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Research

PHARMACOGNOSTICAL PRELIMINARY PHYTOCHEMICAL EXPLORATION AND ITS *IN-VITRO* ANTIOXIDANT AND ALPHA AMYLASE INHIBITORY EFFECT OF *MUNTINGIA CALABURA* LINN

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Abstract

Muntingia calabura Linn. belongs to the family *Muntingiaceae*, commonly known as Jamaican cherry, is a traditionally valued medicinal plant used in folk medicine for the management of headache, fever, gastric ulcers, inflammation and diabetes. The present study was aimed to evaluate the physicochemical parameters, preliminary phytochemical screening, in-vitro antioxidant activity and alpha-amylase inhibitory effect of *M. calabura* leaves. Physicochemical parameters such as ash values, extractive values and moisture content were determined to assess quality and purity. Preliminary phytochemical screening was carried out using standard qualitative tests. In vitro antioxidant activity was evaluated through established radical scavenging assays, while antidiabetic potential was assessed using the alpha-amylase inhibition method. The extract exhibited significant, concentration-dependent antioxidant activity and α-amylase inhibitory effect, indicating potential to mitigate oxidative stress and postprandial hyperglycemia. Overall, the findings provide scientific validation for the traditional use of *M. calabura* and support its potential as a promising natural source of antioxidant and antidiabetic agents. Further bioassay-guided isolation and mechanistic studies are recommended.

Keywords: Alpha-amylase inhibition, *in-vitro* antioxidant activity, *Muntingia calabura*, physicochemical evaluation, preliminary phytochemical screening.

1. INTRODUCTION

Muntingia calabura Linn. belongs to *Muntingiaceae*, commonly known as the Jamaica cherry, Strawberry tree or Singapore cherry. It is native to the tropical regions of America and the West Indies. It is used as a traditional medicinal herb across several countries in Southeast Asia and South America. It is particularly recognized for its use in Peru, Colombia, Mexico, Vietnam, Malaysia and the Philippines. In Peru, Colombia, Mexico, the leaves of *Muntingia calabura* are traditionally used as herbal infusions to treat gastric ulcers, headaches, colds, inflammation and as a mild sedative, while Vietnam, Malaysia and Philippines, the leaves are commonly prepared as decoctions or teas for managing fever, hypertension, diabetes, pain, inflammation and as an antiseptic and antioxidant remedy in folk medicine [1]. Traditionally, various parts of the plant have been utilized in folklore medicine; the leaves have been steeped or boiled in water to reduce gastric ulcers, alleviate headaches and treat the common cold [2]. This plant reported pharmacological studies such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective and anticancer. Phytochemical screening confirmed the presence of bioactive secondary metabolites, particularly flavonoids and phenolic compounds. [3-6].

To further explore and validate its therapeutic potential, the aim of the present study is to perform pharmacognostical, preliminary phytochemical screening of *Muntingia calabura* leaves and to evaluate its *in-vitro* antioxidant and alpha-amylase inhibitory effects. Investigating these parameters is essential, as the rich phytochemical content of the plant - including flavonoids, phenolics and tannins, may contribute to significant antioxidant activity, which helps combat oxidative stress linked to chronic diseases.

Therefore, exploring its alpha-amylase inhibitory activity can suggest its potential utility in the management of diabetes.

An attempt has been taken to investigate the pharmacognostical studies available in local garden Madurai Medical college campus, Madurai. Tamilnadu.

2. MATERIALS AND METHODS

Authentication and collection of leaves of *Muntingia calabura* Linn.

Muntingia calabura leaves were collected from the local garden, Madurai Medical College campus, Madurai during December 2024 and were authenticated by Dr.Stephen, Department of Botany, The American College, Madurai, Tamilnadu, India.

Macroscopical studies of *Muntingia calabura* Linn.

Leaves were studied separately for its morphological characters by organoleptic test.

Morphological characters are presented in table 1.

Microscopical studies of *Muntingia calabura* Linn.

Thin sections were taken and were stained with routine methods and were observed under microscope. Thin transverse sections of the fresh leaves were taken and it was stained with routine procedure. The microscopical characters of the leaves in those slides were studied using a microscope and was presented in figure 3.

Quantitative microscopy

The quantitative microscopical parameters such as vein islet and vein termination number stomata type are determined as per WHO 2004 guidelines and its resulted are given in table 2.

Physicochemical parameters

The powder is subjected for physicochemical evaluation parameters such as loss on drying, extractive value with different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water in increasing order of polarity, ash value (total, acid- insoluble, and water-soluble ash) as per the standard procedure. The results are presented in table 3.

Phytochemical screening

Phytochemical screening was performed using standard procedures adopted in Indian Pharmacopoeia and its results were given in table 5.

In-vitro antioxidant studies

- **Determination of hydrogen peroxide scavenging effect**

The scavenging effect of hydrogen peroxide was determined as per method [7]. 1ml of extract solution was treated with 0.6 ml of hydrogen peroxide for 10 minutes, absorbance was read at 230 nm against blank, using ascorbic acid as standard and its results are presented in table 6.

- **Determination of ferric reducing power assay**

The reducing power assay was determined by the spectrophotometric method [8].Various concentrations ranging from 40 to 200 microgram was treated with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), 2.5ml of 1% potassium ferricyanide incubated at 50°C for 20 minutes cooled to which 2.5 ml of trichloro acetic acid (TCA) was added and centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution was removed and 2.5 ml of methanol and 0.5 ml of (0.1% ferric chloride) solutions were added, the absorbance of the resulting solution was read at 700 nm. Ascorbic acid

was used as standard and its results are presented in Table 7.

- **Determination of total antioxidant capacity**

The total antioxidant capacity was determined by the spectrophotometric method [9]. Various concentrations of extract 40 to 200µg were taken in Eppendorff tube and 1ml of reagent containing 0.6mM sulphuric acid, 28nM sodium phosphate and 4 mM ammonium molybdate were added. The tubes were incubated at 95°C for 90 minutes, were cooled to room temperature, the absorbance was read at 695 nm. Ascorbic acid was used as standard and the total antioxidant capacity and its results are presented in table 8.

Determination of *In-vitro* alpha amylase inhibitory activity

- **Procedure:** The method adopted as per [10].

To 100 µL of plant extract (2, 4, 8, 10, and 15 µg/mL), 200 µL of 1% starch solution was added, and the mixture was incubated at 37°C for 20 minutes. Then, 100 µL of the enzyme solution was added and further incubated at 37°C for 10 minutes. The reaction was stopped by adding 200 µL of DNSA (dinitrosalicylic acid) reagent. The mixture was kept in a boiling water bath for 5 minutes. After cooling, the reaction mixture was diluted with 2.2 mL of distilled water, and the absorbance was measured at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 µL of distilled water, control was prepared in a similar manner without the extract. All experiments were performed in triplicate and the obtained results are presented in Table 9.

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

3. RESULTS AND DISCUSSION

Table 1: *Macroscopy of Muntingia calabura leaves*

S.no	Particulars	Result
1.	Colour	Dark green
2.	Odour	characteristic
3.	Taste	Bitter and astringent
4.	Length	5-12 cm
5.	Breadth	1-4 cm
6.	Margin	Serrate
7.	Base	Oblique
8.	Apex	Accuminate
9.	Petiole	2- 5mm long
10.	Phyllotaxy	Alternate
11.	Lamina	Ovate lanceolate

Microscopy of *Muntingia calabura* leaves

Epidermis

Leaf of *M. calabura* were hypostomatous and dorsiventral. The epidermis was uniseriate. On the abaxial leaf surface, the ordinary epidermal cells were rectangular in cross-section, except in the vein regions, where they were round to papilliform in shape. On the adaxial leaf surface, the epidermal cells were voluminous, conical in shape, and possessed thick outer periclinal walls. These voluminous cells were bi-compartmentalized by a wall-like septum. The upper portion adjacent to the cuticle was smaller, whereas the inner portion protruded into the mesophyll and contained mucilaginous material. Both glandular and non-glandular trichomes were present.

Ground cells

Mesophyll consisted of two to three layers of palisade parenchyma and 2-3 layers of spongy parenchyma. Druses of calcium oxalate were observed within the bundle sheath cells and in inner colloidal region. The outline of the midrib region was concave on the adaxial surface and convex on the abaxial surface of the leaf. The cortex of the midrib comprised one to three layers of collenchyma followed by 4-8 layers of parenchymatous cells.

Vascular bundles

Vascular system in the midrib showed vascular bundles arranged in a half-moon shape. Mucilage cells were observed in the phloem.


Figure 1.1


Figure 1.2


Figure 2.1


Figure 2.2

Fig 1.1: Habitat of *Muntingia calabura*, **Fig 1.2**: Enlarged view of *Muntingia calabura,* **Fig 2.1:** Dorsal view of *Muntingia calabura* leaves, **Fig 2.2:** Ventral view of *M. calabura* leaves

Microscopic evaluation of *Muntingia calabura* leaves

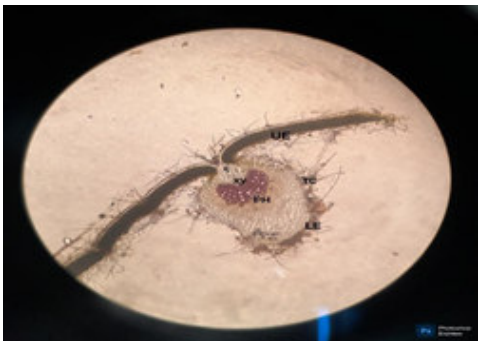


Fig 2: Transverse section view of *Muntingia calabura*

LE-Lower epidermis, UP-Upper epidermis, C- Collenchyma, XY-Xylem, PH-Phloem, TC-Trichomes

Table 2: *Quantitative microscopy of Muntingia calabura leaves*

S.no.	Parameter	Results (cells/mm^2) (Mean ± SEM *)
1.	Vein islet number	7.5/mm^2 ± 0.163304
2.	Vein termination number	6.25/mm^2 ± 0.024496
3.	Stomata type	Paracytic

**Indicates results of triplicate.*

Table 3: *Physio-chemical parameters of Muntingia calabura leaves*

S.no.	Parameter	Results (% w/w) (Mean ± SEM *)
1.	Foreign matter	Nil
2.	Loss on drying	6.58 ± 0.008165
3.	Total solids	93.42 ± 0.461894
4.	Total ash	10 ± 0.17321
5.	Acid insoluble ash	2.10 ± 0.288684
6.	Water soluble ash	4.50 ± 0.51963
7.	Petroleum ether Extractive value	5.6 ± 0.34642
8.	Chloroform Extractive value	10.42 ± 0.008165
9.	Ethanol Extractive value	22.35 ± 0.230947
10.	Water Extractive value	17.50 ± 0.461894

**Indicates results of triplicate.*

Table 4: *Behaviour of M.calibura powder with various reagents*

S.no.	Powder + reagent	Visible	UV light 254nm	UV light 366nm
1.	Powder + Conc.Hydrochloric acid	Greenish-brown	Brown	Black
2.	Powder + Conc.Sulphuric acid	Dark brown	Brown	Black
3.	Powder + Conc.Nitric acid	Blackish-brown	Black	Black
4.	Powder + Acetic acid	Yellowish-brown	Black- brown	Golden yellow
5.	Powder + 20% Sodium hydroxide	Brown	Dark brown	Black
6.	Powder + Hydrochloric acid + Water	Green	Green	Black
7.	Powder + Sulphuric acid + water	Brown	Black	Black
8.	Powder + Nitric acid + Water	Orange	Greenish-yellow	Yellow

Table 5: *Determination of phytochemical analysis of Muntingia calabura*

S.no.	Test for	Observation
1.	Flavanoids	+
2.	Saponins	+
3.	Tannins	+
4.	Steroids	+
5.	Alkaloids	+
6.	Triterpenoids	+
7.	Anthraquinone	-
8.	Reducing sugar	+
9.	Cardiac glycosides	+
10.	Phenolic compounds	+
11.	Gum	-
12.	Mucilage	-
13.	Volatile oil	-
14.	Protein	-
15.	Amino acid	-

(+) - Positive, (-) – Negative

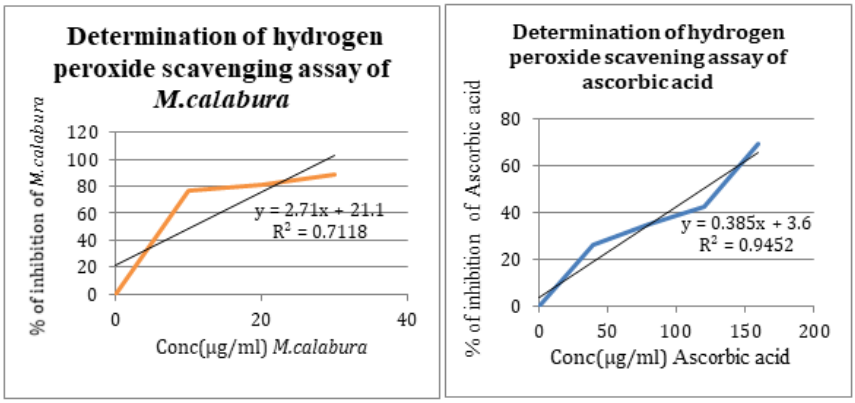
Determination of Hydrogen peroxide scavenging assay

Table 6: *Determination of hydrogen peroxide scavenging assay of Muntigia calabura and ascorbic acid*

S.no.	*M.calabura* (µg/ml)	Ascorbic acid (µg/ml)	% of inhibition of *M.calabura* (Mean ± SEM *)	% of inhibition of ascorbic acid (Mean ± SEM *)
1	10	40	77 ± 0.230947	26 ± 0.461894
2	20	80	81 ± 0.34642	35 ± 0.17321
3	30	120	89 ± 0.51963	42 ± 0.288684
4	----	160	----	69 ± 0.816521
IC_{50}			10.6µg/ml	120 µg/ml

**Indicates results of triplicate.*

Fig 5: *Determination of hydrogen peroxide scavenging assay of Muntigia calabura and ascorbic acid*



It is observed that *M.calibura* exhibited hydrogen peroxide scavenging effect at the concentration of IC_{50} - 10.6µg/ml in comparison with ascorbic acid used as standard IC_{50}-120 µg/ml.

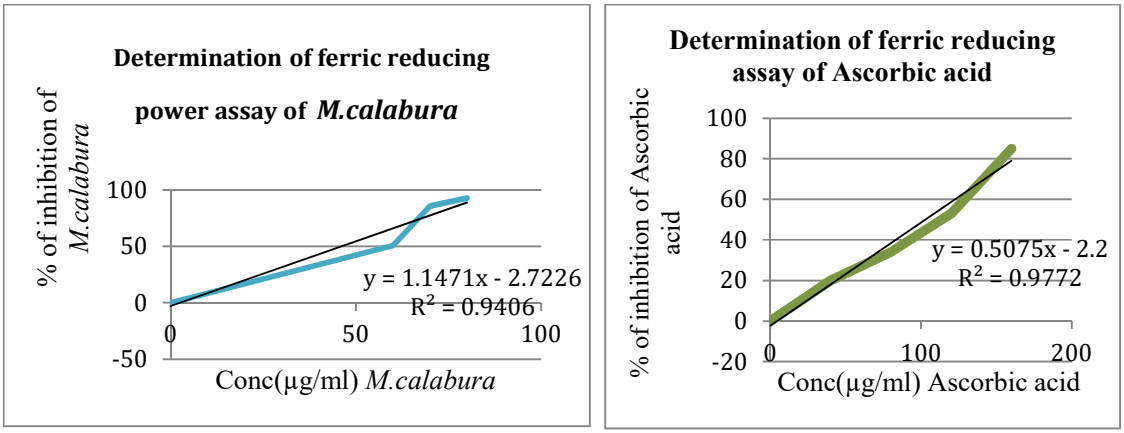
Determination of Ferric reducing power assay

Table 7: *Determination of ferric reducing assay of Muntingia calabura and ascorbic acid*

S.no.	Conc(µg/ml) *M.calabura*	Conc(µg/ml) Ascorbic acid	% of inhibition of *M.calabura* (Mean ± SEM *)	% of inhibition of ascorbic acid (Mean ± SEM *)
1	60	40	51 ± 0.461894	20 ± 0.51963
2	70	80	86 ± 0.17321	34 ± 0.816521
3	80	120	93 ± 0.461894	53 ± 0.34642
4	**----**	160	--------	85 ± 0.230947
IC_{50}			46µg/ml	103µg/ml

Indicates results of triplicate*.**

Fig 6: ***Determination of ferric reducing assay of Muntingia calabura and ascorbic acid***



It is observed that *M.calibura* exhibited ferric reducing power assay at the concentration of IC_{50} - 46µg/ml in comparison with ascorbic acid used as standard IC_{50} - 103µg/ml.

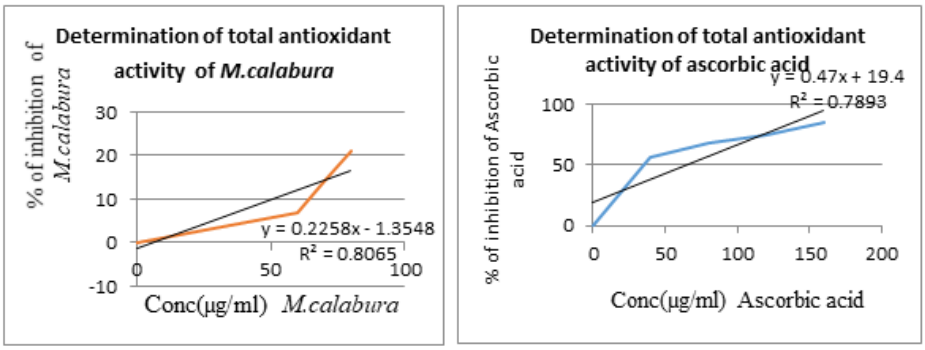
Determination of Total antioxidant activity assay

Table 8: *Determination of total antioxidant activity of Muntingia calabura and ascorbic acid*

S.no.	Conc(µg/ml) *M.calabura*	Conc(µg/ml) Ascorbic acid	% of inhibition of *M.calabura* (Mean ± SEM *)	% of inhibition of ascorbic acid (Mean ± SEM *)
1	60	40	7 ± 0.461894	57 ± 0.17321
2	70	80	14 ± 0.230947	68 ± 0.34642
3	80	120	21 ± 0.288684	75 ± 0.816521
4	----	160	**----**	85 ± 0.51963
IC_{50}			227µg/ml	5µg/ml

**Indicates results of triplicate.*

Fig 7: *Determination of total antioxidant activity of Muntigia calabura and ascorbic acid*



It is observed that *M.calibura* exhibited total antioxidant activity at the concentration of IC_{50}-227µg/ml in comparison with ascorbic acid used as standard IC_{50}- 5µg/ml

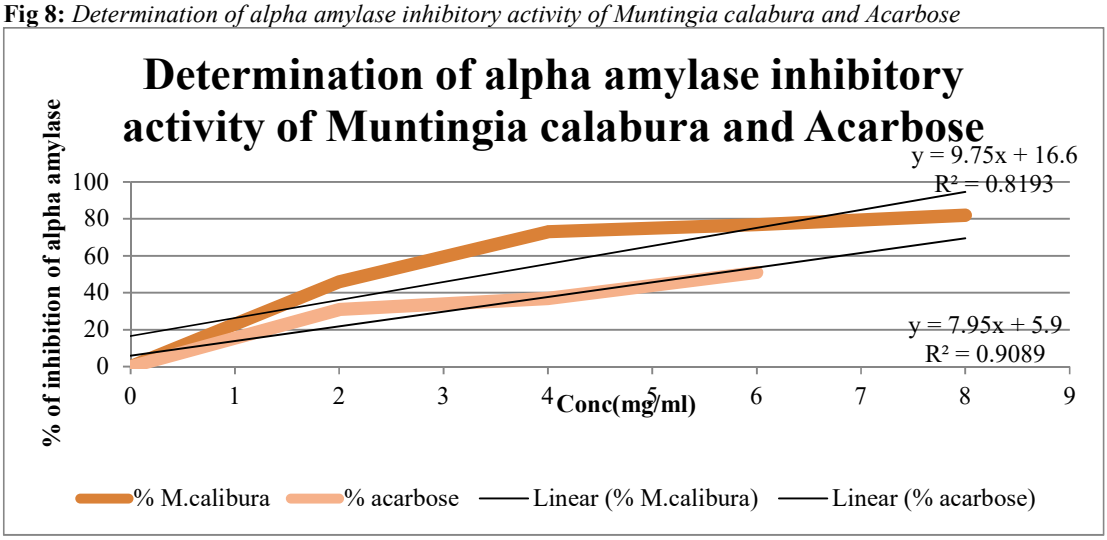
Determination of Alpha amylase inhibitory activity

Table 9: *Determination of alpha amylase inhibitory activity of Muntingia calabura and Acarbose*

..no.	Conc(µg/ml)	% of inhibition (*M.calibura*) (Mean ± SEM *)	% of inhibition (acarbose) (Mean ± SEM *)
1.	0	0	0
2.	2	46 ± 0.17321	31 ± 0.461894
3.	4	73 ± 0.288684	37 ± 0.230947
4.	6	77 ± 0.34642	51 ± 0.51963
5.	8	82 ± 0.230947	-
	IC_{50}	3.42µg/ml	6µg/ml

**Indicates results of triplicate.*

Fig 8: *Determination of alpha amylase inhibitory activity of Muntingia calabura and Acarbose*



It is observed that *M.calibura* exhibited alpha amylase inhibitory activity at the concentration of IC_{50} -3.42µg/ml in comparison with ascorbic acid used as standard IC_{50}-6µg/ml

4. Conclusion

Pharmacognostical parameters of *Muntingia calabura* were established through detailed evaluation of macroscopic, microscopic and powder characteristics using various reagents. The plant demonstrated significant antioxidant activity, which may be attributed to its rich phytochemical constituents such as flavonoids, phenolic compounds and tannins. These antioxidant properties play an important role in reducing oxidative stress associated with various chronic diseases. Furthermore, the extract exhibited α-amylase inhibitory activity, indicating its potential role in the management of diabetes mellitus. The overall findings scientifically support the traditional medicinal uses of the plant. However, further *in-vivo* studies and well-designed clinical trials are required to validate its therapeutic efficacy and safety profile.

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