



Assessment of Phytochemical screening and Antifungal Activity of *Parthenium hysterophorus* L.

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ABSTRACT: Phytochemical screening and Antifungal activities of *Parthenium hysterophorus* L (Asteraceae) was carried out in laboratory. Distilled water and methanol extracts of the leaves of plant was prepared. Five phytopathogenic fungi: *Alternaria brassicae*, *Botrytis cinerea*, *Fusarium oxysporum*, *Phytophthora capsici* and *Sclerotium rolfii* were tested at different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml) of plant extracts. The phytochemical screening depicted the presence of terpenoids, saponins, flavonoids, tannins and alkaloids. The antifungal activity of extracts was determined by poisoned food technique; and linear mycelium growth reduction (LMGR) percentage was calculated. Methanol crude leaf extract had higher antifungal potential than the distilled water extract.

Key words: *Parthenium hysterophorus*, antifungal activity, poisoned food technique and linear mycelium growth reduction

INTRODUCTION

Parthenium hysterophorus L. is an aromatic annual and obnoxious invasive herb. It is native to the subtropics of North and South America and first reported in Nepal in 1982 (Tiwari *et al.* 2005). It can colonise degraded natural ecosystems and produce inhibitory effect on surrounding herbaceous vegetation. *Parthenium* weed rapidly colonizes arable land, disturbed areas along roadsides and heavily grazed pasture. It is found more profuse in central and western Nepal (Tiwari *et al.* 2005). *Parthenium hysterophorus* L. is also known as congress weed, carrot weed, star weed, white top, chatak chandani, bitter weed, ramphool and gajar grass (Yadav *et al.*, 2010). *Parthenium hysterophorus* L. is an erect herb with alternate, deeply dissected leaves, growing up to 2 m tall with much branched inflorescences. Bearing white flower heads and numerous obovoid, smooth and black achenes (Srivastava and Singh, 2011).

Development of new drugs from natural sources is highly influenced by its ethnobotanical uses. Though *Parthenium hysterophorus* L. is a weed and causes severe allergies to human being, It is reported with many therapeutic applications. Decoction prepared from its roots has been used by American Indians in traditional medicine to treat amoebic dysentery (Uphof, 1959). Ramos *et al.* (2001) reported its applications in treating neurologic disorders, fever, urinary infections, dysentery and malaria. Rastogi and Mehrotra (1991) describe *Parthenium hysterophorus* L.

as a medicinal plant. This is very much resistant to plant pathogenic microorganisms; this could be attributed to presence of antimicrobial metabolites in plant parts. In present investigation an attempt was made to ascertain the effectiveness of leaves extract which imparts antimicrobial effect to the whole plant extracts.

MATERIAL AND METHODS

Collection of Plant Material. Leaves of *P. hysterophorus* was collected from different areas of Kirtipur, TU., during October 2011 to January 2012 at flowering stage.

Drying and preservation of plant samples. Fresh and healthy leaves were collected and washed properly with tap water. The leaves were cut into small pieces and were shade dried. The dried leaves were ground into fine powder with the help of electric grinder. The ground plant samples were preserved into zipper bag for the further analysis.

Preparation of extract. The ground plant leaf sample of 25 gm was soaked in 250 ml of distilled water and methanol (99 %) separately in a conical flask for 72 h. Each mixture was stirred at 24 h interval using a sterilized glass rod. The samples were filtered using three layers of muslin cloth. Distilled water extract was evaporated on heating mantle using water bath till the thick residue was formed (Mahida and Mohan 2007) and methanol was evaporated using rotary evaporator at 60°C.

It was made into semisolid form by evaporation to water bath. After the solvent evaporation, each of the solvent extract was weighted and preserved in air tight bottles until further use in the refrigerator at temperature 4-10°C.

Antifungal Activity. Antifungal activity of the extracts was evaluated by measuring mean linear mycelium growth reduction (LMGR) percentage. The test fungi were inoculated for 7 days at 27°C.

Collection of Test organism. The pure fungal strains were collected from Nepal Agriculture and Research Council (NARC), Khumaltar, Kathmandu. The five strains used for the test were *Sclerotium rolfsii*, *Phytophthora capsici*, *Alternaria brassicae*, *Fusarium oxysporum* and *Botrytis cinerea*.

Phytochemical screening. The phytochemical screening of crude extracts from the leaves of *P. hysterophorus* was carried out to determine the presence of active secondary plant metabolites. The plant extracts were screened for the presence of tannins, saponin, cardio-glycosides, terpenoids, steroids, flavonoids and alkaloids according to the established procedures. Preliminary qualitative phytochemical screening was carried out on the powdered samples applying the following standard procedures described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) and for the result sharp change in color was noted.

Preparation of different concentrations (extract). Distilled water and methanol semisolid leaf extract of *Parthenium hysterophorus* were used for the preparation of concentrations viz. 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml. These concentrations were diluted in distilled water and

methanol separately hence distilled water and methanol were used as negative control.

Transfer of the fungus strains. A volume of 1ml concentration to be used was poured aseptically into 9 ml of PDA and was swirled to mix the content completely. 10 ml of this mixture (PDA and extract) was then poured into the pre sterilized and well labeled petriplates (Samie and Nefefe 2012). The plates were then were allowed to solidify for 15-20 min. The negative control plates received only distilled water and methanol in PDA without extract, while for positive control; fungicide Bavistin (Systemic fungicide) and Mancozeb (contact fungicide) were used. 4 mm inoculums from actively growing margin of fungus culture isolate (7 day old culture) was placed face down in the center of the petriplate with the help of sterilized needle. Each time the needle was sterilized with flame and the whole process was done in a very aseptic and sterilized condition. The test fungi were inoculated for 7 days at 27°C. Control petriplates were run following the same process. The fungal colony was measured on the 7th day of incubation for the data analysis. Minimum and maximum readings of the colony diameter were taken using millimetre ruler. For each treatment seven replicates were used and mean value was taken. The result was compared with the positive control.

RESULTS AND DISCUSSION

Qualitative analysis showed that *Parthenium hysterophorus* showed highest reaction for tannins and saponins in distilled water while terpenoids and flavonoids in methanol extract. Moderate and weak reactions were shown in distilled water in plant leaf extract (Table 1).

Table 1: Preliminary phytochemical screening of Leaf of *P. hysterophorus*.

Plants	Phytochemical Constituents							
	Solvent	Tannin	Saponin	C.glycoside	Terpenoide	Steroids	Flavonoid	Alkaloid
<i>Parthenium hysterophorus</i>	Dist. water	+++	+++	-	-	-	++	+
	Methanol	-	+	++	+++	+	+++	++

Responses to various tests were denoted by (+), (++) and (+++) signs indicating weak, moderate and strong reactions respectively while (-) for no reaction; c. glycoside- cardiac glycoside, Dist. water- distilled water

A. Mean Linear Mycelium Growth (LMG)

In distilled water leaf extract, *Sclerotium rolfsii* had the highest (100 mm) LMG at 50, 100 and 150 mg/ml and lowest (46 mm) at 250 mg/ml concentration and 100 mm LMG was in negative control (Table 2). LMG 46 mm was lower than the LMG in the fungicide bavistin which was 47 mm. *Phytophthora capsici* had 100 mm LMG at all the concentrations. *Alternaria brassicae* had highest (63 mm) LMG at 50 mg/ml concentration and lowest (32 mm) at 250 mg/ml concentrations while

it was 72 mm in negative control. *A. brassicae* had lower mycelium growth 32 mm at 250 mg/ml than bavistin with 45 mm LMG. *Fusarium oxysporum* had 77 mm growth in negative control and had highest (55 mm) and lowest (38 mm) LMG at 50mg/ml and 250 mg/ml concentrations respectively. Similarly, *Botrytis cinerea* had the highest (33 mm) LMG and lowest (15 mm) LMG at 50mg/ml and 250 mg/ml concentrations respectively and growth in negative control was 27 mm.

The lowest (15mm) value was found lower than bavistin having LMG 16 mm (Table 2). There was significant difference ($p < 0.01$) among the LMG of the tested fungi and the different distilled water concentrations used (Table 2).

In methanol crude leaf extract, highest (100 mm) LMG for *S. rolfisii* was found at 50 mg/ml and the growth was found inhibited at 250 mg/ml concentration while 47 mm and 21 mm linear growth was observed in fungicides bavistin and mancozeb respectively. *P. capsici* had the highest (100 mm) growth at 50 and 100 mg/ml and lowest (66 mm) at 250 mg/ml. Both *S. rolfisii* and *P. capsici* had 100 mm growth in negative control as well (Table 3). *A. brassiceae* had highest (43 mm) and lowest (28 mm) mycelium growth at 50 mg/ml and 250 mg/ml concentrations respectively, while growth in negative control it was found 51 mm. LMG at all the concentrations was found lower than

bavistin with mycelium growth 45 mm. *F. oxysporum* had highest (30 mm) LMG at 50 mg/ml and 31 mm in negative control, while its growth was completely inhibited at 250 mg/ml concentration. *B. cinerea* had highest 16 mm and lowest 11 mm LMG at 50 and 250 mg/ml concentrations while it had 22 mm growth in negative control. LMG 14 mm, 13 mm and 11 mm at concentrations 100mg/ml -250 mg/ml was lower (16 mm) than bavistin. *B. cinerea* at 250 mg/ml had lower mycelium growth than mancozeb having LMG 16 mm. Among the fungus *P. capsici* had the largest (100 mm) LMG while *B. cinerea* had the lowest (11 mm) LMG (Table 3). There was significant ($p < 0.01$) difference between mycelium growth of tested fungi and the different methanolic concentrations used. Concentrations among themselves were also found significant given by different letters in the table (Table 3).

Table 2: Mean linear mycelium growth in distilled water crude leaf extract of *Parthenium hysterophorus* in different test fungus.

Linear mycelium growth (mm) in distilled water leaf extract of <i>Parthenium hysterophorus</i>										
Fungal strains	Concentrations (mg/ml)					Control			p	F
	50	100	150	200	250	Negative Dist. water	Positive Bavistin	Mancozeb		
<i>Sr.</i>	100±0d	100±0d	100±0d	79±5c	46±6b	100±0d	47±1b	21±2a	.000	883.07
<i>Pc.</i>	100±0b	100±0b	100±0b	100±0b	100±0b	100±0b	100±0b	33±2a	.000	1.04
<i>Ab.</i>	63±5e	61±1e	54±7d	51±4d	32±1b	72±6f	45±1c	21±1a	.000	108.15
<i>Fo.</i>	55±5e	51±6de	49±7cd	46±1c	38±5b	77±3f	13±1a	13±1a	.000	179.55
<i>Bc.</i>	33±1	30±4e	26±3d	19±2c	15±1ab	27±1d	16±1b	13±1a	.000	90.95

Abbreviations: *Sr* = *Sclerotium rolfisii*, *Pc* = *Phytophthora capsici*, *Ab* = *Alternaria brassiceae*, *Fo* = *Fusarium oxysporum*, *Bs* = *Botrytis cinerea*, values are mean ± SD of seven replicates, Dist. water = distilled water

For each fungal strains significance difference between mean among different concentration are indicated by different letters (Duncan homogeneity test, $p < 0.01$). F and p values were obtained by one way analysis of variance (ANOVA).

Table 3: Mean linear mycelium growth in methanol crude leaf extract of *Parthenium hysterophorus* in different test fungus.

Linear mycelium growth (mm) in methanol leaf extract of <i>Parthenium hysterophorus</i>										
Fungal strains	Concentrations (mg/ml)					Control			p	F
	50	100	150	200	250	Negative Methanol	Positive Bavistin	Mancozeb		
<i>Sr.</i>	100±0f	100±0f	81±5e	60±7d	0±0a	100±0f	47 ±1c	21±2b	.000	1.13
<i>Pc.</i>	100±0d	100±0d	83±5c	78±10c	66±8b	100±0d	100±0a	33±2d	.000	163.66
<i>Ab.</i>	43±1e	36±5d	34±1cd	32±1c	28±4b	51±4f	45±1e	21±1a	.000	79.82
<i>Fo.</i>	30±5d	28±5d	23±2c	16±1b	0±0a	31±5d	13±1b	13±1b	.000	83.39
<i>Bc.</i>	16±3d	15±2cd	14±2bcd	13±3b	11±1a	22±1e	16±1d	13±1bc	.000	20.52

Abbreviations: *Sr* = *Sclerotium rolfisii*, *Pc* = *Phytophthora capsici*, *Ab* = *Alternaria brassiceae*, *Fo* = *Fusarium oxysporum*, *Bs* = *Botrytis cinerea*, values are mean ± SD of seven replicates.

For each fungal strains significance difference between mean among different concentration are indicated by different letters (Duncan homogeneity test, $p < 0.01$). F and p values were obtained by one way analysis of variance (ANOVA).

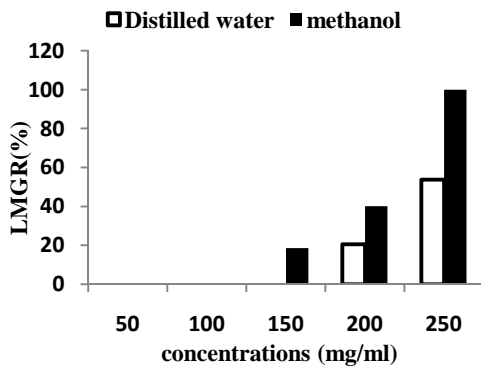
Distilled water extract of *P. hysterophorus* had the highest antifungal activity against *S. rolfsii* and *Alternaria brassiceae* (Table 2). All the tested fungi were found more resistant to methanol extract than distilled water extract (Tables 2 and 3) which can be attributed to antimicrobial properties of the plant parts or the whole plant vary with the type of solvents used to prepare the extracts from respective plant parts (Ganjewala and Devi 2009). There was significant difference ($p < 0.01$) between the linear mycelium growth and the different concentrations of plant extract and the tested fungus. The plant crude extract at concentrations 150 - 250 mg/ml were found more effective in reducing the growth of the fungus than the synthetic fungicides used in the study showing the plant extracts at higher concentrations might have fungicidal properties.

Antifungal activity of the plant extract which might be correlated to the various phytochemicals present in their respective extract (Sule *et al.* 2011) and also this may be due to the reason that the agrochemicals present in the plants are the supply of natural fungicides, insecticides and pesticides (Shu 1998; Cordell 1995). Similarly, Bajpai *et al.* (2012) also reported antifungal

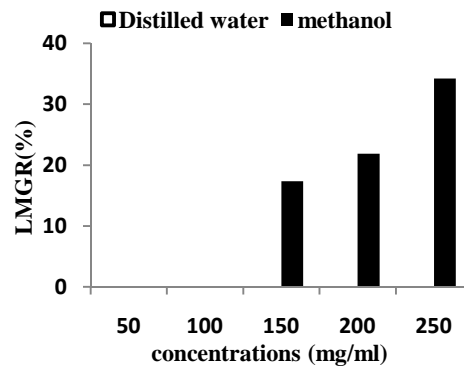
activity of Invasive Alien plants species against *Magnaporthe oryzae*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, *Blumeria graminis f. sp. hordei*, *Colletotrichum coccodes*.

B. Mean Linear Mycelium Growth Reduction (LMGR) percentage

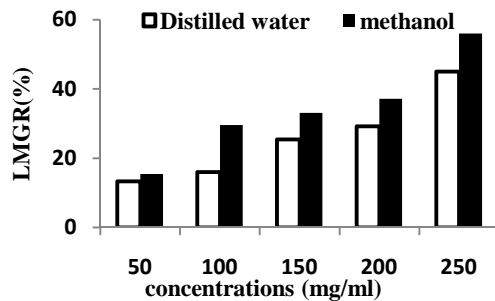
Mean Linear Mycelium Growth Reduction (LMGR) percentage was found higher in methanol leaf extract than in distilled water leaf extract for all the tested fungi at all the concentrations (Figs.1). In distilled water crude leaf extract no LMGR was found for *Sclerotium rolfsii* at concentrations 50, 100 and 150 mg/ml while at 200 mg/ml and 250 mg/ml concentrations 21% and 54% reduction was observed respectively. In methanol extract 100 % reduction was observed at 250 mg/ml concentration (Fig.1a). No LMGR percentage was found in *P. capsici* in distilled water, while in methanol 34% growth reduction was observed at 250 mg/ml concentration (Fig.1b). *A. brassiceae* had LMGR percentage 13-45 % and 15-56 % in distilled water and methanol extract respectively at concentrations 50-250 mg/ml (Fig.1c).



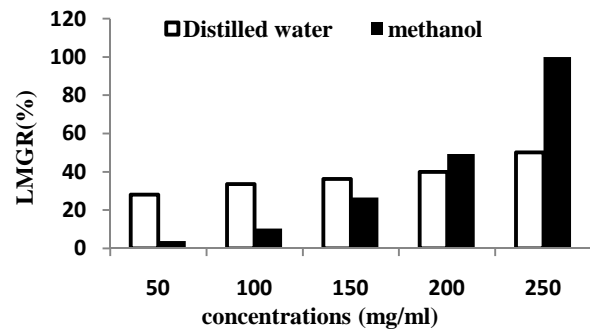
a: Mean LMGR percentage in *S. rolfsii*, n=7



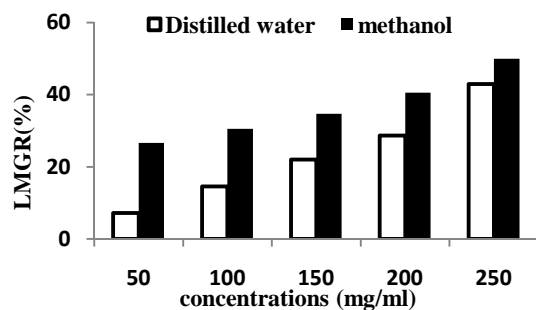
b: Mean LMGR percentage in *P. capsici*, n=7



c: Mean LMGR percentage in *A. brassiceae*, n=7



d: Mean LMGR percentage in *F. oxysporum*, n=7



e: Mean LMGR percentage in *B. cinerea*, n=7

Fig. 1. Mean linear mycelium growth reduction (LMGR) percentage in distilled water and methanol leaf extract of *Parthenium hysterophrous*.

In *F. oxysporum* 28-50 % LMGR was observed in distilled water while methanol extract had 4-100 % LMGR (Fig.1d). *B. cinerea* had 7-43 % and 27-50 % LMGR percentage in distilled water and methanol extract respectively (Fig.1e). In methanol crude leaf extract of *Parthenium hysteophrous* highest (100 %) LMGR was observed in *S. rolfsii* and *F. oxysporum* (Fig. 1a and 1d) while least percentage was found in *P. capsici* (Fig.1b) at 250 mg/ml concentration. In distilled water extract the highest LMGR percentage was found in *S. rolfsii* (54%) and no percentage reduction in *P. capsici* at 250 mg/ml concentration (Figs. 1a and 1b). The result showed that the Linear Mycelium Growth Reduction (LMGR) percentage was found higher in methanol leaf extract than in distilled water leaf extract for all the test fungus at all the concentrations which might be due to the reason that methanol solvent is known with its ability to isolate more antimicrobials compounds from plants than water solvent extracts (Cowan 1999).

The leaf extract was found most effective against *S. rolfsii* by reducing the growth to 54 % in distilled water and 100 % in methanol extract at 250 mg/ml concentration. It was also found most effective against *F. oxysporum* by reducing the growth to 50 % in distilled water and inhibiting the growth to 100 % at concentration 250 mg/ml (Fig. 1).

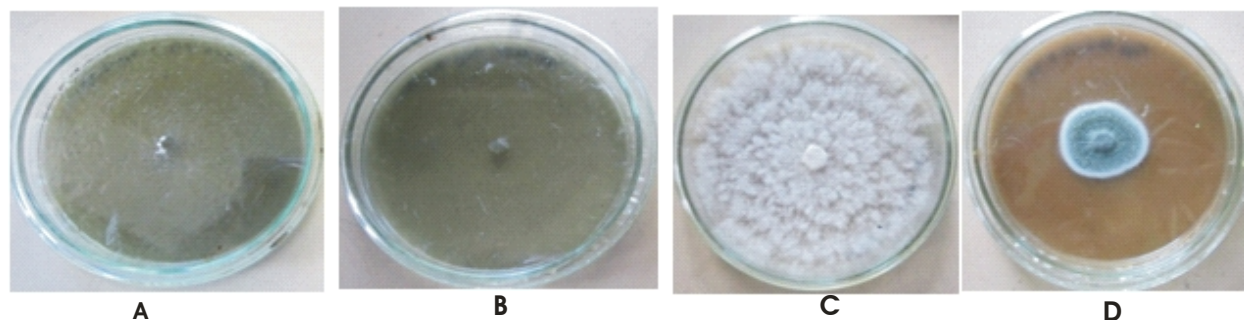


Fig. 2. A. *S. rolfsii* (Meth), B. *F. oxysporum* (Meth) C. *P. capsici* (Meth.) D. *B. cinerea* (DW)

The several important chemical constituents present in the leaves of *P. hysterophrous* have antifungal activity (Kushwaha and Maurya 2012). *P. capsici*, *S. rolfsii* and *F. oxysporum* were found the most susceptible fungus while *A. brassiceae* and *B. cinerea* were the most resistant fungus to the extract at higher concentrations. Similar result for *A. brassiceae* was shown by Mandloi *et al.*, (2013) in the methanol leaf of extract of *Terminalia catappa*. The resistance of fungi to the tested extracts might be due to the presence of more complex cell wall with rigidity and also might be due to the reason that the fungi differ in optimum growth conditions such as pH, production rate of manganese and lignin peroxidases and their resistance to toxic chemicals (Rucksdeschel and Renner 1986). Also, this may be due to their ability to produce extracellular enzymes that helps them to degrade and metabolize

substrate such that the extract becomes a source of food to the fungi instead of inhibiting their growth after they have been rendered nontoxic due to degradation (Gatsing *et al.* 2010) and also fungi are able to degrade chemicals extracellularly using lignase and manganese-dependant enzymes (Michel *et al.* 1991, Perie and Gold 1991). Increasing the concentration of the plant extract had increased the LMGR percentage of the test fungus under study as evident from shorter mycelium length at higher concentration. Similarly the present result was found similar with the results of Suleiman (2011) on different plant and fungus and who found that the inhibitory action of the extracts on mycelial growth increased with increase in concentrations. Bajpai *et al.* (2012) also reports that disease severity increased as the concentrations of the plant extract increased in all tested pathosystem.

Goel and Sharma (2013) found that the fungal growth was inhibited by the extract and the inhibition was directly proportional to the increasing concentration of the extract.

CONCLUSION

Leaves of *Parthenium hysterophorus* was rich source of important antifungal chemicals such as tannins, saponins, terpenoids, flavonoids, alkaloids, cardiac glycosides and steroids which has enabled them to show varying degree antifungal properties. Both distilled water and methanol crude leaf extracts exhibit antifungal action. However, methanol crude leaf extract had higher antifungal potential than the distilled water extract.

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