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(RESEARCH ARTICLE)

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Anti-diabetic activity and anti-oxidant activity of *Syzygium aqueum* leaves

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Abstract

*Syzygium aqueum***,** commonly known as water apple, is a tropical plant from the *Myrtaceae* family, known for its fruit and medicinal properties. The plant has gained attention for its rich phytochemical composition, including alkaloids, flavonoids, tannins, and essential oils, which contribute to its potential therapeutic benefits, such as antimicrobial, anti-inflammatory, antioxidant, and antidiabetic effects. Phytochemical studies on *Syzygium aqueum* have identified several bioactive compounds like *eugenol*, *syzygine*, and flavonoids, which are responsible for its medicinal properties. Research has shown that extracts from various parts of the plant exhibit significant antioxidant, antimicrobial, anti-inflammatory, and anti-cancer activities. Despite promising results, further research is needed to explore its safety and clinical applications.

This study explored the potential of *Syzygium aqueum* (water apple) leaves as a source of natural anti-diabetic and antioxidant agents. Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, is often managed with α-amylase inhibitors, which delay carbohydrate absorption. Oxidative stress plays a crucial role in the development of diabetic complications. Therefore, substances with both anti-diabetic and antioxidant properties are highly desirable.

In this study, the anti-diabetic activity of *Syzygium aqueum* leaf extracts was evaluated using the 3,5-dinitrosalicylic acid (DNS) assay. This method quantifies the inhibition of α-amylase, a key enzyme in the breakdown of carbohydrates. Different concentrations of the leaf extracts were incubated with the enzyme and substrate, and the amount of reducing sugars produced was measured. The percentage inhibition of α-amylase activity was then calculated.

The antioxidant capacity of the *Syzygium aqueum* leaf extracts was assessed using the ferric reducing antioxidant power (FRAP) assay. This method measures the ability of the extract to reduce ferric ions (Fe3+) to ferrous ions (Fe2+). A higher FRAP value indicates a greater antioxidant potential. The extracts were reacted with a ferric tripyridyltriazine (Fe(III)-TPTZ) complex, and the reduction of Fe3+ to Fe2+ was measured spectrophotometrically.

The results of this study will provide valuable insights into the potential of *Syzygium aqueum* leaves as a therapeutic agent for managing diabetes and oxidative stress-related complications. The findings will be discussed in light of existing literature and will contribute to the growing body of knowledge on natural remedies for these conditions. Further research, including the isolation and identification of specific bioactive compounds responsible for the observed activities, as well as in vivo studies, is warranted to fully elucidate the therapeutic potential of *Syzygium aqueum* leaves.

Key words: *Syzygium aqueum*; Anti-diabetic effect; Anti-oxidant activity; FRAP method; α-amylase; DNS method

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1. Introduction

Syzygium Aqueum, commonly referred to as watery apple, one among the foremost valuable medicinal plant species under the Myrtaceae. In Ayurveda, the plant extract has been evidenced to be pharmaceutically active as anti-hyperglycemic activity, anti-inflammatory activity, Antioxidant activity etc. Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication since the earliest times known to man. Several medicinal plants have been found to possess neurobehavioral activity and serves as alternative to modern medicine. The medicinal activities of this genus are such a lot vigorous that a broader range of study is required to be completing to assess the whole pharmacological role in various ailments. *Syzygium* is a flowering plant belonging to Myrtaceae family. There are 1200- 1800 species of *Syzygium* and most of them are mainly shrubs as well as evergreen trees. Generally, the lower part of the tree trunk of *Syzygium* is rough, flaking and cracked while its bark is dark grey in colour, becoming smoother and lighter higher up. The leaves are dark-green and glossy, evergreen, elliptic and either blunt or tapering with pointed apexes. Each fruit contains a single, green or brown and obligate shaped seed.

The tree is about 3 to 10 meters tall height and having wide spreading branches and flaky brown bark. It has stem about 30 cm. The leaves are glossy and narrow. The tree possesses greenish-white or creamy-white flowers with diameter 7.5 to 10 cm and width 2-4 inch (5 to 10 cm). The plant flowers during midsummer (June, August). The plant prefers warm and humid climate with an adequate rainfall and it thrives better in well drained soils. Diabetes mellitus, a chronic metabolic disorder, affects millions worldwide. Conventional treatments often have adverse effects, prompting the search for alternative therapies.

Diabetes is a condition that happens when blood sugar (glucose) is too high. It develops when pancreas doesn't make enough insulin or any at all, or when body isn't responding to the effects of insulin properly. Diabetes affects people of all ages. Most forms of diabetes are chronic (lifelong), and all forms are manageable with medications and/or lifestyle changes. Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia (elevated blood glucose levels), poses a significant global health challenge. The increasing prevalence of diabetes and its associated complications, such as cardiovascular disease, neuropathy, and retinopathy, necessitates the exploration of novel therapeutic strategies. Current management approaches often involve lifestyle modifications, oral hypoglycemic agents, and insulin therapy. However, the search for safer and more effective treatments, particularly from natural sources, continues to be a priority.

One promising avenue of research lies in the investigation of traditional medicinal plants. *Syzygium aqueum* (commonly known as the water apple), a tropical fruit widely cultivated in Southeast Asia, has been used in traditional medicine for various purposes, including the management of diabetes. This traditional use suggests the presence of bioactive compounds with potential anti-diabetic properties within the *Syzygium aqueum* plant.

Hyperglycemia in diabetes often results from impaired insulin secretion, reduced insulin sensitivity, or increased glucose production. One key target for managing hyperglycemia is the inhibition of α-amylase, an enzyme involved in the breakdown of carbohydrates in the small intestine. Inhibiting α-amylase can delay carbohydrate absorption, thereby blunting postprandial glucose spikes and improving glycemic control. This study aims to investigate the anti-diabetic potential of *Syzygium aqueum* leaf extracts by evaluating their α-amylase inhibitory activity. By employing in vitro assays, we seek to quantify the ability of the extracts to inhibit this key enzyme. The findings of this research will contribute to a better understanding of the potential therapeutic benefits of *Syzygium aqueum* in the context of diabetes management. Furthermore, this investigation will add to the growing body of scientific evidence supporting the traditional use of medicinal plants for treating diabetes and may pave the way for the development of novel anti-diabetic agents derived from natural sources.

Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates, plays a critical role in the pathogenesis of various chronic diseases. ROS, commonly known as free radicals, are highly unstable molecules with unpaired electrons, making them prone to reacting with cellular components like lipids, proteins, and DNA, leading to cellular damage. This damage has been implicated in conditions ranging from cardiovascular diseases and cancer to neurodegenerative disorders and diabetes. Therefore, the search for effective antioxidants, which can neutralize these free radicals and prevent oxidative damage, has become a major area of research.

Nature has provided a rich reservoir of potential antioxidants in the form of phytochemicals present in fruits, vegetables, and other plant-based foods. *Syzygium aqueum* (commonly known as the water apple), a tropical fruit belonging to the Myrtaceae family, has been traditionally used in various folk medicines. While *Syzygium aqueum* is consumed for its nutritional value, its potential as a source of natural antioxidants has garnered increasing scientific attention. This

interest stems from the presence of various bioactive compounds, including phenolics, flavonoids, and vitamins, which are known for their potent antioxidant properties.

This study aims to investigate the antioxidant activity of *Syzygium aqueum* leaf extracts. By employing established in vitro assays, we seek to quantify the free radical scavenging capacity and reducing potential of the extracts. The findings of this research will contribute to a better understanding of the potential health benefits associated with *Syzygium aqueum* and may provide a basis for its utilization in functional foods or as a source of natural antioxidants for pharmaceutical applications. Furthermore, this investigation will add to the existing body of knowledge regarding the therapeutic potential of traditionally used medicinal plants.

2. Materials & methodology

2.1. Collection of crude drug

Green leaves plucked from our campus garden. The herbarium was prepared and authenticated at State Medicinalplant board - Kerala by Senior scientist. Organoleptic characters are observed and noted. The collected leaves washed in running water to remove any organic or foreign particle if present. Dried in shade for 2 days and pulverized in mortar and pestle of the laboratory. The resultant powder was sieved to obtain a uniform particle sized crude drug.

2.2. Extraction of chemical constituents

The chemical constituents are obtained by soxhlet extraction method. The coarse powder was weighed and 15 gm was packed in an extraction chamber of the soxhlet apparatus. The RBF was filled with aqueous alcoholic solvent 50% i.e. equal amounts of distilled water and ethanol. The condenser was attached and heated at 40^{O}C for six hrs. The obtained extract was concentrated by simple evaporation at 40^{O}C. % yield of the extract was determined.

2.3. Anti-diabetic activity by DNS method

2.3.1. Materials

- Starch solution: Take 1 g of potato starch and dissolved in 100 ml of 0.02 M phosphate buffer of pH 7.
- DNS reagent: It can be prepared by dissolve at room temperature 1 g of 3, 5- Di Nitro Salicylic Acid in 20 ml of 2N NaOH, add 50 ml of distilled water followed by 30 g of Rochelle Salt make the volume up to 100 ml with distilled water. Protect this solution from CO2 and store at 4°C.
- α-amylase enzyme solution: Dissolve 6 mg of α-amylase in 200 ml of 0.2 M phosphate buffer (pH 7) containing 0.006 M NaCl. From this stock solution take 10 ml, dilute to 100 ml with same buffer solution. The final concentration of enzyme in the solution is 30 µg/ml.
- Maltose standard solution: Dissolve 50 mg of maltose in 50 ml distilled water and store at 4°C.
- NaOH (4.5%): Weigh 4.5 g of NaOH, dissolves in approximately 80 ml distilled water, and make the volume up to 100 ml with distilled water.
- NaOH (2N): Weigh 8 g NaOH, dissolve in approximately 80 ml distilled water, and the final volume up to 100 ml with distilled water.
- Phosphate buffer (0.2 M, pH 7): Take 39 ml of 0.2 M. monobasic sodium phosphate solution and mix with 61ml of 0.2M dibasic sodium phosphate solution and dilute to a total volume of 200 ml.
- Phosphate buffer (0.02 M. pH 7): Take 10 ml of the above phosphate buffer (0.2 M) and dilute it to 100 ml with distilled water.
- **Preparation of maltose calibration curve:**
- Pipette aliquots of 0.1 to 1.0 ml of maltose (100-1000 µg) solution into test tubes and make up the volume to 1ml with suitable addition of distilled water. To each tube add 2 ml of DNS reagent. Cover tubes with marbles. Keep the tubes in water bath for 10 minutes. Cool the tubes and add 10 ml of distilled water to each test tube. The orange red colour formed is measured at 540 nm against a reagent blank.
- **Determination of α-Amylase inhibitory activity**
- Pre incubate the entire reagents for 15 minutes at 37° C in a water bath
- Pipette 0.5 ml of 1% starch solution: add it to 0.25 ml of phosphate buffer (0.2M, pH 7) and 0.25 ml of α-amylase enzyme solution,
- Similarly, a second set of test tubes (blank) by using phosphate buffer in place of enzyme solution. Prepare a third set of test tubes containing 0.5 ml of starch solution, 2 ml of DNS reagent. 0.25 ml of α-amylase enzyme solution; this set is called the zero-time control.

- Incubate all the tubes at 37°C for three minutes. At the end of the incubation add 2 ml of DNS reagent to first and second set of tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes.
- After cooling under cold water, add 10 ml of distilled water, mix thoroughly and take absorbance at 540 nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve.
- One unit of enzyme activity is defined as that amount which liberates 1 **µ**mol of reducing sugars (calculated as maltose) /min from soluble starch at 37°C, pH 7, and. under the specified experimental condition
- **Preparation of extract and quantification of α-amylase inhibitor activity**
- Take 1 g of sample and extract with 75 ml of distilled water and 75 ml of ethanol for 2 hrs., at 40°C.
- Centrifuge the suspension at 5000 rpm. Collect the supernatant. Take 0.25 ml and incubate with 0.25 ml of enzyme solution for 15 minutes at 37°C.
- Incubate all the reagents also at 37°C for three minutes. At the end of the incubation add 2 ml of DNS reagent to first, second and sample tubes to stop the reaction and transfer all the tubes to water bath for 10 minutes.
- After cooling under cold water, add 10 ml of distilled water mix thoroughly and take absorbance at 540 nm against the blank. Liberated reducing sugars are expressed as maltose equivalent using the calibration curve.
- One unit of enzyme activity is defined as that amount which liberates 1 **µ**mol of reducing sugars /min from soluble starch at 37°C, pH 7, and. under the specified experimental condition.

2.3.2. Anti-oxidant activity by FRAP method

- **Preparation of reagents**
- 0.2M phosphate buffer (pH 6.6): 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of disodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate was taken in a 1,000 mL standard flask and add 800 mL of distilled water and adjust the pH 6.6 using hydrochloric acid and adjust the volume with deionised water.
- Potassium ferricyanide (1%): 1 g of potassium ferricyanide was dissolved in 100 mL of deionised water.
- Trichloroacetic acid (10%): 10 g of trichloroacetic acid was dissolved in 100 mL of deionised water.
- Ferric chloride (0.1%): 100 mg of ferric chloride was dissolved in 100 mL of deionised water.
- Ascorbic acid (0.1%): 1 mg of ascorbic acid was dissolved in 1 mL of water.
- **Method**
- Different concentrations of the methanolic extract of M. serratulum and its various fractions (10-50 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution.
- The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker.
- At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min.
- The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride.
- The colored solution was read at 520 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

3. Results

3.1. In vitro anti-diabetic activity by DNS method

Table 1 Maltose calibration curve

CONCENTRATION (µg/ml)	ABSORBANCE
0	0
0.1	0.23
0.2	0.26
0.3	0.30
0.4	0.34
0.5	0.40
0.6	0.43

0.7	0.50
0.8	0.55
0.9	0.61
1.0	0.73

Table 2 α- Amylase inhibitory activity detection

Sample	**Absorbance**
Set 1 (E+S)	0.14
Set 2 (blank)	0.26
Set 3 (E+S+DNS)	0.39
Extract (E+S+DNS)	0.30

Where E = Enzyme, S = Starch, DNS = Di Nitro Salicylic acid reagent

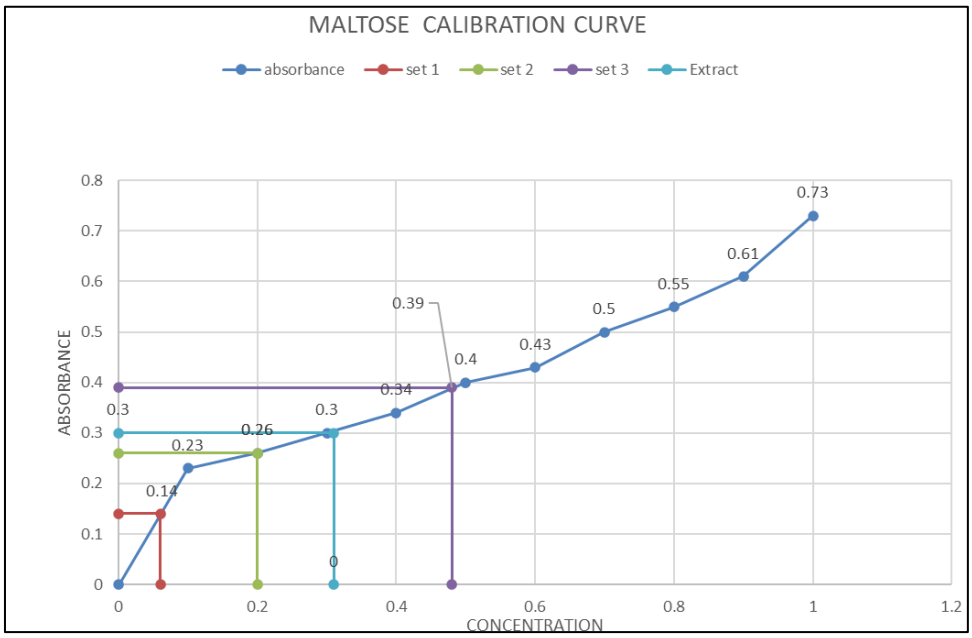

Figure 1 Maltose calibration curve

3.2. % Inhibition of α- Amylase Enzyme

$$\%\ inhibitory\ activity = \frac{[A - C]}{[B - C]} \times 100$$

Where
A= Absorbance of sample
B= Absorbance of blank
C= Absorbance of control

$$\%\ \text{inhibitory activity} = \frac{[0.30 - 0.39]}{[0.26 - 0.39]} \times 100$$

$$= 69.23\%$$

Figure 2 Maltose solution test tube series

3.3. Anti-oxidant activity by FRAP method

Table 3 Anti-oxidant activity by FRAP method observation

Sample	Absorbance
Blank	0
Reference Standard (Ascorbic acid)	1
Plant Extract (*Syzygium aqueum)*	1.06

In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing reference standard with plant extract is found to be **1.06**, so the anti-oxidant activity in *Syzygium aqueum* is more. The antioxidants such as phenolic acids and flavonoids were sent in considerable amount in the extract of *Syzygium aqueum.*

4. Discussion

4.1. Anti-diabetic activity (DNS method)

The 3,5-dinitrosalicylic acid (DNS) method assesses the inhibition of α-amylase, a key enzyme in carbohydrate metabolism. By inhibiting α-amylase, *Syzygium aqueum* leaf extracts can potentially slow down the breakdown of carbohydrates in the digestive system. This can help prevent rapid spikes in blood sugar levels after meals, which is beneficial for managing diabetes. The DNS method is a relatively simple and widely used technique for evaluating α-amylase inhibition. It provides a quantitative measure of the enzyme's activity in the presence of the leaf extracts. . The percentage α- amylase inhibition of *Syzygium aqueum* was found to be **69.23 %.** Because of this high α- amylase inhibitory activity, this extract of *Syzygium aqueum* may have anti-diabetic activity and this compound may have a wide application in future research field of diabetes therapy.

4.2. Antioxidant activity (FRAP method)

The ferric reducing antioxidant power (FRAP) assay measures the ability of the leaf extracts to reduce ferric ions (Fe3+) to ferrous ions (Fe2+). This indicates the presence of antioxidants in the extracts, which can neutralize free radicals and protect cell from oxidative damage. Oxidative stress is known to play a role in the development of diabetes and its complications. The FRAP assay is a straightforward and reproducible method for assessing the overall antioxidant capacity of a sample. It reflects the combined reducing power of various antioxidant compounds present in the extract. In this experiment, the yellow color changes to pale green and blue color depending on the concentration of antioxidants in the samples, by comparing reference standard with plant extract is found to be **1.06**, so the anti-oxidant activity in *Syzygium aqueum* is more. The antioxidants such as phenolic acids and flavonoids were present in considerable amount in the extract of *Syzygium aqueum.*

5. Conclusion

These results suggest that the identified active compounds may possess various medicinal properties. The next step involves quantifying these active constituents, isolating them, and testing their medicinal potential, which could lead to the discovery of significant therapeutic molecules.

Amylase inhibitory activity of *S. aqueum* leaf extract was also assessed using the DNS method, revealing a 69.24% inhibition of amylase enzyme activity. This indicates that *S. aqueum* has potential α-amylase-inhibitory effects. Further isolation and analysis of its phytoconstituents could help identify which specific compounds contribute to its anti-diabetic properties. In this Anti-oxidant is comparing reference standard with plant extract is found to be **1.06**, so the anti-oxidant activity in *Syzygium aqueum* is more. These findings may serve as a foundation for future research and the development of new drugs.

The combined anti-diabetic and antioxidant activities of *Syzygium aqueum* leaves make them a promising natural resource for managing diabetes and related complications. By inhibiting α-amylase and reducing oxidative stress, the leaf extracts may help improve blood sugar control and protect against cellular damage.

Further research**:** While the DNS and FRAP methods provide valuable preliminary data, further research is needed to fully understand the therapeutic potential of *Syzygium aqueum* leaves. This includes identifying the specific bioactive compounds responsible for the observed activities, investigating other potential anti-diabetic mechanisms, and conducting in vivo studies to evaluate the efficacy and safety of the leaf extracts in animal models or clinical trials.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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