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Evaluation of Allelopathic Activity of *Hibiscus sabdariffa* L.

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Abstract: The aqueous methanol extracts prepared from dried medicinal plants of *Hibiscus sabdariffa* L. inhibited both shoot and root growth of 12 test plant_@species, cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.), Italian ryegrass (*Lolium multiflorum* Lam.), ryegrass (*Lolium rigidum* Gaud.), crabgrass (*Digitaria sanguinalis* L.), buckwheat (*Eriogonum compositum* Douglas ex Benth.), Chinese sprangletop (*Leptochloa chinensis* [L.] Nees.), jungle rice (*Echinochloa colona* [L.] Link.), barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) and sand fescue (*Festuca myuros* L.) at concentrations greater than 0.03 g dry weight equivalent extract/mL. The inhibition increased with increasing extract concentration. The concentrations required for 50% growth inhibition of test plants were 0.0012-0.1351 g dry weight equivalent extract/mL. Barnyard grass, cress and alfalfa seedlings were the most sensitive to the extract. These results suggesting that *H. sabdariffa* may contain growth inhibitory substances and possess allelopathic activity. Therefore, *H. sabdariffa* may be the candidates for isolation and identification of allelochemicals. Further studies, however, should be conducted under greenhouse and field conditions to help evaluate the implications of these potential species.

Key words: Allelopathy · *Hibiscus sabdariffa* L. · Growth inhibitor · Aqueous methanol extract · Weed control

INTRODUCTION

Widespread use of synthetic herbicides has resulted in herbicide-resistant weeds and environmental concerns about the safety of synthetic herbicides, require alternative weed management systems which are less synthetic herbicide dependent or based on naturally occurring compounds [1, 2]. Allelopathy holds promise for the environmentally friendly weed management. Allelopathy is the ability of plant to inhibit the germination of other plants through the production of allelochemicals which may be present in any parts of the plants, i.e. leaves, roots, fruits, stems, rhizomes and seeds, from where they are released to the soil through volatilization, root exudation, leaching and decomposition of plant residues [3]. The importance of allelopathy in biological control of weeds and crop productivity has been highly recognized and various methods have been suggested to know the allelopathic effects [4-6]. The role of allelochemicals in agro-ecosystem has attracted the attention of many scientists. Numerous plants are reported to possess allelopathic potential and efforts have been made to apply them for weed control.

Allelopathic effect of medicinal species is of special interest in recent years [7, 8]. Fujii *et al*. [9-11] evaluated the allelopathic potentials of 239 medicinal species using the "Plant Box Method" and 223 species of them were found to suppress tested plant growth, whereas 17 species were enhancing lettuce radicle growth. They also surveyed allelopathic potential of 387 Japanese medicinal plants to find out possible candidates as natural herbicides 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*. [12] also surveyed allelopathic potential of 81 Japanese medicinal plants to find out possible candidates as natural herbicides. Nazir *et al*. [13] evaluated allelopathic potential

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of 3 herbal species (*Rheum emodi, Saussaurea lappa* and *Potentilla fulgens*) against some traditional crops. Germination of all crops was reduced significantly by aqueous extracts of *S. lappa* and *P. fulgens*. Khan *et al*. [14] found the allelopathic effects of 4 medicinal plants by using various methods. Aziz and Fujii [15] examined allelopathic activities of 14 medicinal plant species grown in plain areas of Pakistan with semi-arid conditions on growth of lettuce (*Lactuca sativa*).

Hibiscus sabdariffa L. (family Malvaceae), commonly known as roselle or red sorrel in English and as karkadeh in Arabic, is widely grown in tropics and subtropics of both hemispheres and has become naturalized in many areas of Central and West Africa, South East Asia, many areas of America and elsewhere [16]. *H. sabdariffa* is an annual, erect, bushy, herbaceous sub-shrub that grows to 8 ft (2.4 m) in height. The thick, red and fleshy, cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot drink (sour tea). *H. sabdariffa* extracts are also used in folk medicine which include remedies for high blood pressure, liver diseases and fever [17, 18]. It is also used against inflammation [19] and mutagenicity [20]. The red anthocyanin pigments present in their calyces are used as food coloring agents [21]. Chemical composition of *H. sabdariffa* has been widely reported for long time such as quercetin, luteolin, chlorogenic acid, protocatechic acid, palargonic acid, β-sitosterol and ergosterol, Hydroxycitric acid, delphinidin-3-sambubioside, cyaniding-3-sambubioside have been previously detected as main components in the aqueous extracts of *H. sabdariffa* [22-26]. The present study is to examine the allelopathic effects of aqueous extract of *H. sabdariffa* on seedling growth of 12 tested plant species for the purpose of alternative weed management systems.

MATERIALS AND METHODS

Plant Materials: Whole plants (leaves, stem and roots) of *Hibiscus sabdariffa* L. were collected from Chiang Mai province, Thailand in August 2010. The plants were washed several times by tap water, dried under the sunlight until the materials dried and then ground into powder. Cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.), were chosen as test plants for bioassay because of their known seedling growth behavior. Italian ryegrass (*Lolium multiflorum* Lam.), ryegrass (*Lolium rigidum* Gaud.), crabgrass (*Digitaria sanguinalis* L.), buckwheat (*Eriogonum compositum* Douglas ex Benth.), Chinese sprangletop (*Leptochloa chinensis* [L.] Nees.), jungle rice (*Echinochloa colona* [L.] Link.), barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) and sand fescue (*Festuca myuros* L.) were chosen as test plants for bioassay because there are common weeds and distributed throughout the cultivated fields.

Extraction: Plant powder (100 g) was extracted with 1 L of 80% (v/v) aqueous methanol for two days. After filtration using one layer of filter paper (No. 2; Toyo Ltd., Tokyo, Japan), the residue was extracted again with 1 L of cold methanol for one day and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Bioassay: An aliquot of the extract (final assay concentration was 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL) was evaporated to dryness at 40°C *in vacuo* by rotary evaporator, dissolved in 3 mL of methanol and added to a sheet of filter paper (No. 2) in a 2.8 cm Petri dish. The methanol was evaporated in a draft chamber then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan), which was used for surfactant and did not cause any toxic effects. Ten seeds of cress, lettuce, alfalfa, or 10 germinated seeds of timothy, sand fescue, buckwheat, crabgrass, barnyard grass, jungle rice, Italian ryegrass, ryegrass or Chinese sprangletop were arranged on the filter paper in Petri dishes. Timothy, sand fescue and buckwheat were germinated in the darkness at 25°C for 48 h, crabgrass, barnyard grass and jungle rice were germinated in the darkness at 25°C for 120 h and Italian ryegrass, ryegrass and Chinese sprangletop were germinated in the darkness at 25°C for 72 h. The shoot and root lengths of seedlings was measured at 48 h after incubation in the darkness at 25°C. Control seeds were sown on the filter paper moistened with the aqueous solution of Tween 20 without the extract. The percentage length of seedlings was then determined by reference to the length of control seedlings. The bioassay was repeated three times with 10 plants for each determination. The inhibition percentage was calculated as follows: Inhibition (%) = [1- (sample extracts/ control)] ×100.

Table 1: Inhibition percentage of aqueous methanol extracts of *H. sabdariffa* on the growth of test plant seedlings

	Inhibition (%)							
	Shoot				Root			
	Concentration (g dry weight equivalent extract/mL)							
Test plant species	0.01	0.03	0.1	0.3	0.01	0.03	0.1	0.3
Cress	38.67a	96.96b	100.00b	100.00b	53.01a	97.42b	100.00b	100.00b
Lettuce	4.42a	78.14b	100.00b	100.00b	29.67a	84.57b	100.00b	100.00b
Alfalfa	36.35a	96.89b	100.00b	100.00b	46.12a	95.00b	100.00b	100.00b
Timothy	3.59a	29.31a	56.31ab	100.00b	5.70a	33.28ab	66.56bc	100.00c
Crabgrass	-16.13a	21.64ab	73.86bc	100.00c	-20.82a	28.09ab	73.70bc	100.00c
Italian ryegrass	7.85a	37.93ab	75.68bc	100.00c	44.73a	58.41ab	78.21b	100.00c
Ryegrass	2.05a	39.25ab	58.53b	78.17b	-10.66a	27.20ab	64.50b	85.86b
Buckwheat	17.01a	39.30a	48.48ab	74.86b	32.56a	55.81ab	70.85b	83.86b
Chinese sprangletop	29.68a	55.00b	80.42c	92.37c	30.25a	56.13b	84.55c	95.56c
Jungle rice	12.02a	37.91b	54.42b	90.96c	23.68a	63.47b	79.38bc	94.15c
Barnyard grass	17.23a	69.26b	86.38c	96.05c	44.01a	81.94b	89.15b	97.05b
Sand fescue	13.09a	55.76b	81.08c	100.00c	24.93a	52.42b	90.67c	100.00c

Mean with same letters in a row is not significantly different at $P<0.001$

Table 2: I_{50} values of aqueous methanol extracts of *H. sabdariffa* for shoots and roots of test plants

	I_{50} (g dry weight equivalent extract/mL)	
Test plant species	Shoot	Root
Cress	0.0058	0.0058
Lettuce	0.0068	0.0065
Alfalfa	0.0058	0.0058
Timothy	0.1351	0.0706
Crabgrass	0.0393	0.0267
Italian ryegrass	0.0438	0.0988
Ryegrass	0.0168	0.0287
Buckwheat	0.0939	0.0217
Chinese sprangletop	0.0254	0.0254
Jungle rice	0.1243	0.0078
Barnyard grass	0.0012	0.0041
Sand fescue	0.0154	0.0267

The values were determined by a logistic regression analysis after bioassays

Concentration-Response Curves: The concentrations required for 50% inhibition (defined as I_{50}) of the test plants were determined by concentration-response curves. Filter paper (No.2) was placed into Petri dish and different amounts of the extract were added on it. Final concentrations of the extract were 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract/mL. After the methanol evaporated, 10 seeds of cress, lettuce, alfalfa or 10 germinated seeds of timothy, crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass, or sand fescue were arranged on the filter paper in Petri dishes. Control seeds were sown on the filter paper moistened with the aqueous solution of Tween 20 as described above. The shoot and root lengths of the seedlings were measured at 48 h after incubation in darkness at 25°C. The concentrations required for 50% inhibition (defined as I_{50}) of the test plants in the assay was calculated from the regression equation of the concentration-response curves. The values of I_{50} and coefficient of correlation were summarized in Table 2.

Statistical Analysis: Each treatment of this experiment was carried out with three replications and repeated twice. Treatments were prepared in a completely randomized design. Data were analyzed by SPSS version 16.0 using One-way ANOVA.

RESULTS

Allelopathic activity of *H. sabdariffa* Extract: Figure 1 shows the effects of aqueous methanol extracts of *H. sabdariffa* on shoot and root growth of test plants. The inhibitory effect was increased with increasing concentrations of the extracts. Significantly inhibited shoot and root growth of all test plant species was observed at the concentration greater than 0.03 g dry weight equivalent extract/mL ($P<0.001$).

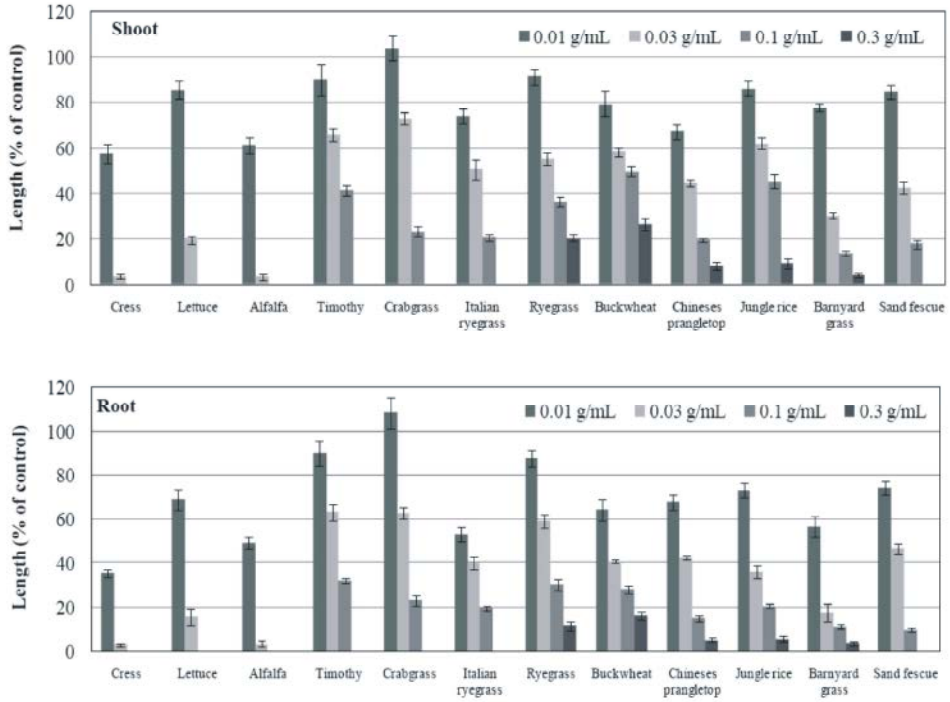

Fig. 1: Effects of aqueous methanol extracts on shoot and root growth of cress, lettuce, alfalfa, timothy, crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass, and sand fescue seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.01, 0.03, 0.1 and 0.3 g dry weight of *H. sabdariffa*. Shoot and root lengths of these seedlings were determined after 48 h of incubation in the dark at 25°C. Means ± SE from three independent experiments with 10 seedlings for each determination are shown ($P<0.001$).

Effect of Aqueous Methanol Extracts of *H. sabdariffa* on Shoot Growth: The inhibitory activity of the extracts on shoot growth of test plant species was summarized in Table 1. The extracts obtained from 0.1 g dry weight of the *H. sabdariffa* plants completely inhibited shoot growth of cress, lettuce and alfalfa seedlings (100%) and shoot growth of timothy and crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass or sand fescue were inhibited by 56.31, 73.86, 75.68, 58.53, 48.48, 80.42, 54.42, 86.38 and 81.08%, respectively. Exposure to the concentration of 0.3 g/mL, shoot growth of timothy, crabgrass, Italian ryegrass and sand fescue were completely inhibited.

Comparing the concentration required for 50% inhibition, barnyard grass shoots were the most sensitive to the extracts follow by cress and alfalfa. On the other hand, shoot growth of timothy was the less sensitive to the extracts (Table 2).

Effect of Aqueous Methanol Extracts of *H. sabdariffa* on Root Growth: The extracts obtained from 0.1 g dry weight of the *H. sabdariffa* plants completely inhibited root growth of cress, lettuce and alfalfa seedlings (100%) and root growth of timothy and crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass or sand fescue were inhibited by 66.56, 73.70, 78.21, 64.50, 70.85, 84.55, 79.38, 89.15 and 90.67%, respectively (Table 1). At the concentration of 0.3 g/mL, the inhibition of timothy, crabgrass, Italian ryegrass and sand fescue were completely inhibited. Comparing the concentration required for 50% inhibition, barnyard grass roots were the most sensitive to the extract of *H. sabdariffa* followed by cress and alfalfa. Italian ryegrass roots were less sensitive to the extracts (Table 2).

DISCUSSION

Results reported in this study demonstrated that aqueous methanol extract of *H. sabdariffa* inhibited shoot and root growth of all test plant species at the concentrations greater than 0.03 g dry weight equivalent extract/mL and increasing the extract concentration increased the inhibition. Such inhibition on the growth of test plant species might be due to the presence of allelochemicals in *H. sabdariffa.* Similar results were reported by Randhawa *et al*. [27], who found that the

germination of *Trianthema portulacastrum* was suppressed by higher concentration of the sorghum water extract. The results are in agreement with earlier studies reporting that effectiveness of receiver plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold [28-33]. The test plants responded differently to the aqueous methanol extract exhibiting a differential species-specificity. The aqueous methanol extract therefore had an inhibitory effect on a wide range of plant species, both of the monocotyledonous (timothy, crabgrass, Italian ryegrass, ryegrass, Chinese sprangletop, jungle rice, barnyard grass and sand fescue) and the dicotyledonous plants (cress, lettuce, alfalfa and buckwheat). Such allelochemicals are both species-specific and concentration-dependent. These characteristics may influence the density and the composition of individual plant communities [34]. In addition to that, aqueous methanol extract of *H. sabdariffa* had higher root growth inhibition than that of shoot growth of the test plant species. Salam and Kato-Noguchi [35] 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. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue [36]. Results of this study also identified that inhibitory effects of *H. sabdariffa* on test plant species were different. This unequal susceptibility to different extracts could be due to inherent differences in various biochemicals involved in the process. The species specificity of phytotoxins has also been demonstrated for other allelopathic plants species [37].

The seedlings of each test species used in these experiments were grown in a single Petri dish without intra-species competition for resources, as young seedlings withdraw nutrients from the seeds and light is unnecessary in the developmental stage [38]. Thus, growth inhibitions of the test plant species are likely to have been caused by the allelopathic reaction rather than by competitive interference. It is important to note that *H. sabdariffa* had strong inhibitory effect on the growth of noxious paddy weeds barnyard grass. Based on the results of this study, it could therefore be concluded that the aqueous methanol extract of *H. sabdariffa* may possess allelopathic potential and may contain growth inhibitory substances. These results might have value in enabling weed control based on natural plant extracts and hence this plant could be used for the development of bioherbicide in weed management. Further studies will focus on isolation and characterization of the allelopathic compounds from the aqueous methanol extract of *H. sabdariffa*.

CONCLUSION

An aqueous methanol extract of *H. sabdariffa* plants showed allelopathic effects on all test plant species at the concentration greater than 0.03 g dry weight equivalent extract/mL and increasing the extract concentration increased the inhibition. Therefore, *H. sabdariffa* may contain growth inhibitory substances and possess allelopathic potential. The effective natural products from *H. safdariffa* could be used as environmentally friendly herbicides to control weeds. Further evaluation of allelopathic potential of *H. safdariffa* plants under field conditions is required.

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