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Research Article

ASSESSMENT OF ANTIFUNGAL ACTIVITIES OF SOME INVASIVE ALIEN SPECIES OF ASTER FAMILY

A Devkota* and A Sahu
Central Department of Botany Tribhuvan University, Kathmandu, Nepal
**Author for Correspondence: devkotaa@gmail.com*

ABSTRACT
In present study, the antifungal activity and phytochemical screening of the leaves of three invasive alien plant species of Aster family *viz. Ageratum haustonianum, Ageratina adenophora* and *Parthenium hysterophorus* were carried out in laboratory. Eight phytopathogenic fungi; *Alternaria alternate, Alternaria brassicae, Botrytis cinerea, Exserohilium sp, F. moniliforme, Fusarium oxysporum, Phytophthora infestans,* and *Sclerotium rolfsii* were tested against leaves extract of three plants. Aqueous and methanolic extracts of three plants at four different concentrations viz. at 50 mg/ml, 100 mg/ml, 150 mg/ml & 200 mg/ml were tested on phytopathogenic fungi using the disc diffusion method. *In vitro* antifungal activity were screened by using Potato Dextrose Agar (PDA) media. The qualitative phytochemical analysis depicted the presence of saponins, alkaloids, glycosides, flavonoids, glycosides and coumarins in the plants. Results showed broad spectrum antifungal activity against tested fungi. Results from *in vitro* study revealed that the antifungal activity might have been influenced by the solubility of active compound (s) in extracting solvent. Methanol extracts were relatively more effective than aqueous extract.
The demonstration of activity against all these organisms had shown that all three alien invasive species; *Ageratum haustonianum, Ageratina adenophora* and *Parthenium hysterophorus* can be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity.

Keywords*: Invasive Alien species, Antifungal activity, Phytochemical screening*

INTRODUCTION
An invasive plants referred to as indigenous or non-native, is one that has been introduced by humans intentionally or otherwise through human agency or accidently from one region to another. The diverse bioclimatic zones of Nepal range from tropical to alpine favor the introduction of several alien species (Tiwari *et al.* 2005). These species have been spreading aggressively by colonizing several landscapes and ecosystems displacing the native ones (Lockwood *et al.* 2001).
Invasive plants have important ecological and economic impacts world-wide and increasing attention is now being paid to eradication and management efforts (Pimental *et al.* 2000). Antimicrobial activities and phytochemical constituents of several medicinal plants have already been studied (Parekh *et al.* 2005; Parekh and Chand 2007). But there are very few references regarding the invasive alien species. The impact of the invasion can be site-specific and is linked, among other factors, to soil properties in the novel habitat prior to the invasion. Management of such alien invasive plant is necessary for conservation of biodiversity and native species. Antimicrobial study of alien plants helps to find the potentialities of effect of these plants on bacterial and fungal growth. Further phytochemical study also help to find the chemical constituents that may be useful for pharmaceutical formulation. So the result of this study may have great significance in the management of such invasive alien weed.
The present study focused on the qualitative screening of secondary metabolites from the different invasive alien weeds and their antimicrobial assay. The result of this type of study may provide potential health applications at affordable cost. This type of study also keenly represents one of the best opportunities in searching for new economically important plants for medicine. Hence the study deals with the screening and scientific evaluation of bioactive compound possessing a diverse range of

pharmacological properties that may in turn prove beneficial for the mankind along with the management of the weeds.

MATERIALS AND METHODS

Collection of plant materials

Three invasive alien plant species namely *Ageratum haustonianum, Mikania micrantha*, and *Parthenium hysterophorus* were collected for study in their vegetative stages. *Parthenium hysterophorus* was collected from roadside fallow land of Kirtipur (27º 40.20' N 85º 17.32' E, alt. 1300 m asl) *Ageratum haustonianum* was collected from the grassland of Kasara village Chitwan and *Mikania micrantha* from Chitwan National Park during the month of June to August , 2012. Leaves of all the selected plants were collected and shade dried. Dried samples were chopped, pulverized to powder and stored in zipper plastic for further analysis.

Test Organism

Eight phytopathogenic fungi *viz. Alternaria alternate*, *Alternaria brassicae*, *Botrytis cinerea*, *Exserohilium sp. Fusarium moniliforme, F. oxysporum, Phytophthora infestans* and *Sclerotium* ***rolfsii.*** were used as test organism of extract of invasive alien plants. The pure fungal strains were brought from NAARC, Khumaltar.

Laboratory Analysis

Laboratory work was carried out in Laboratory of Central Department of Botany, Tribhuvan University, Kirtipur.

Extraction of plant sample

Plant samples from each species were individually extracted by soaking 25 g of finely ground plant material with 250 ml of distilled water and methanol solvents separately in conical flasks, plugged with cotton and kept on a rotary shaker at 180-200 rpm for 24 h. It was filtered through 3 layered muslin cloths and the supernatant was filtered through Whatman No.1 filter paper. Each of the solvent extract was concentrated separately under reduced pressure. After complete solvent evaporation, each of the solvent extract was weighed and preserved at 5℃ in air tight bottles until further use.

Phytochemical screening

The samples were grinded in a blender and used for the phytochemical screening test. The extracts of all test plants were screened for the phytochemical constituents by using standard chemical test methods (Harborne 1998) with slight modifications.

Antifungal assay

For the antifungal assay, different concentrations (50, 100, 150, 200 mg/ml) of each extracts were prepared in respective solvents. The ability of various extracts to inhibit the growth of phytopathogenic fungi were determined by disc diffusion method (Bauer *et al.*1996, Parekh and Chand 2007) with some modifications considering the access and availability of equipments and chemicals.

Preparation of Potato Dextrose Agar (PDA)

250 ml of PDA was prepared by taking 100 g freshly sliced potato in 250 ml of water. The potato was boiled until it became soft. This was then filtered into a beaker using muslin cloth. This was slowly heated and 10 g Type 1 agar - agar and 10g dextrose was added slowly and was completely dissolved. The final volume of PDA was made 500 ml by adding extra water. The whole media was allowed to boil; this was then transferred into the conical flask and was sterilized by autoclaving at 15 lbs pressure and 121^{o}C for 2 h.

Preparation of strain

Potato Dextrose Agar (PDA) media was used for the fungal culture.100 g potato was sliced into small pieces and it was boiled into 250 ml water. This was slowly heated and 10 g Type 1 agar - agar and 10 g dextrose was added slowly and was completely dissolved. The final volume of PDA was made 500 ml by adding extra water and then it was boiled. Then 5ml of the prepared media was poured in a test tube and

plugged the mouth using cotton plug. Same process was repeated for other 9 test tube. After that they were autoclaved at 15 lbs and 121°C for 30 min and let them cool in slanted position in sterilized laminar flow.

Preparation of test discs

The test disc (4 mm diameter) was made by punching the filter paper (Whatman no, Grade 292). The discs were prepared by dipping and saturating sterilized filter paper in different concentrations of the plant extracts (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml and control). The discs were air dried in the laminar flow.

Inoculation of fungal strain on petriplates

Inhibition of fungal growth was tested by using disc diffusion method (Bauer *et al.*1996 Parekh and Chand 2007) with some modifications according to the lab facility and the time. PDA plates for the assay were prepared by drawing in each petriplates 6 chamber and labeling them with the date, code name of the fungi and the dices code. The inoculums of fungi were transferred into petriplates containing solidified media using sterile cotton swab. The sterile cotton swab was dipped into well mixed distilled water test culture and was spread on the media by moving the swab in Z –shape. One swab was used for one fungal strain. Seven replicates were used for each fungus. The culture plates were allowed to dry for 5-10 minutes. Then in each petriplate's in each chamber different concentrated dices was put with the help of sterile forceps. The plates were then incubated at 27±1 ^{o}C for 5 days. Microbial growth was determined by measuring the diameter of zone of inhibition (ZOI), indicated by the clear zone around the disc after incubation. The readings were taken in three different fixed directions in all seven replicates and the average values were recorded.

Statistical analysis: Each treatment of this experiment was carried out with seven replications . Treatments were prepared in a completely randomized design. Data was analyzed by SPSS version 11.5 using One-way ANOVA. Comparison on mean inhibition zone among plant samples against bacterial strains was carried out by Duncan multiple range test.

RESULTS

Phytochemical Screening

Phytochemical screening of the plants under study is given in Table 1. Alkaloids, flavonoids tannins, terpenoids, glycosides and saponins were the phytochemicals present in the plants. Alkaloids were present in all studied plant samples in varied amount. Carotene was absent in all samples. Responses to various tests were denoted by +, ++ and +++ signs indicating weak, moderate and strong reactions respectively while - for no reaction.

Table 1: Phytochemical constituents in leaves of three invasive alien plants studied

Plants species	Family	Phytochemical constituents						
		Alkaloids	Flavonoid	Carotene	Tannins	Terpenoid	Glycoside	Saponins
Ageratum haustonianum	Asteraceae	++	+	-	-	+++	-	+
Mikania micrantha	Asteraceae	+	+	_	++	+	++	+
Parthenium hysterophorus	Asteraceae	+	+	_	+	+	+	+

If PPT is slight: +, Medium: ++, Heavy: +++, Not: -

Antifungal activity

The effects of plant extracts from three invasive alien plants species on growth of fungal strains varied with plant species, and concentration of extract and type of solvent used. Aqueous extract at all concentration of three alien invasive plant species had no effect on growth of fungal phytopathogens.

Methanolic extract of some species had effect on growth of certain fungi. Growth of *Alternaria alternata* was effected by methanolic extract of all three plants; with maximum zone of inhibition was exhibited by *Parthenium hysterophorus* , with maximum zone of inhibition (10.5 mm) at 200 mg/ml concentration (Table 2) .

Table 2: Effect of three alien invasive species on growth of fungal strain- *Alternaria alternata;*

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Mean zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	7.5 ±1.3 ^{b}	7.2±1.14^{c}	8.0±1.32^{b}	8.27±0.88^{b}
Mikania micrantha	0.00	0.00	0.00	0.00	7.0±1.3 ^{b}	8.78±1.04^{d}	8.5±0.34^{c}	8.9 ±1.04^{b}
Parthenium hysterophorus	0.00	0.00	0.00	0.00	6.5±0.9 ^{b}	6.25±0.35^{b}	7.21±0.91^{b}	10.5±0.65^{d}
Control	0.0	0.00	0.00	0.00	0.00 ^{a}	0.00^{a}	0.00^{a}	0.00^{a}
F value	-	-	-	-	512	234	617	167
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

Methanolic extract of all three plant extracts showed inhibitory effect on growth of *Alternaria brassicae*; with highest effect was exhibited by extract of *Ageratum haaustonianum* with maximum zone of inhibition (10.0 mm) at 200 mg/ml concentration (Table 3).

Table 3: Effect of three alien invasive species on growth of fungal strain - ***Alternaria brassicae***

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	6.0 ±1.60^{b}	7.0±1.43 ^{b}	7.5±1.61 ^{b}	10.0±1.31^{d}
Mikania micrantha	0.00	0.00	0.00	0.00	8.0±1.19 ^{d}	8.5±1.10 ^{c}	9.0±1.45^{c}	9.2±1.73^{c}
Parthenium hysterophorus	0.00	0.00	0.00	0.00	7.5±1.0 ^{d}	7.8±2.10 ^{bc}	7.3±1.17 ^{b}	8.4±1.13^{b}
Control	0.0	0.00	0.00	0.00	0.00 ^{a}	0.00^{a}	0.00^{a}	0.00^{a}
F value	-	-	-	-	891	567	134	312
P value		-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

Methanolic extract of all three alien invasive plant species had inhibitory effect on growth of *Botrytis cinerea*. Maximum zone of inhibition (10.5 mm) was shown by extract of *Ageratina adenophora* (Table 4).

Table 4: Effect of three alien invasive species on growth of fungal strain - *Botrytis cinerea*

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	6.70 ±1.0 [b]	7.5±1.41 [d]	8.0±1.30 [c]	10.0±1.34 [c]
Mikania micrantha	0.00	0.00	0.00	0.00	7.0±1.34 [b]	8.0±1.10 [de]	8.5±1.18 [c]	10.3±1.10 [c]
Parthenium hysterophorus	0.00	0.00	0.00	0.00	0.0 [a]	0.00	5.8±1.61 [b]	7.9±1.20 [b]
Control	0.0	0.00	0.00	0.00	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
F value	-	-	-	-	456	312	902	126
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA) (n=7).

Besides aqueous extract, methanolic extract of *Ageratum haustonianum* and *Mikania micrantha* also had no effect on growth of *Exserohilium* sp. Maximum effect was shown by *Parthenium hysterophorus* (ZOI; 14.34mm) (Table 5) at 200 mg/ml concentration.

Table 5: Effect of three alien invasive species on growth of fungal strain - *Exserohilium* sp.

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
Mikania micrantha	0.00	0.00	0.00	0.00	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
Parthenium hysterophorus	0.00	0.00	0.00	0.00	0.00 [a]	0.00 [a]	9.5±1.1 [c]	14.34 ±1.9 [d]
Control	0.0	0.00	0.00	0.00	0.00 [a]	0.00 [a]	0.00 [a]	0.00 [a]
F value	-	-	-	-	456	629	521	568
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

Table 6. Effect of three alien invasive species on growth of fungal strain - *F. moniliforme*.

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	9.0±1.09 ^{d}	8.8±1.41^{c}	10.0±1.13 ^{d}	14.5±2.18^{e}
Mikania micrantha	0.00	0.00	0.00	0.00	10.0±1.09^{e}	11.0±1.1 ^{d}	11.5±1.31^{e}	13.0±1.15^{d}
Parthenium hysterophorus	0.00	0.00	0.00	0.00	6.8±1.10^{b}	8.2±1.16^{c}	7.8±1.14 ^{b}	9.6±1.16^{c}
Control	0.0	0.00	0.00	0.00	0.00 ^{a}	0.00^{a}	0.00^{a}	0.00^{a}
F value	-	-	-	-	302	478	982	367
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

The antifungal activity of methanolic extract of three alien invasive plant on *Fusarium oxysporum* was ranged from inhibition diameter of 0.00 mm at lower concentration to 14.0 mm at higher concentration (Table 7). The highest diameter of inhibition zone (14.0 mm) was recorded from methanolic extract of *Ageratum haustonianum* at 200 mg / ml concentration that was statistically different from other plant extract and concentration.

Growth of *Phytophthora infestans* was effected by methanolic extract of selected plant species. Maximum zone of inhibition was shown by methanolic extract of *Ageratum haustonianum* (14.6 mm), while growth was not effected by methanolic extract of *Mikania micrantha* (Table 8) .

Growth of *Sclerotium rolfsii* was effected significantly with methanolic extract of *Ageratum haustonianum* and *Parthenium hysterophorus* .*M*aximum zone of inhibition was exhibited by *Ageratum haustonianum* with zone of inhibition (10.54 mm). Growth was not effected by extracts of *Mikania micrantha* (Table 9).

Table 7: Effect of three alien invasive species on growth of fungal strain - *Fusarium oxysporum*

Aqueous extract (mg/ml)					**Methanolic extract (mg/ml)**			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	10.0±1.81	10.5±1.1 ^{d}	12.0±1.1 ^{e}	14.0±1.19^{e}
Mikania micrantha	0.00	0.00	0.00	0.00	0.00	9.5±1.1^{c}	10.0±1.13^{c}	11.0±1.20^{c}
Parthenium hysterophorus	0.00	0.00	0.00	0.00	0.00 ^{a}	0.00 ^{a}	6.8±1.15^{b}	9.2±1.05^{b}
F value	-	-	-	-	286	134	561	309
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

Table 8: Effect of three alien invasive species on growth of fungal strain - *Phytophthora infestans.*

Aqueous extract (mg/ml)					Methanolic extract (mg/ml)			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	12.0±1.34[d]	12.5±1.9[d]	14.0±2.13[e]	14.6±2.19[d]
Mikania micrantha	0.00	0.00	0.00	0.00	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Parthenium hysterophorus	0.00	0.00	0.00	0.00	0.00[a]	0.00[a]	8.90±1.34[b]	10.5±1.50[b]
Control	0.0	0.00	0.00	0.00	0.00[a]	0.00[a]	0.00[a]	0.00[a]
F –value	-	-	-	-	128	316	209	159
P- value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA) (n=7)

Table 9: Effect of three alien invasive species on growth of fungal strain - *Sclerotium rolfsii*.

Aqueous extract (mg/ml)					Methanolic extract (mg/ml)			
Plant species	**50**	**100**	**150**	**200**	**50**	**100**	**150**	**200**
	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)							
Ageratum haustonianum	0.00	0.00	0.00	0.00	6.0±0.98[b]	7.0±1.56	7.5±1.15[c]	10.54±2.1[d]
Mikania micrantha	0.00	0.00	0.00	0.00	0.00[a]	0.00[a]	0.00[a]	0.00[a]
Parthenium hysterophorus	0.00	0.00	0.00	0.00	5.8±0.69[b]	6.2±0.59[b]	6.5±0.98[b]	8.8±1.11[c]
Control	0.0	0.00	0.00	0.00	0.00[a]	0.00[a]	0.00[a]	0.00[a]
F value	-	-	-	-	145	613	261	213
P value	-	-	-	-	0.000	0.000	0.000	0.000

Significant difference between mean zone of inhibition (ZOI) among three invasive alien plants are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=7).

DISCUSSION

Phytochemical analysis of the plant extract showed that it contains alkaloids, flavonoids, tannins, terpenoids glycosides and saponins (Table 1). The methanol, and aqueous extracts of the leaves of *Ageratum haustonianum, Mikania micrantha* and *Parthenium hysterophorus* were subjected to a preliminary screening for antimicrobial activity against eight phytopathogenic fungi .

It was clear from the present results, that methanol leaves extracts of *Ageratum haustonianum, Mikania micrantha,* and *Parthenium hysterophorus* exhibited pronounced activity against almost all tested microbes in a concentration depended manner, but it was ineffective against aqueous extract of the same (Tables 2-9). However antifungal effect varied with species of plants and species of micro-organisms. The present study is in accordance with the works of Barsagade and Wagh (2010) , who reported that methanol leaf extract of common plants and weeds exhibited antifungal activities. Among the fungal strains *Phytopthora infestans* was the most susceptible fungus (ZOI; 14.6mm) at methanolic extract of *Ageratina adenophora* at concentration of 200 mg/ml) and *Sclerotium rolfsii* was the most resistant fungus among the tested fungal strains. It has been suggested that the antimicrobial activity of plant secondary metabolites is mainly due to presence of essential oil , flavonoids and tri- terpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas *et al.* 1992). The observed antifungal effects in present study also may be due to the presence of phytochemicals (alkaloids, tannins and saponins) in the extract.

The results of this study support the antifungal activities of three invasive alien plants as a broad spectrum antifungal agent since they inhibited the growth of some fungi (*A. alternate, A. brassicae, B. cinerea, Exserohilium sp, F. moniliforme, F. oxysporum, P. infestans and S. rolfsii*)).

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). The present study is in accordance with the works of Okunola *et al*. (2012), who reported higher antimicrobial activities of extracts of *Carica papaya* in fungal starins.

Results of present study is also supported by Barsagade and Wagh (2010), who indicated antimicrobial activities of plants and weed extracts may exist in a variety of different components , including aldehyde and phenolic compounds . Variation in effectiveness of the extract against a target microorganisms depends upon the chemical compositions of the extract and membrane permeability of the microbe for the chemicals. Naturally occurring combination of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents. The antimicrobial effects of the extracts could be explained by disturbance of the permeability barrier of the living membrane structure (Cowan 1999).

Results from *in vitro* study revealed that the antifungal activity might have been influenced by the solubility of active compound (s) in extracting solvent. Methanol extracts were relatively more effective than aqueous extract (Tables 2- 9) . This greater effectiveness of methanol extract compared to aqueous extract may be due to differences in constituents and amount of extraction of phytochemicals , which are toxic to targeted pathogens, present in leaf parts of tested invasive alien plants. The leaf extracts of selected plants contain alkaloids, coumarins and tannins (Table 1). Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg *et al.* 1983), also Eloff (1998) and Cowan (1999) found that methanol was more efficient than distilled water in extracting phytochemicals from plant materials. The absence or less effect of antimicrobial activity of aqueous extracts of plant indicates the insolubility of the active ingredients in this solvents. Similar observations were reported from nimbolide isolated from neem seed oil showing antibacterial activity against *S. aureus* and *Staphylococcus coagulase* (Nazma and Rao 1977).

Plant products, particularly extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. Results of this study revealed very significant antifungal activity with the

extracts demonstrating broad spectrum of activity against fungal starains (*A. alternate, A. brassicae, B. cinerea, Exserohilium sp, F. moniliforme, F. oxysporum, P. infestans and S. rolfsii*). The organisms used in this study are associated with various forms of infections in plants like leaf spot of spinach (*A. alternate*), leaf spot and blight of crucifers (*A. brassicae*), Noble rot of fruits, botrytis disease of chickpea (*Botrytis cinerea*), Leaf spot of rice (*Exserohilium sp)*, Foot rot disease of rice (*F. moniliforme*), Fusarial wilt (*F. oxysporum),* Late blight of potato (*Phytophthora infestans*), and Southern blight of vegetables (*Sclerotium rolfsii*). The demonstration of activity against all these organisms had shown that all three alien invasive species; *Ageratum haustonianum, Mikania micrantha* and *Parthenium hysterophorus* can be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity.

Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to identity of the antimicrobial compounds from within these plants and also to determine their full spectrum of efficacy. However the present study of *in vitro* antifungal evaluation of invasive alien plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

Conclusion

Phytochemical analysis of the plant extract showed that it contains alkaloids, flavonoids, tannins, terpenoids glycosides and saponins . Methanolic and aqueous extract of showed antifungal property against almost all tested microbes in a concentration depended manner Methanol extracts were relatively more effective than aqueous extract. The demonstration of activity against all these organisms had shown that all three alien invasive species; *Ageratum haustonianum, Mikania micrantha* and *Parthenium hysterophorus* can be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity.These findings can form the basis of further studies to isolate compounds, to find new therapeutic principles.

Invasive alien species are considered as one of the greatest threat to natural ecosystem of the earth. Alien species are known to have become aggressive and rapidly colonized in Nepal, displacing the native species by predation, parasitism or by competition for space and nutrients. Growing and harvesting promising plants would increase the cost substantially. Also, combating invasive plants is difficult and costly. Invasive alien plants and weeds known for their characteristics as aggressive growers could aid in developing a less expensive plant source if explored for bioactivity. Exploitation of these rapidly growing species can be done on making the different pharmaceutical products in one hand while proper management on the other side.

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