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Antimicrobial Activity of Alcoholic Extracts of Medicinal Plants against Phytopathogenic Fungi

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Authors' contributions

This work was carried out in collaboration among all authors. Author CTBS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AKN, WPL and LCO discussed the results and corrected of the manuscript. Author OAL gave away the medicinal plants used in the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work 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*. Those are fungi that cause 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.

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Methodology: The extracts were prepared with 1.0 g of powdered plant material and 10 mL of commercial ethyl alcohol 92.8º (0.1 g mL^{-1}) under constant agitation 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 passionfruit, is one of the most important crops. Almost all commercial orchards are represented by *Passiflora edulis* Sims f. *flavicarpa* Deg. (yellow passionfruit). 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, such as *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 the resulting plant base rot are characterized by rot and plant death due to root and plant base tissues rotting [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, because, 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 research aimed 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. MATERIALS 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 herbarium specimens were

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 40ºC 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 92.8º 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 4ºC, 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 28ºC.

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 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 *C. gloeosporioides* growth.

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 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%.

In the evaluation of the effects of extracts on *F. solani*, only the *E. angulosa* extract was positive, differing 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*.

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.

In this study, the antimicrobial potential of the alcoholic extracts of studied 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*.

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 [16]. Thus, the antifungal activity of *E. angulosa* alcohol extract may be due to the presence of one or more compounds of these chemical classes. The antifungal activity of phenolic compounds of plants of the genus *Eucalyptus*, for example, was demonstrated by Oh, et al. that isolated

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*

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*

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*

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*

the gallic acid from *E. darlympleana* methanol extract and it was found to be effective in mycelial growth and spore germination of *Botrytis cinerea*, the fungal pathogen causing kiwifruit soft rot decay during postharvest storage [17].

The activity of the extracts of *Z. officinale* and *L. alba* can also be highlighted. After the extract of *E. angulosa*, the extracts appeared to cause frequently the greatest inhibitions of the three studied fungi growth (*C. gloeosporioides*, *F. oxysporum* f. sp. *passiflorae* and *R. solani*). Among the results presented, the extract of *Z. officinale* against *R. solani* showed higher results than those reported by Choudhury, et al. [18], 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. [19], the aqueous extract of *L. alba* at a 8% concentration inhibited of *C. gloeosporioides* growth by 41.9%.

These results provides important information for further isolation and characterization studies of active compounds of species *E. angulosa*, *Z. officinale* and *L. alba*, necessary for the development of biopesticides for the control de plant diseases caused by fungi.

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 *P. edulis* diseases caused by phytopathogenic fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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