BIOASSAY SCREENING OF THE ESSENTIAL OIL AND VARIOUS EXTRACTS FROM 4 SPICES MEDICINAL PLANTS

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ABSTRACT

Four commonly used spices plants in Iran were evaluated for cytotoxicity effect using Brine Shrimp Lethality (BSL) assay. Essential oils and various extracts of *Heracleum persicum*, *Nigella arvensis*, *Cinnamomum zeylanicum and Zingiber officinale* were assessed by two methods of disk and solution of BSL. Data were processed in probit-analysis program to estimate LC_{50} values. All of the tested fractions have exhibited more cytotoxicity in the solution method. Essential oils of *H. persicum* and *C. zeylanicum* have shown the most cytotoxicity with LC_{50} values 0.007 and 0.03 µg/ml respectively. None of aqueous extracts showed significant cytotoxicity. The analysis of the essential oil of *H. persicum* showed the hexyl butyrate and octyl acetate as the main compounds. These results suggest some limitation for using of these spices in diet. Furthermore, these plants could be considered as a source of cytotoxic compounds which might be studied in more details.

Keywords: Cytotoxicity; *Artemia salina*; spice; brine shrimp; essential oil.

INTRODUCTION

Spices and herbs are a part of the daily food in several parts of the world, comprise the most important products used for flavoring foods and play a major role in concepts of illness and curing. Next to their importance for general well-being, they are quite often parts of traditional formulae (Mandeel and Al-Laith, 2007). Furthermore diets rich in bioactive phytochemicals reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular disease and oxidative dysfunction (Bazzano *et al*., 2003; Lee *et al*., 2004; Sherry *et al*., 2003). Foods containing these phytochemicals not only can provide our diet with certain antioxidant vitamins like vitamin C, vitamin E and provitamin A, but also a complex mixture of other natural substances with antioxidant capacity. Epidemiological, biological and clinical studies have provided various lines of evidences that dietary factors have a profound impact on etiology and prevention of human cancers (Surh and Ferguson, 2003). Therefore, chemoprevention of cancers by nutraceuticals and phytochemicals has become a flourishing research field in the past decade (Surh and Ferguson, 2003; Manson, 2003; Gosslao and Chen, 2004). The potential toxicity of regularly consumed spices, whether as a condiment or a medicinal treatment, is an important consideration when studying the traditional use of the plants.

In Iran, the dietary inclusions of cinnamon, ginger, siahdaneh, turmeric, onions, etc. have been practiced for centuries. The present study was therefore designed to assess the cytotoxicity of four most common spices of parsnip (*Heracleum persicum Desf. ex Fischer,)* cinnamon *(Cinnamomum zeylanicum Blume.,* Lauraceae), ginger (*Zingiber officinale* Rosc., Zingiberaceae) and siahdaneh (*Nigella arvensis* L., Ranunculaceae) using Brine Shrimp Lethality (BSL) assay. These four plants include parts of a common daily diet in Iran, and this will provide a rational basis for their use in phytomedicine as a positive health food supplement or with some precaution as food supplements.

Simple bioassays developed for screening plant extracts to detect plant compounds with relevant biological activities may be used as a guide for fractionating plant extracts. As a rapid preliminary indication of possible adverse effects of spices medicinal remedies, the BSL assay has been used for screening of the plant fractions. The eggs of the brine shrimp, *Artemia salina*, have been used in a simple bench top bioassay which has yielded good results. *A. salina*, with same purine metabolism as that of mammalian cells, has been shown to have a good correlation with anti tumor activity, although drugs that require metabolic activation in the liver may not be detected by *A. salina*. The DNA- dependent RNA polymerases of *A. salina* are also similar to the

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mammalian type (Mc Laughlin *et al*., 1998; Solis *et al*., 1993). The aim of this study was to investigate and compare the cytotoxicity of the essential oils and various extracts of these four spices plants.

MATERIALS AND METHODS

Plant materials

Ginger is one of the widely used spices and has been used in traditional oriental medicines as antimicrobial and antioxidant (Habsah *et al*., 2000), anti-inflammatory (Tjendraputra *et al*., 2001), anti-fungal (Ficker *et al*., 2003), inhibitor of nitric oxide synthesis (Ippoushi *et al*., 2003) and an agent for protecting neuronal cells from amyloid insult (Kim and Kim, 2004). The plant of parsnip is native to Iran and grows in moist ground, floodlands and thickets. Its fruits and stems locally known as "golpar" in Iran, has been used in traditional medicine as antiflatulence. In traditional medicine it has been used for treatment of epilepsy (Kermani, 1988). Its anticonvulsant activity has been reported previously (Sayyah, *et al*., 2005). Immunostimulant properties of *Heracleum maximum* has been reported by Webster and his colleagues (2006). Cinnamon is one of the most widely used spices. Its extracts and essential oil have shown a variety of biological activities including antioxidant (Jayaprakasha *et al*., 2006), antimicrobial (Matan *et al*., 2006), anti-diabetic (Kim, *et al*., 2006) and ovicidal activities (Yang *et al*., 2005). The seeds of *N. arvensis* known as "siahdaneh" have been used in folk medicine in Iran. Its biologic activity and chemical composition has not been known yet, but according to a study carried out by Kokdil and Ylmaz (2005), the percent and composition of fixed oils in various species of *Nigella* have been shown to be similar.

Plant collection and authentication

Ginger and cinnamon were purchased from a local market; siahdaneh and parsnip were collected from Lalehzar and dehbakri region in Kerman province, Iran (June and July, 2006 respectively). Their scientific names were authenticated in department of Pharmacognosy, Faculty of Pharmacy. A voucher specimen of siahdaneh and parsnip (KP1143 and KP1185 respectively) was deposited in herbarium center in Department of Pharmacognosy, Kerman University of Medical Sciences, Faculty of Pharmacy, Kerman, Iran.

Phytochemical screening

These 4 plants were subjected to phytochemical studies for searching the flavonoids, alkaloids, tannins and saponins (Trease and Evans, 1983).

Plant extraction

Preparation of the various extracts

Dried medicinal parts of the plants were ground in a grinder with a 2 mm in diameter mesh, about 500g of dry powder of each plant extracted with petroleum ether, chloroform, diethyl ether, methanol and water consecutively by percolation method for 72h for each solvent. Solvent removal carried out under vaccum to drying.

Isolation of the essential oils

100 g of the air-dried and ground parts of the tested plants were submitted for 4 h to water-distillation using a Cleavenger apparatus. The obtained essential oils were dried over anhydrous sodium sulphate, then stored at 4°C until tested and analyzed.

Gas chromatography/mass spectrometry analysis

GC–MS analysis of the essential oil of parsnip was performed with GC (column, oven temperature, flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC–MS system and literature data (Adams, 2001). Alkanes were used as reference points in the calculation of relative retention indices (RRI).

Toxicity testing against the brine shrimp

Hatching shrimp

Brine shrimp eggs (*Artemia salina* leach) were prepared from the Schillat center in Hormozgan province and were hatched in artificial seawater which prepared by dissolving 38 g of sea salt (Sigma chemicals Co., UK) in 1 L of distilled water. The two glass compartments chamber with several holes on the divider was used for hatching. One compartment was illuminated. After 48 hours incubation at room temperature (25-29°C), nauplii (larvae) were collected by pipette from the lighted side whereas their shells were left in the dark side.

Brine shrimp assay

The collected nauplii were treated with selected concentrations (three dilutions of 10,100 and 1000 µg/ml) of plant extracts and essential oils. Potassium dichromate was used as positive control. This bioassay was done in two modified methods of disk and solution. In the disk method, various concentrations of the fractions were loaded on paper disks (d=0.5cm), air dried and placed in test tubes. 5 ml of artificial sea water was added to the tubes and were shook to give homogenous solution. 10 Active larvae was placed to each tube and subjected under light. Survivors were counted after 24h and the percent of deaths were determined. In solution method, various concentrations of each fraction were dissolved in Dimethylsulfoxide (DMSO) 1%, placed in test tubes. 5 ml of artificial sea water was added and 10 active larvae was placed to the tubes and subjected under light. Survivors were counted after 24h. and the percent of deaths were determined.

STATISTICAL ANALYSIS

The 50% lethal concentration (LC_{50} value) at 95% confidence interval was calculated for each fraction using the probit analysis method described by finney (Meyer *et al*., 1982).

RESULTS

Results of phytochemical screening of the plant

The results of primary phytochemical screening of tested plants show the presence of high amounts of alkaloid and lacking the saponins in all of four tested plants. Two plants of *Z. officinale* and *C. zeylanicum* were found to be rich in flavonoid content (table 1).

Chemical composition of the essential oil

Air-dried fruits of *H. persicum* were subjected to hydrodistillation using a Clevenger apparatus and the pale yellow-colored essential oil was obtained (yield 1.6% v/w). The results of analysis of this essential oil have given in table 2. Nineteen compounds were identified, representing 97.74% of the total oil. The oil profile exhibits that the hexyl butyrate and octyl acetate were the main compounds (38.99% and 22.34% respectively).

The results of extraction

The results of extraction show that the most percent of *H. persicum* seed composed of methanol (ME) and petroleum ether (PTE) extracts (19.34% w/w and 17.47% w/w respectively). This plant has given 1.6% w/w essential oil. Extraction of *N. arvensis* resulted the PTE extract with the most percent (20.63% w/w), and the essential oil about 1.3%w/w. PTE and ME extracts constitute the most percent of the bark of *C. zeylanicum* (17.34% w/w and 14.41% w/w respectively). In the rhizome of *Z. officinale*, chloroform extract (CHE) and ME extracts have shown to be the most (20.88% w/w and 13.76% w/w respectively). This plant has the less percent of the essential oil and the most percent of CHE extract among the tested plants (table 3).

Table 1: Results of the primary phytochemical screening of 4 tested plants

Plant species	alkaloid	flavonoid	saponin	tannin
H. persicum	High	Low	Negative	Medium
N. arvensis	High	Negative	Negative	Low
C. zeylanicum	High	Medium	Negative	High
Z. officinale	High	Medium	Negative	High

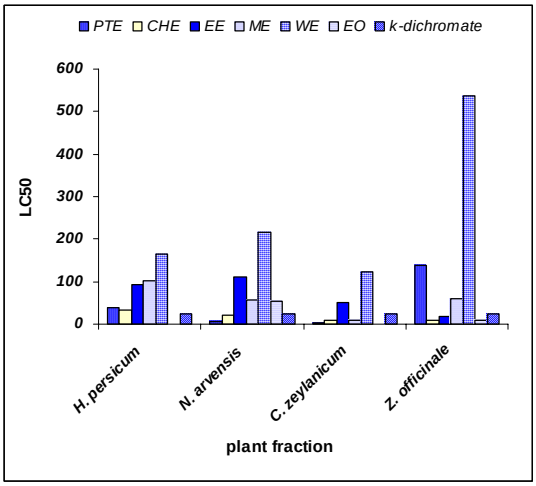

Fig. 1: The mean LC_{50} (µg/ml) confidence interval for the essential oil and various extracts (PTE: Petroleum ether ex.; CHE: Chloroform ex.; EE: Ether ex.; ME: Methanol ex.; WE: Water ex.; EO: Essential oil) from 4 plants screened using BSL assay- Solution method. The 50% lethal concentration (LC_{50} value) at 95% confidence interval was calculated for each plant extract, using the probit analysis method described by finney.

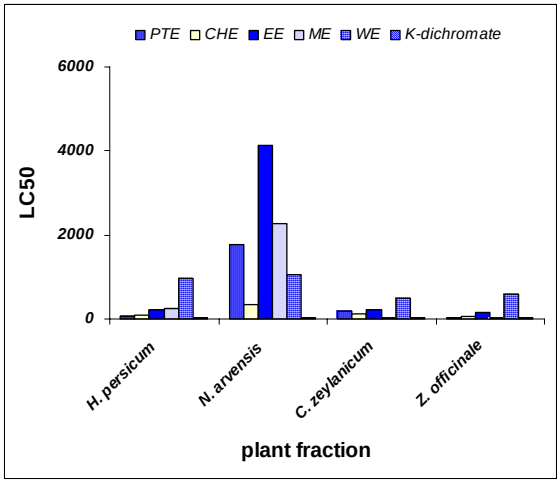

Fig. 2: The mean LC_{50} (µg/ml) confidence interval for the various extracts (PTE: Petroleum ether ex.; CHE: Chloroform ex.; EE: Ether ex.; ME: Methanol ex.; WE: Water ex.; EO: Essential oil) from 4 plants screened using BSL assay- Disk method. The 50% lethal concentration (LC_{50} value) at 95% confidence interval was calculated for each plant extract, using the probit analysis method described by finney.

Table 2: Chemical composition of *Heracleum persicum* essential oil (diluted 1/100 in acetone v/v).

No.	Component	% composition	KI
1	Hexanol	1.07	867
2	Butanoic acid butyl ester	0.84	990
3	Octanal	0.77	998
4	Hexyl acetate	0.59	1005
5	Butyl isovalearate	0.51	1042
6	1- octanol octilin	1.36	1065
7	L- linalool	1.73	1092
8	Hexyl iso butyrate	4.58	1139
9	1- hexanol	0.68	1154
10	Hexyl butyrate	38.99	1182
11	E-4- dodecenyl acetate	7.81	1186
12	Hexyl isovalerate	0.51	1192
13	Octyl acetate	22.34	1198
14	Hexyl-2-methyl butyrate	4.27	1223
15	1- octanol	0.67	1256
16	Octyl isobutyrate	2.02	1326
17	Hexyl caproate	1.57	1366
18	Octyl butyrate	0.9	1369
19	N- octyl-2-methyl butyrate	2.25	1410

Relative percentages of the compounds were obtained electronically from FID area percent data
KI: Kovats index on non-polar DB-5 ms column in reference to n-alkanes

The results of bioassay

The results of the toxicity of essential oils and various extracts of 4 medicinal plants against brine shrimp in two methods of disk and solution are shown in Fig: 1 and 2. *In vitro* cytotoxicity of the studied plants in the BSL assay, expressed as LC_{50} values, alongside the confidence limits at 95% interval. A total of 24 extracts and essential oils were tested for their toxicity using the BSL assay. The cytotoxicity values ranged from 0.007 to 535.6µg/ml in the solution method and 28.3 to 7.3µg/ml in the disk method. The upper and lower LC_{50} in two methods are considerable. Among the tested fractions, the essential oils of *H. persicum* and *C. zeylanicum* have shown the most significant cytotoxicity against brine shrimp larvae with LC_{50} values 0.007 and 0.03µg/ml respectively. PTE extracts of *C. zeylanicum* and *N. arvensis*, ME extract of *C. zeylanicum*, and CHE extract and essential oil of *Z. officinale* showed significant activity with LC_{50} values 4.03, 7, 7.9, 9 and 10 µg/ml respectively in comparison to k-dichromate with LC_{50} value of 25.5 µg/ml. None of the aqueous extracts showed significant cytotoxicity (figs. 1, 2).

DISCUSSION

Chemoprevention of cancers by neutraceuticals and phytochemicals has become a flourishing research field in the past decade (Surh and Ferguson, 2003; Manson, 2003; Gosslao and Chen, 2004). As a part of our interest in the safety of Iranian herbal spices and condiments, we have evaluated cytotoxicity of various extracts and essential oils of 4 plants which have been used widely as spices by using the BSL assay. None of these plants have been assessed for in vitro cytotoxicity. The essential oil, PT and CH extracts of the *H. persicum* showed strong cytotoxicity with LC_{50} value of 0.007, 38.4 and 33.8 µg/ml respectively in comparison to K-dichromate (LC_{50} = 25.5 µg/ml). The other extracts exhibited lesser activity. The analysis of the chemical composition of the essential oil of *H. persicum* shows the octyl acetate and hexyl butyrate as the main compounds. There is a report in the literature about the cytotoxicity of the *Heracleum sphondylium*. The major compounds of the latter oil have been reported to be similar to the *H. persicum* (Ugur, 1998). However more studies are needed to determine cytotoxic compounds of the oil of *H. persicum*. These investigations have been done and their results will be reported later. The presence of furocoumarines has also been reported in the genus of *Heracleum* (Doi *et al*., 2004). Among the various fractions of the *N. arvensis*, PT and CH extracts have exhibited the most cytotoxicity (LC_{50} of 7.2 and 21.4µg/ml respectively). The PT extract (LC_{50} of 7.2 µg/ml) constitutes high percent of the *N. arvensis*, therefore this plant has high potency of cytotoxicity. The phytochemical screening of this plant has exhibited high percent of alkaloids. The low cytotoxicity of this plant in disk method may be attributed to its fatty acid content. There are many reports in literature for cytotoxicity of the another species, *N. sativa* (Islam *et al*., 2004; Thabrew *et al*., 2005). Almost all

Table 3: The percentage (w/w) of essential oil and each extract from tested plants

Plant species	Used part	Essential oil	Petroleum ether ex.	Chloroform ex.	Ether ex.	Methanol ex.	Aqueous ex.
H. persicum	Fruit	1.6	17.47	1.95	12.50	19.34	1.32
N. arvensis	Seed	1.3	20.63	1.92	6.87	8.16	4.12
C. zeylanicum	Bark	1.7	1.7	3.93	2.20	14.41	8.55
Z. officinale	rhizome	0.67	1.84	1.84	6.90	13.76	1.95

fractions of *C. zeylanicum* have exhibited strong cytotoxicity. The essential oil of the plant has shown the most activity (LC_{50} = 0.03). The presence of trans cinnamicaldehyde in the essential oil of *C. zeylanicum* might have been responsible for the observed cytotoxicity. The cytotoxicity of cinnamicaldehyde acid has been reported previously (Kwon *et al*., 1998). The high cytotoxicity of the PT and CH extracts of this plant may be due to their essential oil content. The ME extract of this plant has shown the most activity among the ME extracts of four tested plants (LC_{50}= 7.9 µg/ml). This activity of ME extract of *C. zeylanicum* can be related to the presence of its high amounts of flavonoids, tannins and polyphenols. Water extract of the plant is the only fraction with little cytotoxic activity. Finally all of the extracts and essential oil of *Z. officinale* have exhibited strong cytotoxicity except the PT and aqueous extracts. Anti tumor activity of two compounds of zingiberene and gingerol has been studied (Chrubasik *et al*., 2005) and it seems that at least some of the toxicity of the oil of *Z. officinale* may be attributed to these compounds. The cytotoxicity of the species of *Z. cassumunar* and *Z. zerumbone* has been reported (Han, 2004; Kluwer, 2003).

CONCLUSION

In general, the results drawn from the present work show that, all of these 4 tested plants have exhibited considerable cytotoxicity by BSL assay. Most of the activity of these plants is due to their essential oils. For determining the limits of safety of these plants, extensive *in vitro* and *in vivo* toxicological studies and animal assays must be undertaken. The results of this work suggested that the use of these plants, as spices in a food or drug for humans or as neutraceutical, should be treated cautiously and caution should be exercised in the use of the other herbal spices preparations until exhaustive phytochemical and bioassay-guided fractionation of the components have been achieved. It is also probable to access some compounds with cytotoxicity activity from these herbal sources, however more investigations are needed for logical conclusion.

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REFERENCES

Adams RP (2001). *Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy*. Illinois, Allured Publishing Corporation, USA.

Bazzano LA, Serdula MK and Liu S (2003). Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Current Atherosclerosis Reports*, **5**: 492-499.

Chrubasik S, Pittler M.H, Roufogalis BD (2005). Zingiberis rhizome: A comprehensive review on the ginger effect and efficacy profile. *Phytomedicine*, **12**: 84-701.

Doi M, Nakamori T, Shibano M, Taniguchi M, Wang NH, Baba K and Candibirin A (2004). A furanocoumarin dimer isolated from *Heracleum candicans*. *Acta Crystallographica Section C,* **60**: 833-835.

Ficker C, Smith ML, Akpagana K, Gbeassor M, Zhang J, Durst T, Assabgui A and Arnason JT (2003). Bioassay-guided isolation and identification of antifungal compounds from ginger. *Phytotherapy Research,* **17**: 897-902.

Gosslao A and Chen, KY (2004). Neutraceuticals, apoptosis and disease prevention. *Nutrition,* **20**: 95-102.

Habsah M, Amran M, Mackeen MM, Lajis NH, Kikuzaki H, Nakatani N, Rahman, AA and Ghafar Ali, AM (2000). Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *Journal of Ethnopharmcology*, **72**: 403-410.

Han AR (2004). A new cytotoxic phenyl butenoid dimmer from the rhizomes of *Z. cassumnar. Planta Medica,* **70**; 1095-1097.

Ippoushi K, Azuma K, Ito H, Horie H and Higashio, H (2003). [6]-Gingerol inhibits nitric oxide synthesis in activated J774.1 mouse macrophages and prevents peroxynitrite-induced oxidation and nitration reactions. *Life Science*, **73**: 3427-3437.

Islam SN, Begum P, Ahsan T, Huque S and Ahsan M (2004). Immuno suppressive and cytotoxic properties of *Nigella sativa*. *Phytotherapy Research,* **18**: 395-398.

Jayaprakasha GK, Ohnishi-Kameyama M, Ono H, Yoshida M and Jaganmohan Rao L (2006). Phenolic constituents in the fruits of *Cinnamomum zeylanicum* and their antioxidant activity. *J. Agric. Food Chem*., **54**: 1672-1679.

Kermani MKK (1988). *Daghayegh-ol-Eladj*, Saadat Press, Kerman, p.365.

Kim DSHL and Kim JY (2004). Side-chain length is important for shogaols in protecting neuronal cells from β-amyloid insult. *Bioorganic and Medicinal Chemistry Letters,* **14**: 1287-1289.

Kim SH, Hyun SH and Choung SY (2006). Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacol.*, **104**: 119-123.

Kluwer W (2003). *Guide to popular natural products*. 3rd ed., St. Louis, Fact and Comparison, pp.115-115.

Kokdil G and Ylmaz H (2005). Analysis of the fixed oils of the genus *Nigella* (Ranunculaceae) in Turkey. *Biochemical Systematics and Ecology,* **33**: 1203-1209.

Kwon BM, Lee SH, Choi SU, Park SH, Lee CO, Cho YK, Sung ND and Bok SH (1998). Synthesis and *in vitro* cytotoxicity of cinnamaldehydes to human solid tumor cells. *Archives of Pharmacal. Research*, **21**: 147-152.

Lee KW, Lee HJ and Lee CY (2004). Vitamins, phytochemicals, diets, and their implementation in cancer chemoprevention. *Critical Review Food Science Nutrition*, **44**: 437-452.

Mandeel QA and Al-Laith AA (2007). Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. *Journal of Ethnopharmacol.*, **110**: 118-129.

Manson MM (2003). Cancer prevention: the potential for diet to modulate molecular signaling. *Trends in Molecular Medicine*, **9**: 11-18.

Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V and Parker M (2006). Anti-microbial activity of cinnamon and clove oils under modified atmosphere conditions. *International Journal of Food Microbiology,* **107**: 180-185.

Mc Laughlin JL, Roger LL and Anderson JE (1998). The use of biological assays to evaluate botanicals. *Drug Information Journal*, **32**: 513-524.

Meyer BN, Ferrigni NR and Putnam JE (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, **45**: 31-34.

Sayyah M, Moaied S and Kamalinejad M (2005). Anticonvulsant activity of *Heracleum persicum* seed. *Journal of Ethnopharmacol.,* **98**: 209-211.

Sherry E, Sivananthan S, Warnke PH and Eslick GD (2003). Topical phytochemicals used to salvage the gangrenous lower limbs of type 1 diabetic patients. *Diabetes Research and Clinical Practice, **62***: 65-66.

Solis PN, Wright CW, Anderson M, Gupta MP and Phillipson JD (1993). A microwell cytotoxicitiy assay using *Artemia salina* (brine shrimp). *Planta Medica,* **26**: 250-252.

Surh Y-J and Ferguson LR (2003). Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential -highlights of a symposium. *Mutation Research*, **523/524**: 1-8.

Thabrew MI, Mitry RR, Morsy MA and Hughes RD (2005). Cytotoxic effects of a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. *Life Science*, **77**: 1319-1330.

Tjendraputra E, Tran VH, Liu-Brennan D, Roufogalis BD and Duke CC (2001). Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorganic Chemistry*, **29**: 156-163.

Trease GE and Evans WC (1983). Pharmacognosy. Bailliere Tindall Press, London, pp.309-706.

Ugur MS (1998). Cytotoxicity assay and fibrinolytic evaluation of *H. sphondylium* and *Ferula thirreana. Fitotherapia,* **4**: 338-340.

Webster D, Taschereau P, Lee TD and Jurgens TJ (2006). Immunostimulant properties of *Heracleum maximum* Bartr. *Journal Ethnopharmacol.*, **106**: 360-363. Epub 2006 Feb 28.

Yang YC, Lee HS, Lee SH, Clark JM and Ahn YJ (2005). Ovicidal and adulticidal activities of *Cinnamomum zeylanicum* bark essential oil compounds and related compounds against *Pediculus humanus capitis* (Anoplura: Pediculicidae). *International Journal of Parasitoogy,* **35**: 1595-600.