Journal of Natural Pesticide Research 12 (2025) 100116

Contents lists available at ScienceDirect

Journal of Natural Pesticide Research

journal homepage: www.journals.elsevier.com/journal-of-natural-pesticide-research

In vitro analysis of antifungal activity of the selected weed species against *Rhizoctonia solani* Kuhn

Sanduni Iresha Vimalaveera [a,*], Jeyagowri Nimalan [a], Ashoka Gamage [b], Othmane Merah [c,d,**], Terrence Madhujith [e]

[a] Department of Bioscience, Faculty of Applied Science, University of Vavuniya, Vavuniya, Sri Lanka
[b] 118, Riverbend Research Center North 110 Riverbend Road Athens, GA 30605 USA
[c] Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRAe, INPT, Toulouse 31030, France
[d] Département Génie Biologique, IUT A, Université Paul Sabatier, Auch 32000, France
[e] Department of Food Science and Technology, University of Peradeniya, Peradeniya 20420, Sri Lanka

ARTICLE INFO

Keywords:
Antifungal activity
Bio fungicide
Oryzae sativa
Phytochemical
Rhizoctonia solani
Sheath blight

ABSTRACT

The fungus *Rhizoctonia solani* Kuhn (*R. solani*) is the causative agent of the infection of sheath blight disease, which has harmful effects on rice (*Oryzae sativa*) cultivation in Sri Lanka. The approach to combating the disease by applying fungicide is expensive and builds a hazardous environment for humankind, fauna and flora. The present study aimed to assess the *in vitro* antifungal activity of extracts of *Calatropis gigantea* (L.) W.T.Aiton (giant milkweed), *Antigonon leptopus* Hook. and Arn. (coral vine), and *Parthenium hysterophorus* L. leaves and flowers against *R. solani* to control the sheath blight disease in rice cultivation. To substantiate the antifungal properties, various chemical tests were performed on the dry powder of the leaves and flowers to detect certain phytochemicals, including glycosides, tannins, saponins, proteins, flavonoids, terpenoids, and phenol. The extraction process was done by using different solvents, such as hexane, acetone and distilled water. Stock solutions were prepared by adding 10 ml of the solvent to the crude extracts. The antifungal assay and finding the Minimum Inhibition Concentration (MIC) value were performed using the poisoned food technique and IC 50 and IC 90 values were calculated by probit analysis. Data were analyzed using IBM SPSS Statistics (one way ANOVA and DMRT test) at 0.05 significant level. The majority of tested phytochemicals were found in the leaves and flowers. Compared to the hexane extracts of the studied weed species, acetone and distilled water extracts demonstrated antifungal activity against *R. solani* and the acetone extract of the flowers of *A. leptopus* exhibited the strongest antifungal properties. The acetone extract of *A. leptopus* flowers had the MIC value of 4.85 mg/ml, and IC 50 and IC 90 values were 1.74 mg/ml and 4.66 mg/ml, respectively. More than 60 % of growth inhibition was reported by the distilled water extract of the leaves of *A. leptopus* and *P. hysterophorus* and these extracts can be applied as a homemade fungicide. The acetone extract of the flowers of *A. leptopus* is an appropriate agent to scrutinize the potential of formulating a novel bio fungicide to manage sheath blight disease in rice cultivation.

1. Introduction

Twofold of the world's population consume rice on a daily basis, and it is the single most important crop in the world. About 20 % of the energy in food comes from rice, while more than 70 % of the calories in some Asian nations are obtained from rice (Rubaiyath and Zhang, 2023). More than 100 nations cultivate rice, with Asian nations producing 90 % of the world's total (Fukagawa and Ziska, 2019). As a consequence of global population expansion, rice consumption is rising gradually, and general agricultural production will need to expand by 60 % in order to fulfill the global food demand by 2050. Farmers are experiencing challenges producing sufficient quantities of rice to meet demand because of urbanization and fungal rice diseases like rice blast and sheath blight (Durgeshlal et al., 2019).

Rice diseases (rice blast and sheath blight) are one of the main biological factors accountable for rice yield loss globally. The diseases that have a harmful economic impact in Sri Lanka are bacterial leaf blight, rice blast, sheath blight, and brown spot (Mithrasena et al., 2010).

* Corresponding author.
** Corresponding author at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRAe, INPT, Toulouse 31030, France.
E-mail addresses: sanduni.i@kelanicables.com (S.I. Vimalaveera), othmane.merah@ensiacet.fr (O. Merah), tmadhujith@agri.pdn.ac.lk (T. Madhujith).

https://doi.org/10.1016/j.napere.2025.100116
Received 31 December 2023; Received in revised form 7 February 2025; Accepted 17 February 2025
Available online 18 February 2025

Sheath blight affects 15–20 million hectares of rice annually in China alone and can result in losses of up to six million metric tons of rice, or nearly 1 % of the world's total rice crop. According to the estimates, the annual losses brought by sheath blight in India and Thailand range from 10 % to 20 % (Tsiboe et al., 2017). There are no genetically modified resistant varieties against this disease, and the only available disease management method is fungicide application (Attanayake et al., 2010). Most Sri Lankan rice types are easily infected with the disease (Deparment of agriculture Sri Lanka, 2022).

World Health Organization mortality database indicates more than seven lakh of Unintentional Acute Pesticide Poisoning (UAPP) cases are reported annually, and approximately 7500 fatal cases occur. About 44 % of farmers suffer UAPP annually, notably the region with the highest estimated number of UAPP cases is southern Asia, followed by Southeast Asia (Boedeker et al., 2020). Sri Lankan agriculture department has recommended some fungicides, such as hexconazole, propiconazole, thiophanate, pencicuron and tebuconazole for control of sheath blight disease (Deparment of agriculture Sri Lanka, 2022). Overuse of some recommended fungicides (hexconazole) has an effect on soil microorganisms, reducing soil fertility and increasing the risk of nitrogen loss from paddy soil (Ju et al., 2017). Hexconazole poisons the human nerve system, causing neurotoxic symptoms such as trembling, jittering, and shaking (David et al., 2008). Plant-based products and biocontrol agents are sustainable and nonchemical disease management strategies. These can act as affordable and environmentally safe substitutes for synthetic products (Sharf et al., 2021). The use of natural agents to manage fungal diseases in plants is beneficial instead of synthetic chemicals. Botanical pesticides are less expensive, and even farmers can easily make their own crude extracts (Cherkupally et al., 2017). Currently, researchers are looking into environmentally friendly ways to manage plant diseases. Among these, use of naturally occurring plant-based antifungal compounds is becoming significant against plant pathogens (Afzal et al., 2023).

The leaves and flowers of *P. hysterophorus*, *C. gigantea* and *A. leptopus* were chosen for the study. These plants are abundant in the environment and some of them are invasive species in Sri Lanka. These weeds have potential antimicrobial properties. Antifungal bioassays were carried out by using aqueous, methanol and n-hexane extracts of roots, shoots and leaves of *P. hysterophorus* against *Fusarium solani* and this study revealed that n-hexane extract was more efficient at inhibiting growth of *F. solani* than aqueous and methanol extracts of leaves and flowers (Zaheer et al., 2012). *In vitro* antifungal activity of leaf extracts from five invasive plant species including *A. leptopus* was assessed against the fungal pathogen *Macrophomina phaseolina*. All of the plants' methanolic extracts had good activity, with MIC values varying from 0.078 to 2.5 mg/ml (Rashmi and Rajkumar, 2012). The inhibitory effect was assessed for ethanolic extract of the latex of *C. gigantea* against various human pathogen fungi (*Aspergillus niger*, *Candida albicans*) and this study disclosed a considerable antifungal effect with MIC values ranging from 1 to 8 mg (Saratha and Subramanian, 2010). Therefore, these three weed species were selected for this study.

Due to the current economic crisis (2022) in Sri Lanka, agrochemical importation is restricted, and available agrochemicals are expensive. Purchasing these agrochemicals is challenging for local farmers. Antifungal extracts derived from plant materials can be easily prepared at household level at minimum expenses and technology. The Sri Lankan government attempts to go towards organic farming. In this concept, it is appropriate to find a suitable antifungal source to formulate a plant based fungicide to control sheath blight disease in rice cultivation. Therefore, the present study was conducted to evaluate the antifungal activity of different solvent extracts of the leaves and flowers of the selected weed species against *R. solani*.

Fig. 1. Studied weed species. (a) *P. hysterophorus* (b) *A. leptopus* (c) *C. gigantea*.

Table 1
Plant specimens' voucher numbers.

Plant species	Voucher number
Parthenium hysterophorus L.	PDA00120445
Antigonon leptopus Hook. and Arn.	PDA00120444
Calatropis gigantea (L.) W.T.Aiton	PDA00120443

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

Leaves and flowers of *P. hysterophorus* and *C. gigantea* were collected from Salambukulam, Vavuniya, Sri Lanka. Leaves and flowers of *A. leptopus* were collected from Kurumankadu, Vavuniya, Sri Lanka (Fig. 1).

2.1.2. Microbial culture

R. solani was obtained from the pathology division of the Rice Research and Development Institute (RRDI) Kurunegala, Sri Lanka. The fungus was isolated from the infected plants and identified by Koch's postulates method.

2.1.3. Chemical materials

All chemicals used for the study were obtained from the Environmental Chemistry Laboratory and Environmental Biological Laboratory, Department of Bio-Science, Faculty of Applied Science, University of Vavuniya, Sri Lanka.

Folicur tebucinazol Emulsion-in- Water (EW) fungicide (positive control) was bought from a CIC agrochemical shop in Vavuniya, Sri Lanka.

2.2. Methodology

2.2.1. Plant identification

The plants were authenticated by the National Herbarium, Department of National Botanic Gardens in Peradeniya, Sri Lanka, and the voucher specimens were deposited in the same herbarium. Plant specimens' voucher numbers are listed in Table 1.

2.2.2. Preparation of plant powder

Leaves and flowers of the selected weed species were washed by tap water and dried under shade for seven days. Dried plant materials were ground by an electrical grinder (Mixer grinder-big boss model). Ground plant powder was sieved (mesh size-8) and stored in airtight containers until used.

2.2.3. Phytochemical analysis

The leaves and flowers plant powder of the selected weed species were subjected to various chemical analyses to detect the presence of phytochemicals and confirm the antimicrobial Property. Tannins, saponins, and phenols were tested using the method described by Yadav and Agarwala, 2011. Glycosides, flavonoids and terpenoids were tested according to the method described by Gul et al., 2017. Protein was

tested as described in Ali et al., 2018.

2.2.4. Extraction procedure

Plant extracts were prepared according to the method described by Rashmi and Rajkumar, 2012 with some modifications (excess solvent evaporated in the fume cupboard). Ground powder of Leaves and flowers (20 g) from each weed species were mixed with 200 ml of hexane, acetone and distilled water (W/V=1:10) separately in 200 ml conical flasks. The flasks' mouths were covered by aluminum foil and tied up by rubber bands. The mixtures were kept in a rotary shaker (Gemmy orbit shaker-VRN-480 model) for 24 h, at 180–200 rpm (revolutions per minute) at room temperature. After 24 h, the mixtures were filtered by two-layer cotton cloths and the supernatants were filtered by Whatman No. 1 filter paper. The filtrations were concentrated in a fume cupboard at room temperature and crude extracts were weighted. The percentage yield was calculated using the following formula:

Extraction yield (%) $= \frac{\text{Weight of crude extract}}{\text{Weight of sample}} \times 100\ \%$ (Sheneni et al., 2018)

Then 10 ml of relevant solvents were added to make stock solutions. Stock solutions were stored in airtight McCartney bottles and preserved at 4 ^{0}C in the refrigerator.

2.2.5. Culturing of fungus

Fungal mycelium was obtained by culturing the sclerotia of *R. solani*. A sclerotium was inoculated on the center of the petri dish which contained sterilized Potato Dextrose Agar (PDA). The petri dish was incubated at room temperature. Seven-day-old mycelium was used for antifungal analysis.

2.2.6. Antifungal assay

The antifungal assay was performed following the poisoned food technique as described in Gupta and Tripathi, 2011. Treatment plates were prepared by adding 1 ml of plant extracts from each stock solution to 8 ml of sterilized PDA media and control sets contained 1 ml of relevant solvent (hexane, acetone and sterilized distilled water) with 8 ml of sterilized PDA media separately. The negative control set consisted solely of PDA culture media. The positive control petri dish set was made by mixing 1 ml of tebuconazole fungicide with 8 ml of sterilized PDA media. The content in the petri dishes was agitated in circular motions to mix the extracts or solvents in the culture medium homogenously. Mycelial disks (7 mm diameter) obtained from the periphery of a seven-day-old culture and inoculated upside down at the center of petri dishes and incubated at room temperature (28^0 C-32^0 C) for 48 h. Each set of treatments was replicated three times. The diameter of the mycelium was measured by a ruler. The percentage of mycelial growth inhibition was calculated using the following formula:

Inhibition of mycelial growth (%) $= \frac{dc-dt}{dc} \times 100$ (Gupta and Tripathi, 2011)

Where.

dc = Mean diameter of fungal mycelial in control petri dishes

dt = Mean diameter of fungal mycelial in treatment petri dishes

2.2.7. Determination of minimum inhibition concentration (MIC) value

Acetone extracts of *A. leptopus* (leaves and flowers) and *C. gigantea* (flowers) were demonstrated 100 % of mycelial growth inhibition; therefore, further analysis was progressed on these extracts to find MIC value. The MIC value was determined by the poisoned food technique as described by Gupta and Tripathi, 2011. The antifungal assay was conducted for the selected volumes (0.25 ml, 0.5 ml and 0.75 ml) of stock solutions. Further, an assay was conducted for the acetone extract of *A. leptopus* flowers by reducing volume (below 0.25 ml) until observing the mycelium growth.

2.2.8. Determination of IC50 and IC 90 values

The term "IC50 value" refers to the concentration at which 50 % inhibition of mycelia growth occurs and "IC90" value refers to the concentration at which 90 % inhibition of mycelia growth occurs in the tested fungus and IC50 and IC90 values were determined by probit analysis using IBM SPSS statistics 26.

Table 2
Summary of the phytochemicals presence in the weed species.

Phytochemical	*P. hysterophorus*		*A. leptopus*		*C. gigantea*	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
Glycosides	+	+	+	+	+	+
Tannins	+	-	+	+	-	-
Saponins	+	-	-	-	+	+
Proteins	+	+	+	+	+	+
Flavonoids	+	-	+	+	+	+
Terpenoids	-	-	+	+	+	+
Phenol	+	-	+	+	-	-

(+ present of phytochemicals, - absent of phytochemicals)

2.2.9. Data analysis

IBM SPSS statistics 26, Minitab 17 and MS Excel 2016 were used for data analysis. Statistical analysis was carried out by the one-way analysis of variance (ANOVA) test using the IBM SPSS statistics 26 statistical package and the significant difference between means of mycelium growth diameter was determined by Duncan's multiple range test (DMRT) at $p < 0.05$ significant level. The analysis was carried out in triplicate. Data were expressed as the mean ± Standard Deviation (SD) of triplicate measurements. Graphs were prepared by Mini table 17 and MS Excel 2016.

3. Results and discussion

3.1. Phytochemicals analysis

Phytochemicals are secondary metabolite chemical components that are present in plants and have a variety of beneficial properties. Table 2 summarizes the presence of phytochemicals in the flowers and leaves of the studied weed species. The results indicate that all leaves and flowers contained glycosides and proteins. The leaves and flowers of *A. leptopus* and the leaves of *P. hysterophorus* contained most of the studied phytochemicals. Terpenoids were absent in the leaves of *P. hysterophorus*, while saponins were absent in the leaves and flowers of *A. leptopus*. Only proteins and glycosides are found in the flowers of *P. hysterophorus*. The phenols and tannins were not detected in the flowers and leaves of *C. gigantea*. Certain phytochemicals (tannis, saponins, flavonoids and phenol) are found in the leaves of *P. hysterophorus* but are absent from the flowers. High color intensity was observed as the outcome of some phytochemical tests conducted for flowers of *A. leptopus*. Such a finding suggests that *A. leptopus* flowers may contain more phytochemicals quantitatively than others. However, it needs to be confirmed through quantitative phytochemical analysis by the High Performance Liquid Chromatography (HPLC) method.

The study conducted by Marimuthu and Ravi, 2014 confirmed the presence of glycosides, saponins, flavonoids, tannins and phenols in the leaves of *P. hysterophorus* which is similar to the present study. The present study identified the presence of glycosides and proteins in the flowers of *P. hysterophorus* but Iqbal et al., 2022 detected the presence of phenolic compounds and flavonoids in the flowers of *P. hysterophorus* by using HPLC.

The detection of the tested phytochemicals in the leaves and flowers concludes their antimicrobial properties. Antimicrobial chemicals that are effectively utilized against various plant infections and diseases are frequently made from the crude sap, volatile, and essential oil collected from whole plants or specific plant parts, including roots, stems, leaves, flowers, fruits, and seeds. Six broad phytochemicals, including flavonoids, saponins, steroides, tannins, and phenolic acids effective against

Table 3
Extraction yield (%).

Solvent	*P. hysterophorus*		*A. leptopus*		*C. gigantea*	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
Hexane	0.24 g	0.07 g	0.1 g	0.03 g	0.3 g	0.42 g
	1.2 %	0.35 %	0.5 %	0.15 %	1.5 %	2.1 %
Acetone	0.72 g	0.86 g	0.84 g	0.97 g	0.88 g	1.09 g
	3.6 %	4.3 %	4.2 %	4.8 %	4.4 %	5.43 %
Distilled water	2.65 g	3.28 g	2.15 g	3.5 g	2.73 g	4.93 g
	13.25 %	16.40 %	10.75 %	17.5 %	13.65 %	24.65 %

plant pathogens. (Gurjar et al., 2012).

According to Gurjar et al., 2012, phytochemicals exert various kinds of mode of actions against plant pathogenic microbes. Such as, Phenolic compounds are caused by membrane disruption and substrate deprivation of microorganisms. Phenolic acids are bound to adhesions and form complexes with the cell wall that ultimately inactivate enzymes. Tannins bind to proteins and prevent enzymes from working by depriving them of substrates. Flavonoids interact with the cell wall, and render enzymes inactive.

3.2. Calculation of the extraction yield

The polarity of the solvents (such as hexane, acetone, and distilled water were used as non-polar, intermediate polar and polar, respectively) was considered when preparing the extracts for this study. The extraction yield (%) is stated in Table 3. The highest quantity of yield was obtained from the distilled water. Hexane was given the lowest quantity of extraction yield. The highest yield (4.93 g) was obtained from the distilled water extract of the flowers of *C. gigantea* while lowest yield (0.03 g) was given by the hexane extract of the flowers of *A. leptopus*. The highest acetone extraction yield was obtained from the flowers of *C. gigantea* and the lowest yield was given by the leaves of *P. hysterophorus*. According to the results, as the polarity of the solvent increases, the quantity of crude extracts increases; the results confirmed the presence of a high number of polar components in the studied weed species. In all three solvents, the highest yield was observed for *C. gigantea* flowers. Both acetone and distilled water extraction yields of flowers were higher than leaves in each weed species. Further studies are required to confirm the presence of phytochemicals in different solvent extracts.

According to the study done by Truong et al., 2018 the quantity of extraction yield depends on the polarity of the solvents and a higher extraction yield is obtained from polar solvents. This finding is similar to the results of the present study because, with the increases in solvent polarity, the extraction yield increased.

Low toxicity, ease of evaporation at low heat, promotion of quick physiologic absorption of the extract and preservation action are qualities of a good solvent in plant extractions Gurjar et al., 2012). In the present study, hexane and acetone evaporated easily, but an extra period of time was needed for distilled water evaporation at room temperature. However, water confers many benefits, including being highly polar, inexpensive, harmless, and able to dissolve a large variety of compounds. Although, it has a significant potential for microbial growth during the water evaporation. According to the Environmental Protection Agency (EPA) of the United States, there is insufficient data to determine if acetone causes cancer in people. Acetone has not been categorized as carcinogenic by the National Toxicology Program (NTP) or the International Agency for Research on Cancer (IARC).

Table 4
Antifungal activity of the hexane extracts against *R. solani*.

Weed extract		*Mycelium diameter (cm) Mean ± SD	Inhibition percentage (%)
P. hysterophorus	Leaves	9.00 ± 0.00	0.00
	Flowers	9.00 ± 0.00	0.00
A. leptopus	Leaves	9.00 ± 0.00	0.00
	Flowers	9.00 ± 0.00	0.00
C. gigantea	Leaves	9.00 ± 0.00	0.00
	Flowers	9.00 ± 0.00	0.00
Control (hexane)		9.00 ± 0.00	0.00
Negative Control		9.00 ± 0.00	0.00
Positive Control		0.00 ± 0.00	100

* Values were performed in triplicate and represented as the mean ± Standard deviation (SD).

3.3. Antifungal assay for weed extracts

3.3.1. Antifungal activity of hexane extracts

The growth of *R. solani* was not inhibited by hexane extracts from

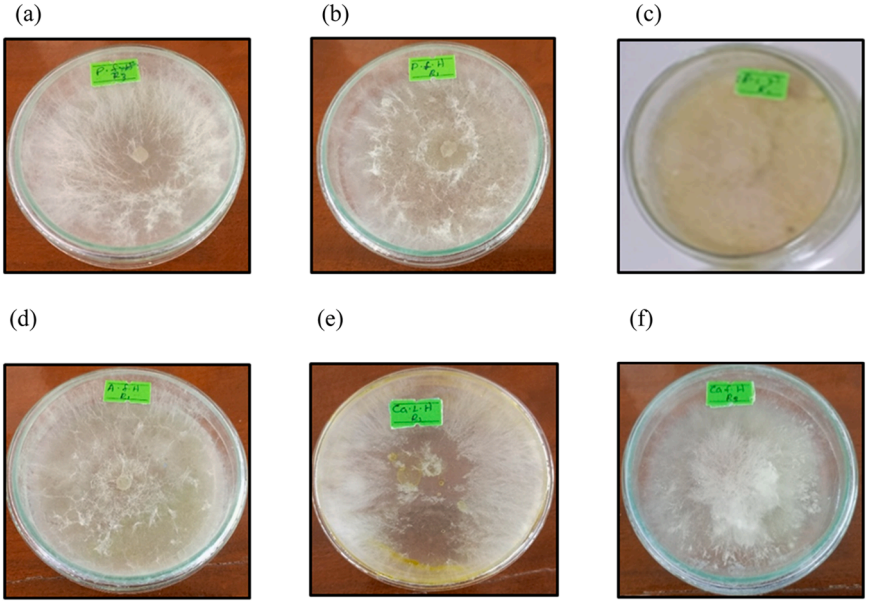

Fig. 2. Antifungal activity of the hexane extracts against growth *R. solani*. (a) *P. hysterophorus* leaves extracts (b) *P. hysterophorus* flowers extracts (c) *A. leptopus* leaves extract. (d) *A. leptopus* flowers extract (e) *C. gigantea* leaves extract (f) *C. gigantea* flowers extract.

Table 5
Antifungal activity of the acetone extracts against *R. solani*.

Weed extracts		Mycelium diameter (cm) *Mean ± SD	Inhibition percentage (%)
P. hysterophorus	Leaves	5.90 ± 0.173^{b}	34.44
	Flowers	6.00 ± 0.000^{bc}	33.33
A. leptopus	Leaves	0.00 ± 0.000^{a}	100
	Flowers	0.00 ± 0.000^{a}	100
C. gigantea	Leaves	6.10 ± 0.173^{c}	32.22
	Flowers	0.00 ± 0.00^{a}	100
Control (acetone)		9.00 ± 0.00^{d}	0.00
Negative control		9.00 ± 0.00^{d}	0.00
Positive Control		0.00 ± 0.00^{a}	100

* Values were performed in triplicate and represented as the mean ± Standard deviation (SD). Mean values followed by different superscript alphabets in the rows indicate statistically significant differences at $p < 0.05$ level according to the DMRT test.

either the flowers or the leaves of the all studied weed species, which showed complete growth of mycelium in petri plates (Fig. 2). The complete growth of mycelium is observed in control plates that contained hexane in the media. This proves that the hexane solvent has no effect on the growth of *R. solani* (Fig. 3). The inhibition percentages of the hexane extracts are illustrated in Table 4.

According to the results of this study, hexane extracts do not show any antifungal activity against *R. solani.* A study found that the hexane extract of the leaves of *A. leptopus* showed the antifungal effect on the growth of *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans* (Sravanthi et al., 2017) but according to the present study, the antifungal activity of the hexane extract of *A. leptopus* leaves is not effective in suppressing the growth of *R. solani*. It may be that the biochemical components did not extracted by the hexane or extracted components did not possess properties.

3.3.2. Antifungal activity of acetone extracts

As shown in Table 5, acetone extracts had various growth inhibitory percentages. Mycelium growth was entirely suppressed by acetone extracts of the leaves and flowers of *A. leptopus* and the flowers of *C. gigantea*. The extract of the flowers of *P. hysterephorus* exhibited 33.33 % growth inhibition. The extract of the leaves of *C. gigantea* showed 32.22 % inhibition and the leaves of *P. hysterephorus* showed 34.44 % growth inhibition.

According to the DMRT test ($p < 0.05$), there is no statistically significant difference between the means of mycelium growth diameter given by the acetone extract of *P. hysterophorus* leaves and flowers. In addition, there is no statistically significant difference between acetone extracts of *C. gigantea* leaves and *P. hysterophorus* flowers. Acetone extracts of the leaves and flowers of *A.leptopus* and the flowers of *C. gigantea* showed 100 % inhibition and no significant differences between them. Zero percentage of growth inhibition (Fig. 3) exhibited in control petri plates, which contained PDA with acetone. It showed that acetone has no influence on growth of *R. solani* and these results were already reported by Meela et al. (2019). As per the study, acetone is recommended as an effective extraction solvent. Fig. 4 shows the antifungal activity of the acetone extracts against tested fungi. All acetone extracts exhibited more than 30 % growth inhibition.

According to the study done by Kim et al., 2019 acetone extracts of tomato leaves, immature green fruits of tomato and red fruits of tomato show higher growth inhibition against *R. solani* compared to hexane, dichloromethane and methanol extracts. The results of the current study also show high growth inhibition of *R. solani* by some acetone extracts. Hemalatha et al., 2011 revealed that the acetone extract of *C. gigantea* leaves has antimicrobial activity against some human pathogens. According to their study, the extract showed more significant antibacterial effects than fungicidal effects. However, the present study discovered the significant fungicidal effect of acetone extract of the flowers of *C. gigantea* against plant pathogenic fungi.

3.3.3. Antifungal activity of distilled water extracts

All distilled water extracts except the extract of the flowers of *C. gigantea* showed more than 40 % growth inhibition against *R. solani*. Table 6 shows the growth inhibition percentages of the distilled water extracts.

Distilled water extracts of the leaves of *P. hysterophorus* and *A. leptopus* showed 66.00 % and 67.11 % growth inhibition respectively. According to the results of DMRT test, there is no statistically significant difference between the means of mycelium growth diameter given by the extract of the leaves of *P. hysterophorus* and *A. leptopus* at 0.05 level of significance. Distilled water extracts of the leaves of *P. hysterophorus* and *A. leptopus* showed the highest growth inhibition percentage

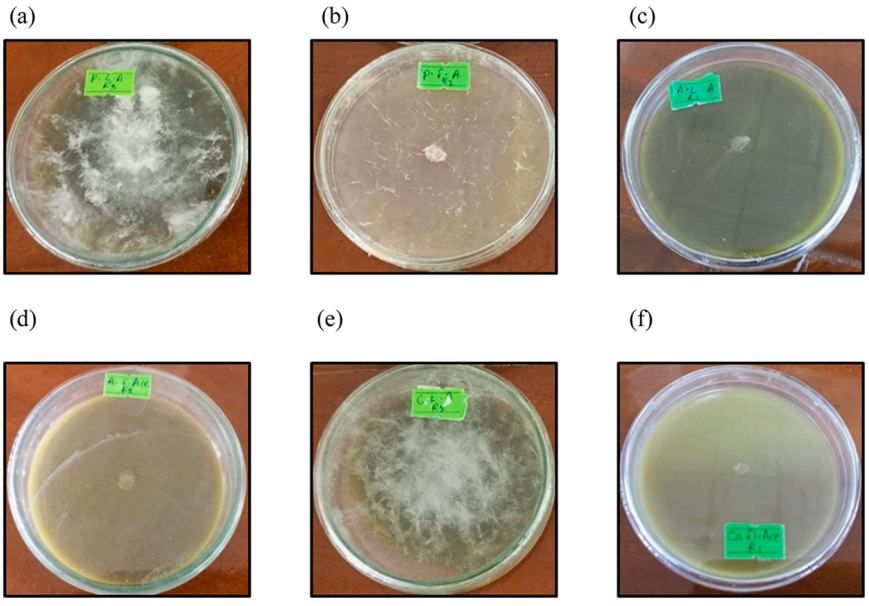

Fig. 3. Antifungal activity of the acetone extracts against mycelium growth of *R. solani*. (a) *P. hysterophorus* leaves extract (b) *P. hysterophorus* flowers extract (c) *A. leptopus* leaves extract. (d) *A. leptopus* flowers extract (e) *C. gigantea* leaves extract (f) *C. gigantea* flowers extract.

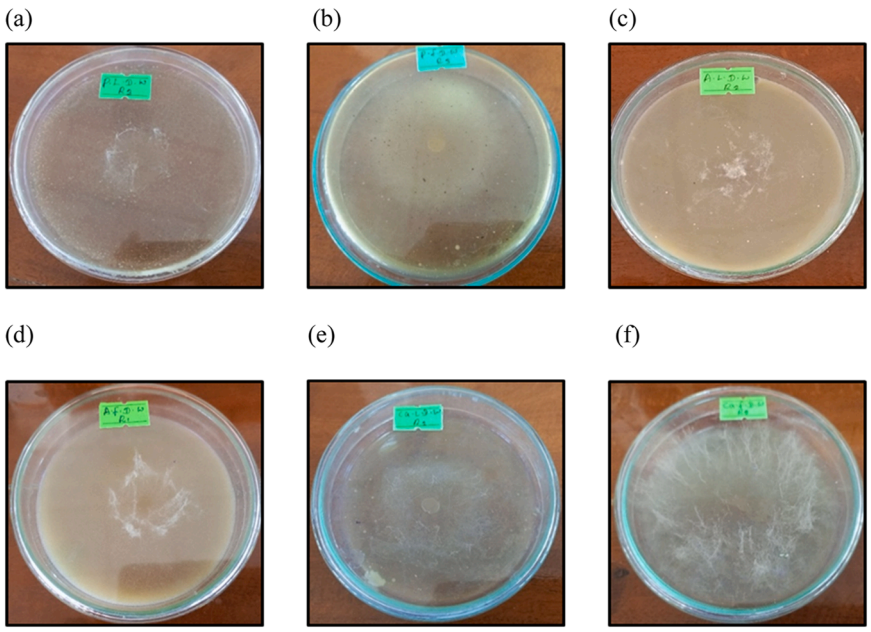

Fig. 4. Antifungal activity of the distilled water extracts against mycelial growth of *R. solani.* (a) *P. hysterophorus* leaves extract (b) *P. hysterophorus* flowers extract (c) *A. leptopus* leaves extract. (d) *A. leptopus* flowers extract (e) *C. gigantea* leaves extract (f) *C. gigantea* flowers extract.

Table 6
Antifungal activity of the distilled water extracts against *R. solani*.

Weed extract		*Mycelium Diameter (cm) (Mean ± SD)	Inhibition percentage (%)
P. hysterophorus	Leaves	2.96 ± 0.057^{a}	67.11
	Flowers	5.06 ± 0.115^{c}	43.77
A. leptopus	Leaves	3.06 ± 0.115^{a}	66.00
	Flowers	3.60 ± 0.361^{b}	60.00
C. gigantea	Leaves	5.06 ± 0.115^{c}	44.44
	Flowers	9.00 ± 0.000^{d}	0.00
Control (Distilled water)		9.00 ± 0.000^{d}	0.00
Negative Control		9.00 ± 0.000^{d}	0.00
Positive Control		0.00 ± 0.000^{e}	100

* Values were performed in triplicate and represented as mean ± Standard deviation (SD). Mean values followed by different superscript alphabet in the rows indicate statistically significant differences at $p < 0.05$ level according to the DMRT test.

Table 7
Antifungal activity of acetone extracts of *A. leptopus* and *C. gigantea* against *R. solani*.

Weed extracts	Volume (ml)	Concentration of the weed extract (mg/ml)	*Mycelium diameter (cm) (Mean ± SD)	Inhibition percentage (%)
A. leptopus leaves	0.25	21	6.66 ± 0.577	26.00
	0.5	42	3.33 ± 0.288	63.00
	0.75	63	1.83 ± 0.288	79.66
A. leptopus flowers	0.25	24.25	0.00 ± 0.000	100
	0.5	48.5	0.00 ± 0.000	100
	0.75	72.75	0.00 ± 0.000	100
C. gigantea flowers	0.25	27.25	3.20 ± 0.200	64.44
	0.5	54.5	1.00 ± 0.000	88.88
	0.75	81.75	0.00 ± 0.000	100

* Values were performed in triplicate and represented as mean ± Standard deviation (SD).

compared to other distilled water extracts. The *C. gigantea* flower extracts showed the lowest inhibition percentage. The extract of the flowers of *P. hysterophorus* showed 43.77 % growth inhibition, while the leaves of *C. gigantea* extract showed 44.44 % growth inhibition, but according to the DMRT test, there is no statistically significant difference found between the means of mycelium growth diameter at the 0.05 level of significance. In the present study, it was noticed that the distilled water extract of the leaves of *P. hysterophorus* and the leaves of *A. leptopus* revealed considerable antifungal activities against the tested fungus.

The antifungal activity of the distilled water extract of the leaves of *P. hysterophorus* has been reported in a study done by Cherkupally et al., 2017. *C. gigantea* and *Calatropis procera* (Ait) are closely related species and belong to the Apocynaceae family (Ali-seyed and Ayesha, 2020). Manoorkar et al., 2015 proved the fungicidal effects of aqueous and ethanol extracts of latex and leaves of *C. procera* on *R. solani.* The present study found fungicidal effects of distilled water extracts of the leaves of *C. gigantea*. Latex of *C. gigantea* may have antifungal effects, but further studies are needed to confirm antifungal effects on *R. solani*.

Control petri plates with sterilized distilled water showed zero

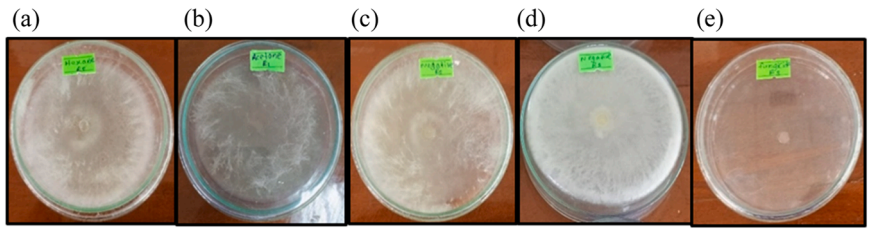

Fig. 5. Mycelium growth in control petri plates. (a) Hexane (b) Acetone (c) Distilled water (d) Negative control (e) Positive control.

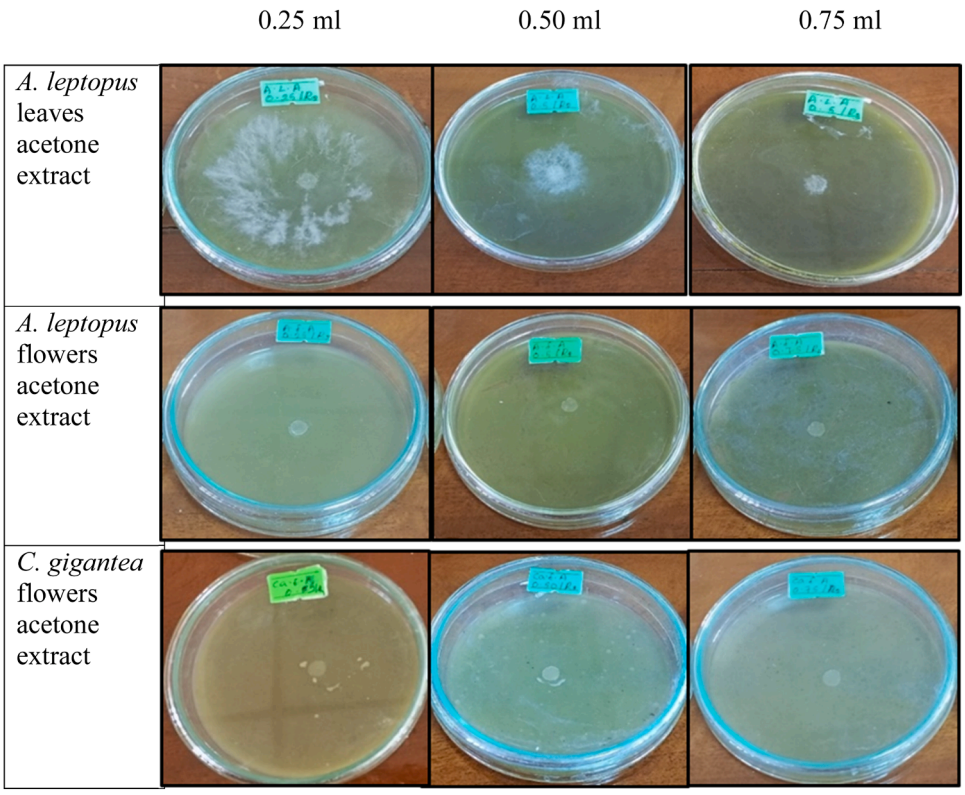

Fig. 6. Antifungal activity of acetone extracts of *A. leptopus* and *C. gigante* against growth of *R. solani*.

percentage of growth inhibition (Fig. 3). It was confirmed that distilled water has no influence on the growth of *R. solani*. As per the study, distilled water could be a suitable solvent to extract phytochemicals at a low cost and without using any chemicals.Fig. 5 shows the antifungal activity of the distilled water extracts against tested fungi.

3.3.4. *Mycelium growth in control petri plates*

All studied solvents did not influence the growth of *R. solani*. (Fig. 3)

3.4. *Determination of MIC value*

The MIC value is the lowest concentration of an antifungal agent required to prevent fungus growth that is visible. Mycelium growth was entirely prevented by 1 ml of *A. leptopus* (leaves and flowers) and 1 ml of *C. gigantea* (flowers); therefore, the study proceeded by reducing the volume to 0.25 ml, 0.5 ml, and 0.75 ml. Table 7 illustrates the inhibition percentages of the plant extracts at the pertinent concentrations.

The growth of mycelium completely inhibited by 1 ml of the acetone extract of the leaves of *A. leptopus* and 0.75 ml showed 79.66 % inhibition. Therefore, the MIC value of the acetone extract of the leaves of *A. leptopus* was between 0.75 and 1 ml, and the corresponding concentration was 63–115 mg/ml. Growth inhibition of acetone extract of the leaves of *A. leptopus* were 26.00 % and 63.00 % at 0.25 ml and 0.5 ml of applied volume respectively. The growth of mycelium was entirely suppressed by 1 ml and 0.75 ml of acetone extract of the flowers of *C. gigantea* and 88.88 % inhibited by 0.5 ml. Therefore, the MIC of the acetone extract of the flowers of *C. gigantea* is between 0.5 and 0.75 ml. MIC value ranges from 54.5 to 81.75 mg/ml. Acetone extract of the flowers of *A. leptopus* exhibited significant inhibition activity whereas 100 % growth inhibition was observed at 1 ml as well as 0.25 ml. Hence, the study was narrowed down to find the MIC value of the acetone extract of the flowers of *A. leptopus*. Fig. 6 shows the antifungal activity of acetone extracts of *A. leptopus* and *C. gigantea* against *R. solani*.

Due to the entire growth inhibition by 0.25 ml of acetone extract of the flowers of *A. leptopus*, further study was carried out with volumes of 0.20 ml, 0.15 ml, 0.10 ml, and 0.05 ml. However, the lowest volume (0.05 ml) showed 100 % inhibition. The study proceeded with volumes of 0.04 ml, 0.03 ml, 0.02 ml, and 0.01 ml of acetone extract of the flowers of *A. leptopus* and mycelium growth was observed in 0.01 ml as well as 0.04 ml. The following Table 8 shows the inhibition percentages at various concentrations.

Table 8
Antifungal activity of acetone extracts of the flowers of *A. leptopus* at different concentrations against *R. solani*.

Volume (ml)	Concentration of the weed extract (mg/ml)	*Mycelium Diameter (cm) Mean ± SD	Inhibition percentage (%)
0.01	0.97	6.76 ± 0.252^{e}	24.88
0.02	1.94	3.93 ± 0.115^{d}	56.33
0.03	2.91	2.93 ± 0.115^{c}	67.44
0.04	3.88	1.93 ± 0.115^{b}	78.55
0.05	4.85	0.00 ± 0.000^{a}	100

* Values were performed in triplicate and represented as mean ± Standard deviation (SD). Mean values followed by different superscript alphabet in the rows indicate statistically significant differences at $p < 0.05$ level according to the DMRT test.

When reducing the volume of acetone extract of the flowers of *A. leptopus* at 0.04 ml mycelium growth could be observed with 78.55 % inhibition and 100 % inhibition was observed at 0.05 ml. The MIC value of the acetone extract of the flowers of *A. leptopus* was 4.85 mg/ml against *R. solani*. According to the DMRT test, there is a significant difference between all concentrations at the 0.05 significant level. The

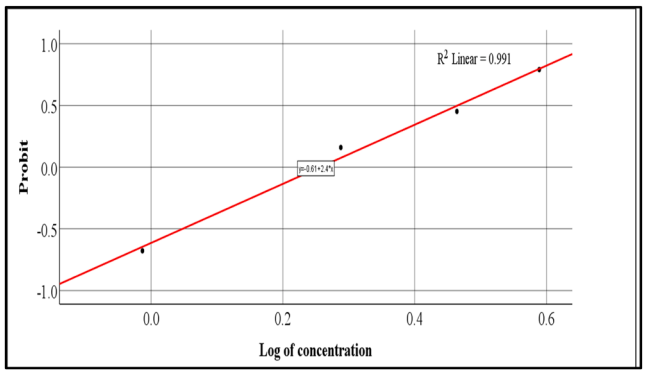

Fig. 7. Probit transform response.

Table 9
Probit regression line parameters and inhibition concentrations.

IC50 (LFL – UFL)	IC90 (LFL – UFL)	Regression equation	Chi-square (df)	R^2 (%)
1.74 (0.73 – 2.53)	4.66 (3.07 – 23.84)	$Y = 0.61 + 2.4 \times X$	16.71 (3)	99.1

LFL = Lower Fiducial Limits, UFL = Upper Fiducial Limits, df = degree of freedom,
R^2 = Regression coefficient.

lowest MIC value was given by the acetone extract of the flowers of *A. leptopus*. As per the study, the acetone extract of *A. leptopus* flowers showed more antifungal activity compared to other extracts.

3.5. Determination of IC50 and IC90 values of the acetone extract of the flowers of A. leptopus

The IC50 value is the required concentration of fungicide for 50 % of mycelium growth inhibition and the IC90 value is the required concentration of fungicide for 90 % of mycelium growth inhibition. IC50 and IC90 values were calculated by probit analysis. Probit transform responses as shown in Fig. 7:

The results of probit analysis indicate that the chi square test has significantly higher heterogeneity in the test population (Table 9). The IC50 value of the acetone extract of *A. leptopus* flowers was 1.74 mg/ml and the IC90 value was 4.66 mg/ml against *R. solani*.

Antifungal activity against *R. solani* depends on the selected weed species. According to Nguyen et al., 2021, acetone extract of the areal parts of *Ageratum conyzoides* L. strongly suppressed the growth of *R. solani* with a low IC50 value (250–275 μg/ml). In the present study, IC50 value of the acetone extract of the flowers of *A. leptopus* is 1740 μg/ml. The present study was limited to *in vitro* analysis and detected higher antifungal activity of the acetone extract of the flowers of *A. leptopus* against *R. solani*. Thus, this finding opens the pathway to further studies focusing on the preparation of bio fungicide to control sheath blight disease at field application.

4. Conclusion

Acetone extracts of the flowers of *A. leptopus* exhibit excellent antifungal activity against *R. solani*. However, further studies are required to conduct *in vivo* assessments of the acetone extract of the flowers of *A. leptopus* under field conditions in order to clarify their efficacy in the natural environment. Furthermore, distilled water extracts of the leaves of *A. leptopus* and *P. hysterophorus* can be used as a bio control agent at the residential level, which can be prepared with minimum production costs and less technology. The findings of this study will provide a conceptual framework for the development of a fungicide using the acetone extract of the flowers of *A. leptopus* to combat sheath blight in rice cultivation. Additionally, it gives an economic value to some invasive weed species.

Funding

This research did not receive any specific grant from funding agencies in public, commercial or not-for-profit sectors.

CRediT authorship contribution statement

Madhujith Terrence: Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Merah Othmane:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Vimalaveera Sanduni Iresha:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Gamage Ashoka:** Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Nimalan Jeyagowri:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

Authors declare no potential interest

Acknowledgement

We would like to express our sincere gratitude to Rice Research and Development Institute (RRDI), Kurunegala, Sri Lanka, for facilitating the obtaining of *R. solani* fungi for the study.

Data availability

Data will be made available on request.

References

Afzal, M., Ahmed, E., Sharif, A., Javaid, A., 2023. Antifungal potential of two new triterpenoidal glycosides from the *Albizia kalkora* against *Fusarium oxysporum* f. sp. lycopersici. Int. Allelopath. J. 60 (1), 83–92. https://doi.org/10.26651/allelo.j/2023-60-1-1455.

Ali, S., Khan, M.R., Sajid, M., Zahra, Z., 2018. Phytochemical investigation and antimicrobial appraisal of *Parrotiopsis jacquemontiana* (*Decne*) Rehder. BMC Complement. Altern. Med. 18 (1), 1–15. https://doi.org/10.1186/s12906-018-2114-z.

Ali-seyed, M., Ayesha, S., 2020. Calotropis - a multi-potential plant to humankind: special focus on its wound healing efficacy. Biocatal. Agric. Biotechnol. 28, 101725. https://doi.org/10.1016/j.bcab.2020.101725.

Attanayake, M.B.N.W.M., Weerasekara, W.M.I., Weeratunga, D.B., Chithral, G.M.W., 2010. Past achievements and future directions of the seed paddy sector in Sri Lanka. Rice Cong. 205–218.

Boedeker, W., Watts, M., Clausing, P., Marquez, E., 2020. The global distribution of acute unintentional pesticide poisoning estimations based on a systematic review. BMC Public Health 20 (1), 1–19.

Cherkupally, R., Kota, S.R., Amballa, H., Reddy, B.N., 2017. In vitro antifungal potential of plant extracts against *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Ann. Plant Sci. 6 (9), 1676–1680.

David, D., Prabhakar, A., Peter, J.V., Pichamuthu, K., 2008. Human poisoning with hexastar ™: a hexaconazole-containing agrochemical fungicide. Clin. Toxicol. 46 (7), 692–693. https://doi.org/10.1080/15563650701447012.

Deparment of agriculture Sri Lanka, Rice Diseases-sheath Blight. ⟨https://doa.gov.lk/rrdi_ricediseases_sheathblight/⟩, 2022 (Accessed 26 July 2022).

Durgeshlal, C., Khan, S.M., Prabhat, S.A., Aaditya, P.Y., 2019. Antifungal activity of three different ethanolic extract against isolates from diseased rice plant. J. Anal. Tech. Res. 01 (01), 47–63. https://doi.org/10.26502/jatri.007.

Fukagawa, N.K., Ziska, L.H., 2019. Rice: importance for global nutrition. J. Nutr. Sci. Vitaminol. 65, S2–S3. https://doi.org/10.3177/jnsv.65.S2.

Gul, R., Jan, S.U., Faridullah, S., Sherani, S., Jahan, N., 2017. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Sci. World J. 2017, 7. https://doi.org/10.1155/2017/5873648. Article ID 5873648.

Gupta, S.K., Tripathi, S.C., 2011. Fungitoxic activity of *Solanum torvum* against *Fusarium sacchari*. Plant Prot. Sci. 47 (3), 83–91.

Gurjar, M.S., Ali, S., Akhtar, M., Singh, K.S., 2012. Efficacy of plant extracts in plant disease management. Agric. Sci. 03 (03), 425–433. https://doi.org/10.4236/as.2012.33050.

Hemalatha, M., Arirudran, B., Thenmozhi, A., Mahadeva, U.S., 2011. Antimicrobial effect of separate extract of acetone, ethyl acetate, methanol and aqueous from leaf of milkweed (*Calotropis gigantea* L.). Asian J. Pharm. Res. 1 (4), 102–107.

Iqbal, J., Khan, A.A., Aziz, T., Ali, W., Ahmad, S., Rahman, S.U., Iqbal, Z., Dablool, A.S., Alruways, M.W., Almalki, A.A., Alamri, A.S., 2022. Phytochemical investigation, antioxidant properties and *in vivo* evaluation of the toxic effects of *Parthenium hysterophorus*. Molecules 2022 27, 4189. https://doi.org/10.3390/molecules27134189.

Ju, C., Xu, J., Wu, X., Dong, F., Liu, X., Tian, C., Zheng, Y., 2017. Effects of hexaconazole application on soil microbes community and nitrogen transformations in paddy soils. Sci. Total Environ. 609, 655–663. https://doi.org/10.1016/j.scitotenv.2017.07.146.

Kim, D.S., Kwack, Y., Lee, J.H., Chun, C., 2019. Antimicrobial activity of various parts of tomato plants varied with different solvent extracts. Plant Pathol. J. 35 (2), 149–155. https://doi.org/10.5423/PPJ.OA.07.2018.0132.

Manoorkar, V.B., Manadge, S.V., Gachande, B.D., 2015. Antifungal activity of leaf and latex extracts of *Calotropis procera* (Ait.) against dominant seed-borne storage fungi of some oil seeds. J. Biosci. Discov. 6 (1), 22–26.

Marimuthu, K., Ravi, D., 2014. Phytochemical analysis of *Parthenium hysterophorus* L. leaf. World J. Pharm. Res. 3 (6), 1066–1074.

Meela, M.M., Mdee, L.K., Masoko, P., Eloff, J.N., 2019. Acetone leaf extracts of seven invasive weeds have promising activity against eight important plant fungal pathogens. South Afr. J. Bot. 121, 442–446. https://doi.org/10.1016/j.sajb.2018.12.007.

Mithrasena, Y.J.P.K., Wijesundera, W.S.S., Wijesundera, R.L.C., Dissanayake, D.M.N., 2010. Investigations on pathotype genetic variation of rice blast fungus (*Magnaporthe grisea*) isolates in agro ecological zones in Sri Lanka. *3rd International rice congress*. Vietnam 8–12.

Nguyen, C.C., Nguyen, T.Q.C., Kanaori, K., Binh, T.D., Dao, X.H.T., 2021. Antifungal activities of *Ageratum conyzoides* L. extract against rice pathogens *Pyricularia oryzae* Cavara and *Rhizoctonia solani* Kühn. Agriculture 2021 11 (11), 1169. https://doi.org/10.3390/agriculture11111169.

Rashmi, S., Rajkumar, H.G., 2012. Phytochemical analysis and in vitro evaluation of antifungal activity of five invasive plant species against *Macrophomina Phaseolina* (Tassi) Goid. Int. J. Plant Res. 1 (1), 11–15. https://doi.org/10.5923/j.plant.20110101.02.

Rubaiyath, B.R., Zhang, J., 2023. Trends in rice research: 2030 and beyond. Food Energy Secur. 12 (2), e390. https://doi.org/10.1002/fes3.390.

Saratha, V., Subramanian, S.V., 2010. Evaluation of antifungal activity of *Calotropis gigantea* latex extract: an *In vitro* study. Int. J. Pharm. Sci. Res. 1 (9), 88–96.

Sharf, W., Javaid, A., Shoaib, A., Khan, I.H., 2021. Induction of resistance in chili against sclerotium rolfsii by plant-growth- promoting rhizobacteria and *Anagallis arvensis*. Egypt. J. Biol. Pest Control 31, 16. https://doi.org/10.1186/s41938-021-00364-y.

Sheneni, V.D., Usman, O.S., Musa, Q., 2018. Phytochemical constituent, percentage yield and phenolic content estimation of different solvent system of Carica papaya leaves. Korean J. Food Health Converg. 4 (2), 17–23. https://doi.org/10.13106/kjfhc.2018.vol4.no2.17.

Sravanthi, M., padmaja, M., Muni, K.D.H.K., 2017. *In vitro* antimicrobial properties and phytochemical screening of crude extracts of *Antigonon leptopus* Hook. & Arn. leaf. Int. J. Innov. Pharm. Sci. Res. 5 (02), 29–41. https://doi.org/10.21276/IJIPSR.2017.05.02.511.

Truong, V. D., Avula, R. Y., Pecota, K. V., Yencho, G. C., 2018. Sweetpotato production, processing, and nutritional quality. In: Siddiq, M; Uebersax, M. A. (Eds), Handbook of vegetables and vegetable processing, Volume II, 2nd Edition. John Wiley & Sons, Ltd.https://doi.org/10.1002/9781119098935.ch35.

Tsiboe, F., Lanier, L., Durand, A., Thoma, G., Shew, A., 2017. The economic and environmental benefits of sheath blight resistance in rice. J. Agric. Resour. Econ. 42 (2), 215–235.

Yadav, R.N.S., Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. J. Phytol. 3 (12), 10–14.

Zaheer, Z., Shafique, S., Shafique, S., Mehomood, T., 2012. Antifungal potential of *Parthenium hysterophorus* L. plant extracts against *Fusarium solani*. Sci. Res. Essays 7 (22), 2049–2054. https://doi.org/10.5897/sre12.082.