Potential Usage of an Ancient Plant *Lawsonia inermis* (Henna) and *Murraya koenigii* (Curry leaves) as Biofungicides against Damping off Disease Pathogens

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Abstract: Malaysia is one of the inheritance nations with rich medicinal plant and herbs species. Henna (*Lawsonia inermis*) and Curry leaves (*Murraya koenigii*) has been used in traditional herbal medicine for decades. Henna leaves have been traditionally used for dying hair, skin and nails since antiquity were found to exhibit strong fungi toxicity and non-phytotoxicity. Meanwhile, the leaves of *M. koenigii* also known as Kare, with aromatic smell are commonly used for food flavoring in some Indian and Asian cooking. Leaf extracts of *M. koenigii* have also been reported to possess antifungal, antineoplastic and antioxidant activities. However, the benefits of these plants have been discussed only in a few publications in regard to medical usage. The potential usage of this plant against plant pathogens was none. Thus, this study was conducted to evaluate the antifungal activity of the leaves extracts of *L. inermis* and *M. koenigii* in vitro against the *Rhizoctonia solani, Fusarium sp,* and *Pythium ultimum* pathogens of damping off disease. The presence of phytochemicals was also investigated. Leaves of *L. inermis* and *M. koenigii* were extracted with hexane, chloroform and methanol. The antifungal activity of both leaves extracts against damping off disease pathogen was determined by using well diffusion method on Potato Dextrose Agar (PDA). Among treatments, maximum in vitro inhibition was scored in methanol extracts of *L. inermis and M. koenigii* which offered inhibition zone of 100% (at 25,000ppm) and 58% (at 100,000ppm) against *R.solani;* 54% and 50% inhibition against *Fusarium sp* and 44% and 6% inhibition against *Pythium sp* respectively. However, *L. inermis* hexane extract showed higher inhibition against *Fusarium sp* (61.57%) compared to *R. solani* (29.89%) and *Pythium sp* (32.98%). Unfortunately the hexane extracts of *M. koenigii* did not show any inhibition. Chloroform extracts of Henna and Curry leaves were found to be ineffective or showed poor inhibition against all tested pathogens. The phytochemical screening revealed the presence of alkaloid, glycoside and tannin (in methanol extract) in both leaves extracts except flavonoid and triterpenoid which is only present in Henna extracts. These results suggested that *L. inermis* and *M. koenigii* leaves extracts potentially could be used to control damping off disease caused by *Pythium sp, Fusarium sp* and *R.solani.*

Keywords: Antifungal activity, Damping off disease pathogens, *Lawsonia inermis, Murraya koenigii,* Phytochemical analysis

1. Introduction

Thousands years ago, Malaysia had an extensive variety of plant species and traditional medicinal plant. The Malaysian traditional medicinal were influenced by Indonesian, Chinese, Indian, and Orang Asli traditional practices (Alsarhan, et al., 2014). Herbal medicines are in great demand in the developing countries because of their wide biological and medicinal activities and lesser cost (Agarwal, et al., 2014; Goswani, et al., 2011). In Malaysia, the value of herbal related products is more than RM 4.5 billion a year (Mardi, 2014). Malaysian plants are widely valued for their treasured components of medicines, seasonings, beverages, cosmetics and dyes and many of these plants are used to treat various human illnesses. Qader, et al. (2011) stated that screening botanical extracts for potential toxins is a main step to assess their suitability for the market.

Damping off disease caused by *Pythium sp, Phytophthora sp, Rhizoctonia solani, Sclerotium rolfsii,* and *Fusarium oxysporum* (Alaei and Rostami, 2013), is a conventional disease of vegetable seedlings which affects seed proliferation (Islam and Faruq, 2012) and infestations can occur at the age of seedling from pre-emerge, post emerge or even achieve the mature or woody stage (Wieland, 2012). For many years, a variety of different chemical and synthetic compounds have been used to control this disease. However, these chemical fungicides usually take a long time to be degraded completely, which may cause heavy toxicity to human and domestic animals (Damalas & Eleftherohorinos, 2011). In way to reduce the dependency to chemical fungicide, the alternative control using plant extract was introduced to provide the cheapest, biodegradable and environmental friendly fungicide (Darvin, 2013).

Lawsonia inermis (Lythraceae) commonly called "henna" or "inai" in Malay is abundantly available in tropical and subtropical areas and widely used in traditional medicine to treat various diseases and for cosmetic use. The leaves of *L. inermis* have been reported to contain various compounds like coumarins, flavonoids, gallic acid, naphthoquinones, naphthalene derivatives, triterpenoids, phenolic glycosides and xanthones (Chaudhary et al., 2010; Hsouna et al., 2011). Henna leaf has an orange-red dye and leaf paste or powder whih are widely used for decorating hands, nails, feet and hair in some occasions, such as weddings and religious festivals (Abdulmoneim, 2007). Henna has the potential for antibacterial activity against microorganism such as *Bacillus subtilis*, *E. coli*, and *Proteus sp*, (Elmanama et al., 2011) *Streptococcus gordonii*, *Klebsiella pneumonia* and *Micrococcus* (Mastaniah et al., 2011). Henna is also present in a good act in antifungal activity against microorganism such *C. albicans* and *Microsporum spp* (Elmanama, et al., 2011) *Colletotrichum notatum* and *Rhizopus stolonifer* (Jeyaseelan et al., 2012)

Curry leaf (*Murraya koenigii*) belongs to subfamily "Rutaceae", is a native of India and South-East Asia. Leaves of the plant are called "curry leaves" in English, or "Daun kari" in local Malay language. It is a small deciduous tree measuring about 6–9m height×15-40cm diameter with highly pungent aromatic leaves and characteristic taste and commonly used in some Asian cuisine (Parthasarathy, et al., 2008). Jain, et al., (2012) reported that chemical substances isolated from the fresh leaves of *Murraya koenigii* consist of carbazole alkaloids, triterpene and carotenoids in form of volatile oil have been used in pharmacological. *International Food Research Journal,* (2011) reported that curry leaves contain carbazole alkalois. Cheng, (2007) informed that these alkaloids have an antimicrobial activity against gram positive and gram negative bacteria and fungi. Vats, et. al. (2011) reported that the root extracts of *Murraya koenigii* possed remarkable antibacterial activity against *Staphylococus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Asperigillus niger*. Meanwhile, Sree, et al. (2012) found that the leaf extract of *Murraya koenigii* exhibited antifungal activity against many fungal strains, which include plant pathogens such as *Collentotrichum capsici, C. errassipes, Cladosporium and Armillaria melea.*

Hence, the objectives of the present study are (i) to evaluate the potential antifungal activity of *Lawsonia inermis* and *Murraya Koenigii* leaves extracts against damping off disease pathogens. (ii) to determine the phytochemical constituents in active *Lawsonia inermis* and *Murraya koenigii* leaves extract that act as antifungal activity.

2. Materials and Methods

2.1 Collection of plant sample

The fresh henna and curry leaves were collected at herbal nursery of UiTM Jengka, Pahang. The fresh leaves were air dried for seven days at room temperature. Then, the dried leaves were grinded into powder form and kept in the air tight container for further used.

3. Isolation of damping off disease pathogen

Damping off disease samples were collected from infected plant at share farm, UiTM Jengka. The samples were cut into small pieces and soaked in 10% bleach for five minutes and rinsed three times with sterile distilled water. They were then blotted dry with sterile tissue paper and three or two pieces of the sample were put on the media culture i.e Potato Dextrose Agar (PDA), Corn Meal Agar (CMA) and Water agar (WA). The plates were then incubated at 28-30°C for 2-3 days. The appearance of mycelium or hyphae on the media was observed daily and sub-cultured to the fresh media for pure culture.

4. Plant sample extraction (solvents extraction)

Henna and curry leaves powders were extracted by using a sequential extraction. The extractions were carried out with three differences polarities of organic solvents i.e. hexane, chloroform and methanol. About 600 gram of henna and curry leaves powder was soaked in hexane respectively for 24 hours and filtered with Whatman No1 filter paper. Later, hexane filtrates were collected and dried by rotary evaporator to get hexane extract. The residues of each samples were then soaked again with chloroform and methanol for 24 hours following the same procedure.

5. *In vitro* antifungal activity of *L. inermis* and *M. koenigii* leaves extracts against damping off disease fungal pathogens

The antifungal activity screening of henna and curry leaves extracts against damping off disease pathogen were conducted by well diffusion method. A plug of 7 day-old culture of damping off disease fungal pathogen was placed on the center of PDA plate. Then, two parallel holes with 5cm diameter were made at 2.5cm from the center of the plate with sterile cork borer. About 10µl of leaves extract were then pipetted into holes respectively. After that, the plates were incubated at 25°C for 7-day incubation. The radial growth of fungal pathogen in the plant extract was observed and measured daily. The percentages of inhibition radial growth (PIRG) were calculated using the following formula.

$$\text{Percentage inhibition of radial growth} = \frac{(R2-R1)}{R2} \times 100, \text{ where,}$$

R1 = The radial growth of the fungal pathogen in treatment plate.
R2 = The radial growth of the fungal pathogen in control plate.

6. Phytochemical screening of active plant extract

The phytochemical analysis was conducted to determine the presence of alkaloids, flavonoids, saponin, glycosides and tannins (Harborne, 1998).

7. Experimental design and statistical data analysis

Complete randomized designs (CRD) with 5 replications were used as the experimental design. The antifungal activity of henna and curry leaves extract against damping off diseases pathogens was carried out using one-way ANOVA with Minitab16 software. The Least significantly Different (LSD) were used to determine the mean comparison.

8. Results and Discussion

Antifungal activity of Henna and Curry leaves extracts against damping off disease pathogens

Results of the antifungal activity of henna (*L. inermis*) and curry leaves (*M. koenigii*) leaves extracts are shown in Table 1. Results show that methanol leaves extracts of Henna showed the highest growth inhibition against *Rhizoctonia solani* (100%) followed by chloroform leaves extracts with 59.79% and hexane leaves extract 29.89%. Furthermore, methanol leaves extracts of henna only gave 57% of growth inhibition against *Fusarium sp* compared to chloroform extracts (55.2%) and hexane extracts (61.57%). However, all the henna leaves extracts showed only little inhibition against *Pythium ultimum* with 44.325 (methanol extract), 34.03% (chloroform extract) and 32.98% (hexane extract). This indicated that henna (*L. inermis*) leaves extracts showed a good inhibition against *R. solani* and *Fusarium sp*. Meanwhile, methanol leaves extract of *Murraya koenigii* also gave the highest inhibition against *Rhizoctonia solani* (58%) compared to *Fusarium sp* (50%) and *Pythium ultimum* (6%). Moreover, chloroform leaves extract of *M koenigii* showed slightly inhibition on the growth of damping off disease pathogens with 47% (*R. solani*), 37% (*Fusarium sp*) and 14.5% (*P. ultimum*). However, hexane leaves extract of *M. koenigii* did not show almost any inhibition against all tested fungal pathogens.

Table 1. The percentage of inhibition of *Lawsonia inermis* and *Murraya koenigii* leaves extract against damping off disease pathogens

Type of extracts	Percentage radial growth inhibition (%)					
	Rhizoctonia solani		*Fusarium sp*		*Pythium ultimum*	
	Lawsonia inermis	*Murraya koenigii*	*Lawsonia inermis*	*Murraya koenigii*	*Lawsonia inermis*	*Murraya koenigii*
Methanol	100d	58c	57a	50 bc	44.32a	6 ab
Chloroform	59.79b	47b	55.26a	37b	34.03a	14.50a
Hexane	29.89a	8ab	61.57a	0a	32.98a	8a
+ ve control	85c	85c	80c	80c	80a	80a

*Lower case letter indicates significant differences (LSD<0.001)

Of all the extracts, *L. inermis* leaves extracts gave a good inhibition to all tested fungal pathogen compared to *M. koenigii* leaves extracts. This indicates that *L. inermis* leaves have potential as biofungicides to control damping off disease pathogens. According to Gull et al. (2013), *Lawsoia inermis* methanol leaves extract gave a higher inhibition against *Rhizoctonia sp* than chloroform leaves extract due to metabolites solubilised in solvent on the bases of polarity. Studied by Mohammedi and Atik (2011) reported that, crude extract or fraction expressing good biological capacity indicates that the substance with powerful biological effect exists in the *Lawsonia inermis* leaves extract and purified to confirm its pharmacological and medical use. Rajnikant, et al. (2015) stated that methanol extract of *Murraya koenigii* leaves extract showed higher antifungal activity against *Rhizoctonia solani* than against *Fusarium Oxysporum*. Meawhile, Balamurugan (2014) reported that crude aqueous and alcoholic extract of *Murraya*

koenigii exhibited strong fungi toxicity against *Rhizoctonia solani*. The findings of present study therefore coincide with previous study.

Phytochemical analysis of active leaves crude extracts

The phytochemical screening revealed that alkaloid, glycoside and tannin (methanol) were present in both leaves extracts except for flavonoid, triterpenoid which were only present in Henna extracts. However, glycoside and tannin (chloroform) and tannin (hexane) were only present in the *L. inermis* and *M. koenigii* leaves extract respectively (Table 3.2). Argal et al. (2011) stated that leaves extract of *Murraya koenigii* contain carbazole alkaloids and glycosides play a role to protect this plant from pathogenic microorganism. Every antioxidant activity of extract depends fully on its solvent because of different potential of antioxidant with different polarity (Zohra and Fawzia, 2011).

Table 2. Phytochemical constituents in *Lawsonia inermis* and *Murraya koenigii* leaves extracts

Phytochemical constituents	*Lawsonia inermis*			*Murraya koenigii*		
	Methanol	Chloroform	Hexane	Methanol	Chloroform	Hexane
Saponin test	-	-	+	-	-	-
Alkaloids	-	-	+	+	-	-
Triterpenes	-	-	+	-	-	-
Tannin	-	+	+	+	+	+
Flavonoids	+	+	+	-	-	-
Glycosides	-	-	+	+	+	-

4. Conclusion

It can be concluded that methanol leaves extract of *Lawsonia inermis* and *Murraya koenigii* gave the highest antifungal activity against *Rhizoctonia solani* inhibition as compared to *Fusarium sp* and *Pythium ultimum.* Furthermore, this study showed that the effective dosage of methanol leaves extract of Henna was at 25000 ppm against *R. solani*, for *Fusarium sp* was at 5000 ppm of hexane extract and *Pythium sp* was at 50000 ppm. Besides that, the effective concentration of Curry leaves extracts showed that methanol leaves extracts inhibit the growth of *Rhizoctonia solani* was at 100,000ppm and for *Fusarium sp* was at 250,000ppm. For the microscopic observation of antifungal activity, it showed the swollen of mycelium, crinkled and stunted of the hyphae and lysis of the spore, hyphae retarded and stunted spore. The phytochemical screening revealed that alkaloid, glycoside and tannin (methanol) were present in both leaves extracts except for flavonoid and triterpenoid which presence were only in Henna extracts. However, glycoside and tannin (chloroform) and tannin (hexane) was only present in the *L. inermis* and *M. koenigii* leaves extract respectively. Thus, this study reveals that *Lawsonia inermis* and *Murraya koenigii* have the alternative potential bio fungicide for antifungal activity in order to control the incidence of damping off disease in any seedling stage plant.

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