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Potent herbicidal activity of *Sapindus mukorossi* Gaertn. against *Avena fatua* L. and *Amaranthus retroflexus* L.

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ABSTRACT

The evaluation of herbicidal activity and the isolation of plant allelopathic substances can lead to the discovery of new herbicide. This study has confirmed that the ethanol extract of *S. mukorossi* leaves was inhibitory to growth of *Avena fatua* L. and *Amaranthus retroflexus* L. both in petri dish experiment and pot culture assay, with the fresh weight inhibition varied between 47.60% and 62.05% at a concentration of 40 g/L of the leaf extract, being comparable to that of a commercialized natural product herbicide pelargonic acid at a concentration of 4 g/L. Five herbicidal compounds, oleanolic acid **(1)**, lupeol **(2)**, d-pinitol **(3)**, hexadecanoic acid **(4)** and octadecanoic acid **(5)** were isolated from the most active fraction, petroleum ether extract. D-pinitol **(3)** showed the highest growth inhibitory activity against both the shoot and root of *A. fatua*, with the half maximal inhibitory concentration (IC_{50}) values of 53.61 and 56.43 μg/mL, respectively. Furthermore, oleanolic acid **(1)** and d-pinitol **(3)** also inhibited the growth of *A. retroflexus* with the IC_{50} value of 56.05 and 61.89 μg/mL against shoot growth, and 58.07 and 66.70 μg/mL against root growth, respectively. Thus, oleanolic acid **(1)** and d-pinitol **(3)** are the main phytotoxin constituents of *S. mukorossi*, and these compounds have the potential for further development as a botanical herbicide.

1. Introduction

In agricultural fields, weeds can cause greater reduction in both quantity and quality of crop production than the presence of any other agricultural pest (Ervin and Jussaume, 2016; Wang et al., 2015). Not surprisingly, crop protection relies heavily on the use of synthetic herbicides for controlling weeds in modern agriculture (Cerecetto et al., 2000; Wang et al., 2012). However, long-term application of synthetic herbicides has contributed to increased resistance in weeds, also leading to soil and groundwater contamination, and to hazards to human health (Akbar et al., 2014; Masi et al., 2017; Sheng et al., 2010). In the last two decades, botanical herbicides have received a recent surge in interest due to their potential safety and environmental friendliness (Dayan and Duke, 2014; Dayan et al., 2009). And more importantly, botanical phytotoxins are also a source for the discovery of new herbicide target sites that can serve as the focus of traditional herbicide discovery efforts (Duke and Dayan, 2015; Grosso et al., 2010; Uddin et al., 2014).

Sapindus mukorossi Gaertn (Sapindaceae, *Sapindus*), well known as soap nut tree, is widely distributed in tropical and subtropical regions (Jiang et al., 2013). As an herbal medicine, it is used in the treatment of snakebite, asthma, piles, tooth disorders, hepatic disorders, and dermatological disorders (Pelegrini et al., 2008; Rao et al., 2012; Upadhyay and Singh, 2012). The main ingredients of *S. mukorossi* galls and pericarp are saponins, triterpenoids, flavonoids and fatty acids (Kuo et al., 2005; Sharma et al., 2013; Suhagia et al., 2011; Tanaka et al., 1996). Saponins isolated from *S. mukorossi* fruit have antibacterial and anticancer activity (Sarethy et al., 2015; Wu et al., 2014), and sesquiterpene glycosides display strong antimicrobial activity against dermatophytes (Ibrahim et al., 2006). In addition, crude saponins from the seed of *S. mukorssi* had insecticidal and molluscicidal activity (Huang et al., 2003; Lu et al., 2010), and potential herbicidal property (Singh, 2010). In searching for suitable herbicidal agents from Chinese herbs, it was found that ethanol extract of the leaves of *S. mukorossi* exhibited the growth inhibitory activity against *Avena fatua* L. Meanwhile, a preliminary examination of the chemical constituents showed that there is hardly any saponin in the leaves of *S. mukorossi*. However, there is no report concerning the isolation of herbicidal constituents from *S. mukorossi* leaves.

In the present study, the herbicidal activities of the leaf extract of *S. mukorossi* against *A. fatua* L. and *Amaranthus retroflexus* L. were evaluated using a combination of petri plate test and pot culture assay. Herbicidal compounds were also isolated by bioassay-guided fractionation.

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2. Materials and methods

2.1. Plant materials and chemical

Leaves of *Sapindus mukorossi* Gaertn were collected in botanical garden of Northwest A&F University, PR China, in November 2014. The plant was identified by Dr. Li Yan, College of Life Sciences, Northwest A &F University and then air-dried. A voucher specimen (RDCBP-S067) was deposited in the laboratory (Research & Development Center of Biorational Pesticide, Northwest A&F University).

Avena fatua L. and *Amaranthus retroflexus* L. plants were identified by Dr. Li Yan and seed were collected from the experimental farm of Northwest A&F University, PR China.

Technical grade 97% pelargonic acid was purchased from Aladdin Industrial Corporation (Shanghai, China).

2.2. Organic solvent extraction

Dried leaves of *S. mukorossi* (4.0 kg) were crushed, and extracted three times with 95% ethanol (10 L) (3 × 3 days) at room temperature (25 °C) (Emam et al., 2009). The extract were filtered through Whatman No.1 filter paper and evaporated under reduced pressure (20 kPa) to yield crude extract (521 g). The ethanol extract was dissolved in water. Subsequently, three organic solvents, viz. petroleum ether, ethyl acetate and butyl alcohol, were successively used for extraction, in order of their increasing polarity. These phases were collected and evaporated under vacuum (20 kPa) in a rotary evaporator, then dried through the electric drum wind drying oven (50 °C) to yield petroleum ether fraction (170 g), ethyl acetate fraction (65 g) and butyl alcohol fraction (85 g), respectively.

2.3. Petri plate assay

The growth inhibitory activity of the testing samples (ethanol extract, crude fractions and compounds) against *A. fatua* and *A. retroflexus* were tested using seed germination method in petri plates (Cerecetto et al., 2000).

A. fatua and *A. retroflexus* seed were surface-sterilized in 0.525 g/L sodium hypochlorite for 15 min, and then rinsed four times with deionized water. Ten seed were separately placed on two layers of filter paper (Whatman No.1) in petri plates (9 cm diameter). Each sample was first dissolved in dimethyl sulfoxide (DMSO) and then diluted with distilled water to the desired concentration. In order to ensure DMSO did not interfere with the assay, the maximum final concentration of DMSO was 0.5%. Five milliliter of respective samples were applied as per treatment. Weed seed applied with distilled water including 0.5% DMSO were used as control. Treated seed were incubated at 25 ± 1 °C in dark. Treatments were arranged in a completely randomized design with three replications. Shoot and root length of weed seedlings were measured 5–7 days (according to the length of seedlings) after sowing. The inhibition rate was calculated according to Eq. (1):

$$\text{inhibition rate (\%)} = (1 - T/C) \times 100, \quad (1)$$

where T is the average shoot or root length of the tested seedlings, and C is the average shoot or root length of the control seedlings.

Further toxicity test for potent herbicidal samples was also evaluated. Five concentrations were arranged for every potent sample with three replications. Half maximal inhibitory concentration (IC_{50}) and 95% confidence intervals were calculated by SPSS 19.0.

2.4. Pot culture assay

2.4.1. Pre-emergence herbicide tests

The ethanol extract was dissolved in DMSO, and then diluted with distilled water to give 40, 20 and 10 g/L concentrations. *A. fatua* and *A. retroflexus* seed were surface sterilized with 0.525 g/L sodium hypochlorite for 15 min, and then rinsed four times with deionized water. Sand was sterilized by dry heat, and then placed in plastic pots (15 cm depth), and ten sterilized seed were placed on top of the sand, which were then covered with 1 cm of additional sand. The ethanol extract (5 mL) was applied directly to the soil surface, using a laboratory spray bottle. Commercialized botanical herbicide pelargonic acid was applied as positive control. After treatment, the pots were placed in a greenhouse (20 °C), where they received regular watering (overhead). The experiment was divided into two groups, and one group was used for assay of 14 days, the other was used for 21 days. *A. fatua* and *A. retroflexus* were harvested 14 and 21 days after sowing and fresh weights were recorded. Three replications were conducted for each treatment. Fresh weight inhibition rate was calculated using the formula Eq. (2):

$$\text{fresh weight inhibition rate (\%)} = (1 - T/C) \times 100, \quad (2)$$

where T is the average fresh weight of the tested weed, and C is the average fresh weight of the control weed.

2.4.2. Post-emergence herbicide tests

An identical preparation experimental method was used for post-emergence herbicide tests. After the *A. fatua* and *A. retroflexus* had two to three true leaves, the plants were sprayed with the ethanol extract (5 mL) each treatment. Subsequently, the plants were returned to the greenhouse and water was applied directly to the sand surface. Three replications were conducted for each treatment. The experiment was also divided into two groups, and one group was used for assay of 14 days, the other was used for 21 days. Fresh weights were measured after 14 and 21 days, respectively. Fresh weight inhibition rate was used to describe the curative efficiency and was calculated according to Eq. (2).

2.5. Isolation of active compounds

Bioassay-guided fractionation was used for crude petroleum ether fraction (170 g), the most active fraction among crude organic fractions. The petroleum ether fraction were eluted through a silica gel (200–300 mesh) column with gradient mixtures of petroleum ether—acetone (100:0-0:100), yielding six fractions (Fr1 through Fr6) according to TLC (Thin-Layer Chromatography) analysis. Fraction 3 (Fr3), the most active fraction in petri plate assay, was further separated through a silica gel column with gradient mixtures of petroleum ether—acetone (100:1, 80:1, 60:1, 30:1, 20:1), and six fractions (Fr3.1 through Fr3.6) were obtained. Fraction 3.3 (Fr3.3) was further separated by silica gel column to give compounds **1** (0.32 g), **4** (0.22 g), and **5** (0.48 g). Fraction 3.4 (Fr3.4) was separated by silica gel column and recrystallizing to give compounds **2** (0.46 g) and **3** (0.18 g). The structures of these compounds were determined from infrared spectroscopy (IR) (Nicolet, USA), mass spectrometry (MS) (Waters, USA), and nuclear magnetic resonance (NMR) (^{1}H and ^{13}C) (Bruker, Germany) spectral data.

2.6. Data analysis

The shoot or root length inhibition rates of different sample concentrations were gained with Microsoft Excel, and SPSS 19.0 was used to calculate IC_{50} and 95% confidence intervals. Fresh weight inhibition rates were subjected to one-way analysis of variance (ANOVA) to determine significant differences among mean values ($P < 0.05$).

3. Results

3.1. Herbicidal activity of S. mukorossi ethanol extract

S. mukorossi ethanol extract showed high inhibitory effects against

Table 1
Growth inhibitory activity of *S. mukorossi* ethanol extract against *A. fatua* and *A. retroflexus*.

Weed	Weed parts	IC_{50}(95% CL) (g/L)	Slope ± SE	χ^2
A. fatua	Shoot	0.42 (0.38–0.47)	0.98 ± 0.11	4.64
	Root	0.24 (0.21–0.29)	0.83 ± 0.06	2.43
A. retroflexus	Shoot	0.44 (0.41–0.53)	1.125 ± 0.12	0.93
	Root	0.27 (0.22–0.35)	0.87 ± 0.07	3.07

In bioassay, all data represent the mean value of triplication and is corrected for control inhibition ratio using the Abbott's formula; IC_{50} value was determined by log-probit analysis. $\chi^2_{0.05(3)} = 7.81$, χ^2 values less than 7.81 were considered as significant.

both the shoot and root growth of *A. fatua*, with IC_{50} values of 0.42 and 0.24 g/L, respectively. It also had effective inhibition against the shoot and root of *A. retroflexus*, with IC_{50} values of 0.44 and 0.27 g/L, respectively (Table 1).

On the 14th and 21st day after treatment, *S. mukorossi* ethanol extract at a concentration of 40 g/L showed effective pre-emergence inhibition, with 58.42% and 62.05% against *A. fatua*, and 55.34% and 58.09% against *A. retroflexus*, respectively, equivalent to those of pelargonic acid at a concentration of 4 g/L (Table 2). Post-emergence herbicidal activity of *S. mukorossi* ethanol extract (40 g/L) was potent, with the inhibition rate of 52.89% and 54.54% against *A. fatua*, and 47.60% and 52.65% against *A. retroflexus*, respectively, less of an effect than those of pelargonic acid at a concentration of 4 g/L. However, inhibition rate of *S.mukorossi* ethanol extract at a concentration of 20 g/L and 10 g/L was low (average inhibition varied between 8.50% and 24.45%) against both *A. fatua* and *A. retroflexus*.

3.2. Growth inhibitory activity of crude organic fractions

The petroleum ether fraction had the highest inhibition rate among the four fractions, with the result of 87.54% and 92.60% against the shoot and root of *A. fatua*, and 84.68% and 89.22% against *A. retroflexus*, respectively (Table 3). There were statistically significant ($P < 0.05$) in the treatments for different fractions

Further bioassay of the petroleum ether fraction showed that IC_{50} values were 0.29 g/L and 0.16 g/L against the shoot and root of *A. fatua*, and 0.37 g/L and 0.23 g/L against *A. retroflexus*, respectively (Table 4).

3.3. Identification of active compounds from S.mukorossi

The compounds isolated from *S. mukorossi* (Fig. 1.) were soluble in chloroform.

Oleanolic acid **(1)**: $C_{30}H_{48}O_3$, obtained as white needle crystal. The compound was identified as oleanolic acid by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data and by comparison with published results (Seebacher et al., 2003).

Lupeol **(2)**: $C_{30}H_{50}O$, obtained as white powder. The compound was identified as lupeol by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data and by comparison with published results (Imam et al., 2007).

D-pinitol **(3)**: $C_7H_{14}O_6$, obtained as white crystal. The compound was identified as d-pinitol by ^{1}H NMR, ^{13}C NMR and MS and by comparison with published results (Lovina et al., 1992).

Hexadecanoic acid **(4)**: $C_{16}H_{32}O_2$, obtained as white crystal. The compound was identified as hexadecanoic acid by ^{1}H NMR, ^{13}C NMR and MS and by comparison with published results (Wang et al., 2002).

Octadecanoic acid **(5)**: $C_{18}H_{34}O_2$, obtained as yellow waxy solid. The compound was identified as octadecanoic acid by ^{1}H NMR, ^{13}C NMR and MS spectral data and by comparison with published results (Muhammad et al., 2002).

3.4. Growth inhibitory activity of compounds

All five compounds were effective against *A. fatua* (Table 5). D-pinitol was the most active of the five compounds tested against both the shoot and root of *A. fatua*, with the IC_{50} values of 53.61 and 56.43 μg/mL, respectively. However, growth inhibitory activity of all five compounds was a little lower than commercialized botanical herbicide pelargonic acid. Growth inhibitory activity of the five compounds against *A. retroflexus* was also significant (Table 6). Oleanolic acid, lupeol and d-pinitol had higher activity against both the shoot and root of *A. retroflexus* than that of hexadecanoic acid and octadecanoic acid. Among them, d-pinitol had the lowest IC_{50} value, that is, 56.05 and 58.07 μg/mL, respectively. Surprisingly, the herbicidal activity of d-pinitol was similar to that of pelargonic acid.

4. Discussion

The ethanol extract of *S. mukorossi* leaves exhibits herbicidal activity against *A. fatua* and *A. retroflexus* both in petri dish experiment and pot culture assay. Singh (2010) reported that saponins from *S. mukrossi* seed had herbicidal activity, but it was found that the leaf extract of *S. mukorossi* with hardly any saponin also exhibited the growth inhibitory activity. It is surprising that the herbicidal activity of *S. mukorossi* leaves extract is more active than other plant extracts: *Achillea biebersteinii* extract (1.0 mg/mL) showed inhibitory effect of 17.39% and 72.20% against the shoot and root of *A. retroflexus* (Kordali et al., 2009); the half-inhibitory concentrations of crude extract of chicory root exceeded 0.5 g/L against *Echinochloa crusgalli* L. Beauv and *Amaranthus retroflexus* L. (Wang et al., 2011); the fresh weight inhibition ratio was less than 50%, with *Echinochloa colona* (L.) treated by *Ageratum conyzoides* L. leaf extract at a concentration of 100 g/L (Lim

Table 2
Fresh weight inhibition effect of *S. mukorossi* ethanol extract against *A. fatua* and *A. retroflexus*.

Weed	Treatment	Dosage (g/L)	Fresh weight inhibition rate (%)			
			Pre-emergence		Post-emergence	
			14d	21d	14d	21d
A. fatua	*S. mukorossi* ethanol extract	40	58.42 ± 3.34a	62.05 ± 4.09a	52.89 ± 2.90a	54.54 ± 4.82a
		20	23.60 ± 0.64b	24.45 ± 1.08b	17.37 ± 0.56b	21.43 ± 1.23b
		10	14.72 ± 0.30c	20.53 ± 0.86b	7.35 ± 0.07c	11.09 ± 0.53c
	Pelargonic acid	4	52.09 ± 4.38a	58.53 ± 3.57a	54.67 ± 3.79a	57.54 ± 5.06a
A. retroflexus	*S. mukorossi* ethanol extract	40	55.34 ± 3.56a	58.09 ± 1.34a	47.60 ± 3.09b	52.65 ± 2.65b
		20	18.17 ± 0.27b	21.89 ± 1.64b	14.91 ± 0.34c	18.90 ± 0.78c
		10	8.50 ± 0.07c	11.86 ± 0.63c	–	8.65 ± 0.23d
	Pelargonic acid	4	57.42 ± 4.08a	60.55 ± 5.85a	68.54 ± 4.12a	71.05 ± 4.31a

Data are the mean of three replicates and are represented as mean ± standard deviation. Means in the same column followed by the same lower case letter are not significantly different ($p < 0.05$) in a Tukey test.

Table 3
Growth inhibitory activity of crude extracts against *A. fatua* and *A. retroflexus*.

Extracts	Inhibition rate (%)			
	A. fatua		*A. retroflexus*	
	Shoot	Root	Shoot	Root
Petroleum ether	87.54 ± 4.33a	92.60 ± 5.34a	84.68 ± 4.56a	89.22 ± 5.84a
Ethyl acetate	44.36 ± 3.21b	43.42 ± 4.36b	40.26 ± 3.63b	40.35 ± 4.72b
Butyl alcohol	13.27 ± 1.16c	11.33 ± 1.01c	17.95 ± 1.42c	17.87 ± 1.36c
Aqueous phase	9.82 ± 0.89c	12.80 ± 1.19c	11.63 ± 1.67c	18.93 ± 1.04c

The concentrations of extracts were 1 mg/mL. Data are the mean of three replicates and are represented as mean ± standard deviation. Means in the same column followed by the same lower case letter are not significantly different ($p < 0.05$) in a Tukey test.

Table 4
Growth inhibitory activity of the petroleum ether fraction against *A. fatua* and *A. retroflexus*.

Weed	Weed parts	IC_{50}(95% CL) (g/L)	Slope ± SE	χ^2
A. fatua	Shoot	0.29 (0.25–0.34)	0.98 ± 0.09	2.01
	Root	0.16 (0.11–0.19)	0.69 ± 0.05	0.83
A. retroflexus	Shoot	0.37 (0.31–0.47)	0.90 ± 0.08	1.03
	Root	0.23 (0.18–0.28)	0.71 ± 0.09	1.20

In bioassay, all data represent the mean value of triplication and is corrected for control inhibition ratio using the Abbott's formula; IC_{50} value was determined by log-probit analysis. $\chi^2_{0.05(3)} = 7.81$, χ^2 values less than 7.81 were considered as significant.

et al., 2017). In consideration of its long history as a traditional herb in Chinese medicine (Chen et al., 2013; Li et al., 2012; Nia and Krivenchuk, 1964; Wang et al., 2016), *S.mukorossi* extract should be relatively safe to the environment. Thus, *S.mukorossi* has the potential to be developed into a natural herbicide for the control of *A. fatua*, *A. retroflexus* and potentially other weeds.

During the isolation of the active compound(s) of *S.mukorossi*, petroleum ether fraction was subjected to bioassay-directed isolation, which led to the identification of five herbicidal compounds (oleanolic acid, lupeol, d-pinitol, hexadecanoic acid and octadecanoic acid). D-pinitol and oleanolic acid, the most active compounds, presented the same potent herbicidal activity against *A. fatua* as pelargonic acid, a commercial botanical herbicide, regarded as effective for many weeds (Coleman and Penner, 2008; Stratford, 1999). D-pinitol has been confirmed to various homeopathic applications (Selvaraj and Sorimuthu, 2009; Selvaraj et al., 2010; Thamaraiselvan et al., 2013, 2014; Jun et al., 2007a, 2007b), as does oleanolic acid (Farina et al., 1998; Liu, 1995, 2005; Ma et al., 2005). Meanwhile, only a few studies on the pesticidal activities of these two compounds have been reported, until now. The antifungal activity of d-pinitol against powdery mildew (*Podosphaera xanthii*) in cucumber was been reported by Chen and Dai (2014), Chen et al. (2014a, 2014b). D-pinitol can also inhibit *Alfalfa cautiva*, *Lotus tennis* and *Porotos vaina* seed germination (Sosa et al., 2009). Furthermore, although there were previous reports about isolation of oleanolic acid from allelopathic plants, such as *Grewia optiva* (Uddin et al., 2011), *Pyrenacantha staudtii* (Falodun et al., 2009) and *Salvia moorcraftiana* Wall (Khan et al., 2002), its toxicity on weed growth was not confirmed in these studies. The herbicidal activities of d-pinitol, oleanolic acid and the other three compounds extracted from *S. mukorossi* against *A. fatua* and *A. retroflexus* were reported in this study. Thus, based on the high toxicity to weeds, further investigation of the chemical constructions and herbicidal mechanisms of these compounds is warranted.

5. Conclusion

S. mukorossi has potential to be developed into biological herbicidal agent. Five herbicidal compounds were isolated from *S. mukorossi* and characterized as oleanolic acid, lupeol, d-pinitol, hexadecanoic acid and octadecanoic acid. D-pinitol and oleanolic acid were the main active constituents and were effective to *A. fatua* and *A. retroflexus*.

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Competing interests

The authors declare that they have no competing financial interests.

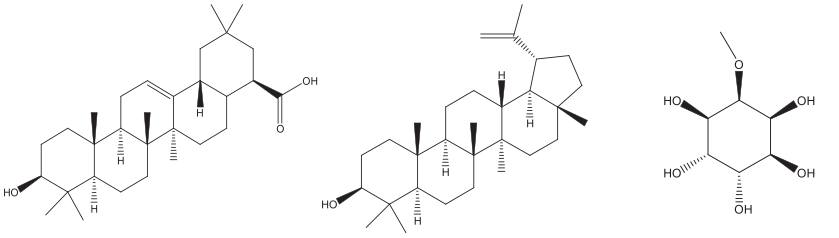

Fig. 1. The structure of compounds soluble in chloroform isolated from *S. mukorossi*.

Table 5
Growth inhibitory activity of compounds from *S.mukorossi* against *A. fatua.*

Compounds	Shoot			Root		
	IC_{50}(95% CL) (μg/mL)	Slope ± SE	χ^2	IC_{50}(95% CL) (μg/mL)	Slope ± SE	χ^2
Oleanolic acid	58.67 (51.67–64.75)	1.59 ± 0.13	1.05	59.65 (48.41–68.81)	1.05 ± 0.11	0.84
Lupeol	80.12 (73.45–88.00)	1.71 ± 0.21	1.83	78.78 (74.00–84.06)	2.41 ± 0.19	3.26
D-pinitol	53.61(40.99–66.29)	0.75 ± 0.08	1.00	56.43 (35.00–78.40)	0.46 ± 0.05	0.29
Hexadecanoic acid	126.76 (91.70–156.07)	0.95 ± 0.07	2.08	184.43 (143.68–220.78)	0.85 ± 0.06	2.52
Octadecanoic acid	152.61 (108.14–189.64)	0.93 ± 0.10	1.59	143.38 (113.65–162.93)	0.71 ± 0.08	0.33
Pelargonic acid	56.48 (43.54–76.03)	1.43 ± 0.12	1.79	57.74 (45.21–74.20)	0.92 ± 0.10	1.06

In bioassay, all data represent the mean value of triplication and is corrected for control inhibition ratio using the Abbott's formula; IC_{50} value was determined by log-probit analysis. $\chi^2_{0.05(3)} = 7.81$, χ^2 values less than 7.81 were considered as significant.

Table 6
Growth inhibitory activity of compounds from *S.mukorossi* against *A. retroflexus.*

Compounds	Shoot			Root		
	IC_{50}(95% CL) (μg/mL)	Slope ± SE	χ^2	IC_{50}(95% CL) (μg/mL)	Slope ± SE	χ^2
Oleanolic acid	61.89 (53.03–69.66)	1.26 ± 0.15	1.23	66.70 (56.05–76.77)	1.05 ± 0.11	0.80
Lupeol	81.80 (76.42–88.02)	2.20 ± 0.28	1.09	95.84 (89.20–104.48)	2.27 ± 0.19	1.78
D-pinitol	56.05 (45.93–66.31)	0.95 ± 0.09	1.95	58.07 (44.94–71.50)	0.74 ± 0.04	0.95
Hexadecanoic acid	220.88 (170.24–272.23)	0.71 ± 0.06	1.63	152.88 (108.14–189.64)	0.78 ± 0.09	1.59
Octadecanoic acid	218.28 (183.32–253.24)	0.99 ± 0.11	3.30	187.07 (136.62–231.35)	0.71 ± 0.07	1.69
Pelargonic acid	35.76 (25.46–48.54)	0.82 ± 0.09	1.72	31.75 (20.54–48. 05)	1.13 ± 0.14	1.95

In bioassay, all data represent the mean value of triplication and is corrected for control inhibition ratio using the Abbott's formula; IC_{50} value was determined by log-probit analysis. $\chi^2_{0.05(3)} = 7.81$, χ^2 values less than 7.81 were considered as significant.

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