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Evaluation of allelopathic potential and phytochemical screening of some selected medicinal plant species of Nepal

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Abstract

In the present work four medicinal plants (*Ageratum conyzoides, Cannabis sativa, Eclipta prostata, and Woodfordia fruticosa*) were evaluated for their allelopathic action. Toxicity and non toxicity was assessed by recording their effects on germination and percentage growth of root and hypocotyle of test plants. Tested extracts reduced the germination of the test seeds. However, root and hypocotyle elongations of wheat and pea seedlings were significantly inhibited by the extract of studied plant parts, with the percentage of inhibition increased as the concentration of the extract increased. The observed allelopathic activity of the extract of selected medicinal plants on the seed germination and seedling growth of wheat and pea was attributed to the presence of the allelopathic phytochemicals in medicinal plants. The results showed that *Ageratum conyzoids* and *Woodfordia fruticosa* had strong inhibitory effect on germination as well as root and hypocotyls growth of test seeds. Phytochemical screening of selected medicinal plants was also carried out by using standard methods and it revealed that the extract contained alkaloid, terpenoids, flavonoids, tannins and saponins in different proportions; with more of alkaloids flavonoids and terpenoids.

Key words: Allelopathy, medicinal species, phytochemical screening, filter paper.

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1.0 Introduction

A variety of plant species are being used by individuals and communities for the treatment of diseases. It is now clear that the medicinal values of these plants lie in the bioactive phytochemical constituents that produce physiological effects on human body [1]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and some other phenolic compounds [2]. The effects of these chemicals are not limited to animals and human body alone, but also on other plants. Many plants including medicinal plants were reported to interact chemically with other plant species. Such chemical interaction is known as allelopathy. The use of plants with strong allelopathic properties for weed control has shown promising results and allelopathy holds great prospect for meeting some of these demands [3]. Many crop and weed species have been observed to have allelopathic properties [4]. Allelochemicals (inhibitors) are present in plants as end products, by-products, and metabolites. These chemicals are present in different parts of plants like stem, leaves, roots, flowers, inflorescence, fruits and seeds. Out of these plant parts, leaves seem to be the most consistent producers of these allelochemicals. These allelochemicals are often times released from the plants by volatilization, leaching, exudation and decomposition from plant residues. The concept of allelopathy was further supported and developed by [5, 6]. In allelopathy, a major tool for research is bioassay, which controls laboratory condition; high sensitivity gives reproducible result, and takes relative short time to perform. There are many ways to evaluate the herbal aqueous extracts of allelopathic activities. These are hydroponic culture methods, Ratoon screening method, Plant box method [7], a plastic tray with 6 holes [8], Dish pack method - a new bioassay for volatile allelopathy [9], Sandwich method [10] and Filter paper [11]. The filter paper is a more suitable method because it can tolerate the moderate temperature during incubation (25°C) in the laboratory. The aqueous extracts remain fresh for longer period of time. Millipore filter paper is used to make the method sterile. The reason for the use of filter paper in techniques is that, it is easily available and free from contamination. It is easily handled and a good media for germination, it has high flow rate for movement of extracts and porosity [12]. Allelopathic potential of some selected species had been studied by various researchers [13, 14]. Allelopathic effect of medicinal species against temperate crop is well studied [15, 16].

Nepal is endowed with a great diversity of indigenous medicinal plants. The local communities of Nepal have been using the medicinal plant species for curing various diseases for a long time [17]. The beneficial medicinal effects of these plants typically result from the secondary compounds in the plants which are specific in certain taxa, such as family, genus and species [18]. However, information on the allelopathic effects of medicinal herbs on many vegetables and cereals is limited . The purpose of this study is to carry out an evaluation on allelopathic activity and phytochemical screening of Nepalese medicinal plants for future chemical analyses. Thus, six species of medicinal plants were collected from the preliminary survey and assessed for the effects of their extracts on the growth of two tested plant species, wheat and pea.

2.0 Materials and Methods

To study the allelopathic activities, four different medicinal plant species (Table-1) were collected from different localities (*Ageratum conyzoides* and *Cannabis sativa* were collected from Kathmandu valley and *Eclipta prostata* and *Woodfordia fruticosa* were collected from Chitwan district) of Nepal and shade dried. The medicinal plants with fully expanded parts were selected as donor of allelochemicals. Then used parts of plants were sorted out to make aqueous extraction .

2.1 Test plants

Pea and wheat seeds were used as receptor plants for initial screening of species to check allelopathic potentialities. Seeds were obtained from Botany Division, NARC (National Agriculture Research Center, Khumaltar Lalitpur) as the seeds of these two species germinate easily, easy to handle, with high fecundity rate, showed pronounced effects after the application of aqueous extracts. Filter paper was used as growth medium for germination [19]. For sterilization of the medium from dust particles or fungal attack on petri plates of 9 cm, cleaned ethanol dipped cotton was used, and then filter paper was placed. Different percentage of aqueous extracts of all selected medicinal plant species were applied on the test/receptor plant (pea and wheat).

2.2 Experiments

Ten gram of air dried selected plant parts were ground, mixed with 100 ml distilled water and left for 24 h in dark at the room temperature (average during day: 25ºC) for extraction. Aqueous extract was obtained as filtrate of the mixture and final volume was adjusted to 100 ml; this gave 10% aqueous extract. The extract was considered as stock solution and a series of solution with different strengths (2, 4, 6 and 8%) were prepared by dilution. Fifteen uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of wheat (*Triticum aestivum*) and ten seeds of pea (*Pisum sativum*) were kept for germination in sterilized petri-dishes lined double with blotting paper and moistened with 10 mL of different concentrations of aqueous extracts (2 to 10%). Each treatment had three replicas for wheat and control and four replicas for pea (total number of test seeds: 15 x 3 = 45 wheat seeds; 10 x 4=40 pea seeds). One treatment was run as control with distilled water only. The Petri-dishes were maintained under laboratory conditions (room temperature 25°C at mid day, and diffused light during day) for one week. Equal volume of distilled water was added in the dishes when moisture content of the blotting paper declined. After one week, number of germinated seeds were counted and, the root and shoot length were measured. This experiment was repeated twice and data were pooled together before analysis.

2.3 Phytochemical screening methods

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 [20] with slight modifications.

3.0 Results and discussion

3.1 Allelopathy

Effects of aqueous extracts of four medicinal plants on the seed germination of wheat and pea.

All studied medicinal plants had significant effect on seed germination of wheat and pea (Table-2a,2b). The inhibitory effect on germination was increased with increasing concentrations of the extracts. Among these plants, *Woodfordia fruticosa* had highest inhibitory effects on germination; that was followed by *Ageratum conyzoids* . Least effect was shown by *Cannabis sativa* on tested seeds.

Effects of aqueous extracts of four medicinal plants on the seedling growth of wheat.

All studied medicinal plants had significant effect on seedling growth of wheat. Table - 3a and 3b shows the effects of aqueous extracts of four medicinal plants on hypocotyl and root growth of wheat. The inhibitory effect was increased with increasing concentrations of the extracts and inhibition of the roots was greater than that of the hypocotyls. At concentration of 2% the extract of *W fructicosa* showed the highest inhibitory effect on root and hypocotyl growth of wheat seedlings, followed by *A conyzoids* (Table-3a & 3b).

Effect of plant extracts on seedling growth of pea

Effects of aqueous extracts of four medicinal plants on the growth of pea seedlings: Table-4a and 4b showed the effect of aqueous extracts of four medicinal

plants on hypocotyl and root growth of pea. At concentration of 2%, aqueous extract of all medicinal plants slightly inhibited root and hypocotyl of pea. When the concentration was increased to 10% , the inhibitory effects were increased. The aqueous extract of Woodfordia fruticosa had exhibited the greatest inhibition, that was followed by Ageratum conyzoids.

3.2 Phytochemical Screening

The phytochemical characteristics of four medicinal plants tested are summarized in the table-5. The results revealed the presence of medically active compounds in the six plants studied. From the table-5, it could be seen that, alkaloids, flavonoids, terpenoids and tannins were present in all the plants. Species *Ageratum conyzoids* ,*Eclipta prostata* and *Woodfordia fruticosa* contained high amount of alkaloids. Screening for the flavonoids of the plants *Ageratum conyzoids* and *Woodfordia fruticosa* gave the highest positive test. Likewise *Ageratum conyzoids, Cannabis sativa* and *Eclipta prostrata* contained high amount of tannins and terpenoids.

4.0 Conclusion

Allelopathy in agricultural practices has become more important with the main objectives of using this phenomenon in biological control of weeds [21]. As a possible approach, this fact shall be further evaluated and utilized for screening allelopathic plant species [22, 23]. The growth inhibitory effects on four Nepalese medicinal plants were confirmed by two test plant species in the present research. Plants exhibit allelopathic activity due to release of allelochemicals of different chemical classes mainly polyphenolic compounds (flavonoids and tannins), cyanogenic glycosides and alkaloids [24]. The inhibitory effect of the test extract on seed germination and radicle length may be due to the presence of putative allelochemicals. Preliminary phytochemical analysis revealed the presence of flavonoids,, alkaloids, tannins, saponins in aquoues extracts of all four medicinal plants (Table-5). In the present study, allelopathic effect of six medicinal plants can be attributed to its alkaloid and flavonoid contents. The effect may be due to synergistic effect rather than single constituent. The inhibitory effect augmented with increasing concentrations of aqueous extracts (Tables-2 a,b - 4a,b). It was also reported that effectiveness of receiver plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold [25-28]. Inhibitory effects of these medicinal plants were different on test plant species. The variation might be attributed to the differences in kind, total amount as well as properties of allelochemicals produced by different species used in this study. Chon et al. [29[reported that the extracts from lettuce plant had potent allelopathic activity and the activity differed depending on cultivar, extract or fraction. However, the extracts of *W fruticosa* and *A conyzoids* respectively showed the highest inhibitory effects on wheat and pea seedlings (Tables-3a,b & 4a,b).

In addition, the inhibition of the aqueous extract of four Nepalese medicinal plants on the root growth of two test plant species was greater than that on hypocotyls growth. These results are in agreement with the results of Stachon and Zimdahl [30], which reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on hypocotyl growth because root is the first organ to absorb allelochemical from the environment. Similar kinds of results were reported which indicated that root length was the best indicator of allelopathic effects of plant extracts because root growth has been reported to be more sensitive to phytotoxic compounds than hypocotyl growth in alfalfa. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue [32]. The present research suggests that the extracts of *Ageratum conyzoids*, and *Woodfordia fruticosa* have showed higher allelopathic effects among all studied Nepalese medicinal plants. These two

plants, therefore, may be the candidates for isolation and identification of allelochemicals.

4.1 Phytochemical screening

Phytochemical screening was carried out on four traditionally used medicinal plants of Nepal. Investigation revealed the presence of plant secondary metabolities in all the species but their concentration varied (Table -5). All these constituents are known to exhibit medicinal as well as physiological activities [33]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [34]. They possess biological properties such as antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [35, 36]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.. Several workers have reported on the analgesic properties of alkaloids [37, 38] as well as the anti-inflammatory and anti-bacterial properties of tannins [39]. These classes of compounds are known to show curative activity against several bacteria and it is not surprising that these plant extracts are used traditionally by herbalists to cure bacteria related ill-health. Tannins with its protein-precipitating and vasoconstriction effect could be advantageous in preventing ulcer development [40]. The diuretic and antibacterial activity of plant extracts containing flavonoids have been documented [41, 42].. The alkaloids contained in plants are used in medicine as anaesthetic agents [43]. The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs [44]. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the parts of the plants studied.

Allelopathic potentialities and phytochemical screening of *Ageratum conyzoides*, *Cannabis sativa*, *Eclipta prostate* and *Woodfordia fruticosa* was studied. The present research suggests that the extracts of *Ageratum conyzoids* and *Woodfordia fruticosa* have showed higher allelopathic effects among all studied Nepalese medicinal plants. From the present preliminary investigation, it can be concluded that all these plants exhibited remarkable negative allelopathic potential by significantly affecting the germination and hypocotyle and root growth of both *Triticum aestivum* and *Pisum sativum*. The inhibition of the aqueous extract of four Nepalese medicinal plants on the root growth of two test plant species was greater than that on hypocotyls growth.

Table-1: List of the selected medicinal species used for the allelopathy screening

SN	Species Name	Family	Geographic Location of Collection site	Parts use	Uses
1	Ageratum conyzoides L.	Asteraceae	27º 40.20' N 85º 17.32' E	Fresh leaves	Cure wounds, stops bleeding, antidysenteric
2	Cannabis sativa L.	Cannabaceae	27º 40.20' N 85º 17.32' E	Dried branches and resinous exudates from the inflorescence	Tonic, intoxicant, stomachic, analgesic
3	Eclipta prostata L.	Asteraceae	27º21'-27º52'N 83º54'-84º48'E	Whole plants	Tonic, hepatic trouble, liver disorders, skin- and hair care
4	Woodfordia fruticosa Kurz	Lythraceae	27º21'-27º52'N 83º54'-84º48'E	Leaves	Leaf paste used in skin disease and conjunctivitis

Table- 2a: Effect of plants aqueous extracts on germination of wheat seed. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90)

Species name	Germination %						F-Value	P Value
	Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	99 ± 0.50^{a}	99.8 ± 0.10^{a}	98 ± 0.78^{b}	98.5 ± 0.50^{b}	94 ± 0.4^{c}	92 ± 1.0^{d}	8.93	0.000
Cannabis sativa L.	99 ± 0.50^{a}	98 ± 0.50^{b}	98.5 ± 0.20^{b}	96 ± 0.16^{c}	98 ± 1.0^{b}	90 ± 2.0^{d}	34.89	0.000
Eclipta prostata L.	99 ± 0.50^{a}	99 ± 0.50^{a}	98 ± 1.30^{b}	98 ± 1.50^{b}	94 ± 2.0^{d}	95 ± 1.0^{c}	4.14	0.002
Woodfordia fruticosa Kurz	99 ± 0.5^{a}	99 ± 0.50^{a}	97 ± 1.0^{b}	98 ± 1.0^{c}	90 ± 2.0^{d}	86 ± 2.0^{e}	8.07	0.000

Table –2b: Effect of plants aqueous extracts on germination of pea seed. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=80).

Species name	Germination %						F-Value	P Value
	Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	99.9 ± 0.10^{a}	99.8± 0.10^{a}	98± 1.0^{b}	98.5± 0.5^{b}	96± 2.0^{c}	94± 1.4^{d}	8.93	0.000
Cannabis sativa L.	99.9±0.10^{a}	98 ± 1.0^{c}	98.5± 1.0^{b}	99± 0.10^{b}	98± 1.5^{c}	92± 2..50^{d}	4.89	0.003
Eclipta prostata L.	99.9± 0.1^{a}	99± 0.20^{a}	98± 1.50^{b}	98± 1.5^{b}	96± 2.5^{c}	96± 2.40^{c}	5.14	0.006
Woodfordia fruticosa Kurz	99.9± 0.1^{a}	99± 0.5^{a}	97± 2.20^{c}	98± 1.2^{b}	90± 2.50^{d}	85± 3.0^{e}	8.07	0.000

Table-3a: Effect of plants aqueous extracts on hypocotyle length of wheat. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90).

Species name	Plant parts	Hypocotyle length (cm) at different concentration						F-Value	P Value
		Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	Leaf	8.68 ± 1.31^{a}	5.92± 2.12^{b}	3.86 ± 2.36^{c}	3.47 ± 2.67^{c}	1.78 ± 1.28^{d}	0.86± 0.19^{d}	34.89	0.000
Cannabis sativa L.	Leaf	8.68 ± 1.33^{a}	7.12 ±1.33^{a}	5.92 ± 2.12^{b}	5.40 ± 3.31^{b}	3.92 ± 2.90^{c}	2.22±1.41^{c}	42.89	0.000
Eclipta prostata L.	Leaf	8.68 ± 1.32^{a}	7.18 ± 1.10^{a}	6.82 ± 1.00^{b}	3.64 ± 0.89^{c}	2.63 ± 0.68^{d}	2.06 ± 0.87^{d}	16.67	0.000
Woodfordia fruticosa Kurz	Leaf	8.68 ±1.33^{a}	3.03 ±1.21^{b}	2.49 ±2.03^{b}	1.98 ± 1.02^{c}	1.40 ±1.02^{c}	0.61± 0.23^{d}	8.07	0.000

Table-3b: Effect of plants aqueous extracts on root length of wheat. For each parameter, significant difference between mean among the sites are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=90)

Species name	Plant parts	Root length (cm) at different concentration						F-Value	P Value
		Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	Leaf	5.78 ± 1.33^{a}	2.78 ± 3.12^{b}	2.81 ± 1.50^{b}	1.52 ± 0.90^{c}	1.45 ± 1.20^{c}	0.88 ± 0.32^{d}	15.64	0.000
Cannabis sativa L.	Leaf	5.78 ± 1.33^{a}	3.30 ± 1.23^{b}	3.41 ± 2.13^{b}	3.00 ± 2.34^{b}	2.07 ± 1.31^{c}	1.07 ± 0.21^{d}	4.21	0.001
Eclipta prostata L.	Leaf	5.78 ± 1.33^{a}	5.49 ± 1.21^{a}	4.25 ± 1.02^{a}	4.53 ± 1.21^{a}	2.79 ± 0.94^{b}	1.03 ± 0.34^{c}	20.006	0.000
Woodfordia fruticosa Kurz	Leaf	5.78 ± 1.33^{a}	1.032 ± 0.52^{b}	0.62 ± 2.12^{c}	0.66 ± 0.13^{c}	0.41 ± 0.22^{c}	0.34 ± 0.12^{d}	16.43	0.000

Table-4a: Effect of plant extracts on hypocotyle length of pea. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=80).

Species name	Plant parts	Hypocotyle length (cm) at different concentration						F-Value	P Value
		Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	Leaf	7.86 ± 1.86^{a}	7.67 ± 1.78^{a}	5.80 ± 1.30^{b}	4.54 ± 0.13^{b}	3.01 ± 1.11^{c}	3.01 ± 1.21^{c}	11.99	0.03
Cannabis sativa L.	Leaf	7.86 ± 1.86^{a}	7.01 ± 1.43^{a}	7.52 ± 1.93^{a}	7.46 ± 1.49^{a}	6.06 ± 1.31^{b}	4.35 ± 1.26^{c}	9.58	0.028
Eclipta prostata L.	Leaf	7.86 ± 1.86^{a}	6.36 ± 1.15^{a}	5.76 ± 1.38^{b}	6.26 ± 1.34^{b}	4.10 ± 1.21^{c}	4.80 ± 1.30^{c}	10.68	0.000
Woodfordia fruticosa Kurz	Leaf	7.86 ± 1.86^{a}	5.06 ± 0.91^{b}	2.47 ± 0.56^{c}	3.22 ± 0.91^{c}	1.72 ± 0.74^{d}	1.17 ± 0.42^{d}	9.87	0.004

Table-4b: Effect of plant extracts on root length of pea. For each parameter, significant difference between mean among the treatments are indicated by different letters (Duncan homogeneity test, á = 0.05). F and P values were obtained by one way analysis of variance (ANOVA). (n=80).

Species name	Plant parts	Root length (cm) at different concentration						F-Value	P Value
		Control (0%)	2 %	4 %	6%	8 %	10 %		
Ageratum conyzoides L.	Leaf	4.40[a]	4.07 ± 0.45[a]	4.71 ± 0.12[a]	3.74 ± 0.16 [a]	2.09 ± 0.23 [b]	0.72 ±0 .23[c]	4.65	0.000
Cannabis sativa L.	Leaf	4.40 [a]	3.58 ±0.40[b]	5.0 ± 0.56 [a]	3.74± 0.42[b]	3.55 ± 0.36 [b]	3.02 ± 0.27 [b]	2.009	0.078
Eclipta prostata L.	Leaf	4.40 [a]	4.09 ± 1.02 [a]	4.15 ±0.53 [a]	3.53 ±0.63[b]	2.04 ±0.76[b]	1.37 ± 0.58 [c]	14.95	0.008
Woodfordia fruticosa Kurz	Leaf	4.40[a]	4.61 ± 0.46[a]	4.25 ± 0.87[a]	3.65 ± 1.08[b]	1.04 ±0.72 [c]	0.67± 0.28[d]	18.94	0.000

Table-5: Phytochemical constituents of six medicinal plants studied.

Species name	Plant parts	Phytochemical constituents						
		Alkaloids	Flavonoid	Carotene	Tannins	Terpenoid	Glycoside	Saponins
Ageratum conyzoids	Leaf	+++	+++	+	+++	+	++	+
Cannabis sativa	Leaf	+	+	+	++	++	_	+
Eclipta prostata	Leaf	++	+	_	++	+	++	_
Woodfordia fructicosa	Leaf	++	++	_	+	+	+	+

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

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. There was definite co- relation between traditional application of plants and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. These results may be useful to future workers to select a group of plants having similar constituents to isolate biologically active principle or prepare remedies for particular case.

The traditional medicinal practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

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