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# Antifungal Activities and Phytochemical Screening of *Xanthium strumarium*

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**ABSTRACT: Antifungal activities and phytochemical screening of *Xanthium strumarium* (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 rolfsii* were tested at different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml) of selected 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. The distilled water extracts inhibited the growth of fungal mycelium while methanolic extracts completely inhibited (100%) the growth of some selected fungi at higher concentrations. *Fusarium oxysporum* was the most susceptible fungus while *A. brassicae* and *B. cinerea* were the most resistant fungi.**

**Key words**: Antifungal activities, phytochemical screening, *Xanthium strumarium*, Linear Mycelium Growth Reduction (LMGR)

## INTRODUCTION

Cocklebur (*Xanthium strumarium* L.) is an herbaceous annual plant of worldwide distribution. The plant is erect, up to 2.5 m tall, and presents blotched purple stems. Its leaves are dark green on the upper surface, similar in shape to grape leaves, 15 cm in diameter and roughly textured with minute bristles. It is one of the twenty one Invasive Alien Species (IAS) of Nepal ranked by IUCN (Tiwari *et al*. 2005). It is found to be problematic in agricultural field. Plant is also found to be dominating in roadsides and open dry pastures. The principal compounds isolated from *X. strumarium* include xanthanol, isoxanthanol, hydroquinone, caffeyolquinic acids, alkaloids, and thiazinedione (Han *et al*. 2007; Ying-Tsun *et al*. 1998).

All parts of plant possess sedative, diaphoretic and diuretic properties. The plant also shows its efficacy in mitigating longstanding cases of malarial fever (Sharma 2003). Some biological properties of *Xanthium strumarium* L. have been reported, such as antiulcerogenic (Favier *et al.*, 2005), anthelmintic (Sharma 2003), anti-inflammatory (Kim *et al*., 2005; Yadava & Jharbade, 2007), diuretic (Nieves *et al*., 1999), antileishmanial, and a significant depressant action on the central nervous system (Mandal *et al*., 2001). However, there are no reports about the antifungal activities of *X. strumarium* extracts against phtopathogenic microorganisms such as *Alternaria brassicae, Botrytis cinerea, Fusarium oxysporum, Phytophthora capsici* and *Sclerotium rolfsii.* In present study antifungal activity of leaf extract of *X. strumurium* was tested against these five phytopathogenic fungi in laboratory.

## MATERIALS AND METHODS

*A. Collection of plant material and extraction procedure*

The fresh and healthy leaves of *Xanthium strumarium* were collected at the flowering stage from the Kirtipur of Kathmandu. The leaves were washed under running tap water and kept under shade on the newspaper till they become completely dry. The completely dried leaves sample was grinded into fine powder with the help of electric grinder and stored in air tight zipper bag.

A fixed weight (25 g) of dried powdered leaf sample of plant material was soaked separately in 250 ml of methanol (95%) and distilled water for 72 h. Each mixture was stirred at 24 h interval using a sterile glass rod (Alagesaboopathi 2011). The plant samples squeezed and then filtered with the help of triple layered cotton cloth. Water content of distilled water extract was evaporated on heating mantle using water bath till the solution reduced to semisolid form (Bhattarai and Shrestha 2009). The round bottom flask containing methanolic extract was fitted with rotary vaccum evaporator under negative pressure. The flask was constantly heated in rotating condition by using water bath below 55°C. Extracts were then transferred into sterile labeled bottles and they were made into semisolid form by evaporation to water bath at 50°C. The crude extracts were weighted and made the bottles air tight and stored in a refrigerator at temperature 4°C until further use (Mahida and Mohan 2007).

*B. Antifungal assay*

The antifungal activity of plant extracts was evaluated against food-associated fungi by using poisoned food technique applying the method of Nene and Thapliyal (1979). For this potato dextrose agar media was applied. Potato Dextrose Agar (PDA) when cooled at 50°C, a volume of 1 ml of each concentration was aseptically poured into the well labelled and sterilized petriplate followed by the addition of 9 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents (Singh and Singh 2013). The plates were then allowed to solidify for 15-20 minutes. After solidification of PDA, Mycelia discs of 5 mm diameter were cut with sterilized needle from the periphery of 7 day old culture of the test organisms were aseptically inoculated upside down on the center of the PDA. Each time the needle was sterelized with flame and the whole process was done in a very aseptic and sterilized condition. Seven replicates for each extract was incubated for 7 days at 27°C.

The fungal growth was measured on the 7th day (Singh and Singh 2013). Minimum and maximum readings of the colony diameter were taken using transparent millimetre ruler. The percentage of fungal growth inhibitation ( growth in control – growth in treatment)/ growth in control x 100) was calculated using the formula given by Khalil and Dababneh (2007).

*C. Phytochemical Screening*

Phytochemical screening of the extracts was done for saponins, tannins, alkaloids, terpenoids and flavonoids using standard phytochemical screening methods with slight change.

*D. Qualitative analysis*

Preliminary qualitative phytochemical screening was carried out on aqueous and methanol extract applying the standard protocols described by Sofowara (1993), Trease and Evans (1989), and Harborne (1973) and for the result sharp change in color was noted.

**Terpenoids (Salkowski test):** five ml of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated $H_2SO_4$ was added carefully to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

**Saponin:** One gram of the powered samples were boiled in 10 ml of water and methanol separately in a water bath and filtered. Five ml of the filtrate was mixed with 2.5 ml of distilled water and shaken vigorously for a stable persistent froth.

**Flavonoids:** Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated $H_2SO_4$. A yellow coloration observed in extract indicated the presence of flavonoids. The yellow coloration disappeared after some time.

**Tannins**: About 0.5 g of the dried powered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Alkaloid**: Plant sample of 200 mg was taken in 10 ml methanol and was filtered. Mayer's Test: In 2 ml filtrate 1% HCL was added and was steamed and 1 ml of filtrate was treated with 6 drops of Mayer's reagents.

Formation of yellow coloured precipitate indicated the presence of alkaloids. Wagner's Test: Filtrate was treated with Wagner reagent.

Formation of brown/ reddish coloured precipitate indicated the presence of alkaloids. Dragondroff's Test: Filtrate was treated with Dragondroff's reagents Formation of red coloured precipitate indicated the presence of alkaloids.

*E. Data Analysis*

The values were expressed as mean ± standard deviation (SD). Each value was a mean of seven replicates. The One–way Analysis of Variance (ANOVA) was used to determine the significant differences between the parameters and the Tukey HSD test was done to compare the differences of letters at $p < 0.05$ using statistical package SPSS version 16.

## RESULTS AND DISCUSSION

*A. Antifungal activity*

Linear mycelium growth reduction (LMGR) of D/W extract of *X. strumarium* ranged from 90 mm-13 mm diameter at 50 mg/ml-250 mg/ml concentrations respectively. Among fungi *P. capsici* showed the highest LMG (90 mm) diameter at all concentrations and *B. cinerea* showed the lowest LMG (24 mm-13 mm) diameter at 50 mg/ml-250 mg/ml concentrations respectively (Table 1).

**Table 1: Linear mycelium growth (mm) diameter in distilled water crude leaf extract of *Xanthium strumarium.***

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P<0.05.

| | Concentrations(mg/ml) | | | | | control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fungal strain | 50 | 100 | 150 | 200 | 250 | Negative | Positive | | | |
| | | | | | | DW | Bavistin | Mancozeb | P | F |
| *A.b.* | 61±2 e | 57±3 e | 40±1 cd | 39±2 c | 28±3 b | 72±6 f | 44±1 d | 21±1 a | .000 | 204.55 |
| *B.c.* | 24±2 c | 17±3 b | 15±3 ab | 14±.8a | 13±.9 a | 27±c | 16±.7 ab | 13±1 a | .000 | 55.8 |
| *F.o.* | 72±2 d | 60±9 c | 32±1 b | 27±1 b | 16±2 a | 76±3 d | 13±1 a | 15±.9 a | .000 | 326.06 |
| *P.c.* | 90±0 b | 90±0 b | 90±0 b | 90±0 b | 90±0 b | 90±0 b | 90±0 b | 32±1 a | .000 | 7.61 |
| *S.r.* | 90±0 e | 90±0 e | 90±0 e | 86±.4 d | 84±.9 c | 90±0 e | 46±1 b | 21±1 a | .000 | 7.56 |

Abbreviations: *A.b. = Alternaria brassicae, B.c.= Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.=Phytophthora capsici, S.r.=Sclerotium rolfsii,* values are mean ± SD of seven replicates.

As compared with positive controls (Bavistin and Mancozeb), *A. brassicae* had (40 mm, 39 mm, 28 mm) diameter at 150 mg/ml-250 mg/ml concentrations respectively which was lower LMG than Bavistin which was (44 mm). *Botritis cinerea* had (15 mm, 14 mm and 13 mm) diameters a LMG at 150 mg/ml-250 mg/ml concentrations which was lower LMG than Bavistin (16mm). ANOVA result showed there was significant differences in mean value of LMG of fungi in different concentration of plant leaf extract (Table 1).

Linear mycelium growth reduction (LMGR) of methanol crude leaf extract of *X. strumarium* ranged from 90 mm-0 mm diameter at 50 mg/ml-250 mg/ml concentrations respectively among all fungi. *Phytophthora capsici* showed the highest LMG, ranged from 90 mm-27 mm diameter at 50 mg/ml-150 mg/ml concentrations while from 200 mg/ml-250 mg/ml the growth of fungus completely inhibited by plant extract, followed by *Sclerotium rolfsii* ranged from 87 mm-80 mm diameter at 50 mg/ml-100 mg/ml concentrations but at 150 mg/ml-250 mg/ml concentrations the growth was completely inhibited. The lowest LMG was observed in *Botrytis cinerea* (16 mm-10 mm) at 50 mg/ml-250 mg/ml (Table 2).

In the comparison of positive controls Bavistin and Mancozeb with different concentrations of plant extract, *Alternaria brassicae* had mycelium growth 37 mm diameter at 50 mg/ml which was lower than Bavistin with mycelium growth 44 mm and at 250 mg/ml concentration (20 mm) diameter which was also lower than Mancozeb (21) mm diameter. Similarly, *Botritis cinerea* had LMG (12 mm) at 100 mg/ml concentration which was found lower than Mancozeb (13 mm). *F. oxysporum* showed 0 mm diameter at 150 mg/ml which was lower than both Bavistin (13 mm) and Mancozeb (15 mm) diameter. In the fungus *Phytophthora capsici* the lowest mycelium growth was observed 31mm at concentration 100 mg/ml which was lower than both Bavistin (90 mm) and Mancozeb (32 mm) respectively.

**Table 2: Linear mycelium growth (mm) diameter in methanol leaf extract of *Xanthium strumarium.***

The data were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. For each fungal strains significance between mean among different concentrations are indicated by different letters (Tukey HSD multiple comparison test, P< 0.05.

| | Concentrations | | | | | control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fungal strain | 50 | 100 | 150 | 200 | 250 | Negative | Positive | | | |
| | | | | | | Methanol | Bavistin | Mancozeb | P | F |
| *A.b.* | 37±1 b | 35±3 b | 26±5 a | 25±5 a | 20±.8 a | 50±4 d | 44±1 c | 21±1 a | .000 | 76.86 |
| *B.c.* | 16±1 d | 12±2 bc | 11±.2abc | 11±.5 ab | 10±.2 a | 22±1 e | 16±.7 d | 13±1 c | .000 | 89.19 |
| *F.o.* | 28±.8 d | 22±1 c | 0±0 a | 0±0 a | 0±0 a | 31±4 d | 13±1 b | 15±.9 b | .000 | 354.7 |
| *P.c.* | 90±0 d | 31±1 c | 27±.8 b | 0±0 a | 0±0 a | 90±0 d | 90±0 d | 32±1 c | .000 | 1.61 |
| *S.r.* | 87±1 e | 80±6 d | 0±0 a | 0±0 a | 0±0 a | 90±0 e | 46±1 c | 21±1 e | .000 | 1.94 |

Abbreviations: *A.b.= Alternaria brassicae, B.c.= Botrytis cinerea, F.o.= Fusarium oxysporum, P.c.= Phytophthora capsici, S.r.= Sclerotium rolfsii,* values are mean ± SD of seven replicate.

In *Sclerotium rolfsii* the LMG was completely inhibited from 150 mg/ml which was also lower than both Bavistin (46 mm) and Mancozeb (21mm). ANOVA result showed that there was significant difference p<0.05 in mean value of LMG of fungi in different concentrations of plant leaf crude extract (Table 2).

*B. Linear mycelium growth reduction percentage (LMGR) of Xanthium strumarium*

In methanolic leaves extract *Xanthium strumarium* showed 100% inhibition on *F.oxysporum* (Fig.3), *P.capsici* (Fig. 4) and *S. rolfsii* (Fig. 5) at higher concentration and least LMGR percentage was found in *A.brassicae* (60%) Fig.1 and *B. cineria* (54%) Fig. 2 at 250 mg/ml concentration

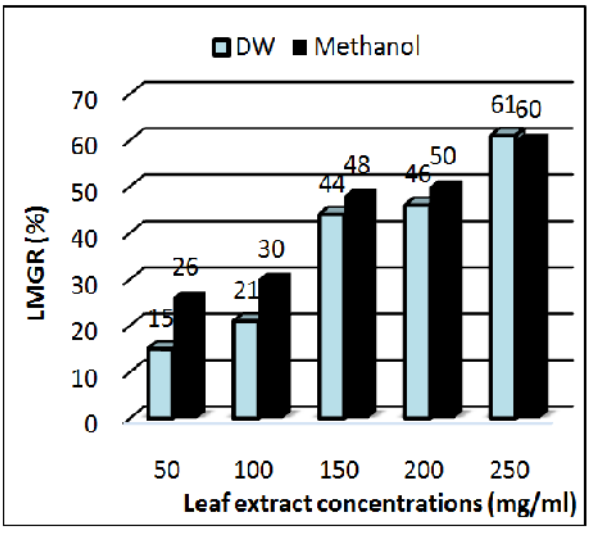


**Fig. 1.** Mean Linear mycelium growth reduction (LMGR) percentage in *A. brassicae*, n=7

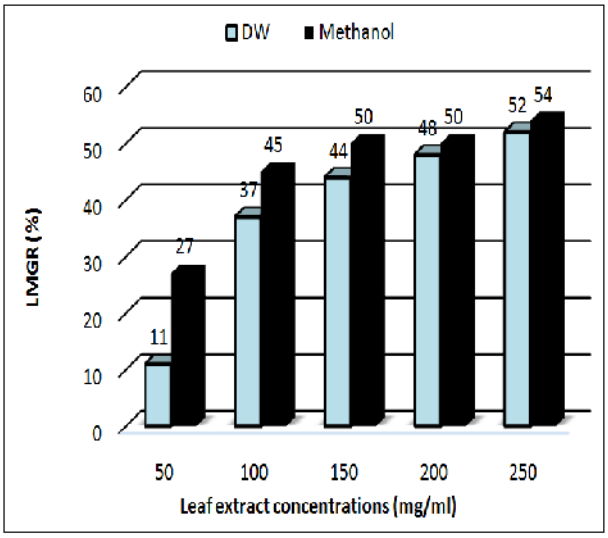


**Fig. 2.** Mean Linear mycelium growth reduction (LMGR) percentage in *B. cinerea*, n=7.

In DW leaf extract *X. strumarium* showed the highest percentage of LMGR on *F. oxysporum* (79%). Fig. 3 and the lowest percentage in *S. rolfsii*. (6%) Fig. 5 at 250 mg/ml concentration (Table 3) while there was no LMGR% was found in *P. capsici* from 50 mg/ml-250 mg/ml (Fig. 3) and in *S. rolfsii* at 50 mg/ml-150 mg/ml concentration (Fig. 4). Khuda *et al*. (2012) found that *X. strumarium* inhibited *F. solani* in solvents-chloroform and n-hexane (30% inhibited) and ethyle acetate (20% inhibited). But in aqueous extract there was no inhibition observed.

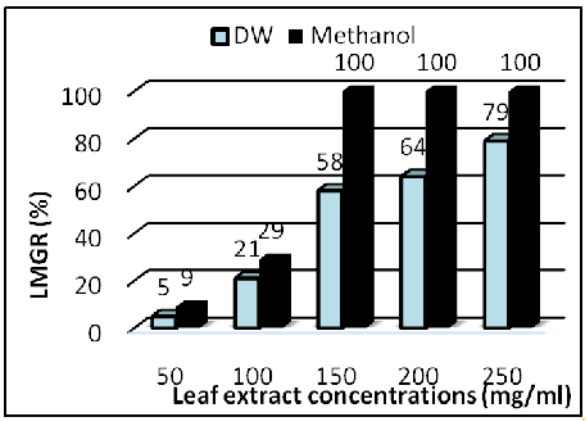


**Fig. 3.** Mean Linear mycelium growth reduction (LMGR) percentage in *F. oxysporum*, n=7.

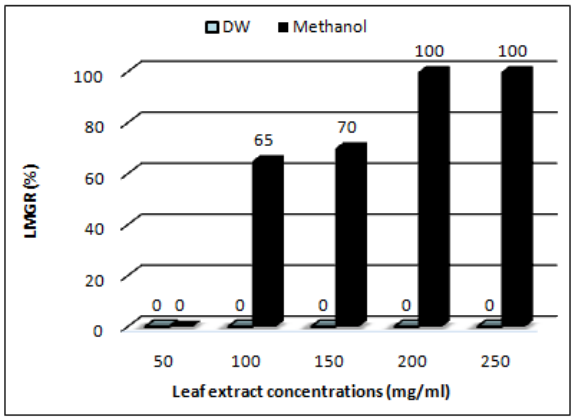


**Fig. 4.** Mean Linear mycelium growth reduction (LMGR) percentage in *P. capsici*, n=7.

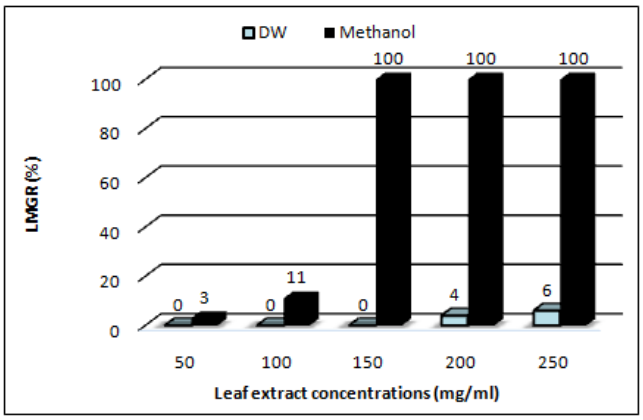


**Fig. 5.** Mean Linear mycelium growth reduction (LMGR) percentage in *S. rolfsii*, n=7.

This dissimilar result may be due to the inhibitory activity of plant extract is largely dependent on the concentration, parts of the plant used and the microbes tested (Kalimuthu *et al.* 2010).

*C. Phytochemical screening*

As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and tri-terpenoids are producing a better opportunity for testing wide range of microorganism (Arora *et al.* 2012).

The phytochemical screening test had shown the presence of active chemical constituents such as terpenoids, saponins, flavonoids, tannins and alkaloids.

Distilled water extract of *Xanthium strumarium* leaves revealed the presence of high concentration of terpenoids (Table 3); while its methanolic extract of revealed the presence of high concentration of terpenoids, saponins, flavonoids and alkaloids. Sravani and Laxshmi (2012) found that the methanol, aqueous and petroleum ether extracts of *X. strumarium* revealed the presence of flavonoids and steroids but negative results were obtained for alkaloids, terpenoids, tannins, glycosides and saponins. The result is somewhat dissimilar with this present study. This dissimilar result may be due to a large extent, the phenological stage of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction and solvent used (Felix 1982).

**Table 3: Preliminary phytochemical screening of leaf extract of *X. strumarium*.**

| Chemical compounds | Solvents | |
|---|---|---|
| | Distilled water | Methanol |
| Terpenoids | +++ | +++ |
| Saponins | ++ | +++ |
| Flavonoids | _ | +++ |
| Tannins | _ | + |
| Alkaloids | ++ | +++ |

Responses to various tests were denoted by +, ++ and +++ signs indicating weak, moderate and strong reactions respectively while - for no reaction.

## CONCLUSION

Both distilled water and methanol extracts had shown varying degrees of antifungal activities on the microorganisms tested. The chance to find antifungal activity was more apparent in methanol than distilled water extracts of *Xanthium strumarium*. *Alternaria brassicae* and *Botrytis cinerea* were found the most resistant fungi against the all concentrations of both methanolic and distilled water extracts of plant and *Fusarium oxysporum* was found the most susceptible fungus among the selected phytopathogenic fungi.


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## AUTHORS' CONTRIBUTION

First author help to analyse data and draft the article while second author help in laboratory work.

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