Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: https://saudijournals.com/

Phytochemical Screening and *in Vitro* Antidiabetic Activity of Plumeria Acuminata Leaves

Gomathi P*[1,2], Shalini T[2], Nazima Farheen[2], Sanjeevkumar A[2]
[1]School of Pharmacy, College of Health Sciences, Mekelle University, Mekelle, Tigray, Ethiopia
[2]Vaagdevi Colleges of Pharmacy, Kishanpura, Hanamkonda, Warangal, Telangana, India

Original Research Article

***Corresponding author**
Gomathi P

Article History
Received: 10.11.2017
Accepted: 18.11.2017
Published: 30.12.2017

DOI:
10.36348/sjmps.2017.v03i12.001

Abstract: The present study was carried out to evaluate the preliminary phytochemical screening and *in vitro* antidiabetic activity of *Plumeria acuminata* leaves. The leaves of *Plumeria acuminata* was extracted with different solvents and phytochemical investigations were done for all extracts using standard procedures. *In vitro* anti-diabetic activity of ethyl acetate extract of *Plumeria acuminata* (MEPA) was evaluated using α-amylase inhibition assay. The percentage inhibition increased in a dose dependent manner. In this study we investigated the better *in vitro* anti-diabetic potential of the *Plumeria acuminata*.
Keywords: *Plumeria acuminata*, *in vitro*, antidiabetic, phytochemical screening, alpha Amylase, dose dependent.

INTRODUCTION

The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history [1]. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals [2]. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated.

Plant derived medicines have been the first line of defense in maintaining health and combating diseases. Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds.

In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants.

Medicinal plants, since times, have been used virtually in all cultures as a source of medicine. Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other problems.

In India, plants like *Abroma augusta* (L.) L.f.,*Aconitum palmatum* D. Don., *Aloe barbadensis* Mill, *Asparagus racemosus* Willd., *Berberis aristata* DC., *Calamus rotang* L.), *Cannabis sativa* (L.), *Catharanthus roseus* (L.) G. Don., *Cinnamomum tamala* (Buch.-Ham.) Nees, *Coccinea grandis* (L.)Voigt.,*Costus speciosus* Sm., *Ficus racemosa* (L.), *Ipomoea batatus* (L.) Lamk., *Momordica chrantia* (L.), *Nardostachys jatamansi* DC., *Picrorhiza kurrooa* Royle ex Benth., *Quercus lanata* Sm., *Swertia chirayita* (Roxb. ex Flem.) Karst., *Syzygium cuminii* (L.) Skeels, *Trigonella foenum-graecum* (L.), *Urtica dioica* (L.), *Zingiber officinale* Rosc., *Allium cepa* L., *Allium sativum* L., *Aloe vera* (L.) Burm.f.,*Cajanus cajan* (L.) Millsp., *Coccinia indica* Wight & Arn., *Caesalpinia bonducella* (L.) Roxb., *Ficus bengalensis* L., *Gymnema sylvestre* R. Br., *Ocimum sanctum* L., *Pterocarpusmarsupium* Roxb., *Tinospora cordifolia* (Willd.) Hook.f etc., are most commonly used species in traditional medicine as antidiabetic agents [3].

The treatment of diabetes with synthetic drugs is costly and chances of side effects are high. Therefore Herbal drugs play an important role as alternative medicine due to less side effects and low cost. *Plumeria acuminata* belongs to the Apocynaceae family. Its common name "Frangipani" comes from an Italian noble family [4]. Also known as the Lei flower. They are recognized as excellent ornamental plants and often seen in the graveyards [5]. *Plumeria* plants are famous for their attractiveness and fragrant flowers. The Plant possess poisonous, milky sap. Contact with the sap may irritate eyes and skin. *Plumeria acuminata* leaves are traditionally being used for diabetes. There is no

scientific evidence on hypoglycemic, anti hyperglycemic activities of the parts (roots, leaves) of this plant. The plant is abundantly available in the southern parts of India. Hence we tried to evaluate the phytochemicals present and *in vitro* antidiabetic activity of *Plumeria acuminata*.

MATERIALS AND METHODS

Plant material

For the present investigation, the plant *Plumeria acuminata* was collected in Warangal district, AP, India. The leaves were shade dried and taxonomically identified by Dr. Vastavya Raju, Head, Department of Botany, Kakatiya University, and Warangal. The voucher specimen (No: PGS-1) was deposited in our laboratory for further use.

Extraction

In present investigation the coarsely powdered leaves (750gm) of *Plumeria acuminata* were extracted successively using petroleum ether (60-80^{o}C) and methanol by soxhlation. The solvent was removed by distillation and a greenish black-sticky residue is obtained. The methanol extract was re-extracted with ethylacetate and chloroform. The extracts thus obtained were weighed and percentage yields were calculated.

Phytochemical screening

These four fractions were evaluated by phytochemical qualitative reactions for usual plant secondary metabolites. The screening was performed for steroids, alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrates and proteins [6-11]. The color intensity or the precipitate formation was used as analytical responses to these tests.

In-vitro antidiabetic activity

Inhibition assay for α-amylase activity

A-amylase is the enzyme in humans that isresponsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose). Carbohydrates are major constituents of human diet and polysaccharides are one of the main components of carbohydrates that play a role in the energy supply. The dietary carbohydrates break down to monosaccharides by some gastrointestinal enzymes, since only manosaccharides can be absorbed from intestinal lumen. In monosaccaride, glucose can be readily absorbed from the gastrointestinal tract into blood stream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch by the enzyme α-amylase and α-glucosidase. Inhibition of these enzymes reduced the high post prandial blood glucose peaks in diabetics [12,13].

Procedure The activity of amylase (Himedia, Mumbai) was assayed with different concentrations of sample, with control. The test tubes contained 2 ml reaction mixture with sodium phosphate buffer (50 mM, pH 7.0-7.3), different concentration of ethylacetate extract of *Plumeria acuminata* (EEPA), starch (in buffer) and enzyme (from 1mg/ml sample in buffer). For every different concentration of sample analyzed, a different blank was maintained. The blank tubes were added with 1 ml of DNS before adding enzyme. The tubes were incubated at 37^{0}C for 10 min followed by addition of 1 ml of DNS. The tubes were incubated in boiling water bath for 10 min, cooled and read for absorbance at 540 nm against blank. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the following formula [13]. Activity was expressed as µmoles/ml/min

$$\textbf{Activity} = \frac{\text{Conc. of Maltose liberated X ml of enzyme used}}{\text{Mol wt of Maltose X Incubation time (min)}} \text{ X Dilution Factor}$$

The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula.

$$\textbf{\% Inhibition/Induction} = \frac{\text{Activity in presence of compound}}{\text{Control activity}} \text{ X } 100$$

STATISTICAL ANALYSIS

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at $p<0.001$.

RESULTS AND DISCUSSIONS

Extraction

Various extracts (Petroleum ether, Chloroform, Ethyl acetate and chloroform extracts) of *Plumeria acuminata* leaves were prepared by successive solvent extraction. The colour, nature and percentage yields of all obtained extracts were recorded in the table 1.

Table-1: Colour, nature and percentage yields of all extracts

S.No	Extract	Weight	Colour	Consistency
1	Petroleum ether	6.2%w/v	Yellowish black	Resinous
2	Methanol extract	11.6%w/v	Brownish black	Resinous
3	Ethylacetate extract	3.7%w/v	Greenish black	Solid
4	Chloroform extract	0.92%w/v	Green	Solid

Phytochemical screening

In present investigation, four different solvent extracts of *Plumeria acuminata* leaves are subjected to phytochemical evaluation. Standard tests and reagents were employed to detect various phytochemical studies. The experimental observations were recorded in table 2.

Table-2: Phytochemical screening of different type of extracts

Name of Test	Petroleum Ether Extract	Methanol Extract	Ethylacetate Extract	Chloroform Extract
Alkaloids	-	-	+	-
Flavanoids	-	+	+	-
Glycosides	-	+	+	+
Saponins	+	+	+	+
Steroids	+	-	+	-
Carbohydrates	-	-	-	-
Proteins	-	-	-	-
Amino acids	-	-	-	-
Phenolics/tannins	-	+	-	+

***In-vitro* antidiabetic activity**

The antidiabetic activity was investigated through the inhibition of α-amylase, an enzyme that made the digestion of starch and so reduced the glucose absorption. Standard Maltose curve is represented in Figure 1. From the standard curve, the concentration of maltose liberated by the treatment of various concentrations of EEPA was estimated. The percentage inhibition of α-amylase is given in Table 3. Inhibitory Activity of EEPA against α-amylase is shown in figure 2 by plotting graph against concentration and activity. The inhibition of enzyme activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes. The percentage inhibition increased in a dose dependent manner. In this study we investigated the better *in-vitro* anti-diabetic potential of the *Plumeria acuminata.* Therefore, effective and nontoxic inhibitors of α - amylase were present in the herb.

CONCLUSION

The present study indicates that treatment of EEPA at the doses of 250mg/kg and 500mg/kg brought the parameters altered to near normal level. From this we can conclude that EEPA can be used as a potent antidiabetic activity.

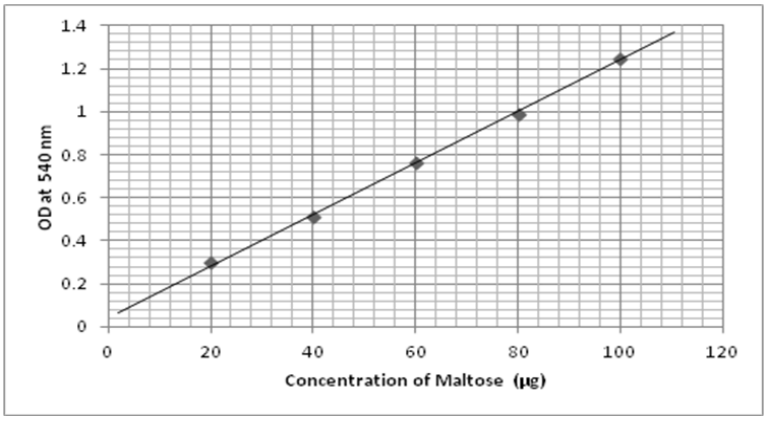

Fig-1: Standard Maltose Curve

Table 3: Percentage Inhibition of Ethylacetate Extracts of *Plumeria Acuminata* against α-Amylase

Sample	Optical Density at 540nm	Conc. of Maltose liberated (µg)	Activity (µmoles/ml/min)	Percentage Activity
Control	1.39	112	0.031	100
100µg	0.86	68	0.018	58.06
75µg	0.92	73	0.02	64.52
50µg	0.99	79	0.021	67.74
25µg	1.14	91	0.025	80.65
10µg	1.29	104	0.028	90.32

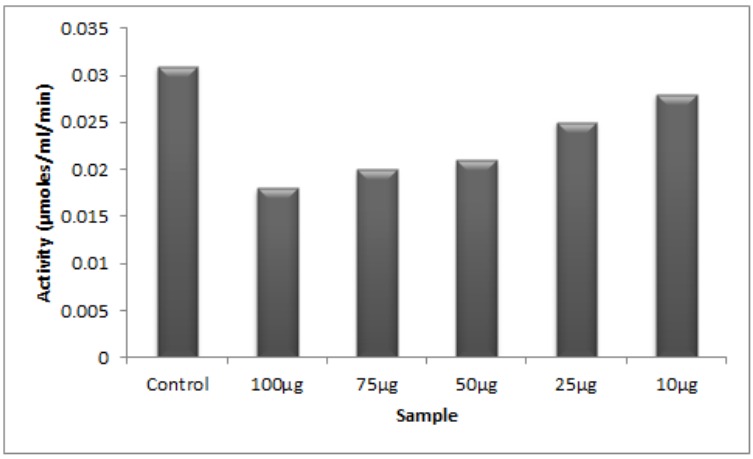

Fig-2: Inhibitory Activity of Ethylacetate Extract of *Plumeria acuminata* against α-amylase

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