

Full Length Research Paper

Extraction of *Jatropha curcas* fruits for antifungal activity against anthracnose (*Colletotrichum gloeosporioides*) of papaya

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Accepted 1 July, 2011

Extracts from seeds and leaves of *Jatropha curcas* have shown molluscicidal, insecticidal and fungicidal properties. *J. curcas* extracts were found to inhibit the mycelium growth of *Colletotrichum musae* that causes anthracnose disease in bananas. The antimicrobial activity of crude methanol extracts of *J. curcas* fruits, pulp and seeds were investigated. *J. curcas* fruits, pulp and seeds were collected from the farm of Universiti Putra Malaysia. The samples were air dried at ambient temperature, then oven-dried to remove the residual moisture. Equivalent amounts of each ground sample of the *J. curcas* fruits, pulp and seeds were soaked in methanol solvent and left to stand for 7 days before being filtered and evaporated. The extract was spread over potato dextrose agar (PDA) medium under an aseptic condition and incubated. The zone of inhibition of mycelial growth (mm) around the disc was measured. Both *J. curcas* seed and pulp extracts had higher antifungal activity than whole fruit extract. *J. curcas* seed extract showed significant antifungal activities with growth inhibition zone of 5.6 mm or equivalent to 78.87% inhibition followed by pulp with zone of 7.4 mm or equivalent to 72.07%, and whole fruits with zone of 14.2 mm or equivalent to 46.42% as compared to the control with zone of 26.5 mm or equivalent to 100%. Active microbial components in *J. curcas* have the potential of an antifungal compound to control *Colletotrichum gloeosporioides* which causes anthracnose disease in papaya *in vitro*.

Key words: Methanol extracts, inhibition zone, postharvest pathogen.

INTRODUCTION

Fungal diseases are the most common among the postharvest pathogens to cause decay and postharvest losses. Many alternatives to chemical control have been investigated to reduce postharvest diseases of fruits and vegetables. These include the use of biocontrol agents (Janisiewicz and Korsten, 2002), irradiation and other physical treatments (Nigro et al., 2002), natural antimicrobial substances (Ippolito and Nigro, 2003) and organic and inorganic compounds (Palou et al., 2002). A vital alternative to fungicides that is promising for managing postharvest diseases of fruits in a wide range

of crops is plant extracts. According to Thangavelu et al. (2004), the mycelial growth of *Colletotrichum musae* was inhibited by the *Jatropha curcas* leaves extracts which are able to control the anthracnose disease in three banana varieties: 'Robusta', 'Rasthali' and 'Ney Poovan'. Sepiah (1993) and Couey et al. (1984) reported that anthracnose of papaya has been studied by the application of fungicides, hot water dip treatment (HWT), or HWT in combination with fungicides at postharvest stage. But, HWT affect the ripening process of papaya as heat treatment enhance fruit softening. Currently, there is a growing public concern on the possible risks of the use of synthetic fungicides on food commodities to human health (Wilson and Wisniewski, 1994). According to Conway et al. (2004), use of commonly used fungicides leads to development of resistance within the population

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of postharvest pathogens.

In many subtropical and semi-arid regions, traditionally, *J. curcas* is used for its medicinal properties and its seeds contained semi dry oil which has been found to be useful for medicinal purposes. According to Rug and Ruppel (2000), seeds and leaves extracts of *J. curcas*, have shown molluscidal and insecticidal properties. They showed that methanol extracts of *J. curcas* had the highest activity against vector snails of the human parasites *Schistosoma* spp. Studies by Daouk et al. (1995) and Kishore et al. (1993) showed that essential oil of mugwort (*Artemisia vulgaris*) have antifungal activities against soil borne pathogens and food storage fungi. The plant can grow in a number of climatic zones of both tropical and sub-tropical regions of the world, especially in areas of low rainfall and poor soils. However, information on the use of extracts of *J. curcas* as an agent against anthracnose (*Colletotrichum gloeosporioides*) is still scarce. Therefore, this study was carried out to investigate the natural antifungal properties of *J. curcas* fruit extracts against anthracnose of papaya.

MATERIALS AND METHODS

Extraction of plant material

J. curcas fruits were collected 8 weeks after the formation of pin-head sized fruits from the Universiti Putra Malaysia farm. The collected fruits were washed with distilled water to remove dust particles. The fruits were air dried on a plastic tray at ambient temperature for 3 days, then dried in an oven (Memmert, ULM 500, Germany) at 50°C for 5 to 7 days to remove residual moisture. Final moisture content of the samples was 12% (MF-50, Moisture Analyzer, US). The plant materials were extracted according to the methods of Okoh et al. (2009) and Obafemi et al. (2006) with some modifications. Pulp and seeds were separated from the whole fruits manually using a knife. The whole fruit, pulp and seeds were ground separately by Polymix grinder (CZ 13, Cullaati MFC grinder, Netherlands). Each 20 g ground samples of whole fruits, pulp and seeds were put into a 250 ml Erlenmeyer flask and then soaked in 120 ml methanol solvent. Each flask was sealed with an aluminum foil and parafilm to prevent evaporation. The suspended solutions were left to stand for 7 days and then filtered (filter paper 90 mm, Toyo Roshi Kaisha, Ltd., Japan) and evaporated by vacuum using a rotary evaporator (Model CA-1310 Eyela, Tokyo Rikakikal Co., Ltd., Japan). The extracts were used as an antifungal agent against *C. gloeosporioides* by using 10 mg ml⁻¹ on the potato dextrose agar (PDA) media.

Isolation and identification of *C. gloeosporioides*

C. gloeosporioides were isolated from naturally infected fruits of papaya obtained from papaya fields of the Malaysian Agrifood Corporation, Lanchang, Pahang, Malaysia. Pieces of tissue were cut from the advancing margin of the lesion; surface sterilized in 5% sodium hypochlorite solution and washed in three changes of sterile distilled water. The tissues were dried on sterilized filter paper and then plated on PDA. The plates were incubated for seven days at 28 ± 2°C and observed every 24 h for 7 days. After the emergence of mycelial growth, each of the fungal colonies were transferred to fresh PDA plates and incubated at room temperature for 2 to 4 days

to obtain pure cultures. Fungal mycelium from fresh cultures were examined under the dissecting and compound microscope and identified by comparing their morphological and cultural distinctiveness with images (Sutton, 1992). PDA, the medium used for antifungal activity, was prepared according to standard instructions provided by the Dickinson and Company (Sparks, MD 21152 USA) and sterilized at 121°C and 15 psi for 15 min in an autoclave. Twenty-five milliliters of pre autoclaved PDA was poured into a 90 mm diameter pre sterilized Petri plates and allowed to solidify at room temperature.

Anti fungal activity of *J. curcas* fruits

10 mg ml⁻¹ of *J. curcas* plant extract, as used by Igbinosa et al. (2009), was spread over each PDA plate using an L-shaped sterile glass rod under aseptic condition in a laminar air flow. Then, a mycelial plug of 6 mm in diameter was taken from a 7 day old culture of *C. gloeosporioides*, using a sterile cork borer. Then, the culture was seeded in the center of each PDA plate. The inoculated plates were incubated for 72 h at 37°C. The diameter of the mycelial growth of *C. gloeosporioides* (colony diameter) on each of the PDA plate was measured using a digimatic caliper (CD-6" CSX, Mitutoyo Corp., Kawasaki, Japan). Antifungal action of the extract was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter of (Control - Treatment)}}{\text{Colony diameter of control}} \times 100$$

Experimental design and statistical analysis

A completely randomized design (CRD), with five replications, was used in this experiment. Data were analyzed using analysis of variance to determine the differences between treatments (SAS-Version 9.1; 2003). Least significant difference test (LSD, P < 0.05) was carried out for mean separation when the F test was significant.

RESULTS AND DISCUSSION

Figure 1 shows that *J. curcas* seeds and pulp extracts have higher antifungal activities than whole fruit extract. Extracts of *J. curcas* seeds showed antifungal activities with growth inhibition zone of 5.6 mm or equivalent to 78.87% followed by pulp with zone of 7.4 mm or equivalent to 72.07% and whole fruits with zone of 14.2 mm or equivalent to 46.42% as compared to the control with zone of 26.5 mm or equivalent to 100%. Figure 2 shows that fungal development was significantly higher in the control as compared to treatments with *J. curcas* extracts. Fungal development in extracts from the whole fruit was significantly higher from day 4 until day 7 as compared to extracts from seeds and pulp. Throughout the 4 days of treatment, there was a significantly lower fungal development in treatment containing whole *J. curcas* fruit extract as compared to the control.

After 4 days, there was a sharp increase in fungal development in the control, while there was only

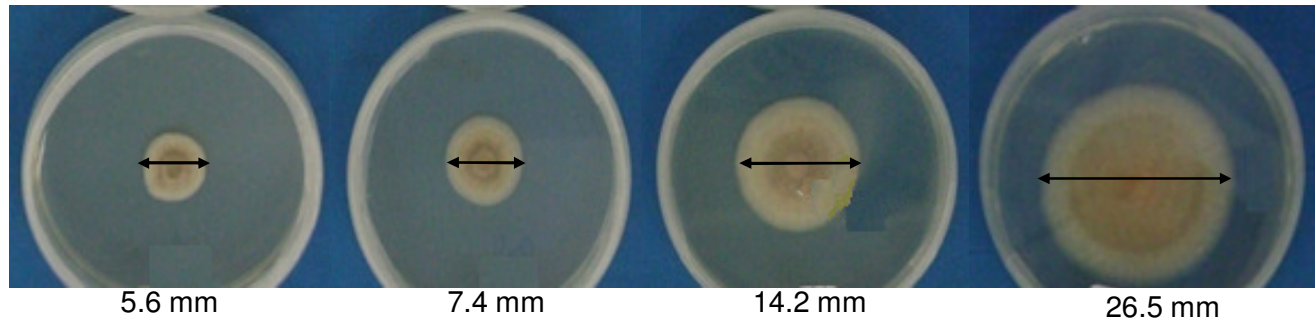


Figure 1. Zone of growth inhibition (mm) of *C. gloeosporioides* after incubation for 72 h at 37°C on potato dextrose agar medium containing *J. curcas* extracts from (A) seeds, (B) pulp, (C) whole fruit and (D) control.

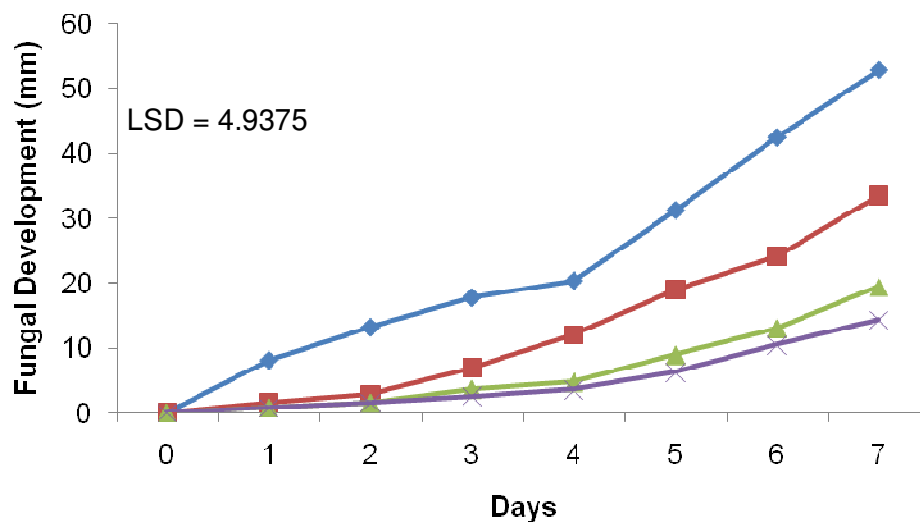


Figure 2. Development of mycelia growth of *C. gloeosporioides* during 7 days of inoculation on potato dextrose agar containing *J. curcas* extracts from (X) seed, () pulp, () whole fruit and () control (water only).

moderate fungal development in the whole fruit treatment until 7 days. However, treatment containing extracts from *J. curcas* pulp and seed showed significant differences in fungal development throughout the seven days after inoculation. Extracts of roots of *Jatropha podagrica* was reported to have moderate antifungal activity against *Candida albicans* (Aiyelaagbe et al., 1998). Our result is in agreement with the study of Ogbemor et al. (2007) who reported that the *J. curcas* leaves extract inhibited the mycelium growth of *C. gloeosporioides* in 'Para' rubber tree. Seed cake of *J. curcas* extracts caused complete inhibition of the mycelium growth of *C. gloeosporioides*, which is responsible for anthracnose, dieback, root rot, leaf spot, blossom rot and seedling blight of tropical fruit (Saetae and Suntornsuk, 2010). Extracts of *J. curcas* stem bark inhibit the growth of several bacterial and fungal species which is an indicator of the antimicrobial potential of this plant (Igbiosa et al., 2009). *J. curcas* fruits and seeds could be used as antifungal compound to control major postharvest diseases of fresh

horticultural produce *in vitro*. In this study, *J. curcas* seed and pulp extracts were more effective than whole fruit extract. *J. curcas* seed and pulp extracts have the potential to be natural fungicide against fungal phytopathogens, to replace synthetic fungicides in agricultural applications. Since this study was done under laboratory condition, further studies need to be done to determine if similar results could be obtained *in vivo*.

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