ejpmr, 2024, 12(4), 217-222

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

α-AMYLASE INHIBITORY ACTIVITY & ANTI-OXIDANT ACTIVITY OF *BOUGAINVILLE GLABRA* LEAVES

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Article Received on 27/01/2025 **Article Revised on 18/02/2025** **Article Accepted on 10/03/2025**

ABSTRACT

Bougainvillea glabra leaves have been studied for their potential health benefits, including α-amylase inhibitory activity and antioxidant activity.

- **α-Amylase inhibitory activity:** α-Amylase is an enzyme that breaks down starch into sugars. Inhibiting α-amylase can help regulate blood sugar levels, which may be beneficial for people with diabetes or those who are at risk of developing diabetes. Studies have shown that extracts from *Bougainvillea glabra* leaves can inhibit α-amylase activity.

- **Antioxidant activity:** Antioxidants are substances that can protect cells from damage caused by free radicals. Free radicals are unstable molecules that can contribute to various diseases, including cancer and heart disease. Studies have shown that *Bougainvillea glabra* leaves contain compounds with antioxidant properties.

More research is needed to confirm the potential health benefits of *Bougainvillea glabra* leaves.

Bougainvillea glabra leaves may interact with certain medications. It is important to talk to your doctor before using *Bougainvillea glabra* leaves, especially if you are taking any medications.

Bougainvillea glabra leaves may cause side effects in some people. It is important to start with a low dose and increase the dose gradually as needed

INTRODCTION

India is the habitat to more than 50,000 plant species, the majority of which are employed in folk and traditional herbalism. Many medicinal plants are used directly to treat illnesses or heal wounds, while some natural or pure compounds are consumed every day as a source of vital nutrients. Asia, the largest continent in the world, has diverse plant flora but species richness is concentrated mainly in tropical and subtropical regions, one of which is the Philippines. Medicinal plants are the major bio resource of drugs for both traditional and conventional systems of medicine.

Bougainville glabra belongs to the Nyctaginaceae family. It has been determined to be native to South Asia, specifically to Bangladesh, India, and Sri Lanka. *Bougainvillea glabra*, commonly known as paperflower or lesser bougainvillea, is a vibrant, evergreen climber that belongs to the Nyctaginaceae family. Native to Brazil, this species is widely cultivated in tropical and subtropical regions around the world for its showy, colorful bracts, which come in shades of pink, purple, magenta, and sometimes white. Although the actual flowers are small and white, they are surrounded by bright bracts that make the plant strikingly attractive.

Characterized by its hardy nature and vigorous growth, *Bougainvillea glabra* is ideal for use as a decorative climber on trellises, walls, and fences, or as ground cover in warmer climates. It thrives in full sun, tolerates drought well once established, and requires minimal maintenance, making it a popular choice in both private gardens and urban landscapes. Besides its ornamental appeal, this plant also provides ecological benefits, attracting pollinators like bees, butterflies, and birds.

Medicinal plants are of great significance to the health of individuals and communities. India is well known as the "Emporium of Medicinal Plants". Due to their great importance, demand of medicinal plants has increased

numerous folds *Bougainvillea* genus is an incredibly widespread group throughout the world. It belongs to the family Nyctaginaceae and, according to the "The Plant List", contains approximately 18 species. The objectives of this review are to provide recent update on *Bougainvillea glabra* with emphasis on their morphological characteristics features along with their phytochemistry and pharmacological activity. *Bougainvillea* was named after the world traveler, Louis de Bougainville, who discovered it in Brazil in 18th century and brought it to Europe where it became both widespread and popular. The leaves of *Bougainvillea glabra* are simple, ovate to elliptic, and grow alternately along the stems.

Bougainvillea glabra leaves have been traditionally used in some cultures for their purported medicinal properties. Preliminary scientific investigations suggest that extracts from these leaves may possess both anti-diabetic and antioxidant activities. Studies have explored the potential of *Bougainvillea glabra* extracts to inhibit α-amylase, an enzyme involved in carbohydrate breakdown. Inhibiting α-amylase can help slow down the digestion of carbohydrates and potentially contribute to better blood sugar control, which is crucial in diabetes management. Furthermore, research has examined the antioxidant potential of *Bougainvillea glabra* leaves, identifying the presence of compounds that may scavenge free radicals and protect against oxidative stress.

While the initial findings are promising, it's important to emphasize that more research is needed to fully understand the mechanisms of action and the therapeutic potential of *Bougainvillea glabra* leaves. Clinical trials are necessary to evaluate the efficacy and safety of *Bougainvillea glabra* extracts in humans, particularly in the context of diabetes management and antioxidant defense. This introduction provides a foundation for exploring the scientific evidence related to the potential benefits of *Bougainvillea glabra* leaves, while acknowledging the need for rigorous research to validate these claims.

MATERIALS AND METHODOLOGY

➢ Collection of crude drug

Green leaves plucked from our campus garden. The herbarium was prepared and authenticated at State Medicinal plant 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.

➢ 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.

❖ α-AMYLASE INHIBITION BY DNS METHOD 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.

- hosphate 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.

❖ **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 [K3Fe(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.

RESULTS

❖ **α-AMYLASE INHIBITION 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)	0.28

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

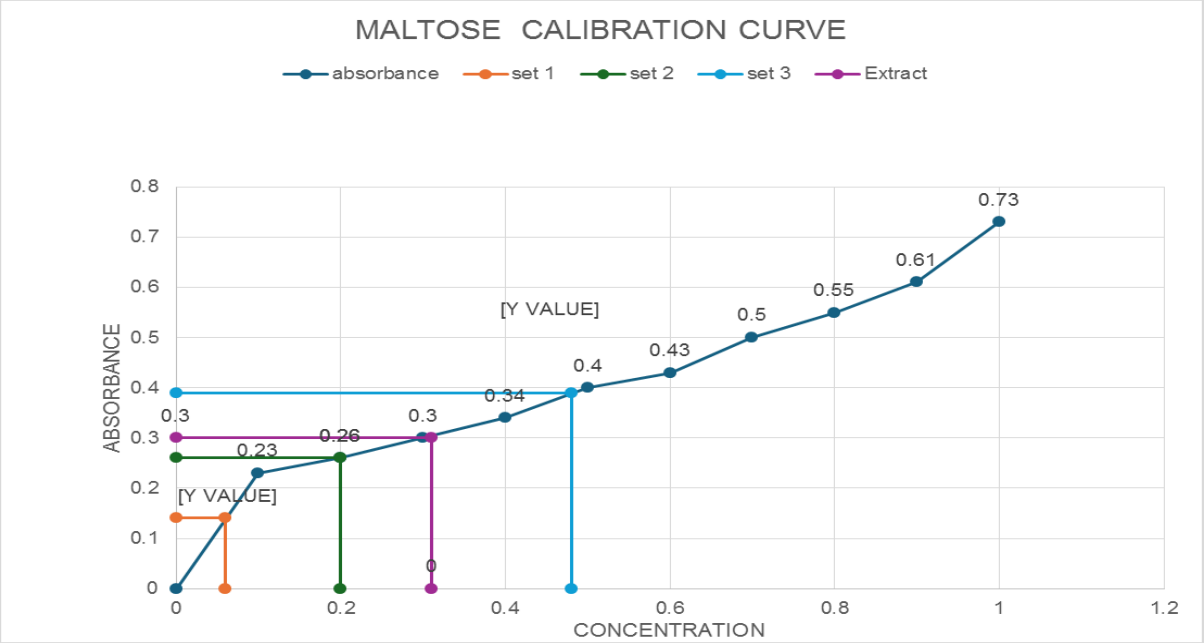

Figure 1: Matose Calibration Curve.

% 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

$$\%\ \text{inhibitory activity} = \frac{[0.28 - 0.39]}{[0.26 - 0.39]} \times 100$$

= 84.615%

Figure 2: Soxhlet Apparatus.

Figure 3: Maltose solution test tube series.

❖ **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 (*Bougainvillea glabra)*	1.43

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 **1.43**, so the anti-oxidant activity in *Bougainvillea glabra* is more. The antioxidants such as phenolic acids and flavonoids were sent in considerable amount in the extract of *Bougainvillea glabra.*

DISCUSSION

This study investigated the potential of *Bougainvillea glabra* leaf extracts to inhibit α-amylase activity and scavenge free radicals, two key factors in the management of diabetes and oxidative stress-related disorders.

α-Amylase Inhibition

The results of this study demonstrate that *Bougainvillea glabra* leaf extracts exhibit significant α-amylase inhibitory activity. This suggests that the extracts may have the potential to regulate postprandial hyperglycemia by slowing down the digestion and absorption of carbohydrates. The degree of α-amylase inhibition observed in this study is comparable to that of maltose, a commonly used α-amylase inhibitor drug. This finding supports the traditional use of *Bougainvillea glabra* leaves in the management of diabetes and suggests that the leaves may contain natural compounds with potent anti-diabetic properties. The percentage of α-amylase inhibition in flower of was found to be 84.615% because of this have α– amylase inhibitory activity this compound may have anti diabetic activity and this compound have a wide applications in future research field.

Anti-oxdiant Activity

In addition to α-amylase inhibition, *Bougainvillea glabra* leaf extracts also exhibited significant free radical scavenging activity. This indicates that the extracts possess potent antioxidant properties, which can help to protect against oxidative stress-related damage. The free radical scavenging activity of *Bougainvillea glabra* leaves can be attributed to the presence of various bioactive compounds, including polyphenols and flavonoids. These compounds have been shown to have strong antioxidant properties and can effectively scavenge free radicals, reducing oxidative stress and protecting against cellular damage. 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 1.43, so the anti-oxidant activity in *Bougainvillea glabra* is more. The antioxidants such as phenolic acids and flavonoids were sent in considerable amount in the extract of *Bougainvillea glabra*.

CONCLUSION

Bougainvillea glabra leaves have been studied for their potential health benefits, including α-amylase inhibitory activity and antioxidant activity.

- **α-Amylase inhibitory activity:** α-Amylase is an enzyme that breaks down starch into sugars. Inhibiting α-

amylase can help regulate blood sugar levels, which may be beneficial for people with diabetes or those who are at risk of developing diabetes. Studies have shown that extracts from Bougainvillea glabra leaves can inhibit α-amylase activity.

- **Antioxidant activity:** Antioxidants are substances that can protect cells from damage caused by free radicals. Free radicals are unstable molecules that can contribute to various diseases, including cancer and heart disease. Studies have shown that Bougainvillea glabra leaves contain compounds with antioxidant properties.

- **Standardization and Dosage:** One of the biggest challenges in using natural products like *Bougainvillea glabra* for medicinal purposes is standardization. The concentration of active compounds can vary depending on factors like the growing conditions, harvesting time, and extraction method. Therefore, developing standardized extraction protocols and determining optimal dosages are crucial.

- **Safety and Toxicity:** While preliminary studies may suggest some benefits, it's essential to thoroughly investigate the safety and potential toxicity of *Bougainvillea glabra* extracts. Long-term studies are needed to assess any potential side effects or adverse reactions.

- **Interactions with Medications:** *Bougainvillea glabra* extracts may interact with other medications, including those used to manage diabetes. Therefore, it's crucial for individuals with diabetes to consult with their doctor before using *Bougainvillea glabra* or any other herbal remedy.

- **Mechanism of Action:** A deeper understanding of the mechanisms by which *Bougainvillea glabra* exerts its anti-diabetic and antioxidant effects is needed. This will help in developing targeted therapies and optimizing the use of *Bougainvillea glabra* extracts.

In conclusion, *Bougainvillea glabra* leaves hold promise as a potential source of anti-diabetic and antioxidant compounds. However, much more research is needed to fully understand their therapeutic potential and ensure their safety and efficacy. Rigorous scientific investigation, including clinical trials, is crucial before any definitive conclusions can be drawn about the use of *Bougainvillea glabra* in the management of diabetes or oxidative stress-related conditions.

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