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BIOCHEMICAL STUDIES ON *PLANTAGO MAJOR* AND *CYAMOPSIS* *TETRAGONOLOBA*

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ABSTRACT

Plantago major (seeds and leaves) and *Cyamopsis tetragonoloba* (guar beans) were analyzed for their chemical components including, fatty acids and amino acids. Guar beans had high contents of proteins, fats and total hydrolysable carbohydrates. *Plantago* leaves contained high percentage of linolenic acid (56.19%). While, *Plantago major* seeds and Guar beans had high percentages of linoleic acid (25.41 and 48.99%, respectively). Guar beans had high amounts of glutamic, arginine, aspartic and leucine. Total phenol, total flavonoid and tannin contents 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 plants had 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, antioxidant Activity, anti-tumor.

INTRODUCTION

There is a growing trend to use medicinal plants because of their medical effectiveness and low toxicity, there are many natural anticancer agents derived from these plants (Ozaslan *et al.*, 2007) *Plantago major* L. is a perennial plant from Plantaginaceae family. 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. *P. major* L has been used to cure from many diseases varying from cold to viral hepatitis (McCutcheon *et al.*, 1995). *P. major* has been also used as an anesthetic, antiviral, anti-inflammatory, astringent, anti-helminthic, analgesic, analeptic, antihistaminic, antirheumatic, antitumor, anti-ulcer, diuretic, expectorant and hypotensive in traditional medicine (Grigorescu *et al.*, 1973; Matev *et al.*; 1982 and 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. hot water extract induced inhibition effect on Ehrlich Ascites Tumor (EAT) and prevented tumor extension (Ozaslan *et al.*, 2007). In addition, its hot water extract inhibits tumor activity on the Ehrlich ascites tumors (EAC cells) (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 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).

Cyamopsis 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 antiulcer, antisecretory, cytoprotective, hypoglycemic, hypolipidemic and antihyperglycemic 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 *Cyamopsis tetragonoloba* L. and to evaluate their antioxidant and anticancer effects.

MATERIALS AND METHODS

Chemicals and raw materials:

Aerial parts of the *Plantago 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.

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, ethanol and methanol were purchased from Merck Co. (Germany). The solvents were purified and bidistilled before use.

Chemical analysis:

Proximate chemical analysis, crude protein, fat, ash and crude fiber contents of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans were determined as described in AOAC (2005). Total hydrolysable carbohydrate was 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 (2005).

Identification of fatty acids:

The fatty acids of the oil were converted to methyl esters according to the method of Glass (1971) and identified using a Capillary gas chromatography (HP 6890) equipped with DB-23 (50%-cyano propyl)-methylpoly siloxane) capillary column (60m × 0.32 mm × 0.25 µm film thickness) and a flame ionization detector. Nitrogen flow rate was 0.6 ml/min, hydrogen and air-flow rates were 45 and, 450 ml/min, respectively. The oven temperature was isothermally heated at 195°C. The injector and the detector temperatures were 230°C and 250°C, respectively.

Preparation of plant extracts:

Plant materials were air dried at room temperature and ground in a mortar. Fifty grams of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans powder were 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 *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans (50 g) were boiled in 100 ml distilled water, and then filtrated. This procedure was repeated 3 times. The aqueous extracts were combined and concentrated under vacuum, and then lyophilized.

Total flavonoid determination

Total flavonoid was 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 Singleton and Rossi (1965) using gallic acid as standard.

Determination of tannin content

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

Identification of phenols and flavonoids by HPLC

Phenols and flavonoids were fractionated using HPLC (HP 1100), using a hypersil C₁₈ reversed – phase column (250x 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/99.5ml distilled water), mobile phase B (0.5 ml acetic acid/99.5 ml acetonitrile). The separation was performed at ambient temperature 25°C . (Daigle and Conkerton, 1983)

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 15 min at room temperature, the absorbance was measured at 517 nm. The experiment was repeated for three times. BHT and quercetin were used as standard controls.

Viability of Tumor Cells

This study was performed on cells harvested from adult leukemia patients or healthy relatives admitted to the National Cancer

Institute, Cairo University. International protocols governing the ethical treatment of patient were followed.

The cytotoxicity of each extract on AML cells was determined by the MTT assay (Mosmann, 1983). AML were diagnosed by peripheral blood and bone marrow examination, cytochemistry and immunological markers in some cases. Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient (Pharmacia, Uppsala, Sweden). 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.

Calculation:-

The effect of extracts on the proliferation of human AML cells was expressed as the cytoviability %, using the following formula :-

$$\text{cytoviability \%} = (A_{570} \text{ of treated cells} / A_{570} \text{ of control cells}) \times 100.$$

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of *P. major* L. and *C. tetragonoloba* L. is shown in Table (1).

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

Investigated samples	Crude Protein	Fat	Ash	Crude fiber	Total hydrolysable carbohydrate
<i>P. major</i> L. leaves	14.50±0.06	0.9±0.01	16.8±0.1	13.7±0.13	48.89±0.44
<i>P. major</i> L. seeds	15.01±0.07	1.45±0.02	7.14±0.06	26.9±0.3	45.87±0.36
<i>C. tetragonoloba</i> L. beans	25.58±0.11	2.94±0.02	3.6±0.05	10.3±0.10	56.90±0.6

Values are means ± SD of three measurements.

The results showed that, the *C. tetragonoloba* L. beans contained higher contents of protein, fat and total hydrolysable carbohydrate

compared to *P. major* L. leaves and seeds. While, it had lower values of ash and crud 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 trend 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, Bukhsh *et al.* (2007) reported that leaves and seeds of *P. ovata* contained crude proteins of 21.87% and 13.12%, respectively. While, Hendawy (2008) found that *Plantago arenaria* seeds contained 46.25 % carbohydrates.

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, oleic acid.

In the mean time, arachidic acid recorded 3.49 and 0.41% for *P. major* L. seeds and leaves, respectively. These results are in agreement with Samuelson *et al.* (2000) who reported that, arachidic

acid was isolated from *P. major* L. only, and not from any other investigated *Plantago* species.

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

Fatty acid	<i>P. major</i> L. leaves	<i>P. major</i> L. seeds	<i>C. tetragonoloba</i> L. beans
C _{12:0}	-	10.18	-
C _{14:0}	-	9.27	-
C _{16:0}	17.99	15.48	14.72
C _{16:1}	0.73	0.37	0.07
C _{18:0}	1.62	3.84	7.81
C _{18:1}	4.08	11.89	21.85
C _{18:2}	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
Total unsaturated F.A	79.98	57.74	76.31
Total saturated F.A	20.02	42.26	23.69
Unsaturated F.A/ Saturated F.A	3.22	1.37	3.99

Amino Acid composition:

In order to obtain more information about the chemical composition of the *P. major* L. and *C. tetragonoloba*, the 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* leaves (6.01%) and seeds (3.87%). On the other hand, *C. tetragonoloba* L. beans comprised high amounts of the amino acids glutamic, arginine, aspartic and leucine (4.41, 2.41, 2.22 and 1.31%, respectively).

These results are in agreement with those obtained by Khalil (2001) who mentioned that, histidine of *P. major* L. leaves and seeds were 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 *Plantago ovata* contained high amount of glutamic and aspartic amino acids .

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	<i>P. major</i> L. leaves	<i>P. major</i> L. seeds	<i>C. tetragonoloba</i> 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±0.03	2.41±0.09
Total non-essential amino acids	7.55	6.69	13.55

Values are means ± Estimated Uncertainty (U).

Biological active compounds:

Total phenols, total flavonoids and tannin contents of *P. major* L. leaves and seeds, and *C. tetragonoloba* L. beans are shown in Table (4).

Table (4): Biological active compounds of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans as mg/100g dry weight

Constituent	<i>P. major</i> L. leaves	<i>P. major</i> L. seeds	<i>C. tetragonoloba</i> beans
Total phenol	1305.75±10.08	743.00±7.17	376.00±3.889
Total flavonoid	641.20±6.84	303.44±2.94	83.98±1.10
Tannins	562.90±6.04	243.06±3.035	76.68±0.72

Values are means ± SD of three measurements

The results in Table (4) indicate that *P. major* L. leaves contained the highest total phenol content (1305.75 mg/100g) followed by *P. major* L. seeds (743 mg/100g) and *C. tetragonoloba* L. beans (376 mg/100g).

It is also clear that, flavonoids and tannins are present in lower levels in *C. tetragonoloba* L. beans (83.98 and 76.68 mg/100g, respectively) compared to *P. major* L. leaves (641.2 and 562.9 mg/100g, respectively) and *P. major* L. seeds (303.44 and 243.06 mg/100g).

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. Souiri *et al.* (2008) reported that the phenols content of *plantago major* L. seeds were 672 mg/100g.

Also, Grubešić *et al.* (2005) demonstrated that the tannins content in *plantago* species leaves was ranged from 0.56 to 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 flavonoid 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 acid and vanillic acid, and flavonoid compound ,i.e., luteolin. Ferulic acid was the *major* phenolic compound in the *P. major* L. seeds and leaves while, in *C. tetragonoloba* L. beans colchicin 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 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* contain the following biologically active compounds (vanillic acid), flavonoid (luteolin), phenolic compounds (caffeic acid and ferulic acid).

In addition Kaushal and Bhatia (2006) reported that, gallic acid, gallic acid 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.

Table (5): Phenolic and flavonoide compounds of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. beans as mg/100g dry weight

Compounds	<i>P. major</i> leaves	<i>P. major</i> seeds	<i>C. tetragonoloba</i> beans
Gallic	4.8096	0.686	1.090
pyrogallic	-	-	-
Catechol	0.241	2.356	4.103
Catechin	15.924	3.684	1.003
Caffeic acid	8.164	9.832	0.222
Vanillic acid	2.478	1.365	-
Syrengeic	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
Colchicin	25.026	1.828	6.851
Luteolin	10.079	7.107	1.04
Quercetin	2.089	1.563	0.030
Isorhomonnetin	-	-	-
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

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 Table (6).

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases (Koleva *et al.*, 2002).

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 (Table6).

Further more, the data illustrated in Table 6 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 ethanolic, hot and water 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 the antioxidant activity *plantago major* L. leaves methanol extract was 89.3%

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

Table (6) Antioxidant activity of ethanolic, hot and cold water extracts of *P. major* L. (seeds and leaves) and *C. tetragonoloba* L. (guar beans)

Plant extract	Concentration ($\mu\text{g/ml}$)				
	20	40	60	80	100
<i>plantago</i> leaves ethanolic	77 \pm 1.15	85.7 \pm 1.28	92.6 \pm 1.39	93.8 \pm 1.41	94 \pm 1.41
<i>plantago</i> leaves hot water	20.84 \pm 0.29	46.19 \pm 0.69	76.3 \pm 1.06	78.5 \pm 1.09	84.5 \pm 1.26
<i>plantago</i> leaves cold water	17.8 \pm 0.26	37.29 \pm 0.55	62.37 \pm 0.93	63.9 \pm 0.95	65.9 \pm 0.98
<i>plantago</i> seed ethanolic	26 \pm 0.39	62 \pm 0.93	86 \pm 1.29	88 \pm 1.32	89 \pm 1.33
<i>plantago</i> seed hot water	15.7 \pm 0.23	30.9 \pm 0.46	34.6 \pm 0.51	35.49 \pm 0.53	37.7 \pm 0.56
<i>plantago</i> seed cold water	12.74 \pm 0.19	24.61 \pm 0.36	25.93 \pm 0.38	27.03 \pm 0.40	30.10 \pm 0.45
guar beans ethanolic	17.3 \pm 0.25	20 \pm 0.3	24 \pm 0.36	31 \pm 0.46	33.2 \pm 0.49
guar beans hot water	14.6 \pm 0.21	18.18 \pm 0.27	19.4 \pm 0.29	25.3 \pm 0.37	29.66 \pm 0.44
guar beans cold water	12.9 \pm 0.19	14.8 \pm 0.22	18.6 \pm 0.27	20 \pm 0.30	24.8 \pm 0.37
quercetin	95 \pm 1.42	95.7 \pm 1.43	96.9 \pm 1.45	98.47 \pm 1.47	98.5 \pm 1.47
BHT	80 \pm 1.2	80 \pm 1.18	81 \pm 1.21	82 \pm 1.23	83 \pm 1.24

Values are means \pm SD of three measurements

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 (7).

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% \pm .35) followed by hot water extract of *P. major* L. leaves (Dead 54.6 % \pm 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 (Post and Varma, 1992; Ryu *et al.*, 1994; Le Bail *et al.*, 1998).

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

Table (7): The effect of samples on NCI 20 (male – 65y) cell after incubation for 24 h

Plant extract	Concentration ($\mu\text{g/ml}$)		
	50	75	100
	Dead %	Dead	Dead
<i>C. tetragonoloba</i> L hot water	14.2 \pm 2.66	30.7 \pm 1.68	39.3 \pm 0.43
<i>C. tetragonoloba</i> L cold water	11.8 \pm 1.4	26.31 \pm 2.85	28.4 \pm 2.35
<i>C. tetragonoloba</i> L ethanolic	24.6 \pm 2.58	31 \pm 3.56	33.4 \pm 2.29
<i>P. major</i> L. seeds hot water	27.5 \pm 1.3	31.00 \pm 2.66	31.6 \pm 1.57
<i>P. major</i> L. seeds cold water	16.3 \pm 1.17	22.8 \pm 1.4	29.9 \pm 2.26
<i>P. major</i> L. seeds ethanolic	19.2 \pm 2.35	35.5 \pm 2.58	34.7 \pm 0.32
<i>P. major</i> L. leaves hot water	22.2 \pm 1.92	37.4 \pm 1.3	48.7 \pm 1.23
<i>P. major</i> L. leaves cold water	26.5 \pm 2.57	39.2 \pm 1.17	43.5 \pm 1.2
<i>P. major</i> L. leaves ethanolic	47 \pm 1.76	62.1 \pm 2.35	74.6 \pm 0.35

Values are means \pm SD of three measurements

Conclusion

This investigation shows that the *Plantago major* and *Cyamopsis tetragonoloba* contained an important biologically active compounds and *Plantago 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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دراسات كيميائية حيوية علي لسان الحمل والجوار

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تمت دراسة التركيب الكيماوي لبذور واوراق نبات لسان الحمل وبذور نبات الجوار متضمنة الاحماض الدهنية والامينية. وقد اظهرت الدراسة ان بذور نبات الجوار قد سجل اعلي محتوى من البروتين والدهن والكاربوهيدرات. كما وجد ايضا ان اوراق لسان الحمل تحتوي علي نسبة عالية من حامض اللينولينك (56.19%) بينما اوضحت النتائج ان كلا من بذور لسان الحمل والجوار تحتوي علي كمية كبيرة من حامض اللينولينك (25.41-48.99%) علي التوالي. وايضا بينت الدراسة ان بذور نبات الجوار ذات محتوى عالي من كلا من حامض الجلوتاميك والاسبارتيك والليوسين

كما اظهرت الدراسة ان اوراق لسان الحمل ذات محتوى عالي من الفينولات والفلافونيدات الكلية والتانينات وايضا تمت دراسة التأثير المضاد للتأكسد للمستخلص الايثانولي والمائي البارد والساخن لاوراق وبذور لسان الحمل وبذور الجوار حيث اظهرت ان مستخلصات اوراق لسان الحمل اعطت اعلي تأثير مضاد للتأكسد مقارنة بمستخلصات بذور لسان الحمل وبذور الجوار.

كما اوضحت الدراسة ايضا النشاط المضاد لسرطان الدم للمستخلص الكحولي والمائي البارد والساخن للنباتات موضع الدراسة ولكن بدرجات متفاوتة . حيث بينت الدراسة ان المستخلص المستخلص الايثانولي لاوراق لسان الحمل كان له التأثير الاكبر علي موت الخلايا السرطانية (74% نسبة الموت) يليه المستخلص المائي لاوراق لسان الحمل (54.6% نسبة موت).

الكلمات الدالة: لسان الحمل الكبير ، بذور الجوار ، مضاد للتأكسد ، مضاد للسرطان.