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PHYTOCHEMICAL SCREENING AND EVALUATION OF IN VITRO ANTI DIABETIC ACTIVITY OF *CATHARANTHUS ROSEUS*

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ABSTRACT

The present study aims to investigate the phytochemical profile and evaluate the in vitro anti-diabetic potential of various extracts of *Catharanthus roseus* leaves. Extracts were prepared using petroleum ether, methanol, and water. The methanolic extract showed the highest percentage yield (8.45%). Phytochemical screening revealed the presence of key bioactive compounds such as alkaloids, flavonoids, glycosides, phenols, proteins, and diterpenes, particularly in methanolic and aqueous extracts. Quantitative estimations confirmed that the methanolic extract possessed the highest total phenolic (0.652 mg/100 mg) and flavonoid content (0.745 mg/100 mg). Antioxidant activity, evaluated via the DPPH free radical scavenging assay, showed dose-dependent inhibition, with the hydroalcoholic extract achieving a maximum inhibition of 59.95% and an IC_{50} value of 114.01 µg/ml, compared to 80.23% for ascorbic acid (IC_{50} = 19.73 µg/ml). Furthermore, α-amylase inhibitory assays demonstrated significant enzyme inhibition, with the extract showing 76.65% inhibition at 500 µg/ml (IC_{50} = 228.83 µg/ml), compared to the standard drug acarbose (IC_{50} = 25.32 µg/ml). The results suggest that *Catharanthus roseus* contains multiple phytoconstituents with potential antioxidant and anti-diabetic properties. Further *in vivo* studies and compound isolation are warranted to explore its utility as a natural therapeutic agent in diabetes management.

Keywords: *Catharanthus roseus*, Phytochemical screening, Anti-diabetic activity, α-Amylase inhibition, DPPH assay, Phenolic content, Flavonoids, Herbal medicine, Antioxidant, Type 2 diabetes.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is associated with long-term complications affecting the eyes, kidneys, nerves, and cardiovascular system (American Diabetes Association, 2022). The global prevalence of diabetes has reached epidemic proportions, with the World Health Organization estimating that over 500 million people will be affected by 2030 (WHO, 2021). Despite the availability of synthetic antidiabetic drugs, their long-term use is often associated with side effects such as hypoglycemia, gastrointestinal disturbances, and hepatotoxicity (Maruthur *et al.*, 2016). Hence, there is a growing interest in identifying alternative, safer, and cost-effective remedies derived from medicinal plants.

Medicinal plants have historically served as a significant source of therapeutic agents, and their bioactive compounds known as phytochemicals play an essential role in disease management (Rates, 2001). Among

these, *Catharanthus roseus* (commonly known as Madagascar periwinkle), a plant belonging to the Apocynaceae family, has garnered considerable attention due to its wide range of pharmacological properties including anticancer, antimicrobial, antioxidant, and antidiabetic effects (Van Der Heijden *et al.,* 2004).

C. roseus is known to contain various bioactive constituents such as alkaloids (vincristine, vinblastine), flavonoids, tannins, phenolics, and saponins, many of which have shown potential in regulating blood glucose levels through mechanisms like inhibition of carbohydrate-digesting enzymes (e.g., α-amylase and α-glucosidase) and improvement in insulin sensitivity (Niranjan *et al.,* 2018). Preliminary studies suggest that the ethanolic and aqueous extracts of *C. roseus* exhibit significant in vitro antidiabetic activity (Chaudhary *et al.,* 2012).

The present study aims to conduct a comprehensive phytochemical screening of *Catharanthus roseus* leaf extracts and evaluate their in vitro antidiabetic activity, particularly focusing on the inhibition of key carbohydrate-metabolizing enzymes. This research may contribute to the development of plant-based therapeutic agents for the management of diabetes mellitus.

MATERIALS AND METHODS

Successive solvent extraction of plant drugs

All Collected plant drugs were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (250 gm of each plant drug namely leaves of *Catharanthus roseus* and packed in Soxhlet apparatus. Each plant drug was defatted with petroleum ether (40°-60°C) for about 12 hrs separately & complete defatting was censured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted plant drugs were subjected to extraction by methanol and water as solvent. The process was carried out for about different timings for different solvents. The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Palaiogiannis *et al.,* 2023).

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of leaves of Catharanthus roseus, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Sharma *et al.,* 2020).

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

The total phenolic content was estimated according to the standard method [6]. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added. After

mixing, solution was incubated at 90oC for one minute and the absorbance was recorded at 725 nm against the reagent blank. Using catechol, a standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as Catechol equivalent in µg/mg of extract (Dwivedi *et al.,* 2019).

Estimation of total flavonoid contents

Total flavonoid contents of all the extracts were determined by the method of Zhishen (1999) and expressed as catechol equivalent in µg/mg of extract. An aliquot (1ml) of extracts or standard solution of catechol (20, 40, 60, 80 and 100mg/ml) was added with 0.3ml of 5% NaNO2, 0.3 ml of 10% AlCl3. The mixture was incubated for 5 min at room temperature then it was added with 2ml NaOH. The total volume was made up to 10ml by adding distilled water. The solution was mixed well and the absorbance was measured at 510 nm. Using the standard curve, the total flavonoid content was calculated (Acharya *et al.,* 2019).

DPPH radical scavenging

The different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in dark. Changes in absorbance of samples were measured at 517 nm and methanol was read as blank. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula and the results are expressed as EC50, which is the amount of antioxidants necessary to decrease the initial concentration by 50%. Ascorbic acid was used as the standard (Chaudhari *et al.,* 2022).

$$\% \text{ of Inhibition} = \frac{(A0 - A1)}{A0} x100$$

Where, A0 = absorbance of the control (without test samples)
A1 = absorbance of test samples.

Inhibition of alpha amylase enzyme

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-1000µg/ml were prepared in methanol. 10 mg of dried extract was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (100- 500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent (Dayma *et al.,* 2020).

The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

The present study investigated the phytochemical composition, antioxidant potential, and in vitro anti-diabetic activity of various *Catharanthus roseus* leaf extracts. The findings provide meaningful insights into the plant's therapeutic potential, particularly for diabetes management.

As shown in Table 1, the methanolic extract of *C. roseus* yielded the highest percentage (8.45%), followed by the aqueous (7.74%) and petroleum ether extracts (6.85%). The higher yield from methanol suggests that polar solvents are more efficient in extracting a broad range of phytochemicals, especially phenolics and flavonoids, which are known for their antioxidant and anti-diabetic activities.

Phytochemical analysis (Table 2) revealed the presence of various bioactive compounds across the extracts. The methanolic extract tested positive for alkaloids, glycosides, flavonoids, diterpenes, phenols, proteins, and carbohydrates, indicating a rich phytochemical profile. The aqueous extract contained flavonoids, glycosides, diterpenes, saponins, tannins, and proteins, whereas the chloroform extract showed a limited presence of proteins only. The absence of sterols across all extracts aligns with some previous reports, though this can vary based on extraction methods and plant maturity.

Flavonoids and phenolic compounds, identified predominantly in the methanolic and aqueous extracts, are particularly noteworthy due to their role in scavenging free radicals and modulating carbohydrate metabolism. These compounds can inhibit key digestive enzymes and reduce postprandial hyperglycemia.

Table 3 shows that the methanolic extract had the highest total phenolic (0.652 mg/100 mg) and flavonoid (0.745 mg/100 mg) content. These findings correlate well with the qualitative screening results and support the strong antioxidant and potential anti-diabetic activity observed in subsequent assays.

As shown in Table 4, the hydroalcoholic extract demonstrated dose-dependent DPPH radical scavenging activity, with a maximum inhibition of 59.95% at 100 µg/ml. However, this was lower than the standard ascorbic acid (80.23%). The IC_{50} value of the extract (114.01 µg/ml) was significantly higher than that of ascorbic acid (19.73 µg/ml), indicating comparatively moderate antioxidant potential. Nonetheless, the extract's radical scavenging ability suggests it can help combat oxidative stress a major contributor to diabetic complications.

In Table 5, the hydroalcoholic extract exhibited promising α-amylase inhibitory activity, which also increased with concentration. At 500 µg/ml, the extract showed 76.65% inhibition compared to 92.23% for acarbose. While the extract's IC_{50} (228.83 µg/ml) was significantly higher than that of the standard (25.32 µg/ml), the result still supports its potential to delay carbohydrate digestion and glucose absorption. This mechanism is clinically relevant for controlling postprandial hyperglycemia in type 2 diabetes.

Table 1: Results of percentage yield of leaf extracts of *Catharanthus roseus*

Plant Name	**Percentage Yield (%)**		
Catharanthus roseus	**Pet. Ether**	**Methanol**	**Water**
	6.85	8.45	7.74

Table 2: Result of Phytochemical screening of extracts of *Catharanthus roseus*

S. No.	Constituents	Chloroform extract	Methanolic extract	Aqueous extract
1.	**Alkaloids**			
	Wagner's Test:	-ve	+ve	-ve
	Hager's Test:	-ve	+ve	-ve
2.	**Glycosides**			
	Conc. H_2SO_4 Test:	-ve	+ve	+ve
3.	**Flavonoids**			
	Lead acetate Test:	-ve	+ve	+ve
	Alkaline test:	-ve	+ve	+ve
4.	**Diterpenes**			
	Copper acetate Test:	-ve	+ve	+ve
5.	**Phenol**			
	Ferric Chloride Test:	-ve	+ve	-ve
	Folin Ciocalteu Test:	-ve	+ve	-ve
6.	**Proteins**			
	Xanthoproteic Test:	+ve	+ve	+ve
7.	**Carbohydrate**			
	Fehling's Test:	-ve	+ve	-ve
	Benedict's Test	-ve	+ve	-ve
8.	**Saponins**			
	Froth Test:	-ve	-ve	+ve
9.	**Tannins**			
	Gelatin test:	-ve	-ve	+ve
10.	**Sterols**			
	Salkowski Test:	-ve	-ve	-ve

+Ve = Positive, -Ve= Negative

Table 3: Estimation of total phenolic and flavonoids content of *Catharanthus roseus*

S. No.	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Methanol	0.652	0.745
2	Aqueous	0.458	0.658

Table 4: % Inhibition of ascorbic acid and *Catharanthus roseus* extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract *Catharanthus roseus*
1	10	31.25	12.25
2	20	58.89	19.95
3	40	65.45	26.65
4	60	73.32	38.85
5	80	78.85	46.65
6	100	80.23	59.95
IC50 Value		**19.73**	**114.01**

Table 5: Results of % Inhibition of Acarbose and extract

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Hydroalcoholic extract *Catharanthus roseus*
1.	100	52.23	36.65
2.	200	68.85	49.98
3.	300	74.45	56.66
4.	400	82.23	63.32
5.	500	92.23	76.65
$IC_{50\ Value}$ (µg/ml)		**25.32**	**228.83**

CONCLUSION

The methanolic and aqueous extracts of *Catharanthus roseus* contain a range of bioactive phytochemicals, including alkaloids, flavonoids, phenols, and proteins. These constituents are likely responsible for the observed antioxidant and enzyme inhibitory activities. Although the activity was lower than synthetic standards, the natural origin, lower toxicity, and multi-targeted actions of plant extracts make them valuable candidates for further development as complementary therapies in diabetes management.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Acharya, R., Sharma, B., Singh, R. & Jain, P. (2019) Phytochemical and high-performance liquid chromatography analysis of extract of Vernonia cinerea. *Journal of Drug*

Delivery and Therapeutics, 9, 229–232.

- American Diabetes Assoc. (2022) Standards of medical care in diabetes—2022. *Diabetes Care*, 45 (Supplement 1), S1–S2.
- Chaudhari, R.N., Jain, A.K. & Chatap, V.K. (2022) Investigation of antioxidant and antimicrobial activity of bark extract of Muntingia Calabura. *Journal of Medical Pharmaceutical and Allied Sciences*, 11, 4214–4217.
- Chaudhary, A., Goyal, S. & Poonia, P. (2012) Evaluation of antidiabetic activity of Catharanthus roseus Leaf extracts in alloxan induced diabetic rats. *International Journal of Current Pharmaceutical Research*, 4, 79–82.
- Dayma, V., Chopra, J., Sharma, P., Dwivedi, A., Tripathi, I.P., Bhargava, A., Murugesan, V., Goswami, A.K. & Baroliya, P.K. (2020) Synthesis, antidiabetic, antioxidant and anti-inflammatory activities of novel hydroxytriazenes based on sulpha drugs. *Heliyon*, 6, e04787.
- Dwivedi, S., Ghatuary, S.K., Prasad, S., Jain, P.K. & Parkhe, G. (2019) Phytochemical screening and in vivo anti-inflammatory activity of hydroalcoholic extract of Embelia ribes Burm. *F. Journal of Drug Delivery and Therapeutics*, 9, 386–397.
- Maruthur, N.M., Tseng, E., Hutfless, S., Wilson, L.M., Suarez-Cuervo, C., Berger, Z., Chu, Y., Iyoha, E., Segal, J.B. & Bolen, S. (2016) Diabetes medications as monotherapy or metformin-based combination therapy for type 2 diabetes: A Systematic Review and Meta-analysis. *Annals of Internal Medicine*, 164, 740–751.
- Niranjan, A., Tewari, S.K. & Lehri, A. (2018) In vitro antidiabetic activity and phytochemical screening of selected medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 7, 1342–1346.
- Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D.P. & Lalas, S.I. (2023) Successive solvent extraction of polyphenols and flavonoids from Cistus creticus L. leaves. *Oxygen*, 3, 274–286.
- Rates, S.M.K. (2001) Plants as source of drugs. *Toxicon*, 39, 603–613.
- Sharma, S., Jain, P.K. & Parkhe, G. (2020) Extraction, Phytochemical screening and anti-inflammatory activity of hydro-ethanolic extract of roots of Dactylorhiza hatagirea. *Journal of Drug Delivery and Therapeutics*, 10, 86–90.
- Van Der Heijden, R., Jacobs, D.I., Snoeijer, W., Hallard, D. & Verpoorte, R. (2004) The Catharanthus alkaloids: Pharmacognosy and biotechnology. *Current Medicinal Chemistry*, 11, 607–628.
- World Health Organization (2021). *Diabetes*. Available at https://www.who.int/news-room/fact-sheets/detail/diabetes.