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Phytochemical and Biological Investigations of *Annona muricata* L.

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Abstract

Annona muricata L. is a small-evergreen tree with potential pharmacological applications in traditional medicine. Here, the methanolic extract of *A. muricata* leaf was subjected to phytochemical and biological assays. During preliminary phytochemical screening, phytochemicals such as alkaloid, flavonoid, steroid, glycoside, phenol, carbohydrate and tannin were discovered revealing the plant's rich chemical composition. It exhibited strong antioxidant activity which was evident from DPPH radical quenching assay with an IC_{50} of 30.95 µg/ml. The extractive also presented a significant total phenolic content (63.74 mg GAE/g). In an oral glucose tolerance model in mice, the leaf extract of *A. muricata* at the dose of 400 mg/kg revealed a marked and dose-dependent reduction in blood glucose levels, demonstrating a comparable efficacy to standard glibenclamide. These findings suggest that synergistic phytoconstituents in *A. muricata* may modulate oxidative stress and glucose homeostasis, supporting its development as a promising natural agent for antidiabetic intervention.

Key words: *Annona muricata*, phytochemical, antioxidant, hypoglycemic.

Introduction

The rising global incidence of diabetes mellitus represents a significant public health concern (Almalki *et al*., 2025). The diabetic population will increase by approximately 72% worldwide by 2025 (Florence *et al*., 2014). Although modern antidiabetic drugs continue to advance and are validated through rigorous scientific protocols, ongoing research seeks safer and more accessible therapeutic alternatives derived from plant sources, that may offer profound efficacy with minimal side effects (Tran *et al*., 2020).

Annona muricata L., a member of the Annonaceae family is commonly known as "Soursop" or "Sitaphal" (Wahab *et al*., 2018). The plant grows well in the subtropical and tropical regions of Bangladesh, especially in home gardens and small-scale farms. Its adaptability, nutritional value, and medicinal relevance have made this plant species an important resource in rural communities (Mutakin *et al.*, 2022). Traditionally, it has been used to treat hypertension, diabetes, stomach pain, fever, parasitic infections, and vomiting (Soto *et al*., 2023).

In rural areas, topical application of the leaves is also common for reducing inflammation and aiding the management of localized infections (Fajriyah *et al*., 2024). Phytochemical analyses have revealed a diverse array of constituents, including alkaloids, tannins, coumarins, flavonoids, terpenoids, stearic acid, myristic acid, and ellagic acid (Adewole and Ojewole, 2009). Multiple pharmacological studies have demonstrated that *A. muricata* exhibits antihypertensive, vasodilatory, antispasmodic, and cardiodepressive properties (Ismail *et al*., 2018; Feng *et al*., 1962; Bento *et al*., 2018), along with antiplasmodial, antimutagenic, anticonvulsant (N'gouemo, 1997), antiviral (Padma *et al*., 1998),

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antibacterial, antidiabetic, hypolipidemic, and antioxidant activities (Florence *et al.*, 2014). Based on the diverse biological properties, the present study was designed to evaluate the phytochemical profile of *A. muricata* leaf extract and explore the antioxidant and hypoglycemic potentials of the extract using established methods.

Materials and Methods

Plant Collection and Extraction: Fresh leaves of *Annona muricata* were collected from the botanical garden of Jahangirnagar University. Unwanted plant parts and extraneous materials were removed prior to processing. The collected leaves were washed thoroughly with fresh water and first sun-dried for two days, followed by fan-assisted air-drying for an additional fourteen days. The dried leaves were finely powdered using an electric blender, yielding 683 g of dry material, which was stored in an amber glass bottle. The powdered material was macerated in 2 L of methanol and kept in a cool environment for fifteen days with occasional shaking. After the extraction period, the mixture was filtered sequentially through cotton and Whatman filter paper. The filtrate was then concentrated using a rotary evaporator to obtain the crude methanolic extract.

Phytochemical assessment: Preliminary phytochemical screening was carried out to identify major classes of bioactive constituents present in the methanol extract of *A. muricata* leaf using standard qualitative methods (Chowdhury *et al.*, 2021).

Total phenolic content analysis: Total phenolic content was determined ensuing the method described by Škerget *et al.* (2005), using the Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as the standard reference compound for quantification (Hasib *et al*., 2020). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.

Antioxidant activity: The antioxidant activity of the extract was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, as established by Brand-Williams *et al*. (1999). This assay measures the ability of the leaf extract to quench DPPH free radicals, and results were expressed as IC_{50} values.

Animals. Swiss albino mice (20-25 g) of either sex were procured from International Center for Diarrheal Disease Research, Bangladesh (icddr,b). These mice were housed in polypropylene cages within a controlled environment (Humidity: 60-70%, temperature: 24 ± 2ºC) at the Daffodil International University. They were provided with rodent feed specially formulated by ICDDR,B and had access to water *ad libitum*. All experimental procedures were followed in accordance with the guidelines approved by the institutional ethical committee.

Hypoglycemic activity: For oral administration, the crude extract was prepared at doses of 200 mg/kg and 400 mg/kg body weight. Accurately weighed amounts of extract were triturated with a small volume of Tween-80 as a suspending agent, followed by gradual addition of normal saline to prepare a final suspension volume of 3.0 ml. The suspension was homogenized thoroughly using a vortex mixer. The reference drug, glibenclamide, was prepared by dissolving a 10-mg tablet in 3.0 ml of normal saline (0.9% NaCl) to yield a dose of 10 mg/kg body weight. At zero hour, the control group received 1% Tween-80 in saline, the standard group received glibenclamide, and the test groups received extract suspensions, all administered orally using an oral gavage needle with a ball-shaped tip. After 60 minutes, mice were given 10% glucose solution at a dose of 2 g/kg body weight. Blood samples were collected from the tail vein at 30, 90 and 150-minutes post-glucose administration. Blood glucose levels were measured using a digital glucometer (Islam *et al*., 2019).

Results and Discussion

Phytochemicals are naturally occurring, biologically active compounds that play important roles in plant defense, ecological interactions, and potential therapeutic activity in humans. Preliminary phytochemical analysis revealed the presence of multiple bioactive compound classes in the

methanolic leaf extract of *A. muricata*. These included alkaloids, glycosides, tannins, flavonoids, saponins, and steroids confirming the chemical richness of the plant (Table 1). The diversity of these secondary metabolites supports the traditional therapeutic applications of *A. muricata* and suggests potential pharmacological relevance, particularly in metabolic and oxidative stress-related disorders.

The antioxidant potential of the methanolic extract was assessed using a spectrophotometric DPPH radical scavenging assay. The extract displayed substantial free radical-scavenging capacity, with an IC_{50} value of 30.95 µg/ml (blank absorbance: 0.364). Although this activity was lower than the standard BHT, which had an IC_{50} value of 12.52 µg/ml, the test extract still demonstrated considerable antioxidant strength. The total phenolic content of the extract was found to be 63.74 mg gallic acid equivalents (GAE) per gram of extract. Phenolic compounds are known contributors to free radical neutralization, and the substantial phenolic content in *A. muricata* likely contributes to its antioxidative effects. These findings indicate that *A. muricata* leaves contain bioactive phytochemicals with meaningful antioxidant potential.

Table 1. Phytochemical screening of the crud methanol extract of *A. muricata Leaf*.

Phytochemical	Reagent	Result
Alkaloid	Dragendorff's reagent	Positive
Glycoside	Conc. HCl followed by Fehling Solution	Positive
Tannin	5% $FeCl_3$ solution	Positive
Flavonoid	Conc. HCl	Positive
Saponin	Distilled water	Positive
Steroid	H_2SO_4	Positive

Table 2. Average blood glucose levels of test groups of *A. muricata*.

Group	Dose	Blood glucose levels (mmol/L)				
		0 Min	30 Min	60 Min	120 Min	180 Min
NC	1% tween 80	7.00	17.50	15.60	13.00	10.70
Standard	10 mg/kg body weight	5.667	10.667	9.63	7.067	5.40
MEAM 200	200 mg/kg body weight	5.10	9.067	6.53	5.833	4.733
MEAM 400	400 mg/kg body weight	5.933	4.10	2.433	1.90	1.567

Here, MEAM = Methanol extract of *A. muricata* leaf, NC = Negative Control; Standard = Glibenclamide.

The hypoglycemic activity of the methanolic extract at doses of 200 - and 400 mg/kg was evaluated in mice alongside a positive control group treated with standard glibenclamide (10 mg/kg) and a negative control group receiving 1% Tween 80. The plant extract at both doses resulted in a reduction of blood glucose levels following glucose loading in mice. The leaf extract at 400 mg/kg dose exhibited the most pronounced hypoglycemic effect, reducing glucose levels consistently across all measured time points. The activity at this dose was comparable to, and in later time points even stronger than, that of the standard drug. The 200 mg/kg dose of extract also demonstrated significant glucose-lowering ability, although less marked than the higher dose. Overall, the results indicate a dose-dependent hypoglycemic effect of *A. muricata* leaf extract. These findings support the traditional use of *A. muricata* in managing hyperglycemia and highlight its potential as a natural antidiabetic agent.

As shown in figure 1, a dose-dependent reduction in blood glucose levels was observed following the administration of the test sample. The effect was consistent across all evaluated time points, demonstrating the extract's strong hypoglycemic potential.

Similarly, the extracts displayed robust antioxidant activity, with performance approaching

that of the standard control in several assays. This high level of activity highlights the strong free-radical-scavenging capabilities of the methanol fractions.

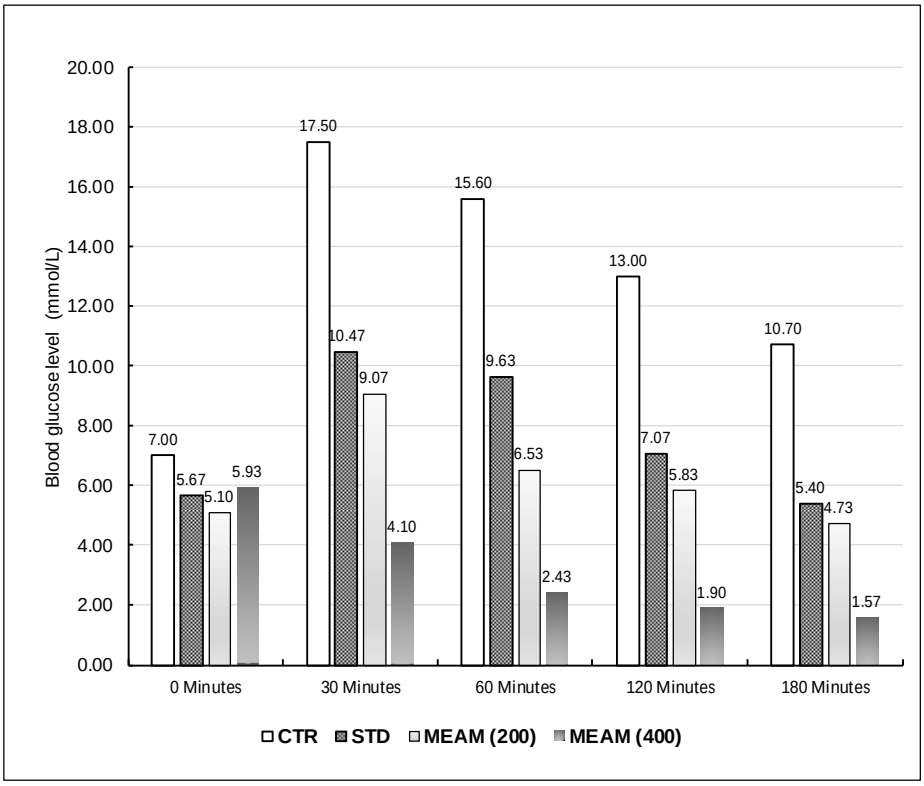

Figure 1. Hypoglycemic activity of methanol extract of *A. muricata* leaf.

The notable hypoglycemic activity of the methanol extract suggests that *A. muricata* contains compounds capable of effectively reducing blood glucose levels. These findings support the traditional use of the plant in managing metabolic conditions and underscore the therapeutic importance of exploring its phytochemical constituents (Parihar 2024). The strong antioxidant activity further builds on its pharmacological promise. This dual activity is particularly valuable as oxidative imbalance plays a central role in the onset and progression of chronic diseases (*Jomova et al.*, 2023). The combined hypoglycemic and antioxidant effects observed in this study highlight the considerable potential of *A. muricata* as a natural antidiabetic candidate. The biological activities suggest that the leaf extract of *A. muricata* may act through multiple complementary pathways, addressing both elevated blood glucose and oxidative stress simultaneously. Such multimodal action is especially important for diabetes management, where single-target therapies often fall short. The results indicate that *A. muricata* may serve as a foundation for future development of plant-based antidiabetic formulations. A limitation of this study is the absence of isolated compound profiling, which prevents precise identification of the active constituents responsible for the observed effects. Future research involving chromatographic isolation, mechanistic studies, and *in vivo* long-term evaluations will be essential for confirming the therapeutic pathways involved. Recent scientific interest has increasingly focused on the metabolic benefits of *A. muricata* (Yajid *et al.*, 2018). Its bioactive compounds-particularly phenolics, flavonoids, and alkaloids-have been linked to mechanisms relevant to glycemic control, including the modulation of carbohydrate-digestive enzymes, enhancement of insulin activity, and attenuation of

oxidative stress (Ojo *et al*., 2022). These biochemical activities underscore the potential of *A. muricata* as a promising candidate for developing plant-based strategies to manage diabetes and its associated complications.

Conclusion

The methanol extract of *A. muricata* exhibit significant hypoglycemic and antioxidant activities. These findings highlight the extract's potential to lower blood glucose and mitigate oxidative stress, supporting its promise as a natural therapeutic agent for managing diabetes and related oxidative-stress-associated disorders.

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