured on seventh and fourteenth day.19Animals were sacrificed by decapitation on 14th day, the Pancreas were isolated and send for histopathology studies . The blood samples (2ml) were also collected and subjected to centrifugation at 5000rpm for 20 minutes. The serum after centrifugation was analyzed for Alkaline Phosphatase (Kind and King's method) Urea (urease, Berthelot, Endpoint method) Creatinine, (Alkaline picrate method), and Total Cholesterol level using diagnostic kits.[16]

STATISTICAL ANALYSIS: Data obtained for antibacterial and antidiabetic activity were written as mean± SEM . Statistical treatment applied is ANOVA two way classifications. The results are significant if $p<0.01$ and $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening: Chemical analysis showed the presence of alkaloids and carbohydrate (Table 1). TLC performed indicates that R_f value for the extract was same as that of standard vinblastine sulphate indicating its presence in the extract (Figure 2).

Table 1: Phytochemical profile of *Catharanthus roseus*

Phytoconstituents	Test	DME
Carbohydrates	Molish's Test	+
Alkaloids	Dragandorff's	+
	Mayer's	+
	Wagner's	+
	Hager's	+
Glycosides	Glycoside test	-
Flavonoids	Flavonoid test(lead acetate solution)	-
Tannins	Tannins test(5% $FeCl_3$ solution)	-
Saponins	Saponin test	-
Protein	Biuret test	-
Amino acids	Ninhydrin solution	-
Fats and Oils	Staining paper	-

DME: dichloromethane: methanol whole plants extract

Thin layer chromatography:

Figure 2: Thin layer chromatogram for extract (ext 1) and standard (VB)

R_f value of standard:

Distance travelled by standard solution = 0.6 cm
Distance travelled by solvent front= 4.5 cm
R_f = 0.13

R_f value for whole plant extract:

Distance travelled by solvent front = 4.5 cm Distance travelled by test sample = 0.65 cm R_f = 0.14

***IN-VITRO* ANTIBACTERIAL ACTIVITY:**

In *in-vitro* study, it is found that dichloromethane: methanol (1:1) whole plant extract of *C. roseus* exert inhibitory activity against all the bacterial strains in the concentration range of 10- 100 mg/ml and showed increased zone of inhibition with increase in concentration (dose dependent). As shown in Table 4, the extract showed significantly more activity against E. *coli* with the zone of inhibition of 21mm for 100mg/ml when compared to standard with a zone of inhibition 18.70mm ($p<0.01$). *P.vulgaris, B. cereus, B. subtilis and S. aureus* also showed more activity with zone of inhibitions of 11.75,13.25, 11.50 and 13.00 mm respectively for 100mg/ml concentration when compared to standard with the zone of inhibition 8.50.11.33,9.50 and 12.66mm respectively ($p<0.01$).*K. pneumoniae* and *P. aeruginosa also* showed to have nearly same activity as that of standard.(Table 2 and Figure 3).

Figure 3: Zone of inhibition at different concentrations against *E. coli* strain in whole plant extract of *C. roseus.*

EFFECT OF AN ANTIDIABETIC EXTRACT OF *CATHERANTHUS ROSEUS* ON ENZYME ACTIVITIES

Hypoglycemic activity was detected by using the dichloromethane:methanol extract (1:1) of the leaves and twigs of *Catharanthus roseus* medicinal plant in streptozotocin (STZ) induced diabetic rat model at the dose of 500 mg/kg that was given orally for 7 and 15 days.The extract showed 48.6 and 57.6% hypoglycemic activity and further treatment for 30 days has provided complete protection against STZ challenge (75 mg/kg/i.p.×1). Enzymic activities of glycogen synthase, glucose 6-phosphate- dehydrogenase, succinate dehydrogenase and malate dehydrogenase was found to be decreased in the liver of diabetic animals which would be significantly improved after treatment with extract at dose

500 mg/kg p.o. for 7 days. Results indicated the increased metabolization of glucose in treated rats with the increased levels of lipid peroxidation.[17] (Table 3, 4, 5).

***IN-VIVO* ANTIDIABETIC STUDIES:**

In-vivo study showed that alloxan induced diabetic rats when treated with dichloromethane: methanol (1:1) whole plant extract (500 mg/kg body wt.) have significant decrease in the glucose levels(Table 3, figure 2) and increase in the body weights(Table 4, chart 3) when compared to normal and standard treated diabetic rats ($p<0.05$).

Table 2: Zone of inhibition of whole plant extract of *Catharanthus roseus* against different bacterial strains
Values are mean± SEM

Sl. No.	Bacterial strain used	Zone of inhibition in mm (Excluding the bore diameter =8mm)					
		Streptomycin (500 µg/ ml)	Whole plant dichloromethane methanol extract				
			10mg/ml	25mg/ml	50mg/ml	75 mg/ml	100 mg/ml
1	*K.pneumoniae*	10.73±0.47	5.23±0.33	6.33±0.67	8.5±0.80	10.15±0.63	10.30±1.32
2	*E. coli*	18.40±0.58	7.13±1.33	9.00±0.00	14.6±2.50	19.75±1.93	21.00±3.08
3	*P.aeruginosa*	12.33±0.33	4.33±0.33	6.57±0.33	10.5±1.19	10.6±1.03	12.00±1.08
4	*P. vulgaris*	8.40±0.29	6.00±0.58	7.00±1.00	8.30±0.72	10.5±0.50	11.55±1.11
5	*B. cereus*	11.33±0.88	4.33±0.33	7.0±0.00	10.0±0.63	11.75±0.73	13.15±1.31
6	*B. subtilis*	9.20±0.29	4.57±0.33	6.23±0.33	8.75±0.25	10.75±0.48	11.5±0.29
7	*S. aureus*	12.36±0.34	3.23±0.33	5.60±0.34	9.30±1.17	11.15±1.25	13.00±0.48

Table 3: Glucose levels of different groups on different days

Sl. No.	0 day	1 day	7 day	14 day
Group I	73.33±1.20	75.00±5.00	76.33±3.76	80.00±5.29
Group II	85.33±7.42	228.67±18.70	192.00±6.11	119.00±6.08
Group III	88.33±12.75	228.33±20.48	179.33±19.68	103.67±1.86
Group IV	78.33±1.20	185.33±7.86	133.00±2.65	82.00±3.33

Table 4: Body weights measured for various group of animals on different days

Sl. No.	0 day	1 day	7 day	14 day
Group I	183.33±8.08	192.33±6.49	187.30±6.23	195.00±7.64
Group II	211.67±1.67	184.00±2.31	174.00±5.03	159.00±14.44
Group III	201.67±7.27	160.33±12.33	173.33±14.53	190.67±6.36
Group IV	200.00±5.77	172.67±7.33	177.30±7.51	195.00±1.20

Values are given as mean±SE

Table 5: Biochemical parameters values measured for all groups from the blood serum using diagnostic kits

Sl. No.	**Urea (mg/dl)**	**Total cholesterol (mg/dl)**	**Alkaline Phosphatase (mg/dl)**	**Creatinine (mg/dl)**
Group I	42.50±0.29	116.00±0.58	81.83±0.73	0.36±0.01
Group II	63.33±0.34	183.67±0.34	136.00±1.53	1.35±2.15
Group III	45.83±0.34	99.33±0.33	90.43±3.65	0.41±3.65
Group IV	43.33±0.60	119.67±0.88	85.33±1.76	0.37±2.31

Values are given as mean±SEM

Biochemical parameters:

It is also observed that biochemical parameters like Urea, Creatinine, Total Cholesterol and Alkaline Phosphatase levels increased considerably in diabetes induced rats but are nearer to normal for standard and extract treated animals. From Table 5 and chart 4 & 5, it is observed that Urea, Creatinine, Cholesterol and Alkaline Phosphatase values for dichloromethane: methanol (1:1) whole plant extract were found to be 43.33, 0.36, 120 and 85 mg/dl respectively which is nearer to standard and normal group values.(Table 5)

CONCLUSION

The study showed that, *Catharanthus roseus* plant dichloromethane methanol extract is found to exhibit a significant antibacterial activity against, almost all the bacterial strains and antidiabetic activity in alloxan induced diabetic rats. So, further work can be done for the isolation and molecular characterization of active constituents responsible for antibacterial and antidiabetic activity, can be assessed for the bioactivity of the *Catharanthus roseus* plant

extract and also its activity against wide spectrum of microbes to develop it into a powerful antibiotic.

REFERENCES

1. Kaneda Y, *et al*. In vitro effects of berberine sulfate on the growth of Entamoeba histolytica, giardia lamblia, and Trichomonas vaginalis. *Annals Trop Med Parasitol, 1991; 85:417-425.*
2. Barbagallo G and Chisari G. Antimicrobial activity of three Hypericum species. *Fitoterapia,* 1987; 58: 175-77.
3. Cooper WC and James J. An observational study of the safety and efficacy of hypericin in HIV + subjects. *Int Conf. AIDS,* 1990; 6: 369 (abstract no. 2063).
4. Furner V and Bek Gold M. A Phase I/II unblended dose ranging study of hypericin in HIV positive subjects. *Int Conf AIDS,* 1991; 7:199 (abstract no. W.B. 2071).
5. Mitscher L, Park Y and Clark D, Antimicrobial agents from higher plants. *Antimicrobial isoflavonoids from Glycyrrhiza glabra. J Nat Products,* 1980;43:259-269.
6. Rang HP and Dale MM .Text Book of Pharmacology. 5th edition; 385-86.
7. Kokate CK and Purohit AP.Text Book of Pharmacognosy. 37th edition; 2007: 484-87.
8. Treas and Evans WC. Text book of Pharmacognosy. 2002; 15th edition: 402-403.
9. Huxley A., ed. (1992). *New RHS Dictionary of Gardening*. Macmillan ISBN 0-333-47494-5.
10. Jayanthi M, Sowbala N,*et al.* Study of Antihyperglycemic effect of *catharanthus roseus* in alloxan induced diabetic rats. *International J. of Pharmacy and Pharmaceutical Sciences*;2010:2(4)
11. Khandelwal KR. Practical Pharmacognosy. 2007: 18th edition: 149-153.
12. Ashutosh kar. Pharmaceutical Drug Analysis. 2005; 2nd edition: 409-13.
13. Pulok K. Mukherjee, Kakali Saha, Murugesan T, *et al. J. Ethnopharmacol.,* 1998; 60: 85-89.
14. Gunakkunru A, Padmanaban K, Thirumal P, *et al. J. Ethnopharmacol.,* 2005; 98: 241-244.
15. 15. Mohammed Fazil Ahmed, Syed Mohammed Kazim, *et al.* Antidiabetic activity of *Vinca rosea* Extracts in Alloxan Induced Diabetic Rats. *International Journal of Endocrinology*, 2010; pp 6
16. Mohammed Ibrahim1, Syeda SughraMehjabeen, et al., Pharmacological Evaluatuin of Catharanthus roseus, International Journal of Pharmaceutical Applications. 2011; 2: 165-173.
17. Som Nath Singh, PraveenVats, Shoba Suri, Radhey Shyam, Kumria MML, Ranganathan S. Sridharan K. Effect of an antidiabetic extract of *Catharanthus roseus* onenzymic activities in streptozotocin induced diabetic rats. Journal of Ethnopharmacology, 2001; 76: 269–277.

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