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Research Article

Phytotoxic and Cytotoxic potential of leaf, stem and root of Sageretia thea (Osbeck) M.C. Johnston

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Abstract

The phytotoxic and cytotoxic potential of leaf, stem and root of *Sageretia thea* carried out to check their weedicidal and anti cancer potential against lemna minor and brine shrimps respectively. The ethanolic extract of stem, root and n-hexane extract of leaf showed significant phytotoxicity while the n-hexane extract of stem, root showed low phytotoxicity. In cytotoxic activity the ethanol and aqueous extracts of root showed significant mortality while n-hexane showed low mortality. The ethanol extract of stem showed good lethality while aqueous and n-hexane extract showed poor cytotoxicity. Similar results also recorded for the leaf.

Key words: Phytotoxic, Cytotoxic, *Sageretia thea*.

INTRODUCTION

Sageretia thea a member of the family Rhamnacea is traditionally used for tea making in Korea and therapeutically used for the treatment of hepatitis in China (Xu et al., 1994) it is an annual shrub. The leaves of S. thea have good antioxidant potential and poor HIV-1 protease inhibitory activities. Herbicides and weedicides are used for the effective control of weeds in order to increase crop yield (Kim, 1994; Santos, 2009). But sometime the misuse and overuse of these synthetic weedicides may create some problems like resistance against the pesticides, water, soil and air pollutions (Judith et al., 2001). These synthetic herbicides have long persistence and some like DDT has the potential of bioaccumulation which is threatful to human health (Clarkson, 1995; Snelder et al. 2008). Similarly the discovery of new and efficient drugs against various cell disease like cancer and skin disease etc are made by screening of active compounds from various parts of plants (Amara et al., 2008). For detection of general toxicity of active compound derived from various parts of the plant, brine shrimps (*Artimia salina*) bioassay has been used for the last 30 years (Persoone and Wallis, 1987). In the present study the phytotoxic and cytotoxic potential of S. thea have been studied to evaluate the preliminary anticancer and phytotoxic potential of the plant.

MATERIALS AND METHODS

Phytotoxicity

The Phytotoxic activity of leaf, stem and root of S. thea were conducted using Lamna minor as test species by following the procedure of (Atta-ur-Rehman et al., 2001) as follows.

Activity Requirements

Lemna minor fronds, flask 250 ml, distilled water, extracts, micropipette (10-100µl, 100-1000 µl), filter paper, E-medium, glass vials, laminar flow hood, brush, oven etc.

E-medium preparation

For Lemna minor bioassay the E-medium was prepared by dissolving specific amount of mineral nutrients in distilled water the required volume of medium was made up to 1000 ml with a pH which was adjusted between (5.5-5.6) by adding KOH pellets.

Procedure

30 mg of respective extract was dissolved in 1.0 ml of 70% ethanol which served as stock solution. From this solution 1000, 100 and 10 µl were transferred to sterilized Petri plates (3 plates for each concentration), which was equivalent to 1000, 100 and 10 µl/ml respectively. Than the petri dishes were placed under sterilized condition in laminar flow and the solvent was allowed to evaporate overnight. Than in each plate 20 ml of E-medium was added. For negative (standard drug, Paraquate) and positive (E-medium) controls three petri plates for each were placed. Than in each petri plate ten plants were transferred with 2-3 fronds and kept in growth cabinet for seven days. After seven days the numbers of fronds were counted. Present growth promotion were recorded with reference to the positive control using the following formula

$$\% \text{ Regulation} = 100 - \frac{\text{Number of fronds in test samlpe}}{\text{Number of fronds in possitive control}} \times 100$$

Statistical analysis

To determine Fl50 values, the results were analyzed with Finny computer program with 95% confidence intervals (Saeed et al., 2010; Barkatullah et al., 2011).

Cytotoxicity

The cytotoxic activity of the etanolic extracts of the leaf, bark and fruit of S. thae were carried out as follows by Atta-ur-Rehman et al., (2001).

Activity Requirement

Brine shrimp eggs, Sea Salt (38 g/L of D/W,pH 7.4), Hatching tray with perforated partition, electric lamp to attract brine-shrimp larvae, Micro pipette (5, 50, 500µl), Vials tray, vials, ethanol.

Preparation of Brine solution

Brine solution was prepared by dissolving thirty eight grams (38 g) of sea salt in one liter of distilled water and then filtered.

Hatching Techniques

The hatching tray was divided into unequal two parts with a center perforated partition. The tray was half filled with filtered brine solution. In smaller part of the tray 25 mg of brine shrimps eggs were sprinkled and it was covered with black paper while the other larger half was left uncovered. For hatching of shrimp's eggs the tray was kept at 25ºC. To illuminate the open portion of the tray a lamp was suspended above the tray. When the eggs hatched it will be noted that the larvae will be moving from the covered dark portion toward the enlightened portion through the perforation of the partition.

Sample preparation

Dissolved 20 mg of an extract test sample was added to 20ml of 70% ethanol and from this solution 5, 50 and 500 µl were transferred to vials (3 vials /concentration). The vials were kept to open and allow the solvent to evaporate over night. After evaporation 5 ml of brine solution was added to each vial. These concentrations were equal to 10, 100 and 1000 µg/ml respectively. After two days of eggs hatching 10 larvae was transferred to each vial by using Pasteur pipette. The vials filled with brine solution for negative and positive controls. All the vials were placed under illumination at room temperature (25-27°C) and

The numbers of live and dead larvae were counted after 24 hours in all the vials and the data was analyzed with Finny computer program for LD50 values determination with 95% confidence intervals (Saeed et al., 2010; Barkatullah et al., 2011).

RESULTS AND DISCUSSION

Phytotoxic bioassay of S. thea

The synthetic drugs are used for the control of herbs and weeds and to increase crop productivity (Kim, 1994; Santos, 2009). But sometime the misuse or overuse of that drug cause air pollution and soil pollution which not only affect the plants but as well humans and animals (Judith et al. 2001). In the present study the aqueous, ethanolic and n-hexane extracts of different parts of S. thea have been applied against lemna minor to checked their herbicidal and weedicidal potential. All of these three parts showed different growth inhibition at different concentrations. The results obtained for the frond proliferation after 7 days shows that the aqueous extract of root showed high growth inhibition which is then followed by ethanol and n-hexane extract with LD50 values of 28825.32, 12005.78 and 98133.72 for aqueous, ethanol and n-hexane extracts respectively. LD70 values for all of these extracts were 2817997.51, 308814.44 and 3575946.52 at 10, 100, 1000µg/ml respectively. The chi square values for aqueous extract was 0.203 with a regression line of Y= 3.82+ 0.26x, for ethanol was 0.627 with a regression line Y= 3.48+ 0.37x and for n-hexane was 0.487 with regression line Y= 3.32+ 0.33x.

Similarly, the stem also showed inhibitory property with the same solvent at 10, 100, 1000µg/ml concentrations. The ethanolic extract show more growth inhibition as compared to n-hexane and aqueous extracts. LD50 values of ethanol, aqueous and n-hexane extract of stem was 53204.58, 6616.60 and 6534654.02 respectively. The LD70 values were 1597910.20, 23333.33 and 2246427047.29 respectively. The chi square values for aqueous extract was 0.257, for ethanol it was 1.26 and for n-hexane it was 0.097 with a regression line were Y= 4.73+ 0.46x, Y= 3.35+0.32x and Y= 3.59+ 0.21x respectively. The leaf also showed same inhibitory property like stem at aqueous extracts while n-hexane showed more inhibition as compared to ethanol. The LD50 and LD70 values for all the three extracts at 10, 100 and 1000µg/ml were 98133.72, 70623.79, 47554.72 and 23333.33, 608728.43, 1334555.60 respectively. The chi square values for aqueous, ethanol and n-hexane were 0.257, 3.548 and 0.012 respectively as shown in table 1.

Our results indicated that all the three extracts of leaves showed high degree of growth inhibition while root and stem showed moderate and low frond inhibition respectively.

Many other researchers also conducted experiments to check the weedicidal and herbicidal affects of different plants like Shanee et al. (2011) tested the phytotoxic effects for aqueous extract of different parts of Euphorbia dracunculoides at two different concentrations on germination and seedling growth of Cicer arietinum (chickpea). Onocha et al. (2011) reported significant phytotoxicity for the methanolic extract of leaves and stems of Acalypha torta, A. hispida, and A. wilkesiana (*Euphorbiaceae*) against lemna minor. Similarly Khan et al. (2012) observed Euphorbia prostrata with significant and dose dependent inhibitory potential on the germination and seedling growth of wheat.
So our current results are strongly supported by the findings of these researchers.

Cytotoxic activity of *Sageretia thea*

The discovery of new drugs against various disease like cancer and skin disease etc are made by screening of active compounds from various parts of plants (Amara et al., 2008). The toxicity of active compound derived from natural products of brine shrimps (Artimia salina) bioassay has been used, because the shrimp's response is similar to that of mammalian tissue (Persoone and Wallis, 1987).

In the present study for brine shrimps cytotoxicity bioassay was carried out to evaluate the cytotoxic potential of S. *thea*. The aqueous, ethanolic and n-hexane extracts of leaf, stem and root of S. thea at three different concentrations (10, 100 and 1000µg/ml) were applied. The results obtained are shown in Table 2. It showed that for all the three

extracts, the lethality of shrimp's larvae was correlated to the dose concentration. The ethanolic extract of the root showed significant mortality with LD50 value of 25.42 which is then followed by aqueous extract with LD50 value of 56.45 and n-hexane with LD50 value of 189.67. The LD70 and LD90 values for aqueous, ethanol and n-hexane were 732.11, 210.46, 3460.56 and 29778.16, 4473.12, 230573.83 respectively. The aqueous extract of the root have a chi square value of 0.003 with a regression line of Y= 4.17+0.47 x, ethanol have 0.002 with a regression line of Y= 4.50+0.57 x and n-hexane have 0.086 with a regression line of Y=4.05+0.41x.

Table1. Phytotoxic potential of *Sageretia thea*

Plant part	Extract	Conc. µg/ml	% Growth inhibition	FI_{50}µg/ml	FI_{70} µg/ml	Regression	χ^2
ROOT	Aqueous	10	19.72	28825.32	2817997.51	Y=3.82+ 0.26x	0.203
		100	24.55				
		1000	36.61				
	Ethanol	10	14.97	12005.78	308814.44	Y= 3.48+	0.627
		100	19.23			0.37x	
		1000	36.56				
	n-hexane	10	8.38	98133.72	3575946.52	Y= 3.32+	0.487
		100	18.64			0.33x	
		1000	24.78				
STEM	Aqueous	10	-	6616.60	23333.33	Y= 4.73+	0.257
		100	7.81			0.46x	
		1000	21.33				
	Ethanol	10	11.59	53204.58	1597910.20	Y= 3.35+0.32x	1.26
		100	13.27				
		1000	29.41				
	n-hexane	10	11.53	6534654.02	2246427047.29	Y= 3.59+	0.097
		100	17.29			0.21x	
		1000	21.07				
LEAF	Aqueous	10	-	-	-	-	-
		100	-				
		1000	-				
	Ethanol	10	-	70623.79	608728.43	Y= 2.28+	3.548
		100	9.27			0.56x	
		1000	13.59				
	n-hexane	10	9.27	47554.72	1334555.60	Y= 3.31+	0.012
		100	17.43			0.36x	
		1000	27.19				

Similar results have also been recorded for the stem. The ethanolic extract of the stem showed significant toxicity while aqueous and n-hexane extracts showed moderate and low toxicity towards brine shrimps. The LD50 values for aqueous, ethanol and n-hexane extracts were 1046.68, 98.00 and 16058.42 while the LD70 values were 29130.93, 1634.00 and 8116242.75 respectively. The LD90 values for aqueous, ethanol and n-hexane extracts were 29130.93, 1634.00 and 8116242.75 respectively. The chi square values for aqueous, ethanol and n-hexane were 0.920, with a regression line of Y= 3.94+0.52x, 0.031 with a regression line of Y=4.29 +0.62x and 0.346 with regression line of Y= 4.34+0.28 x. The Leaf also showed different concentration (10, 100 and 1000µg/ml) ethanolic extract showed significant lethality with LD50 value 60.79, LD70 value 438.57 and LD90 value 438.57 with chi square value of 0.034 and regression line of Y=3.95 +0.88 x. The aqueous extracts at 10, 100 and 1000µg/ml showed good mortality with LD50 value 65.99, LD70 value 1274.83 and LD90 value 1274.83 with chi square value 0.140 and regression line of Y=4.45 +0.59 x. Similarly the n-hexane extract showed low toxicity against brine shrimps at different concentrations (10, 100 and 1000µg/ml) with LD50 value 3.90, LD70 value 115.46 and LD90 value 2447.06. The chi square value for these concentrations was 0.024 with Y= 4.73+ 0.46x regression line.

Table 2. Cytotoxic activity of root, stem and leaf of *Sageretia thea*

Plant part	Extract	Conc. µg/ml	% mortality	LD_{50}µg/ml	LD_{70} µg/ml	LD_{90}µg/ml	Regression	χ^2
ROOT	Aqueous	10	36.7	56.45	732.11	29778.16	Y= 4.17+0.47 x	0.003
		100	55.3					
		1000	72.6					
	Ethanol	10	41.8	25.42	210.46	4473.12	Y= 4.50+0.57 x	0.002
		100	63.3					
		1000	82.9					
	n-hexane	10	29.5	189.67	3460.56	230573.83	Y=4.05+0.41x	0.086
		100	47.3					
		1000	61.2					
STEM	Aqueous	10	27.6	104.92	1046.68	29130.93	Y= 3.94+0.52x	0.920
		100	56.8					
		1000	67.8					
	Ethanol	10	47.6	14.0	98.00	1634.00	Y=4.29 +0.62x	0.031
		100	69.2					
		1000	88.4					
	n-hexane	10	37.3	216.77	16058.42	8116242.75	Y= 4.34+0.28 x	0.346
		100	43.8					
		1000	59.6					
LEAF	Aqueous	10	53.7	8.51	65.99	1274.83	Y=4.45 +0.59 x	0.140
		100	71.5					
		1000	90					
	Ethanol	10	44.6	15.50	60.79	438.57	Y=3.95 +0.88 x	0.034
		100	75.5					
		1000	95.3					
	n-hexane	10	58.1	3.90	115.46	2447.06	Y= 4.73+ 0.46x	0.024
		100	73.4					
		1000	87.5					

CONCLUSION

Many other researchers carried out cytotoxic activity for different plant products like Zaidi et al. (2006) who carried out cytotoxic activity of *Juniperus excels*, Koba et al. (2009) investigated the cytotoxic potential of *Cymbopogon citratus L. and Cymbopogon nardus L* leaves, Ali et al. (2009) studied the effect of *Euphorbia wallichii* root for cytotoxic potential. Our current results make sure that all the three parts of S.thea have cytotoxic potential and can be further use for the control of cancer cell growth and as anti tumor.

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