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ASSESSMENT OF PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF *MUNTINGIA CALABURA* FLOWERS

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ABSTRACT

Medicinal plants have been used as drugs since ancient times and have significant value in primary health care. This study aims to investigate *Muntingia calabura* (Jamaica Cherry), belonging to the family *Elaeocarpaceae*, to explore its potential medicinal properties. From previous studies, we found that traditional medicinal use has been reported from the leaves (for headaches, gastric ulcers, prostate problems), bark (antiseptic) and fruits (respiratory problems, anti-diarrhoea). The present study gives an overview of the flower (antiseptic, reduces swelling) and its antimicrobial activities were documented. The hydroalcoholic extract of flowers was prepared by the maceration technique, which consists of primary and secondary metabolites such as carbohydrates, alkaloids, flavonoids and tannins. The extract exhibited significant antimicrobial activity, which can be further explored against *E. coli* and *S. aureus* microorganisms. Based on this study, we conclude that the extract can serve as an alternative to a marketed preparation.

KEYWORDS

Muntingia calabura, Antimicrobial, Extraction, *E. coli* and *S. aureus.*

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INTRODUCTION

Medicinal plants have long been recognized as a rich source of bioactive compounds with therapeutic potential against a wide range of human diseases. With the growing limitations of synthetic drugs, including drug resistance, toxicity and high production costs, there is renewed global interest in plant-derived natural products as safe and effective alternatives[1].

Medicinal plants *Muntingia calabura L.*, commonly known as Jamaican cherry, Paanchara, or Kersen. Belonging to the family *Elaeocarpaceae*, *Muntingiaceae*, this plant is native to the Southern region of India, Southern Mexico, the Caribbean, Central America and Western South America, South to Bolivia and Argentina[2].

The *Muntingia calabura* is widely cultivated and the most common roadside tree. It is basically known for fast-growing fruit trees; within 2 years, its height reaches a maximum of 7 to 12m. Leaves are evergreen; they contain small flowers with 5 white petals and 5 green sepals, many prominent yellow stamens, and are slightly malodorous. Its fruit is an edible berry with smooth, thin skin. The flavour is fig-like, musky, and quite delicious[2].

The aim and objectives are to determine the morphological characters, preliminary phytochemical screening and antimicrobial activities using the hydro-alcoholic extract of *Muntingia calabura*.

Several researchers around the globe have isolated and identified the compounds of this plant as alkaloids, tannins, phenols and flavonoids. The therapeutic strategies of flavonoids are used for the development of effective future drugs against a variety of bacterial infections[2,3].

The flowers and bark have antiseptic activity and boiled barks can be used to reduce swelling in the lower extremities. The whole plant was used to treat melases, pimples, headaches, and stomach aches in Mexico, and used as a tranquillizer, antispasmodics and antidyspeptics in the Philippines and Colombia. Its leaves, bark, flowers, and roots have been used as a folk remedy for the treatment of fever, incipient cold, liver disease, as well as an antiseptic agent in Southeast Asia. The *Muntingia calabura* roots are effective as an antiproliferative agent, which caused apoptosis in the oral cancer cell line[4,5].

Plant Profile

Kingdom: Plantae
Subphylum: Euphyllophytina
Sub-class: Magnoliidae
Superorder: Rosanae
Order: Malvales
Family: Muntingiaceae
Genus: Muntingia
Species: Calabura

Traditional Uses

Muntingia calabura has recently gained limited traditional uses throughout the world, with records in particular Peru, Colombia, Mexico, Vietnam and the Philippines.

The flowers and barks have antiseptic activity and boiled barks can be used as a wash to reduce swelling in the lower extremities.

The whole plant was used to treat melases, pimples, and stomach ache in Mexico and is used to treat headaches and incipient cold or tranquillizers, antispasmodics and antidyspeptics in the Philippines and Colombia.

All parts of this plant (the leaves, bark, flowers and roots) are used in traditional medicine. People use them to treat fever, early cold symptoms, liver problems and as a natural antiseptic agent in Southeast Asia[2].

MATERIAL AND METHODS

Plant material

Muntingia calabura flowers were collected in April and May from Kamptee, Nagpur. Flower specimens were identified and authenticated at the Department of Botany at RTM Nagpur University, Nagpur 440033. Samples were shade-dried for two weeks before pulverization.

Preparation of extract

Flowers were dried in the shade, pulverized into coarse powder, and about 150g of powder was extracted using ethanol by the maceration technique. The hydro alcoholic extract so obtained was then filtered and evaporated on a water bath to dryness, and the % yield was calculated and stored in a clean container till further use[6].

Preliminary phytochemical screening

The hydro alcoholic extract of *Muntingia calabura* was subjected to phytochemical screening to detect the presence of primary and secondary metabolites like proteins, amino acids, fats and oils, carbohydrates, alkaloids, glycosides, flavonoids,

tannins, terpenoids, steroids, gums and mucilage, etc[7].

Tests for Carbohydrates

Molisch's test (General test)

To 2mL of test solution, a few drops of alcoholic solution of α-Naphthol were added. The mixture was shaken well, and a few mL of concentrated sulphuric acid was added slowly along the sides of the test tube. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

Tests for reducing sugars

Benedict's test

Equal volumes of test solution and Benedict's reagent were mixed. The mixture was heated on a boiling water bath for 5 minutes. Solution appears green, yellow, or red depending on the amount of reducing sugar present in the test solution.

Fehling's Test

1mL of Fehling's A solution and 1mL of Fehling's B solution were mixed, boiled for 1 minute and an equal amount of test solution was added to it. The reaction mixture was heated in a boiling water bath for 5-10 minutes. First yellow, then red precipitate of cuprous oxide indicates the presence of sugar.

Test for monosaccharides

Barfoed's test

Equal volumes of test solution and Barfoed's reagent were mixed, and it was then heated for 1-2 minutes in a boiling water bath and cooled. Formation of red ppt. indicates the presence of monosaccharides.

Iodine Test

0.5mL of Iodine solution was mixed with 1ml of the test solution. Formation of deep blue colour indicates the presence of starch. First yellow, then red precipitate of cuprous oxide indicates the presence of sugar.

Tests for Proteins

Biuret test

To 3mL of the test solution, 2mL of Biuret reagent was added; violet or pink colour indicates the presence of proteins.

Millon's test

To 3mL of the test solution, 5mL of Millon's reagent was added. A white precipitate, which on warming turns brick red, indicates the presence of proteins.

Tests for Amino acids

Ninhydrin Test: Two drops of ninhydrin solution were added to 2mL of test solution. The appearance of purple color indicates the presence of amino acids.

Tests for Fixed Oils and Fats

Spot test

A small quantity of powder was pressed between two filter papers. An oil stain on the paper indicates the presence of fixed oils.

Tests for Alkaloids

Mayer's test

To 2-3mL of test solution, a few drops of Mayer's reagent were added. The appearance of white creamy precipitate indicates the presence of alkaloids.

Wagner's test

A few drops of Wagner's reagent were added to a few mL of the test solution. A reddish- brown precipitate shows alkaloids are present.

Dragendorff's Test

To a 2-3mL test solution, a few drops of Dragendorff's reagent were added. Formation of reddish-brown precipitate shows alkaloids are present.

Hager's Test

To 2-3mL test solution, a few drops of Hager's reagent were added. Formation of yellow-orange precipitate indicates the presence of alkaloids.

Tests for Glycosides

Anthraquinone glycoside

Borntrager's Test

To 3mL of test solution, add a few mL of dil. sulphuric acid was added and boiled for a few minutes, filtered and cooled. To it, 3mL of chloroform was added and shaken; the chloroform layer was separated and 10% ammonia solution was added to it. Appearance of pink or red colour in the ammoniacal layer indicates the presence of anthraquinone glycosides.

Modified Borntrager's test

5mL of test solution was boiled with 5mL of dilute HCL and 5% aqueous ferric chloride solution for

about 5 minutes in a boiling water bath. It was then allowed to cool and then shaken with an equal volume of chloroform. The chloroform layer was separated and a 10% ammonia solution was added to it. Appearance of pink or red colour indicates the presence of C-type anthraquinone glycosides.

Cardiac glycosides

Keller-Killiani test (for deoxy-sugars)

The test solution was shaken with chloroform and evaporated to dryness. 0.4mL of glacial acetic acid containing a trace amount of ferric chloride was added and the contents were then transferred to a small test tube, carefully. 0.5mL of concentrated sulphuric acid was added to the side of the test tube. A reddish-brown colour appears at the junction of the two liquid layers, and the upper layer appears bluish green.

Legal's test

The test solution was treated with 1mL of pyridine and 1mL of sodium nitroprusside solution. Appearance of pink or blood red colour indicates the presence of a cardiac glycoside.

Baljet's test

The test solution was treated with picric acid or sodium picrate; the formation of an orange colour indicates the presence of a cardiac glycoside.

Tests for Phenols and Tannins

Ferric chloride test

2-3mL of the test solution was treated with a few drops of ferric chloride solution. The appearance of blue colour indicates the presence of hydrolysable tannins, and the appearance of green colour indicates the presence of condensed tannins.

Lead acetate Test

To 2-3mL of aqueous test solution, a few drops of lead acetate solution were added; the formation of white precipitate indicates the presence of tannins.

Potassium dichromate test

To the 2-3mL of aqueous test solution, a few drops of Potassium dichromate solution were added. The appearance of red precipitate indicates the presence of tannin.

Tests for Steroids

Salkowski test

To 2mL of test solution, 2mL of chloroform, and 2mL of conc. sulphuric acid was added and shaken well. The chloroform layer shows red colour and the acid layer shows greenish yellow fluorescence.

Tests for Flavonoids

Shinoda test

To the test solution, a few magnesium turnings and concentrated hydrochloric acid were added; pink scarlet, crimson red, or occasionally green to blue colour appears after a few minutes, which indicates the presence of flavonoids.

Alkaline reagent test

To the test solution, a few drops of sodium hydroxide solution were added, and an intense yellow colour was formed, which turned colourless on addition of a few drops of dilute acid, showing the presence of flavonoids.

Lead acetate Test

To the test solution, a few drops of lead acetate solution were added. Yellow precipitate indicates the presence of flavonoids.

Organoleptic evaluation

The macroscopic characters are useful in the quick identification of plant material and also serve as an important standardization parameter. Fresh flowers of *Muntingia calabura* and the alcoholic extract were evaluated for organoleptic characteristics like color, odour, size, shape, and texture[6,7].

Thin-layer chromatography

The hydro alcoholic extract of *M. calabura* flowers was subjected to thin-layer chromatographic techniques. The readymade plates of TLC Silica gel were used. The hydro alcoholic extract of *M. calabura* flowers was separately applied on the various plates, and the chromatograms were developed in a saturated air-tight chromatographic chamber containing different ratios of various solvents. After development of the chromatogram, the plates were dried at room temperature, and the detection was mainly carried out using a UV radiation chamber and an iodine chamber. The colours of the spots and Rf were recorded[8,9].

Antimicrobial Activity of Plant Extract

In order to scientifically verify the traditional uses of the plant species for treating various diseases, their antimicrobial activity was investigated. Plant extracts were screened for *in vitro* antimicrobial activity against two microorganisms, *Staphylococcus aureus* and *Escherichia coli*. Cultures of these bacteria were grown in nutrient broth at 37ºC[10].

Preparation of plant extract for antimicrobial activity

Dried flower extracts obtained by maceration methods of *Muntingia calabura* plant flowers were dissolved in a minimum quantity of distilled water and collected in sterile airtight wells of 10ml. These were used for the determination of the zone of inhibition against different bacteria used in the study. Hydro alcoholic extract was taken and a stock solution was prepared, and different dilutions were prepared according to concentrations of 1mg, 2mg and 3mg, respectively[6].

Preparation of Inoculums

Inoculums of *Staphylococcus aureus* and *Escherichia coli* were prepared in nutrient broth medium and incubated at 37 ℃ for 24 hours[11,12].

Preparation of Nutrient Agar Medium

28g of nutrient Agar was suspended in 1000 mL of distilled water, and the pH was adjusted to (7.3 ± 0.2). The agar was boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lb/sq. inch pressure (121ºC) for 15 minutes and mixed well before pouring[11,12].

Preparation of Petri plates

The melted agar was poured into previously sterile petri plates immediately after it was taken out of the autoclave to prevent it from solidifying, and allowed to settle down. A bacterial lawn was spread on agar plates using a sterile glass loop[11,12].

Antibacterial activity testing of flower extract

The sensitivity of the test organisms against hydro alcoholic extract was tested by using the well diffusion method. A lawn of both cultures of the test organism was prepared by the spread plate technique using a glass loop separately over a nutrient agar plate. Wells were prepared on an agar plate with the help of a 6 mm borer. Solutions of different concentrations of test extract as well as reference drug were then put in each well and incubated at 37℃ overnight. The sensitive microbes grew everywhere except in areas around the well in the medium. Then the resulting inhibition zones were measured by zone measurement scale in mm[10-12].

RESULTS AND DISCUSSION

The *Muntingia calabura* flower is white, odourless, slightly bitter in taste, with a smooth texture and a roughly square outline. This gives a simple morphological profile, but further qualitative or quantitative analysis would help confirm its identity.

The hydro-alcoholic extract of *Muntingia calabura* flowers appeared dark brown in colour with a strong pungent odour and had a pH of 4.7. Its consistency was slightly sticky and the extract yield was 11.47 %.

Table No.3 -ve indicates absence of phytoconstituents, +ve indicates presence of phytoconstituents. The hydro alcoholic extract showed the presence of Carbohydrates, Alkaloids, Flavonoids, and Tannins. While proteins, saponins, glycosides and steroids were not detected. This indicates that the extract is rich in flavonoids and alkaloids, which may contribute to its biological activities, especially antioxidant or antimicrobial effects.

The hydro alcoholic extract showed the separation of one compound in ethyl acetate: methanol (8:2) solvent system with Rf value 0.41.

The hydro alcoholic extract showed the separation of one compound in ethyl acetate (10 ml) solvent system with Rf values 0.30.

The hydro alcoholic extract of *Muntingia calabura* flowers showed significant antibacterial activity against *E. coli* and *S. aureus* microorganisms. The activity was found to increase with an increasing concentration. The extract showed maximum inhibitory effect at 3mg/ml concentration against microorganisms, which is comparable with the standard antibiotic drug Cefixime trihydrate.

Table No.1: Macroscopic characters of *Muntingia calabura* Flower

S.No	Characters	Observations
1	Colour	White
2	Odor	Odourless
3	Taste	Slightly Bitter
4	Texture	Smooth
5	Shape	Square

Table No.2: Organoleptic characteristics of the hydro alcoholic extract of *Muntingia calabura* flowers

S.No	Characteristics	Observations
1	Colour	Dark Brown
2	Odour	Strong Pungent
3	pH	4.7
4	Consistency	Slightly sticky
5	% Yield	11.47%

Table No.3: Phytochemical screening of hydro alcoholic extract of *Muntingia calabura* flowers

S.No	Phytochemical test	Hydro alcoholic extract
1	Carbohydrates Test	+
2	Protein and Amino acid	-
3	Saponins Test	-
4	Alkaloid Test	++
5	Glycosides Test	-
6	Steroid Test	-
7	Flavonoid Test	+++
8	Tannins and Phenol Test	++

Table No.4: TLC of hydro alcoholic extract of *Muntingia calabura* flowers

S.No	Extract	Solvent System	Ratio	No. of Spot	Rf values
1	Hydro alcoholic	Ethyl acetate: Methanol	8:2	1	0.41
2	Hydro alcoholic	Ethyl acetate	10ml	1	0.30

Table No.5: Antimicrobial Activity of hydro alcoholic extract of *Muntingia calabura* flowers

S.No	Compound	Concentration	Zone of Inhibition (mm)	
			Test organisms	
			S. aureus	***E. coli***
1	Solvent	70µg/ml	-	-
2	Antibiotic	Cefixime trihydrate (10mg)	47	19
3	Hydro alcoholic extract	1mg	8	9
		2mg	10	12
		3mg	14	15

Figure No.1: *Muntingia calabura* Flower

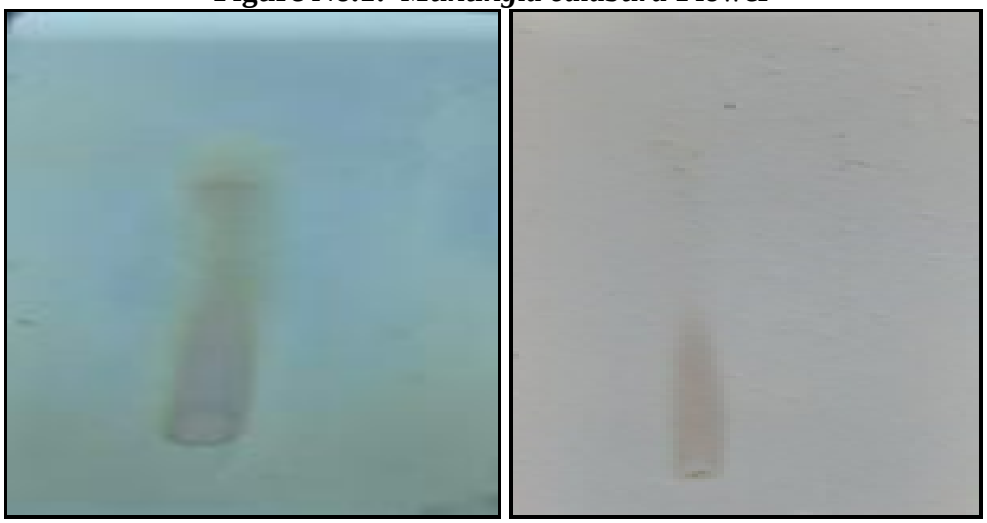

Figure No.2: a. Ethyl acetate: Methanol b. Ethyl acetate

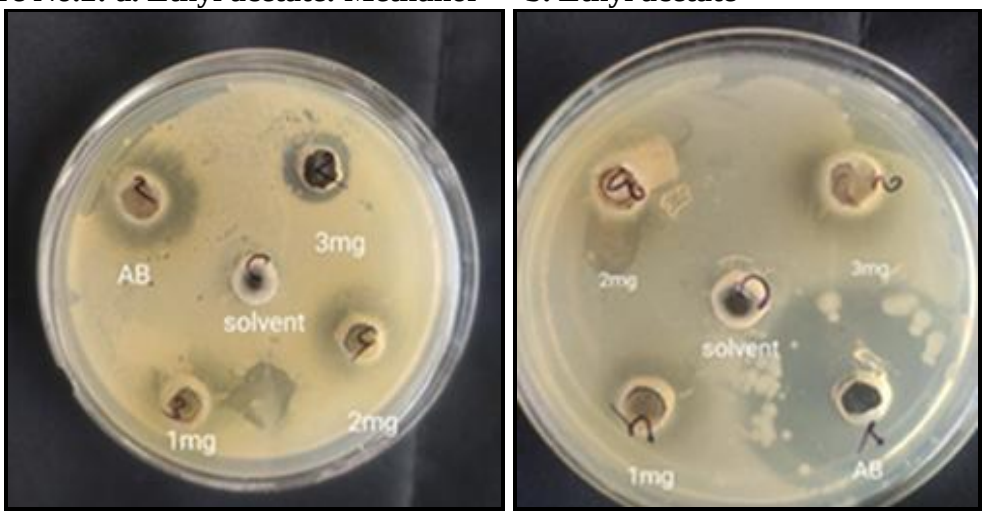

Figure No.3: a. Hydroalcoholic extract (*S. aureus*) b. Hydroalcoholic extract (*E. coli*)

CONCLUSION AND FUTURE SCOPE

The hydro alcoholic extract of *Muntingia calabura* flowers was found to contain primary and secondary metabolites like carbohydrates, alkaloids, flavonoids and tannins. The extract exhibited significant antibacterial activity, wh
ch can be further explored against many more pathogenic microorganisms. In the future, the extract can be suitably formulated as a potent antimicrobial agent. The chemical constituents can be isolated, identified, and characterised.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Nasim N, Sandeep I S, Mohanty S. Plant-derived natural products for drug discovery: Current approaches and prospects, *Nucleus (Calcutta),* 65(3), 2022, 399-411.
2. Upadhye M, Pujari R, Kadam S, Gunjal P. *Muntingia calabura*: A comprehensive review, *J Pharm Biol Sci,* 9(2), 2021, 81-87.
3. Lwin H H. Investigation of morphological and histological characters, preliminary phytochemical examination and antimicrobial activity on leaves of *M. calabura, J Myanmar Acad Arts Sci,* 19(4), 2021, 1-10.
4. Mahmood N D, Salleh M Z, Zakaria Z A. *Muntingia calabura*: A review of its traditional uses, chemical properties and pharmacological observations, *Pharm Biol,* 52(12), 2014, 1598-1623.
5. Kumaran C, Dinesha R, Santhosh Kumar N. Apoptosis inducing activity of proteins isolated from *Muntingia Calabura* plant root on oral cancer cell line: An *in vitro* study, *Saudi J Pathol Microbiol,* 4(12), 2019, 875-877.
6. Kokate C K, Purohit A P, Gokhale S B. Pharmacognosy, *Nirali Prakashan, Pune,* 40th Edition, 2007.
7. Khandelwal K R. Practical Pharmacognosy-techniques and experiments, *Nirali Prakashan,* 19th Edition, 2011, 149-150.
8. Evans W C. Trease and Evans' Pharmacognosy, *Elsevier Health Sciences,* 16th Edition 2009, 116-118.
9. Sethi P D, Charegaonkar D. Identification of drugs in pharmaceutical formulations by thin layer chromatography, *CBS Publishers, New Delhi,* 2nd Edition, 1996, 95-97.
10. Buhian W P C, Rubio R O, Martin-Puzon J J. Chromatographic fingerprinting and free-radical scavenging activity of ethanol extracts of *Muntingia calabura L*. leaves and stems, *Asian Pac J Trop Biomed,* 7(2), 2017, 139-143.
11. Amalia A R, Setiyanto R, Nurrahman A, Utami A R, Sari D W. Antibacterial test of cherry leaves ethanol extract (*Muntingia calabura L*.) against streptococcus mutans, *Crown J Dent Health Res,* 2(1), 2024, 80-86.
12. Hassan Cheong N D, Amran M M, Yusof H. Phytochemical investigation and antimicrobial activity of *Muntingia calabura L*. against selected pathogens, *Malaysian J Med Health Sci,* 18(S15), 2022, 301-307.

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