J. Nat. Hist. Mus. Vol. 28, 2014, 57-65

ALLELOPATHIC POTENTIAL OF MEDICINAL PLANTS: *COSTUS SPECIOSUS* KOEN EX. RETZ AND *JUSTICIA ADHATODA* LINN

ANJANA DEVKOTA[1] ✉ AND SWASTI SHARMA[1]

[1]Central Department of Botany, Tribhuvan University
Kirtipur, Kathmandu, Nepal
devkotaa@gmail.com

ABSTRACT

The study was carried out to evaluate the allelopathic effect of aqueous extract of rhizome and leaves of *Costus speciosus* Koen ex. Retz and *Justicia adhatoda* Linn. respectively on germination and growth of wheat (*Triticum aestivum* Linn.) and pea (*Pisum sativum* Linn.). Effect was assessed by recording their effects on germination and percentage growth of root and hypocotyle of test plants. However, the extent of inhibitory and stimulatory effect of extracts varied with the plant species. The extract 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 both 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 *Justicia adhatoda* Linn. had strong inhibitory effect on germination as well as root and hypocotyls growth of test seeds. Phytochemical screening of both medicinal plants was also carried out by using standard methods. The extract contained alkaloid, terpenoids, flavonoids, tannins and saponins in different proportions; with more of alkaloids, flavonoids and terpenoids.

Keywords: allelopathy, hypocotyle, medicinal plants, phytochemical screening, root length

INTRODUCTION

Allelopathic interaction involves the production and release of chemical substances (allelochemicals) by certain plants that inhibit the growth and development of the individuals of the neighbouring species (Shaukat *et al.,* 2003). These `allelochemicals 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 (Molisch, 1937). The concept of allelopathy was further supported and developed by Bonner (1950), Grummer & Beyer (1960), Whittaker (1970) and Fischer *et al.,* (1978). Allelopathic potential of some selected species had been studied by Hussain *et al*. (1997), Maharjan *et al.* (2007), and Timothy *et al.* (2009). Allelopathic effect of medicinal species against temperate crop is well studied (Rice,1971; Han *et al.,* 2008; Li *et al*., 2009). The beneficial medicinal effects of plants typically result from the secondary compounds in the plants which are specific in certain taxa, such as family, genus and species

(Parekh *et al*., 2005). 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 two Nepalese medicinal plants *Costus speciosus* and *Justicia adhatoda* for future chemical analyses. Thus, two 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.

MATERIALS AND METHODS

To study the allelopathic activities, leaves of *Justicia adhatoda* was collected from Kathmandu valley (27º 40.20' N 85º 17.32' E ; alt. 1350 masl) and rhizome of *Costus specious* was collected from Chitwan district (27º21'-27º52'N 83º54'-84º48'E; alt. 200 masl) of Nepal and shade dried.

Test plants

Pea and wheat seeds were used as receptor plants for initial screening of species to check allelopathic potentialities. Seeds were taken from Botany Division, NARC (National Agriculture Research Council), Khumaltar Lalitpur. 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 (Randhawa *et al*., 1998). 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 the selected medicinal plant species were applied on the test/receptor plant (pea and wheat).

Experiments

Ten grams of air dried selected plant parts were grounded, 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.

Phytochemical screening methods

The samples were grounded 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.

RESULTS

Allelopathy

Effects of aqueous extracts of Costus speciosus and Justicia adhatoda on the seed germination and seedling growth of wheat and pea

Both medicinal plants had significant effect on seed germination and seedling growth of wheat and pea (Table 1, 2 & 3). The inhibitory effect on germination was increased with increasing concentrations of the extracts. Out of these two tested plants, *Justicia adhatoda* had highest inhibitory effects on germination and seedling growth. The inhibitory effect was increased with increasing concentrations of the extracts and inhibition of the roots was greater than that of the hypocotyls; while *Justicia adhatoda* at 2% concentration had stimulatory effect (table 2).

Effect of plant extracts on seedling growth of pea

Table 3 shows the effect of aqueous extracts of *Costus speciosus* and *Justicia adhatoda* 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.

Phytochemical Screening

The phytochemical characteristics of two medicinal plants tested are presented in the table 4. The results revealed the presence of medicinally active compounds in the two plants studied. From the table, it could be seen that, alkaloids, flavonoids, terpenoids and tannins were present in all the plants. *Justicia adhatoda* contained high amount of alkaloids. Screening for the flavonoids, terpenoids and tanins of the plants *Costus speciosus* gave the highest positive test while it gave negative test to sample of *Justicia adhatoda*.

TABLE 1. Effect of plants aqueous extracts on germination of wheat and 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).

Species name	Germination % (wheat seed) (n=90)						F-Value	P Value
	Control (0%)	2 %	4 %	6%	8 %	10 %		
Costus speciosus (Koenig) Smith	99 ± 0.5[a]	99.9± 0.10[a]	99.9± 0.10[a]	98±0.87[b]	98±0.30[b]	99±0.60[a]	2.89	0.08
Justicia adhatoda L	99 ± 0.50[a]	100± 0.20[a]	98±1.0[b]	98±1.0[b]	95± 1.0[c]	96± 2.0[c]	6.67	0.008
		Germination % (pea seed) (n=80)					F-Value	P Value

Costus speciosus (Koenig) Smith	99.9 ±0.1^{a}	100± 0.0^{a}	99.9±0.10^{a}	98± 1.0^{b}	98± 1.5^{b}	98± 1.0^{b}	2.90	0.08
Justicia adhatoda L	99.9± 0.1^{a}	100± 0.0^{a}	98± 1.0^{b}	98± 1.0^{b}	95± 2.5^{c}	94± 2.0^{d}	6.87	0.000

TABLE 2. Effect of plants aqueous extracts on hypocotyle and root 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 %		
Costus speciosus (Koenig) Smith	Rhizome	8.68 ±1.23^{a}	6.97 ±2.31^{b}	6.19 ± 2.01^{b}	3.74 ±1.34^{c}	3.84 ±2.90^{c}	2.94 ± 0.61^{d}	48.14	0.000
Justicia adhatoda L	Leaf	8.68± 1.33^{a}	10.54±1.33^{a}	5.67±1.45^{b}	4.81±1.85^{b}	1.06±1.23^{c}	0.64±0.30^{d}	8.93	0.000
Species name	**Plant parts**	**Root length (cm) at different concentration**							
		Control (0%)	2 %	4 %	6%	8 %	10 %	**F-Value**	**P Value**
Costus speciosus (Koenig) Smith	Rhizome	5.78 ±1.33^{a}	3.63 ± 2.01^{b}	4.19 ± 1.09^{a}	3.73 ±1.50^{b}	2.14 ± 1.02^{c}	1.07 ± 0.22^{d}	29.003	0.000
Justicia adhatoda L	Leaf	5.78± 1.33^{a}	5.91 ± 1.32^{a}	4.34 ± 1.69^{b}	3.09 ±1.13^{b}	1.28 ± 1.01^{c}	0.84 ± 1.96^{d}	25.38	0.000

TABLE 3. Effect of plant extracts on hypocotyle and 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**	**Hypocotyle length (cm) at different concentration**							
		Control (0%)	2 %	4 %	6%	8 %	10 %	**F-Value**	**P Value**
Costus speciosus (Koenig) Smith	Rhizome	7.86 ± 1.86^{a}	7.44 ± 2.10^{a}	5.16±1.14^{b}	5.33 ± 1.85^{b}	4.91 ±1.76^{b}	3.71 ±1.95^{c}	11.12	0.000
Justicia adhatoda L	Leaf	7.86 ± 1.86^{b}	9.53 ± 1.10^{a}	7.41 ±0.97^{b}	5.40± 1.21^{c}	5.22 ± 1.03^{c}	3.98± 0.84^{d}	24.09	0.000
Species name	**Plant parts**	**Root length (cm) at different concentration**							
		Control (0%)	2 %	4 %	6%	8 %	10 %	**F-Value**	**P Value**

Costus speciosus (Koenig) Smith	Rhizome	4.40^{a}	3.34 ± 0.99^{b}	3.69 ± 0.61^{b}	3.12 ± 0.82^{bc}	2.89 ± 0.75^{c}	1.71 ± 0.98^{d}	19.94	0.006
Justicia adhatoda L	Leaf	4.40^{a}	4.69 ± 0.33^{a}	3.71 ± 0.38^{a}	3.84 ± 0.36^{a}	2.89 ± 0.15^{b}	1.52 ± 0.32^{c}	2.47	0.033

TABLE 4. Phytochemical constituents of tested plants.

		Phytochemical constituents						
	Plant parts	Alkaloids	Flavonoid	Carotene	Tannins	Terpenoid	Glycoside	Saponins
Costus speciosus	Rhizome	++	++	□	+	++	+	++
Justicia Adhatoda	Leaf	+++	□	+	□	□	+	+++

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

DISCUSSION

Extracts from rhizome of *Costus speciosus* and leaves of *Justicia adhatoda* showed inhibitory effects on seed germination. The degree of inhibition increased with the extract concentration. At the highest extracts concentration (10%), all aqueous extracts significantly reduced, root and shoot lengths compared with control (table1). Such inhibition on the shoot and root growth of the test plant species might be due to the presence of allelochemicals in both plant extract and the percent of inhibition of the test plant species showed the similar trend with the increase of concentration of the extracts. These types of growth inhibition by the allelopathic plants extract was also reported by Inderjit & Keating (1999), Batlang & Shushu (2007), Islam & Kato-Noguchi (2012).The shoot and root growth inhibition of the test plant species, observed in this experiment might be due to the allelopathic effect of *C. speciosus* and *J. adhatoda* as there was no intra species competition for light, nutrient, space and moisture. The seedlings of each test plant species used in this experiment were grown in a single Petri-dish without supplying any nutrient solution. The young seedlings only get nutrients from their reserved food material into the seeds. From this viewpoint, it is clear that the seedlings growth inhibition of the test plant species is mainly due to the allelopathic reaction rather than by competitive interference (Ashrafi *et al.,* 2008).

The growth inhibition of the test plant species, in the presence of allelochemicals could be for the reason of lower cell division, elongation and expansion rate which are growth pre-requisites (Rice 1984, Einhellig 1996). Moreover, allelochemicals inhibit the respiration (Inderjit & Keating, 1999), ion absorption process (Qasem & Hill, 1989), enzyme activity , germination and thus results in arrested plant growth (Islam & Kato-Noguchi, 2012).

This finding is supported by Turk *et al.* (2003) in black mustard on growth of alfalfa and congress grass on crop plants (Dhawan, 1995). Plants exhibit allelopathic activity due to release of allelochemicals of different chemical classes mainly polyphenolic compounds (flavonoids and tannins), cyanogenic glycosides and alkaloids (Einhelling, 1995a & 1995b). 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 aqueous extracts of both medicinal plants (table 4). In the present study, allelopathic effect of both 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 increased with increasing concentrations of aqueous extracts (tables 1, 2 & 3). It was also reported that effectiveness of recipient plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold (Lovett *et al*., 1989; Caussanel, 1979; An *et al*., 1996; Ashrafi *et al*., 2009; Batlang & Shushu, 2007). 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*. (2005) 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 *J. adhatoda* showed the highest inhibitory effects on wheat and pea seedlings (tables 2 & 3).

In addition, the inhibition of the aqueous extract of both medicinal plants on the root growth of two test plant species was greater than that on hypocotyl growth. These results are in agreement with the earlier findings of many researchers working with other plant materials. Similar findings were also reported by Stachon & Zimdahl (1980), Islam and Kato-Noguchi Hisashi (2012) . The higher root growth inhibition is mainly because the roots are the first organ to absorb allelochemicals from the environment (Salam and Kato-Noguchi, 2010) and the permeability of allelochemicals. Similar kinds of results were reported from the studies of Chon *et al*. (2000), 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 (Nishida *et al*., 2005). The present research suggests that the extracts of *J. adhatoda* had shown higher allelopathic effects than *C. speciosus*. This plant, therefore, may be the candidates for isolation and identification of allelochemicals.

Allelopathic potentialities and phytochemical screening of *C. speciosus* and *J. adhatoda* was studied. The present research suggests that the extracts of *J. adhatoda* had shown higher allelopathic effects among two studied medicinal plants. From the present preliminary investigation, it can be concluded that both 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 both medicinal plants on the root growth of two test plant species was greater than that on hypocotyls growth.

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 (Harborne, 1973). Earlier studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments (Ali *et al.,* 2008). 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. This 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 medicine 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.

ACKNOWLEDGEMENTS

We are thankful to Nepal Academy of Science and Technology (NAST) for providing financial support for this research work.

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