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ORIGINAL RESEARCH ARTICLE

Control of Plant Pathogenic Fungi using Organic Solvent Extracts of Leaf, Flower and Fruit of *Lawsonia inermis* L.

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ABSTRACT

In this study leaf, flower and fruit of *Lawsonia inermis* L. Powders were sequentially extracted with DCM, ethyl acetate and ethanol solvents. The dried extracts were tested for their antifungal activity against *Aspergillus niger*, *Penicillium notatum*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. The results revealed that all the test extracts were able to inhibit the growth of all the tested fungi except *Rhizopus stolonifer*. Ethyl acetate extract of flower revealed significantly ($P < 0.05$) higher activity on *Aspergillus niger*, *Penicillium notatum* and *Colletotrichum gloeosporioides*, while highest inhibition on *Fusarium oxysporum* was expressed by ethyl acetate extract of leaf and on *Rhizopus stolonifer* was by ethanol extracts of leaf and fruit. The ethyl acetate and ethanol extracts of flower and leaf were able to inhibit the growth of most of the test fungi even at 1mg / 100 µl. Among the tested eight phytochemicals at least two of them were detected in all tested extracts. Ethanol extracts had higher number of phytochemicals in all three tested plant parts, it was followed by ethyl acetate extracts and then DCM extracts. All the ethanol extracts had tannins, flavonoids, terpenoids and cardiac glycosides. According to the results of this preliminary study, ethyl acetate and ethanol extracts of both flower and leaf could be used as a source for further screening of bio-pesticides.

Key words: *Lawsonia inermis*, antifungal activity, plant pathogens, bio-pesticides.

INTRODUCTION

Lawsonia inermis L. (Maruthondi - Tamil, Henna - English), member of the family Lythraceae is a popular medicinal plant in Jaffna peninsula, Sri Lanka. It is widely distributed in dry zones of the Sri Lanka, India, Africa, Arabia and Persia. Henna is a glabrous, much branched deciduous shrub. Its lateral branches often ending in spines and leaves are simple, opposite, entire, lanceolate, petioles very short or absent. Flowers white colored, fruits globose capsules with numerous seeds.[1]

Because of its tremendous bioactivity, almost all parts of the plant have been used in various traditional medical practices in North and East part of the Sri Lanka. The seeds are frequently used as an ingredient of medicines for the treatment of intermittent fever, insanity, dysentery, diarrhoea, and amentia.[2] Decoction of the bark is used for the treatment of spleen enlargement and skin diseases, and the root is specific for leprosy.[1] Leaves and flowers are also better sources for the management of various ailments; leaves are useful for the treatment of diarrhoea, dysentery, leprosy, scabies and boils, and the flowers are used in cephalalgia, burning sensation, sardiopathy, anemia, insomnia and fever. [3]

Due to the wide applications of *L. inermis* in traditional medical practices, it has been intensively subjected for screening of pharmaceutical products against a range of diseases and disorders. For instance, phenolic compounds isolated from methanol extract of *L. inermis* leaves inhibited the osteoclastogenesis in murine bone-marrow macrophages,[4] ethanolic extract of seeds showed efficient antioxidant activity,[5] ethanolic extract of whole plant revealed significant antidiabetic effect on rats,[6] aqueous extract can be used as a supplementary agent for cancer treatment,[7] ethanolic extract of leaf have more anti ulcer activity,[8] and there are number of studies revealed the antimicrobial activity of aqueous and different organic solvent

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extracts of *L. inermis* against human pathogenic bacteria and fungi. [9, 10]

The usage of synthetic pesticides in modern agriculture farming have several drawbacks, such as residual effect on environment, very expensive when concern farmers in developing countries, toxic to non-targeted microbes and development of resistance in pathogenic microbes. This background creates a need to find out eco-friendly, less expensive and effective alternative pesticides. Due to the abundant nature of diverse chemicals and their intrinsic resistance properties against pests, medicinal plants are also being as important target for alternative pesticides. [11, 12]

The present study was carried out to reveal the feasibility of using organic solvent extracts of fruit, flower and leaf of *L. inermis* as pesticide against some selected plant pathogenic fungi.

MATERIALS AND METHODS

Collection of plant materials

Fresh and healthy leaves, flowers and fruits of *Lawsonia inermis* were collected from the botanical garden of the Department of Botany, University of Jaffna, Sri Lanka. The collected plant parts were thoroughly washed under running tap water, dried in shade and then ground into fine powders using an electric grinder. These powders were stored in air sealed brown bottles at 4 ^{0}C until used for the extraction.

Preparation of plant extracts

Each powdered plant parts of *L. inermis* was successively extracted with different organic solvents in the increasing polarity order according to Jeyaseelan *et al.* [13]. Briefly, 100 g of each powder was soaked in 300 ml Dichloromethane (DCM) separately with intermittent shaking for three days. They were first filtered with double layered muslin cloth and then through WhatmanNo1 filter paper. The residue was further extracted two times by using fresh DCM solvent. Then all the filtrates were pooled together. The resulting residue was air dried and used for further extraction with ethyl acetate and followed by ethanol similar to the procedure that carried out for the DCM extraction. Finally each filtrate was condensed using rotary evaporator under reduced pressure and low temperature. The extracts were stored at 4 ^{0}C until used for further study.

Test fungi

The test fungal pathogens, *Aspergillus niger, Penicillium notatum, Fusarium oxysporum, Colletotrichum gloeosporioides* and *Rhizopus stolonifer* were isolated from diseased plant materials and identified based on their morphological features and pathogenicity. These fungi were stored on potato dextrose agar (PDA) slants at 4 ^{0}C until used for further study.

Antifungal assay

Test extracts of *L. inermis* leaf, flower and fruit were prepared separately by dissolving 100 mg of extract in the mixture of 100 µl dimethyl sulfoxide (DMSO) and acetone. The *in vitro* antifungal activity of the crude extracts was tested by agar well diffusion method. PDA plates were prepared and 0.1 ml (10^4 spores / ml) of each fungal inoculum was seeded on the entire surface of the agar by spreading the inoculum with the help of sterile glass spreader. 8 mm diameter wells were punctured by using sterile cork borer and 100 µl of each extract was added into the well separately. The antifungal agent, Mancozeb (Dithane M-45) (200 µg/100 µl) was used as standard and the mixture of (DMSO) and acetone (1:1 v/v) was used as control. Plates were incubated at room temperature and the diameter of zone of inhibition was recorded after five days. Each experiment was conducted three times.

Determination of lowest antifungal concentration

Different concentrations of test extracts were prepared by dissolving the extracts in the mixture of dimethyl sulfoxide (DMSO) and acetone (1:1 v/v), 1 mg/100 µl, 25 mg/100 µl, 50 mg/100 µl and 75 mg/100 µl of each extracts were used to test antifungal activity as the method explained above. Each experiment was conducted three times.

Phytochemical screening

All the extracts of leaf, flower and fruit of *L. inermis* were analyzed for the presence of alkaloids, terpenoids, cardiac glycosides, saponins, tannins, flavonoids, phlobatannis and steroids. [14]

Statistical analysis

The results obtained for antifungal activity were given as mean value ± standard deviation and the data were subjected to examine by analysis of variance (ANOVA) ($P<0.05$) followed by Tukey's test ($\alpha = 0.05$) by using a software, SPSS 13.0 for Windows version.

RESULTS

Antifungal activity of different solvent extracts of *L. inermis* flower, fruit and leaf revealed that all the test extracts were found to be inhibitive to the growth of all the test fungi except *R. stolonifer* and the inhibitory effect differed significantly *(P<0.05)* with respect to the type of extract. All three flower extracts and DCM and ethyl acetate

extracts of fruit were failed to inhibit the growth of *R. stolonifer* (**Table 1**).

Ethyl acetate extract of flower showed significantly *(P<0.05)* highest inhibitory effect on *A. niger, P. notatum,* and *C. gloeosporioides* compared to other test extracts of flower as well as fruit and leaf. *F. oxysporum* and *R. stolonifer* were highly inhibited by ethyl acetate extract of leaf and ethanol extract of fruit and leaf respectively (Table 1).

Flower and fruit ethyl acetate extracts and ethanol extracts demonstrated relatively higher activity compared to DCM extracts of both flower and fruit. Among the fruit extracts, the ethanol extract showed better activity on *A. niger, P. notatum, F. oxysporum,* and *R. stolonifer*. But *C. gloeosporioides* was highly inhibited by ethyl acetate extract of fruit than ethanol extract of fruit (Table 1).

Among the leaf extracts, ethyl acetate had better inhibitory effect on *P. notatum*, *C. gloeosporioides* and *F. oxysporum* and it was followed by DCM extract. For *A. niger* and *R. stolonifer* ethanol extract had higher activity.

The standard Mancozeb showed inhibition on all test fungi. Furthermore, the diameter of inhibition zones produced by Mancozeb against test fungi were relatively smaller than that produced by some of the test extracts. The solvent mixture used as control did not inhibit the growth of any of the test fungi (Table 1).

The study with different concentration of test extracts showed that the antifungal effect of the extracts was dose dependent. The ethyl acetate and ethanol extract of flower and leaf were found to be effective even at 1 mg/100 µl against most of the test fungi except *R. stolonifer*. DCM extract of leaf and ethanol extract of fruit also had inhibition on the growth of *P. notatum, F. oxysporum* and *C. gloeosporioides* at the lowest test concentration. Other test extracts revealed inhibitory effect at higher concentrations only (**Table 2**).

The qualitative phytochemical analysis revealed that among the tested eight phytochemicals, at least two of them were able to detect in tested DCM, ethyl acetate and ethanol extracts of fruit, flower and leaf of *L. inermis* (**Table 3**). The number of available phytochemicals in the extracts revealed a tendency with the polarity of organic solvent used, with the increasing polarity of the solvent number of extracted chemicals also increases in all three parts of the plant. All the test extracts showed positive results for cardiac glycosides test and negative results to phlobatannins and steroids.

Among the tested DCM extracts, flavonoids were in leaf and fruit extracts, and the terpenoids were in fruit and flower extracts. However, other phytochemicals excluding cardiac glycosides were not present in any of the tested DCM extracts.

In the case of ethyl acetate extracts of *L. inermis*, flavonoids and cardiac glycosides were present in all three ethyl acetate extracts. Terpenoids were detected only in fruit and flower ethyl acetate extracts. Tannins and alkaloids were found only in ethyl acetate extracts of fruit and leaf respectively.

The phytochemical profiles obtained for the ethanol extracts of leaf, flower and fruit of *L. inermis* was identical to one another except the result produced for saponins. All these three extracts had tannins, flavonoids, terpenoids and cardiac glycosides. However, saponins were present only in fruit and flower extracts.

Table 1: The inhibitory effect of *L. inermis* at 100 mg/100 µl concentration

Plant part	Test extract	Diameter of inhibition zone (mm)*				
		A. niger	***P. notatum***	***C. gloeosporioides***	***F. oxysporum***	***R. stolonifer***
Flower	DCM	14.1 ± 0.6^{f}	13.1 ± 0.5^{f}	24.1 ± 0.6^{e}	12.1 ± 0.7^{h}	-
	EA	32.2 ± 1.2^{a}	32.2 ± 0.8^{a}	37.2 ± 1.3^{a}	18.0 ± 0.6^{f}	-
	EtOH	23.0 ± 0.8^{b}	21.1 ± 0.3^{b}	30.1 ± 0.8^{b}	26.2 ± 0.4^{c}	-
Fruit	DCM	12.0 ± 0.9^{g}	13.1 ± 0.3^{f}	16.1 ± 1.0^{g}	14.1 ± 0.3^{g}	-
	EA	12.1 ± 0.7^{g}	14.1 ± 0.6^{e}	24.1 ± 0.9^{e}	20.1 ± 0.9^{e}	-
	EtOH	20.1 ± 0.4^{d}	18.2 ± 0.4^{d}	20.1 ± 0.6^{f}	24.0 ± 0.7^{d}	22.1 ± 0.9^{a}
Leaf	DCM	16.0 ± 0.6^{e}	19.1 ± 0.3^{c}	26.1 ± 0.7^{d}	28.2 ± 0.9^{b}	14.1 ± 0.3^{c}
	EA	20.1 ± 0.7^{d}	21.1 ± 0.5^{b}	28.1 ± 0.4^{c}	32.1 ± 0.6^{a}	15.1 ± 0.5^{b}
	EtOH	21.2 ± 0.8^{c}	14.2 ± 0.5^{e}	24.0 ± 0.6^{e}	20.1 ± 0.4^{e}	22.1 ± 0.4^{a}
Mancozeb		26.4 ± 0.8	26.4 ± 0.8	26.0 ± 0.6	20.4 ± 0.9	19.4 ± 0.6
Control (DMSO:Acetone)		-	-	-	-	-

**Diameter of inhibition zones includes the diameter of well (8mm); Values = mean ± SD; Values with different superscript in the same column differ significantly (P<0.05); DCM – Dichloromethane; EA – Ethyl acetate; EtOH – Ethanol.*

Table 2: The inhibitory effect of *L. inermis* at different solvent concentrations

Plant parts	Extract	Test concentration (mg/100 µl)	Diameter of zone of inhibition (mm)*				
			An	*Pn*	*Cg*	*Fo*	*Rs*
Flower	DCM	1	-	-	-	-	-
		25	-	-	9.2 ± 0.3	-	-
		50	12.6 ± 0.5	-	14.2 ± 0.9	-	-
		75	13.4 ± 0.9	10.4 ± 0.8	19.2 ± 0.7	9.6 ± 0.3	-
	EA	1	10.1 ± 0.4	14.3 ± 0.6	12.1 ± 0.8	9.4 ± 0.6	-
		25	12.7 ± 0.8	17.5 ± 0.4	16.4 ± 1.1	11.2 ± 0.4	-
		50	18.3 ± 1.2	21.5 ± 0.4	21.2 ± 0.7	13.2 ± 0.8	-
		75	26.7 ± 0.4	28.4 ± 0.7	34.3 ± 0.6	15.7 ± 0.4	-
	EtOH	1	11.2 ± 0.8	9.4 ± 0.5	11.4 ± 0.6	11.1 ± 0.7	-
		25	12.3 ± 0.8	11.2 ± 0.6	15.4 ± 1.0	15.3 ± 0.6	-
		50	16.4 ± 0.4	15.4 ± 0.8	21.4 ± 0.8	18.1 ± 0.7	-
		75	20.1 ± 0.6	20.1 ±0.4	26.2 ± 0.4	21.7 ± 0.8	-
Fruit	DCM	1	-	-	-	-	-
		25	-	-	-	-	-
		50	-	-	-	9.1 ± 0.4	-
		75	11.2 ± 0.6	10.2 ± 0.4	11.2 ± 0.8	11.5 ± 0.4	-
	EA	1	-	-	-	-	-
		25	-	-	11.4 ± 0.7	12.7 ± 0.6	-
		50	-	9.2 ± 0.3	14.2 ± 0.6	14.3 ± 0.9	-
		75	10.3 ± 0.5	11.4 ± 0.7	19.5 ± 0.6	17.2 ± 1.0	-
	EtOH	1	-	9.6 ± 0.6	9.2 ± 0.6	9.4 ± 0.7	-
		25	-	12.2 ± 0.5	11.5 ± 0.8	11.2 ± 0.3	-
		50	11.2 ± 0.7	14.3 ± 0.3	14.3 ± 0.3	15.4 ± 0.5	13.1 ± 1.1
		75	16.2 ± 0.9	16.1 ± 1.2	15.8 ± 0.9	19.1 ± 0.7	17.2 ± 0.7
Leaf	DCM	1	-	9.2 ± 0.6	11.2 ± 0.5	11.3 ± 0.6	-
		25	9.4 ± 0.5	14.7 ± 0.5	18.1 ± 0.4	18.2 ± 1.2	-
		50	11.2 ± 0.4	15.1 ± 0.7	19.7 ± 0.4	21.3 ± 1.1	10.1 ± 0.9
		75	13.4 ± 0.6	16.2 ±0.4	23.1 ± 0.6	24.3 ± 0.4	10.8 ± 0.4
	EA	1	10.4 ± 0.8	10.2 ± 0.6	10.2 ± 0.9	12.3 ± 0.7	-
		25	11.7 ± 0.3	14.7 ± 0.7	15.4 ± 1.0	18.2 ± 0.5	-
		50	15.2 ± 0.4	16.1 ± 0.5	20.7 ± 0.4	24.3 ± 0.4	
		75	18.2 ± 0.9	17.4 ± 0.9	24.1 ± 0.4	27.1 ± 0.9	11.2 ± 0.6
	EtOH	1	9.2 ± 0.5	-	10.3 ± 0.7	9.5 ± 0.5	-
		25	11.1 ± 0.9	-	13.1 ± 0.5	11.2 ± 0.6	-
		50	14.2 ± 0.7	10.1 ± 0.4	17.2 ± 0.3	14.3 ± 0.4	11.2 ± 0.8
		75	18.2 ± 0.6	13.3 ± 0.7	20.1 ± 0.7	17.3 ± 0.4	14.3 ± 0.4

**Diameter of inhibition zones includes the diameter of well (8mm); Values = mean ± SD; DCM – Dichloromethane; EA – Ethyl acetate; EtOH – Ethanol. An - Aspergillus niger; Pn - Penicillium notatum; Cg- Colletotrichum gloeosporioides; Fo- Fusarium oxysporum; Rs - Rhizopus stolonifer*

Table 3: Phytochemical constituents of different organic solvent extracts of flower, fruit and leaf of *L. inermis*

Phytochemicals	Leaf			Fruit			Flower		
	DCM	EA	EtOH	DCM	EA	EtOH	DCM	EA	EtOH
Tannins	-	-	+	-	+	+	-	-	+
Phlobatannins	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	+	-	-	+
Flavonoids	+	+	+	+	+	+	-	+	+
Steroids	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	-	-	-	-	-	-	-

+ =Present; - = Absent; DCM – Dichloromethane; EA – Ethyl acetate; EtOH – Ethanol.

DISCUSSION

In the present study sequentially extracted different organic solvent extracts of leaf, flower and fruit of *L. inermis* were tested for their antifungal activity against some selected phytopathogenic fungi. The outcomes of this study provide further knowledge on bioactivity of *L. inermis* and it may be useful for people who interested on alternative bio – pesticides.

Number of previous studies with different plants and extracting solvents showed that the successful extraction of active compounds from plant powder is greatly depends on the type of the solvent used for the extraction. [15] In the present study, the plant materials were subjected for organic solvent extraction in sequential manner. Even though in the traditional practice water is the most widely using solvent, in the present study only organic solvent extraction was carried because of several reasons; certain enzymes like phenol oxidase inactivate phenol in aqueous medium but not in organic solvent medium, water act as a better

medium for the occurrence of the micro-organisms as compared to ethanol,[16] and also lawsone, the active principle present in henna, is much soluble in organic solvent like ethanol and methanol than in water.[17] The sequential extraction with solvents in increasing polarity was done to ensure extraction of wide polarity range of compounds, because extraction of phytochemicals depends on the polarity of extracting solvent and the polarity of the chemical being extracted. [15]

All the test extracts of fruit, flower and leaf of *L. inermis* revealed better antifungal activity against all tested fungi, except *R. stolonifer*. This implies the presence of effective antifungal compounds in the extracts. The ability of leaf extracts to inhibit the growth of all the tested fungi including *R. stolonifer* indicates their broad spectrum of activity.

In most cases ethyl acetate and ethanol extracts exhibited greater activity compared to DCM extracts. That may be due to the differences in the type or amount of active constituents present in the extracts. The better activity of ethanol extracts of *L. inermis* have already been reported in several studies.[5, 6, 8] Even though ethyl acetate is not a very commonly using extracting solvent in antimicrobial studies as ethanol, some studies reported the higher antimicrobial activity of the ethyl acetate extract in different plants. [13] Therefore, like ethanol and methanol, ethyl acetate also can be widely used for antimicrobial screening purpose.

The results obtained for the phytochemical test shows correlation with previous studies in some ways and contradiction in some other ways.[17, 18, 19] The variations might be due to the differences in mode of extraction, solvents used for the extraction or differences in the climatic conditions where the plants grow. [20] As observed in the present study, Koruthu *et al*. [21] reported that with the increasing polarity order the amount of extracting phytochemicals also increases. Therefore, this result reinforces the influence of solvent polarity on the type of extracting phytochemical.

Phytochemiclas are secondary metabolic products of plants; they play several roles in the growth and development of plants as well as they protect the plant from pathogenic microorganisms. These pytochemicals act in different ways on pathogenic microorganisms [22]. In the present study, higher antifungal activity of ethyl acetate and ethanol extracts of *L. inermis* could be due to the available phyotchemicals. However, further study *in vivo* condition with the above crude extracts or purified compounds from the extracts is needed to confirm the feasibility of using these extracts as bio-pesticide to control plant pathogenic fungi.

CONCLUSION

In conclusion, present preliminary study for the antifungal activity of different parts of *L. inermis* clearly revealed that the ethyl acetate and ethanol extracts of flower and leaves are suitable sources for further screening of bio-pesticides.

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