

Original Research Article

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Solvent Extraction and Evaluation of Antifungal Activity of *Muntingia calabura* Root against Fungal Phytopathogens

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ABSTRACT

Keywords

Muntingia calabura,
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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 *A. solani*, *Fusarium oxysporum* f.sp *lycopersici*, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* sp. Results of this study showed that the methanol leaf extract of *M. calabura* was effective against *A. solani*, *Fusarium oxysporum* f.sp *lycopersici*, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* sp. with inhibition zone of 2.3, 2.0, 2.0, 1.8, 1.5, 1.6 and 1.7cm respectively. The chloroform and petroleum ether extracts showed comparatively less zone of inhibition against the selected pathogens. Finally, it is concluded that *M. calabura* possesses a potential antifungal property and the results also suggested the presence of more potent polar antifungal compound in the *Muntingia calabura* plant material.

Introduction

Muntingia calabura L. (Kerukupsiam), also known locally as Jamaica cherry, is a plant of the family Elaeocarpaceae (Morton, 1987). It is native to the American continent and is widely cultivated in warm areas of Asian region, including Malaysia (Chin, 1989).

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 (Nshimo *et al.*, 1993). 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 tranquillizer and tonic in Colombia (Kaneda *et al.*, 1991).

In addition, the *M. calabura* leaves extracts also possesses antibacterial activity (Zakaria *et al.*, 2006) and antistaphylococcal activity (Zakaria *et al.*, 2007). 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. 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). In this study the methanol, chloroform and petroleum ether extracts of *M. calabura* root is screened against selected fungal pathogens for the presence of antifungal activity using the agar well diffusion assay method.

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 fungal pathogens was identified and certified through Botanical Survey of India (BSI), TNAU, Coimbatore -3, Tamil Nadu.

Preparation of *M. calabura* root extracts

The dried and powdered plant samples root 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 *Alternaria solani* (Ell. and Mart.) Jones and Grout, *Fusarium oxysporum* f.sp. *lycopersici*

W.C. Snyder and H. N. Hansen, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani* J.G. Khunn, *Aspergillus niger* Van Tiegham and *Colletotrichum* sp.

Antimicrobial screening

The sterilized medium seeded with respective fungal 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 cork 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* root using tube dilution method (Claeys *et al.*, 1988). 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.

Results and Discussion

Based on the experiment conducted in the Microbiology lab, TNAU, Coimbatore, *Muntingia calabura* is significant as the

potential source for the control of plant pathogens. The medicinal plant sample *Muntingia calabura* Linn., was identified and certified through Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore for confirmation of the genus and species (Plate 2). The present study was aimed at evaluating the antimicrobial property of *M. calabura* root extract against fungal pathogens.

The antimicrobial compounds from the root 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 fungal 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* root against fungal pathogens

The methanol extract of *M. calabura* root possessed more inhibitory activity against *A. solani*, *Fusarium oxysporum* f.sp *lycopersici*, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* sp. The methanol extract of *M. calabura* root was found to inhibit the pathogens more effectively than the chloroform and petroleum ether extracts. The diameter of inhibition zones produced by the methanol extract of *M. calabura* against *A. solani*- 2.3 cm, *F. oxysporum* f.sp. *lycopersici*- 2.0 cm, *Pythium* sp. - 2.0 cm and *Phytophthora* sp. - 1.8 cm, *Rhizoctonia solani*- 1.5 cm, *Aspergillus niger* - 1.6 cm and *Colletotrichum* sp. - 1.7 cm respectively. Whereas chloroform extract showed inhibition zone of 1.5 cm for both *A. solani* and *F. oxysporum* f.sp. *lycopersici*. In case of petroleum ether extract the inhibition zone was found to be 1.0 cm for *A. solani* and

0.7 cm for *F. oxysporum* f.sp. *lycopersici*. The positive control ketoconazole showed the highest activity of 3.5, 3.0, 2.8, 3.0, 3.2, 3.5 and 2.9 cm of inhibition against *A. solani*, *F. oxysporum* f.sp. *lycopersici*, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* sp. (Table 1).

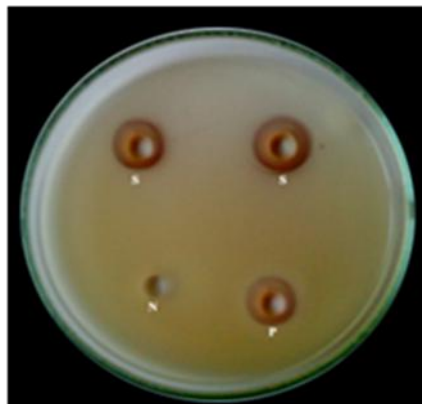
In the present study, *Muntingia calabura* root was tested for its antimicrobial activity by agar well diffusion assay against selected fungal pathogens. Based on the results, the methanol extract of *Muntingia calabura* was considered to be the most active extract than compared to chloroform extract 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) 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) reported the antibacterial effect of *Syzygium aromaticum* and *Allium sativum* against food borne microorganisms. Omojasola and Awe (2004) 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) 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). 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).

Plate.1 Antimicrobial activity of methanol extract of *M. calabura* root against fungal plant pathogens by agar well diffusion assay

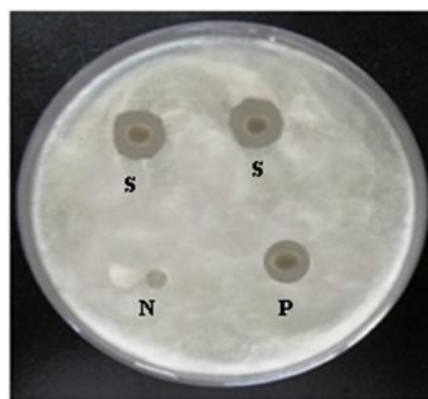
Alternaria solani



Fusarium oxysporum f. sp. *lycopersici*



Pythium sp.



S - Sample (Methanol extract of *M. calabura* root)
P - Positive control (Ketoconazole)
N - Negative control (Ethanol 100%)

Table.1 Antimicrobial activity of *Muntingia calabura* root extract against fungal plant pathogens

Extracts	Zone of inhibition (Diameter in cm)						
	<i>Alternaria solani</i>	<i>F.oxysporum</i> f.sp. <i>lycopersici</i>	<i>Pythium</i> sp.	<i>Phytophthora</i> sp.	<i>Rhizoctonia solani</i>	<i>Aspergillus niger</i>	<i>Colletotrichum</i> sp.
MethanolExtract (100mg/ml)	2.3 (± 0.17)	2.0 (± 0.46)	2.0 (± 0.41)	1.8 (± 0.12)	1.5 (± 0.17)	1.6 (± 0.09)	1.7 (± 0.06)
Chloroform Extract (100mg/ml)	1.5 (± 0.12)	1.5 (± 0.06)	1.5 (± 0.07)	1.2 (± 0.12)	1.0 (± 0.06)	1.2 (± 0.17)	1.2 (± 0.23)
Petroleum ether Extract(100mg/ml)	1.0 (± 0.12)	0.7 (± 0.06)	0.8 (± 0.12)	0.6 (± 0.17)	0.6 (± 0.18)	0.7 (± 0.06)	0.6 (± 0.12)
Ketoconazole (1mg/ml)	3.5 (± 0.64)	3.0 (± 0.29)	2.8 (± 0.55)	3.0 (± 0.29)	3.2 (± 0.64)	3.5 (± 0.52)	2.9 (± 0.58)
Ethanol (Control)	0.3 (± 0.12)	0.3 (± 0.07)	0.4 (± 0.03)	0.3 (± 0.06)	0.4 (± 0.09)	0.3 (± 0.12)	0.4 (± 0.02)

Mean of three replications

Table.2 Minimum inhibitory concentration of methanol extract of *M. calabura* root against fungal plant pathogens

Extracts	Fungal plant pathogens						
	<i>Alternaria solani</i>	<i>F.oxysporum</i> f.sp. <i>lycopersici</i>	<i>Pythium</i> sp.	<i>Phytophthora</i> sp.	<i>Rhizoctonia solani</i>	<i>Aspergillus niger</i>	<i>Colletotrichum</i> sp
MethanolExtract							
10mg/ml	-	-	-	-	+	+	+
1mg/ml	+	+	+	+	+	+	+
0.1mg/ml	+	+	+	+	+	+	+
0.01mg/ml	+	+	+	+	+	+	+
Ketoconazole							
10mg/ml	-	-	-	-	-	-	-
1mg/ml	-	-	-	-	-	-	-
0.1mg/ml	+	+	+	+	+	+	+
0.01mg/ml	+	+	+	+	+	+	+
Solvent control	+	+	+	+	+	+	+
Cells	+	+	+	+	+	+	+

+ Growth; - No growth

Minimum inhibitory concentration of methanol root extract of *M. calabura* against fungal pathogens

The minimum inhibitory concentration was evaluated for the methanol root 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 root extract of *M. calabura* indicated that the extract inhibited the growth against *A. solani*, *Fusarium oxysporum* f.sp *lycopersici*, *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, *Aspergillus niger* and *Colletotrichum* sp. 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. Methanol extract of *Aeglema rmelos* 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). Negi and Jayaprakasha, (2001) 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) 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 fungal pathogens. The root extracts 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* root were tested against the selected fungal pathogens by agar well diffusion assay.

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