CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.6798424

Available online at: http://www.iajps.com *Research Article*

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

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Article Received: May 2022 **Accepted:** June2022 **Published:** July 2022

Abstract:

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion and impaired insulin action. Though diabetes is a global problem so the present study aims to open new avenue to explore the antidiabetic activity of various medicinal plants on a firm scientific footing and different market formulation which add value as novel antidiabetic drug. In present Study Catharanthus roseus and Saraca indica was tested on the different standardization criteria such as organoleptic measurement, percentage yield, phytochemical screening, antioxidant and antidiabetic potential. Popularity of naturopathy, nature healing has huge impact on herbal drugs which are extensively used in many herbal formulations in traditional system of medicine. Hence, it is required to standardize the herbal materials in order to check purity of the materials that will serve as reference for further studies. For standardization three features must be checked; authenticity, purity and assay. Indian history of medicinal plants has proven that herbs have been used to treat topical infections. From the long list of herbs in my research Catharanthus roseus and Saraca indica are some of the common herbs that are found in abundant. The chosen plants develop an effective antioxidant and anti-diabetic which is environmentally safe.

Key words: Catharanthus roseus, Saraca indica, In vitro antioxidant and anti-diabetic activity

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Please cite this article in press Naina Sharma et al, ***Phytochemical Screening And Evaluation Of In Vitro Anti Diabetic Activity Of Catharanthus Roseus And Saraca Indica .,*** *Indo Am. J. P. Sci, 2022; 09(7).*

INTRODUCTION:

Medicinal plants (MPs) played a significant role in various ancient traditional systems of medication such as Ayurveda, Siddha and Uranic in India. Herbal medicine is the oldest and still the most widely used system of medicine in the world today. It is used in all societies and is common to all cultures. According to World Health Organization (WHO), Herbal Medicine is defined as plant derived material or preparation, which contains raw or processed ingredients from one or more plants, with therapeutic values [1].

The WHO estimates that a minimum of 20,000 plant taxa has recorded medicinal uses. It is estimated that up to 70,000 plants species are used in folk medicine and a majority of these species are found in the Asia-Pacific region [2]. Interest in the exploitation of medicinal and aromatic plants as pharmaceuticals, herbal remedies, flavourings, perfumes and cosmetics, and other natural products has greatly increased in the recent years. As with many other economic plants that are still being collected from the wild and exploited by humans unsustainably, threats to genetic diversity and species survival have also increased in the case of medicinal plants as a result of habitat destruction, over- exploitation, land use changes and other pressures [3]. In India alone, less than 10 % of the medicinal plants traded in the country are cultivated, about 90% are collected from the wild, very often in a destructive and unsustainable manner.

The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (Eg. diosgenin, solasodine, and ionone). Not only, that plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion and impaired insulin action. Though diabetes is a global problem so the present study aims to open new avenue to explore the antidiabetic activity of various medicinal plants on a firm scientific footing and different market formulation which add value as novel antidiabetic drug.

MATERIAL 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 *saraca indica* 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.

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* and *saraca indica*, were subjected to the phytochemical tests as per standard methods [4]. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids.

Quantitative estimation of phenols and flavonoids:

Phenolic and flavonoids compounds which are the secondary metabolites in plants are one of the most widely occurring groups of phytochemicals. They have biological and pharmacological properties especially exhibiting antimicrobial activity, antiviral, antimutagenic, anticarcinogenic, antiinflammatory and cytotoxic activities. It has been recognized that phenolic compounds are a class of antioxidant compounds which act as free radical terminators. The compounds such as flavonoids, which contain conjugated ring structures and hydroxyl functional groups, have the potential to function as antioxidants by scavenging or stabilizing free radicals involved in oxidative processes through hydrogenation or complex formation with oxidizing species that are much stronger than those of vitamins C and E [5].

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.

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% $NaNO_2$, 0.3 ml of 10% $AlCl_3$. 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.

Evaluation of in vitro antioxidant activity:
Determination of total antioxidant capacity:
The total antioxidant capacity was measured by spectrophotometeric method of Prieto et al. 1999. At different concentration ranges, extracts were prepared in their respective solvents and mixed with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate and 4mM ammonium Molybdate mixture). The tubes were incubated for 90 min at 95ºC. The mixture was cooled to room temperature and the absorbance was read at 695 nm against blank. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid.

Qualitative antioxidant activities:
Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods and natural products. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods are based on the direct interaction with reactive molecules or on their reactivity with metal ions and the effects are monitored by chemical measurements. Examples are determination of peroxyl radical scavenging, the ORAC assay, total antioxidant scavenging activity, the DPPH test or the FRAP method. In addition to these methods, chemical approaches have been developed which allow the detection of radical specific DNA- modifications in vitro.

DPPH radical scavenging:
Free radical scavenging activity is determined using 1,1-diphenyl-2picryl hydrazyl radical (DPPH), which is a stable free radical having purple color. When free radical scavengers are added, DPPH is reduced and its color is changed to yellow, based on the efficacy of antioxidants. A 100µM solution of DPPH in methanol is added to the drug solution and the absorbance is read at 517 nm after 10 min. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) is calculated as percentage scavenging. Reagents 2,2-Diphenyl-2-picryl hydrazyl solution was prepared by dissolving 19mg in 50ml of methanol and then the total volume was made up to 500ml with methanol in a volumetric flask. Procedure DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong et al. 2006. 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 [7].

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

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

***In vitro* Antidiabetic studies:**
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 [8]. 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. 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 10 ml 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:

In present Study *Catharanthus roseus* and *Saraca indica* was tested on the different standardization criteria such as organoleptic measurement, percentage yield, phytochemical screening, antioxidant and antidiabetic potential. The percentage yield of different extract of leaves of *Catharanthus roseus and Saraca indica* exhibited higher yield in methanolic extract 8.45% and 7.85% respectively. Aqueous extract of leaves of *Catharanthus roseus and Saraca indica* also exhibited comparable yield 7.74% and 6.80% followed by methanolic leaves extract.

Preliminary phytochemical analysis generally helps identify and classify the plant extracts' bioactive constituents. For extracts of all samples, a small portion of the dried extracts of plant leaves underwent phytochemical screening using Kokate (1994) methods for chemical testing of alkaloids, glycosides, flavonoids, saponins, phenolics, proteins and amino acids, diterpenes and tannins separately. Flavonoid was detected in different successively extracted leaves of *Catharanthus roseus*. The *Catharanthus roseus* leaves, pet. ether, methanolic and aqueous extract showed presence of phenols. The methanolic extracts possess almost all the phytochemicals that were tested when compared other solvent. It could be seen from table no. that flavonoids, saponins, and phenolics were present in the *Catharanthus roseus* methanol extract. Because of lower pet ether polarity, leaf phytochemical screening showed negative findings for alkaloids, glycosides, saponins, phenolics, proteins and amino acids, diterpenes and tannins.

The results of phytochemical screening of successively extracted leaves extract of *Saraca indica* were depicted in table. The phytochemical screening results also showed mostly similarity in the phytochemicals composition of different solvents (pet ether, methanol and aqueous). However, the physiological and biosynthetic reactions taking place inside the plant, responsible for absence of some phytochemicals in one parts and its presence in the other part of plant sample. The presence of phytochemicals (Phenols and Flavonoids) was quantitatively screened. The overall flavonoid content of the *Catharanthus roseus* methanol and aqueous extract. The total content of methanol and aqueous flavonoid (equivalent to quercetin) was 4,428, 0.745 and 0.658 mg/100 mg respectively In *Catharanthus roseus*, the quantitative analysis of the total phenolic content showed that methanol extract was the richest source of phenols than aqueous extract. The methanol and aqueous extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 0.652mg/100mg and 0.458 mg/100mg respectively.

The flavonoid content of the *Saraca indica* methanol and aqueous extract. The total content of methanol and aqueous flavonoid (equivalent to quercetin) was 40.615 and 0.581mg/100 mg respectively In *Catharanthus roseus*, the quantitative analysis of the total phenolic content showed that methanol extract was the richest source of phenols than aqueous extract. The methanol and aqueous extract quantitative analysis revealed total phenolic content (equivalent to gallic acid) of 0.741mg/100mg and 0.562 mg/100mg respectively.

The concentration of methanol and aqueous extract of different parts of each medicinal plant taken to inhibit 50% of DPPH was indicated in table. Variable DPPH activity was recorded for methanolic extract of *Catharanthus roseus* and *Saraca indica* extract. The DPPH scavenging activity of methanolic extract of *Catharanthus roseus* and *Saraca indica* were showed IC50 value 114.01µg/ml and 73.82 µg/ml as compared to positive control ascorbic acid 19.73µg/ml.

In antidiabetic activity extract of 100, 200, 300, 400, 500 µg doses inhibits the α-amylase enzyme by 51.19%, 70.10%, 74.20%, 85.18%, and 88.75%, respectively. Acarbose of 100, 200, 300, 400, 500 µg doses inhibits the α-amylase enzyme by 17.90%, 35.43%, 43.05%, 48.56%, and 56.76%, respectively. Dose-dependent % inhibition of α- amylase enzyme is observed with the extracts.

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

Plant Name	Percentage yield (%)		
	Pet. ether	Methanol	Water
Catharanthus roseus	6.85	8.45	7.74
Saraca indica	5.36	7.85	6.80

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

S. No.	Constituents	Pet. ether extract	Methanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve -Ve	 +Ve +Ve	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve	 +Ve	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 -Ve -Ve	 +Ve +Ve	 +Ve +Ve
4.	Saponins A) Froth Test:	 -Ve	 +Ve	 -Ve
5.	Phenolics A) Ferric Chloride Test:	 -Ve	 +Ve	 +Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	 +Ve	 +Ve	 +Ve
7.	Carbohydrate A) Fehling's Test:	 -Ve	 +Ve	 -Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve	 +Ve	 +Ve
9.	Tannin A) Gelatin test:	 -Ve	 -Ve	 -Ve

Table 3: Result of Phytochemical Screening of extracts of *Saraca indica*

S. No.	Constituents	Pet. ether extract	Methanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve -Ve	 -Ve -Ve	 -Ve -Ve
2.	Glycosides A) Legal's Test:	 -Ve	 +Ve	 -Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 -Ve -Ve	 +Ve +Ve	 +Ve +Ve
4.	Saponins A) Froth Test:	 -Ve	 +Ve	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 -Ve	 +Ve	 +Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	 -Ve	 +Ve	 -Ve
7.	Carbohydrate A) Fehling's Test:	 -Ve	 +Ve	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve	 +Ve	 +Ve
9.	Tannin A) Gelatin test:	 -Ve	 -Ve	 +Ve

Table 4: Estimation of total phenolic and flavonoids content of *Catharanthus roseus*

		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
		Saraca indica	
3.	Methanol	0.741	0.615
4.	Aqueous	0.562	0.581

Table 6: % Inhibition of ascorbic acid and *Catharanthus roseus* extract using DPPH method

S. No.	**Concentration (µg/ml)**	**% Inhibition**		
		Ascorbic acid	**Hydroalcoholic extract *Catharanthus roseus***	**Hydroalcoholic extract *Saraca indica***
1	10	31.25	12.25	14.65
2	20	58.89	19.95	22.32
3	40	65.45	26.65	29.98
4	60	73.32	38.85	40.25
5	80	78.85	46.65	56.65
6	100	80.23	59.95	63.32
IC_{50}		**19.73**	**114.01**	**73.82**

Table 8: % Inhibition of acarbose and extract

S. No.	**Concentration (µg/ml)**	**% Inhibition**	
		Acarbose	**Methanolic Extract of *Saraca indica***
1	100	51.19	17.90
2	200	70.10	35.43
3	300	74.20	43.05
4	400	85.18	48.56
5	500	88.75	56.76
IC_{50} value		**59.90**	**390.66**

CONCLUSION:

In conclusion herbal medicine is used for treatment against various diseases has gained importance worldwide in the recent years. Popularity of naturopathy, nature healing has huge impact on herbal drugs which are extensively used in many herbal formulations in traditional system of medicine. Hence, it is required to standardize the herbal materials in order to check purity of the materials that will serve as reference for further studies. For standardization three features must be checked; authenticity, purity and assay. Indian history of medicinal plants has proven that herbs have been used to treat topical infections. From the long list of herbs in my research *Catharanthus roseus* and *Saraca indica* are some of the common herbs that are found in abundant. The chosen plants develop an effective antioxidant and anti-diabetic which is environmentally safe.

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