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Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from *Muntingia calabura* L. leaves and stems

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ABSTRACT

Objective: To determine the bioactive phytochemicals and antimicrobial activity of leaf and stem ethanolic extracts from *Muntingia calabura* L. (*M. calabura*).

Methods: Dried leaves and stems of *M. calabura* were extracted with 95% ethanol. The antibacterial and antifungal activities of the extracts were examined using the disc diffusion assay. The minimum inhibitory concentration (MIC) of each extract showing antimicrobial activity was determined. The dried extracts were subjected to phytochemical screening to determine the presence of bioactive components. Total phenolic and flavonoid contents were also determined by the Folin–Ciocalteu method and the aluminum chloride method, respectively.

Results: Varying degrees of antimicrobial activity were exhibited by the leaf and stem extracts against *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium*, *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis*, and *Candida albicans* (*C. albicans*), with minimal activity against *Escherichia coli*. Based on the MIC, the extracts showed the highest activity against *C. albicans*, *S. aureus* and *P. aeruginosa*. Phytochemical screening revealed the presence of sterols, flavonoids, alkaloids, saponins, glycosides and tannins in the leaf extract; however, no triterpenes were detected. In the stem extract, triterpenes were detected along with relative amounts of flavonoids, saponins, glycosides and tannins. Alkaloids and sterols were absent in the stem extract.

Conclusions: *M. calabura* leaf and stem ethanol extracts are potential sources of antibacterial agents against *P. aeruginosa* and *S. aureus*. This study reports for the first time the high degree of antifungal activity of *M. calabura* ethanolic extract, especially against *C. albicans*.

1. Introduction

Traditional medicine encompasses multiple indigenous traditions around the world. In Western and other developed nations, its usage is often in conjunction with, and complementary to, modern medicine. As a testament to its persistence, over 80% of the world's population is known to still utilize traditional medicine for primary healthcare. Most are in underdeveloped countries, many of which are situated in biodiversity hotspots in Southeast Asia, Africa, Central and Southern America among others. Plants are the single largest source for traditional medicines [1]. They account for over 25% of new drugs tested for clinical use [2]. Newman and Cragg noted that in the field of cancer research, almost 80% of new chemical entities discovered were derived from natural sources, or semi-synthetic modifications thereof [3].

Secondary metabolites are the sources of plant natural products used in medicine. These compounds are broadly divided into phenolics which include polyphenols, flavonoids,

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tannins and quinones known for their potent antioxidant, cytotoxic and antimicrobial activities [4–7]; alkaloids which are cytotoxic and have a wide range of physiological effects [8–10]; glycosides; terpenes and other volatile compounds, most of which are endogenously utilized as plant defense compounds [11]. However, the under-utilization of plants remains a challenge for developing drugs. The use of plant-derived compounds and their derivatives is well-established in other medicinal applications, most notably in chemotherapeutic agents [12,13]. Moreover, antimicrobial agents derived from plant metabolites have increasingly gained attention in the past few years [7]. The emergence of multi-drug resistant bacteria and the rise of infectious diseases have led to the reevaluation of the use of antibiotic agents in treatment, and novel solutions such as the use of plant secondary metabolites as resistance-modifying agents. In addition, there is a marked decline in the development of new classes of antibiotics since 1960. These factors underscore the importance of searching for alternative sources of antimicrobial agents from plants [14,15].

Muntingia calabura L. (*M. calabura*) is a shrub introduced from Tropical America to Southeast Asia. It is well-adapted in areas where it is introduced, often growing as roadside trees [1,16]. This plant species is heavily indigenized in most localities. Its leaves are distinctively lanceolate in shape, with margins irregularly serrate. The plant flowers throughout the year; its fruits are berries which turn red when mature with lenticularly shaped seeds [17].

Its leaves, stems and roots have been documented to have traditional medicinal usage in various modes of applications. In Peru, its leaves and bark are used as antiseptics, and to treat swelling in the lower extremities. Leaf decoctions are also popular treatments in South America to reduce gastric ulcers. In the Philippines, the flowers are used to treat headache and for relief of incipient colds. Its roots are also used as emmenagogues in Malaysia and Vietnam, although in these countries, *M. calabura* is considered as a neglected species [1].

A recent review revealed only thirty published studies on *M. calabura*, which focused mostly on leaf ethanolic and methanolic extracts [1,18]. In consonance with its traditional usage, researchers have identified several bioactive properties of the plant. Anti-inflammatory, anti-nociceptive [18–20] and antioxidant activity of the leaves [19], cytotoxicity against leukemia cell lines of the roots [21], and antimicrobial activity of the leaves [22] of *M. calabura* have been observed and demonstrated.

This paper presented the antimicrobial activity of the crude ethanolic extracts from the leaves and stems of *M. calabura* against the following bacteria: *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Bacillus subtilis* (*B. subtilis*); and the fungus *Candida albicans* (*C. albicans*). The phytochemicals of the leaf and stem extracts which are responsible for the bioactive properties are also reported.

2. Materials and methods

2.1. Plant material

M. calabura leaves and stems were collected in April–June, 2015 from Paniqui, Tarlac, Philippines. Leaf and stem specimens were identified and authenticated at the Jose Vera Santos Memorial Herbarium (PUH) at the Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines. Samples were then laid and air-dried for two weeks before pulverization.

2.2. Preparation of crude extracts

Leaves and stems weighting 100 g each were immersed in 95% ethanol at a ratio of 1:10 (w/v) for 72 h. The mixtures were then decanted and filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator (Laborota 4001, Heidolph) with the temperature set at 40 °C. The crude leaf and stem extracts were then air-dried for 14 days. After air-drying, extracts were reconstituted in 95% ethanol, and filtered through a Whatman No. 1 filter paper for further bioassays.

2.3. Phytochemical screening

Ethanolic extracts derived from the leaves and stems of *M. calabura* were subjected to phytochemical screening for the presence of tannins, flavonoids, alkaloids, sterols, triterpenes, saponins, and glycosides following standardized methods [23].

2.4. Estimation of total phenolic and flavonoid contents

Total phenolic content of the ethanolic extracts was determined by the modified Folin–Ciocalteu method [24]. A total of 10 mL Folin–Ciocalteu reagent and 200 μL of Na_2CO_3 (2%, w/v) were added to 100 μL of plant extract solution (1 mg/mL). Then, the resulting mixture was incubated at 45 °C with shaking at 120 r/min for 15 min. The absorbances of the samples were measured at 765 nm using a UV-Vis spectrophotometer. Results were expressed as mg gallic acid equivalent (GAE)/g plant extract. The same procedure was used for making a standard curve of gallic acid with a concentration range of 0–100 μg/mL.

Total flavonoid content was determined using the modified aluminum chloride method [24]. One milliliter of plant extract solution (1 mg/mL) was mixed with 3 mL ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 mol/L potassium acetate, and 5.6 mL of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm using a UV-Vis spectrophotometer. The total flavonoid content was computed from a calibration curve made with rutin as standard (0–200 μg/mL in ethanol). The concentration of total flavonoids was then expressed as mg rutin equivalents/g crude extract.

2.5. Antimicrobial assay

Potential antibacterial and antifungal activities of the plant extracts were examined using the disc diffusion assay. Organisms used to test the antimicrobial activity of the plant extracts were *E. coli*, *S. typhimurium*, *P. aeruginosa*, *S. aureus*, *B. subtilis* (bacteria) and *C. albicans* (fungus). Microbial suspensions in 0.1% peptone at 0.5 McFarland were inoculated on nutrient agar plates for bacterial samples, and glucose yeast peptone agar plates for fungal samples. For each agar plate, three equidistant wells, one for each replicate, were drilled using a cork borer. Then 200 μL of each plant extract (10 mg/mL) was aspirated into each well. In addition to the plant extracts, ethanol