***Short Research Article***

# Antimicrobial activity of alcoholic extracts of medicinal plants against phytopathogenic fungi

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## ABSTRACT

**Aims:** The aimed to evaluate the antimicrobial effects of 14 alcoholic extracts of medicinal plants on the mycelial growth of *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f. sp. *passiflorae*, *Fusarium solani* and *Rhizoctonia solani*, fungi that causes diseases in *Passiflora edulis*.
**Study design:** With the obtained data the mycelial growth rate index (MGRI) was calculated, afterwards the analysis of variance was performed and the means were compared by the Scott-Knott test at 5% probability.
**Place and Duration of Study:** Plant Pathology Laboratory, Embrapa Eastern Amazon, Belém, Pará, Brazil, between May 2014 and April 2015.
**Methodology:** The extracts were prepared with 1.0 g of powdered plant material and 10 mL of commercial ethyl alcohol 92.8° (0.1 g $mL^{-1}$) under constant in an orbital shaker at 200 rpm for 20 minutes. They were then kept in the refrigerator for 24 hours at rest. The extracts were centrifuged and filtered on Millipore membranes with 0.22 µm porosity. The tests with the phytopathogenic fungi were carried out *in vitro* with the alcoholic extracts at 1% concentration. The experimental design was completely randomized with 15 treatments and 5 replicates.
**Results:** All the extracts reduced the growth of the fungi *C. gloeosporioides*. The extracts the *Eucalyptus angulosa*, *Lippia alba*, *Zingiber officinale*, *Cymbopagon citratus*, *Azadirachta indica*, *Plectranthus barbathus*, *Hibiscus sabdariffa*, *Aloe vera*, *Pedilanthus tithymaloides*, *Mansoa alliacea* and *Chenopodium ambrosioides* reduced the mycelial growth of *F. oxysporum* f. sp. *passiflora*e. Only the extract of *E. angulosa* presented reduction in the growth of *F. solani*. Meanwhile the extracts of *E. angulosa*, *Z. officinale*, *L. alba*, *M. alliacea* and *P. barbathus* reduced the mycelial growth of *R. solani*.
**Conclusion:** All extracts presented antimicrobial potential, being that the extract of *E. angulosa* reduced the mycelial growth of all the evaluated fungi.



*Keywords: Antifungal activity; alternative control; medicinal plants; phytopathogenic fungi.*


## 1. INTRODUCTION

In the state of Pará, the species *Passiflora edulis* Sims, popularly known as passion fruit, is one of the most important crops. Almost all commercial orchards are represented by *Passiflora edulis* Sims f. *flavicarpa* Deg. (yellow passion fruit). This species is appreciated for its characteristic taste and aroma. However, it is susceptible to various diseases that may compromise productivity and fruit quality and cause plant death [1].
Anthracnose, caused by *Colletotrichum gloeosporioides*, is widespread in all regions of *P. edulis* cultivation in Brazil and in other countries [2]. This pathogen has a wide range of

hosts. Its symptoms can be observed in all organs of shoots, such as branches, tendrils, leaves, flower buds, and fruits [3].

The fungus *Fusarium oxysporum* f. sp. *passiflorae* W.L. Gordon (FOP) is specific to the Passifloraceae family. Responsible for fusariosis, this fungus colonizes plant vessels by causing small wounds or natural openings in roots, causing xylem obstruction and plant death [4]. The fungus *F. solani* is polyphagous and affects a large number of plant species, among them *Nicotiana tabacum* L., *Phaseolus vulgares* L., *Solanum tuberosum* L., *Beta vulgaris* L., and *Capsicum annuum* L. [2]. Unlike *F. oxysporum* f. sp. *passiflorae*, *F. solani* has no systemic action. The symptoms of plant base rot caused by it are characterized by decay and death of the plant because of the rotting of the root and plant base tissues [5].

In Pará, the fungus *Rhizoctonia solani* affects several crops of economic importance, among them *P. edulis* [6]. In plants of this culture, the occurrence of leaf burn caused by the fungus is noticed by the necrosis in leaves with a yellow halo, which results in withering and fall of the leaves at the final stage of the disease [5].

There is currently a demand for sustainable alternatives to control diseases of various crops, especially in small and medium-sized plantation areas, using active ingredients that do not harm the environment, biodiversity and above all the health of farmers and consumers [7]. In this context, natural plant products may have an antimicrobial activity. They act directly on the pathogen [8]. Thus, the alternative control is a great option to minimize the harmful effects caused by the intensive use of pesticides as sustainable agriculture prioritizes the use of natural products for the control of plant diseases [9].

This work aims to evaluate the antimicrobial effects of 14 alcoholic extracts of medicinal plants on the mycelial growth of *C. gloeosporioides*, *F. oxysporum* f. sp. *passiflorae*, *F. solani* and *R. solani*, fungi that causes diseases in *P. edulis*.

## 2. MATERIAL AND METHODS

Samples of 14 medicinal plants were collected at the Embrapa Eastern Amazon medicinal plant garden. They were packed in plastic bags and taken to the Plant Pathology Laboratory. All species used in this research were identified by the Botanical Laboratory of Embrapa Eastern Amazon by the researcher MSc. Silvane Tavares Rodrigues. The exsiccates are deposited at the IAN Herbarium of the Institution. The species were *Aloe vera* (L) Burm. f. (aloe), *Azadirachta indica* A. Juss (neem), *Chenopodium ambrosioides* L. (wormseed), *Cymbopogon citratus* (D.C.) Stapf. (lemon grass), *Eucalyptus angulosa* Schauer. (eucalyptus), *Hibiscus sabdariffa* L. (roselle), *Lippia alba* (Mill) N.E. Brown (bushy matgrass), *Mansoa alliacea* (Lam.) A.H. Gentry. (garlic vine), *Morinda citrifolia* L. (noni), *Ocimum basilicum* L. (basil), *Ocimum gratissimum* L. (clove basil), *Plectranthus barbatus* Andrews (forskohlii), *Pedilanthus tithymaloides* Poit. (coramine), and *Zingiber officinale* Roscoe (ginger).

### 2.1 Obtaining alcoholic extracts

To obtain alcoholic extracts, only the leaves of the medicinal plants were used. The asepsis of the samples was performed by washing under running water, soaking in 70% alcohol for one minute and in NaClO 1% solution for two minutes. Then, the residual chlorine was removed using sterile distilled water. After removing excess water from the absorbent paper, the material was dried in a forced air oven (Quimis brand, Q.360.14) at 40°C until constant weight, and ground in an electric mill (Tecnal brand, Willye, TE 650) to obtain a powder [10]. The extracts were prepared with 1.0 g of the powdered material and 10 mL of 92.8°

commercial ethyl alcohol (0.1 g $mL^{-1}$), and kept under constant agitation in an orbital shaker (Solab, SL223) at 200 rpm for 20 minutes. They were then transferred to a refrigerator, and kept at rest for 24 hours. Subsequently, the extracts were centrifuged in a centrifuge (Eppendorf, Centrifuge 5430R) at 7,000 rpm for ten minutes at 4°C, and filtered on Millipore® membranes with 0.22 µm porosity, which were used soon after obtaining them [11].

## 2.2 Origin of pathogens

The isolates of *C. gloeosporioides*, *F. oxysporum* f. sp. *passiflorae*, *F. solani* and *R. solani* were obtained from passion fruit plants showing characteristic disease symptoms at the municipalities of Castanhal, Parauapebas, Belém and Tomé-Açu (Pará, Brazil), respectively. They were preserved in mineral oil at the Plant Pathology Laboratory of Embrapa Eastern Amazon. For experimental use, the isolates were grown in potato dextrose agar (PDA) culture medium, and incubated for seven days at 28°C.

## 2.3 *In vitro* tests

To evaluate the *in vitro* antimicrobial activity, the alcoholic extracts were incorporated into the fluxing PDA culture medium, reaching a concentration of 1%. After solidification of the culture medium containing the treatments, an 8-mm diameter disc of mycelium was deposited at the center of each Petri dish. In control plates, the culture medium without the extracts was used. Mycelial growth was evaluated daily using a digital caliper until the fungus in one of the treatments reached the borders of the plate. The experimental design was completely randomized with 15 treatments and five replications. The obtained values were used to calculate the mycelial growth rate index (MGRI) [12]. An analysis of variance was performed, and the means were compared by Scott-Knott test [13] at 5% probability.

## 3. RESULTS AND DISCUSSION

In the assay with the fungus *C. gloeosporioides*, all extracts reduced the mycelial growth of the pathogen, differing significantly from the control (Table 1). The extracts showed inhibitions between 11.73 and 50.66%, and the extract of *E. angulosa* showed the best result, with an inhibition above 50% of the growth of *C. gloeosporioides*.

**Table 1. Effect of alcoholic extracts of medicinal plants on mycelial growth of *Colletotrichum gloeosporioides***

| Treatments | MGRI | Inhibition (%) |
|---|---|---|
| *Eucalyptus angulosa* | 13.03 c* | 50.66 |
| *Ocimum basilicum* | 19.93 b | 24.53 |
| *Zingiber officinale* | 20.08 b | 23.96 |
| *Lippia alba* | 21.02 b | 20.40 |
| *Azadirachta indica* | 21.30 b | 19.35 |
| *Ocimum gratissimum* | 21.49 b | 18.62 |
| *Plectranthus barbathus* | 21.54 b | 18.43 |
| *Aloe vera* | 22.07 b | 16.43 |
| *Cymbopagon citratus* | 22.22 b | 15.86 |
| *Chenopodium ambrosioides* | 22.35 b | 15.37 |

| | | |
|---|---|---|
| *Mansoa alliacea* | 22.45 b | 14.99 |
| *Morinda citrifolia* | 22.52 b | 14.72 |
| *Pedilanthus tithymaloides* | 22.61 b | 14.39 |
| *Hibiscus sabdariffa* | 23.31 b | 11.73 |
| Control | 26.41 a | - |

**Averages followed by same letter do no differ significantly each other by Scott-Knott test at 5% probability*

In the evaluation of the effects of extracts on *F. oxysporum* f. sp. *passiflorae*, the extracts of *E. angulosa*, *L. alba*, *Z. officinale*, *Cymbopagon citratus*, *A. indica*, *P. barbathus*, *H. sabdariffa*, *A. vera*, *P. tithymaloides*, *M. alliacea* and *C. ambrosioides* promoted a reduction in mycelial growth, differing significantly from the control, with inhibitions between 5.28 and 51.73% (Table 2). As for the *C. gloeosporioides* assay, the best result was obtained by the extract of *E. angulosa*, which inhibited pathogen growth by 51.73%.

**Table 2. Effect of alcoholic extracts of medicinal plants on mycelial growth of *Fusarium oxysporum* f. sp. *passiflorae***

| Treatments | MGRI | Inhibition (%) |
|---|---|---|
| *Eucalyptus angulosa* | 14.08 d* | 51.73 |
| *Lippia alba* | 23.79 c | 18.44 |
| *Zingiber officinale* | 25.07 c | 14.05 |
| *Cymbopagon citratus* | 25.70 c | 11.89 |
| *Azadirachta indica* | 26.46 b | 9.29 |
| *Plectranthus barbathus* | 26.68 b | 8.54 |
| *Hibiscus sabdariffa* | 26.73 b | 8.36 |
| *Aloe vera* | 26.78 b | 8.19 |
| *Pedilanthus tithymaloides* | 26.92 b | 7.71 |
| *Mansoa alliacea* | 27.46 b | 5.86 |
| *Chenopodium ambrosioides* | 27.63 b | 5.28 |
| *Ocimum basilicum* | 27.94 a | 4.12 |
| *Ocimum gratissimum* | 28.16 a | 3.46 |
| *Morinda citrifolia* | 28.50 a | 2.30 |
| Control | 29.17 a | - |

**Averages followed by same letter do no differ significantly each other by Scott-Knott test at 5% probability*

In the evaluation of the effects of extracts on *F. solani*, only the *E. angulosa* extract was positive, differing significantly from the control, with a 21.06% inhibition of pathogen mycelial growth (Table 3). All other extracts showed no antifungal activity. The extracts of *A. indica*, *M. citrifolia*, *C. ambrosioides*, *O. gratissimum*, *M. alliacea*, *P. barbathus* and *O. basilicum* stimulated the growth of *F. solani*.

**Table 3. Effect of alcoholic extracts of medicinal plants on mycelial growth of *Fusarium solani***

| Treatments | MGRI | Inhibition (%) |
|---|---|---|
| *Eucalyptus angulosa* | 15.37 b* | 21.06 |
| *Lippia alba* | 18.48 a | 5.08 |
| *Aloe vera* | 18.58 a | 4.57 |
| *Zingiber officinale* | 19.12 a | 1.80 |
| *Cymbopagon citratus* | 19.23 a | 1.23 |

| | | |
|---|---|---|
| *Hibiscus sabdariffa* | 19.26 a | 1.08 |
| *Pedilanthus tithymaloides* | 19.37 a | 0.51 |
| Control | 19.47 a | - |
| *Azadirachta indica* | 19.62 a | - |
| *Morinda citrifolia* | 20.10 a | - |
| *Chenopodium ambrosioides* | 20.13 a | - |
| *Ocimum gratissimum* | 20.44 a | - |
| *Mansoa alliacea* | 20.50 a | - |
| *Plectranthus barbathus* | 20.53 a | - |
| *Ocimum basilicum* | 20.84 a | - |

**Averages followed by same letter do no differ significantly each other by Scott-Knott test at 5% probability*

In the antifungal assay on *R. solani*, extracts of *E. angulosa*, *Z. officinale*, *L. alba*, *M. alliacea* and *P. barbathus* decreased the fungal mycelial growth, differing from the control, with inhibitions between 5.15 and 28.68% (Table 4). All other extracts showed no antifungal activity, and stimulated pathogen growth.

**Table 4. Effect of alcoholic extracts of medicinal plants on mycelial growth of *Rhizoctonia solani***

| Treatments | MGRI | Inhibition (%) |
|---|---|---|
| *Eucalyptus angulosa* | 32.79 f* | 28.68 |
| *Zingiber officinale* | 39.92 e | 13.18 |
| *Lippia alba* | 40.09 e | 12.81 |
| *Mansoa alliacea* | 43.21 d | 6.02 |
| *Plectranthus barbathus* | 43.61 d | 5.15 |
| Control | 45.98 c | - |
| *Azadirachta indica* | 46.64 c | - |
| *Cymbopagon citratus* | 47.46 b | - |
| *Ocimum gratissimum* | 48.19 b | - |
| *Aloe vera* | 48.53 b | - |
| *Pedilanthus tithymaloides* | 48.73 b | - |
| *Ocimum basilicum* | 48.75 b | - |
| *Morinda citrifolia* | 49.84 a | - |
| *Chenopodium ambrosioides* | 50.04 a | - |
| *Hibiscus sabdariffa* | 50.21 a | - |

**Averages followed by same letter do no differ significantly each other by Scott-Knott test at 5% probability*

In this study, the antimicrobial potential of the alcoholic extracts of medicinal plants was evident, with emphasis on the extract of *E. angulosa*, which promoted the inhibition of mycelial growth of all fungi tested.

No studies were found demonstrating the antifungal activity of *E. angulosa* extracts. However, Hedge et al. [14] reported inferior results. The extract of *Eucalyptus* sp. at the concentrations 5 and 10% reduced by 27.07 and 38.70%, respectively, the mycelial growth of *C. gloeosporioides*. Koma et al. [15] reported that the extract of *Eucalyptus* sp. completely inhibited the growth of *R. solani*.

*Eucalyptus* is a large genus of evergreen aromatic trees originating in Australia, Tasmania, New Guinea and neighboring islands. They form a large portion of the forest vegetation, giving it a distinctive appearance [16]. Among the biological activities reported for *Eucalyptus* sp. leaf extracts are antiseptic, antibacterial, anti-hyperglycemic, insecticides, antioxidant, cytotoxic and activity against upper respiratory tract infections [17]. The major classes of secondary metabolites isolated from different species of the genus *Eucalyptus* include floroglucinols, flavonoids and their glycosides, terpenes (monoterpenes, sesquiterpenes, triterpenes) and their glycosides, phenolics and their superior glycosides, steroids, tannins and polyphenols [18]. Thus, the antifungal activity of *E. angulosa* alcohol extract may be the result of the presence of one or more compounds of these chemical classes.

The activity of the extracts of *Z. officinale* and *L. alba* can also be highlighted. After the extract of *E. angulosa*, the extracts appeared frequently causing the greatest inhibitions of the growth of three fungi studied (*C. gloeosporioides*, *F. oxysporum* f. sp. *passiflora* and *R. solani*). Among the results presented, the extract of *Z. officinale* against *R. solani* showed slightly higher results than those reported by Choudhury et al. [19], who reported that *Z. officinale* hexane extract inhibited the growth of the pathogen by 9.26%. Hedge et al. [14] obtained superior results. The aqueous extract of *Z. officinale* at a concentration of 5% inhibited the growth of *C. gloeosporioides* by 39.99%. As reported by Ferreira et al. [20], the aqueous extract of *L. alba* at a 8% concentration inhibited the growth of *C. gloeosporioides* by 41.9%.

## 4. CONCLUSION

According to the results, it can be inferred that alcoholic extracts of medicinal plants represent a viable and ecologically correct strategy in the management of plant diseases through the antimicrobial action that they can exert against pathogens. All extracts tested had an antimicrobial potential. The extract of *E. angulosa* reduced the mycelial growth of all evaluated fungi. Thus, the *E. angulosa* extract represents a potential alternative for the control of diseases of *P. edulis* caused by phytopathogenic fungi.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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