International Journal of Basic and Applied Sciences, 5 (1) (2016) 75-81

International Journal of Basic and Applied Sciences

Website: www.sciencepubco.com/index.php/IJBAS
doi: 10.14419/ijbas.v5i1.5631
Research paper

Evaluation of allelopathic activity of 178 Caucasian plant species

Hossein Mardani [1]*, Elena Kazantseva [2], Vladimir Onipchenko [2], Yoshiharu Fujii [3]

[1] *Department of Biological Production Science, United Graduate school of Agricultural science*
[2] *Department of Geobotany, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia*
[3] *Department of International Environmental And Agricultural Science, Tokyo University of Agriculture and Technology, Japan*
**Corresponding author E-mail: hmardani26@yahoo.com*

Abstract

Seeking for new plant species as the main resources of bioactive chemicals is one of the fundamental steps in biological production science. The main objective of this paper was to screen for the allelopathic activity of Caucasian plant species in order to select the strongest allelopathic species for future studies. Dried leaves of 178 plant species collected from the Teberda State Reserve in the Caucasus region were assayed by the Sandwich method for allelopathic activity, using *Lactuca sativa* (lettuce) as the test plant. To evaluate allelopathic activity, standard deviation (SD) and SD of variance (SDV) of radicle growth inhibition were calculated. The highest (100%) inhibition was observed for *Artemisia austriaca* Jacquin, followed by *Oxalis acetosella* L., *Convallaria majalis* L. and *Polygonatum odoratum* (Miller). Among plant families, members of the Fabaceae caused greatest inhibition of radicle growth. Plants classified as "poisonous" had the highest allelopathic activities, followed closely by those designated "medicinal". Results of this study will guide the identification of novel phytotoxic chemicals useful in medicinal and/or industrial applications.

Keywords: *Allelopathy; Caucasian Plant; Inhibitory Activity; Sandwich Method.*

1. Introduction

Interactions between plants-plants, and between plants and other organisms, have long been of fundamental interest to plant scientists. Among these interactions a particular one, allelopathy, has focused attention on release of plant-produced toxins from aerial parts into the phyllosphere or from underground parts into the rhizosphere. These toxicants are released through by exudation from roots, by leaching of shoots, or by volatilization of decaying plant tissue [1]. The global demand for organic products has boomed during the last decades. The use of allelopathic compounds as bio-herbicides or bio pesticides in agricultural systems has several benefits in contrasted with common synthetic products. Because of the natural origin of allelochemicals, researchers have suggested that most will be biodegradable and less harmful than traditional pesticides [2]. Many plant species are not dominant competitors in their natural systems, yet compete aggressively when introduced to new territories [3, implying that applied allelopathic research has potential for weed control. As bioherbicides, allelopathic plants might be used in cultural practices as cover crops, or as green manure in cropping patterns, or as sources of new natural products with herbicidal activities [4] [5]. Therefore, it is important to identify new species with allelopathic activity in order to facilitate plant protection strategies.

The Teberda State Nature Reserve in the Caucasus region was chosen as the source of plants for this survey because it occupies a relatively small geographic area, yet has an unusually rich diversity of flora concentrated within it. More importantly, this area is located in one of the world Origin of Cultivated Plants called "Asian Minor Center or Persian center" [6] providing a perfect diversity of plants. A recently revised checklist included 1,133 vascular plant species confined to an area of 86,000 ha [7]. However, there is no documented study of Caucasian plants for allelopathic capacities. The objective of the present study was to survey the allelopathic activity of some Caucasian plants.

The Caucasus region covers 500,000 km^2 in Armenia, Azerbaydzhan and Georgia, the North Caucasian portion of the Russian Federation, NE Turkey, and a small part of NW Iran [8]. The Teberda State Nature Reserve is located in the northwestern part of the northern slope of the Greater Caucasus mountain range. The great diversity of plant life within the reserve is due to the highly diverse climates found there, which result from the complex mountain topography and large changes in elevation: the lower parts of the Reserve are at 1,300 m, while the highest point (Mt. Dombai-Ulgen) is 4,046 m above sea level. Variously oriented steep slopes exacerbate differences in vegetation between, and even within, altitudinal zones. Repeated Quaternary glaciations also contributed to plant species heterogeneity concentrated in the relatively small area represented by the Reserve [9].

2. Materials and methods

We have used a new procedure, called the "Sandwich Method" [10], [11], [12], [13], to screen plant allelopathic activity. This bioassay was previously developed by Fujii et al [10] to determine allelopathic activity of plant leaf leachates. A variation of the method using an agar growth medium was employed to screen large numbers of tree species, as well as herbaceous plants of medicinal or herbal value [11], [12], and [14].

2.1. Plant samples and preparation

Green leaves of 178 Caucasian plant species were collected fresh from the Teberda State Natural Biosphe Reserve. All the plant samples were identified by the plant science experts in Teberda State Natural Biosphe Reserve. To confirm Latin binomials, plant identities were also checked against the Reserve's data base [7] and were confirmed by The International Plant Names Index (IPNI) website. Samples were placed individually in a drying machine (Ezidri Snackmaker FD500) at 60 °C for approximately 4 hours.

2.2. Sandwich method (SW)

According to the previous study, agar medium (containing 0 carbohydrates) is best for lettuce seedling growth in this assay. We used powdered agar (Nacalai Tesque Inc., gelling temperature 30-31 ºC) (0.75% w/v) to prepare the medium, which was sterilized by autoclaving at 121 °C for 15 min. Desiccated leaf samples (3 replicates/sample) from each species were placed in 3 wells of a six-well (area of each well ~10 cm^2) microplate (12.7 cm X 8.45 cm, Thermo Fisher Scientific Inc.). Amounts (10 mg or 50 mg suspended in 5 ml of 0.7 w/v agar medium) of desiccated leaf of each sample to use in the assay were calculated based on conditions of fallen leaves in nature [11](Fujii et al. 2003). Three repli cates were used for each 10 mg or 50 mg suspension. Each 5 ml suspension was placed in a well of a 6-well multi dish plate, followed by an overlay of molten agar (5 ml/well). Seeds of lettuce (Lactuca sativa L., Great Lakes No. 366, Takii Co.) were placed on the surface of the top layer, which provided a physical barrier between the sample and test seeds. Lettuce was chosen as a test plant because it is highly sensitive to inhibition by allelochemicals (Fig.1) [10].

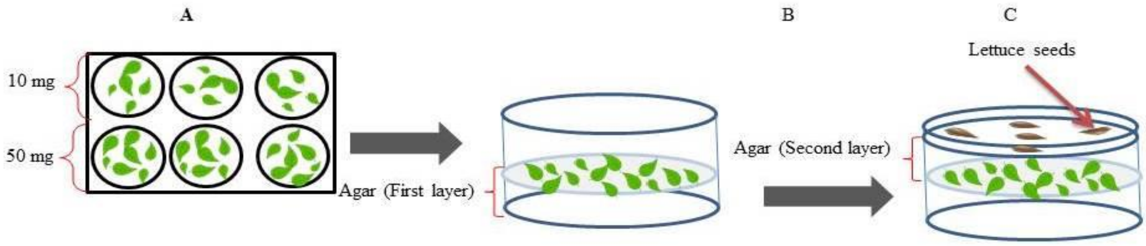

Fig. 1: Sandwich Method: (A) 10 or 50 Mg Dried Leaves Placed in Each Well of A Six-Well Multidish Plastic Plates; (B) Addition of 5 Ml Plus 5 Ml Agar in Two Layers on the Dried Leaves; (C) Five Seeds (*Lactuca Sativa* Var. Great Lakes 366) Lettuce Seeds Vertically Placed,) Covered with Plastic Tape and Labeled Multidish for Incubation in Dark Conditions.

2.3. Data collection

Each multi dish plate was sealed with plastic tape, labeled, and incubated in the dark at 25ºC for 3 days. Lengths of hypocotyls and radicles were measured and percent inhibitions (compared to controls) were calculated. Means and standard deviations (SDs) were evaluated by SD variation (SDV). All data, from both 10 mg and 50 mg samples of all 187 species tested, conformed to a normal distribution.

$$\text{Elongation \%} = \frac{\text{(Average length of treatment radicle/hypocotyl)}}{\text{(Average length of control radicle/hypocotyl)}} \times 100 \quad (1)$$

3. Results and discussion

Table 1 indicates lettuce seedling radicle and hypocotyl growth (1) after exposure of germinating seeds to plant leaf samples. Some plant samples were inhibitory, others were stimulatory, and some had no effect. Among all screened plants, 32 samples showed 50% inhibitory activity lettuce seedling growth. In the present study, the radicle elongations percentages of lettuce seedlings were in the range 0-111% and 0-91% in comparison to the control when respectively treated with 10 mg and 50 mg dried leaves. The most abundant species included in this study belonged to the families Asteraceae (23 species), Fabaceae (19 species), Poaceae (14 species), Lamiaceae and Apiaceae (9 species). Moreover, 19% of plants samples (10mg) showed 50% inhibition on root elongation of lettuce seedling.

Our study showed *Artemisia austriaca* Jacquin to have the greatest inhibitory activity (10 mg caused 100% inhibition of both radicals and hypocotyls), followed by *Oxalis acetosella* L., *Convallaria majalis* L. and *Polygonatum odoratum* (Miller) (Table 1). Nonetheless, these plants contain some chemical compounds that are likely to be phytotoxins and the inhibition activities observed in these species may be due to these compounds. Inhibition of seed germination by *A. austriaca* was even greater in our hands .This study than has been reported previously by others [15, 16]. Furthermore, *A. austriaca* can be toxic to animals [17]). GuÈ venalp et al. [18] showed that the main chemical compounds found in *A. austriaca* essential oil are: camphor (45.5%), 1, 8-cineole (30.4%), camphene (6.5%), α-terpineol (3.2%), α -pinene (3.0%) and terpinen-4-ol (2.9%) respectively. Some of these materials might be of future use as bioherbicides; for example, it is known that 1, 8-cineole (Eucalyptol) is a fungal growth inhibitor [19]. Therefore, the inhibitory effect of *A. austriaca* could result from toxicity of 1, 8-cineole. The third most allelopathic plant found in our study *Convallaria majalis* L. (Lily-of-the-Valley), reflects its toxic properties i.e., it is known to be both poisonous and medicinal, and is considered an invasive alien species [17, 20, 21]. Our designation of plant species as poisonous or medicinal (Table 1) was based on previous classifications [17 and 21]. Since medicinal and/or poisonous plants have high inhibitory activity on hypocotyls and radicle, we assessed plants in these two categories for presence of allelochemicals. Almost 10% of screened medicinal or poisonous species caused more than 50% elongation decline on lettuce radicle while most of the other plants were shown less activity (Fig 2). Perusal of the Table 1 data showed that 50 % of the plants used in our survey have medicinal properties while 23 % of them are poisonous. Each of the top eleven plants with strong allelopathic activity have been reported as medicinal [17], and eight of these are poisonous. We also observed that medicinal plants and poisonous plant expressed almost the same inhibitory activity, regardless to the number of screened species. However, poisonous species showed slightly greater inhibitory activity than medicinal ones (Table 2).

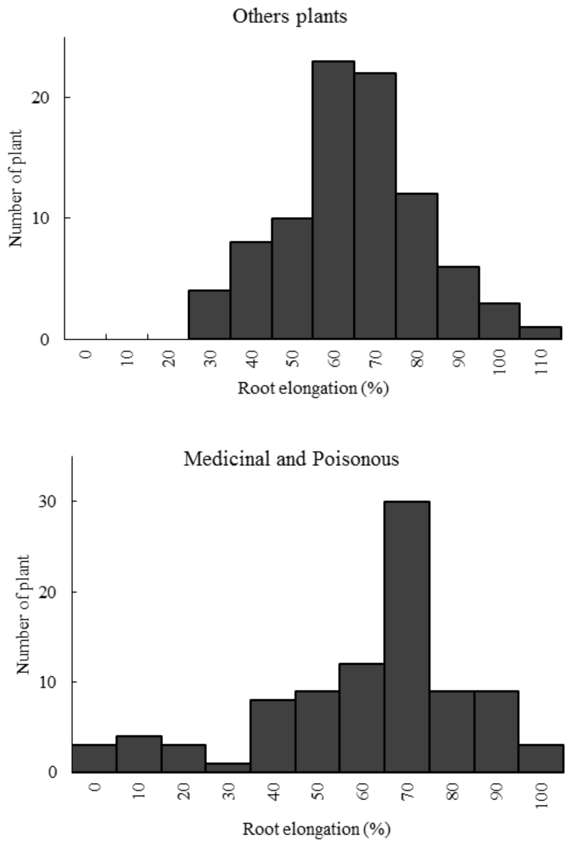

Fig. 2: Normal Distribution of Screened Plants with Medicinal or Poisonous Properties in Comparison with other Screened Plant Using Sandwich Method.

Table 1: Effects of Leaf Litter of 178 Caucasian Plants on Lettuce (*L. Sativa*) Radicle and Hypocotyl Lengths (%).

Family	Scientific Name	Poisonous	Medicinal	10mg R %	H %	50 mg R %	H %	Criterion $
Asteraceae	*Artemisia austriaca* Jacquin	P	M	0	0	0	0	****
Oxalidaceae	*Oxalis acetosella* L.	-	M	4	0	3	7	****
Liliaceae	*Convallaria majalis* L.	P	M	9	35	8	21	****
Liliaceae	*Polygonatum odoratum* (Miller) Druce	P	M	10	44	8	37	****
Fabaceae	*Melilotus albus* Medikus	P	M	13	42	1	1	****
Fabaceae	*Melilotus officinalis* L.	P	M	15	42	7	21	****
Poaceae	*Anthoxanthum odoratum* L.	-	M	16	3	2	0	****
Trilliaceae	*Paris incompleta* Bieb.	P	M	23	81	11	57	****
Fabaceae	*Vicia cracca* S. F. Gray	-	M	25	68	17	69	****
Melanthiaceae	*Veratrum album* L.	P	M	29	72	12	49	***
Papaveraceae	*Chelidonium majus* L.	P	M	33	48	25	40	***
Aceraceae	*Acer trautvetteri* Medw.	-	-	36	133	17	69	***
Fabaceae	*Vicia truncatula* Fischer ex Bieb.	-	-	36	96	22	104	***
Fabaceae	*Hedysarum caucasicum* Bieb.	-	-	37	94	14	70	**
Asteraceae	*Taraxacum stevenii* DC.	-	-	39	118	29	98	**
Brassicaceae	*Cardamine acris* Griseb.	-	-	40	120	16	42	**
Liliaceae	*Polygonatum orientale* Desf.	-	-	40	75	16	37	**
Aceraceae	*Acer negundo* L.	-	-	41	90	9	35	**
Rubiaceae	*Galium odoratum* (L.) Scop.	-	M	41	94	13	30	**
Ranunculaceae	*Aconitum orientale* Miller	P	M	42	75	21	76	**
Campanulaceae	*Campanulla collina* Bieb.	-	-	42	90	18	50	**
Cyperaceae	*Carex sempervirens* Vill.	-	-	42	105	85	133	**
Ericaceae	*Rhododendron caucasicum* Pallas	-	M	43	89	10	58	**
Sambucaceae	*Sambucus ebulus* L.	P	M	45	75	24	80	**
Poaceae	*Hyalopoa pontica* (Bal.) Tzvel.	-	-	45	110	25	91	**
Lamiaceae	*Clinopodium vulgare* L.	-	M	46	88	14	69	**
Fabaceae	*Vicia nissoliana* L.	-	-	48	101	22	79	*
Lamiaceae	*Stachys officinalis* (L.) Trev.	P	M	48	116	30	80	*
Geraniaceae	*Geranium robertianum* L.	P	M	48	72	8	41	*
Caryophyllaceae	*Silene vulgaris* (Moench) Garcke	-	-	49	130	26	106	*
Onagraceae	*Chamaenerion angustifolium* (L.) Scop.	-	M	49	102	17	75	*
Apiaceae	*Bupleurum polyphyllum* Ledeb.	-	-	50	112	18	65	*
Fabaceae	*Trifolium polyphyllum* C.A. Mey.	-		50	76	15	42	*

Family	Scientific Name	Poisonous	Medicinal	10mg R %	H %	50 mg R %	H %	Criterion $
Fabaceae	*Vicia abbreviata* Fisch. ex Spreng.	-	M	50	123	23	122	*
Pinaceae	*Picea orientalis* (L.) Link	-	M	51	77	13	39	*
Fabaceae	*Vicia sepium* L.	-	M	52	92	44	85	*
Asteraceae	*Centaurea cheiranthifola* Willd	-	-	52	101	21	66	*
Liliaceae	*Lilium kesselringianum* Miscz	-	-	53	103	27	100	*
Pinaceae	*Abies nordmaniana* (Stev.) Spach	-	M	53	105	9	46	*
Apiaceae	*Carum caucasicum* (Bieb.) Boiss.	-	-	53	104	30	91	*
Fabaceae	*Vicia tenuifolia* Roth subsp. subalpina (Grossh.) A. Zernov	-	-	53	132	26	80	*
Asteraceae	*Anthemis cretica* L.	-	-	54	101	26	58	*
Lamiaceae	*Leonurus quinquelobatus* Gilib.	-	M	55	78	32	54	*
Solanaceae	*Datura stramonium* L.	P	M	56	128	26	84	*
Fabaceae	*Anthyllus vulneraria* L.	-	M	57	112	25	66	*
Fabaceae	*Lupinus polyphyllus* L.	-	-	57	94	20	71	*
Polygonaceae	*Polygonum bistorta* L.	-	M	59	95	32	84	
Asteraceae	*Senecio taraxcifolius* (Bieb.) DC.	-	-	59	92	30	76	
Thymelaeceae	*Daphne glomerata* Lam.	P	M	59	79	45	59	
Fabaceae	*Oxytropis kubanensis* Leskov	-	-	59	127	27	91	
Rosaceae	*Sibbaldia procumbens* L.	-	-	60	80	34	70	
Caryophyllaceae	*Minuartia aizoides* (Boiss.) Bornm.	-	-	60	114	34	109	
Asteraceae	*Kemulariella caucasica* (Willd.) Tamamsch.	-	-	60	105	22	66	
Geraniaceae	*Geranium gymnocaulon* DC.	-	-	60	104	24	60	
Hypericaceae	*Hypericum perforatum* L.	-	M	60	100	37	104	
Poaceae	*Catabrosella variegata* (Boiss.) Tzvel.	-	-	61	93	25	61	
Poaceae	*Nardus stricta* L.	-	M	62	98	50	110	
Lamiaceae	*Salvia glutinosa* L.	-	M	62	68	44	61	
Fabaceae	*Astragalus glycyphyllus* L.	-	M	63	76	39	47	
Asteraceae	*Taraxacum confusum* Schischk.	-	-	63	96	29	88	
Brassicaceae	*Draba hispida* Willd.	-	-	64	104	33	106	
Campanulaceae	*Campanula tridentata* Schreb.	-	-	64	132	44	123	
Scrophulariaceae	*Melampyrum arvense* L.	P	M	64	110	33	103	
Ranunculaceae	*Anemona speciosa* Adams ex G. Pritz.	-	-	64	96	40	80	
Primulaceae	*Androsace albana* Stev.	-	-	64	133	23	77	
Salicaceae	*Populus tremula* L.	-	M	65	74	43	69	
Ranunculaceae	*Aquilegia olimpica* Boiss.	P	-	66	95	28	69	
Scrophulariaceae	*Veronica gentianoides* Vahl.	-	-	66	107	41	84	
Poaceae	*Deschampsia flexuosa* (L.) Trin.	-	-	66	101	41	91	
Asteraceae	*Inula orintalis* Lam.	-	-	66	120	51	126	
Primulaceae	*Lysimachia verticillaris* Spreng.	-	-	67	83	52	92	
Fabaceae	*Coronilla varia* L.	-	-	67	119	17	87	
Poaceae	*Bromus variegatus* Bieb.	-	-	67	144	54	114	
Apiaceae	*Osmorhiza aristata* (Thunb.) Rydb.	P	M	67	135	54	135	
Rosaceae	*Filipendula vulgaris* Moench	-	M	67	126	38	127	
Orchidaceae	*Traunsteinera globosa* L.	-	-	68	112	40	85	
Asteraceae	*Solidago virgaurea* L.	P	M	68	103	18	48	
Primulaceae	*Primula ruprechtii* Kusu.	-	-	68	80	35	57	
Caryophyllaceae	*Minuartia recurva* (All.) Schinz et Thellung	-	-	68	82	41	67	
Cyperaceae	*Carex atrata* L.	-	-	68	116	43	103	
Polygonaceae	*Rumex alpestris* Jacq.	-	-	68	107	44	93	
Ericaceae	*Rhododendron luteum* Sweet	-	M	68	84	46	79	
Scrophulariaceae	*Pedicularis condensata* Bieb.	-	-	69	93	34	78	
Asteraceae	*Pyrethrum coccineum* (Willd.) Worosch.	P	M	69	120	37	88	
Poaceae	*Calamagrostis arundinacea* (L.) Roth	-	-	69	144	38	89	
Thymelaeceae	*Daphne mezereum* L.	P	M	70	104	33	51	
Caryophyllaceae	*Saponaria officinalis* L.	P	M	70	103	49	81	
Dipsacaceae	*Cephalaria gigantea* (Ledeb.) Bobr.	-	M	70	104	43	91	
Asteraceae	*Anthemis marshalliana* Willd.	-	-	71	94	30	80	
Asteraceae	*Antennaria dioica* (L.) Gaertn.	-	M	71	118	55	119	
Poaceae	*Festuca brunnescens* (Tzvel.) Galushko	-	-	71	124	32	116	
Fabaceae	*Lotus corniculatus* L.	-	M	71	126	30	105	
Plantaginaceae	*Plantago atrata* Hoppe	-	-	71	110	57	105	
Botrychiaceae	*Botrychium lunaria* (L.) Sw.	-	M	71	109	29	80	
Asteraceae	*Achillea nobilis* L.	-	M	72	127	45	111	
Lamiaceae	*Thymus nummularis* Bieb.	P	M	72	114	56	100	
Rosaceae	*Alchemilla vulgaris* L. aggr.	-	M	72	124	23	81	
Geraniaceae	*Geranium renardii* Trautv.	-	-	72	137	31	117	
Apiaceae	*Seseli libanotis* (L.) W.D.J.Koch	P	M	73	110	33	85	
Ranunculaceae	*Pulsatilla aurea* (Somm. et Levier) Juz.	-	-	73	111	45	87	
Athyriaceae	*Athyrium filix-femina* (L.) Roth	P	M	73	135	32	109	
Ranunculaceae	*Actaea spicata* L.	P	M	73	110	28	77	
Sambucaceae	*Sambucus nigra* L.	P	M	73	110	28	80	
Polypodiaceae	*Polypodium vulgare* L. aggr.	P	M	73	104	48	80	
Berberidaceae	*Berberis vulgaris* L.	-	M	73	118	32	81	

Family	Scientific Name	Poisonous	Medicinal	10mg R %	50 mg H %	R %	H %	Criterion $
Rubiaceae	*Galium verum* L.	P	M	74	126	62	108	
{lichen}	*Pseudevernia* sp.	-	-	74	79	54	67	
Boraginaceae	*Echium vulgare* L.	-	M	74	149	36	102	
Boraginaceae	*Eritrichium caucasicum* (Albov) Grossh.	-	-	75	122	61	120	
Poaceae	*Festuca ovina* L.	-	M	75	112	37	93	
Orchidaceae	*Platanthera chlorantha* (Cust.) Reichub	-	M	75	87	71	89	
Asteraceae	*Senecio caucasicus* (Bieb.) DC.	-	-	76	125	40	105	
Asteraceae	*Aetheopappus caucasicus* Sosn.	-	-	76	119	35	87	
Ranunculaceae	*Anemona fasciculata* L.	P	-	76	93	42	83	
Cupressaceae	*Juniperus communis* L.	-	M	77	95	25	48	
Poaceae	*Agrostis vinealis* Schreb.	-	-	77	117	37	87	
Hypericaceae	*Hypericum linarioides* Bosse	-	-	77	127	45	121	
Asteraceae	*Erigeron caucasicum* Stev.	-	-	77	181	27	109	
Asteraceae	*Lapsana grandiflora* Bieb.	-	-	77	149	42	131	
Lamiaceae	*Mentha longifolia* (L.) Hudson	P	M	77	109	44	86	
Gentianaceae	*Gentiana pyrenaica* L.	-	-	77	135	51	102	
Cyperaceae	*Carex oreophila* C.A. Mey.	-	-	77	91	34	84	
Rosaceae	*Potentilla verna* L.	-	-	78	145	76	131	
Asteraceae	*Ambrosia artemisiifolia* L.	P	M	78	126	59	174	
Scrophulariaceae	*Digitalis ciliata* Trautv.	P	M	78	121	43	74	
Polygonaceae	*Polygonum panjiutinii* Charkev.	-	-	78	116	25	75	
Rosaceae	*Agrimonia eupatoria* L.	-	M	78	128	78	128	
Ranunculaceae	*Aconitum nasutum* Fuscher ex Reichenb.	P	M	78	126	20	80	
Poaceae	*Phleum alpinum* L.	-	M	78	143	29	77	
Valerianaceae	*Valeriana alpestris* Stev.	-	-	79	106	47	78	
Betulaceae	*Corylus colurna* L.	-	-	79	97	35	91	
Plantaginaceae	*Plantago major* L.	-	M	79	108	62	103	
Valerianaceae	*Valeriana alliariifolia* Adams	-	-	79	95	35	89	
Asteraceae	*Gnaphalium supinum* L.	-	-	79	94	46	88	
Polygonaceae	*Polygonum bistorta* L.	-	M	79	129	36	99	
Asteraceae	*Artemisia absinthium* L.	-	M	79	123	66	131	
Poaceae	*Helictotrichon versicolor* (Vill.) Pilger	-	-	79	132	49	114	
Geraniaceae	*Geranium sanguineum* L.	-	M	80	112	18	54	
Pinaceae	*Pinus sylvestris* L	p	M	80	92	77	77	
Asteraceae	*Tanacetum vulgare* L.	P	M	80	132	55	108	
Apiaceae	*Tussilago farfara* L.	-	-	81	104	70	113	
Fabaceae	*Trifolium badium* Schreb.	-	-	81	133	51	127	
Apiaceae	*Conium maculatum* L.	P	M	81	128	54	123	
Boraginaceae	*Myosotis alpestris* F.W. Schmidt	-	-	82	164	46	132	
Cyperaceae	*Carex umbrosa* Host	-	-	82	127	28	81	
Crassulaceae	*Sedum tenellum* Bieb.	-	-	82	103	67	97	
Scrophulariaceae	*Verbascum densiflorum* Bertol.	P	M	83	112	43	103	
Lamiaceae	*Origanum vulgare* L.	-	M	83	106	76	122	
Asteraceae	*Anthemis macroglossa* Somm. et Levier	-	-	84	115	49	97	
Ranunculaceae	*Ranunculus oreophilus* Bieb.	-	-	84	116	47	97	
Fabaceae	*Trifolium trichocephalum* Beib.	-	M	85	133	45	110	
Brassicaceae	*Murbeckiella huetii* (Boiss.) Rothm.	-	-	85	110	59	104	
Caryophyllaceae	*Arenaria lychnidea* Bieb.	-	-	87	100	65	91	
Poaceae	*Festuca varia* Haenke	-	-	87	160	63	131	
Boraginaceae	*Symphytum asperum* Lepech.	-	M	89	146	58	139	
Fagaceae	*Fagus orientalis* Lipsky	-	M	89	138	47	107	
Poaceae	*Calamagrostis arundinacea* (L.) Roth	-	-	89	145	45	107	
Lamiaceae	*Betonica macrantha* C. Koch.	-	-	89	137	63	118	
Taxaceae	*Taxus baccata* L.	P	M	90	98	66	133	
Betulaceae	*Corylus avellana* L.	P	M	90	128	56	104	
Valerianaceae	*Valeriana officinalis* L.s.1.	-	M	91	180	40	132	
{lichen}	*Lobaria pulmonaria* (L.) Hoffm.	-	-	92	105	71	72	
Rosaceae	*Alchemilla caucasica* Buser	-	-	92	132	55	133	
Apiaceae	*Carum meifolium* (Bieb.) Boiss.	-	-	93	127	55	88	
Campanulaceae	*Asyneuma campanuloides* (Bieb. ex Sims) Borum	-	-	93	144	33	108	
Fabaceae	*Galega orientalis* Lam.	P	M	93	144	48	107	
Cucurbitaceae	*Echinocystis lobata* (Michk.) Torrey et Gray	-	M	94	180	27	139	
Celastraceae	*Euonymus latifolia* (L.) Miller	-	-	95	136	70	112	
Ericaceae	*Vaccinium vitis-idaea* L.	-	M	95	110	53	79	
Betulaceae	*Betula raddena* Trautv.	-	-	96	133	67	140	
Betulaceae	*Carpinus betulus* L. s.l.	-	M	96	145	31	89	
Lamiaceae	*Thymus marschallianus* Willd.	-	M	97	131	79	107	
Rosaceae	*Potentilla gelida* C.A. Mey.	-	M	98	139	79	142	
Polygonaceae	*Polygonum hydropiper* L.	-	M	100	142	91	138	
Cyperaceae	*Carex pyrenaica* Wahlenb.	-	-	102	161	55	114	
Scrophulariaceae	*Pedicularis nordmanniana* Bunge	-	-	104	143	62	106	
Apiaceae	*Leontodon hispidus* L.	-	-	105	157	71	138	
Onocleaceae	*Matteuccia struthiopteris* (L.) Todaro	P	M	106	138	50	130	
Apiaceae	*Sanicula europaea* L.	P	M	106	125	31	54	
Asteraceae	*Matricaria caucasica* (Willd.) Poir.	-	-	111	144	46	95	

$ Indicates stronger inhibitory activity in the radicle by deviation value: *m–0.5 (sd), **m–1 (sd), ***m–1.5 (sd), ****m–2 (sd) m: mean of radicle length, sd: standard deviation of radicle. R: radicle, H: hypocotyl, %: percentage of control growth. M: Medicinal plant, P: poisonous plant.

Table 2: Inhibition of Lettuce (L. Sativa) Radicle Growth by Leaf Leached from Medicinal and Poisonous Plant Families.

Type		Average Family (%)	
	n	10 mg R	50 mg R
Medicinal and Poisonous	131	67± 26	38± 19
Medicinal	89	65± 23	37± 20
Poisonous	42	61± 17	34± 16
Others	47	70± 20	40± 18
All	178	67± 20	40± 18

±: indicates the standard deviation and n: shows number of plants in each category, R: radicle, % = percentage of control growth.

The prominence of medicinal plants in the field of allelopathy has been noted [11], [22], [23] our survey is the first to report allelopathic activity for several species, including *Hedysarum caucasicum* Bieb. *Polygonatum odoratum* Pallas and *Paris incompleta* Bieb. However, previous studies have made a variety of observations relevant to our discoveries in case of other species:
Shinwari et al. [24] recently reported allelopathic activity of another Fabaceae family member, *Melilotus albus* Medikus, which is also known for its medicinal properties and poisonous effects. Lan et al. [25] reported allelopathic activities of Polygonatum species, which have been used as folk medicines in the Caucuses region [17]. Putnam et al. [26] reported sorghum (Sorghum bicolor L.), a species in the Poaceae family, has good weed killing potential. Moreover, *Paris incompleta* is known as rare plants [27] in a number of areas, including the Caucasus region. *P. incompleta* is known as a medicinal plant in its center of evolutionary origin [17] and as a poisonous plant when used in high amounts. This effect of P. incomplete could be due to high concentration of secondary metabolites in leaves. Over all, most of the plants screened in this study showed either strong or weak inhibitory activity. However, we also observed that some plants, such as *Carex pyrenaica* (Cyperaceae) and *Leontodon hispidus* (Apiaceae), can stimulate lettuce seedling growth. Since there are no prior reports of growth stimulation by extracts of these plants, further studies are necessary.

4. Conclusion

This is the first comprehensive report on screening a large number of Caucasian plant species for allelopathic activity. Several species were found to have high toxicity toward the receptor plant, lettuce. Future research will be applied to identify the allelochemicals responsible for the allelopathic activities of these plants, and to understand the biological roles of these compounds in natural ecosystems. Such information could provide further insight for researchers on development of new bioactive chemicals from natural products. Knowledge of allelopathic chemicals and their biological functions will be important for biological control of species that can negatively impact agriculture and forestry projects

Acknowledgements

This research was supported by the grant-inaid for research on Agriculture and Food Science (25029AB) from the Ministry of Agriculture, Forestry and Fisheries of Japan. This work was also supported by Japan Society for Promotion of Science (JSPS) KAKENHI Grant Number 26304024.We acknowledge the organization Field-Oriented Leaders in Environmental Sectors in Asia and Africa (FOLENS) in Tokyo University of Agriculture and Technology for providing the core funding for this research. We are much indebted to Hirokazu Ozaki for his great help and support during this project. Special thanks to Prof. Olen Yoder for his kind supports during the writing procedure of this paper. We are much indebted to Aliy Kipkeev, Fatima Salpagarova for their kind help during plant collection.

5. Conflict of interest

The authors declare that there is no conflict of interest associated with this publication.

References

[1] Rice EL (1984) Allelopathy, 2th end, *Academic Press*, New York.

[2] Daizy, R., Batish, D.R., Singh, H., Kohli, R.K., Saxena, D.B., Dakora, F.D. (1995) Plant flavonoids: biological molecules for useful explotation, *Australian Journal of Plant Physiology*, 22:87–99. http://dx.doi.org/10.1071/PP9950087.

[3] Van Driesche, R.G., Bellows, J. (1996) Pest Origins, Pesticides and the History of Biological Control. In: *Biological Control*,Chapman and Hall, New York, USA, pp 1–20. http://dx.doi.org/10.1007/978-1-4613-1157-7_1.

[4] Singh, H.P., Batish, D.R., Kohli, R.K. (2003) Allelopathic Interactions and Allelochemicals: New Possibilities for Sustainable Weed Management, *Critical Reviews in Plant Sciences*, 22:239–311. DOI: 10.1080/713610858H

[5] Shennan C (2008) biotic interactions, ecological knowledge and agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363:717–739. http://dx.doi.org/10.1098/rstb.2007.2180.

[6] Vavilov, N. I. (1926) Studies on the Origin of Cultivated Plants. *Bulletin. of Applied Botany*, 2-16.

[7] Onipchenko,V.G., Zernov, A.S., Vorob'eva, F.M. (2011) Vascular plants of Teberda Reserve (annotated list of species), *Flora and fauna of natural reserves*, 2nd edn. 99A, MAKS Press, Moscow.

[8] Zazanashvili, N., Gagnidze, R., Nakhutsrishvili. G. (2010) Main types of vegetation zonation on the mountains of the Caucasus, *Institute of Botany of the Georgian Academy of Sciences* 85:7–16.

[9] Onipchenko, G.V. (2004) Alpine ecosystems in the northwest of Caucuses, In: *Alpine plant communities syntaxonomy, floristic composition and richness*, Kluwer academic publisher, Dordrecht, Netherland, pp 55–75.

[10] Fujii, Y., Matsuyama, M., Hiradate, S., Nakatani, K. (2000) Developments of new bioassay and analysis method for volatile allelochemicals, *Journal of Weed Science and Technology* , 45:80–81. http://dx.doi.org/10.3719/weed.45.Supplement_80.

[11] Fujii, Y., Parvez, S.S., Parvez, M.M., Ohmae, Y., Iida, O. (2003) Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. *Weed Biology and Management*, 3: 233–241. http://dx.doi.org/10.1046/j.1444-6162.2003.00111.x.

[12] Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S., Parvez, M.M. (2004) Assessment method for allelopathic affect from leaf litter leachates. *Weed Biology and Management*, 4:19–23. DOI: 10.1111/j.1445 6664.2003.00113.x

[13] Jung, K., Fujii, Y., Yoshizakiand, S., Kobori, H. (2010) Evaluation of total allelopathic activity of heartseed walnut (*Juglans ailanthifolia* Carr.) and its potential to control black locust (*Robinia pseudoacacia* L.) *Allelopathy Journal*, 26:243–254.

[14] Morita, S., Ito, M., Harada, J. (2005) Screening of an allelopathic potential in arbor species. *Weed Biology and Management*, 5:26–30. http://dx.doi.org/10.1111/j.1445-6664.2005.00151.x.

[15] Samedani, B., Baghestani, M.A. (2005) Comparison of allelopathic activity of different Artemisia species on seed germination rate and seedling growth of *Avena ludoviciana, Journal of Pajouhesh & Sazandegi*, 68:69–74.

[16] Zhang, R.M., Zuo, Z.J., Gao, P.J., Houa, P., Wen, G.S., Gaoa,Y. (2012) Allelopathic effects of VOCs of *Artemisia frigida* Willd. on the regeneration of pasture grasses in Inner Mongolia *Journal of Arid Environments*, 87: 212–218. http://dx.doi.org/10.1016/j.jaridenv.2012.04.008.

[17] Budantsev, A.L., Lesiovskaya, E.E. (eds) (2001) Useful Wild Plants of Russia. Saint Petersburg Chemical-Pharmaceutical State Academy, Russia, pp. 662 (in Russian).

[18] GuÈ venalp, Z.È., CË akir, A., Harmandar, M., Gleispach, H. (1998) The Essential Oils of *Artemisia austriaca* Jacq. And *Artemisia spicigera* C. Koch. From Turkey. *Flavour and Fragrance Journal*, 13: 26–28. DOI: 10.1002/(SICI)1099-1026(199801/02)13:1<26:AID-FFJ686>3.0.CO;2-P

[19] Vilela, G.R., Almeida, G.S., D'Arce, M.A.B.R. et al. (2009) Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globules* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. *Journal of Stored Products Research*, 5: 108–111. http://dx.doi.org/10.1016/j.jspr.2008.10.006.

[20] Cymbaly, L. (2007) Invasive Alien Plant Species Found in the Carolinian Zone:Inventory and Management Options for rare Charitable Research Reserve. *Rare Charitable Research Reserve*, pp. 3–74.

[21] Zernov, A.S. (2010) Plants of the Russian Western Caucasus, *Field atlas*. KMK Scientific Press Ltd. Moscow (in Russian).

[22] Gakuya, D.W., Itonga, S.M., Mbaria, J.M., Muthee, J.K,, Musau, J.K. (2013) Ethnobotanical survey of biopesticides and other medicinal plants Traditionally used in Meru central district of Kenya. *Journal of Ethnopharmacol* 1455:47–553. http://dx.doi.org/10.1016/j.jep.2012.11.028.

[23] Latif Khan A, Hamayun M, Hussain J, Khan H, Gilani SA, Kikuchi A, Watanabe KN, Jung EH, Lee IJ (2009) Assessment of allelopathic potential of selected medicinal plants of Pakistan. *African Journal of Biotechnology*, 8:1024–1029.

[24] Shinwari, M.I., Shinwari, M.I., Fujii, Y (2013) Allelopathic evaluation of shared invasive plants and weeds of Pakistan and Japan for environmental risk assessment. *Pakistan Journal of Botechnology,* 45: 467–474.

[25] Lan G, Chen H, Chen S, Tian (2012) Chemical composition and physicochemical properties of dietary fiber from Polygonatum odoratum as affected by different processing methods. Food Res Int 49:406-410. http://dx.doi.org/10.1016/j.foodres.2012.07.047.

[26] Putnam, A.R., DeFrank, J., Barnes, J.P. (1983) Exploitation of allelopathy for weed control in annual and perennial cropping systems. *Journal of Chemical Ecology*. 9:1001–1010. http://dx.doi.org/10.1007/BF00982207.

[27] Terziglu S, Cofikunçeleb K, Zeki Bafikent E (2008) *Paris quadrifolia* L. (Liliaceae): A New Record for the Flora of Turkey. Turk J Bot 32:75–177.