



## SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF PLANT *MUNTINGIA CALABURA*

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### ABSTRACT

The present study includes the extraction and screening of various active chemical constituents' whole plant of *Muntingia calabura* that exhibit anti-microbial activity. The condensate obtained was subjected to preliminary phytochemical screening and revealed the presence of various phyto- constituents like, alkaloids, carbohydrates, steroids, glycosides, flavonoids, tannins, gums and mucilage; sterols. The antimicrobial activity of the ethanol extract of whole plant of *Muntingia calabura* was studied against both gram positive (*Staphylococcus*) and Gram negative (*Escherichia coli*) organism and fungus (*Rhizopus stolonifera*) at 100mg concentration and the antibacterial activity was compared with that of the standard drugs Ampicillin, Tetracycline and Nystatin at 100 microgram concentration.

## INTRODUCTION

The 17th-century discovery of living forms existing invisible to the naked eye was a significant milestone in the history of science, for from the 13th century onward it had been postulated that "invisible" entities were responsible for decay and disease. A **microorganism** or **microbe** is an organism of microscopic size which may exist in its single-celled form or as a colony of cells. Medicinal microbiology is the study of microbes that infect the humans, the diseases they cause, and their diagnosis, prevention and treatment. It also deals with the response of the human host to microbial and other antigens. Microorganisms are beneficial in producing oxygen, decomposing organic material, providing nutrients for plants, and maintaining human health, but some can be pathogenic and cause diseases in plants and humans.

**Classification of Microorganisms:** The major groups of microorganisms namely

bacteria, archaea, fungi (yeasts and molds), algae, protozoa, and viruses are summarized below. Links to the more detailed articles on each of the major groups are provided<sup>[2]</sup>

**Useful Microorganism:** These are mainly used in the baking industry for the preparation of cakes, bread, pastry, etc. Mainly used in the production of milk products. Example: In the formation of curd from the milk, lactobacillus bacteria is used. These are used in the production of alcohol. This is used in the preparation of organic acids such as citric acid, lactic acid, fumaric acid, gluconic acids. Steroids are prepared by using microorganisms. Vitamins are also produced with the microorganisms such as Vitamin B complex or Riboflavin by *Ashbya gossypii*, *Eremothecium ashbyii* and *Clostridium butyricum*. A known Vitamin C or Ascorbic acid is produced by species *Acetobacter*. Enzymes such as lipase, lactase, amylase, pectinase, penicillinase are synthesized by

microorganisms. The soil fertility is increased by the microorganisms and by fixing nitrogen. Used in pest control.

### **Harmful Microorganisms<sup>[3]</sup>**

Microorganisms grow on food and spoil it. Some pathogens are transmitted directly from an infected person. Through air, water and food, the pathogens can enter our bodies easily. Pathogens cause diseases in living beings

### **ANTIMICROBIAL ACTIVITY**

After an exhaustive literature survey is carried out on the plant *Muntingia calabura*, it is understood that the various parts of the plant are in use to cure many diseases. It is also understood that many important phytoconstituents are present in the plant. If such important phytoconstituents are isolated and their pharmacological activities are trapped, those can be used in the treatment of various diseases<sup>[4]</sup>. Plants are the major source of therapeutic agents in any traditional system of medicine. Several chemotherapeutic agents have been developed in the allopathic system of medicine as a result of screening the medicinal plants used in various parts of the world. The present work has been aimed to screen and evaluate different phytoconstituents present in the various extracts of plant. The anti-microbial activity for the extract of the plant is also aimed.

### **The objectives of the study include**

Extraction of the whole plant powder by solvent extraction using Soxhlet apparatus.

Preliminary phytoconstituent screening to detect the presence of various compounds.

Determination of anti microbial activity by filter paper disc method by preparation of suitable stock culture medium.

Determination of zone of inhibition of the plant extracts in both gram negative and gram positive bacterial species of *Staphylococcus*, *E. coli* and *Rhizopus* fungus species.

### **Materials and methods:**

#### **Collection And Authentication Of The Plant Material**

The whole plant of *Muntingia calabura* was collected in the month of March 2022 from forest area of HMT Medchal-malkajgiri district. It was shade dried away from sunlight and stored suitably.

### **Extraction**

The whole plant of *Muntingia calabura* was powdered with a mechanical grinder and passed through Sieve no. 40 which was stored in an air tight container for further use. Powdered calabura whole plant of *Muntingia calabura* was extracted with ethanol, water and methanol by continuous soxhlet extraction method. The solvent was removed by rotary vacuum evaporator, the remaining mass of extract was concentrated and dried. The extract was stored in desiccator for further phytochemical studies<sup>[5]</sup>.

### **Preliminary Phytochemical Screening (Kokate et al., 2006)**

The whole plant extract of *Muntingia calabura* was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as carbohydrates, proteins, amino acids, steroids, tannins, flavonoids, alkaloids, saponins, mucilage, glycosides.

### **Collection and authentication**

The whole plant of *Muntingia calabura* was collected in the month of March 2022 from forest area of HMT Medchal-malkajgiri district. It was shade dried away from sunlight and stored suitably. It was authenticated by botanist

### **Preparation of stock culture (Ashok Rathan, 2000)**

From the cultures, which were maintained on nutrient agar slants, one loopful of the respective organisms were taken and aseptically transferred to 100ml of sterile nutrient broth in a flask, which was shaken thoroughly and incubated at 37 ° C for 24hrs<sup>[6]</sup>.

### **Standardization of stock culture<sup>[7]</sup>**

1ml of this seeded broth was then diluted with 9ml of sterile water in a culture tube. This was shaken thoroughly and about 1ml of this suspension was transferred to a second culture tube which in addition contains 9ml of sterile water. This was shaken thoroughly and thus was further diluted 10 times with sterile water till 10 :10 dilution was obtained (up to 10 culture tubes)<sup>[8]</sup>. Standardization of the seeded broth was done by inoculating 0.2ml of each dilution on solidified nutrient agar medium by spread plate method. After incubation at 37 ° C for 48 hours, the number of well-formed

colonies on the plates was counted. The seeded broth was then suitably diluted to contain between 10<sup>7</sup>-10<sup>8</sup> microorganism

#### **Procedure (Ashok Rathan, 2000)<sup>[9,10]</sup>**

Antibacterial activity of methanol extract was screened by filter paper disc method. A previously liquefied medium, appropriate for the test is inoculated with the requisite quantity of the suspension of the microorganism, the suspension was added to the medium at a temperature between 40° -50° C and the inoculated medium was poured immediately into dried Petri dishes to occupy a depth of 3 to 4 mm.

The paper disc (No.2 Whatmann) was cut down into small disc (6mm diameter) and sterilized at 180 ° C/30' min in hot air oven impregnated with the test solution and the standard solution.

#### **RESULTS AND DISCUSSION:**

##### **Phytoconstituent screening**

##### **Anti-microbial screening**

The antimicrobial activity of the ethanol extract of whole plant of *Muntingia calabura* was studied against both gram positive (*Staphylococcus*) and Gram negative (*Escherichia coli*) organism and fungus (*Rhizopus stolonifera*) at 100mg concentration and the antibacterial activity was compared with that of the standard drugs Ampicillin, Tetracycline and Nystatin at 100 microgram concentration. The results showed that the ethanol extract of whole plant of *Muntingia calabura* at 100mg concentrations exhibited a significant antibacterial activity against both Gram positive, gram negative organisms and anti-fungal activity against fungus. It has exhibited a more significant antimicrobial activity than that of standards Ampicillin, Tetracycline and Nystatin. The figure shows that the ethanol extracts of *Muntingia calabura* at 100mg concentration exhibited a significant antimicrobial activity against both Gram

c.f.u./ml (colony forming unit per ml). This was designated as the working stock that was used for antibacterial studies

positive, gram negative and fungus. It has exhibited a significant antimicrobial activity comparing with that of standards Ampicillin, Tetracycline and Nystatin. The whole plant extract of *Muntingia calabura* was tested for antimicrobial study using aqueous, methanol, ethanol extracts. Among these three extracts ethanol extract is more active than othersolvents like aqueous and methanol.

#### **Antimicrobial activity of the ethanol extract of whole plant of *Muntingia calabura***



**Antimicrobial activity of the ethanol extract of whole plant of *Muntingia calabura***

Name of the organism	Diameter of Zone of Inhibition in mm produced by ethanol extract
Gram positive bacteria	4
Gram negative bacteria	3
Fungus	3.5

S.NO	Chemical tests	Observation	Aqueous extract	Methanolic extract	Ethanol extract
1	Alkaloids Dragendorff's Test Mayer's test Hager's test Wagner's test	Reddishbrown precipitate White precipitate Yellow precipitate Reddish brown precipitate	+	+	+

2	Carbohydrates Molisch's test Fehling's test Barfoed's test Benedict's Test	Violet color ring BrickRed precipitate Orange color precipitate Yellow/Red color	-	+	+
3	Proteins Biuret Test(General) Millon's test Xanthoprotic Test	Violet /pink color Red color precipitate Orange color	-	-	+
4	Amino Acids Ninhydrin test	Purple or bluish color	+	-	-
5	Steroids Salkowski reaction Liebermann-Burchard reaction Liebermann's Reaction	Yellow Flourescence Green Color Blue Color	-	+	+
6	Glycosides Cardiac glycosides Baljset's test Legal's test Saponin glycosides Foam test Hemolytic test	Yellow or orange color Pink Red Color Foam Observed Hemolytic zone appears	+	+	+
7	Flavonoids Shinoda test	Pink color	+	+	+
8	Tannins Test 1 & Test 2	White Precipitate White Precipitate	+	.	+
9	For Gums & Mucilage	Swelling observed	+	-	+
10	For Sterols	Pink color	+	+	+

### INFERENCE:

Preliminary phytochemical screening of *Muntingia calabura* was carried out with different solvents and the data are represented in Table. The preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, tannins, gums and mucilage; sterols were shown in ethanol extract.

### CONCLUSION:

The present study includes the extraction and screening of various active chemical constituents' whole plant of *Muntingia calabura* that exhibit anti-microbial activity. The dry powder was subjected to extraction using ethanol as solvent by soxhlation. The condensate obtained was subjected to preliminary phytochemical screening and revealed the presence of various phyto-

constituents like, alkaloids, carbohydrates, steroids, glycosides, flavonoids, tannins, gums and mucilage; sterols which is evidence for its reported pharmacological activities.

Based on the ethnomedical claims of the plant *Muntingia calabura* which has reported to have beneficial uses in treating various diseases like inflammation, hyperuricemia, hepatic damage, diabetes, ulcer, and also cancer. The traditional folklore uses of the plant reported to have disinfectant property, tranquilizer, anti-spasmodic, anti-septic based on its anti-oxidant profile. Further, the extract was subjected to anti-microbial activity screening using Ampicillin, Tetracycline and Nystatin as standards. Hence the above study reported to have a tremendous anti-microbial potential

by determining the inhibitory zone of culture mediums of both gram +ve and gram -ve bacterial strains of *Staphylococcus*, *E. coli* and fungus *Rhizopus stolonifera* group using ampicillin, tetracycline and nystatin as standard drugs. The procedure followed was filter paper disc method. The results show that the ethanol extract of whole plant of *Muntingia calabura* at 100mg concentrations exhibited a significant antimicrobial activity against various organisms. It has exhibited more significant antimicrobial activity than that of standards Ampicillin, Tetracycline and Nystatin. This would be an ideal model for understanding the underlying mechanism involved in the pharmacological activity of the plant extract and the possible role of oxidative stress and ROS in the pathogenesis of various diseases and ailments. This antimicrobial model may reflect the appropriate choice to understand pathogenesis, diagnosis, prevention and even treatment of various diseases in the future studies as it is less time consuming and in-expensive method. Hence, the study concludes that the potential use of medicinal plants in treating various diseases have been gained a remarkable importance and advantage due to its limited adverse effects and desired therapeutic effects

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