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Full Length Research Paper

Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L.

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***Plantago major* (seeds and leaves) and *Cyamopsis tetragonoloba* (guar beans) were analyzed for general chemical components including, fatty acids and amino acids. Guar beans had high contents of proteins, fats and total hydrolysable carbohydrates. *Plantago* leaves high percentage of linolenic acid was characterized by 56.19%. While, *P. major* seeds and Guar beans had high percentages of linoleic acid (25.41 and 48.99%, respectively). Essential and non essential amino acids were present in all samples and Guar beans had high amounts of glutamic, arginine, aspartic and leucine. Total phenols, total flavonoids and tannin content were the highest in *Plantago* leaves. Antioxidant activity of ethanolic, hot and cold water extracts of *Plantago* leaves and seeds and guar beans were evaluated. *Plantago* leaves extracts exhibited higher antioxidant activity than *plantago* seeds and guar beans extracts. The ethanolic, hot and cold extracts of plant induced anticancer activity with various degrees. Ethanolic extract of *P. major* leaves possessed the greatest effect on tumor cell growth (Dead 74%) followed by hot water extract of *P. major* leaves (Dead 54.6%).**

Key words: *Plantago major*, Guar beans, flavonoids, phenolic compounds antioxidant activity, antileukemia.

INTRODUCTION

Cancer is the leading cause of death in the developing and developed countries. Cancer claims over six million lives globally each year (Pezzuto, 1997).

There is a growing trend in the use of medicinal plant because of their medical effectiveness, low toxicity and the many natural anticancer agents derived from these plants (Ozaslan et al., 2007). *Plantago major* L. is a perennial plant from Plantaginaceae family.

It is introduced to the Nordic countries parallel to the introduction of the first primitive cultivated fields in the stone age nearly 4000 years ago (Jonsson, 1983). *P. major* spread by man from Europe throughout the world. The Indians named it 'White man's footprint' because it is found everywhere the Europeans had been. This adapted into the genus name *Plantago* that is from Latin *planta*, meaning sole of the foot.

It is an old medicinal plant that has been known for centuries, but it is regarded as weed by many people (Samuelsen, 2000). It is renowned as a traditional herbal medicine throughout the world (McCutcheon et al., 1995).

P. major has also been used as an anesthetic, antiviral, anti-inflammatory, astringent, anti-helmintic, analgesic, analeptic, antihistaminic, anti rheumatic, antitumor, anti-ulcer, diuretic, expectorant and hypotensive in traditional medicine (Grigorescu et al., 1973; Matev et al., 1982; Franca et al., 1996). Moreover, water soluble compounds isolated from *Plantago* spp. (especially *P. major*) have been reported to induce an immunostimulating activity on human lymphocyte proliferation (Chiang et al., 2003).

Pathological studies showed that, *P. major* L. extract induced inhibitive effect on ehrlich ascites tumor (EAT) and prevented tumor extension (Ozaslan et al., 2007). In addition, its crude extract inhibits tumor activity on the ehrlich ascites tumors (EAC) (Ozaslan et al., 2009).

Guar or cluster bean (*Cyamopsis tetragonoloba* L.) belongs to the family Leguminaceae and is grown in tropical Africa and Asia. *C. tetragonoloba* bean is commercially grown for its seeds as a source of natural

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polysaccharide (galactomannan), commercially known as guar gum. It has a number of uses in food and other industries, such as paper, textiles, oil well drilling and pharmaceuticals (Whistler and Hymowitz, 1979).

C. tetragonoloba L. is a well-known traditional plant used in folklore medicine. It acts as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia Anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anti-hyperglycemic effects (Mukhtar et al., 2006). In addition, Guar beans are potentially high sources of additional phytochemicals (Wang and Morris, 2007). The aim of this study was to determine the main components beside biologically active compounds of *P. major* L. and *C. tetragonoloba* L. and to evaluate their antioxidant and anticancer effects.

MATERIALS AND METHODS

Chemicals and raw materials

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, tert-butyl-4-hydroxy toluene (BHT), Folin-Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany). Aerial parts of the *P. major* L. (leaves and seeds) were collected from Medicinal and Aromatic Plants Farm in Kanater, Agriculture Research Center, Egypt. Guar (*C. tetragonoloba* L.*)* beans were obtained from Crop Research Institute, Agriculture Research Center, Giza, Egypt.

Chemical analysis

Proximate chemical composition of crude proteins, fats, ash and crude fiber contents were determined as described in Association of Official Analytical Chemists (AOAC) methods (2005). Total hydrolysable carbohydrates were determined as glucose using phenol sulphuric acid reagent (Dubois et al., 1956).

Amino acid profiles were determined using amino acid analyzer (Beckman, 7300/G300) according to the method mentioned by AOAC (2006).

Identification of fatty acids

Fatty acid profiles were determined according to the method of (Aura et al., 1995) utilizing a gas chromatograph (HP 6890).

Preparation of plant extracts

Plant materials were air dried at room temperature and ground in a mortar. Fifty grams of each plant powder was extracted in 500 ml of ethanol by maceration (48 h). The solvent was removed under vacuum at temperature below 50°C, and then the extracts were freeze-dried.

Hot water extract of samples was prepared from plants according to the standard methods of Chang and Yeung (1988). Dried whole plants (100 g) were boiled in 1000 ml of distilled water for 1 h and filtered by gauze. The filtrate was collected and the residue was further extracted with 1000 ml of distilled water. The same procedure was repeated three times. The aqueous extract obtained from three successive extraction was mixed, concentrated *in vacuo*, and then lyophilized. The dried extract was collected and stored at 4°C until use.

Cold water samples (100 gm) were suspended in (1 L) of distilled water, stirred for 12 h at about 25°C, left to stand at the same temperature for another 12 h. The solution in each case was passed through folded muslin. The process was repeated twice. The aqueous extract obtained was mixed, concentrated *in vacuo*, and then lyophilized. The dried extract was collected and stored at 4°C until use.

Total flavonoid determination

Total flavonoids were determined using aluminum chloride colorimetric method as reported by Chang et al. (2002).

Total phenol determination

Total phenol content was estimated by Folin-Ciocalteu method of McDonald et al. (2001) and gallic acid was used a standard.

Determination of tannin content

Quantitative estimation of tannin for each sample was carried out as catechin equivalents using the vanillin–HCl/methanol (Price et al., 1978).

Identification of phenols and flavonoids by HPLC

Phenols and flavonoids were fractionated using HPLC (HP 1100), using a hypersil C_{18} reversed–phase column (250 × 4.6 mm) with 5 µm particle size. UV Detector was set at 254 nm. Flow rate was 0.3 ml/min. Two mobile phase: A (0.5 ml acetic acid/99.5 ml distilled water), mobile phase B (0.5 ml acetic acid/99.5 ml acetonitrile). The separation was performed at room temperature 25°C (Merfort et al., 1997).

Free radical scavenging activity determination

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Koleva et al., 2002). Different concentrations of each herbal extract were added, at an equal volume, to ethanolic solution of DPPH (0.025 g/l). After 30 min at room temperature, the absorbance was measured at 517 nm absorption of blank sample containing the same amount of ethanol and DPPH. The experiment was repeated three times. BHT and quercetin were used as standard controls.

Inhibition (antioxidant activity) was calculated by the following formula:

$$\% \text{ Inhibition} = [(AB - AA)/ AB] \times 100$$

where
AB—absorption of blank sample (t=0 min)
AA—absorption of tested extract solution (t=30 min).

Viability of tumor cells

This study was performed on cells harvested from adult leukemia patients or healthy relatives admitted to the National Cancer Institute (NCI), Cairo University.

The cytotoxicity of each extract on Acute Myeloblastic Leukemia (AML) cells was determined by the MTT assay (Selvakumaran et al., 2003). The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance relying on the

Table 1. Chemical composition of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (% on dry weight basis).

	P. major leaves	*P. major* seeds	*C. tetragonoloba*	L.S.D. at 5%
Crude Protein	14.50^{c}±0.06	15.01^{b}±0.07	25.58^{a}±0.11	0.1639
Fat	0.9^{c}±0.01	1.45^{b}±0.02	2.94^{a}±0.02	0.0370
Ash	16.8^{a}±0.1	7.14^{b}±0.06	3.6^{c}±0.05	0.1442
Crude fiber	13.7^{b}±0.13	26.9^{a}±0.3	10.3^{c}±0.10	0.3929
Total hydrolysable carbohydrate	48.89^{b}±0.44	45.87^{c}±0.36	56.90^{a}±0.6	0.9627

Values are means ± SD of three measurements. Means in the same raw with different letters are significantly different ($p < 0.05$).

conversion of yellow MTT to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells**.** Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient (Pharmacia, Uppsala, Sweden). AML cells were then washed with three changes of PBS. The cell counts were adjusted to 3×10^3 cell /well and plated in 100 µl of medium/well in 96-well plates (Costar Corning Rochester, NY). After overnight incubation, extracts were in various concentrations (100, 200 µg/ml) with cytotoxicity to human normal lymphoid cell line (reported in elsewhere); 3 wells were included in each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) were added per well and cultivated for another 4 h; the supernatant fluid was removed, then 100 µl DMSO were added per well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without cells as blanks. All experiments were performed in triplicate.

Cytoviability % = A_{570} of treated cells / A_{570} of control cells × 100.

Statistical analysis

Statistical analysis was carried out according to (Fisher, 1970) LSD (Least squares difference) test was used to compare the significant differences between means of treatment (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of *P. major* L. and *C. tetragonoloba* L. is shown in Table 1. The results showed that, the C. *tetragonoloba* L. beans contained higher contents of proteins, fats and total hydrolysable carbohydrates compared to *P. major* L.leaves and seeds; meanwhile, it had lower values of ash and crude fibers.

In this respect Kays et al. (2006) found that the protein, fat, carbohydrate and ash contents of C. *tetragonoloba* L. beans were ranged between 22.9 - 30.6%, 2.88 - 3.45%, 50.2 - 59.9% and 3.04 - 3.53%, respectively. Al-Hafedh and Siddiqui (1998) reported that the C. *tetragonoloba* L. beans had 32.81% crude proteins, 3.18% crude fats, 4.19% ash and 10.87% crude fibers. In addition Khatta et al. (1988) examined four cultivars of C. *tetragonoloba* L. and found 24.5 - 32.9% crude proteins, 2.4 - 3.3% crude fats, 3.2 - 4.0% ash and 9.0 - 10.2% crude fibers.

Plantago seeds had higher contents of crude fibers and proteins than that of *plantago* leaves. These data are in the line with those obtained by Romero-Baranzini et al. (2006) who mentioned that, *plantago ovata* seeds contained crude proteins (17.4%), crude fats (6.7%), ash (2.7%) and carbohydrates (48.6%). It is well known that protein content differs among cultivars due to differences in genotype and environmental conditions during developing and maturation of the grains. On the other hand, leaves and seeds of *P. ovata* contained crude proteins of 21.87% and 13.12%, respectively (Romero-Baranzini et al., 2006).

Fatty acid composition

The saturated and unsaturated fatty acids of the *P. major* L. seeds and leaves and *C. tetragonoloba* L. beans are shown in Table 2.

From the tabulated data, it could be stated that *P. major* L. and *C. tetragonoloba* L. are rich in unsaturated fatty acids. *C. tetragonoloba* L.and *P. major* seeds contained high amounts of linoleic acid $C_{18:2}$ (48.99 and 25.41%**,** respectively), while *plantago* leaves had the highest amounts of linolenic acid $C_{18:3}$ (56.19%). These results are in agreement with those obtained by Liu et al. (2002) who reported the major fatty acids of *P. major* L. leaves were linolenic, linoleic and palmitic acid, but smaller amounts of stearic and oleic acid.

In the meantime, $C_{20:0}$ recorded 3.49 and 0.41% for *P. major* L. seeds and leaves, respectively. These results are in agreement with Samuelsen et al. (2000) who reported that arachidic acid was isolated from *P. major* L. only, and not from any other investigated *Plantago* species.

Amino acid composition

In order to obtain more information about the chemical composition of the *P. major* L. and *C. tetragonoloba*, the

Table 2. Fatty acid composition % of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (% of total lipid).

Fatty acid	*P. major* L. leaves	*P. major* L. seeds	C. *tetragonoloba* L. beans
$C_{12:0}$	-	10.18	-
$C_{14:0}$	-	9.27	-
$C_{16:0}$	17.99	15.48	14.72
$C_{l6:l}$	0.73	0.37	0.07
$C_{18:0}$	1.62	3.84	7.81
$C_{l8:l}$	4.08	11.89	21.85
$C_{l8:2}$	18.73	25.41	48.99
$C_{18:3}$	56.19	20.07	5.11
$C_{20:0}$	0.41	3.49	1.16
$C_{20:1}$	0.26	-	0.28
Unsaturated	79.98	57.74	76.31
Saturated	20.02	42.26	23.69
Unsaturated F.A/ Saturated F.A	3.22	1.37	3.99

Table 3. Amino acid composition of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (g/100g sample) on dry weight basis.

Amino acids	*P. major* L. leaves	*P. major* L. seeds	C. *tetragonoloba* L. beans
Lysine	0.79±0.03	0.51±0.008	1.05±0.04
Threonine	0.64±0.01	0.48±0.009	0.75±0.01
Valine	0.92±0.03	0.70±0.01	0.81±0.03
Isoleucine	0.92±0.03	0.45±0.008	0.79±0.03
Leucine	1.30±0.05	0.77±0.03	1.31±0.05
Phenylalanine	0.80±0.03	0.55±0.01	0.89±0.03
Tyrosine	0.64±0.01	0.41±0.008	0.87±0.03
Total essential amino acid	6.01	3.87	6.47
Serine	0.62±0.02	0.59±0.01	1.07±0.04
Proline	0.64±0.03	0.62±0.03	0.71±0.04
Glycine	0.70±0.02	0.70±0.01	1.27±0.05
Alanine	0.75±0.04	0.59±0.02	0.93±0.05
Aspartic	1.85±0.03	1.05±0.04	2.22±0.04
Glutamic	1.97±0.03	2.16±0.05	4.41±0.08
Histidine	0.32±0.006	0.26±0.006	0.53±0.01
Arginine	0.70±0.02	0.72±.03	2.41±0.09
Total non-essential amino acids	7.55	6.69	13.55

Values are means ± estimated uncertainty (U).

amino acids were determined and presented in Table 3.

The results showed that, the C. *tetragonoloba* L. beans contained lightly higher total essential amino acids (6.47%) compared with *plantago* seeds (3.87%).

These results are in agreement with those obtained by Khalil (2001) who mentioned that, histidine of *P. major* L. leaves and seeds are (0.32 and 0.256%, respectively). However, it contained high amounts of other amino acids especially glutamic acid, aspartic acid, leucine, and valine. In the same line, Romero-Baranzini et al. (2006) found that *P. ovate* contained high amount of glutamic and aspartic amino acids.

Biological active compounds

Total phenol, total flavonoid and tannin contents of *P. major* L. leaves and seeds, and *C. tetragonoloba* L. beans are shown in Table 4.

The results in Table 4 indicate that *P. major* L. leaves contained the highest total phenol content (13.05. mg/g) followed by *P. major* L. seeds (7.43 mg/g) and

Table 4. Biological active compounds of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (mg/g) on dry weight basis.

Constituent	*P. major* L. leaves	*P. major* L. seeds	C. *tetragonoloba* beans	L.S.D. at 5%
Total phenol (mg gallic/g)	13.05^{a}±0.10	7.43^{b}±0.07	3.76^{c}±0.04	0.1496
Total flavonoid (mg Quercetin/g)	6.41^{a} ±0.04	3.03^{b} ±.03	0.83^{c} ±0.01	0.05652
Tannins (mg Catechine /g)	5.63^{a} ±0.06	2.43^{b} ±0.03	0.76^{c} ±0.01	0.07838

Values are means ± SD of three measurements. Means in the same raw with different letters are significantly different ($p < 0.05$).

Table 5. Phenolic and falvonoide compounds of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (mg/100 g).

Compounds	*P. major leaves*	*P. major seeds*	*C. tetragonoloba beans*
Gallic	4.8096	0.686	1.090
pyrogallic	-	-	-
Catechol	0.241	2.356	4.103
Catechine	15.924	3.684	1.003
Caffeic acid	8.164	9.832	0.222
Vanillic acid	2.478	1.365	-
Syrengic	4.158	3.074	1.788
Caffeine	-	-	3.158
Ellagic	132.110	-	1.055
Coumarin	2.347	0.4387	-
Ferulic acid	32.439	48.584	1.885
Colchicine	25.026	1.828	6.851
Luteolin	10.079	7.107	1.04
Quercetin	2.089	1.563	0.030
Isorhhomnetin	-	-	-
Rutin	4.323	4.071	0.338
Quercetrin	0.323	0.542	1.056
sakaruntin	-	-	-
Naringenin	0.086	0.892	0.045
Hypersoid	0.992	-	-
Chrisin	0.0224	0.013	0.043
Kaempferol	0.825	0.347	0.026

C. *tetragonoloba* L. beans (3.76 mg/g).

It is also clear that, flavonoids and tannin are present in lower levels in C. *tetragonoloba* L. beans (0.83 and 0.76 mg/g, respectively) compared to *P. major* L. leaves (6.41 and 5.63 mg/g, respectively) and *P. major* L. seeds (3.03 and 2.43 mg/g).

These results are in agreement with those obtained by Gálvez et al. (2005) who found that the flavonoids content ranged between 0.69 - 3.09% in *Plantago* species**.** Souri et al*.* (2008) reported that the phenols content of *P. major* L. seeds were 672 mg/100 g.

Also**,** Grubešić et al. (2005) demonstrated that the tannins content in *Plantago* species leaves was (0.56 - 2.26%). Kaushal and Bhatia (2006) outlined that guar beans contained flavonoids in the range of 0.13 to 0.23%.

Identification of phenols and flavonoid

The data of phenolic acids and falvonoid compounds of *P. major* L. seeds and leaves, and *C. tetragonoloba* L. beans are shown in Table 5.

The data indicate that the *P. major* L. (leaves and seeds) and C. *tetragonoloba* L. comprised biologically active phenolic compounds ferulic, caffeic and vanillic acids, and flavonoid compound, that is, luteolin. Ferulic acid was the *major* phenolic compound in the *P. major* L. seeds and leaves, while in *C. tetragonoloba* L. beans colchicine was the *major* phenolic compound. On the other hand, luteolin was the *major* flavonoid compound in the *P. major* L. seeds and leaves.

The aforementioned data are in line with data of

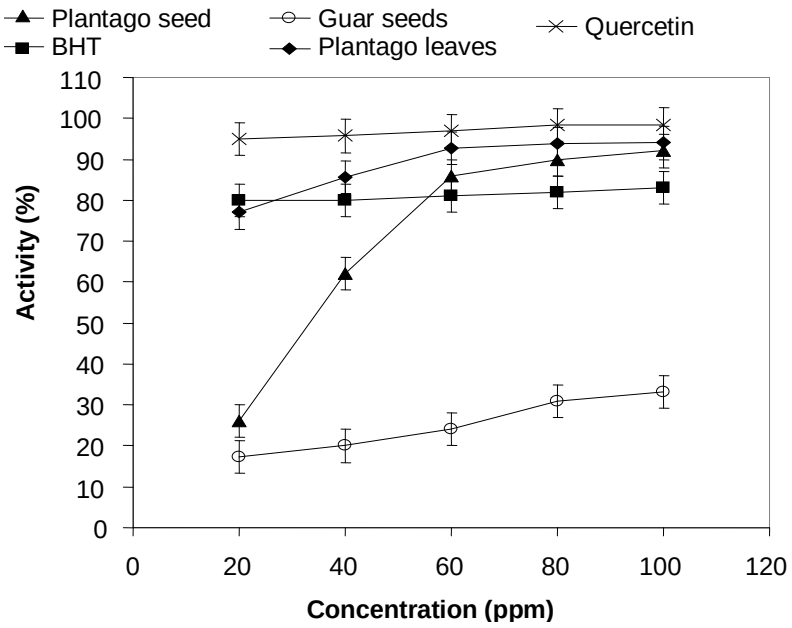

Figure 1. Antioxidant activity of ethanolic extract of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. (guar beans).

Kawashty et al. (1994) who studied the flavonoid profile of 18 species of *Plantago.* They indicated that, the flavonoid compound luteolin was the major component in most of *Plantago* species.

In this context Samuelsen et al. (2000**)** reported that, *Plantago* contains the following biologically active compounds (vanillic acid), flavonoid (luteolin) and phenolic compounds (caffeic and ferulic acids).

In addition, Kaushal and Bhatia (2006) reported that gallic acid as well as its derivatives, kaempferol, caffeic acid and ellagic acid were derived from *C. tetragonoloba* L. beans. Furthermore, Wang and Morris (2007) stated that C*. tetragonoloba* L. beans seem to be a good source of quercetin and kaempferol.

Antioxidant activity

The antioxidant activity of ethanolic, hot water and cold water extracts of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. (guar beans) are illustrated in Figures 1, 2 and 3, respectively.

It could be observed that the highest antioxidant activity was found in ethanolic extract of *P. major* L. leaves even at low concentration of 20 ppm, and increased with increasing the concentration up to 60 ppm which is also the most effective concentration of *P. major* L. seeds. It is worthy to mention that C*. tetragonoloba* L. beans exhibited low antioxidant activity at different concentrations of the ethanolic extract (Figure 1).

Furthermore, the data illustrated in Figures 2 and 3 clearly indicated that, either hot or cold water extract of *P. major* L. leaves exhibited the most active ones, when compared with *P. major* L. and C*. tetragonoloba* L. seeds. It is also observed that, ethanolic extracts were more active than the hot and cold water extracts of the samples under investigation. In general the activity increased with increasing the concentration of extract.

However, the radical scavenging activity of the plant extracts can be arranged in the following order: *P. major* L. leaves > *P. major* L. seeds > *C.tetragonoloba L.* beans.

Pourmorad et al. (2006) reported that antioxidant activity *P. major* L. leaves are 89.3% at 0.8 mg/ml concentration.

The results of the present study are in agreement with Gálvez et al. (2005), Souri et al. (2008) and Beara et al. (2009) who examined *Plantago* species for anti oxidant activity and reported that it could be regarded as a possible new sources of natural antioxidants.

Anti tumor

The results of the cytotoxic effect of ethanolic, hot and cold extracts from *P. major* L. leaves and seeds and *C. tetragonoloba* L. beans on AML cells are summarized in Table 6.

The results showed that all extracts possessed anticancer activity with differing degrees. A dose-dependent inhibition of cell proliferation was observed for most of the all extracts tested in this study.

It is clear that ethanolic extract of *P. major* L. leaves had the greatest effect on tumor cell growth (Dead 74% ±

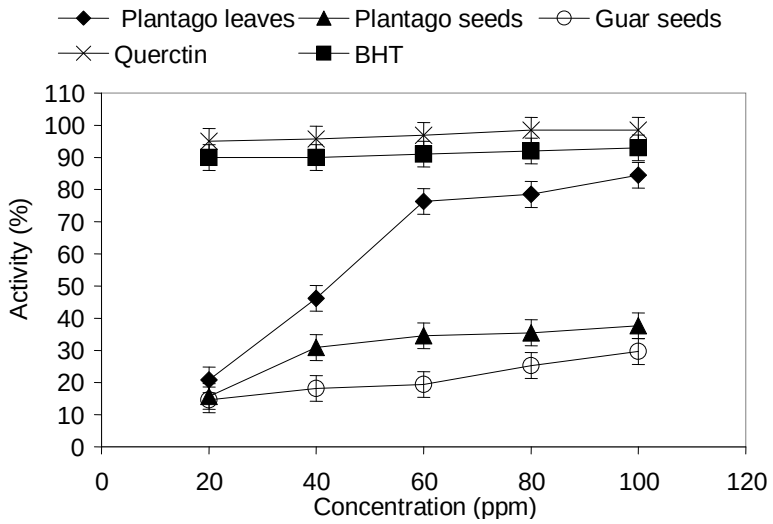

Figure 2. Antioxidant activity of hot water extract of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. (guar beans).

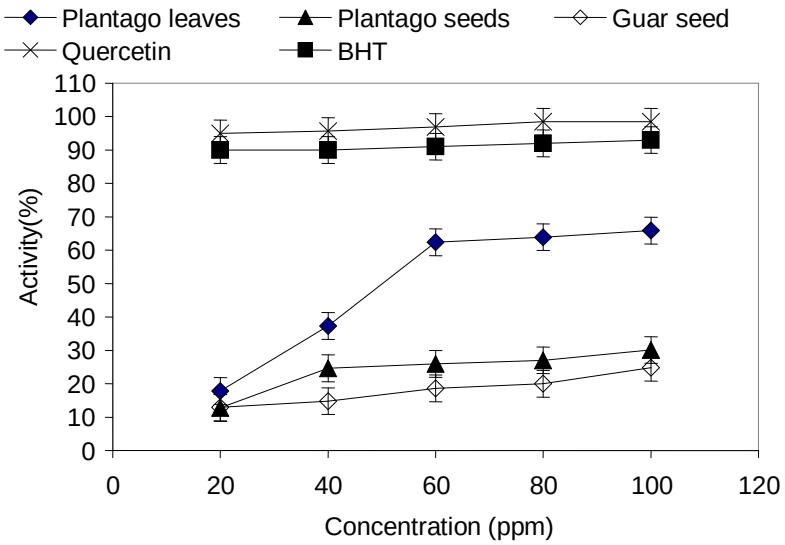

Figure 3. Antioxidant activity of cold water extract of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. (guar beans).

0.35) followed by hot water extract of *P .major* L. leaves (Dead 54.6% ± 1.21).

In this respect, Gomez et al. (2000) observed that *P. major* L. leaf extracts activate nitric oxide and TNF-α production of macrophages-mediated lymphocyte proliferation. Macrophages play an important role in modulating humoral and cellular immunity against infectious diseases and cancer.

Gálvez et al. (2003) demonstrated that *Plantago* spp. extracts have shown growth inhibitory and cytotoxic effects on melanoma cell lines and breast adenocarcinoma. It is thought that cytotoxic activity depends basically on flavonoids, flavone and luteolin.

Luteolin exhibited inhibitory effect on various human cancer cell lines, such as renal A-549, ovary SK-OV-3, melanoma SK-MEL-2, XF-498, HCT15, gastric HGC-27, breast MCF-7 and human leukemia cells (Le Bail et al., 1998; Post and Varma, 1992; Ryu et al., 1994).

Chiang et al. (2003) showed that hot water extracts of *P. major* L. and *Plantago asiatica* possessed effects of immunomodulatory activity on human mononuclear cells proliferation.

In addition, Okazaki et al. (2002) reported that fibers had a remarkable effect in preventing colorectal cancer

Table 6. The effect of extracts on National Cancer Institute (NCI) 20 (male – 65 years old) cell after incubation for 24 h.

Plant extract	Concentration (µg/ml)		
	50	75	100
	Dead %	Dead %	Dead %
C. tetragonoloba L Hot water	14.2^{fg}±2.66	30.7^{c}±1.68	39.3^{d}±0.43
C. tetragonoloba L cold water	11.8^{g}±1.40	26.31^{d}±2.85	28.4^{h}±2.35
C. tetragonoloba L ethanolic	24.6^{bc}±2.58	31.0^{c}±3.56	33.4^{ef}±2.29
P. major L. seeds Hot water	27.5^{b}±1.30	31.0^{c}±2.66	31.6^{fg}±1.57
P. major L. seeds cold water	16.3^{ef}±1.17	22.8^{d}±1.40	29.9^{gh}±2.26
P. major L. seeds ethanolic	19.2^{de}±2.35	35.5^{b}±2.58	34.7^{e}±0.32
P. major L. leaves hot water	22.2^{cd}±1.92	37.4^{b}±1.30	48.7^{b}±1.23
P. major L. leaves cold water	26.5^{b}±2.57	39.2^{b}±1.17	43.5^{c}±1.20
P. major L. leaves ethanolic	47^{a}±1.76	62.1^{a}±2.35	74.6^{a}±0.35
L.S.D. at 5%	3.4948	3.9573	2.6628

Values are means ± SD of three measurements. Means in the same row with different letters are significantly different ($p < 0.05$).

and other cancer risks.

Conclusion

This investigation shows that the *P. major* and *C. tetragonoloba* contained important biologically active compounds and *P. major* leaves had the highest total phenol, flavonoid and tannin content. In addition, ethanol, cold and hot extracts of the same plants showed antioxidant activity, but the highest antioxidant activity was found in ethanolic extract of *P. major* leaves .Also, ethanolic extract of *P. major* leaves had the greatest effect on tumor cell growth followed by hot water extract of *P. major* leaves.

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