

QUALITATIVE ANALYSIS AND α -AMYLASE INHIBITION ASSAY OF AQUEOUS FOLIAR EXTRACT OF *LANTANA CAMARA* (LINN)**Debapriyo Bhowmick¹, Dr. Garima Bartariya² and Abhishek Kumar*³**^{1,3}Department of Biotechnology, ITM University, Gwalior, Madhya Pradesh, India.²Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India.Article Received on
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Biotechnology, ITM
University, Gwalior,
Madhya Pradesh, India.**ABSTRACT**

Lantana camara (Linn) is native to the American tropics. It belongs to verbena family. It also displays antimicrobial, fungicidal, insecticidal properties. It has also been used in traditional herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosy, rabies, chicken pox, measles, asthma and ulcers. The present study was carried out on foliar extract of *Lantana camara* (Linn) to find their significance in diabetic therapeutic studies. The conducted work was based on phytochemical analysis and in-vitro α -amylase activity. High concentration of glucose in the blood causes a metabolic disorder which is known as diabetes. High concentration of glucose in blood

may damage many of the body's systems that is why diabetes is a chronic disease. Result of qualitative analysis exhibited that this plant contains flavonoids and phenol in leaves but amino acid, alkaloids, and saponins were absent. The phytochemicals present in plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy to understand the underlying mechanism of their action. Result of this study revealed that leaves extract showed α -amylase inhibition in a dose dependent manner. The extracts showed maximum inhibition at a concentration of 5 mg/ml and which is decreased with decreasing concentrations i.e. 2.5 and 1 mg/ml. In conclusion, more research is required for developing a potential and valuable antidiabetic therapy by using α - amylase inhibition.

KEYWORDS: *Lantana camara*, Flavonoids, Alkaloids, α -amylase, Phytochemical.

INTRODUCTION

Lantana camara (Linn) belongs to verbena family. It also known as big-sage (Malaysia), wild-sage, red-sage, white-sage (Caribbean) and tickberry in South Africa. Studies have shown that *L.camara* can display antimicrobial, fungicidal and insecticidal properties.^[1] *L.camara* has also been used in traditional herbal medicines for treating a variety of ailments, including cancer, skin itches, leprosy, rabies, chicken pox, measles, asthma and ulcers. There are also some scientific studies which have shown beneficial effects of *L.camara*, such as one by R. Satish who found that an extract from the plant reduced ulcer development in rats.^[2] Extracts from the plant have also been used to treat respiratory infections in Brazil.^[3]

High concentration of glucose in the blood causes a metabolic disorder which is known as diabetes. It is chronic disease and spreading world wise.^[4] Diabetes is mainly caused by the deficiency in production of insulin or ineffectiveness of the insulin produced by pancreas. The deficiency of insulin results in high concentration of glucose in blood. High concentration of glucose in blood may damage many of the body's systems. Diabetes is mainly classified into two categories i.e. Type 1 and Type 2. Type 1 is also known as insulin dependent diabetes mellitus and Type 2 is also known as non-insulin dependent diabetes mellitus.^[5]

A therapeutic approach for treating diabetes is to decrease the carbohydrates hydrolyzing enzyme such as α -amylase and α -glucosidase. Both of these enzymes are important for the digestion of carbohydrates. Amylase is a digestive enzyme that involves in the breakdown of carbohydrates, α -amylase involves in the breakdown of long chain of carbohydrates. It breaks the bonds between sugar molecules in polysaccharides through hydrolysis reaction and decreases the level of glucose in the blood.^[4,5]

MATERIALS AND METHODS

The experiment was performed in June- July 2017 in ITM University, Gwalior, Madhya Pradesh, India. Fresh and disease free leaves of *Lantana camara* (Linn) were collected from the botanical garden and used for solvent extraction. Collected leaves were washed with distilled water and shed dried at room temperature. The shed dried leaves were ground and stored in air-tight container for the further use of extraction with solvents.

Qualitative Analysis

Qualitative analysis was done by following the methodology of.^[6,7,8,9]

Alkaloids

Mayer's test- Few drops of Mayer's reagent (1.36gm of Mercuric Chloride and 5gm of Potassium Iodide in 100ml distilled water) were added in 2-3 ml of test extract. Appearance of cream colour is observed in the sample. This change in colour of extract indicated the presence of alkaloids.

Amino acid

Two millilitre Million's reagent (Mercuric nitrate) was mixed with two-three ml of test sample. Formation of white precipitate indicated that amino acid was present in the sample extract.

Saponin

Saponin content was tested by Froth formation test. For this two ml of test extract was shaken vigorously with distilled water in a test tube. Persistent foam formed at the surface indicated the presence of saponin in the extract.

Flavonoids

Flavonoids were tested by performing alkaline reagent test. Few drops of sodium hydroxides solution (NaOH) was added in 2 ml of test extract. Intense yellow colour formed which turned into colour less solution on addition of few drops of dilute sulphuric acid (H₂SO₄). This change indicated that extract possesses flavonoids in it.

Phenol

Two millilitre of test extract was treated with two ml of 5% ferric chloride solution. Formation of blue colour indicated the presence of phenol.

 α - Amylase assay by DNSA Method

α - amylase assay was performed by following methodology of Abhishek Kumar et al. 2017^[5] and Abhishek Kumar et al. 2018.^[10] 120 micro-litter of plant extract was mixed with 480 μ l of distilled water and 1.2 ml of starch solution (1g starch in 0.02M sodium phosphate buffer containing 0.0067 M of sodium chloride in 100ml) was added. The reaction was initiated by adding 600 μ l of enzyme solution (1mg of α - amylase in 10ml of 0.02M of sodium phosphate at PH 6.9) were added into the mixture and kept at room temperature for 3 minutes. After 3 minutes 600 μ l of the mixture was transferred into separate test tube which contains 300 μ l of DNSA colour reagent (1g 3,5- Dinitrosalicylic acid, 30g sodium potassium tartrate and

20 μ l of sodium hydroxide to final volume of 100 ml in distilled water), test tube was kept into the water bath for 15 minutes at 85-90 $^{\circ}$ C. After water bath sample was allowed to cool down at room temperature and 2.7 ml of distilled water was added into each test tube. The absorbance was recorded at 540nm by using UV- visible Spectroscopy (PerkinElmer). The control was prepared by using 120 μ l of solvent in place of plant extract. The inhibition % was calculated by using formula.

$$\text{Inhibition \%} = \frac{(\text{control}_{540} - \text{sample}_{540}) \times 100}{\text{Control}_{540}}$$

RESULTS AND DISCUSSION

Qualitative Analysis

Phenolics are largest found compounds and most widely distributed phytochemical of plant. Phenols were found to be present. This result was supported by findings of Jain et al. 2017.^[11]

Saponins are groups of secondary metabolites found in plants and regarded as high molecular weight compounds. Saponins were absent. Saponins were screened in Naz et al. 2013.^[12]

Amino acid was not shown. This can be in very trace amount during qualitative estimation of selected tests. Naz et al. 2013^[12] also found its absence in some tests.

Flavonoids, polyphenolic compounds, made up of more than one benzene ring were present. The findings of Naz et al. 2013.^[12] Alkaloids are natural products that contain heterocyclic nitrogen atoms, significant in protection and survival of plants, it was not present. this qualitative phytochemical screening was subjected to show the preliminary test of bioactive compound in plant.

Table 1: Qualitative Screening of Phytochemical in Foliar Extract of *Lantana camara* (Linn) by using various solvents.

S.No	Phytoconstituents	Extract
1	Phenol	+
2	Saponins	-
3	Amino acid	-
4	Flavanoids	+
5	Alkaloids	-

‘+’= present ‘-’= absent

α - Amylase inhibition assay

α - amylase is a carbohydrate hydrolysing enzyme which cleaves carbohydrate and produce monohydrates. In the present study, aqueous foliar extract of *Lantana camara* (Linn) has been used to find out the inhibition activity of α - amylase by using standard method of Naz et al. 2013.^[12] Result of this study exhibited the leaves extract of *Lantana camara* (Linn) significantly inhibits the α -amylase in a dose dependent manner. Three different concentration i.e. 1 mg/ml, 2.5mg/ml and 5mg/ml of aqueous foliar extract were used for the present study. The extract showed maximum inhibition at a concentration of 5mg/ml and it is decreased with decreasing concentration i.e. 2.5 mg/ml and 1 mg/ml. At a dose of 1 mg/ml, 2.5 mg/ml and 5 mg/ml the aqueous foliar extract of this plant showed inhibition of 54.2%, 61.2% and 71.7% respectively.

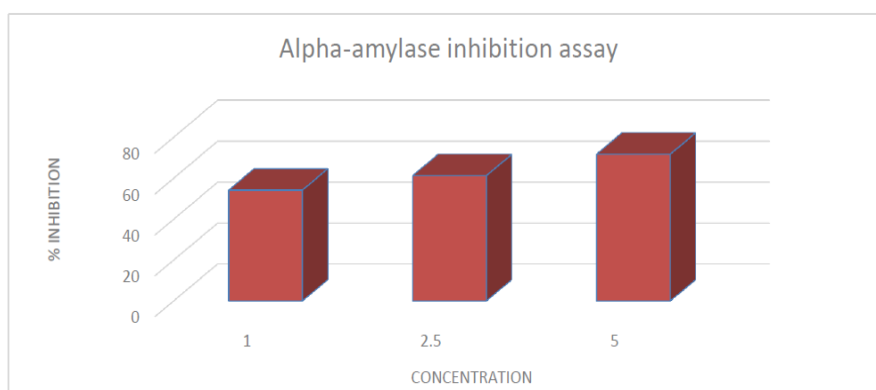


Fig. 1: α - Amylase inhibition assay of *Lantana camara* (Linn) leaves extract.

Table 2: α -Amylase Inhibition Assay in Distilled Water Foliar Extract of *Lantana camara*.

Sample (concentration)	Volume (μ l)	Distilled water (μ l)	Starch (ml)	Enzyme solution (μ l)	DNSA (μ l)	Distilled water (ml)	Absorbance at 540nm \pm SD
Control (solvent)	120	480	1.2	600	300	2.7	0.428 \pm SD 0.260
Test 1 (1 mg/ml)	120	480	1.2	600	300	2.7	0.196 \pm SD 0.011
Test 2 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.166 \pm SD 0.007
Test 3 (5 mg/ml)	120	480	1.2	600	300	2.7	0.121 \pm SD 0.009

μ l= microlitre; ml= millilitre; SD= standard deviation.

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