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PHYTOTOXIC STUDIES OF MEDICINAL PLANT SPECIES OF PAKISTAN

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Abstract

Allelopathic screening of 81 medicinal plant species, collected from North West Frontier Province (NWFP) Pakistan, was carried out to identify significantly higher allelopathic species for future phytochemical analyses. For this purpose, sandwich method was used to test allelopathic potentials of leaf leachates of these plant species against lettuce seeds (*Lactuca sativa* L.). Two different concentrations of 10 mg and 50 mg of leaf leachates were used in the study. The radicle and hypocotyl growths were measured and compared with control treatments. It was observed that an endemic species *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* were the stronger phytotoxic plants as compared to the other test species.

Based on the current screening, three potential medicinal plants are recommended for future bioassay guided isolation of allelochemicals and for genetic diversity studies. It would also be interesting to see correlation between genetic markers and isolated allelochemicals.

Introduction

There is convincing evidence that allelopathy has a potential role in natural and agricultural ecosystems. Thus, extensive research has been conducted to exploit this phenomenon in improving the production of agricultural ecosystems through different ways including using natural products from plants as pesticides instead of synthetic chemicals. One way to exploit allelopathic phenomenon is to screen plant species for their allelopathic and or medicinal potential and to select the most bioactive ones for chemical analyses (Fujii *et al*., 1990, 1991, 2003).

Pakistan has a diverse range of climatic and phytogeographic conditions which results in diverse flora containing several medicinal plant species. Estimated total flora of Pakistan is comprised of 6000 species (Shinwari *et al*., 2000). More than 4000 plant species grow in mountainous regions of one of the four provinces of Pakistan i.e., North West Frontier Province (NWFP) and northern region of Hindukush–Himalayas of Pakistan (Shinwari *et al*., 2002). The total number of estimated species in Kurram Agency are 1200 (Shinwari *et al*., 2002) and in Swat are 1550 (Stewart, 1967). Wide ranges of forests from dry subtropical to alpine grow in NWFP and especially in District Swat (Shinwari & Gilani, 2003). Due to mountains, the districts of Kohat & Karak (previously part of District Kohat) are the only entrances into south regions of NWFP for the people of north region of NWFP. The characteristic flora of District Kohat and Karak are comprised of Dry sub-tropical forest. Similarly, the Kurram Agency is the tribal zone near Afghanistan border towards west of NWFP. It has unique flora and more than 100 endemic as well as threatened medicinal plants have been reported from Kurram Agency (Shinwari *et al*., 2002, Shinwari, 2010).

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The local communities in Pakistan have used medicinal plants species for curing various diseases for a long time (Gilani *et al*., 2009; Mohy-ud-Din *et al*., 2010; Shinwari, 2010). However, little information is available on the allelopathic potentials of medicinal plants as well as the possible variations in the allelopathic activities of the plants species growing in different environmental habitats. In Japan, extensive research has been carried out by Dr. Fujii and his group to test allelopathic activities of 387 Japanese medicinal plant species (Fujii *et al*., 1990, 1991, 2003) and they found that considerable numbers of the test species showed higher allelopathic potentials where toxicity varied within the same plant species growing in different habitats (Gilani *et al*., 2010).

With this in mind, NWFP with 80% of the total flora of Pakistan was selected as the focal region where all the phytogeographic regions are situated. Eighty one medicinal plants were collected from the districts of Kohat, Karak and Swat and the tribal zone, Kurram Agency covering all the phytogeographic regions. These medicinal plants were tested for their allelopathic activities in order to select the most allelopathic species for future studies.

Materials and Methods

Plant collection: Medicinal plants species were collected from fields of North West Frontier Province (NWFP) Pakistan, Districts Kohat, Karak and Swat and Federally Administered Tribal Areas (FATA) - Kurram Agency. The plants were dried in oven at 60°C for 24 hours, packed in polythene bags with silica gel and stored at 10ºC until use.

Bioassay: For allelopathic studies, sandwich method of Fujii *et al*., (2003 & 2004) was followed. Five ml of 0.75% (w/v) agar (Nakalai Tesque, gelling temperature 30–31°C) was poured in each well of the six-well (10 cm^2 area per well) multi-dish plastic plate. The agar solution was left for solidification. Dried leaves of each test species @ 10 and 50 mg were placed in appropriate wells of the plate and were covered by a thin layer of 0.75% (w/v) agar. After solidification, five seeds of lettuce (*Lactuca sativa* L. Great Lakes No. 366, Takii Seed Co. Ltd, Japan) were placed on agar gel in each well of the plate. Each plate was then sealed with the plastic tape and incubated for 72 h at 24ºC under dark conditions. The lengths of radicle and hypocotyl were recorded. In the control treatment, only agar gel without dried leaves was used as a seed bed for lettuce seeds. Each treatment was replicated thrice. Percentages of root and hypocotyl lengths of each medicinal plant were calculated by comparing with control. The normal distribution pattern was evaluated for both the 10 mg and 50 mg of plant materials with root and hypocotyl growths of 81 medicinal plants.

Results

In case of dicotyledonous plants, 74 species were comprised of 34 families (Table 1). In these 34, there were 20 families with a single species; 5 with twin species and 9 with more than two species that were included in our study for allelopathy. Among these dicotyledonous families, Asteraceae, Lamiaceae and Solanaceae were comprised of 10, 9 and 7 medicinal plants species, respectively. While a single medicinal plant species, *Arisaema flavum,* was available from a monocotyledonous family Araceae. A single species each was also included in the study from two gymnospermous families, Pinaceae and Taxaceae, and from Pteridophytes.

Table 1. List of families and number of medicinal plants from the families collected for allelopathic studies.

No.	Family	Total	No.	Family	Total
	Dicotyledonous families				
1.	Acanthaceae	1	2.	Lamiaceae	9
3.	Adoxaceae	1	4.	Leguminosae	3
5.	Amaranthaceae	3	6.	Paeoniaceae	1
7.	Apiaceae	1	8.	Plantaganaceae	2
9.	Apocynaceae	2	10.	Podophyllaceae	1
11.	Araliaceae	1	12.	Polygonaceae	2
13.	Asclepiadaceae	1	14.	Rhamnaceae	2
15.	Asteraceae	10	16.	Rosaceae	3
17.	Bergeniaceae	1	18.	Sapindaceae	1
19.	Celastraceae	1	20.	Scrophulariaceae	1
21.	Chenopodiaceae	4	22.	Solanaceae	7
23.	Cruciferae	1	24.	Tamaricaceae	1
25.	Cucurbitaceae	1	26.	Thymeliaceae	1
27.	Euphorbiaceae	3	28.	Urticaceae	1
29.	Geraniaceae	1	30.	Valerianaceae	1
31.	Hippocastaceae	1	32.	Verbenaceae	1
33.	Hypericaceae	2	34.	Zygophyllaceae	2
	Monocotyledonous family				
35.	Araceae	1			
	Gymnosperms				
36.	Pinaceae	2	37.	Taxaceae	1
	Pteridophytes				
38.	Adiantaceae	1	39.	Fern	2

It was observed that 66 species showed inhibitory effects while 15 species showed stimulatory effects at 10 mg concentration for radicle length (Table 2) while only 3 species, *Taxus wallichiana, Pinus wallichiana* and *Dryopteris juxtaposita* showed stimulatory effects at 50 mg concentration. These species have also shown stimulatory effects for radicle growth at 10 mg and hypocotyl growths at both the concentrations. None of the angiospermic species showed stimulatory effects at 50 mg concentration for radicle growth of lettuce seeds. However, 33 species showed stimulatory effects at 10 mg concentration for lettuce root growth.

From our results, top 10 medicinal plants of highest inhibitory effects among all 81 plants in descending order of toxicity were: *Seriphidium kurramense* (Asteraceae), *Andrachne cordifolia* (Euphorbiaceae), *Foeniculum vulgare* (Apiaceae), *Rhazya stricta* (Apocynaceae), *Solanum surratense (*Solanaceae), *Thymus linearis* (Lamiaceae), *Mentha* sp., (Lamiaceae); *Aerva javanica* (Amaranthaceae), *Withania coagulans* (Solanaceae) and *Paeonia emodi* (Paeoniaceae) (Table 1). However**,** *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* showed stronger allelopathic effects than any other medicinal plant species (Fig. 1). Inhibition was recorded in both the concentrations of leaf leachates at 10 and 50 mg / well. It was observed that inhibitory effect was increased with increasing concentration (Table 1); for example, leaf leachates of *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* at 10mg inhibited growth of radicle lengths of lettuce seedling by 61%, 93%, and 82% and hypocotyls lengths by 65%, 89% and 55%, respectively. While 50 mg leaf leachates of *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* inhibited the radicle lengths by 99, 94 and 90% and hypocotyls lengths by 99, 89, and 77%, respectively.

Table 2. Allelopathic activities of medicinal plant species of Pakistan on radicle (R) and hypocotyls (H) length of lettuce seedling.

No.	Botanical name	Family	R 10	H 10	R 50	H 50
1.	*Seriphidium kurramense* Qazilb. (Y. R. Ling)	Asteraceae	61	35	99	99
2.	*Andrachne cordifolia* (Wall. ex DC.) Mull. Arg.	Euphorbiaceae	93	89	94	89
3.	*Foeniculum vulgare* Mill.	Apiaceae	45	53	93	89
4.	*Rhazya stricta* Decne.	Apocynaceae	82	55	90	77
5.	*Solanum surattense* Burm. f.	Solanaceae	51	4	87	66
6.	*Aerva javanica* (Burm. f.) Juss. ex J.A. Schultes	Amaranthaceae	41	8	84	72
7.	*Mentha spicata*	Lamiaceae	13	13	84	76
8.	*Thymus linearis* Benth.	Lamiaceae	10	-35	84	63
9.	*Withania coagulans* (Stocks) Dunal	Solanaceae	50	-1	82	48
10.	*Paeonia emodi* Wall. Ex Royle	Paeoniaceae	33	61	81	55
11.	*Chenopodium ambrosioides* L.	Chenopodiaceae	59	-26	80	9
12.	*Melothria heterophylla* Lour.	Cucurbitaceae	55	4	80	9
13.	*Geranium wallichianum* D. Don ex Sweet	Geraniaceae	30	-6	80	46
14.	*Arisaema flavum* (Forsk.) Schott.	Araceae	56	-7	79	14
15.	*Sageretia theezans* (L.) Brongn.	Rhamnaceae	20	2	79	43
16.	*Justicia adhatoda* L.	Acanthaceae	52	-20	78	31
17.	*Valeriana jatamansi* Jones	Valerianaceae	18	-2	78	39
18.	*Datura alba* Nees	Solanaceae	7	-110	78	-42
19.	*Podophyllum hexandrum* Royle	Podophyllaceae	37	13	77	22
20.	*Datura strammonium* L.	Solanaceae	41	3	76	4
21.	*Peganum harmala* L.	Zygophyllaceae	32	-73	76	-45
22.	*Alternanthera pungens* Kunth	Amaranthaceae	51	-36	75	-20
23.	*Solanum nigrum* L.	Solanaceae	46	-43	75	-53
24.	*Xanthium sp.*	Asteraceae	25	-19	74	-11
25.	*Withania somnifera* Dunal	Solanaceae	28	8	73	36
26.	*Ricinus communis* L.	Euphorbiaceae	44	-1	72	9
27.	*Fagonia indica* Burm. f. var. indica	Zygophyllaceae	59	51	71	48

Table 2 (Cont'd.).

No.	Botanical name	Family	R 10	H 10	R 50	H 50
28.	*Ocimum basilicum* L.	Lamiaceae	47	-6	71	-2
29.	*Sisymbrium irio* L.	Cruciferae	38	13	71	31
30.	*Urtica dioica* L.	Urticaceae	37	-9	71	-2
31.	*Bistorta amplexicaulis* (D. Don) Green	Polygonaceae	51	4	70	9
32.	*Senecio chrysanthemoides* DC.	Asteraceae	40	52	70	51
33.	*Plantago lanceolata* L.	Plantaganaceae	21	2	70	32
34.	*Salvia lanata* Roxb.	Lamiaceae	42	45	69	32
35.	*Calotropis procera* (Ait.) R. Br.	Solanaceae	34	-52	69	-37
36.	*Salsola drummondii* Ulbr.	Chenopodiaceae	24	-28	69	15
37.	*Rubus ellipticus* Smith	Rosaceae	20	34	68	35
38.	*Achyranthes aspera* L.	Amaranthaceae	46	42	66	25
39.	*Fragaria nubicola* Lindl.	Rosaceae	36	25	66	23
40.	*Vitex negundo* L.	Verbenaceae	59	66	64	54
41.	*Artemisia sp 2*	Asteraceae	36	-37	62	-52
42.	*Prosopis juliflora* (Sw.) DC.	Leguminosae	48	21	61	13
43.	*Plantago major* L.	Plantaganaceae	31	0	61	11
44.	*Hedera nepalensis* K. Koch.	Araliaceae	31	-4	60	0
45.	*Chenopodium botrys* L.	Chenopodiaceae	29	-52	58	-31
46.	*Artemisia vulgaris* L.	Asteraceae	35	25	57	32
47.	*Mentha royleana* Benth.	Lamiaceae	-27	-95	52	44
48.	*Hypericum perforatum* L.	Hypericaceae	6	-44	51	-50
49.	*Rosa webbiana* Wall. Ex Royle	Rosaceae	33	7	50	-5
50.	*Viburnum grandiflorum* Wall. Ex. DC.	Adoxaceae	19	26	48	26
51.	*Periploca aphylla* Decne.	Asclepiadaceae	20	4	47	8
52.	*Artemisia scoparia* Waldst. & Kit.	Asteraceae	27	33	46	22
53.	*Bergenia ciliata* (Haw.) Sternb.	Bergeniaceae	20	-9	46	-20
54.	*Teucrium stocksianum* Boiss.	Lamiaceae	18	1	46	8

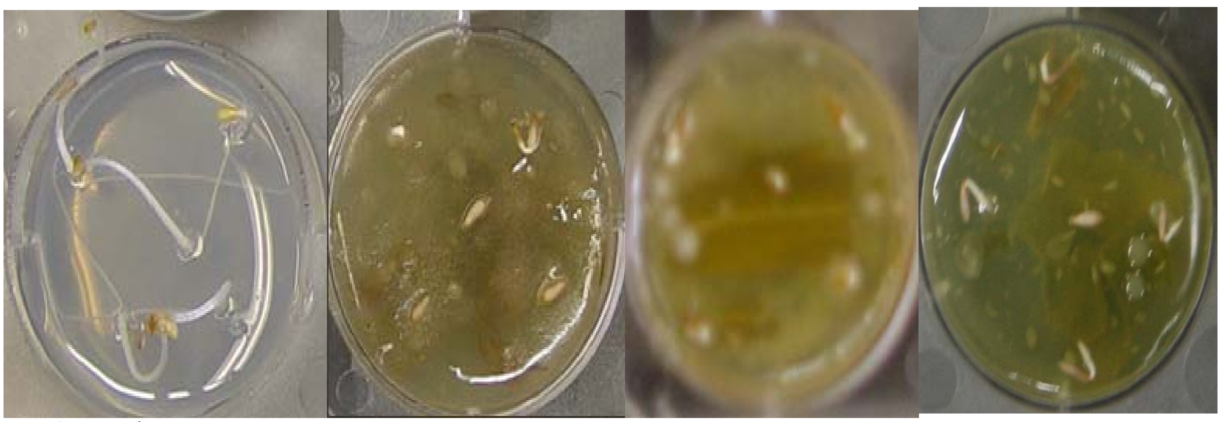
Table 2 (Cont'd.).

No.	Botanical name	Family	R 10	H 10	R 50	H 50
55.	*Chenopodium album* L.	Chenopodiaceae	20	-43	44	3
56.	*Aesculus indica* (Wall. Ex Comb.) Hook.f.	Hippocastaceae	10	25	44	22
57.	*Conyza Canadensis* (L.) Cronquist.	Asteraceae	18	-10	43	-4
58.	*Xanthium strumarium* L.	Asteraceae	-1	26	43	12
59.	*Artemisia vulgaris* L.	Asteraceae	12	-10	42	-11
60.	*Tamarix indica* Willd.	Tamaricaceae	24	12	39	-2
61.	*Gymnosporia royleana* (Wall.) Lawson	Celastraceae	20	-4	39	-49
62.	*Abies pindrow* Royle	Pinaceae	16	-32	36	-26
63.	*Verbascum thapsus* L.	Scrophulariaceae	7	-127	36	-100
64.	*Astragalus sp.*	Leguminosae	2	11	36	15
65.	*Artemisia sp 1*	Asteraceae	-7	-52	34	-10
66.	*Ocimum sp.*	Lamiaceae	-8	-34	33	-42
67.	*Chrozophora obliqua* (Vahl.) Sprengel	Euphorbiaceae	2	-68	31	-55
68.	*Adiantum capillus – veneris* L.	Adiantaceae	-3	-45	25	-62
69.	*Plectranthus rugosus* Wall. ex Benth.	Lamiaceae	-22	-118	25	-100
70.	*Sageretia thea* (Osbeck) M.C. Johnst var. *brandrethiana* (Aitch.) Qaiser & S. Nazimuddin	Rhamnaceae	-2	37	23	30
71.	*Marrubium vulgare* L.	Lamiaceae	24	30	20	34
72.	*Indigofera heterantha* Wall. ex Brand.	Leguminosae	-7	-27	19	-61
73.	*Daphne mucronata* Royle	Thymeliaceae	9	-56	17	-56
74.	*Dodonaea viscosa* (L.) Jacq.	Sapindaceae	-3	-9	13	-38
75.	*Nerium oleander* L.	Apocynaceae	-1	-27	11	-27
76.	*Polygonum aviculare* L.	Polygonaceae	5	-15	5	-22
77.	*Hypericum oblongifolium* Choisy	Hypericaceae	-6	-24	3	-39
78.	*Pteris vittata* L.	Fern	-22	-17	3	-22
79.	*Dryopteris juxtaposita* Christ	Fern	-22	-7	-2	-7
80.	*Taxus wallichiana* Zucc.	Taxaceae	-22	-44	-2	-42
81.	*Pinus wallichiana* A. B. Jackson	Pinaceae	-5	27	-6	15

10mg

Control ***Seriphidium kurramense*** ***Rhazya stricta*** ***Andrachne cordifolia***

50mg



Control ***Seriphidium kurramense*** ***Rhazya stricta*** ***Andrachne cordifolia***

Fig. 1. Photographs show allelopathic effects of *Seriphidium kurramense, Rhazya stricta* and *Andrachne cordifolia* on lettuce seedling, at 10 mg leaf leachate (above) and 50 mg leaf leachate (below).

Discussion

The current results suggest that the test species have allelopathic potentials and also have stimulatory effects. Similar results were also reported by Fujii *et al*., (2004) who screened 239 medicinal plant species for their allelopathic potential in Japan. Statistical analysis of our results showed that 50 mg leaf leachates of all medicinal plant species were the only concentrations which showed normal distribution pattern (data not shown). Numata (1969) had reported that an average amount of fallen leaves of a particular species per tree per year is 5 t ha year^{-1} which was calculated by Fujii *et al*., (2004) as 50 mg of leaves per 10 cm^2 of each well in 6-well multidish. In natural environment, the leaves liter falls on the soil and affects the neighboring plants by releasing chemicals into the soil. In our study, we used agar gel as the seed bed instead of soil. In agar gel, the chemicals ooze out of the leaves and probably move faster as compared to their movements in soil. In that case, some plants may give higher allelopathic effects. Therefore, we reduced the amount of the leaves to 10 mg to see our prediction whether the plant had the same kind of higher allelopathic effects.

Toxicity levels of medicinal plants increased with increasing concentration from 10 mg to 50 mg. Gilani *et al*., (2002 & 2003) also reported the similar findings in other plants that toxicity of leaf leachates increased with increasing concentrations. On the other hand, stimulatory effects of medicinal plants were also decreased with increasing concentration. Similar kinds of results were reported from the studies of *Pinus sylvestris* by Bulut & Demir (2007). Lower concentrations (10g/L) of the aqueous leaf extracts of *Pinus sylvestris* also showed stimulatory effects against roots of rye grass and shoots of creeping red fescue while higher concentrations of leaf extracts showed inhibitory effects (Bulut & Demir, 2007). The lettuce seeds are highly sensitive to inhibitory and stimulatory chemical compounds (Fujii *et al*., 1990 & 2003). Better explanation for decrease in stimulatory effects of the plants with increasing concentration of plant material may be the presence of lower amount of allelochemicals in addition to stimulatory chemicals at 10 mg is appropriate for promotion of radicle and hypocotyl growths of lettuce seedlings. As the concentration of leaf leachates increases to 50 mg, the intensity of allelochemicals also increase which possibly have inhibited the growths of lettuce seedlings. *Xanthium strumarium* showed undistributed pattern of toxicity in results for hypocotyl growth. However, aqueous extracts of leaves and stems of *Xanthium strumarium* significantly reduced the germination, root and shoot lengths of corn, canola, sesame, lentils and chickpea (Shajie & Saffari, 2007).

Seriphidium kurramense is an endemic medicinal plant of Kurram Agency and adjoining Afghanistan border areas (Ghafoor, 2002). It is also narrowly distributed as reported in case of *Cadaba hterotricha* by Abbas *et al*. (2010) which is included in IUCN Red Data List as threatened species but no attention has been paid to categorize this valuable endemic species as threatened. Till date, no allelopathic studies have been reported from the species. *S. kurramense* is commercially traded from the area for higher santonin contents. Being, the member of Asteraceae, the plant also contains essential oils which also showed higher toxic effects against lettuce seeds (Gilani *et al*., 2010). Several species of *Artemisia* have been reported to contain essential oils, especially terpenoids. Gilani *et al*., (2008, 2010) reported 19 essential oils from *Seriphidium kurramense* through GC-MS analysis. Among these essential oils, the major chemical constituents were α-thujone (45.2%), 1, 8-cineole (15.73%), β-thujone (14.38%) and camphor (12.40%) that constituted 87.7% of the total essential oil composition. Terpenoids have also been reported as allelopathic in nature (Wang *et al*., 2008).

Current literature search showed that allelopathic effects of *Andrachne cordifolia, Solanum surratense, Aerva javanica, Withania coagulans* and *Paeonia emodi* are not reported. Essential oils of *Foeniculum vulgare* showed inhibitory effects against common weeds (Azirak *et al*., 2008). Essential oils of Lamiaceae members have already shown toxic effects against different plant e.g., *Raphanus sativus* L., *Lactuca sativa* L., and *Lepidium sativum* L. (Armenante *et al*., 2006). Crude methanolic extracts and subsequent fractions of *Andrachne cordifolia* have recently shown significantly high antibacterial and antifungal properties (Ahmad *et al*., 2007). The presence of triterpenes (Mukherjee & Mukhopadhyay, 2005) and rare occurrence of Bis (benzulisoquinoline) (Khan *et al*., 1983) from *Andrachne cordifolia* have been reported.

The toxic effects of *Rhazya stricta* for 10 mg leaf leachates were in line with the results of Gilani *et al*., (2007 a & b) who reported that *Rhazya stricta* showed toxic effects against lettuce seeds. *Rhazya stricta* contains 89 indole alkaloids (Gilani *et al*., 2007b). Some of these indole alkaloids have shown pharmacological properties including antibacterial activity (see reviews of Ali *et al*., 2000 and Gilani *et al*., 2007a & b). Most probably, some or one of these alkaloids may also be responsible for allelopathic and for antibacterial activities also (Shinwari *et al*., 2009).

In summary, *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* showed higher toxic effects as compared to the rest of the 81 medicinal plants in the present study. It would be necessary to initiate the studies from crude extracts with targeted isolation of responsible allelochemicals in *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta*. So far no studies on genetic diversity and genetic resources of these plants are available. It would also be interesting to evaluate their genetic diversity patterns within and among populations of *Rhazya stricta* (Gilani *et al*., 2009) that may be utilized as future potential genetic resources for commercial exploitation with sustainable utilization.

Based on two types of results i.e., inhibitory and stimulatory effects, it is recommended that the plants with highly significant toxicity and stimulating effects should be utilized for future studies. Stressing top ten (10) highly allelopathic plants, it is recommended that

1. Targeted isolation of responsible allelochemicals may be conducted, initiating studies on the crude extracts.
2. Essential oil rich medicinal plants should be evaluated from allelopathic effects of essential oils.
3. GC-MS analysis of these essential oils should be carried out (Gilani *et al*., 2010). Similarly LC-MS or HPLC analyses of important chemical compounds (for example, indole alkaloids in *Rhayza stricta* or withanolides in *Withania coagulans*) should also be conducted, depending on the availability of standards in the market.
4. Genetic diversity studies of populations of important medicinal plants (Gilani *et al*., 2009) based on their allelopathic levels, endangered nature, commercial and medicinal values, ethnobotanical importance etc., should be evaluated.

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References

Abbas, H., M. Qaiser and J. Alam. 2010. Conservation status of *Cadaba heterotricha* Stocks (Capparaceae): An endangered species from Pakistan. *Pak. J. Bot.,* 42: 35-46.

Ahmad, B., S.M.H. Shah, S. Bashir, M. Nisar and M.I. Chaudry. 2007. Antibacterial and antifungal activities of *Andrachne cordifolia* Muell. *J. Enzyme Inhibition & Med. Chem*., 22: 726-729.

Ali, B.H., A.A. Qarawi, A.K. Bashir and M.O. Tanira. 2000. Phytochemistry, pharmacology and toxicity of *Rhazya stricta* Decne: A Review. *Phytotherap. Res*., 14: 229-234.

Azirak, S. and S.L. Karaman. 2008. Allelopathic effect of some essential oils and components on germination of weed species. *Acta Agriculturae Scandinavica, Section B: Soil & Plant Science*, 58: 88-92.

Bulut, Y. and M. Demir. 2007. The allelopathic effects of Scots Pine (*Pinus sylvestris* L.) leaf extracts on turf grass seed germination and seedling growth. *Asian J. Chem*., 19: 3169-3177.

Fujii, Y., T. Shibuya and T. Yasuda. 1990. Survey of Japanese weed and crops, for the detection of water-extractable allelopathic chemicals using Richards' function fitted to lettuce germination test. *Weed Res. Japan*, 35: 362-370 (In Japanese with English summary).

Fujii, Y., M. Furukawa, Y. Hayakawa, K. Sugawara and T. Shibuya. 1991. Survey of Japanese medicinal plants for the detection of allelopathic properties. *Weed Res. Japan*, 36: 36-42 (in Japanese with English summary).

Fujii, Y., S.S. Parvez, M.M. Parvez, Y. Ohmae and O. Iida. 2003. Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *Weed Biol. and Manag*., 3: 233-241.

Fujii, Y., T. Shibuya, K. Nakatani, T. Itani, S. Hiradate and M.M. Parvez. 2004. Assessment method for allelopathic effect from leaf litter leachates. *Weed Biol. and Manag*., 4: 19-23.

Ghafoor, A. 2002. Asteraceae (I), In: *Flora Pak*., (Eds.): S.I. Ali and M. Qaiser. 207: 144-160.

Gilani, S.S., S.M. Chaghtai, M.A. Khan and I.K. Wazir. 2002. Study of the allelopathic potential of *Eucalyptus microtheca* F. Muell. *Hamdard Med.*, 45: 25-30.

Gilani, S.S., S.M. Chaghtai and U. Khan. 2003. Phytotoxicity of *Eucalyptus microtheca* F, Muell. on *Pennisetum glaucum* cv. BARI-Hairy. *Pak. J. For*., 53: 87-97.

Gilani, S.A., A. Kikuchi, Z.I. Khattak, Z.K. Shinwari and K.N. Watanabe. 2007a. Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. *Phytotherap. Res*., 21: 1-7.

Gilani, S. A., Z. K. Shinwari and K. N. Watanabe. 2007b. Monograph on *Rhazya stricta*. *Plant Genetic Resources Monograph Series*, 1. Mimatsu Corp., Tokyo, Japan. pp. 81. ISBN 978-4-903242-21-7.

Gilani, S.A., Y. Fujii and K.N. Watanabe. 2008. Monograph on endemic medicinal plant, *Seriphidium kurramense* Y. R. Ling, of Kurram Agency, NWFP, Pakistan. *Plant Genetic Resources Monograph Series*, 2. Mimatsu Corp., Tokyo, Japan, pp. 51. ISBN 978-4-903242-23-1.

Gilani, S. A., A. Kikuchi, K. N. Watanabe. 2009. Genetic variation within and among fragmented populations of endangered medicinal plant, *Withania coagulans* (Solanaceae) from Pakistan and its implications for conservation. *Afr. J. Biotechnol.*, 8: 2948-2958.

Gilani, S.A., M. Sugano, Y. Fujii and K.N. Watanabe. 2010. Chemotypic variations and phytotoxic studies of essential oils of endemic medicinal plant, *Seriphidium kurramense*, from Pakistan. *J. Med. Plant Res*., 4: 309-315.

Khan, M.I., M. Ikram and S.F. Hussain. 1983. Bisbenzylisoquinoline alkaloids from *Andrachne cordifolia*. *Planta Medica*, 47: 191-192.

Mohy-ud-Din, A., Z. Khan, M. Ahmad and M. A. Kashmiri. 2010. Chemotaxonomic value of alkaloids in *Solanum nigrum* complex chemotaxonomy of *Solanum nigrum* complex. *Pak. J. Bot.,* 42: 653-660.

Mukherjee, K.S. and B. Mukhopadhyay. 2005. A new triterpenic constituent from *Andrachne cordifolia*. *J. Ind. Chem. Soc.*, 82: 936-937.

Shajie, E. and M. Saffari. 2007. Allelopathic effects of cocklebar (*Xanthium strumarium* L.) on germination and seedling growth of some crops. *Allelopathy J*., 19: 501-506.

Shinwari, Z. K. 2010. Medicinal plants research in Pakistan. *J. Med. Plant Res.,* 4: 161-176.

Shinwari, Z.K and S.S. Gilani. 2003. Swat profile, pp. 9-17. In: *Medicinal and other useful plants of District Swat Pakistan*. (Eds.): Z.K. Shinwari, A.A. Khan and T. Nakaike. April 2003. Al-Aziz Comm. Peshawar, Pakistan.

Shinwari, Z.K., S.S. Gilani, M. Kohjoma and T. Nakaike. 2000. Status of medicinal plants in Pakistani Hindukush Himalayas. Proc. Nepal – Japan Joint Symposium, pp. 235-242.

Shinwari, Z.K., S.S. Gilani and M. Shoukat. 2002. Ethnobotanical resources and implications for curriculum, pp. 21-33, In: *Proc. Workshop Curr. Develop. Appl. Ethnobot*., (Eds.): Z.K. Shinwari, A. Hamilton and A.A. Khan. WWF Peshawar, Pakistan.

Shinwari, Z. K., I. Khan, S. Naz and A. Hussain. 2009. Screening of medicinal plants of Pakistan for their antibacterial activity. *Afr. J. Biotechnol*., 8: 7082-7086.

Stewart, R.R. 1967. Checklist of the plants of South North West Pakistan. *Pak. J. For.*, 45: 457-528.

Wang, P., Y. Wang and C. H. Kong. 2008. Allelopathy of plant volatile monoterpenes mediated by soil: a case study of *Ambrosia trifida* L. *Shengtai Xuebao*. 28: 62-68.

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