

Short report

**In vitro activities of *Maesa lanceolata* extracts
against fungal plant pathogens**

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Abstract

In vitro tests were carried out using extracts of *Maesa lanceolata* var. *goulungensis* weir against a broad range of fungal plant pathogens such as *Phytophthora cryptogea*, *Trichoderma virens*, *Aspergillus niger*, *Phoma* sp., *Fusarium oxysporium*, *Pythium ultimum*, *Cochliobolus heterostrophus*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pyrenophora teres*. *M. lanceolata* extracts were very active against all the pathogens tested except *P. ultimum* and *R. solani*.

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Plant. *Maesa lanceolata* Forsskal var *goulungensis* weir (Myrsinaceae) [1,2] stem bark (500 g) collected from Kakamega forest in Western Kenya and authenticated by Mr Andrew Chapya (a taxonomist affiliated to the University of Nairobi Herbarium). A voucher specimen is held at the Herbarium, in Nairobi, Kenya (Ref. 306/2001).

Uses in traditional medicine. Fruits of *M. lanceolata* are widely used in East Africa to treat a variety of ailments, such as sore throat, tapeworms, hepatitis and

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cholera [3]. In Central Africa, it is used against *Entamoeba histolytica* infections [4], while in Saudi Arabia a decoction of the heated fresh leaves is used to alleviate rheumatic arthritis [5].

Previously isolated classes of constituents. Saponins from the leaves [6], benzophenons from the fruits [7]. *M. lanceolata* extracts are active against *Bacillus subtilis* [8].

Tested material. Hexane, chloroform and methanol extracts (yields: 1.02, 1.91 and 2.99%, respectively), 17 fractions obtained by Si-gel CC of the methanol extract eluting with ethyl acetate–methanol, three fractions obtained from fractions 15–17 (yields: 0.005, 0.009 and 0.015%, respectively) above by PHPLC, performed with a C¹⁸ column eluting with MeOH–water mixtures (POA detection).

Studied activity. Antifungal. Dispensed amount of the tested material: 0.25–50 µg.

Micro-organisms used. Isolated and identified fungal strains stored and maintained by normal sub-culture techniques at Colorado State University (USA) were used for the bioassays. The following fungal isolates were tested: *Trichoderma virens*, *Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora cryptogea*, *Fusarium oxysporium*, *Aspergillus niger*, *Phoma* sp., *Sclerotium rolfsii*, *Pyrenophora teres* and *Cochliobolus heterostrophus*.

Results. All 10 fungi tested were inhibited strongly by the methanolic extract (Table 1). Only two fungi (*P. ultimum* and *R. solani*) were moderately inhibited.

The methanolic extract was separated through open-air chromatography and 17 fractions were collected and bioassayed. With the exception of fractions 15, 16 and 17, which showed strong inhibitions, the other fractions displayed no activity. Upon pooling the three active fractions together and passing them through HPLC, three peaks were recovered. These peaks were tested to determine if activity was retained (Table 2). Peak 1 demonstrated strong activity for *T. virens*, *P. cryptogea* and *A. niger*, moderate activity against *F. oxysporium* and slight activity against *C. heterostrophus*, *S. rolfsii* and *P. teres*. Peak 2 also retained activity except against *P. ultimum* and *R. solani*. Fungal inhibition was not observed in peak 3 against any of the fungi tested. It was interesting to note that there was no activity against *P. ultimum* and *R. solani* in any of the three peaks, although activity was clearly seen in the pooled active tubes 15, 16 and 17 of the open-air chromatography (Table 1). It was also clear from the results that the activity against *C. heterostrophus*, *S. rolfsii* and *P. teres*, which were seen in the pooled sample, were no longer there in 1st and 3rd peaks. The inhibition was nonetheless retained in the 2nd peak. This

Table 1
Antifungal activity of stem bark extracts of *M. lanceolata*^a

Fungi	Hexane	Chloroform	Methanol
<i>Phytophthora cryptogea</i>	—	++	+++
<i>Trichoderma virens</i>	+	+	+++
<i>Aspergillus niger</i>	—	—	+++
<i>Fusarium oxysporium</i>	—	—	+++
<i>Pythium ultimum</i>	—	—	++
<i>Cochliobolus heterostrophus</i>	+	—	+++
<i>Rhizoctonia solani</i>	—	+	++
<i>Sclerotium rolfsii</i>	—	—	+++
<i>Pyrenophora teres</i>	—	—	+++
<i>Phoma</i> sp.	+	—	+++

^a Data are presented as follows: (—)-no inhibition of fungal growth, (+)-slight inhibition, (++)-moderate inhibition, (+++)-strong inhibition, and are the average of two separate experiments with five replicates in each treatment. In the table, each (+) represents 5 mm from the filter disc. (—) Depicts no fungal inhibition.

Table 2
Antifungal activity of HPLC isolated of *M. lanceolata*^a

Fungi	Peak 1 (500 µg)	Peak 2 (500 µg)	Peak 3 (500 µg)
<i>Trichoderma virens</i>	+++	+++	—
<i>Pythium ultimum</i>	—	—	—
<i>Rhizoctonia solanii</i>	—	—	—
<i>Phytophthora cryptogea</i>	+++	+++	—
<i>Fusarium oxysporium</i>	++	+++	—
<i>Aspergillus niger</i>	+++	+++	—
<i>Cochliobolus heterostrophus</i>	+	+++	—
<i>Sclerotium rolfsii</i>	+	+++	—
<i>Pyrenophora teres</i>	+	+++	—
<i>Phoma</i> sp.	—	+++	—

^a The visible absorbance at 210/280 nm was measured by a PDA-100 photodiode array variable UV/VIS detector. Mobile phase solution A consisted of double distilled water and solution B (methanol). The multistep gradient was as follows: 0–5 min 5.0% B; 5–10 min 20.0% B; 15–20 min 20.0% B; 20–40 min 80.0% B; 40–60 min 100% B; 60–70 min 100% B; and 70–80 min 5.0% B.

lack of activity against *P. ultimum* and *R. solani* in all peaks is worth noticing since the compounds in the extracts had not changed from the original pooled extract where there was activity.

Discussion and conclusion. It was previously reported [6] that *M. lanceolata* does not have antifungal activity. However, our results clearly show the contrary. One possible explanation for this contradictory result is that we used stem bark instead of leaves [4,9].

P. ultimum and *R. solani* were inhibited by methanolic extracts. However, the purified fractions lost the inhibitory activity. This phenomenon is currently observed in natural product separation [4,6]. A possible explanation is that the compounds in methanol that inhibited the growth of the other fungi were not the same ones inhibiting *P. ultimum* and *R. solani*. Additionally, some form of activator or cofactor might be removed by the chromatographic purification procedure, which renders the rest of the compounds inactive [10].

The diminishing biological activity of natural products against fungal plant pathogens is a problem throughout the world [11–13]. We were not able to fully characterize the responsible antimicrobial metabolites in *M. lanceolata* extracts because of the abundance of tannins and glycosides in the eluted fractions. Our results show that even crude plant extracts can be used to protect plants against damages inflicted by fungal infections. Various studies have shown a similar strategy to use crude plant extracts against plant pathogens for broad commodity uses [7,14,15].

In conclusion, the study has not just confirmed folklore uses of *M. lanceolata* as a plant that contains possible chemotherapeutic compounds. It is evident from this study that the plant's uses can be extended to protection of other plants against fungal infections. In view of this finding and the work done by many other researchers [3,4,16], it is proposed that *M. lanceolata* be placed on a high priority list for propagation and conservation.

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