

Bioactive polyphenol profiling and *in-vitro* antioxidant activity of *Tinospora cordifolia* Miers ex Hook F and Thoms : A potential ingredient for functional food development

T. A. Khan¹, A. H. Ipshita², R. M. Mazumdar¹, A. T. M. Abdullah¹, G. M. R. Islam² and M. M. Rahman^{1*}

¹*Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh.*

²*Department of Food Engineering & Tea Technology, Shahjalal University of Science & Technology, Sylhet, Bangladesh*

Abstract

Antioxidant activities and specific bioactive polyphenol content of methanol and ethanol extracts of the stem of *Tinospora cordifolia* Miers were evaluated in the present investigation. Phenolic compounds were identified by High Performance Liquid Chromatography (HPLC). Results revealed that absolute methanol extract had the lower IC₅₀ value. DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) was scavenged 50% by 9.36±1.75 mg/ml methanol extract. Flavonoid, tannin and phenolic contents were found comparatively higher in methanol extract. The study implied that the methanol extract had higher antioxidant activity than the ethanolic extract and *T. cordifolia* stem can be used as a source of biologically active polyphenolic compounds as well as antioxidants to develop functional food.

Received: 08 January 2019

Revised: 18 June 2019

Accepted: 01 July 2019

DOI: <https://doi.org/10.3329/bjgir.v55i1.46729>

Keywords: *Tinospora cordifolia*; Antioxidant activity; DPPH; Functional food; Polyphenol

Introduction

The natural oxidation process produces energy to operate biological cycles and causes the formation of free radicals or reactive oxygen species (ROS). The uncontrolled and excessive production of free radicals such as hydroxyl radical, hydrogen peroxide, etc. causes damage to the body as well as assistive oxidative stress (Rao *et al.*, 2011). Many diseases like arthritis, atherosclerosis, Alzheimer's, Parkinson's, gastro intestinal dysfunctions, tumor promotion and carcinogenesis are caused by free radicals (Bagchi *et al.*, 2000). Chronic deteriorative diseases such as diabetes, cancer, hypertension, coronary artery disease, etc. are also caused by oxidative damage in DNA, protein and lipids (Lee *et al.*, 2000).

Antioxidant is very effective to destroy the activity of free radicals as antioxidants inhibit the initiation of oxidizing chain reaction and delay the oxidation of molecules. Natural antioxidants comprised of phenolic and nitrogen compounds, carotenoids, etc. (Kaneria *et al.*, 2009). The antioxidant effect of plant products is functional mainly because of phenolic

compounds like flavonoids and tannins (Nagavani *et al.*, 2010) which trap or scavenge free radicals directly or through a series of coupled reactions with the help of enzymes. As a result, a lot of biological consequences such as anti-ageing, anti-mutagenicity and protective effects on oxidative stress take place (Caputo *et al.*, 2004). Flavonoids are polyphenolic compounds that occur ubiquitously in plant origin exhibit various physiological activities. Interest in medicinal plants has gradually grown in the form of natural cosmetics or medications for their biological impacts on humans. Herbal products are mainly supplemented to the diet with the intention of improving life quality as well as preventing diseases (Maffei, 2003).

Tinospora cordifolia Miers. (Fam.: Menispermaceae), a large deciduous climbing shrub has been used mainly in folk and Ayurvedic medicines. It is found in Bangladesh, India, China, Myanmar and Sri Lanka (Gurav *et al.*, 2014). Locally it is known as Gulancha. It is widely used for its antioxidant, anti-diabetic, anti-spasmodic,

*Corresponding author e-mail: sharifbmb@yahoo.com

anti-allergic and anti-inflammatory properties (Singh *et al.*, 2003). From earlier studies, some other properties of *T. cordifolia* were confirmed, such as: anti-HIV and anti-tumor (Kalikar *et al.*, 2008, Jagetia *et al.*, 2006).

Finding new and safe antioxidants from natural sources is of great interest for applications in functional foods and nutraceuticals. Phytochemical screening is one of the methods to explore antioxidant compounds in plants. The purpose of the present study was to evaluate the antioxidant activities of the methanolic and ethanolic extract of *T. cordifolia* stems and to identify the specific polyphenols from both the extracts by HPLC.

Materials and methods

Sample extraction

The collected plant parts (stems) were separated from undesirable materials or parts manually. The sample was first oven dried at 60°C for 3 days. Then the dried sample was powdered by grinding machine. From this powder 5 g sample in duplicate was taken and dissolved in each of 50 ml methanol and ethanol separately in a round joint conical flask and mixed by a table top shaker for 48 hours. Then the sample was centrifuged and filtered and concentrated by vacuum rotary evaporator. The concentrate was increased to 10 ml by adding extra amount of respective solvents to make the stock solution. Samples were stored at 4°C for further analysis.

Chemicals and reagents

Analar grade reagent of 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH), Folin-Ciocalteu reagent, Aluminum Chloride (AlCl₃), Sodium Acetate, Vitamin C, 28 mM Tri-Sodium Hydrogen Phosphate (Na₃PO₄), 35% Sodium Carbonate (Na₂CO₃), Ammonium Molybdate, 0.6 M H₂SO₄, Potassium Di-Hydrogen Phosphate (KH₂PO₄) from Sigma Aldrich were used in the experiment.

Determination of total flavonoid content

In this experiment, all the spectrophotometric operations were carried out with the help of a double beam Thermo Scientific UV-VIS spectrophotometer (Evolution 300, USA). The flavonoid content was determined with the help of spectrophotometer following Jay *et al.* (1975). For the calibration curve, 0.01 g of Quercetin was dissolved in 100 ml of methanol using ultrasonic bath and vortex to make 100 µg/ml stock solutions. Then 80, 60, 40 and 20 µg/ml solutions were prepared by serial dilution of standard solution from the stock solution and a calibration curve was obtained (Fig. 1). Total flavonoid content was determined as mg of equivalent

per gram using the equation $y=0.009x+0.002$, $R^2=0.999$ of calibration curve (Fig. 2). The results of flavonoids are expressed in terms of Quercetin equivalent (mg per g of dry mass), which is a common reference compound.

Determination of total tannin content

The tannins were determined using the Folin-ciocalteu Phenol reagent after Amorim *et al.* (2008). A set of standard solutions of tannic acid was read against a blank. The results of tannins were expressed in terms of tannic acid in mg/100g of dry extract. Total tannin content was determined as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve $y=0.008x+0.005$, $R^2=0.997$ (Fig. 3).

Determination of total phenolic content

The contents of total phenolic were determined using the Folin-ciocalteu Phenol reagent (Amorim *et al.* 2008). The results of phenolics were expressed in terms of Gallic acid in mg/100g of dry extract. Total phenolic content was determined as mg of Gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve $y=0.009x-0.010$, $R^2=0.999$.

Determination of antioxidant activity

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay method which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition (Prieto *et al.*, 1999). The absorbance (OD) of the solution was measured at 695 nm with help of spectrophotometer against appropriate blank. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid. Antioxidant activity was determined as mg of AAE equivalent per gram using the equation obtained from a standard AAE calibration curve $y=0.008x-0.013$, $R^2=0.996$.

Determination of DPPH radical scavenging activity

The stable DPPH radical-scavenging activity was measured using the modified method described by Chang *et al.* (2001). A quantity of 2 ml of 0.2 mM methanol DPPH solutions was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for few seconds. Then the solutions were allowed to stand at dark place at room temperature for 10 minutes for reaction to occur. After 10 minutes the absorbance was measured against a blank at 517 nm. The percentage of DPPH radical-scavenging activity of each plant extract was calculated as: DPPH radical-

scavenging activity (%I), $= \frac{A_0 - A}{A_0} \times 100$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extracts); A is the absorbance of the DPPH solution containing plant extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50% (IC_{50}). Ascorbic acid was used as positive control standard.

Specific polyphenols identification by HPLC

Arbutin (AR), Caffeic acid (CFA), (+)-Catechin Hydrate (CA), trans-Cinnamic acid (TCA), Ellagic acid (EA), (-)-Epicatechin (ECA), Trans-Ferulic acid (TFA), Gallic acid (GA), Hydroquinone (HQ), Kaempferol (KAEM), Myricetin (MYR), p-Coumaric acid (PCA), Quercetin (QH), Rosmarinic acid (ROS), Rutin Hydrate (RH), Syringic acid (SYR), Vanillic acid (VA), Vanillin (VAN) were used as standards and purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade Acetonitrile, Methanol and Acetic acid were obtained

from E. Merck, Germany. For the preparation of sample MeOH and EtOH were used. The compounds were identified by comparing with standards. Each compound was identified using the retention time, the absorbance spectrum profile and also by running the samples after the addition of pure standards.

Standard preparation

A stock standard solution (100 $\mu\text{g/ml}$) of each phenolic compound was prepared in methanol by weighing out approximately 5 mg of the analyte into 50 ml volumetric flask. The mixed standard solution was prepared by dilution using the mixed stock standard solutions in methanol to give a concentration of 5 $\mu\text{g/ml}$ for each polyphenols except (+)-catechin hydrate (4 $\mu\text{g/ml}$), caffeic acid (4 $\mu\text{g/ml}$), rutin hydrate (4 $\mu\text{g/ml}$) and quercetin (3 $\mu\text{g/ml}$). All standard solutions were stored in the dark at 4°C. The calibration curves of the standards were made by a dilution of the stock standards with methanol to yield 1.0-5.0 $\mu\text{g/ml}$. The calibration curves were constructed from peak area vs. concentration of standard.

Table I. Chromatographic conditions of HPLC

Sl no.	Retention (min)	Flow (ml/min)	% Solvent B	% Solvent A	% Solvent C
1	0.00	1.00	95.0	0.0	5.0
2	0.00	1.00	95.0	0.0	5.0
3	10.00	1.00	90.0	0.0	10.0
4	15.00	1.00	85.0	5.0	10.0
5	20.00	1.00	70.0	15.0	15.0
6	25.00	1.00	70.0	15.0	15.0
7	30.00	1.00	60.0	20.0	20.0
8	35.00	1.00	40.0	30.0	30.0
9	40.00	1.00	0.0	0.0	100.0
10	44.00	1.00	95.0	0.0	5.0
11	45.00	1.00	95.0	0.0	5.0

Sample preparation

From the stock solution (MeOH and EtOH respectively), 1 ml sample was taken and the volume was increased to 10 ml by adding respective solvent. Around 1ml sample was filtered by 0.45 μm filter (Nylon) into a vial.

HPLC system

Chromatographic analyses were carried out on a Rapid Separation LC (RSLC) systems (Dionex UltiMate 3000, Thermo Fisher Scientific Inc., MA, USA), coupled to a quaternary rapid separation pump (LPG-3400RS), Ultimate 3000RS auto sampler (WPS-3000) and rapid separation diode array detector (DAD-3000RS). Phenolic compounds were separated on a Acclaim® Polar Advantage II (PAII) C18 (4.6 \times 250 mm; 5 μm ; 120 $^{\circ}\text{A}$) column (Dionex, USA) which was controlled at 30 $^{\circ}\text{C}$ using a temperature controlled column compartment (TCC-3000). Data acquisition, peak integration, and calibrations were performed with Dionix Chromeleon software (Version 6.80 RS 10).

Chromatographic conditions

The phenolic composition of the stems of *T. cordifolia* was determined by HPLC, as described by Sarunya and Sukon (2006) with some modifications. The mobile phase consisted

of Acetonitrile (solvent A), Acetate buffer pH 3.0 (solvent B) and Methanol (solvent C). The system was run with the following gradient elution program Table I:

There was a 5 min post run at initial conditions for equilibration of the column. The flow rate was kept constant throughout the analysis at 1 ml/min and the injection volume was 20 μl . For DAD detection, the wavelength program was optimized to monitor phenolic compounds at their respective maximum absorbance. The detection and quantification of GA, CH, VA, CA, and EC was done at 280 nm, of PCA, RH, and EA at 320 nm, and of QU at 380 nm, respectively.

Peak characterization and quantification

Quantification was performed by establishing calibration curves for each compound determined, using the standards. Linear calibration curves for standards (peak area vs. concentration) were constructed with R^2 exceeding 0.995 (Figs 1,2,3,4 and 5).

Statistical analysis

The results were calculated as mean \pm SD. Appropriate statistical analyses were performed by using SPSS software version 16. $p < 0.05$ were considered as statistically significant.

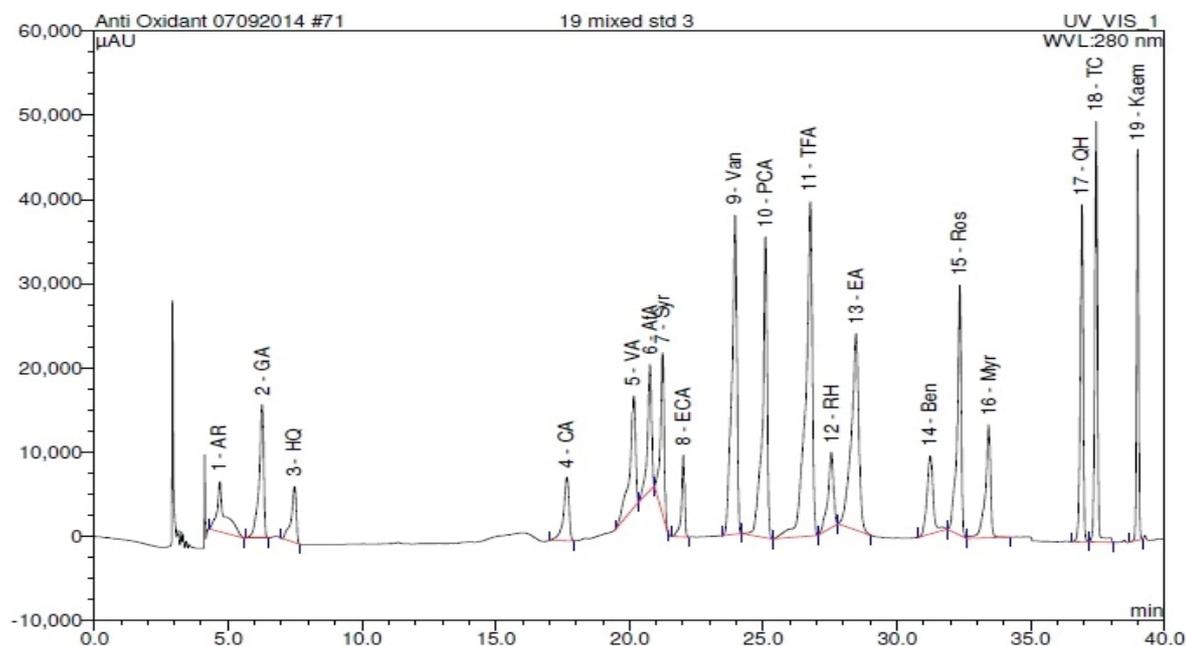


Fig. 1. HPLC chromatogram of Standard phytochemicals

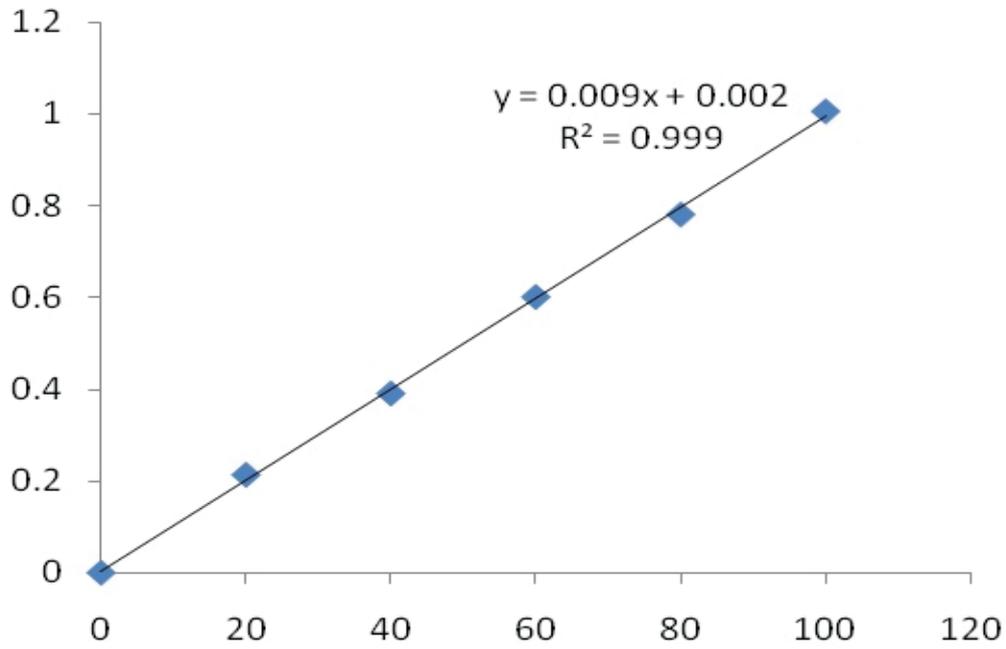


Fig. 2. Standard curve of Quercetin

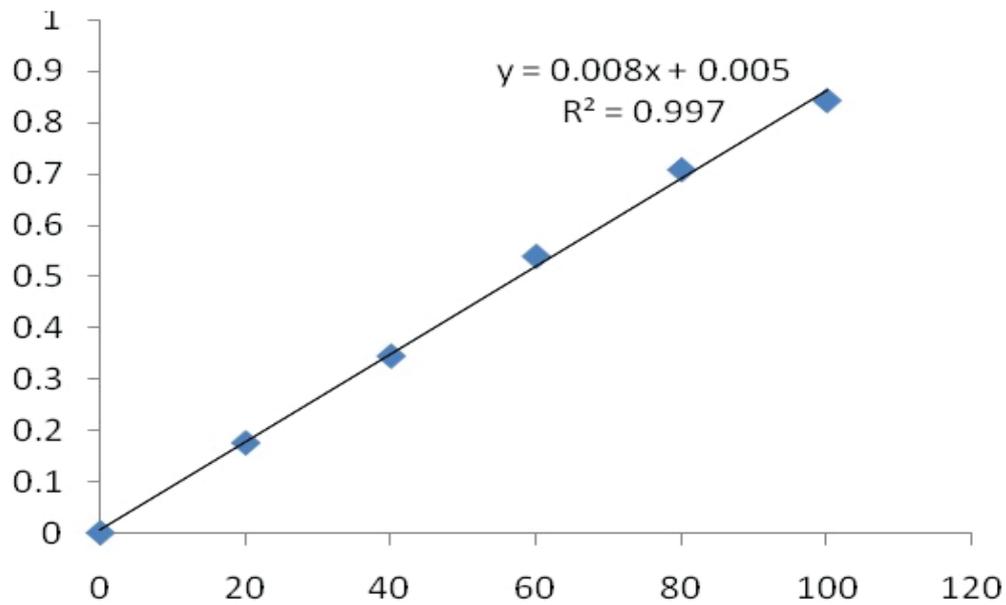


Fig. 3. Standard curve of Tannic acid

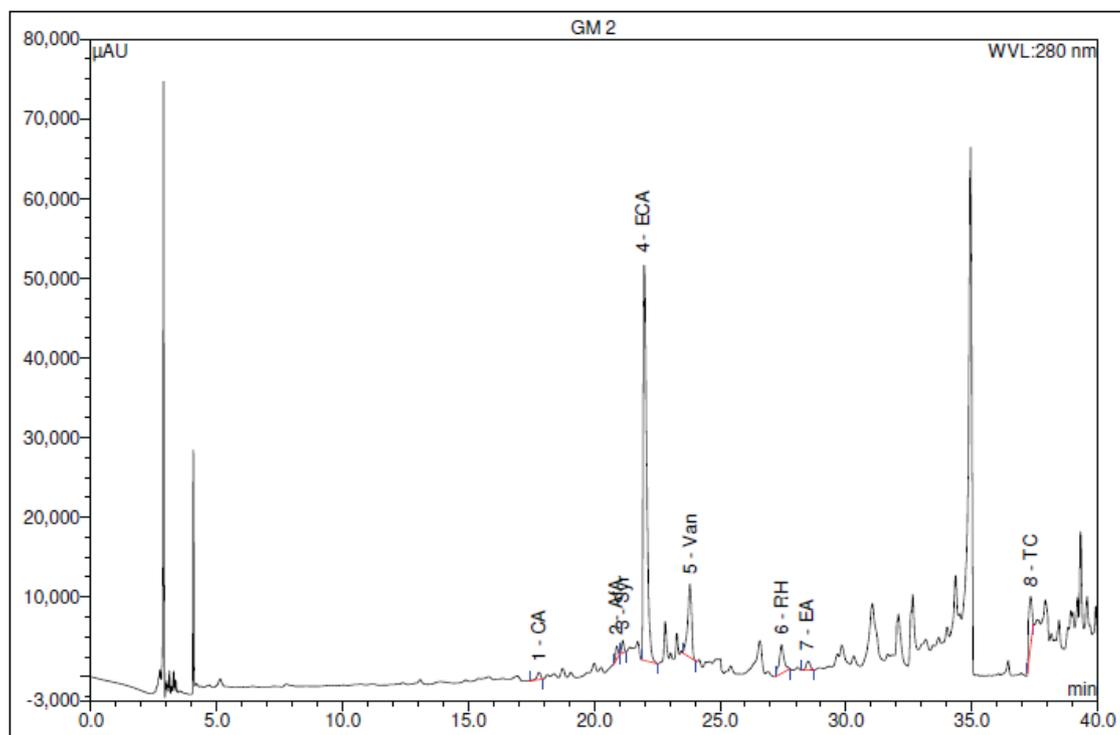


Fig. 4. HPLC Peak diagram of *T. cordifolia* stem MeOH extract

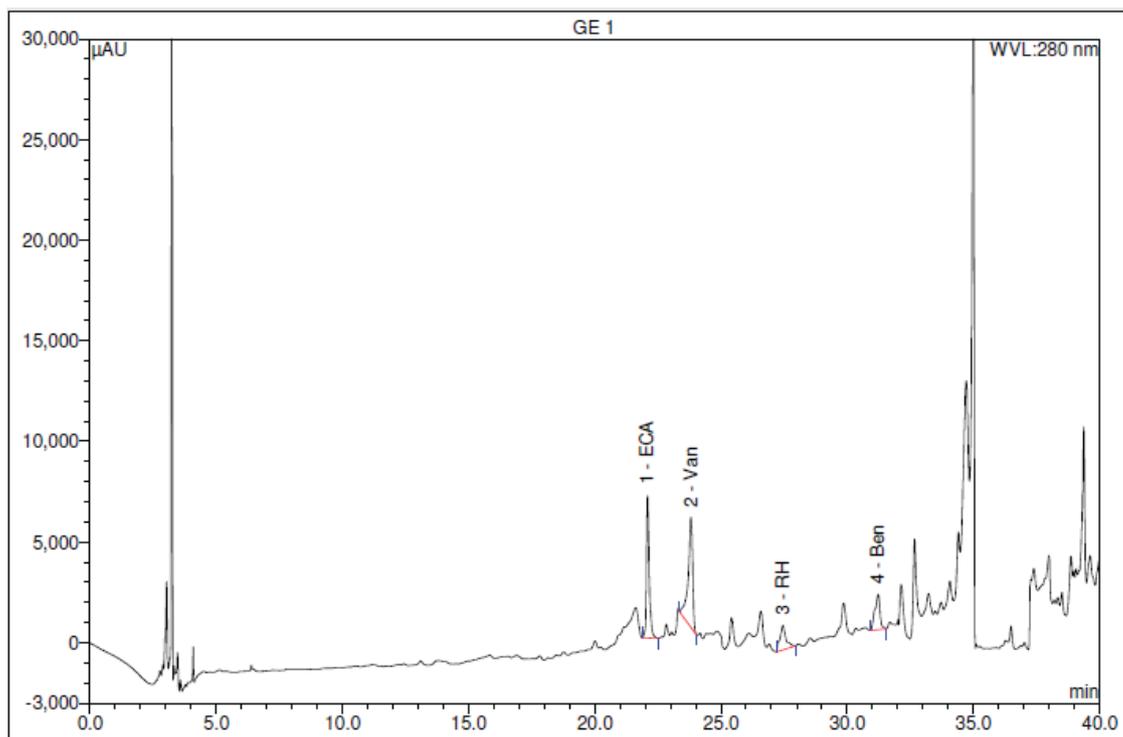


Fig. 5. HPLC Peak diagram of *T. cordifolia* stem EtOH extract

Results and discussion

Result of Table II shows that methanol extracted higher amount of polyphenol (195.45 ± 18.88 mg GAE/g extract). Similarly the tannin (204.39 ± 19.66 mg TAE/100g extract) and flavonoid (21.97 ± 0.98 mg QE/100g) content also showed to be higher in methanolic extract compared to ethanol extract. Specific polyphenols Arbutin (AR), Caffeic acid (CFA), (+)-Catechin Hydrate (CA), trans-Cinnamic acid (TCA), Ellagic acid (EA), (-)-Epicatechin (ECA), Trans-Ferulic acid (TFA), Gallic acid (GA), Hydroquinone (HQ), Kaempferol (KAEM), Myricetin (MYR), p-Coumaric acid (PCA), Quercetin (QH), Rosmarinic acid (ROS), Rutin Hydrate (RH), Syringic acid (SYR), Vanillic acid (VA), Vanillin (VAN) in *T. cordifolia* stem were measured from both the extracts. Sivakumar *et al.* (2010) reported high amounts of glycosides, alkaloids, tannins, phenolics, and all other principal secondary metabolites in methanolic extract of *T. cordifolia* stem. In the determination of total flavonoid content, methanolic extract showed the presence of most flavonoids (21.97 ± 0.98), followed by ethanol extract (12.51 ± 1.18). So, it is certain that methanol serves as a better solvent for the extraction of phenolics and flavonoids than ethanol (Table III).

The methanolic extract exhibited better free radical scavenging activity compared to ethanol extracts. Sivakumar *et al.* (2010) found 85% antiradical scavenging activity of the methanolic extract of *T. cordifolia* stem. The total phenolic content calculated in methanol extract of *T. cordifolia* was higher (195.45 ± 18.88 mg/g of Gallic acid equivalent) than ethanol extract (121.67 ± 6.54). Phytochemical components, especially polyphenols, such as flavonoids, phenylpropanoids, phenolic acids, etc. are responsible for the free radical scavenging and antioxidant activities of plants. Certain flavonoids are also reported as potent free-radical scavengers (Hossain *et al.* 2016).

Antioxidant activity and IC_{50} value

Total antioxidant activity of extracts was analyzed by the formation of phosphomolybdenum (PM) complex based on the reduction degree of Mo (VI) to Mo (V). MeOH extract showed higher antioxidant capacity equal to 277.97 ± 16.72 AAE/100g extract compared to 160.62 ± 6.91 AAE/100g activity of EtOH extract.

DPPH radical scavenging activity of the stem extracts were evaluated (Table 2). The IC_{50} value was calculated and in general, the samples with the low IC_{50} are potent scavenger than the samples with high IC_{50} value. Methanolic extract was found to be the effective radical scavenger of DPPH radical with the lower IC_{50} value (9.36 ± 0.01 mg/ml) of the stem

extracts. In the present study, methanolic extract showed higher antioxidant activity than the ethanol extract as it contains high concentration of total phenols and flavonoids. The finding is in consistent with the study of Ilaiyaraja and Khanum (2011).

T. cordifolia stem exhibited excellent antioxidant activity in methanol extracts (Fig 6). They were effective in scavenging superoxide anion radical and inhibited deoxyribose degradation induced by hydroxyl radical, scavenging them directly rather than via chelating iron ion. In addition, they possess phytochemicals such as polyphenols, flavonoids and tannins which attribute to a strong free radical scavenging activity. Antioxidant rich plant extracts serve as source of nutraceuticals that alleviate the oxidative stress and therefore prevent or reduce the onset of degenerative diseases. It is well-known that plant phenolics, in general, are highly effective free radical scavengers and antioxidants. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups (Aberoumand and Deokule 2008). The result also showed that the methanol extract had the least IC_{50} value (Table II). The lower the IC_{50} value of a compound, the higher its radical scavenging activity (Maisuthisakul *et al.*, 2007). Thus the methanol extract of this plant possesses the stronger ability to scavenge DPPH radical as compared to ethanol extract.

Antioxidant capacity may be associated with high phenol content, as Mansouri *et al.* (2005) reported that most of the antioxidant activity of plants is derived from phenols. The antioxidant activity of this type of molecule is due to their ability to scavenge free radicals and other properties like donating hydrogen atoms or electrons or chelating metal cations (Amarowicz *et al.*, 2004).

The major peaks for the specific polyphenols were identified by comparing with authentic standards (Fig. 1). The major phenolic compounds identified in the methanol extract of *T. cordifolia* stem were (-)-Epicatechin (ECA), Vanillin (VAN), Rutin Hydrate (RH), Syringic acid (SYR), Caffeic acid (CFA), (+)-Catechin Hydrate (CA), Ellagic acid (EA). In EtOH extract ECA, VAN, and RH were identified comparing with the standards. Both the extracts of *T. cordifolia* showed presence of (-)-Epicatechin (ECA), Vanillin (VAN), Rutin Hydrate (RH). Sharma *et al.* (2010) reported the presence of many compounds like Flavonoids, glycosides, saponins and some amount of phytosterols. These active constituents alone or in combination may be responsible for the observed antioxidant activity (Onkar *et al.*, 2012). The physiological activity and importance of the compounds have been reported in other studies.

Table II. Comparison of flavonoid content, tannin content, phenolic content, antioxidant activity and IC₅₀ value

Parameters	MeOH Extract	EtOH Extract
Flavonoid Content (mg QE/100g of dry extract)	21.97±.98 ^a	12.51±1.18 ^b
Tannin Content (mg TAE/100g of dry extract)	204.39±19.66 ^a	125.90±6.69 ^b
Phenolic Content (mg GAE/100g of dry extract)	195.45±18.88 ^a	121.67±6.54 ^b
Antioxidant activity (mg AAE/100g of dry extract)	277.97±16.72 ^a	160.62±6.91 ^b
IC ₅₀ (mg/ml)	9.36±1.75 ^b	27.40±1.09 ^a

Table III. Contents of polyphenolic compounds in the extracts

Phenolic compounds	MeOH Extract mg/100g (Mean±SD)	EtOH Extract mg/100g (Mean±SD)
(-)-Epicatechin (ECA)	120.91±5.90	14.02±.36
Vanillin (VAN)	2.57±2.57	1.52±.09
Rutin Hydrate (RH)	8.57±.06	3.50±.15
(+)-Catechin Hydrate (CA)	3.95±.58	-
Caffeic acid (CFA)	0.82±.02	-
Syringic acid (SYR)	0.67±.11	-
Ellagic acid (EA)	1.25±.17	-
trans-Cinnamic acid (TCA)	0.79±.12	-

From methanol extraction ECA 120.91 mg/100g, VAN 2.57 mg/100g, RH 8.57 mg/100g were found. However, ECA, VAN and RH were found 14.02 mg/100g, 1.52 mg/100g, 3.50 mg/100g, respectively in ethanol extract. It indicates that methanol is better extractor than ethanol. The nature and content of polyphenolics varies among plants, which possess beneficial properties, such as antioxidant, immune modulatory actions and anticancer and antibacterial activity (Yusri *et al.* 2012).

(-) Epicatechin (ECA) was identified and quantified by HPLC found with higher concentration in methanol extract

compared to ethanol extract which is in agreement with Ghasemzadeh *et al.*, (2011) who indicated that the methanol extraction had high content of Epicatechin. High content of phenolic compounds in the studies showed that the extraction yield of phenolic compounds is greatly depending on the solvent polarity.

Presence of (-) Epicatechin in the methanolic extract of stem of *T. cordifolia* was also reported by Pushp *et al.*, (2013). Epicatechin has been extensively researched for its diverse actions on human health. It has been reported as potent antioxidant, antiviral, antimalarial and anti carcinogenesis

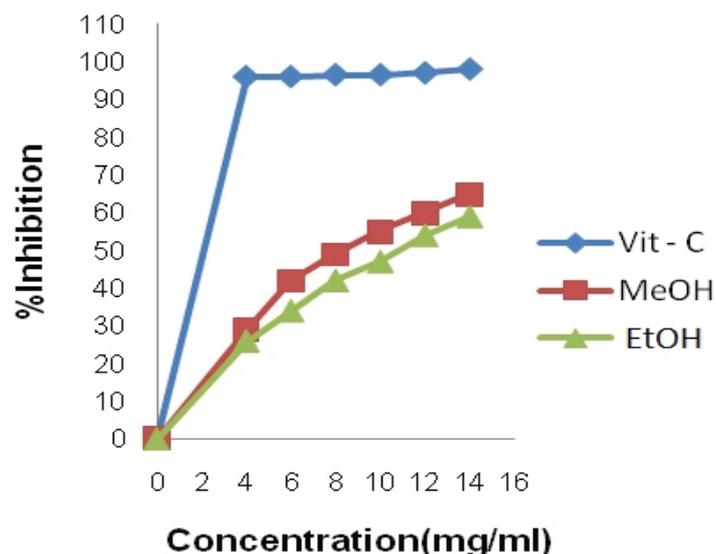


Fig. 6. The DPPH scavenging activity of *Tinospora cordifolia*

properties. They have pharmacological effects such as anti-hyperlipidemic, anti-inflammatory, antioxidative effects, anti carcinogenic, and cytoprotective (Bernatova 2018).

Catechin, Rutin hydrate and Ellagic acid have role in the anti-inflammatory activity (Kleemann *et al.*, 2011). HPLC studies confirmed the presence of these antioxidant chemicals in *Syzygium jambos* (Hossaint *et al.*, 2016). Ellagic acid was also identified and quantified in methanol extracts. Other studies (Hossain *et al.*, 2015,) reported about Ellagic acid in extracts of different parts of plants. Ellagic acid, an investigational drug studied for treatment of Follicular Lymphoma, protection from brain injury of intrauterine growth restricted babies, improvement of cardiovascular function in obese adolescents.

In the present study, vanillin and catechol, two bioactive and flavouring agents were found in a moderate amount in the stems of *T. cordifolia*. Vanillin, a common phenolic compound found in Chinese herbs or roots used as medicine also reported to exhibits antimicrobial activity (Rakchoy *et al.*, 2009) and anticarcinogenic activity (Ho *et al.*, 2009). According to Kumar *et al.*, (2004) neurodegenerative diseases such as Alzheimer's and Parkinson's diseases can be inhibited by Vanillin.

In the present study, methanol was found more effective for extraction of analytes from stems which supports the result by Kallithraka *et al.*, (1995) where methanol was reported as the most effective solvent for extraction of CA, EPC, and EG.

Potential of *T.cordifolia* in anti-inflammatory and antioxidant activities could also possibly be due to the presence of bioactive polyphenolics compounds like, Catechin hydrate, Rutin hydrate, Ellagic acid. Catechins are among the top 10 ingredients with health claim/index compounds for quality control in permitted health foods of Taiwan (Wu 2015).

The polyphenolic compound Catechin, Rutin hydrate, Vanillin in *T. cordifolia* has been determined and reported in this study for the first time ever.

Data from *in vitro* and *in vivo* laboratory studies, epidemiological investigations, and human clinical trials indicate that phenolic compounds are beneficial for human health. A large number of studies have identified cellular targets that could be involved in the health promoting actions of dietary plant phenolic compounds (Zuzana 2011). Tresserra-Rimbau *et al.*, (2014) reported that high polyphenol intake, showed a reduced risk of overall mortality. According to Guarrera *et al.*, (2007),

flavonoid-rich diet modulates the expression of specific genes that have been clearly related to disease risk and provides beneficial health effects. The findings suggests *Tinospora cordifolia* a source of this compounds and possibility of the plant to be used as an ingredient for nutraceutical and functional food development.

Conclusion

T. cordifolia stem was found to have substantial amount of phenolic compounds which were responsible for its marked antioxidant activity as assayed through in-vitro methods. Materials of *T. cordifolia* when extracted with methanol found to be more efficient in antioxidant potentiality. About 50% DPPH was scavenged by 9.36±1.75 mg/ml MeOH extracts of *T. cordifolia* while 27.40±1.09 mg/ml EtOH extracts needed to scavenge the same. For the first time ever Catechin, Rutin hydrate, Vanillin in *T. cordifolia* have been determined and reported. Based on these results, (-)-epicatechin, Catechin, Rutin hydrate, Vanillin were found as the dominating flavonoids of the stem. These bioactive compounds may contribute to a great deal to the antioxidant activity. As a source of many physiologically active compounds with protective activity, *T. cordifolia* stem is very potential to be used as an ingredient for functional food development.

References

- Aberoumand A and Deokule SS (2008), Comparison of phenolic compounds of some edible plants of Iran and India, *Pakistan J Nut.* **7**: 582–585.
- Amarowicz R, Pegg RB, Rahimi M, Barl B and Weil JA (2004), Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies, *Food Chem.* **84**: 551–562.
- Amorim ELC, Nascimento JE, Monteiro JM, Peixoto, Sobrinho TJS, Araújo TAS, and Albuquerque UP (2008), A simple and accurate procedure for the determination of tannin and flavonoid levels and some applications in ethnobotany and ethnopharmacology, *Functional Ecosystem Community*, **2**(1): 88-94.
- Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS and Pruess HG (2000), Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention, *Toxicology* **148**: 187-197.
- Bernatova, I. (2018). Biological activities of (-)-epicatechin and (-)-epicatechin-containing foods: Focus on cardiovascular and neuropsychological health, *Biotechnology Advances* **36**(3): 666–681. DOI: 10.1016/j.biotechadv.2018.01.009
- Caputo M, Sommella MG, Graziani G, Giordano I, Fogliano V, Porta R and Mariniello L (2004), Antioxidant profiles of Corbara small tomatoes during ripening and effects of aqueous extracts on J774 cell antioxidant enzymes, *Journal of Food Biochemistry* **28**: 1-20.
- Chang ST, Wu JH, Wang SY, Kang PL, Yang NS and Shyur LF (2001), Antioxidant activity of extracts from *Acacia confusa* bark and heartwood, *J Agric Food Chem.* **49**: 3420-24.
- Ghasemzadeh A, Hawa Z E and Jaafar AR (2011), Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts, *Journal of Medicinal Plants Research* **5**(7): 1147-1154.
- Guarrera S, Sacerdote C, Fiorini L, Marsala R, Polidoro S, Gamberini S, Saletta F, Malaveille C, Talaska G, Vineis P, Matullo G (2007), Expression of DNA repair and metabolic genes in response to a flavonoid-rich diet, *Br J Nutr.* **98**: 525–533.
- Gurav A, Mondal DB and Vijayakumar H (2014), In Vitro Qualitative And Quantitative Phytochemical Analysis of Ethanolic And 50% Ethanolic Extracts Of *Tinospora Cordifolia*, *Momordica Charantia*, *Cucurbita Maxima* And *Raphanus Sativus*, *International Journal of Pharmaceutical science and Research* **5**(5): 1937-1941. DOI: 10.13040/IJPSR.0975-8232.5(5).1937-41
- Ho K, Yazan LS, Ismail N and Ismail M (2009), Apoptosis and cell cycle arrest of human colorectal cancer cell line HT-29 induced by vanillin, *Cancer Epidemiology* **33**(2): 155–160. DOI: 10.1016/j.canep.2009.06.003.
- Hossain H, Akbar PN, Rahman SE, Yeasmin S, Khan TA, Rahman MM and Jahan IA (2015), HPLC profiling and antioxidant properties of the ethanol extract of *Hibiscus tiliaceus* leaf available in Bangladesh, *European Journal of Medicinal Plants* **7**(1): 7.

- Hossain H, Rahman SE., Akbar PN, Khan TA, Rahman MM and Jahan IA (2016), HPLC profiling, antioxidant and in vivo anti-inflammatory activity of the ethanol extract of *Syzygium jambos* available in Bangladesh, *BMC Research Notes*, **9**:191. DOI: org/10.1186/s13104-016-2000-z
- Ilaiyaraja N and Farhath K (2011), Antioxidant Potential of *Tinospora cordifolia* Extracts and their Protective Effect on Oxidation of Biomolecules, *Pharmacognosy Journal* **3**(20): 56-62. DOI: 10.5530/pj.2011.20.11
- Jagetia GC and Rao SK (2006), Evaluation of cytotoxic effects of dichloromethane extract of Guduchi (*Tinospora cordifolia* Miers ex Hook F & Thoms) on cultured HeLa cells, *Evidence Based Complementary and Alternative Medicine* **3**(2): 267-72.
- Jay M, Gonnet JF, Wollenweber E and Voirin B (1975), Sur l'analyse qualitative des glycosides flavoniques dans une optique chimiotaxonomique, *Phytochemistry* **14**: 1605-1612.
- Kalikar MV, Thawani VR, Varadpande UK, Sontakke SD, Singh RP and Khiyani R K (2008), Immunomodulatory effect of *Tinospora cordifolia* extract in human immunodeficiency virus positive patients, *Indian Journal of Pharmacology* **40**(3): 107-110.
- Kallithraka S, Garcia-Viguera C, Bridle P and Bakker J (1995), Survey of solvents for the extraction of grape seed phenolics. *Phytochem Anal.* **6**: 265-267.
- Kaneria M, Baravalia Y, Vaghasiya Y and Chanda S (2009), Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra Region, India. *Indian Journal of Pharmaceutical Sciences* **71**(4): 406-412.
- Kleemann R, Verschuren L, Morrison M, Zadelaar S, Van Erk MJ, Wielinga PY and Kooistra T (2011), Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models, *Atherosclerosis* **218**(1): 44-52.
- Kumar SS, Priyadarsini KI and Sainis KB (2004), Inhibition of peroxynitrite-mediated reactions by vanillin, *Journal of Agricultural and Food Chemistry* **52**(1):139-145. DOI: 10.1021/jf030319d.
- Lee KG, Mitchell AE and Shibamoto T (2000), Determination of antioxidant properties of aroma extracts from various beans, *Journal of Agricultural and Food Chemistry* **48**: 4817-4820.
- Maffei M (2003), Dietary supplements of plant origin-Nutrition and health approach, Taylor and Francis, p 18.
- Maisuthisakul P, Suttajit M, Pongsawatmanit R (2007), Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants, *Food Chem.* **100**: 1409-1418.
- Mansouri A, Embared G, Kokkalou E and Kefalas P (2005), Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*), *Food Chem.* **89**: 411-420.
- Nagavani V and Rao TR (2010), Evaluation of antioxidant potential and qualitative analysis of major polyphenols by RP-HPLC in *Nymphaea nouchali* Burm flowers, *International Journal of Pharmacy and Pharmaceutical Sciences* **2**(Suppl 4): 98-104.
- Onkar P, Bangar J and Karodi R (2012), Evaluation of Antioxidant activity of traditional formulation Giloy satva and hydroalcoholic extract of the *Curculigo orchioides* gaertn, *J. App. Pharma. Sci.* **2**: 209-213.
- Prieto P, Pineda M and Aguilar M (1999), Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.* **269**: 337-341.
- Pushp P, Sharma N, Joseph GS and Singh RP (2013), Antioxidant activity and detection of (-) epicatechin in the methanolic extract of stem of *Tinospora cordifolia*, *Journal of food science and technology*, **50**(3): 567-572. DOI: 10.1007/s13197-011-0354-8
- Rakchoy S, Suppakul P and Jinkarn T (2009), Antimicrobial effects of vanillin coated solution for coating paper board intended for packaging bakery products, *Asian Journal of Food & Agro-industry* **2**(4): 138-147.
- Rao PS, Kalva S, Yerramilli A and Mamidi S (2011), Free radicals and tissue damage: role of antioxidants, *Free radicals and antioxidants* **1**(4): 2-7.

- Sarunya C and Sukon P (2006), Method development and determination of phenolic compounds in Broccoli seeds samples, *Chiang Mai Journal of Science* **33**(1): 103-107.
- Sharma A, Gupta A and Batra SSA (2010), *Tinospora cordifolia* (Willd.) Hook. F. and Thomson-A plant with immense economic potential, *J. chem. pharm. Res.* **2**: 327-333.
- Singh SS, Pandey SC, Srivastava S, Gupta VS and Patro B (2003), Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi), *Indian Journal of Pharmacology* **35**: 83-91.
- Sivakumar V, Rajan MS and Riyazullah MS (2010), Preliminary phytochemical screening and evaluation of free radical scavenging activity of *Tinospora cordifolia*, *Int J Pharm Pharm Sci.* **2**: 186-8.
- Tresserra-Rimbau A, Rimm Eric B, Medina-Remón A, Martínez-González M A, López-Sabater M C, Covas María I et al. (2014), Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial, *BMC Med.* **12**:77. DOI: 10.1186/1741-7015-12-77
- Wu, P.-W. (2015). A review on the analysis of ingredients with health care effects in health food in Taiwan. *Journal of Food and Drug Analysis*, **23**(3): 343–350. DOI: 10.1016/j.jfda.2015.03.007
- Yusri NM, Chan KW, Iqbal S and Ismail M (2012), Phenolic content and antioxidant activity of *Hibiscus cannabinus* L. seed extracts after sequential solvent extraction, *Molecules* **17**: 12612-21.
- Zuzana K (2011), Toxicological aspects of the use of phenolic compounds in disease prevention, *Interdiscip Toxicol.* **4**(4): 173–183. DOI: 10.2478/v10102-011-0027-5.