

ORIGINAL RESEARCH ARTICLE

Antagonistic Characteristics and Phytochemical Screening of Invasive Alien Species of Nepal Himalaya

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ABSTRACT

The bioclimatic diversity of Nepal favors many alien species to become naturalized invading and dominating intact natural communities. The ability of these plants to tolerate severe harsh conditions and become invasive lights on the potentiality of their use in the pharmacology. In the present study, a bioassay directed phytochemical analysis of the five major invasive alien species of Nepal was carried out. The extracts of the alien species (*Ageratina adenophora*, *Eichhornia crassipes*, *Lantana camara*, *Mikania micrantha* and *Parthenium hysterophorus*) were prepared by hot and cold process using methanol and water as solvents. The antimicrobial (against 10 clinical bacteria and 9 phytopathogenic fungi) activity of different concentration (50, 100, 150, 200 mg/ml) of various plant extracts was studied. The qualitative phytochemical analysis depicted the presence of polyoses, saponins, polyphenols, reducing compounds, alkaloids, glycosides, quinones, flavonic glycosides and coumarins in the plants. The extracts were found to be more effective against the fungal pathogens that may be due to the presence of different bioactive compounds in the plants and their action towards the pathogens. This study improves our knowledge on the scope and use of the plants to control the different pests, to isolate the bioactive compounds and the further strategies on the effective management of these invasive species.

Key words: antibacterial, antifungal, plant extracts, zone of inhibition

INTRODUCTION

An alien species has been defined as a species that is non-native, non-indigenous, exotic, and foreign and/or introduced to an ecosystem other than its natural home. Such species may occur in locations beyond its known historical natural ranges. Many introduced species have been naturalized in a new environment and form a part of existing landscapes and ecosystems. Some alien species colonize unmanageably out competing native species known as invasive alien species. The diverse bioclimatic zones of Nepal range tropical to alpine favor the introduction of several alien species^[1]. These species have been spreading aggressively by colonizing several landscapes and ecosystems displacing the native ones^[2].

In search for characteristics of exotic plants that might serve as predictors of invasiveness, most studies have examined life-history traits rather than attributes that more directly reflect interactions with potential natural enemies and competitors in the plants' new ranges^[3]. Recent

advances have shown that such interactions are critical in determining invasiveness. Resistance of invasive plants to antimicrobial agents has become an increasingly important and pressing global problem. Exotics escaping pathogens to a greater extent are more invasive than those that are more heavily attacked by natural enemies in their new ranges, suggesting that invasive exotics may be better defended from enemies than non-invasive plants^[4]. Interactions with native competitors are also important. These species being aggressive compete for water, light, space and nutrients, and hence are present in large quantities. By virtue of their better defenses and enhanced competitive ability, the invasive alien species may exhibit phytochemically unique exotics^[2]. Systematic phytochemical survey work is an adjunct to knowledge of traditional folk medicine and plays an important role in identifying new and possibly biologically active compounds^[5, 6]. Invasive plants have important ecological and economic impacts world-wide and

increasing attention is now being paid to eradication and management efforts^[7].

The present study was concentrated on the qualitative screening of secondary metabolites from the different invasive alien weeds and their antimicrobial assay. This type of study provides the health applications at affordable cost and also keenly represents one of the best avenues in searching new economical plants for medicine. So, it may not be surprising that in response to antimicrobial resistance, major pharmaceutical companies have tended to concentrate their efforts on improving antimicrobial agents in established classes^[8]. 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

Plant material

Some of the major invasive alien species of Nepal namely *Ageratina adenophora*, *Eichhornia crassipes*, *Lantana camara*, *Mikania micrantha* and *Parthenium hysterophorus* were selected for study. The whole parts of all the selected plants were collected from several regions of Kathmandu and Pokhara Valley. These were shade dried, chopped, pulverized to powder and subjected for further analysis.

Preparation of extracts

Each powdered plant material was extracted by soxhlet extraction and wet reflux condensation processes using methanol and water as the extracting solvents. The extracts obtained were concentrated for further studies at reduced pressure and temperature in a rotary evaporator and were then examined for the presence of secondary metabolites by different phytochemical tests.

Preliminary qualitative phytochemical analysis

The extracts of all tested plants were screened for the phytochemical constituents using standard chemical test method^[9] with slight modifications.

Antimicrobial assay

For the antimicrobial assay, different concentrations (50, 100, 150, 200 mg/ml) of each extracts were prepared in Dimethyl Sulphoxide (DMSO). The ability of various extracts to inhibit the growth of clinical bacteria and phytopathogenic fungi were determined by agar well diffusion method^[10].

Antibacterial assay

Clinical bacterial strains namely *Salmonella* Typhi, *Acinetobacter* sp., *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Schigella dysenteriae*, *Klebsiella pneumoniae*, *Salmonella* Paratyphi, *Proteus mirabilis* and *Escherichia coli* were used for the screening of antibacterial properties. Inoculums of each bacterial culture were prepared as compared to 0.5 McFarland standards (1.5×10^8 cfu/ml) on Nutrient broth (NB) and swabbed on the surface of Mueller-Hinton agar plates (MHA). 50 μ l of different concentrations of the extracts and the solvent DMSO itself as control were loaded on the wells (4 mm diameter) made on the swabbed agar plates and left undisturbed for few minutes. The plates were then incubated at $37 \pm 1^\circ\text{C}$ for 24 h. The zone of inhibition, indicated by the clear zone around the well was measured after incubation.

Antifungal assay

Candida albicans, *Curvularia* sp., *Exserohilium* sp., *F. eridiforme*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum*, *Sclerotium rolfsii* and *Stenophyllum* sp. were used for the antifungal test. Inoculums of each fungal culture were maintained to $1 \times 10^6 - 5 \times 10^6$ spores/ml^[11] and swabbed on the surface of Potato dextrose agar plates (PDA). 50 μ l of different concentrations of the extracts and the solvent DMSO itself as control were dispensed on the wells (6 mm diameter) made on the swabbed agar plates and left undisturbed for few minutes for diffusion. The plates were then incubated at $27 \pm 1^\circ\text{C}$ for 5 days. The zone of inhibition, indicated by the clear zone around the well was measured after incubation.

The readings were taken in three different fixed directions in all 3 replicates and the average values were recorded.

RESULTS

In the qualitative phytochemical analysis, the tested plants showed the presence of various tested groups as shown in (Table 1). The potential sensitivity of the extracts was obtained against all the microorganisms tested that were detected by the zone of inhibition (Tables 2-11). In the different experiments conducted the higher concentration of the extracts (200 mg/ml) showed the significant antimicrobial activity (with some exceptional cases). The variation in antimicrobial potential of the hot and wet methanol and aqueous extracts occurred among the different plant species and organisms. Among the extracts tested, the methanolic extract was effective against bacterial pathogens than aqueous extracts except

E. crassipes. Similarly, the extracts obtained by hot process were more effective towards bacterial and fungal pathogens than cold percolation except that of *P. hysterophorus* for bacterial ones. For the fungal pathogens, the antifungal activity of the methanolic and aqueous extracts varied. Of the tested 10 bacterias, *S. Typhi*, *Acinetobacter* sp. and *B. subtilis* were inhibited by one or more concentration of different plant extracts. *P. mirabilis* was the most resistant bacteria being inhibited by *E. crassipes* extract only. The fungal

pathogens were found to be more sensitive towards the extracts tested than the bacterial ones. The *L. camara* extract was most effective towards the fungal pathogens inhibiting all the tested ones. Of the 9 tested fungi, *F. eridiforme*, *F. moniliforme*, *F. proliferatum* and *S. rolfsii* were inhibited by one or more concentration of each plant extracts. Among the fungi, *C. albicans* was most resistant being inhibited by the extract of *L. camara* only.

Table 1: Qualitative phytochemical assay of extracts of different plants

Tested group	Plants used for Phytochemical assay				
	<i>Lantana camara</i>	<i>Ageratina adenophora</i>	<i>Parthenium hysterophorus</i>	<i>Mikania micrantha</i>	<i>Eichhornia crassipes</i>
Volatile oil	--	--	--	--	--
Sterol & triterpenes	--	--	--	--	--
Carotenoids	--	--	--	--	--
Fatty acids	--	--	--	--	--
Polyoses	--	+	--	--	+
Saponins	--	--	--	--	+
Polyphenols	+	--	+	--	--
Red. compound	--	+	--	--	+
Alkaloids	--	+	--	--	+
Glycosides	+	+	--	+	--
Quinones	+	--	+	+	--
Anthocyanosides	--	--	--	--	--
Anthracyanosides	--	--	--	--	--
Flavonic glycosides	+	--	+	--	--
Coumarins	+	+	--	--	--

+ = presence, -- = absence

Table 2: Antibacterial activity of *Lantana camara* against different clinical bacteria

Bacterial strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI:mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>Salmonella Typhi</i>	14	7	12	7	16	8	14	8	12	10	13	10	16	13	14	14
<i>Acinetobacter</i> sp.	14	11	13	10	14	14	13	10	15	15	14	12	16	20	15	14
<i>Bacillus subtilis</i>	14	13	12	9	15	17	12	10	18	22	9	10	13	23	12	13
<i>Staphylococcus aureus</i>	7	7	--	--	9	7	--	--	11	9	--	--	14	12	--	--
<i>Enterococcus faecalis</i>	8	8	7	6	11	9	9	8	13	11	10	9	15	12	13	12
<i>Shigella dysenteriae</i>	--	6	--	--	--	9	--	--	--	11	--	--	--	14	--	--
<i>Klebsiella pneumoniae</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Salmonella Paratyphi</i>	10	9	--	--	13	11	--	--	13	12	--	--	14	15	--	--
<i>Proteus mirabilis</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Escherichia coli</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 3. Antifungal activity of *Lantana camara* against different phytopathogens

Fungal strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>F. oxysporum</i>	9	6	7	6	--	7	8	8	--	7	10	12	14	10	14	14
<i>F. moniliforme</i>	--	--	10	9	--	--	11	12	--	--	13	13	13	--	17	16
<i>F. eridiforme</i>	8	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>F. proliferatum</i>	13	6	--	7	--	9	8	10	10	10	13	13	12	13	16	15
<i>Curvularia</i> sp.	12	--	8	--	9	--	--	10	12	--	11	--	--	--	--	--
<i>Stenophyllum</i> sp.	10	--	--	--	10	7	--	--	13	8	--	--	10	10	--	--
<i>Sclerotium rolfsii</i>	--	--	--	--	--	--	--	--	9	--	--	--	12	--	--	--
<i>Exserohilum</i> sp.	--	13	7	7	--	14	11	10	14	16	12	12	--	16	8	14

<i>C. albicans</i>	8	--	--	--	13	--	--	--	16	--	--	--	--	--	--	--
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Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 4: Antibacterial activity of *Ageratina adenophora* against different clinical bacteria

Bacterial strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>Salmonella Typhi</i>	16	--	--	--	7	--	7	8	18	--	--	--	18	--	--	--
<i>Acinetobacter sp</i>	18	7	8	--	21	14	9	10	20	14	--	9	22	17	--	10
<i>Bacillus subtilis</i>	14	13	7	--	15	14	7	10	18	14	--	8	20	20	--	--
<i>Staphylococcus aureus</i>	12	--	--	--	16	--	--	--	18	--	--	--	20	--	--	--
<i>Enterococcus faecalis</i>	--	--	--	--	--	--	--	8	--	--	--	--	--	--	--	--
<i>Schigella dysentriae</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Klebsiella pneumoniae</i>	--	6	--	--	--	7	--	--	--	8	--	--	--	11	--	--
<i>Salmonella Paratyphi</i>	7	13	8	--	8	7	9	--	20	7	--	--	12	8	--	--
<i>Proteus mirabilis</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Escherichia coli</i>	7	--	--	--	7	--	--	--	7	--	--	--	8	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 5 : Antifungal activity of *Ageratina adenophora* against different phytopathogens

Fungal strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>F. oxysporum</i>	--	6	8	9	--	9	7	8	12	10	8	13	13	12	10	9
<i>F.moniliforme</i>	6	15	--	--	12	19	8	9	--	20	10	11	--	21	--	--
<i>F. eridiforme</i>	9	--	10	8	14	--	12	11	13	--	--	--	14	--	13	12
<i>F.proliferatum</i>	11	--	8	--	8	--	9	--	14	--	12	12	--	--	--	--
<i>Curvularia sp.</i>	8	10	10	13	--	12	9	10	--	15	12	14	12	16	8	10
<i>Stenophyllum sp.</i>	--	--	--	--	13	--	12	13	13	--	13	14	9	--	10	9
<i>Sclerotium rolfsii</i>	14	6	12	14	10	8	13	14	--	10	9	--	9	14	--	--
<i>Exserohilium sp.</i>	13	16	--	--	10	17	8	9	14	20	--	--	10	21	--	13
<i>C. albicans</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 6: Antibacterial activity of *Parthenium hysterophorus* against different clinical bacteria

Bacterial strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>Salmonella Typhi</i>	9	14	--	--	10	14	--	--	13	19	--	--	15	22	--	--
<i>Acinetobacter sp.</i>	--	9	--	--	--	11	--	--	--	14	--	--	--	16	--	--
<i>Bacillus subtilis</i>	--	12	10	17	--	12	11	17	--	13	13	19	--	14	13	22
<i>Staphylococcus aureus</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Enterococcus faecalis</i>	--	7	--	12	--	8	8	14	--	12	9	15	--	13	9	16
<i>Schigella dysentriae</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Klebsiella pneumoniae</i>	--	6	9	16	--	7	10	17	--	7	12	19	--	10	11	21
<i>Salmonella Paratyphi</i>	--	12	--	8	--	14	--	9	--	14	8	10	--	15	--	12
<i>Proteus mirabilis</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Escherichia coli</i>	--	7	--	--	--	10	--	--	--	10	--	--	--	13	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 7: Antifungal activity of *Parthenium hysterophorus* against different phytopathogens

Fungal strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW

<i>F. oxysporum</i>	12	6	15	14	--	9	16	14	12	9	16	14	13	10	14	16
<i>F.moniliforme</i>	11	--	13	12	12	--	15	13	9	--	16	15	14	--	16	16
<i>F. eridiforme</i>	--	9	12	12	14	13	14	15	8	16	17	16	--	17	17	17
<i>F.proliferatum</i>	8	--	--	--	--	--	--	--	13	--	--	--	15	--	--	--
<i>Curvularia</i> sp.	--	--	11	10	--	--	12	12	--	--	16	15	--	--	16	16
<i>Stenophyllum</i> sp.	12	--	16	15	13	--	18	17	14	--	18	17	8	--	17	17
<i>Sclerotium rolfsii</i>	--	10	--	--	14	14	--	--	9	14	--	--	14	16	--	--
<i>Exserohilium</i> sp.	--	--	7	6	--	--	9	8	--	--	11	12	--	--	13	14
<i>C. albicans</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 8: Antibacterial activity of *Eichhornia crassipes* against different clinical bacteria

Bacterial strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>Salmonella</i> Typhi	8	6	--	--	7	6	--	--	--	7	--	--	12	10	--	--
<i>Acinetobacter</i> sp.	11	6	--	--	9	7	--	--	--	7	--	--	--	9	--	--
<i>Bacillus subtilis</i>	--	6	--	--	--	6	--	--	8	7	--	--	8	8	--	--
<i>Staphylococcus aureus</i>	9	--	--	--	11	--	--	--	11	7	--	--	--	9	--	--
<i>Enterococcus faecalis</i>	--	--	--	--	8	--	--	--	--	--	--	--	--	--	--	--
<i>Schigella dysentriae</i>	8	--	--	--	8	--	--	--	12	--	--	--	--	--	--	--
<i>Klebsiella pneumoniae</i>	12	--	--	--	--	--	--	--	8	9	--	--	7	10	--	--
<i>Salmonella</i> Paratyphi	7	--	--	--	--	--	--	--	11	7	--	--	9	8	--	--
<i>Proteus mirabilis</i>	--	7	--	--	--	7	--	--	--	8	--	--	--	11	--	--
<i>Escherichia coli</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 9: Antifungal activity of *Eichhornia crassipes* against different phytopathogens

Fungal strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>F. oxysporum</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>F.moniliforme</i>	6	8	8	11	8	9	10	13	8	12	13	14	13	13	13	15
<i>F. eridiforme</i>	7	9	--	--	10	12	--	--	10	15	--	--	12	15	--	--
<i>F.proliferatum</i>	6	7	--	--	8	8	--	--	10	13	--	--	11	15	--	--
<i>Curvularia</i> sp.	--	--	--	7	--	--	--	10	--	--	--	13	--	--	--	14
<i>Stenophyllum</i> sp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Sclerotium rolfsii</i>	9	15	--	6	12	15	6	7	12	15	8	9	13	16	10	13
<i>Exserohilium</i> sp.	--	--	--	--	--	--	--	7	--	--	--	8	--	--	--	10
<i>C. albicans</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 10: Antibacterial activity of *Mikania micrantha* against different clinical bacteria

Bacterial strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>Salmonella</i> Typhi	7	--	9	7	8	--	11	9	9	8	12	9	7	7	10	8
<i>Acinetobacter</i> sp.	7	--	11	8	9	--	12	9	10	9	13	10	9	8	12	9
<i>Bacillus subtilis</i>	--	--	12	10	--	--	14	10	--	--	16	12	--	--	15	11
<i>Staphylococcus aureus</i>	--	--	10	8	--	--	11	8	--	--	13	11	--	--	12	10
<i>Enterococcus faecalis</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Schigella dysentriae</i>	--	--	10	7	--	--	12	8	--	--	15	11	--	--	13	10
<i>Klebsiella pneumoniae</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

<i>Salmonella</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Paratyphi																
<i>Proteus mirabilis</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Escherichia coli</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

Table 11: Antifungal activity of *Mikania micrantha* against different phytopathogens

Fungal strains	Zone of inhibition displayed by different types and concentration of extracts (ZOI: mm)															
	50 mg/ml				100 mg/ml				150 mg/ml				200 mg/ml			
	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW	MH	MW	AH	AW
<i>F. oxysporum</i>	6	--	--	--	16	12	17	14	8	--	17	13	9	--	14	10
<i>F. moniliforme</i>	12	7	10	8	22	15	20	12	21	15	13	11	18	12	12	9
<i>F. eridiforme</i>	7	--	--	--	11	8	--	--	12	10	10	7	13	9	8	--
<i>F. proliferatum</i>	7	--	--	--	13	8	--	--	13	10	--	--	15	9	8	--
<i>Curvularia</i> sp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Stenophyllum</i> sp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>Sclerotium rolfsii</i>	--	--	--	--	14	10	15	12	13	11	14	11	9	7	8	--
<i>Exserohilium</i> sp.	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
<i>C. albicans</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Values represent the mean of three replicates

MH: Methanolic hot extraction; MW: Methanolic cold extraction; AH: Aqueous hot extraction; AW: Aqueous wet extraction

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Plants and their secondary metabolites have shown great potential as antibacterial and antifungal source. The first step towards this goal is the *in-vitro* antimicrobial activity. The phytochemicals are known to have antimicrobial activity. The curative properties of plants are perhaps due to the presence of various secondary metabolites which are the non nutritive plant compounds [12,13,14]. These molecules possess interesting biological activities which attracted several researchers to their elucidation to provide knowledge that will lead to the advancement medicine [15].

The results obtained in the present investigation show the presence of phytochemicals which take part in defense mechanism of the plants. This suggests a phytochemical basis for the escape of these species from that has been shown from previous studies to be correlated with invasive potential in exotic plants [4,16,17].

CONCLUSION

The activity of the extracts obtained varied upon the type of solvents used for extraction, the way of extraction and the type of pathogens used for the assay. The optimal effectiveness of a plant may not be due to one main active constituent, but to the combined action of different compounds originally present in the plant [18]. With regard to natural products, it is generally accepted that

phytochemicals are less potent anti-infective than agents of microbial origin, i.e., antibiotics [14]. Theoretically, the increase in concentration of the extracts would probably increase the inhibition zone but it was found to some extent only that may be due to the inability in diffusion of the concentrated extracts into the solid medium.

Hence a complete study conducted with the purpose of finding these chemicals in these alien species is worthwhile as the output is "best out of waste". It can be suggested that these invasive alien species are the potential source of phytochemicals and interesting source of therapeutic activities. 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 sustainably. Also, combating invasive plants is difficult and costly. Alien invasive 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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