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Anti-fungal activity of cold and hot water extracts of spices against fungal pathogens of Roselle (*Hibiscus sabdariffa*) *in vitro*

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ABSTRACT

Crude extracts of seven spices, *viz.* cardamom, chilli, coriander, onion, garlic, ginger, and galangale were made using cold water and hot water extraction and they were tested for their anti-fungal effects against the three Roselle pathogens i.e. *Phoma exigua, Fusarium nygamai* and *Rhizoctonia solani* using the 'poisoned food technique'. All seven spices studied showed significant anti-fungal activity at three concentrations (10, 20 and 30% of the crude extract) *in-vitro.* The cold water extract of garlic exhibited good anti-fungal activity against all three tested fungi. In the case of the hot water extracts, garlic and ginger showed the best anti-fungal activity. Of the two extraction methods, cold water extraction was generally more effective than hot water extraction in controlling the pathogens. Against *P. exigua*, the 10% cold water extracts of galangale, ginger, coriander and cardamom achieved total (100%) inhibition of pathogen mycelial growth. Total inhibition of *F. nygamai* mycelial growth was similarly achieved with the 10% cold water extracts garlic. Against *R. solani*, the 10% cold water extract of galangale was effective in imposing 100% inhibition. Accordingly, the 10% galangale extract effectively controlled both *P. exigua* and *R. solani in vitro*. None of the hot water extracts of the spices succeeded in achieving 100% inhibition of the pathogen mycelial growth.

1. Introduction

In Malaysia cultivation of Roselle is subjected to a number of diseases outbreaks some of the common diseases of Roselle reported were root rots, stem rot, leaf spot and *Fusarium* wilt caused by, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Cercospora hibisci* and *Fusarium oxysporum* [1]. The use of chemicals has helped increase of yields obtained but using of chemical products is discouraged as it causes environmental pollution, leaves toxic residues in the soil, water and food, harmful to non-target organisms leading to ecological imbalance and development of fungicidal resistant strains. All these limitations brought about changes in thinking for developing alternatives to plant disease management such as biological control [2,3]. Biological method of control has been preferred in some cases because it is selective with no side effect and cheap. Resistance to biological control is rare and biological control agents are self-propagating and self-perpetuating. It is becoming increasingly apparent that some plant are resistant to diseases caused by certain pathogens because of one or more inhibitory antimicrobial compounds, known as phytoanticipins which are present in the cell before infection. Chemically these are glycosylated steroidal or triterpenoid compounds having anti-fungal membranolytic activity [4]. In an attempt to reduce the use of synthetic pesticides, extensive investigations into the possible exploitation of plant compounds as natural disease control, which are safe for humans and the environment [5]. Plants are the sources of natural pesticides that make excellent leads for new pesticide development [6,7]. Many scientists have reported use of plant extract for the control of fungal diseases [8,9]. Plant extracts that have been used for the production of phytofungicides include "Fitoekols-IF" from pine (*Pinus sylvestris*) and spruce (*Picea abies*) greens extract, "Fitosativum" from garlic (*Allium sativum*) extract, "Fitocapsicum" from chilli pepper (*Capsicum annuum*) extract, "Fitokrisanthemium" from chrysanthemum (*Chrisanthemum* sp.) leaf extract, "Fitoarmoracium" from wild horse radish (*Armoracia rusticana*) root and leaf extract, "Fitotabacum" from tobacco (*Nicotiana tabacum*, *Nicotiana rustica*) extracts, "Fitopelargonium" from geranium (*Pelargonium* sp.) leaf extract and "Fitosinepium"-from white mustard (*Sinapis alba*) plant and seed extract [10]. Citrus fruits are well endowed with a variety of phytofungicides that help to inhibit fungal growth and development [11]. Some citrus extracts have proven to be effective as anti-fungal substances

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in vitro, for example against the growth of fungi such as *Phythophtora citrophthora*, *Verticillium dahliae*, *Penicillium* spp. and *Colletotrichum gloeosporioides.* Bianchi et al. [12], determined the effects of garlic on the development of mycelium in the following phytopathogenic fungi i.e., *Fusarium solani*, *R. solani*, *Pythium ultimum* and *Colletotrichum lindemuthianum*. The bio efficacy of Neem Seed Kernel Powder (NSKP), Karanj (*Pongamia pinnata*) Seed Kernel Powder (KSKP), Neem Leaf Powder (NLP) and Neem Seed Oil (NSO) against spermoplane mycoflora of neem seeds was studied to control seed-borne fungi. Neem seeds treated with NSO and NLP showed an inhibition in the growth of *Aspergillus flavus, Penicillium* spp. and *Mucor* spp. completely [13]. Harsh [14] conducted trials for the control of damping-off and wilt disease of *Albizia lebbek* caused by *Fusarium pallidoroseum.* It was found that application of *Cuscuta reflexa* extract was most effective in controlling the disease in the field by inhibiting the conidial germination and mycelial growth of the fungus. Diffusates of seeds from 32 plants were tested for antifungal properties against seed-borne fungi of sunflower (*Alternaria alternata, Emericellopsis terricola, F. solani, Macrophomina phaseolina* and *Stemphylium helianthi*) growing in Petri dishes [15]. Bajwa et al. [16] performed *in vitro* fungal toxicity assays with different concentrations of aqueous root and shoot extracts of four allelopathic grass species *Dicanthium annulatum, Imperata cylindrica, Cenchrus pennisetiformis* and *Desmostachya bipinnata* and found considerable susceptibility of both *Fusarium moniliforme* and *F. oxysporum* mycelial growth to the extracts. John et al. [17] evaluated the influence of tamarind leaf extract on the growth of several polypathogenic fungi (*Phythophtora palmivora*, *C. gloeosporioides*, *Alternaria solani*, *F. solani*, *Rhizoctonia bataticola*, *S. rolfsii*, *Pellicularia filamentosa* and *M. phaseolina*), which cause widespread crop damage. Tamarind leaf extract suppressed the growth of all the fungi spp. three days after inoculation and caused maximum suppression in *P. palmivora* and *R. bataticola*. The tamarind leaf extract offers great opportunities in its application as an anti-fungal botanical to control seed, soil and air borne phytopathogenic fungi. Jasso de Rodriguez et al. [18] evaluated leaf pulp liquid fraction of *Aloe vera* for inhibitory effects on the mycelial growth of *R. solani, F. oxysporum*, and *C. coccodes* that were isolated from a potato crop. The liquid fraction reduced the rate of colony growth of these pathogens. Haikal [19], evaluated the aqueous extract of three plant species (*Azadirichta indica, Ziziphus spina-Christi* and *Zygophyllum coccineum*) against the pathogenic fungus *F. solani*, the causal agent of root rots in cucumber seedlings. Somda et al. [20] tested aqueous extracts of *Cymbopogon citratus* (lemon grass), *Eucalyptus camaldulensis* and *A. indica* for inhibitory activity against *Colletotrichum graminicola*, *Phoma sorghina* and *F. moniliforme* in naturally infested sorghum seed. *C. citratus* extract exhibited the best control effect on seed infection by *C. graminicola* and *P. sorghina.* Eucalyptus and neem seed aqueous extracts were not effective in similarly controlling seed infection. Lemon grass extract has therefore good potential as a sorghum seed disinfectant against *C. graminicola* and *P. sorghina*.

2. Materials and methods

2.1. Pathogens isolation

The three fungal pathogens used in this study were *Phoma exigua*, *Fusarium nygamai* and *R. solani.* They were isolated from the leaves, fruits and petioles from Roselle plants in Penang, Malaysia [21].

2.2. Extractions of plants

A list of spice extracts used is given in Table 1. For the *in vitro* evaluation of the extracts, seeds and other parts of nine different plants bought from the vegetable market were used for the fresh preparation of extracts.

Table 1
List of plant extracts used to control pathogenic fungi.

Common name	Botanical name	Parts used
Cardamom	*Elettaria cardamonium*	Seeds
Chilli	*Capsicum annum*	Fruits
Coriander	*Coriandrum sativus*	Seeds
Onion	*Allium cepa*	Flax
Garlic	*Allium sativum*	Cloves
Ginger	*Zingiber officinale*	Rhizomes
Galangale	*Kaempferia galanga*	Rhizomes

2.2.1. Preparation of crude extractions

Extracts were prepared from fresh and dried plants parts according to methods described by Silva et al. [16]. The plant parts of each species were thoroughly washed in running tap water, surface disinfected in 90% ethanol for 2 min, and homogenized to a paste using a blender. A cold water extract was prepared by adding to each 100 g of paste 100 mL of sterile distilled water in a 250 mL Erlenmeyer flask. The mixture was stirred vigorously and allowed to stand for 24 h at room temperature. Hot water extracts were obtained by infusing the plant paste from each plant separately with 100 mL sterile distilled water using 250 mL Erlenmeyer flask in water bath at 90 °C for 1 h. The supernatant was passed through a Whatman® No. l filter paper, then a membrane filter (0.2 μm) to avoid bacterial or fungal contamination. The filtrate was regarded as 100% concentration and this was diluted with PDA medium in the ratio of 5:45 (10%), 10:40 (20%) up to 15:35 (30%) at about 50 °C. The mixtures were poured into sterile Petri plates and allowed to set. They were then used in *in vitro* tests against fungi mycelium growth.

2.3. Effect of crude plant extracts on mycelial growth of the pathogens

To evaluate toxicity of the extracts against the pathogens, Poisoned Food Technique or Radial Growth (RG) Test described by Golembiewski et al. (1995) was used. Six mm diameter discs of the test fungi were placed in the center of Petri dishes, which were then incubated at room temperature for eight days. Petri dishes of PDA medium without plant extract served as controls. Five replicates were used for each of the isolates tested. The colony diameter was measured and the mycelium inhibition percentage was calculated using following equation as given by Deans and Svoboda (1990).

$$\text{Inhibition}(\%) = [(C - T)/C] \times 100$$

Where

C is the colony diameter of the mycelium on the control dish (mm)
T is the colony diameter of the mycelium on treatment dish (mm)

2.4. Statistical analysis

The experimental data were analyzed using SPSS 15 for Windows (SPSS Inc. Chicago, U.S.A), employing basic statistical techniques. One-way (ANOVA) and Independent-Samples *T* Test were applied to determine the difference between the treatment groups and Tukey's Multiple Range test was used to find the significant difference among means at the probability level ≤ 0.05.

3. Results

3.1. Efficacy of crude plant extracts on mycelial growth of fungal pathogens

Seven crude spices extract at three concentrations (10, 20 and 30%), obtained by cold and hot water extraction, and were tested