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Screening of antibacterial activity of *Muntingia calabura* leaves extracts against bacterial pathogens

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Abstract

The aim of the present study is to determine the *in vitro* antimicrobial activity of various extracts of *Muntingia calabura* (Elaeocarpaceae) leaves against a selected panel of microorganisms. Antimicrobial testing was carried out using the agar well diffusion assay method. The microbes targeted were *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora* and *Agrobacterium tumefaciens*. Results of this study showed that the methanol leaf extract of *M. calabura* was effective against *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora* and *Agrobacterium tumefaciens* with inhibition zone of 1.2, 0.8 and 0.6 cm respectively. The chloroform and petroleum ether extracts showed comparatively less zone of inhibition against the selected pathogens. Finally, we concluded that *M. calabura* possesses a potential antibacterial property and the results also suggest the presence of more potent polar antibacterial compound in the *Muntingia calabura* plant material.

Keywords: *Muntingia calabura*, Antibacterial, Agar well diffusion, bacterial pathogen, MIC

1. Introduction

The knowledge about the use of plants possessing antimicrobial properties has been accrued through centuries and such plants are still valued today. Plant, being a major source of natural therapeutic remedies, has been used in various part of the world to treat various infectious diseases and play an important role in health care in many developing countries (Vahidi *et al.*, 2002). Medicinal plants constitute a group of industrially important crops that are of great values for domestic use and export. They are known for their rich sources of secondary metabolites such as triterpenes, glycosides, flavonoids, tannins, alkaloids and other aromatic compounds (Sindhan *et al.*, 1999) [18]. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principle, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies (Rios and Recio, 2005) [17]. Crude extracts of some well-known medicinal plants are used to control the plant pathogens. During the past few years, there is a growing trend all over the world to shift from synthetic to natural products including medicinal plants (Parimaladevi and Marimuthu, 2011) [15].

Muntingia calabura L. (Kerukupsiam), also known locally as Jamaica cherry, is a plant of the family Elaeocarpaceae (Morton, 1987) [9]. It is native to the American continent and is widely cultivated in warm areas of Asian region, including Malaysia (Chin, 1989) [1]. Its leaves, barks and flowers are believed to possess medicinal value as reported in Peru folklore medicinal uses. Various parts of this tree have several documented medicinal uses in both Southeast Asia and tropical America (Kaneda *et al.*, 1991; Nshimo *et al.*, 1993) [7, 11]. The roots have been employed as an emmenagogue in Vietnam and as an abortifacient in Malaysia. In the Philippines, the flowers of this species have been used to treat headaches, and as an antidyspeptic, antispasmodic and diaphoretic. Infusions of the flowers of this plant are drunk as a tranquilizer and tonic in Colombia (Perez-Arbelaez, 1975; Kaneda *et al.*, 1991) [7]. In addition, the *M. calabura* leaves extracts also possesses antibacterial activity (Zakaria *et al.*, 2006) [23] and antistaphylococcal activity (Zakaria *et al.*, 2007) [24]. In this study the methanol, chloroform and petroleum ether extracts of *M. calabura* is screened against selected bacterial pathogens for the presence of antibacterial activity using the agar well diffusion assay method.

2. Materials and Methods

Plant materials

The plant samples taken for this study were collected from Eastern Block Farm in Tamil Nadu Agricultural University, Coimbatore-3. The plant sample, obtained after initial screening studies performed against bacterial pathogens was identified and certified through Botanical Survey of India (BSI), TNAU, Coimbatore -3, Tamil Nadu.

Preparation of *M. calabura* extracts

The dried and powdered plant samples leaf were extracted by percolation with methanol, chloroform and petroleum ether at the rate of 1:5 at room temperature for overnight. The extracts were then filtered with country filter paper and concentrated under vacuum in a rotary evaporator to get 6-11 per cent of gummy residue as a percentage of powdered plant materials. All the extracts were kept in a tightly stoppered bottle in a refrigerator. All the extracts then assayed for antimicrobial activity.

Microorganisms tested

Microorganisms tested in this study were *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora*, and *Agrobacterium tumefaciens*.

Antimicrobial screening

The sterilized medium seeded with respective human pathogen was poured into the petriplates and allowed to solidify. Then each petriplate was divided into four equal quarters using a marker pen. Using a sterile corn borer, wells of 6 mm in diameter were made in each quadrat of the plate containing the media. For each organism, 20 µl of the prepared plant sample was loaded in each well. Two replications were maintained for each treatment. For each test pathogen, the positive control and the negative control (two replications each) were also loaded in a separate well. The plates were incubated for 24 h and the observations were taken. The observations were made by measuring the inhibition zone (or halo like area), which indicates the absence of microbial growth around the well. The diameter of inhibition zone (DIZ) was measured and the mean DIZ was calculated.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC assay was performed to test the antimicrobial activity of the methanol extract of *M. calabura* leaf using tube dilution method (Claeys *et al.*, 1988) [2]. The MIC was defined as the lowest concentration of antibiotics or plant extracts that did not show any growth of tested pathogens. This test was performed at four concentrations of the plant extract *viz.*, 10 mg/ml, 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml.

3. Results and Discussion

Plants still continue to be among the source of drugs for the majority of the world's population. Plant pathogens are a major problem in the cultivation of crop plants around the world. In the present study antibacterial activity of *Muntingia calabura* leaves extracts against bacterial pathogens. The experiment was conducted in the Microbiology laboratory, Tamil Nadu Agricultural University, Coimbatore, *Muntingia calabura* is significant as the potential source for the control of selected bacterial pathogens. The present study was aimed at evaluating the antimicrobial property of *M. calabura* plant against bacterial pathogens. The antimicrobial compounds from the leaves of *M. calabura* were extracted separately by using three different solvents *viz.*, methanol (polar), chloroform (medium polar) and petroleum ether (least polar). The results of the studies on antimicrobial activity against bacterial pathogens revealed that the methanol extract of *M. calabura* possessed broad spectrum of antimicrobial activity compared to other solvent extracts.

Determination of different solvent extracts of *M. calabura* leaf against bacterial pathogens

The methanol extract of *M. calabura* leaves showed antimicrobial activity against bacterial plant pathogens by agar well diffusion assay. The inhibition zone of 1.2, 0.8 and 0.6 cm against *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora* and *Agrobacterium tumefaciens* respectively. The positive control chloramphenicol showed the inhibition zone of 3.5, 2.9 and 3.0 cm for *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora* and *Agrobacterium tumefaciens* respectively (Table 1).

Table 1: Antimicrobial activity of *Muntingiacalabura* leaves against bacterial plant pathogens

Extracts	Zone of inhibition (Diameter in cm)		
	<i>Xanthomonas campestris</i> pv. <i>oryzae</i>	<i>Erwinia amylovora</i>	<i>Agrobacterium tumefaciens</i>
Methanol Extract (100mg/ml)	1.2 (± 0.12)	0.8 (± 0.06)	0.6 (± 0.17)
Chloroform Extract (100mg/ml)	0.8 (± 0.06)	0.6 (± 0.17)	0.4 (± 0.12)
Petroleum ether Extract (100mg/ml)	0.6 (± 0.06)	0.4 (± 0.12)	0.3 (± 0.03)
Chloramphenicol (1mg/ml)	3.5 (± 0.17)	2.9 (± 0.26)	3.0 (± 0.06)
Ethanol (Control)	0.3 (± 0.03)	0.3 (± 0.06)	0.3 (± 0.09)

Mean of three replications

In the present study, *Muntingia calabura* leaves was tested for its antimicrobial activity by agar well diffusion assay against selected bacterial pathogens. Based on the results, the methanol extract of *Muntingia calabura* was considered to be the most active extract than compared to chloroform extract and petroleum ether extract.

Since many years, medicinal plants have been used extensively as sources for the study and research on active compounds against several bacterial strains. Parimaladevi (2008) [14] reported that the chloroform extract of *Polygonum minus* exhibited antimicrobial activity against *A. solani*, *Fusarium oxysporum* f.sp *lycopersici* and *A. Niger* under *in*

vitro condition. Ram Kumar *et al.* (2010) [16] reported the antibacterial effect of *Syzygium aromaticum* and *Allium sativum* against food borne microorganisms. Omojasola and Awe (2004) [13] reported the antibacterial activity of leaf extract of *Anacardium occidentale* and *Gossypium hirsutum* against *Staphylococcus aureus*, *E.coli* and *P. aeruginosa*. Khalid *et al.* (2010) [8] reported the *Achillea fragrantissima* antibacterial activity of these extracts against several numbers of bacterial pathogens. The hydroalcoholic (80% ethanol) extract of *Plumbago indica* roots exhibited antibacterial activity against *Staphylococcus aureus*, *P. aeruginosa*, *E.coli* and *Bacillus subtilis* (Valsaraj *et al.*, 1997).

Some plants may be alternatives to currently used disease control agents, since they constitute a rich source of bioactive chemicals (Swain 1977; Wink 1993) [20, 22]. The substances, which can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered as candidates for developing new antimicrobial drugs (Waccaro *et al.*, 1996).

Minimum inhibitory concentration of methanol leaf extract of *M. calabura* against bacterial pathogens

The minimum inhibitory concentration was evaluated for the methanol leaf extract of *M. calabura* against the selected pathogenic cultures *viz.*, *A. solani*, *Fusarium oxysporum* f.sp

lycopersici, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus Niger* and *Colletotrichum* sp. The results of the minimum inhibitory concentration assay of the methanol leaf extract of *M. calabura* indicated that the extract inhibited the growth against *Xanthomonas campestris* pv. *Oryzae*, *Erwinia amylovora* and *Agrobacterium tumefaciens* at a concentration of 10 mg/ml (Table 2). Whereas growth was observed in the other three dilutions/concentrations (1 mg/ml, 0.1 mg/ml and 0.01 mg/ml). Chloramphenicol (positive control) showed no growth at 10 mg/ml and 1 mg/ml concentrations, but growth was observed in the other two dilutions. The cells and solvent control (negative control) showed growth in all the dilutions for all the organisms.

Table 2: Minimum inhibitory concentration of methanol extract of *M. calabura* leaves against bacterial plant pathogens

Extracts	Bacterial pathogens		
	<i>Xanthomonas campestris</i> pv. <i>oryzae</i>	<i>Erwinia amylovora</i>	<i>Agrobacterium tumefaciens</i>
Methanol Extract			
10mg/ml	-	-	-
1mg/ml	+	+	+
0.1mg/ml	+	+	+
0.01mg/ml	+	+	+
Chloramphenicol			
10mg/ml	-	-	-
1mg/ml	-	-	-
0.1mg/ml	+	+	+
0.01mg/ml	+	+	+
Solvent control	+	+	+
Cells	+	+	+

+ Growth - No growth

Methanol extract of *Aegle marmelos* showed MIC at 5% (w/v) level against *Alternaria solani*, *Fusarium moniliforme* and *Pythium* sp. and methanolic extracts of *Achillea fragrantissima* possessed the MIC of 1.2 - 2.9 mg mL⁻¹ against *E.coli* and *P. aeruginosa* (Khalid *et al.*, 2010) [8]. Negi and Jayaprakasha, (2001) [10] reported that the ethyl acetate extract of kaffir lime (*Citrus hystrix* DC.) peel showed minimum inhibitory concentration (MIC) values of 0.28 and 0.56 mg/ml against *S. cerevisiae* var. *sake* and *B. cereus*, respectively. Khalid *et al.* (2010) [8] reported that the minimum inhibitory concentration of *Teucrium polium* against *Staphylococcus aureus*, *E.coli* and *P. aeruginosa* was 2 mg mL⁻¹. In the present study, the minimum inhibitory concentration of methanol extract of *M. calabura* root was found to be 10 mg/ml against the tested pathogens. In conclusion, the present study has revealed the antimicrobial activity of *M. calabura* root extracts against selected human pathogens. Further investigations are needed to characterize the active compounds in order to determine the structure and antimicrobial potential under *in vivo* studies.

In conclusion the medicinal plant *Muntingia calabura* was chosen for the study to test the antimicrobial activity against the selected bacterial pathogens. The extracts leaves of the medicinal plant were assessed for their antimicrobial activity. The antimicrobial compounds of the medicinal plants were extracted with three different solvents *viz.*, methanol, chloroform and petroleum ether of varying polarity. The extracts were filtered using Whatmann No. 44 filter paper and concentrated using a rotary vacuum evaporator to get 6-11 per cent of gummy residue. Antimicrobial activities of the *M. calabura* leaves were tested against the selected bacterial pathogens by agar well diffusion assay.

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