Muntingia calabura L. (Muntingiaceae) bark hydroalcoholic extract: organoleptic, fluorescent, qualitative, quantitative, and biological validity

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Abstract

A wild herb known as kersen (*Muntingia calabura L.*) is utilised both medicinally and as food. It contains a lot of phytochemicals, particularly the antioxidant-producing phenolic group. Screening for phytochemicals and determining their biological assessment are essential. This investigation aimed to assess the anthelmintic, phytochemical, and pharmacognostic characteristics of hydroalcoholic extract from cherry bark. Flavonoids, tannins, steroids, and saponins were found in the extract. Above-mentioned anthelmintic activity was evaluated using earthworms. Albendazole, a common anthelmintic, was employed as a positive control. Tannins, steroids, and flavonoids are examples of phytochemicals that may contribute to the extract's anthelmintic effect.

Keywords: *Muntingia calabura* L., phytochemicals, anthelmintic activity, earthworms, Tannins.

1. Introduction

Growing plants has many uses, and similarly, healing through plants[1,2] has been practiced in India for a long time. For humans to survive, plants and trees play a critical function. According to some theories, the only things keeping humanity and other animals alive on Earth today are plants and trees; without them, climate change would have wiped them all. People are therefore encouraged to grow saplings. It is well known that plants and trees provide for humans' basic requirements, which include clothes, food, and shelter [3,4]. Phytoconstituents derived from plants have been used to cure a variety of ailments in indigenous cultures for thousands of years.[5,6] As a result, the market for medical plants is expanding for use in medicines, phytochemicals, nutraceuticals, cosmetics, and other related products. [7,8] Research on the pharmacological action of herbs is the main focus of trends in herbal remedies, which seek to verify the claims made in official books.[9] In warm regions of Asia, *Muntingia calabura* L., a member of the *Muntingiaceae* family,[10] is often known as Nakkaraegu in Telugu states. It

is indigenous to Central America, the Caribbean, Southern Mexico, and, to the west, South America. Since birds may swiftly disseminate this plant to the Asian mainland, hummingbirds are also familiar with cherries.

These plants are drought-tolerant, acid- and alkaline-tolerant, and can grow in poor soil. The dried leaves are used to create tea, while the fruit is used to make jam and tarts. Its beneficial protective impact against stomach damage, antibacterial, antioxidant, antidiabetic, hepatoprotective, antinociceptive, and antiproliferative properties have all been shown in recent studies.[11] Despite the fact that parasitic worm infections are a severe problem, there aren't many studies on anthelmintic medications.[12] In this study, we tried to look into the anthelmintic activity and pharmacognostic validation of *M. calabura's* hydroalcoholic extract.

Fig. 1: *Muntingia calabura* L Plant

1.1 Research Objective

- To scientifically study the various properties of Muntingia calabura, organoleptic, fluorescent, qualitative, quantitative, and biological validity.
- To study and analyse the anthelmintic properties of Muntingia calabura.

2. Methods

Plant collection and authenticity:

Dr. K. Srinivasa Reddy, M. Sc., Ph. D., Assistant Professor, Department of Botany, Govt. Degree College for Women, Nalgonda, and Telangana State, authenticated the *Muntingia calabura* L. plant, which was gathered from the hills of Ananthagiri, Kodad regions of Suryapet District. A voucher specimen was kept for future use. After being dried in the shade, the plant bark was ground into a coarse powder and subjected to extraction.

Organoleptic evaluation:

The term "organoleptic" refers to something that can be detected by the senses of taste, smell, touch, and appearance, among others. Sense and therefore establish certain properties of the material, which can be regarded as a preliminary step in determining its identity and level of purity.[13,14] The *Muntingia calabura* plant's dried bark and extract were collected,

examined, and tabulated for a number of characteristics, including shape, size, colour, taste, texture, surface characteristics, fracture characteristics, and cut surface appearance.

Analysis of foreign matters:

Spread out a thin layer of the drug sample to be evaluated (100–500 g) or the maximum amount specified in the monograph. The foreign material should be identified by inspection using the unaided eye or a 10x lens. Calculate the percentage present after separating and weighing it.[15]

Physical evaluation:

Medicinal plants should be free, as much as possible, of mould, insects, and other animal contaminants. According to the pharmacopeia monograph, the amount of foreign matter should not be higher than the standard regulated for each product. Several physico-chemical methods are used to determine different physical constants in order to perform the physical evaluation of crude medicines.

Following the official procedures outlined in WHO guidelines on quality control methods for medicinal plant materials, the ash values, extractive values, and drying loss were determined.[15]

Index of froth and swelling:

Gums and medications that contain a significant amount of components like mucilage, pectin, or hemicelluloses are particularly well-suited for therapeutic or medicinal use due to their swelling capabilities. The foaming index is used to quantify how well an aqueous decoction of medicinal plants and their extracts foams. When an aqueous infusion is agitated, saponins may produce a prolonged froth.[16] The swelling and foaming indices were computed using the method described in WHO guidelines.[15]

Analysis of fluorescence:

When plant material is treated with different chemicals and solvents, the chemical components present exhibit a phenomenon known as fluorescence. The fluorescence of powdered *M. calabura* plant bark was examined in the current study under UV and visible light/daylight conditions after being treated with various agents and solvents. Various solvents, alkaline solutions, and freshly made acids were applied to a small amount of dried and finely powdered material. In addition to alkaline solutions like 1N alcoholic NaOH and various solvents like chloroform, distilled water, petroleum ether, methanol, and ethanol, the powder sample was treated with acids like 50% HCl, 50% HNO3, and 50% H2SO4 and acetic acid. They were examined for fluorescence in both visible and ultraviolet light.[17,18]

Phytochemical screening: Making a plant extract via Soxhlet extraction:

Soxhlation is required when the contaminant is not soluble in a solvent and the desired product has limited solubility in that solvent. If the desired component is highly soluble in a solvent, it can be separated from the insoluble material using a simple filtration procedure.

Instead of passing many portions of heated solvent through the sample, this method has the advantage of recycling a single batch of solvent. This method cannot be used for thermolabile compounds since prolonged heating can degrade the substance. The bark of the *M. calabura* plant was effectively dried and extracted with hydro-alcohol in a Soxhlet system. The concentrated extract was put through a qualitative test in accordance with standard protocols to identify different phytochemical ingredients. There were found to be phytochemicals like flavonoids, tannins and phenolic compounds, and saponins.[19,20,21]

Quantitative estimation of constituents: [22]

Entire Phenol estimation:

The entire phenolic estimation of the extract was assessed using the FC assay. One ml of the FCR was combined with one millilitre of the sample (1 mg/ml). Five minutes later, the mixture was thoroughly combined with ten milliliters of a 7% Na_2Co_3 solution and thirteen milliliters of deionised purified water. After ninty min. at 23^0C in the dark, the mixture's absorbance measured at 760 nm. The entire phenolic estimation was determined by extrapolating the calibration curve following the creation of a GA solution. Three separate analyses were performed to evaluate the phenolic chemicals. The entire phenolic estimation was expressed in mg of GA equal (GAE)/g of dried material.

Finding the total quantity of flavonoids:

Typical solution preparation:

After weighing 10 mg of quercetin, 10 ml of methanol was added to a 10 ml vol. flask (1 mg/ml). One millilitre of the quercetin standard solution (1 mg/ml) was pipetted out and combined with ten millilitres of methanol to create a 100 mcg/ml stock solution. The stock solution was used to create solutions with concentrations of 25, 50, 75, 100, 125, and 150 mcg/ml. Each one received 0.3 millilitres of 5% sodium nitrite and four millilitres of water. After five min, 0.3 ml of a 10% $AlCl_3$ solution was added, and at the sixth minute, 2 ml of 1M sodium hydroxide was added. The entire volume was brought up to 10 millilitres using distilled water. Without using the $AlCl_3$ solution, a blank was created. Once the solutions were thoroughly mixed, the absorbance at 510 nm in comparison to the blank was measured using a UV-visible spectrophotometer. Using different quercetin concentrations and the associated absorbance, a standard graph was created.

Preparing a testing compound:

The overall flavonoid content of the extract was estimated by the method described by Zhi Shen et al. This method involved mixing 1.0 ml of the test sample with 0.30 ml of a 10% NaNO2 solution and 4 ml of distilled water. Five minutes later, 2.0 ml of 1% sodium hydroxide solution and 0.30 ml of 10% AlCl3 solution were attached to the mixture. Absorbance at 510 nm was measured in relation to a blank after the mixture was well mixed. The flavonoid content was

assessed three times. Quercetin equivalents (mg quercetin/g dried extract) were used to express the results.

Assessment of the overall tannin content:

To identify the tannins, the Folin-Ciocalteu technique was employed. A 10-ml vol. flask filled with 7.5 ml of distilled water, 0.5 ml of FCR, 1 ml of 35% Na_2Co_3 solution, and 0.1 ml of the sample extract. Purified water was then used to make up the volume up to 10 ml. After a good shake, wait for 30 minutes at room temperature. The previously indicated technique was used to create standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/ml). A UV/visible spectrophotometer was used to measure the absorbance of both solutions at 700 nm with the blank. The tannin content was assessed three times.

Assessment of anthelmintic action: [23,24]

With small modifications, the efficacy of *M. calabura* hydroalcoholic extract as anthelmintics against *Pheretima posthuma* was investigated in accordance with Neha Shekhawat et al. The paralysis and death periods of the worms were assessed using a bioassay at extract concentrations of 10, 25, and 50 mg/ml. The control was saline water, and the standard reference was albendazole. Adult Indian earthworms were used for the test. Before being employed for anthelmintic research, earthworms were thoroughly cleaned with normal saline after being removed from damp soil. The earthworms were divided into four groups of six each. Small amounts of the extract were mixed with water, and the volume was then adjusted to 20 millilitres using saline water. In petri dishes, extract and standard drug solutions were added. All of the earthworms were placed in a 20 ml solution that contained hydroalcoholic extract and albendazole in different concentrations. The duration required for individual worms to become paralysed and die was recorded. It was time for paralysis when there was no movement keep when the worms were agitated violently. When immersed in heated water (50^0C), the worms lost their ability to move, and their body colours started to fade, indicating their death.

3. Results

Organoleptic evaluation:

For the qualitative identification of the morphological and sensory profiles of plant bark and extract, it is an important parameter. The qualities, colour, taste, scent, size, and touch that are detailed in Tables 1 and 2 were discovered during the study.

Table 1: *M. calabura's* organoleptic characteristics

Attributes	**Bark from stems**
Colour	Brownish-gray
Smell	Characteristic
Taste	Agreeable

Size	Vari in sizes depend on age
Touch	Fibrous and rough

Table 2: Hydroalcoholic extract's organoleptic characteristics

Attributes	**Hydroalcoholic extract**
Colour	Brownish black
Smell	Characteristic
Consistency	Sticky
Yield	5 g

Physical evaluation:

Physical evaluation techniques are appropriately used in order to achieve the many objectives of the evaluation of crude medicinal products, namely to ascertain their identity, purity, and quality. Foreign matter analysis, drying loss, ash values, extractive values in different solvents, swelling index, and foaming index data are shown in Table 3.

Table 3: Physical Specifications

Attributes	**Values**
Analysis of foreign matters	1.02±0.4221% (W/W)
LOD	11.42±0.7831% (W/W)
Total amount of ash	8.18±0.1319% (W/W)
Index of Swelling	Not present
Index of Foaming	Below 100
Values of Extraction (w/w)	
Petroleum ether	0.111±0.1355%
Chloroform	0.497±0.4873%
Acetone	0.809±0.1987%
Methanol	3.100±0.6040%
Hydro-alcohol	4.909±0.1637%

Fluorescence analysis:

The powder sample was treated with a variety of chemicals in order to examine its fluorescence behaviour. Both visible and ultraviolet light are used to observe it; Table 4 summarises the findings.

Table 4: Analysis of fluorescence observations

Treatment	**Presence of visible light**	**Presence of UV light**
Powder	Brownish black	Yellow
Powdar +50% H_2 So_4	Dark brown	Yellow-green
Powder + Distilled Water	Brownish black	Yellowish green
Powdar + 50% HCl	Dark brown	Brown

Powdar+50% HNo_3	Dark brown	Brown
Powdar + Ethanol	Yellowish brown	Yellowish green
Powder +Chloroform	Yellowish brown	Greenish yellow
Powder + 1N NaOH (Alcoholic)	Golden brown	Yellow-green
Powder+ Acetic acid	Greenish brown	Greenish yellow
Powder+ Pet. ether	Greenish brown	Yellowish green
Powder + Methanol	Reddish brown	Yellowish green

When exposed to the aforementioned reagents under visible light, the hydroalcoholic extract of *M. calabura* bark displayed a distinctive luminous brown hue among the different chemical treatments. The characteristic bright yellow colour was produced under UV light by 50% H2So4, ethanol, distilled water, chloroform, 1N NaOH (alcohol), acetic acid, petroleum ether, and methanol (Table 4).

Testing for phytochemicals:

The bioactive components varied depending on the extract type and extraction method. Thus, the unique extract was subjected to phytochemical screening using the conventional procedure outlined in the methodology section. The results indicated that the hydroalcoholic extract had the highest concentration of secondary metabolites, as presented in Table 5.

Table 5: Screening for phytochemicals in *M. calabura*

Parameters	Hydroalcoholic extract
Carbo-hydrates	-
Proteins	+++
Amino-acids	-
Tannins & Phenolic Cpds	++
Glicosides	-
Alkaloids	-
Flavonoids	+++
Steroids	+++
Saponins	+

(Where + Slightly significant, ++ Moderately significant, +++ Highly significant, - Absent)

Quantitative determination of phytoconstituents:

Medicinal plants have been valued for their numerous pharmacological benefits since ancient times. This may be because they contain secondary plant metabolites including phenols, flavonoids, and tannins. Around the world, their diverse chemical and biological activities have assisted in the prevention and treatment of numerous human illnesses.

Table 6: Displays the findings of the quantitative analysis of tannins, flavonoids, and phenolic substances

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Extract 100 µg/ml	**Total phenolic content GAE mcg/ml**
Hydroalcoholic extract	21.55± 0.201
Extract 100 µg/ml	**Content of flavonoids Quercetin equivalent mcg/ml**
Hydroalcoholic extract	42.05± 0.065
Extract 100 µg/ml	**Tannin content-Tannic acid equivalent mcg/ml**
Hydroalcoholic extract	25.123± 0.123

N=3, values are given in SEM

Anthelmintic efficacy:

Table 7 shows that the extract of *M. calabura* exhibited dose-dependent anthelmintic activity, with a dosage of 50 mg/ml producing the shortest time for paralyzing (P) and death (D).

Table 7: *In vitro* anthelmintic property of bark extract from *M. calabura*

Group	**Concentration (mg/ml)**	**Paralysis (min)**	**Death (min)**
Normal	----------	----------	----------
Hydro alcoholic extract	10 25 50	90 65 38	119 97 65
Albendazole	10 25 50	77 58 32	108 85 55

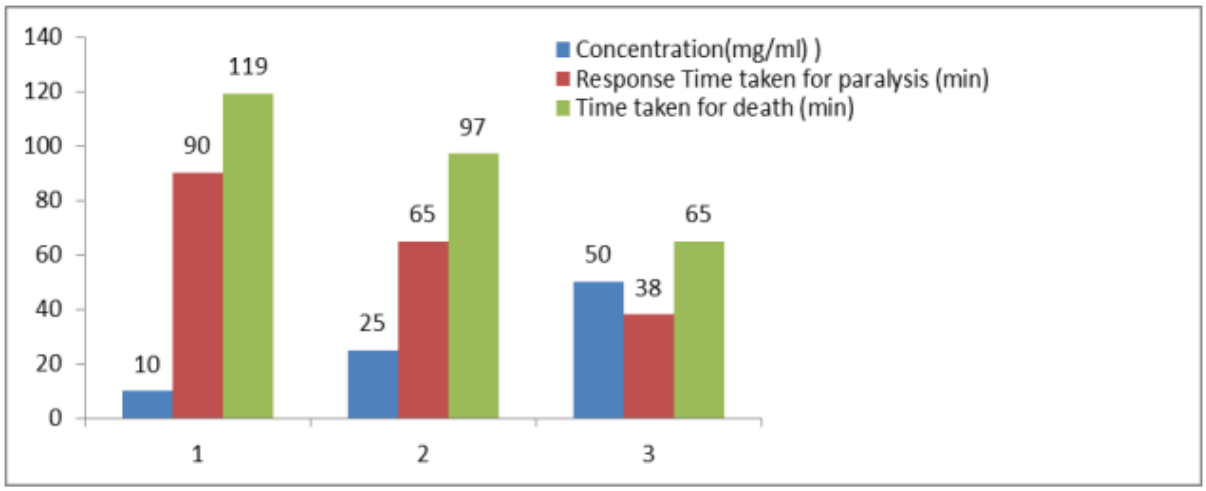

Fig. 2: Graphical representation of anthelmintic property of bark extract from *M. calabura*

3. Discussion

This study identified a number of phytoconstituents in the coarse powder of *M. calabura* plant bark using a hydroalcoholic solvent in the Soxhlet extraction process. The hydroalcoholic extract and bark's organoleptic properties were noted in Tables 1 and 2. To accomplish the several goals of evaluating crude drugs, such as determining their accurate source, the existence of the necessary quantity of active ingredients, and the absence of adulterants, physical assessment techniques are effectively employed. Because of fluorescence analysis's high sensitivity, components can be accurately identified. Without the need for many time-consuming dilution steps prior to doing further pharmaceutical sample analysis, it produces accurate results over a wide enough concentration range. Each type of substance has a unique bright colour. When treated with different chemicals and solvents, different plant material produces distinct colours.[25, 26]

The bark's hydroalcoholic extract, which is shown in Table 5, contained phytochemicals such as flavonoids, phenolic compounds, tannins, steroids, and saponins after the research using the previously mentioned methods. The hydroalcoholic extract underwent quantitative phytochemical analysis. The total amounts of phenolics, tannins, and flavonoids were presented in Table 6.

Tannins: Condensed tannins, in particular, have been found to inhibit the development and motility of various helminth stages. The worm may become immobile and die as a result of direct harm to its cuticle and hypodermis.

Phenolic Compounds: These compounds exhibit strong antioxidant properties, which can enhance the overall health of the host and indirectly contribute to the anthelmintic effect. They can also interfere with the metabolic processes of the parasites.

Flavonoids: Flavonoids, which are well-known for their anti-inflammatory and antioxidant qualities, can interfere with helminths' energy metabolism, reducing their viability and simplifying their removal. The current study indicated that a higher concentration of extract caused paralysis much early and shortened the time to death for all worms. At a concentration of 50 mg/ml, the hydroalcoholic extract showed the shortest time of paralysis (P) and death (D), indicating dose-dependent anthelmintic action. The anthelmintic activity was assessed using albendazole as a reference standard (Table 7).

These findings suggest that plant extracts rich in these compounds like phenolic compounds, flavonoids, and tannins could be a valuable alternative to synthetic anthelmintic drugs, offering a natural and potentially less harmful option for controlling parasitic infections.

4. Conclusion

Non-nutritive plant elements with disease-preventive or protective aspects are called active compounds of plants. Although it's commonly known that plants produce these substances to

protect themselves, recent research indicates that they can also protect humans and other living things from disease.

A hydroalcoholic extract of the bark of the *M. calabura* plant shows considerable anthelmintic activity when compared to a conventional drug, as per the previously described data. This extract's wormicidal action against earthworms indicates that it works well against human parasite infections. The traditional usage of this plant as an anthelmintic may be supported by the experimental data gathered in the lab model. The phytochemical profile of the plant may be investigated to identify the active ingredients liable for the anthelmintic action.

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Conflicts of Interest

The authors declare no conflicts of interest relevant to this article.

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