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Research Article

**IN VITRO ANALYSIS OF SECONDARY METABOLITES FROM  
MUNTINGIA CALABURA CHLOROFORM EXTRACT.**

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**Abstract:**

*In recent times, focus on medicinal plants research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. For many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Therefore, research has developed into investigating the potential properties and uses of terrestrial plant extracts for the preparation of potential drugs for treating human cancers. Many plant species are already being used to treat or prevent development of cancer. Multiple researchers have identified species of plants that have demonstrated anticancer properties with a lot of focus on those that have been used in herbal medicine in developing countries. Hence, the present study envisage that the presence of Phytochemicals from the chloroform extract of Muntingia calabura.*

**Key words:** *Muntingia calabura, Plant based treatments, Developing countries, Chemotherapy, Side effects.*

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**INTRODUCTION:**

Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterised by cells in the human body continually multiplying with the inability to be controlled or stopped. Consequently, forming tumours of malignant cells with the potential to be metastatic. Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer [1]. Chemically-derived drugs have been developed and other cancer treatments pre-exist. However, current methods such as chemotherapy have their limitations due to their toxic effects on non-targeted tissues furthering human health problems. Therefore, there is a demand for alternative treatments with naturally-derived anticancer agents with plants being the desired source [2]. *Muntingia calabura* L. belongs to the family of *Elaeocarpaceae* and is a small, evergreen tree growing in tropical regions of Asia. The plant has been reported to possess antiproliferative, antioxidant, antinociceptive, cardioprotective and antipyretic effects. A total of 42 volatile compounds has been identified in the vacuum distillation extract of ripe fruits. Various parts of this plant contains flavonones, flavones, flavans and biflavans which exhibited cytotoxic effects [3]. The determination of biologically and pharmacologically active compounds from plants and their pharmaceutical potential for human use is necessary to challenge the life threatening diseases like human malignancies. Therefore, it is of interest to investigate the phytochemical or secondary metabolite analysis of various extracts of *Muntingia calabura* was undertaken.

**MATERIALS AND METHODS:****Collection of samples**

The medicinal plants used for the experiment were aerial parts of the plant *Muntingia calabura* were used for this study..

**Preparation of extracts**

250 grams of dried powder of the aerial parts of the plant material was packed in separate round bottom flask for sample extraction using different solvents namely ethanol, methanol, chloroform, ethyl acetate and water. The extraction was conducted with 750 ml of each solvent for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

**Phytochemical analysis**

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature [4-8].

**Test for alkaloids**

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acid. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragendorff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

**Test for saponins**

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

**Test for tannins**

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

**Test for steroids**

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**Test for flavanoids**

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavanoides and orange colour for flavanoids.

**Test for anthraquinones**

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

**Test for cardiac glycosides**

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardiac glycosides.

**Test for Proteins**

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO<sub>4</sub> solution was added. A violet color indicated the presence of peptide linkage of the molecule.

**Test for Amino Acids**

To 2ml of sample was added to 2ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

**Test for Tri-Terpenoids**

5ml of each extract was added to 2ml of chloroform and 3ml of con. H<sub>2</sub>SO<sub>4</sub> to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

**Test for Triple Sugar**

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of con. of con. H<sub>2</sub>SO<sub>4</sub> was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

**Test for Polyphenols**

To 2ml of sample was added to 2ml of ferric chloride solution and kept in the room temperature. Appearance of violet color indicated the presence of phenolic compounds in the sample.

**RESULTS AND DISCUSSION:****Table 1: Preliminary phytochemical constituents of *Muntingia calabura*.**

S. no	Phytochemicals	Chloroform extract	Aqueous extract	Ethyl acetate extract
1.	Flavanoids	++	++	++
2.	Alkaloids	++	++	++
3.	Saponins	++	++	++
4.	Tannins	--	++	++
5.	Amino acids	--	--	--
6.	Proteins	--	--	--
7.	Terpenoids	--	--	--
8.	Reducing sugars	++	--	++
9.	Cardiac glycosides	++	++	++
10.	Anthroquinones	++	--	++
11.	Steroids	--	--	--
12.	Poly phenols	--	--	--

“++” - Positive, “--” - Negative.

Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ. A wide range of medicinal plants parts are used as raw drugs as they possess varied medicinal properties. Bioactive substances from plant origin could be employed in the formulation of antimicrobial agents for the treatment of various bacterial and mycotic infections [9]. The present study aims to focus the presence of Phytochemicals in the chloroform extract of *Muntingia calabura* contains

alkaloids, flavanoids, saponins, triple sugars, anthroquinones and cardiac glycosides. Ethylacetate extract contains cardiac glycosides, triple sugars, anthroquinones, tannins, saponins, alkaloids and flavanoids. In aqueous extract of plant extract possess alkaloids, flavanoids, saponins, tannins and cardiac glycosides. A strong antibacterial activity of the plant was observed against *M. luteus*, causative agent of bloodstream infections followed by *P. aeruginosa* responsible for nosocomial infections. Significant activity was observed with *B. cereus*. Effective antistaphylococcal activity of methanolic extract of this plant was reported [10].

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