Original Article

DETERMINATION OF NUTRITIVE VALUE FOR CERTAIN SOUTH INDIAN INDIGENOUS SPECIES

*Subasini U[1], Sundaraganapathy R[1], Ananda Thangadurai S[1], Malathy R[1], Victor Rajamanickam G[2]

[1]*Swamy Vivekanandha College of Pharmacy, Tiruchengode, India–637205.*

[2]*SriSairam Medical College and Research, West Tamaram, Chennai, India–600 044.*

Abstract

South India is a rich source for various herbs. Plants are commonly used by Indians as culinary herbs, spices and also as folk medicine. Knowledge related with the nutritive value of herbs is of the essence to increase their utility. With that aim, we have selected certain Indian medicinal herbs used by traditional practitioners for various pharmacological activities. Samples of thirteen Indian medicinal plants were analyzed for their Phytochemical composition and vitamin constituents. The yield of extract from each plant has also been calculated to increase their utility in pharmaceutical industry. HPTLC analysis of all the extracts has been carried out. The results reveal that, phytoconstituents like phenol in *Nelumbo nucifera* (NN), Tannin in *Clerodendron inermae* (CI), Carbohydrate in *Enicostemma littorale (*EL), Vitamin C in *Corallocarpus epigaeus* (CE) and Vitamin E in *Clerodendron inermae* (CI). HPTLC data furnished in the present study might be useful for the authentification of those herbs. In conclusion, the presence of phytoconstituents and vitamins such as CI, EL, CH, NN and CE is in higher concentration can be exploited by the pharmaceutical industries and traditional practitioners.

Key words: Carbohydrate, HPTLC, phenol, tannin, vitamin C, vitamin E.

Introduction

In India many indigenous plants are used as spices, food or medicine. Medicinal use of these plants is comparatively higher than that of other uses [1]. Consuming higher level of fruits is inversely correlated with risk of various diseases [2]. Various parts of different plants are commonly used in the preparation of syrups and infusions in traditional medicine as cough suppressant and in the treatment of liver cirrhosis and hepatitis [3]. Phytoconstituents present in these plants are highly responsible for their various medicinal properties like anti-diabetic, anti-cancer and cardioprotective activity [4]. Fruits and vegetables are rich source of phytoconstituents. Diet rich in phytoconstituents or consuming decoction of herbs rich in phytoconstituents can protect the people from various hazardous conditions [5]. Oxidative stress is responsible for developing age related diseases. Consuming plants with higher concentration of phenols and flavonoids can exhibit a better antioxidant activity and prevent the development of diseases related with free radicals. Knowledge related with the concentration of herbs or spices which are used in day-to-day life as a food or consuming as decoction is imperative. With this aim we have appraised the quantitative estimation of phenols, tannin, carbohydrate, Vitamin C and Vitamin E of various herbs, yield of extract from 70 % ethanolic extract and number of spots observed in HPTLC.

Author for Correspondence:

Dr. U.Subasini,M.Pharm,Ph.D
Principal, Department of Pharmacognosy,
Swamy Vivekanandha College of Pharmacy,
Tiruchengode, Namakkal
Tamilnadu, India-637 205.
Email: oviya_subashini@yahoo.co.in

Materials and Methods

Collection of plant material

In order to evaluate the phytochemical constituents of different herbs collected from different region of South India, we have collected the plant from different location of Tamilnadu. The plants selected, parts used and area of collection are mentioned in the Table 1. The collected plant was identified and authentified at Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Tamilnadu, India.

Table 1 Details of plant used for the present study

Botanical name	Part used	Area of collection
Nelumbo nucifera (NN)	Flowers, seeds	Thovalai
Saussurea lappa (SL)	Bark	Madurai
Tribulus terrestris (TT)	Whole plant	Thuraiyur
Withania somnifera (WS)	Root	Madurai
Nardostachys jatamansi (NJ)	Root	Madurai
Terminalia arjuna (TA)	Bark	Thirunelveli
Plumbago zeylanica (PZ)	Root	Madurai
Cardiospermum halicacabum (CH)	Root, leave and seed	Madurai
Indigofera aspalathoides (WS)	Whole plant	Madurai
Enicostemma littorale (EL)	Root	Madurai
Corallocarpus epigaeus (CE)	Leaves and root	Madurai
Acalypha fruticosa (AF)	Whole plant	Madurai
Clerodendron inermae (CI)	Whole plant	Madurai

Extraction of plant material

The plant material was coarsely powdered and shade dried for 15 days. The plant material was soaked in 70% Ethanol. The extract was concentrated *In-vaccuo*. The concentrated extract was stored in a refrigerator until used for experiments.

Quantification of total polyphenols

Total polyphenols were determined by Folin–Ciocalteu procedure [6]. 70% ethanolic extract of each plant material was dissolved in the same solvent. 0.5 ml of sample was

mixed with 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20 % aqueous sodium carbonate solution. Then the sample mixtures were vortexed and absorbance of blue colored mixtures recorded after 40 min at 725 nm against a blank containing 0.5 ml of 70 % ethanol, 0.25 ml of Folin–Ciocalteu reagent and 1.25 ml 20 % aqueous sodium carbonate solution. The calibration curve was prepared from Phenol. Measurements were done in triplicate.

Quantification of tannins

Tannin content of 70 % ethanolic extract of each plant material was determined by following the method of Okwu [7]. 500 mg of 70 % ethanolic extract was weighed in to 100 ml bottle. 50 ml of the same solvent was added and shaken for 1 hr in a mechanical shaker. This was filtered in to a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was pipetted out in to a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 630 nm wavelength. A blank sample was prepared and the colour developed was read at the same wavelength. Different concentration of tannic acid was used as standard. Measurements were done in triplicate.

Estimation of Total Carbohydrate

Total carbohydrate content of plant material was estimated by following the method of Dubois [8]. 50 mg of each sample was ground well with 2-3 ml of 5 % TCA. To the de-proteinized supernatant 10 ml of 45 % ethanol was added to precipitate the polysaccharides. After setting it to stand overnight in cold, the tube was centrifuged for 10 minutes at 4000 rpm. The dried precipitate was analyzed for total carbohydrate by dissolving in 2 ml of 1N NaOH. The sample was made up to 1ml with water. 1 ml of 5 % Phenol and 5 ml of concentrated sulphuric acid were added. The mixture was mixed thoroughly with a glass rod. The solution was allowed to stand for 10 minutes at room temperature and its optical density was read at 490 nm in a spectrophotometer and standard graph was prepared by using different concentration of D-Glucose ranging from 10 to 100 µg/ ml.

Estimation of Vitamin C

Vitamin C in the plant was estimated according to the method of Sarojini[9] with slight modifications. 1.0 gm of plant material was soaked in 70 % ethanol for 24 hours. The extract was filtered. The filtrate was used as sample for the estimation of vitamin C. 0.1 ml of sample was made up to 1.0 ml with water. Different concentrations of ascorbic acid from 4 – 20 µg/ml were used as the standard. Blank contains 1.0 ml of water. 0.5 ml of dinitro phenyl hydrazine (0.2 %) was added to all the tubes including blank, test and standard samples in test tubes. Incubate all the tubes at room temperature for 3 hours. 2.5 ml of 85 % sulphuric acid was measured out in to all the tubes. The absorbance was measured at 520 nm.

Estimation of Vitamin E

Vitamin E in the plant was estimated according to the method of Jayashree[10] with slight modifications. 1.0 gm of plant material was soaked in 100 ml of ethanol: petroleum ether (1.5:2.0) for 24 hours. The petroleum ether fraction was separated and evaporated to dry. The precipitate formed was mixed with 5.0 ml of ethanol. 0.2 ml of extract was made up to 1 ml with ethanol. To this, 0.2 ml of 2, 2', ∝-dipyridyl reagent (0.2 %), 0.2 ml of ferric chloride (0.5 %) solution and 2.0 ml of butanol were added. For blank, 0.2 ml of ethanol, 0.2 ml of 2,2'- dipyridyl reagent, 0.2 ml of ferric chloride and 2.0 ml of butanol were put in to that The red colour developed exactly after 15 minutes was read at 520 nm. Different concentrations of α-tocopherol were used as standard.

HPTLC Profile

Chromatography was performed on preactivated (110^{0}C) silica gel HPTLC plate silica 60GF_{254}. 70 % hydroalcoholic extract of all the extracts dissolved in methanol at the concentration of 10 mg/ml was applied on the silica gel coated plate with an automatic applicator Linomat 5 with N_2 flow (CAMAG, Switzerland). Initially the TLC chamber was saturated with the solvent as mentioned in the Table 5 and the sample applied plate was developed up to 80 mm of the plate. The HPTLC runs were in laboratory conditions of 25 ± 5^{0}C and 50 % relative humidity. After development the plate was withdrawn and air dried and spots were visualized in UV light (UV cabinet, CAMAG, Switzerland). The number of spots and their Rf value were determined by scanning using the scanner, CAMAG, Switzerland. The number of spots observed and the Rf value for each spot is mentioned in the Table 5.

Results and Discussion

Since ancient time, herbs are commonly used for their medicinal values in the daily life either as culinary herb or as a spice. Studies related with nutritive value of herbs are important. This nutritive value knowledge may enhance their utility. With the aim of increasing the utility, we have selected certain medicinal herbs available in India which are commonly used either as a folk medicine, or as a culinary herb.

Before evaluating the nutritive value, knowledge related with the yield of extract from each herb is important. Lower extract yielding plants are not commonly preferred by the pharmaceutical industry though they are rich in their potency. So, the work was carried out with yield calculation. We have selected both ethanol and water in the ratio of 70:30 as solvent for extracting the plant. The organic solvent (ethanol) is eluted the most of the phytoconsituents from the plant. Moreover, it can prevent the activity of phenolic enzymes which are involved in the hydrolysis of phenolic compounds and other related enzymes. By increasing the polarity of the solvent system with water, the elution of glycosides from plant can be improved. The yield of extracts for all herbs is mentioned in the Table 2. The yield from TA is found to be high, followed by SL and NN.

Phenolic compounds as electron donors are readily oxidized to form phenolate ion or quinine, an electron acceptor. This gives rise to practical uses. Protonated phenol is used as

cleaning agents [5]. Phenols are responsible to block certain enzymes that cause inflammation. They also modify the prostaglandin synthesis and thereby, protect platelets from clumping.

Table 2
Yield of extracts of certain Indian medicinal herbs different

Sample	% yield
NN	8.49–10.52
SL	14.19–15.42
TT	4.46 – 6.52
WS	3.83 – 5.28
NJ	4.57 – 6.52
TA	27.70 – 32.25
PZ	1.36 - 1.54
CH	0.72 - 0.89
IA	0.66 - 0.75
EL	0.36 - 0.43
CE	1.11 - 1.21
AF	0.91 - 1.12
CI	1.15 - 1.31

Data of Table 3 shows the presence of phenolic content of all the selected medicinal herbs. Phenolic content of SL is found to be very high (14.40 mg/100 gm) followed by NN (10.20 mg/100 gm) and by TA (8.05 mg/100 gm). The result also displays the presence of the lowest concentration of phenolic content in AF (1.34 Mg/100 gm). Phenolic constituents include flavonoids, phenolic acids, diterpenes and tannins. The presence of higher content of phenolic compound in SL indicate that the above mentioned various phenolic constituents might be present in SL in higher concentration. These phenolic compounds are especially worthy of notice due to their high antioxidative activity [11]. These phenolic compounds are of great value in preventing the onset and/or progression of many human diseases [12].

The tannin content of CE is very high (5.89 mg/100 gm), followed by TA (5.10 mg/100 gm) and TT (5.0 mg/100 gm) (Table 3). This result reveals that the maximum of the phenolic compounds present in CE, TA and TT are tannins. Natural tannins are commonly divided into condensed tannins and hydrolyzable tannins [13]. Some medicinal plants contain complex mixtures of both hydrolyzable and condensed tannins. Condensed tannins are mainly the oligomers and polymers (e.g., monomers, dimers, and trimers) of flavan-3-ols (catechin derivatives), also known as proanthocyanidins. Several flavan- 3-ols are monomers of condensed tannins. The polymerized products of flavan-3,4-diols are considered to be another category of condensed tannins, also called leucoanthocyanidins. Hydrolyzable tannins, including gallotannins and ellagitannins, possess a central core of polyhydric alcohol such as glucose and hydroxyl groups that esterified either partially or wholly by gallic acid (gallotannins) or by hexahydroxy-diphenic acid (HHDP) and other substituents (e.g., chebulic acid) (ellagitannins) [14]. Ellagitannins also have monomeric, dimeric, trimeric or polymeric forms. Being one of the phenolic compounds, the tannin plays an important role in free radical scavenging. The highest concentration of tannins in CE point out that it must be a rich source of proanthocyanidins, leucoanthocyanidins, gallotannins and ellagitannins.

Table 3
Phytochemical constituents of certain Indian medicinal herbs

Sample	Phenolic Content (mg/100 g)	Tannin (mg/100 g)	Carbohydrate (g/100 g)
NN	10.20 ± 0.1	4.30 ± 0.3	0.672 ± 0.23
SL	14.40 ± 0.2	4.00 ± 0.2	0.620± 0.35
TT	7.04 ± 0.3	5.00 ± 0.1	0.725 ±0.42
WS	4.00 ± 0.3	2.70 ± 0.3	0.796 ± 0.5
NJ	5.31 ± 0.2	4.90 ± 0.2	0.984 ± 0.56
TA	8.05 ± 0.2	5.10 ± 0.1	1.154 ±0.53
PZ	2.34± 0.27	1.68 ± 0.34	3.8 ±0.45
CH	1.83± 0.36	1.26 ± 0.87	4.2 ±0.78
IA	3.63 ± 0.56	3.94± 0.78	4.4 ±0.31
EL	1.87 ± 0.46	2.23± 0.45	8.0 ±1.23
CE	2.67 ± 0.54	1.47 ± 0.36	6.2 ±0.65
AF	1.34 ± 0.38	1.78 ± 0.25	4.8 ±0.43
CI	7.56 ± 1.37	5.89 ±0. 23	5.4 ±1.12

Values are Mean ± SD of triplicate

Carbohydrate and Glycosides play an important role in immunomodulatory reactions and their free radical scavenging activity has earlier been reported by Morelli [15]. The cardio-protective effect of carbohydrate isolated form certain plants have also been reported earlier. This made us to estimate the level of glycosides in different extracts. The carbohydrate content of EL is very high (8.0 mg/100 gm) followed by CE (6.2 mg/100 gm) and CI (5.4 mg/100 gm).

Though the vitamins are present in trace amount, their deficiencies adversely affect the metabolism of the body. Vitamin C is essential for the synthesis of intercellular substances including collagen, bone matrix and tooth dentine, and also required for normal wound healing. Normal physiological functions required Vitamin C for transformation of cholesterol in to bile acid, hydroxylation of praline to hydroxyproline and of lysine to hydroxylysine. Iron is absorbed in the ferrous (Fe^{2+}) state rather than the ferric (Fe^{3+}) form and the reducing capability of ascorbate accounts for the enhanced absorption of the metal in the presence of the vitamin. Nitrate upon oxidation offer nitrite which is carcinogenic in nature. The antioxidant activity of Vitamin C can prevent the formation of nitrite from nitrate. Deficiency of Vitamin C causes, scurvey, hemorrhage from mucous membrane of the mouth and gastro intestinal tract, anaemia, pains in the joints and defects in skeletal calcification. In our present study, Vitamin C content of CH, CI are calculated as 2.96 and 2.94 mg/100 gm, respectively followed by IA (1.97 mg/100 gm) in higher concentration (Table 4). So, consuming a higher concentration of CH or CI can prevent the development of various above mentioned diseases [4].

Vitamin E, a major lipid-soluble antioxidant belonging to tocopherols, is the most effective chain-breaking antioxidant within cell membrane. It is able to repair oxidizing radicals directly, preventing the chain propagation step during lipid peroxidation [16]. It also inhibits the oxidative modification of LDL that is responsible for development and progression of atherosclerosis [17]. Moreover, high levels of Vitamin E have been measured in the mitochondria, golgi apparatus, lysosomes, and endoplasmic reticulum [18], and recent studies

have shown that Vitamin E possesses a variety of cardiovascular effects including decreased platelet aggregation [19], arterial superoxide generation and increased eNOS mediated NO production [20]. This importance of Vitamin E made us to estimate the level in selected medicinal plants. SL contains higher concentration of Vitamin E (4.40 mg/100 gm) followed by CH (2.61 mg/100 gm) and CI (2.27 mg/100 gm).

The results of the present study reveals that yield of extract, Vitamin E and phenolic content of SL are observed to be high. Ethanol extract exhibits in-vitro anti-helicobacter pylori which is able to induce gastritis and gastric ulcer [21]. Extract exhibits cyto-static effects on gastric AGS cancer cells. Plant extract is able to inhibit *Trypanosoma cruzi* [22].But consuming higher concentration of SL is not advisable. Earleir references stated that SL is a rich source of aristolochic acid [23]. Aristolochic acid is highly toxic in nature causes various hazardous diseases like nephropathy and urinary tract cancer [24-25].

Table 4
Vitamin content of certain Indian medicinal herbs

Sample	Vitamin C (mg/100 g)	Vitamin E (mg/100 g)
NN	0.36 ± 0.2	0.42 ± 0.2
SL	0.54 ± 0.3	4.40 ± 0.3
TT	0.19 ± 0.1	0.21 ± 0.5
WS	0.26 ± 0.2	0.46 ± 0.6
NJ	0.23 ± 0.3	0.44 ± 0.2
TA	1.47 ± 0.1	0.58 ± 0.3
PZ	1.44±0.98	0. 89 ±0.08
CH	1.31±0.76	2.61 ±0.45
IA	1.97±1.23	1.98 ±0.23
EL	1.27±0.98	1.45 ±0.08
CE	2.96±1.46	1.31 ±0.32
AF	1.58±2.31	0. 98 ± 0.12
CI	2.94±1.65	2.27 ± 0.12

Values are Mean ± SD of triplicate

Data of Table 5 showed HPTLC profile of various herbs. The mobile phase selected for the present study, the obtained Rf value and the number of spots observed in each extract might be useful for the authentification of all the herbs in future. The number of spots observed in AF is found to be high followed by CE and TT (Table 5).

Conclusion

The yield of extract from TA was high. Various phytoconstituents like tannin, phenolic constituents and carbohydrate are high in CI, SL and EL respectively. Likewise, Vitamins like Vitamin C and Vitamin E are present in higher concentration in CE and SL respectively. Since SL is banned drug, NN and CH are considered as rich source of phenolic and Vitamin E content. HPTLC analysis showed the maximum number of spots in AF [12] and CE [11]. CI, EL, CH, NN and CE are observed as rich source of nutrients in the present study.

Table 5
HPTLC profile of certain Indian medicinal herbs different

Sample	Mobile phase	Number of Spots	Rf value of all spots
NN	n-Butanol, Acetic acid, Water (4:1:1)	7	0.33, 0.42, 0.51, 0.63, 0.67, 0.74, 0.89
SL	Chloroform, Ethyl acetate (1:1)	6	0.24, 0.39, 0.53, 0.62, 0.67, 0.70,
TT	Hexane, Ethyl acetate, Acetic acid (6.5:3:0.5)	9	0.14, 0.29, 0.53, 0.59, 0.62, 0.64, 0.67, 0.70, 0.72
WS	Chloroform, Methanol, Water (13:3:4)	8	0.34, 0.46, 0.54, 0.63, 0.72, 0.76, 0.84, 0.88
NJ	Toluene, Ethyl acetate (1:1)	6	0.15, 0.19, 0.22, 0.51, 0.78 0.88
TA	Chloroform, Methanol (16:4)	3	0.50, 0.68, 0.78
PZ	Toluene, Ethyl acetate (6:4)	7	0.1, 0.15, 0.19, 0.39, 0.51, 0.78 0.85.
CH	Ethyl acetate, Methanol (7:3)	6	0.08, 0.32, 0.46, 0.54, 0.61, 0.94
IA	Ethyl acetate, Methanol, Acetic acid (5:4:1)	7	0.11, 0.18, 0.25, 0.34, 0.43, 0.54, 0.74
EL	Ethyl acetate, Methanol (7:3)	5	0.09, 0.19, 0.25, 0.77, 0.91
CE	Toluene, Methanol (8:2)	11	0.10, 0.12, 0.22, 0.27, 0.34, 0.46, 0.55, 0.59, 0.67, 0.74, 0.85
AF	Toluene, Ethyl acetate (6:4)	12	0.10, 0.13, 0.18, 0.21, 0.26, 0.38, 0.43, 0.47, 0.53, 0.65, 0.75, 0.95
CI	Ethyl acetate, Ethyl methyl ketone, Acetic acid (5:3:2)	6	0.15, 0.26, 0.37, 0.53, 0.83, 0.94

Acknowledgement

The authors record their thanks to Prof.R.Sethuraman, Vice chancellor for allowing us to complete this work successfully. We also place our thanks to the Drugs and Pharmaceuticals Division of Department of Science and Technology, Government of India, New Delhi for their financial support during the course of this work. We also thank our colleagues in CARISM, SASTRA University, for their extraordinary support and encouragements at every stage of this study.

Reference

1. Okwu DE. Investigation in to the medicinal and nutritional potential of Garcinia kola (Bitter kola) (Dissertation). Umudike, Nigeria: Michael Okapara University of Agriculture: 2003.
2. Okwu DE. Phytochemicals and vitamin content of indigenous species of south eastern Nigeria. *J. Sustain. Agric. Environ. 6*, 2004, 30-34.
3. Iwu MM. Research finding on the possible application's of Nigeria's raw materials on the pharmaceutical industries in industrial potentials of Nigeria raw materials. In the proceedings of the 1986 Onoh JK. 1986, pp. 251-264.

4. Irvine FR. Woody parts of Ghana, with special reference to their uses. London: Oxford University press London: 1961, pp 9.
5. Uruquiaga I, Leighton F. Plant polyphenol antioxidants and oxidative stress: *Biol. Res.* 33, 2000, 159-165.
6. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic*. 28, 1977, 49-55.
7. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants: *Int. J. Mol. Med. Adv. Sci.* 1, 2005, 375- 381.
8. Dubois M, Giles MK, Hamilton JK, Reber PA, Smith R. Colorimetric methods for the determination of sugar and related substances: *Anal. Chem*. 28, 1954, 350-356.
9. Sarojini Y, Nittala SS. Vitamin C content of some macroalgae of Visakhapatnam. East coast of India: *Indian. J. Mar. Sci.* 28, 1999, 408 – 412.
10. Jayashree VS, Kamat SY. Distribution of tocopherol (Vitamin E) in marine algae from Goa, West coast of India: *Indian. J. Mar. Sci.* 14, 1985, 228–229.
11. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants: *Crit. Rev. Food. Sci. Nutr.* 32, 1992, 67–103.
12. Tsao R, Akhtar MH. Nutraceutical and functional foods: Current trend in phytochemical antioxidant research. *J. Food. Agr. Environ*. 3, 2005, 10–17.
13. Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer: *Life. Science*. 74 (17), 2004, 2157–2184.
14. Khanbabaee K, Van Tee T. Tannins: classification and definition. *Nat. Prod. Repts.* 18(6), 2001 641-649.
15. Morelli R, Russo-Volpe S, Bruno N, Scalzo RL. Fenton-dependent damage to carbohydrates: free radical scavenging activity of some simple sugars. *J. Agri. Food. Chem*. 51, 2003, 7418–7425.
16. Zhang JG, Nicholls-Grzemski FA, Tirmenstein MA, Fariss MW. Vitamin E succinate protects hepatocytes against the toxic effect of reactive oxygen species generated at mitochondrial complexes I and III by alkylating agents: *Chem. Biol. Interact*. 138, 2001, 267–284.
17. Munteanu A, Zingg JM, Azzi A. Anti-atherosclerotic effects of vitamin E-myth or reality: *J. Cell. Mol. Med.* 8, 2004, 59-76.
18. Drevon CA. Absorption, transport and metabolism of vitamin E: *Free. Radic. Res. Commun.* 14, 1991, 229-246.
19. Saldeen T, Li D, Mehta JL. Different effects of alpha- and gamma-tocopherol on LDL oxidation, superoxide activity, platelet aggregation and arterial thrombogenesis: *J. Am. Coll. Cardiol.* 34, 1999, 1208-1215.
20. Li D, Saldeen T, Romeo F, Mehta JL. Relative effects of alphaand gamma-tocopherol on LDL oxidation, superoxide dismutase and nitric oxide synthase activity and protein expression in rats: *J. Cardiovasc. Pharmacol. Ther*. 4, 1999, 219-26.
21. Yang L, Chen X, Qiang Z, Jun LY, Ren TX. Invitro anti – helicobacter pylori action of 30 Chinese Herbal medicines used to treat ulcer diseases: *J. Ethnopharmacol*, 98 (3), 2005, 329–333.
22. Lirussi PJ, Li JM, Gennari M, Busehiaizzo H, Rios JL, Zaidenburg A. Inhibition of Trypanosoma cruzi by plant extracts used in Chinese medicine: *Fitoterapia*. 75(7-8), 2004, 718–723.
23. http://potency.berkeley.edu/pdfs/FDAAristolochicAcidLetter.pdf
24. Nortier JL, Muniz Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Vereerstraeten P, Vanherweghem JL. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi): *N. Engl. J. Med*. 342, 2000, 1686-1692.
25. Lord GM, Cook T, Arlt VM, Schmeiser HH, Williams G, Pusey CD. Urothelial malignancy and Chinese herbal nephropathy: *Lancet* 358, 2001, 1515-1516.