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Research Article

**QUALITATIVE ANALYSIS AND α -AMYLASE INHIBITION
ASSAY OF AQUEOUS FOLIAR EXTRACT OF *SYZYGIUM
CUMINI* (L.)**Ashutosh Nath Jha¹, Dr. Garima Bartariya² and Abhishek Kumar³¹Department of Biotechnology, ITM University, Gwalior, Madhya Pradesh, IndiaEmail- ashutoshprashantjha@gmail.com whtsaap- 9755240482²Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, IndiaEmail- drgarimabartariya@gmail.com whtsaap- 9977188900³Department of Biotechnology, ITM University, Gwalior, Madhya Pradesh, IndiaEmail- abhishekchaudhary964@gmail.com whtsaap- 9752770781**Abstract:**

Syzygium cumini is one of the important medicinal plants found in all parts of India. *Syzygium cumini* (L.) is commonly known as jambolan, Java plum or black plum. It is an evergreen tropical flowering plant belongs to family Myrtaceae. This plant has many pharmaceutical activities such as anti-diabetic, anti-oxidant, CNS depressant and etc. The present study was carried out on foliar extract of *Syzygium cumini* 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. The result of qualitative analysis exhibited that this plant contains alkaloids, flavonoids, saponins and phenol in leaves but amino acid was 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. The 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 1 mg/ml and which is decreased with increasing concentrations i.e. 2.5 and 5 mg/ml. In conclusion, more research is required for developing a potential and valuable antidiabetic therapy by using α -amylase inhibition.

Keywords: A-Amylase Inhibition, Foliar Extract, *Syzygium Cumini* (L.)**Corresponding author:****Abhishek Kumar,**

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INTRODUCTION:

Syzygium cumini (L.) belongs to the family Myrtaceae and commonly known as Jamun, Java plum, Indian Blackberry and etc [1]. This plant has various medicinal values such as anti-diabetic, anti-oxidant, anti-inflammatory, anti-microbial, anti-HIV and many others [2]. It is reported that all the parts of this plant used as medicine in ancient time [3]. The leaves of this plant are simple, opposite, smooth, glossy and elliptic of oblong [4].

High concentration of glucose in the blood causes a metabolic disorder which is known as diabetes. It is a chronic disease and spreading worldwide [5]. Diabetes is mainly caused by the deficiency in the production of insulin or ineffectiveness of the insulin produced by the 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 [6].

A therapeutic approach to 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. It breaks the bonds between sugar molecules in polysaccharides through hydrolysis reaction and decreases the level of glucose in the blood [5, 6].

MATERIALS AND METHODS:

The experiment was performed in June- July 2017 at ITM University, Gwalior, Madhya Pradesh, India. Fresh and disease free leaves of *Syzygium cumini* 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 performed by following methods [7, 8, 9, 10]

Alkaloids:

Alkaloids were tested by 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. The appearance

of cream color is observed in the sample. This change in color extract indicated the presence of alkaloids.

Amino acid:

Two milliliters 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. The 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. The intense yellow color formed which turned into colorless solution on addition of few drops of dilute sulphuric acid (H₂SO₄). This change indicated that extract possesses flavonoids in it.

Phenol:

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

 α - Amylase assay by DNSA Method:

α - amylase assay was performed by following methodology of Abhishek Kumar et al. 2018, Juvekar et al. 2014 and Gayathri et al. 2013 [6, 11, 12]. One hundred twenty 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 color 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 were kept in the water bath for 15 minutes at 85-90 OC . After water bath sample was allowed to cool down to 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.

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

RESULTS AND DISCUSSION:

Qualitative Analysis:

Phenolics are largest found compounds and most widely distributed phytochemical of plants. Phenols were found to be present in the extract. This result was supported by Gowri and Vasantha 2010 [13].

Saponins are groups of secondary metabolites found in plants and regarded as high molecular weight compounds. Saponins were found to present in

aqueous extracts. This result was supported by Gowri and Vasantha 2010 [13].

Flavonoids were present in the aqueous extract of this plant. This result was supported by findings of S. Shyamala Gowri and K. Vasantha 2010 [13].

Alkaloids are natural products that contain heterocyclic nitrogen atoms which have significant role in protection and survival of plant. Alkaloids were found to be present in the foliar aqueous extract. Amino acid was absent in extract of this plant. This result was supported by findings of Kumar et al 2009 [14].

Table 1: Qualitative Screening of Phytochemical in Foliar Extract of *Syzygium cumini* 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 hydrolyzing enzyme which cleaves carbohydrate and produce monohydrates. In the present study, aqueous foliar extract of *Syzygium cumini* has been used to find out the inhibition activity of α - amylase by using standard method of Jevekar et al.2014 [11]. Result of this study exhibited the leaves extract of *Syzygium cumini* significantly inhibits the α -amylase in a dose

dependant 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 1mg/ml and it is decreased with concentration i.e 2.5 mg/ml and 5 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 57%, 49.53% and 44.62% respectively.

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

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	0.244 \pm SD 0.010
Test 2 (2.5mg/ml)	120	480	1.2	600	300	2.7	0.212 \pm SD 0.007
Test 3 (5 mg/ml)	120	480	1.2	600	300	2.7	0.191 \pm SD 0.008

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

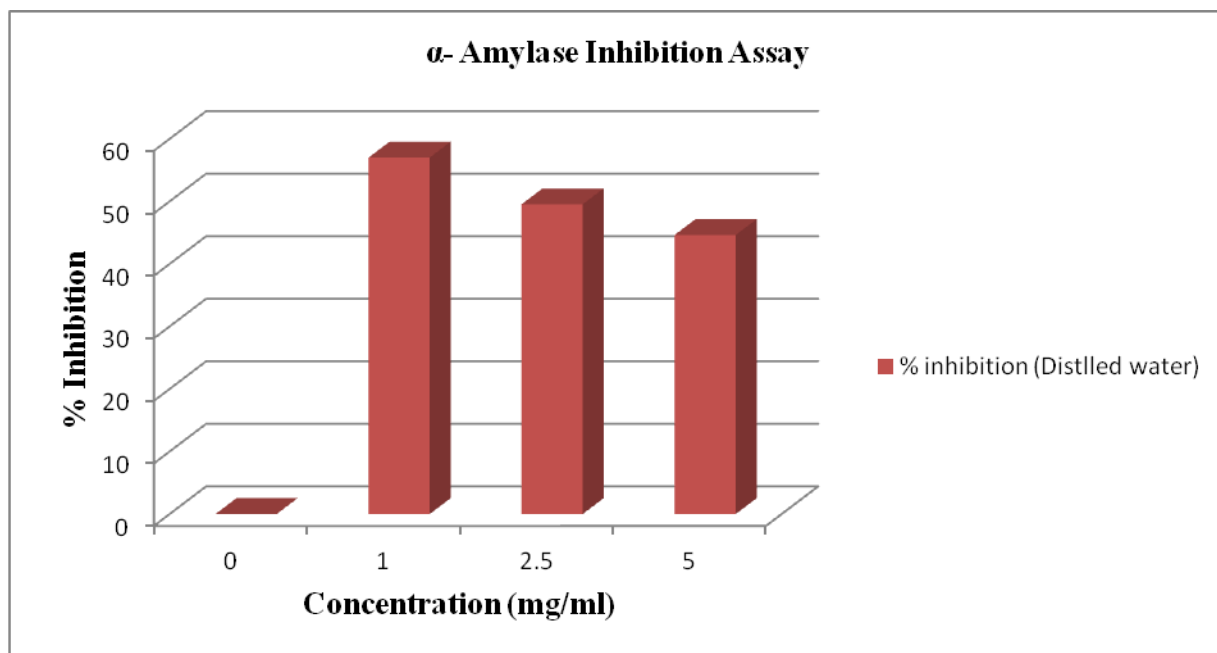


Fig 1: α - amylase enzyme inhibition assay in aqueous foliar extract of *Syzygium cumini*

CONCLUSION:

Syzygium cumini is one of the important medicinal plants found in all parts of India. This plant is well known for its medicinal value. Recently research on medicinal plant is the subject of great interest, which is the reason to explore the medicinal importance of this plant. The present study was carried out on foliar extract of *Syzygium cumini* to find their significance in diabetic therapeutic studies. The conducted work was based on phytochemical analysis and in-vitro α -amylase activity. The qualitative phytochemical study of *Syzygium cumini* foliar extract by using distilled water shown the presence of following bioactive compounds viz. alkaloids, flavonoids, saponins, amino acid and phenols. The presence of various bioactive compounds has justified the use of *Syzygium cumini* leaves extract for various ailments by traditional practitioners. The study also supports the view that the plant has the ability to overcome many incurable diseases such as diabetes. The experiments were also carried out for in-vitro α -amylase activity by using the distilled water extract of leaves of *Syzygium cumini*. The absorbance was recorded at 540 nm by using UV-spectroscopy. The result of this study stated that the aqueous extract of this plant significantly inhibit the α -amylase. In conclusion, more research is required for developing a potential and valuable antidiabetic therapy by using α - amylase inhibition.

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