



Evaluation of Biochemical Contents, Trace Elements, Nutritive Value and HPTLC Profiling in Two Edible Food Plants Based Diets

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ABSTRACT

Background and Objectives: The present study was conducted to evaluate the presence of biochemical contents and trace elements, and detect phytochemicals in the edible part extracts of *Asparagus officinalis DC* and *Chlorophytum comosum Linn* by HPTLC.

Materials and Methods: The biochemical contents and trace elements were determined by different biochemical methods, and trace elements' presence was detected by atomic absorption spectroscopy (AAS), while phytochemicals were detected by HPTLC. Nutrients content of the leaves of *Asparagus officinalis DC* and *Chlorophytum comosum Linn* were analyzed by standard method.

Results: Crude fat and protein content of *Asparagus officinalis DC* and *Chlorophytum comosum Linn* was found to be 3.44, 2.00 and 32.69, 4.54%, respectively. Also their total carbohydrate and crude fiber content was detected as 34.67, 65.84 and 18.5, 17.24%, respectively. Analysis of the edible parts confirmed the presence of phenol steroids, and showed minimum amount of trace elements with moderate nutritive value. Total phenolic content and nutritive value were found to be greater in the stem *Asparagus officinalis DC* as compared to *Chlorophytum comosum Linn*.

Conclusions: Our findings suggest that both *Asparagus officinalis DC* and *Chlorophytum comosum Linn* are endowed with antioxidant phytochemicals and nutritive values.

Keywords: Asparagus officinalis DCChlorophytum comosum Linn, Nutritive values

Introduction

Stem of Asparagus officinalis is edible in Iran. This plant grows in little rainfall localities of South Iran. The plant material was collected from Dezful Agricultural Research Center in South Iran. The young stalks are commonly eaten as a vegetable. It is generally recognized as a safe food. It is a perennial herb with scale-like leaves and muchbranched stem that grows up to 3 meters height. Asparagus is native to Europe and Asia though it is now cultivated throughout the world. The parts used as vegetable consist of the aerial stems or spears, arising from rhizomes. Asparagus spears are widely used as a vegetable, and are frequently blanched before use. Extracts of the seeds and roots have been used in alcoholic beverages, with the maximum levels averaging 16 ppm. The seeds have been used in coffee substitutes, diuretic preparations, laxatives, remedies for neuritis and rheumatism, to relieve toothache, to stimulate hair growth, and to treat cancer. Chinese medicine has used them to treat parasitic diseases. Extracts are said to have served as contraceptives and extracts to cleanse the face (1).

More than 175 species of *Chlorophytum* have been reported in the world. *Chloophytum comosum* is widely used as an ornamental plant where it is commonly known as spider ivy, spider plant, aero-plane plant, or walking anthericum. The plant material is found to be growing in little rainfall localities of South Iran.

Plants have great importance due to their nutritive value. They are the major source of medicines, which play an important role in the human history (2). Plants synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids, etc.) that are used as drugs (3). All human beings require a number of complex organic compounds as added caloric requirements to meet the need

for their muscular activities. Minerals and trace elements are chemical elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health (4). Since plant materials form major portion of the diet, hence, their nutritive value is important. In the present study, HPTLC profile, biochemicals, and nutritive value, as well as the protein and trace element content in the edible parts of Asparagus officinalis DC and Chlorophytum comosum Linn are investigated. These plants belong to the Liliaceae family (4).

Materials and Methods

Experimental section

Plant material: It was collected from the Garden of Shiraz Agricultural College, Shiraz/Iran. it was brought to the Department of Food Science and Technology in cool and dry containers. Tuberous roots were washed with water to eliminate waste material, separated and dried in the shade so as to prevent the composition of chemical compounds. It was then powdered in blender and stored in clean and dry containers for phytochemical screenings (5).

Fresh edible parts of Asparagus officinalis DC and Chlorophytum comosum Linn were collected from the Medicinal Plants Garden belonging to Shiraz University (Shiraz, Iran). They were dried in shade for five days and then crushed to coarse powder. The coarse powder was cold macerated with 50% ethanol (1:1, ethanol: water) and kept for 3 days at room temperature with occasional stirring (6). The suspension was filtered through a fine muslin cloth. Next it was evaporated to dryness at a low temperature (at 40° C) under reduced pressure in a rotary evaporator. Dark brown colored crystals were used for the HPTLC studies. The alkaloids and phenols were detected using HPTLC. Analysis of alkaloids and phenols was carried out in the laboratory of Mumbai University, India, and the rest of the experiments were carried out in the laboratory of Behbahan University, Iran.

Biochemical estimations: Estimation of phenolic compounds and steroids was done by Soni et al (7).

HPTLC: 10μ L of the test solution (plant sample) was applied as 10 mm band on 5x10 pre-coated silica gel 60 F254 thin layer chromatography (TLC) plates with the uniform thickness of 0.2 mm by using Linomat 5 system. The sample loaded TLC plates were kept in TLC twin trough developing chamber with respective mobile phase solvent for 15 minutes (for chamber saturation). The solvent used for HPTLC method was Toluene. The sample loaded plates were developed using respective mobile phase up to 80 mm. The plates were removed and allowed to dry in air. The developed plates were then dried by hot air to evaporate solvents from the plates. The plates were kept in photo-documentation chamber and plate images were captured in white and UV light at 254 and 366 nm. The plates were sprayed with respective spraying reagent

and dried at 110°C in hot air oven. Then they were photo-documented at UV 366 nm and white light using photo-documentation chamber.

Determination of nutritive value and trace elements: Estimation of ash and moisture content were done. Crude fat and crude fiber were estimated (8). To prepare the sample for mineral analysis, the washed and dried materials were ground to fine powder. One gram of the sulphated ash was dissolved in 100mL of 5% HCl to obtain the solution ready for determination of mineral elements (Zn, Fe) through atomic absorption spectroscopy (AAS) (9). AAS was used as a convenient method for the measuring the samples' Na, K and Ca.

Total protein was estimated by Kejeldal method. Carbohydrate content and nutritive value were calculated by the following formulae:

Percentage of carbohydrate was given by (10):

100–(percentage of ash + percentage moisture + percentage fat + percentage protein).

Nutritive value was finally determined by:

Nutritive value = 4x percentage of protein + 9x percentage of fat + 4x percentage carbohydrate.

Estimation of Alkaloids: 5 g the sample was weighed into a 250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. Then it was filtered and the extract was concentrated by using a water bath to one-quarter of the original volume. Then the concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (11).

Determination of Total Phenolic Compounds:

Preparation of Plant Extracts (Method B: Grounded dry plant material (500 mg) was weighed into a test tube. A total of 10 ml of 80% aqueous methanol was added, and the suspension was stirred slightly. The tubes were sonicated for 5 min and centrifuged for 10 min (1500g), and finally, the supernatants were collected. The plant materials were re-extracted twice.

The amount of total phenolic in the extracts was determined according to the Folin-Ciocalteu's procedure (12). The samples (0.5 ml, two replicates) were introduced into test tubes; 2.5 ml of Folin-Ciocalteu's reagent and 2 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured, and the total phenolic content was expressed as tannic acid equivalents in milligrams per gram dry material.

Statistical analysis: Experiments were performed in triplicate and results were expressed as mean \pm SD and were analyzed by one way ANOVA test using SPSS statistical programe.

Results

Table 1 shows the nutritive value of the leaves of Asparagus officinalis DC and Chlorophytum comosum Linn. The ash and moisture content of the edible parts was 6.2, 5.6 and 5.2, 3.6%, respectively. Their crude fat and protein content was found to be 3.44, 2.00 and 32.69, 4.54%, respectively. Total carbohydrate and crude fiber content was detected as 34.67, 65.84 and 18.5, 17.24%. The nutritive value of Asparagus officinalis DC and Chlorophytum comosum Linn edible parts was 300.4 and 299.52%, respectively.

Table 1: Evaluation of nutritive value in the edible parts of *Asparagus officinalis DC* and *Chlorophytum comosum*

-		Linn	
S.No	Parameters	Asparagus	Chlorophytum
		officinalis	comosum
		DC (%)	Linn(%)*
1	Ash	10.70±0.56 ^a	10.38±0.86 ^a
2	Moisture	5.2 ± 0.47^{a}	3.6 <u>+</u> 0.89 ^b
3	Crude fat	3.44 ± 0.458^{a}	2.00 ± 0.59^{b}
4	Protein	32.69 ± 0.24^{a}	4.54 <u>+</u> 0.94 ^b
5	Total carbohydrate	34.67 ± 0.58^{a}	65.84 <u>+</u> 0.39 ^b
6	Crude fibre	18.5±0.59 ^a	17.24 ± 0.49^{a}
7	Nutritive value	300.4 ± 1.23^{a}	299.52±1.67 ^a

*Values are mean of triplicates. Different letters in each row in superscript indicate means are significantly different (p<0.05).

Presence of trace elements was detected in the edible parts of *Asparagus officinalis DC* and *Chlorophytum comosum Linn* by AAS in which zinc level was found to be 2.60 and 0.76 mg/g, respectively. The iron content was 0.19 and 1.89 mg/g, respectively. Potassium was the major constituent in the edible parts of the plants; 10.94 and 4.29 mg/g, respectively (Table 2).

Table 2. Determination of elements in the edible parts of Asparagus officinalis DC and Chlorophytum comosum Linn in percentage

S.No	Parameters	Asparagus	Chlorophytum
		Officinalis DC (%)	Comosum Linn(%)*
1	Sodium (mg)	1.84 <u>+</u> 0.03 ^a	3.95 <u>+</u> 0.04 ^b
2	Potassium (mg)	10.94 ± 0.23^{a}	4.29 <u>+</u> 0.41 ^b
3	Calcium (mg)	0.67 ± 0.45^{a}	13.14 <u>+</u> 0.56 ^b
4	Zinc (µg)	2.60 ± 0.67^{a}	0.76 ± 0.11^{b}
5	Iron (µg)	0.19 <u>+</u> 0.45 ^a	1.89 <u>+</u> 0.57 ^b

*Values are mean of triplicates±SD. Different letters in each row superscript indicate means are significantly different (p<0.05).

The preliminary phytochemical analysis in our laboratory confirmed the presence of secondary metabolites in the 50% hydro ethanolic extract (13). HPTLC profile (Fig. 1) of the 50% hydro ethanolic extracts of *Asparagus officinalis DC* and *Chlorophytum comosum Linn* gave four spots for steroids. The absence of alkaloid was observed in both of the plant extracts but phenols were detected with different amounts (Table 3).

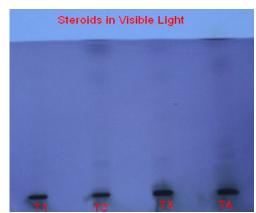


Fig.1. HPTLC analysis of steroids. Steroids after derivatisation with 5% sulphuric acid

T1, T3 – Chlorophytum (10 μl) T2, T4 - Asparagus (10 μl)

Table 3. High performance thin layer chromatography (HPTLC) profile of 50% hydroethanolic parts extracts of *Asparagus officinalis DC* and *Chlorophytum comosum*

			Linn			
Alkaloids		Phenols		Steroids		
	A	В	A	В	A	В
	N.D.	N.D.	0.87 ± 0.67	1.36 <u>+</u> 0.15	-	+

N.D. means not detected.

A- 50% hydro-ethanolic stem extract of Asparagus officinalis DC

B- 50% hydroethanolic stem extract of Chlorophytum comosum Linn (-) mean of the extract of steroids from Asparagus officinalis was not clear.

(+) mean of the extract of steroids from Chlorophytum comosum was clear

Table 4. Proximate chemical composition (g/100 g of dried weight) and energetic value of four the wild edible food plants (mean \pm SD; n = 3)

	Alocacia indica	Asparagus officinalis	Portulaca oleracia	Solanum indicum	Cholorophytum Comosum
Total fat	3.29 ± 0.01^a	3.44 ± 0.02^{a}	5.26 ± 0.02^{b}	13.76± 0.01°	2.00 <u>+</u> 0.59 ^d
Crude protein	$5.7{\pm}~0.18^a$	$32.69 \!\pm 0.03^b$	$23.47 \!\pm 0.02^{c}$	$12.85\!\pm0.03^{d}$	4.54 <u>+</u> 0.94 ^e
Ash	$7.3 {\pm}~0.02^a$	10.7 ± 0.01^{b}	22.6 ± 0.03^{c}	11.0 ± 0.07^{b}	10.38 <u>+</u> 0.86 ^b
Carbohydrates	72.66 ± 0.22^a	$34.67\!\pm 0.17^b$	$40.67\!\pm0.23^{c}$	$38.49 {\pm} 0.04^{c}$	65.84 <u>+</u> 0.39 ^d
Energy (kcal)	$343.05{\pm}\ 1.74^a$	$300.4\!\pm0.73^{b}$	$303.9\!\pm1.04^{b}$	329.2 ± 0.26^{c}	299.52 <u>+</u> 1.67 ^b

Different letters in superscript in each row indicate means are significantly different (p<0.05).

Discussion

Phytochemicals are defined as bioactive non-nutrient plant compounds found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major chronic diseases (14). The medicinal values of plants, i.e. their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, produce a definite physiological action on the human body (15). Medicinal plants contain many antioxidants such as vitamins (A, C & K), carotenoids, flavonoids (flavones, isoflavones. flavonones. anthocyanidin, catechin and isocatechin), polyphenols (ellagic gallic acid and tannin). Several reports say that these compounds possess remarkable antitumor, antidiabetic and antioxidant activity (16-19). Flavonoids are a group of naturally occurring polyphenolic compounds extracted primarily from fruits and vegetables. They are among the most numerous and wide spread groups of phenolic compounds seen in higher plants (20). Several studies have evaluated the cyto-toxic effect of saponins against tumor development. The active components in several herbal medicines that have been used as chemotherapeutic agents in Eastern countries were shown to be saponins. A Chinese herbal drug, 'Yunan Bai Yao' has been used as a hemostatic agent, which is known to promote wound healing (21). The stem of Asparagus officinalis DC and Chlorophytum comosum Linn possesses significant amounts of vitamins (C, E) and phenols. The HPTLC profile shows the presence of secondary metabolites such as steroids, phenols and saponin. Iron is sufficient in all of the studied medicinal plants. It makes the body tendons and ligaments, and certain chemicals of brain are controlled by the presence or absence of iron; it is essential for the formation of hemoglobin (22). Cu is an important component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin (an iron oxidizing enzyme in blood) (23). Cu deficiency has been associated with cardiac abnormalities in both human and animal, and causes anemia and neutropenia (24). Zinc maintains various reactions of the body, which help to construct and maintain DNA. It is required for the growth and repair of body tissues, and is the important element of ligaments and tendons (25). Vitamin B12 exists in several forms, and contains the mineral cobalt (26). Vitamin B12 deficiency is characterized megaloblastic anemia, fatigue, weakness, constipation, loss of appetite and weight loss (27). Neurological changes such as numbness and tingling in the hands and feet, can also occur (27). Both plants studied in this work showed minimum amount of essential minerals and better nutritive value in the stem. HPTLC is an invaluable quality assessment tool for evaluation of

botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. The 50% hydroethanolic stem extract of Asparagus officinalis DC and Chlorophytum comosum Linn showed the presence of phytochemicals. It is concluded that both of these plants have essential minerals with good nutritive value and secondary metabolites. Future goal is to isolate their secondary metabolites in order to study their pharmacological activity.

The mean content of major compounds and nutrients in fresh Asparagus spears (29) has been reported: Protein (1.91%), fat (0.16%), carbohydrates (2.04%), fiber (1.31%), K (45 mg/100g), Ca (26mg/100g), Fe (648µg/100g) and Zn (397 µg/100g). Comparison of the results of this study with those of Souci et al. shows that except for Ca, K, Fe and Zn, the amount of other nutrients in our study was high.

Comparison of nutrient content in the plant foods in Table 4 shows that Asparagus officinalis contains the highest amount of protein (32.69%) and Alocasia indica has the lowest (5.7%) (p<0.05). Solanum indicum contains the highest fat content (13.76%) and Chlorophytum comosum has the lowest (2%) (p<0.05). Also the lowest ash content (7.3%) was found in Alocasia indica and the highest (11%) was in Solanum indicum (p<0.05). Green Asparagus spears were classified into fine (F, ≤8 mm, middle (M, 9-11 mm), thick (T, 12-14 mm), very thick (VT, 15-19 mm) and extra thick (ET, ≥20 mm). This plant food was found to be a good source of protein (> 30% DW), containing most of the essential and nonessential amino acids. However, its arginine, cystine, γ-amino-butyric acid, glutamine, lysine, ornithine, phenylethanolamine, serine and taurine content decreased significantly (P≤0.05) with blanching and canning. Green Asparagus protein showed an adequate amino acid score according to the FAO/WHO recommendations, and seemed to contribute most of the essential amino acids, except histidine and lysine, which were limiting amino acids. In-vitro protein digestibility (IVPD) tended to decrease during the development (77.30% at F to 71.43% at ET), and improved during the processing, mainly after blanching (29,30).

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