Cytotoxicity and Antioxidant Activity of Five Plant Species of Solanaceae Family from Iran

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Received: 16 April 2012 **Accepted:** 9 Oct. 2012

Abstract

Background: The use of natural products as anticancer and antioxidant agents has a long history. Several drugs currently used in chemotherapy were isolated from plant species.

Objective: The aim of this study was to evaluate cytotoxicity and antioxidant activity as well as phenol and flavonoid contents of five plant species of Solanaceae family.

Methods: Five plant species of Solanaceae family were collected from different regions of Iran. Methanol extracts and chloroform fractions of these species were tested by brine shrimp lethality assay in order to detect cytotoxicity. Antioxidant activity was evaluated by DPPH method. The total phenol content was measured using Folin - Ciocalteu method. The flavonoid content was measured by a colorimetric assay.

Results: The extracts of *Datura innoxia* and *Datura stramonium* showed the highest cytotoxicity activities with LC_{50} values of 22.08 and 21.66 µg/ml, respectively. The chloroform fractions of these two species were subjected to cytotoxicity assay with LC_{50} values of 33.00 and 4.29 µg/ml, respectively. In comparing, *Solanum dulcamara* showed the highest antioxidant activity with IC_{50} values of 52.51 µg/ml and the highest phenol and flavonoid content of the dry weight.

Conclusion: It could be seen among five tested plant species that *D. stramonium* had the highest cytotoxic activity and *S. dulcamara* had the highest antioxidant activity, phenol and flavonoid content. Further studies are necessary for chemical composition of the extracts and more comprehensive biological assays.

Keywords: Antioxidant, Cytotoxicity, Flavonoid, Phenol, Solanaceae

Introduction

Over half a century after begining chemotherapy for tumor treatment, phytochemicals have become an important part of antineoplastic agents. About 70% of anticancer drugs approved between 1940 and 2002 are either natural products or developed based on knowledge gained from natural products. Important examples for the success of anticancer drugs originally obtained from plants are the *Vinca* alkaloids from *Catharanthus roseus*, camptothecin from *Camptotheca acuminata*, paclitaxel from *Taxus baccata*, and podophyllotoxin isolated from *Podophyllum peltatum* [1].

The brine shrimp lethality bioassay is a general bioassay that appears capable of detecting a board spectrum of bioactivity present in crude extracts. This bioassay is easily mastered, costs little, and utilizes small amount of test material [2]. This assay has been used successfully to biomonitor the isolation of cytotoxic, antineoplastic, antimalarial, pesticidal, insecticidal, and anti-feedant compounds from plant extracts [3].

Cancer is a complex process where each stage involves different biochemical, molecular and cellular events all of which contribute to malignant transformation. Reactive oxygen species-induced cellular damage underlies key steps during development of the malignant phenotype including evasion of apoptosis, angiogenesis, limitless proliferation, tissue invasion and metastasis [4].

Oxidative stress refers to an imbalance between free radical production and opposing antioxidant defenses. Increasing evidence suggests that antioxidants and natural product-based compounds with antioxidant activity can effectively neutralize oxidative stress and thus suppress ROS-mediated tumorigenesis [4].

A number of *Solanum* species have previously been investigated for their cytotoxicity, antioxidant and antiviral activities, and treatment of protozoal infections [5-13]. In addition antimicrobial, radical scavenging, cytotoxic and nematicidal activities and asthma treatment of some *Datura* species have been reported [14-21]. These studies have shown the potential of effectiveness of the plant species from Solanaceae family especially in cytotoxic and antioxidant activities.

The present study aims to provide data on the cytotoxic potential of five plants belonging to the Solanaceae family from different regions of Iran on developing brine shrimp nauplii. The antioxidant activity has been evaluated using the spectrophotometric 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method. The total phenol content was measured using Folin-Ciocalteu method. The flavonoid content was measured by a colorimetric assay. The other aim of this study is determination of the relationship between antioxidant activity, phenol and flavonoid contents of the species extracts to confirm that phenol or flavonoid constituents are responsible for antioxidant activity of the plants.

Material and Methods

Material

Five plants belonging to the Solanaceae family were collected in May-June 2010 from different regions of Iran. The plant species

were identified and voucher specimens have been deposited at Institute of Medicinal Plants Herbarium (IMPH) (Table 1). The plant parts were air-dried under shade and ground using a laboratory mill. Brine shrimp was obtained from Salt Creek, Inc. Salt Lake City, UT 84104, USA.

Preparation of the crude extracts and fractions

The dried powdered plant samples (20 g) were extracted with methanol in soxhlet apparatus for 12 h. The methanol extracts were concentrated by rotary evaporator 50 ˚C under reduced pressure to get the crude extracts (Table 1) [3, 22].

Among the extracts screened, which extract showed potent activity against brine shrimp, it was resuspended in water and partitioned with chloroform ($CHCl_3$) to separate less polar, water insoluble compounds [3, 22].

Toxicity testing against the brine shrimp
Hatching shrimp

Brine shrimp eggs were hatched in seawater prepared by dissolving 38 g of sea salt in 1 liter of distilled water. After 48 h incubation at temperature (27 – 29 ˚C), the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with pipette [23].

Brine shrimp assay

The bioactivity of the extracts was monitored by the brine shrimp lethality assay [24]; 50 mg of methanol extracts were measured and dissolved in 10 ml of DMSO to get a concentration of 5 mg/ml. From the stock solutions different volume were placed in 22 different vials making the volume up to 5 ml by seawater. The final concentration of the samples, in the vials became 0.1 to 140 µg/ml, respectively. Serial dilutions were made in triplicate [3].

Ten brine shrimp nauplii were then placed in each vial. Both positive (thymol) [25, 26] and negative (seawater containing DMSO) control assays were carried out. After 24 h, the vials were observed and the numbers of survivors in each vial were counted and noted [27]. The data were corrected using Abbott's formula (% deaths = [(test - control) / control] × 100) described by Rasoanaivo and Ratsimamanga-Urverg [28]. The LC_{50} values were determined from the 24 h counts. In cases where data were insufficient for this technique, the dose-response data were transformed into a straight line by means of a logit transformation; the LC_{50} values were derived from the best-fit line obtained by linear regression analysis [24]. The extract or fractions were considered bioactive when LC_{50} value was lower than 30 µg/ml [23].

Antioxidant activity using DPPH method

The DPPH radical-scavenging activity was determined using the method proposed by Afolayan et al. 1.5 ml of 0.135 mM DPPH was mixed with 1.5 ml of extract (2-1000 µg/ml concentration). The reaction mixture was shaken completely and left in the dark at room temperature (30 min). The absorbance was measured by a X-ma 2000 UV-VIS spectrophotometer at the 517 nm. These tests were carried out in triplicate and ascorbic acid

was used as reference [29].

DPPH radical-scavenging activity was calculated using the following formula: DPPH° scavenging activity (%) = [1 - (S - SB)/(C - CB)] × 100%, where S, SB, C and CB were the absorbances of the sample, the blank sample (methanol and extract), the control (1.5 ml of DPPH° solution plus 1.5 ml of methanol), and the blank control (methanol), respectively [30]. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value [3].

Determination of total phenol content

Total phenol content was determined using the Folin-Ciocalteu reagent developed previously with some modification. Briefly, an aliquot (1 ml) of appropriately diluted extracts or standard solutions of gallic acid in water (100, 50, 25, 12.5 and 6.25 µg/ml) was added to a 10 ml volumetric flask containing 5 ml of distilled water. A blank sample was prepared using distilled water. 0.5 ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 3 min, 1 ml sodium carbonate solution (35%) was added to the reaction mixture, which was finally mixed and diluted with water to 10 ml. The absorbance of the solution was measured after 60 min against a blank sample by a X-ma 2000 UV-VIS spectrophotometer at a wavelength of 725 nm. Total phenol contents of extracts were expressed as mg gallic acid equivalents (GAE)/100 g dry weight. All samples were analyzed in triplicate [31].

Determination of total flavonoid content

Total flavonoid content was measured using a colorimetric assay developed previously. 1 ml of the extracts or standard solutions of rutin in methanol (1200, 600, 300, 150 and 75 µg/ml) was added to a 10 ml volumetric flask. 4 ml of distilled water was added. At first, 0.3 ml of 5% (w/v) sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10% (w/v) $AlCl_3$ was added and, then 6 min, 2 ml of 1M NaOH were also added to the mixture, followed by the addition of 3.4 ml distilled water. The absorbance of the pink colour mixture was read at 510 nm against prepared water blank and flavonoid content was expressed as mg rutin equivalents per 100g of dry weight. Samples were analyzed in triplicate [32].

Statistical analysis

All the experimental results were mean ± S.D of three parallel measurements. The data was entered into a Microsoft Excel© database and analyzed using SPSS version 15.0©. The LC_{50} and IC_{50} values were obtained by linear regression analysis. Extracts giving LC_{50} values lower than 30 µg/ml were considered to be cytotoxic [23]. The extracts with IC_{50} values lower than 200 µg/ml showed greater antioxidant activity [33].

Results

Plant species collected from different regions of Iran together with time and area of collection, part used and yields of the methanol extracts are shown in Table 1. Results of the toxicity of methanol extracts or

chloroform fractions against brine shrimp (LC_{50} values) are shown in Table 2.

IC_{50} values for DPPH scavenging activity of extracts are given in Table 3, as calculated from the percent inhibition versus concentration of extract curves. Total phenol and flavonoid contents of the studied species are also given in Table 3.

The relationship between DPPH assay, total phenol and flavonoid content are shown in Figure 1 and 2. Furthermore, the relationship between total phenol content and total flavonoid content is shown in Figure 3.

Discussion

Yield of plant extracts

Percent yield of crude extracts following the removal of solvent using a rotary evaporator, were 13% for *Solanum incanum* to 33% for *Solanum dulcamara* (Table 1).

Cytotoxicity of plant extracts

A total of five methanol extracts were tested for their toxicity against brine shrimp. The extracts of *Datura stramonium* and *Datura innoxia* showed significant cytotoxicity against brine shrimp (LC_{50} < 30 µg/ml) with LC_{50} values of 21.66 and 22.08 µg/ml, respectively, whereas the positive control, thymol showed a LC_{50} value of 1.37 µg/ml. Chloroform fraction of these two species (*D. stramonium* and *D. innoxia*) represented different cytotoxicity against brine shrimp with LC_{50} values of 4.29 and 33 µg/ml, respectively. These results suggested that total extract of *D. innoxia* was more cytotoxic than less polar fraction. But in the case of *D. stramonium*, chloroform fraction had more cytotxic effect than total methanol extract (Table 2).

The extracts of two species including *S. incanum* and *S. dulcamara* presented moderate cytotoxicity (LC_{50} between 30 and 50 µg/ml) against brine shrimp (Table 2). Since in most cases cytotoxicity is associated with pharmacological properties, it was deduced that the extracts from *D. stramonium* and *D. innoxia* had the best bioactivity.

Table 1 - List of plant species collected from different regions of Iran and yields of the methanol extracts

No.	Plant species voucher no.	Time of collection	Area of collection	Part used	Yield (%w/w)
1	*Datura innoxia* Miller. (IMPH[a] 602)	May 2010	Zibakenar, Gilan province	Leaves	27.5
2	*Datura stramonium* L. (IMPH 603)	May 2010	Zibakenar, Gilan province	Leaves	13.5
3	*Solanum dulcamara* L. (IMPH 633)	May 2010	Bandar Anzali, Gilan province	Leaves	33
4	*Solanum incanum* L. (IMPH 634)	June 2010	Bandar Abbas, Hormozgan province	Aerial parts	13
5	*Solanum nigrum* L. (IMPH 635)	May 2010	Bandar Anzali, Gilan province	Aerial parts	22.3

[a] Institute of Medicinal Plants Herbarium

Table 2 - Brine shrimp bioassay results of extracts or fractions of five plant species belong to Solanaceae family

No.	Plant species	Brine shrimp assay LC_{50} (µg/ml)[a]	
		Methanol	Chloroform
1	*Datura innoxia*	22.08 ± 0.52	33.00 ± 0.63
2	*Datura stramonium*	21.66 ± 1.63	4.29 ± 0.09
3	*Solanum dulcamara*	39.99 ± 0.09	-
4	*Solanum incanum*	31.25 ± 0.44	-
5	*Solanum nigrum*	69.59 ± 1.48	-
6	Thymol[b]	1.37 ± 0.005	1.37 ± 0.005

[a] All values are the means of three measurements ± SD
[b] Positive control

Table 3 - Antioxidant activity (DPPH assay IC_{50}), total phenol and flavonoid content of methanol extracts of five plant species belong to Solanaceae family

No.	Plant species	DPPH assay IC_{50} (µg/ml)[a]	Total Phenol (mg Gallic acid/ 100g Dry Weight)	Total Flavonoid (mg Rutin/ 100g Dry Weight)
1	*Datura innoxia*	77.26 ± 0.03	1825.62 ± 48.02	2720.30 ± 152.10
2	*Datura stramonium*	256.20 ± 0.06	567.66 ± 14.72	752.45 ± 47.61
3	*Solanum dulcamara*	52.51 ± 0.02	2118.81 ± 55.69	4816.57 ± 188.28
4	*Solanum incanum*	92.44 ± 0.02	780.82 ± 20.49	1239.10 ± 71.73
5	*Solanum nigrum*	83.88 ± 0.01	1132.53 ± 29.57	2280.20 ± 123.78
6	Ascorbic acid[b]	7.06 ± 0.001	-	-

[a] All values are the means of three measurements ± SD
[b] Positive control

Antioxidant activity of plant extracts

Four species presented high antioxidant activity (IC_{50} < 200 µg/ml) including *S. dulcamara*, *D. innoxia*, *S. nigrum* and *S. incanum* with IC_{50} value of 52.51 to 92.44 µg/ml, whereas the positive control, ascorbic acid showed an IC_{50} value of 7.06 µg/ml. However, *D. stramonium* represented the highest IC_{50} value (256.20 µg/ml) and the lowest antioxidant activity.

Total phenol content

Phenol content of plant materials, calculated as gallic acid equivalent, varied from 567.66 mg/100 g of the dry weight in *D. stramonium* to 2118.81 mg/100g of the dry weight in *S. dulcamara*. These results represent high phenol contents for all studied species (higher than 300mg/100g dry weight) [34]. Antioxidant activity and total phenol content of four species including *S. dulcamara*, *D. innoxia*, *S. nigrum* and *S. incanum* showed a linear relationship with a positive correlation coefficient of $R^2 = 0.8235$. As an explanation, lower IC_{50} value for inhibition of DPPH radical causes higher antioxidant activity (Figure 1).

Total flavonoid content

Flavonoid content of plant materials, calculated as rutin equivalent, varied from 752.45 mg/100 g of the dry weight in *D. stramonium* to 4816.57 mg/100g of the dry

weight in *S. dulcamara*. Antioxidant activity and total flavonoid content of four species including *S. dulcamara*, *D. innoxia*, *S. nigrum* and *S. incanum* showed a linear relationship with a positive correlation coefficient of R^2 = 0.9932 (Figure 2). A good positive correlation was observed between the total phenol and flavonoid content of all five species (R^2 = 0.8862) (Figure 3). The results strongly suggest that phenols and flavonoids are important components of these species, and some of their antioxidant effects could be attributed to the presence of these valuable constituents.

A number of studies have been reported cytotoxic or antioxidant activity of some medicinal plants species of Solanaceae family. For instance, mutagenic and cytotoxic effects of *Solanum paniculatum* L. ethanolic leaf and fruit extracts have been determined using the mouse bone marrow micronucleus test [35]. In another study, alcoholic extracts of seven

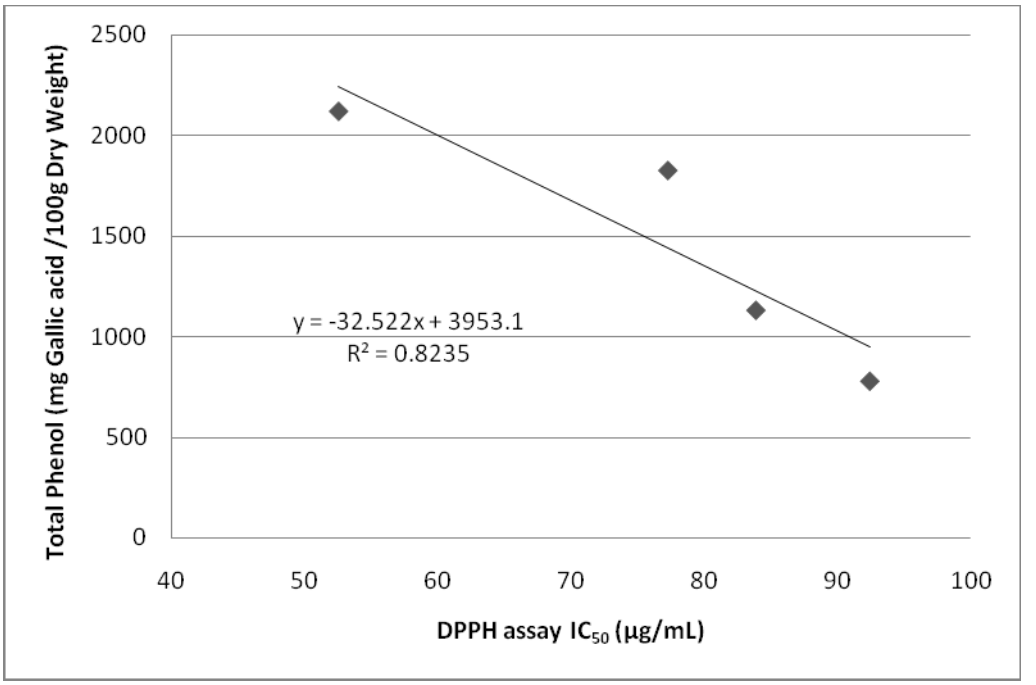

Figure 1- Relationship between DPPH assay and total phenol content

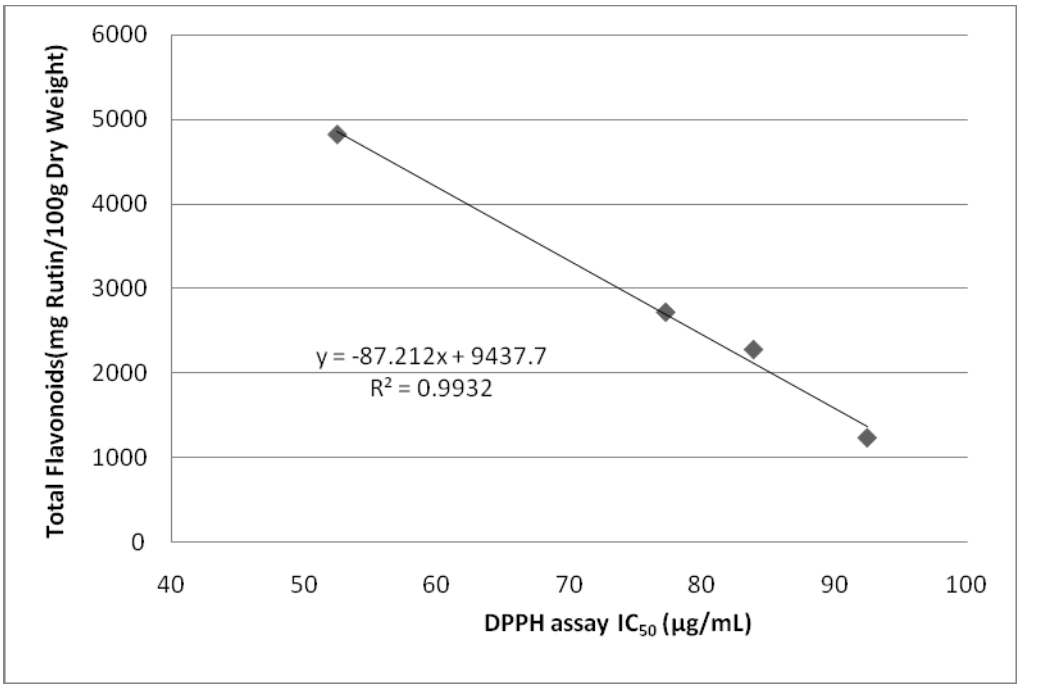

Figure 2 - Relationship between DPPH assay and total flavonoid content

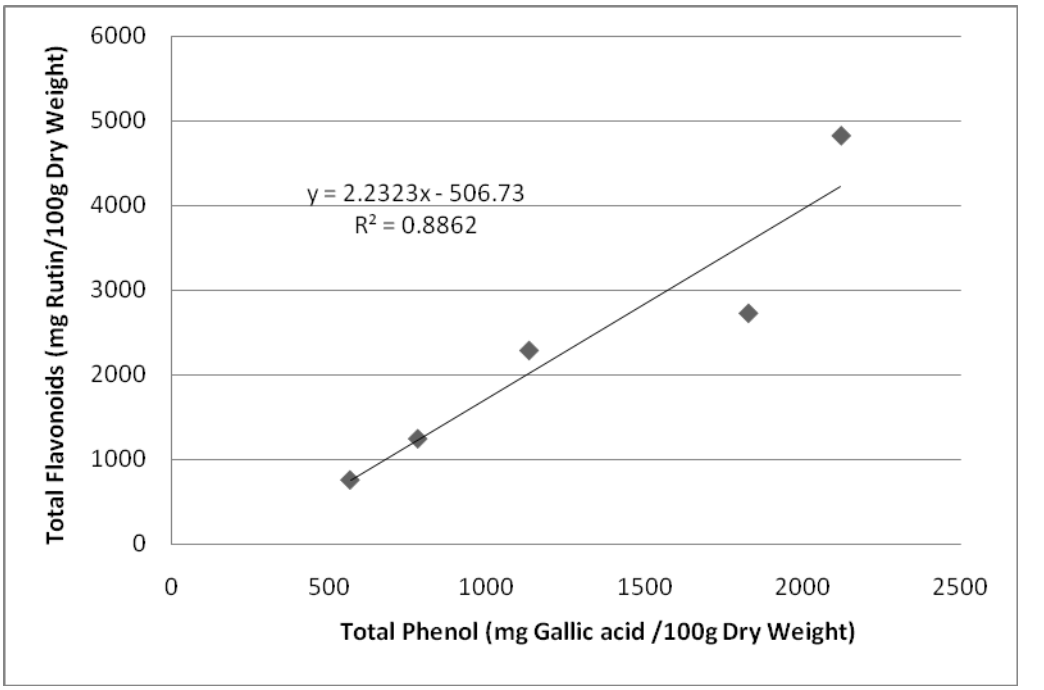

Figure 3 - Relationship between total phenol content and total flavonoid content

plants belonging to the Solanaceae family were phytochemically screened and evaluated for their cytotoxic activity by brine shrimp test; inhibition of cell division test on sea urchin *Loxechinus albus* fertilized eggs and inhibition of crown gall tumors on potato disk bioassay. In this study *Solanum lycioides* did not show any cytotoxicity against brine shrimp [5].

Aqueous extracts from 118 Indian medicinal plants including *Datura metel*, *Solanum indicum*, *S. nigrum*, *S. surattensis* and *S. trilobatum* were screened by the brine shrimp lethality assay and these plants species of Solanaceae family showed weak toxicity to the brine shrimp (LC_{50} between 130 and 4250 µg/ml) [36].

Plants which are used in traditional medicine of Tanzania have been evaluated to obtain preliminary data of their toxicity using the brine shrimps test. The results indicated that *Solanum incanum* exhibited low toxicity with LC_{50} value of 90.2 µg/ml [37]. These results represented lower activity of *S. incanum* than our investigation.

Brine shrimp test was used to screen Kenyan antimalarial plants for their cytotoxicity. Cytotoxicity results showed that organic ($CHCl_3$/MeOH, 1:1) extracts of the leaves and roots of *Solanum incanum* had moderate toxicity to the brine shrimp (LC_{50} values of 31 and 91 µg/ml, respectively). However, aqueous extracts of the leaves and roots of this species did not show any cytotoxicity (LC_{50} values of 273 and 499 µg/ml, respectively) [38]. These results were similar to our study in the case of *S. incanum* with LC_{50} value of 31.25 µg/ml.

Biological investigations of dried fruit of *Solanum nigrum* have been reported. In the brine shrimp lethality test, the extract showed cytotoxicity with LC_{50} value of 63.10 µg/ml [39]. These results were near to our results for cytotoxicity of aerial parts of *S. nigrum* with LC_{50} value of 69.59 µg/ml.

An investigation was conducted to examine the cytotoxicity and the antioxidant activity of some green leafy vegetables consumed in Sri Lanka. In this study, cytotoxicity was tested using brine shrimp lethality bioassay. The results indicated that *Solanum indicum* and *S. nigrum* did not show any cytotoxicity against brine shrimp with LC_{50} value of 519.38 and 268.47 µg/ml, respectively [40]. These results represented lower activity of *S. nigrum* than our investigation.

Methanol plant extracts of three medicinal plants of family Solanaceae, leaves of *Datura innoxia*, *Withania somnifera* and *Solanum surrattense* were screened to investigate the biological activities. Brine shrimps lethality bioassay was used to evaluate the cytotoxic activity. *S. surrattense* showed maximum antioxidant activities i.e. 76%. Cytotoxic effect of methanolic extract of *D. innoxia* and *S. surrattense* on brine shrimps was determined with LC_{50} value of 131 and 290 µg/ml, respectively [17]. These results represented lower activity of *D. innoxia* than our study.

Brine shrimp lethality bioassay is a primary assay to detect cytotoxic property of extracts and fractions and, further studies are required

to establish the cytotoxicity of the extracts against human cancer cell lines. However our results in this study may predict which species of Solanaceae family will give better results on cancer cell lines.

Conclusion

This is the first report on cytotoxicity and antioxidant activity of these five plant species of Solanaceae family from different regions of Iran. However according to the criteria of the American National Cancer Institute, the LC_{50} limit to consider a crude extract promising for further purification is lower than 30 µg/ml [41,23]. Thus, only two species among five tested species of plants presented significant cytotoxicity against brine shrimp. The extracts of *D. stramonium* and *D. innoxia* could be considered as potential sources of anticancer compounds. Chloroform fraction of *D. stramonium* had the highest cytotoxic effect among five tested plant species (LC_{50} = 4.29 µg/ml).

Another mechanism for cancer prevention might be radical scavenging of free radical oxygen and other species associated with cancer cell development. However, the results from radical scavenging activity with DPPH showed that of the five samples tested, *S. dulcamara* was more active (IC_{50} = 52.51 µg/ml).

It could be seen among five tested plant species that *D. stramonium* had the highest cytotoxic activity and *S. dulcamara* had the most radical scavenging activity with the highest total phenol and total flavonoid contents. Further studies are necessary for chemical composition of the extracts and more comprehensive biological assays.

Acknowledgment

This work was supported by grants (713-11) from Institute of Medicinal Plants, ACECR.

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