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Evaluation of Anticancer, Antimycoplasmal Activities and Chemical Composition of Guar (*Cyamopsis tetragonoloba*) Seeds Extract.

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ABSTRACT

The present study investigated the anticancer, antimycoplasmal activities and chemical composition of guar (*Cyamopsis tetragonoloba*) seeds. The anticancer activity of *Cyamopsis tetragonoloba* was determined using sulphorhodamine-B (SRB) assay against human prostate carcinoma cell line (PC3), colon carcinoma cell line (HCT_{116}) and intestinal carcinoma cell line (CACO-2). The results of present study indicated that guar seeds extract possessed anticancer activities against PC-3, HCT_{116} and CACO-2 cell lines with half maximal inhibitory concentration (IC_{50}) of 40.5, 41.0 and 101.0 µg/ml, respectively. The antibacterial activities of the guar seeds crude extract were determined against *mycoplasma bovis* and *mycoplasma gallisepticum*. The chemical constituents were determined by quantifying ash, fat, fibers, moisture, protein and minerals. The results showed that the seeds contained 4.53% ash, 3.32 % fat, 11.06% fiber, 10.0% moisture and 33.25% protein. The most abundant minerals and fatty acids detected in guar seeds were iron (465 ppm) and *cis*-linoleic acid (53.89 %), respectively. Essential and non essential amino acids were present in guar seeds. HPLC analysis of the carbohydrate profile detected the presence of D-glalactose (27.45%) and D-mannose (56.04%) in the seeds. The bioactive compounds found were 2.47 mg/g phenolics and 2.85 mg/g tannins. GC–MS analysis of methanolic extract of guar seeds showed the presence of several metabolites such as 3-hydroxymyristic acid, octadecanoic acid and linolelaidic acid methyl ester.

Keywords: guar seeds; antitumor activity; antimycoplasmal activity; chemical composition.

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INTRODUCTION

Cancer is a major health problem in developing countries and the second leading cause of death [1,2]. Because of high death rate associated with cancer and because of serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary methods of treatment [3]. Terrestrial plants and their extracts have been used as medicines in Egypt and other countries from ancient times and an impressive number of modern drugs have been developed from them [4,5]. On the other hand, *Mycoplasma* refers to a genus of bacteria that lack a cell wall [6]. Without a cell wall, they are unaffected by many common antibiotics such as penicillin or other beta-lactam antibiotics that target cell wall synthesis [7,8]. There are over 100 recognized species of the genus *Mycoplasma*, one of several genera within the bacterial class *Mollicutes*. The *Mycoplasma* which has economic importance in veterinary practice is *mycoplasma gallisepticum* which cause chronic respiratory disease in chickens and turkeys [9]. The *mycoplasma bovis* is also a pathogen causing respiratory disease, otitis media, arthritis, mastitis, and a variety of other diseases in cattle [10]. The application of various antibacterial agents veterinary *Mycoplasma* has been proposed in literature [11]. However, the chemists started to use natural products as antimycoplasmal agents [12].

Legume seeds have received attention as functional foods, because of their nutritive values including amino acid, fiber, trace elements, vitamins, flavonoids, and phenolic acids [13,14]. The guar or cluster bean (*Cyamopsis tetragonoloba*) is basically a legume and the source of guar gum [15]. Several phytochemical works has been carried out on this plant [16-18]. The polyphenol composition of the plant included gallotannins, gallic acid, gallic acid derivatives, myricetin-7- glucoside-3-glycoside, chlorogenic acid, ellegic acid, 2,3,4-trihydroxy benzoic acid, texasin-7-O-glucoside and p-coumaryl quinic acid [19]. The sterols of guar seeds included campesterol, avenasterol, stigmasterol, sitosterol and traces of Delta-7-avenasterol, stigmast-7-enol, brassicasterol and cholesterol [20]. *Cyamopsis tetragonoloba* is a well-known traditional plant used in folklore medicine and acts as an appetizer, digestive aid and laxative. Additionally, it is useful in dyspepsia anorexia, anti-secretory, hypolipidemic and anti-hyperglycemic effects [21]. In addition, Guar seeds are potentially a high source of different natural compounds [22]. In the course of our studying program of medicinal plants with nutritional values [23], we investigated the *Cyamopsis tetragonoloba* seeds. The aim of this study is to evaluate the anticancer, antimycoplasmal activities and determine the chemical composition and of guar (*Cyamopsis tetragonoloba*) seeds.

MATERIALS AND METHODS

Collection of samples and extraction

Guar seeds (*Cyamopsis tetragonoloba)* were obtained from the Crops Research Institute, Agriculture Research Center, Giza, Egypt. The collected seeds (1 Kg) are washed with tap water, dried and then crushed to very fine powder with crusher at the Agricultural Department, Institute of Sufficient Productivity, Zagazig University. For the biological assay, 10.0 g of the powdered guar

seeds was macerated in methanol: water (1: 1) for 72 hours. After filtration and concentration under vacuum, the crude extract was kept in the refrigerator.

Cytotoxicity assay

The cytotoxicity assay of guar seeds extract were carried out and documented in the Cancer Biology Department, Pharmacology Unit, National Cancer Institute, Cairo University. It was carried out using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [24]. Stock solution of 100 mM of guar seed extract in DMSO was prepared and stored at -20 °C. Cells (5×10^4 cells/well) of different cell lines were incubated with various guar seeds extract concentrations (62.5, 125.0, 250.0 and 500.0 µg/ml) at 37 °C in a serum-free medium, before being submitted to MTT assay. The relative cell viability was expressed as the mean percentage of viable cells compared with DMSO-treated cells. The half maximal inhibitory concentration of the cell growth (IC_{50}) was calculated.

Antimycoplasmal assay

Liquid and solid media used for isolation and propagation of *Mycoplasma* were prepared as described by Frey *et al*. [25]. The guar seeds extract were dissolved in DMSO (200 mg/ml) in which the paper disks with a diameter of 6 mm impregnated, dried under sterile flow box and put on an agar plates inoculated with *Mycoplasma* strains (*mycoplasma gallisepticum* and *mycoplasma bovis*). The plates were incubated in CO_2 incubator at 37 °C for 24 hours under. The results were recorded by measuring the size of zone of inhibition. DMSO was used as a negative control.

Chemical composition

Guar seeds powder were analyzed for chemical composition (ash, fat, fibers, moisture and protein) according to the reported method [26, 27]. Estimation of minerals (calcium, phosphorus, sodium, potassium, iron, zinc and copper) in guar seeds was done by inductive coupled plasma ICP "optima 2000" [28]. Estimation of the fatty acids was carried out in accordance with the method of Morrison and Smith [29]. Amino acid profiles of the guar seeds were determined by AOAC protocol [30].

Carbohydrate profile:

Samples of guar seeds and their extract were analyzed for sugar by high performance liquid chromatography (HPLC) according method AOAC [31]. Sugars were extracted in DMSO; the extracted was passed through C_{18} Sep-Pak cartage and stored under refrigerator condition till analysis. Samples were filtered through a 0.45 µm membrane. Analysis of the sugars in the filtrate was performed by using HPLC , Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID -10A Shimadzu detector, LC-16 ADVP binary pump, DC ou-14 A degasser and Shodex PL Hi-Plex Pb column (Sc 1011 No. H706081). Guard column Sc-Lc Shodex, and

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heater set at 65 ° C. Separation and quantitation were carried out on an amino - bonded column with a mobile phase of CH_3CN and H_2O (80:20 V:V).

GC-MS analysis

Approximately 20.0 g of guar seeds powder was extracted with 100 ml methanol. The extract was directly injected into the GC-MS (Model 2010, Shimadzu, Japan). Mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5973-A HP spectrometers. The mass selective detector was operated by electron ionization (EI) at 70 eV, and TR-FAME capillary column (30 m X 0.25 mm i.d, 0.25 µm film thickness). The multi step temperature program was increased from 80 ^{o}C (held for 2 min) to 230 ^{o}C (held for 5 min.) with rate of 3 ^{o}C min^{-1}. The carrier gas was helium at a flow rate of 2 ml min^{-1}, and the sample size was 1 µl of diluted samples (5µl oil/2ml chloroform, v/v). Injector temperature was 250 ^{o}C. A spectral range of 35-500 *m/z* analysis was used.

Determination of total phenolic and tannin Contents

Phenols were determined by the colorimetric method using Folin-Denis' reagent as described by Snell and Snell [32]. Quantitative estimation of tannin was carried out using the modified vanillin–HCl in methanol method by Price *et al*. [33].

RESULTS AND DISCUSSION

Antitumor and Antimycoplasmal Activities

In this study, we investigated the antitumor activity of guar seed extract against intestinal carcinoma (CACO-2) cell line, colon carcinoma cell line (HCT_{116}) and human prostate carcinoma cell line (PC3). The tested concentrations for each cell line were (62.5, 125.0, 250.0 and 500.0 µg/ml), as shown in Figure 1.

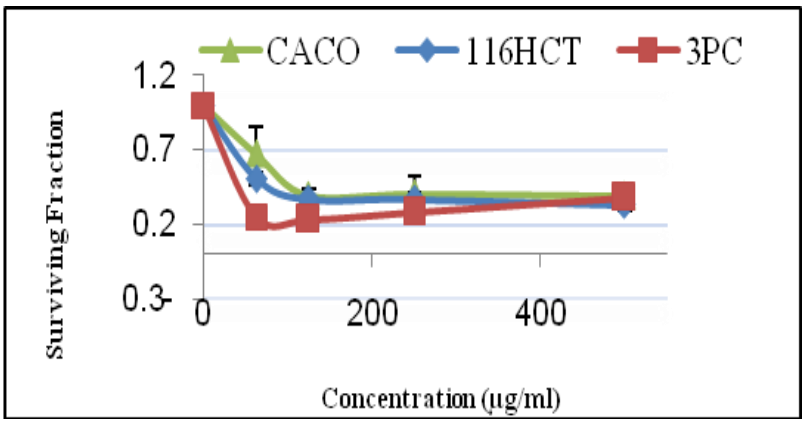

Figure 1: The cytotoxic activity of guar seeds extract against CACO-2, HCT_{116} and PC-3 cell lines

The assay results in Figures 1 showed that guar seeds extract had an inhibitory action against PC-3, HCT_{116} and CACO-2 with IC_{50} values of 40.5, 41.0 and 101.0 µg/ml, respectively. It was reported that guar gum and their sulphated derivative exhibited a significant anticancer activity against human hepatocellular carcinoma cells (Hep G2), and only sulphated derivative was specifically cytotoxic for human breast carcinoma cells (MCF-7) [34]. Additionally, guar gum was found to be used in anticancer medicine for the treatment of colorectal cancer [35]. The cytotoxic activities of the guar seeds extract may be attributed to the presence of different flavonoids such as diadzein, genistein, quercetin and kempherol [36].

The antibacterial activities of the guar seeds crude extract were determined against *mycoplasma bovis* and *mycoplasma gallisepticum*. The extract, at concentration of 200 mg/ml, showed activity against *mycoplasma bovis* with inhibition zone ranging from 30-35 mm. On the other hand, it had no effect on *mycoplasma gallisepticum*. From literature, 480 natural compounds were tested against *mycoplasma bovis*. The results showed that 32 of the 480 compounds tested were able to inhibit growth of *M. bovis* using a tetrazolium salt assay [37]. According to the best of our knowledge, there is no natural antibacterial agent has activity against *mycoplasma gallisepticum*. Therefore, screening of natural compounds and extracts has also yet to be investigated. Determination of the minimum inhibitory concentrations (MIC) of the guar seed extract against *mycoplasma bovis* is really needed.

Chemical composition

The chemical composition of guar (*Cyamopsis tetragonoloba*) seeds is summarized in Table 1. Percentage of ash, fat, fiber, moisture, protein and minerals is calculated based on dry weight of seed. The results showed that guar seeds are rich with protein (33.25 %), fiber (11.06 %) and fat content (3.32 %). Our results showed that guar seeds are a good source for protein. The guar seeds are suitable for animal consumption as a rich source of the protein [38]. Additionally, the seeds are an effective replacement of fish meal protein up to the level of 50% diets without any adverse effects on growth and feed conversion ratio [39]. The moisture and ash content of the guar seeds were found to be 10.0 % and 4.53 %, respectively. The higher percentage of ash content reflects that guar seeds are a rich with minerals. These data are in a good agreement with literature [40].

Table 1: Chemical composition of guar seeds

Components	% (based on dry weight basis)
Ash	4.53
Fat	3.32
Fiber	11.06
Moisture	10.00
Protein	33.25
Minerals	**(ppm)**
Fe^{+2}	465.90
Zn^{+2}	73.31
Cu^{+2}	11.17

The contents of Fe, Zn and Cu in the seeds were measured by inductivity coupled plasma-optical emission spectrometry ICP optima 2000 as shown in Table 1. Iron (465.90 ppm) seems to be predominant elements in the investigated sample. The results suggested that, guar seeds are an important source for iron. Some of the leguminous crops such as soybean are also a rich source of iron [41]. Other elements in the guar seeds turned out to be zinc (73.31 ppm) and copper (11.17 ppm). The presence of zinc and copper may play an important role in the functioning of various enzymes (e.g., copper is incorporated into metalloenzymes involved in hemoglobin formation, drug/xenobiotic metabolism, carbohydrate metabolism, catecholamine biosynthesis and cross-linking of collagen, as well as in the antioxidant defense mechanism [42]. The presence of these mineral elements could thus indicate that guar seeds could be useful in the management of diseases.

Fatty acids composition

The fatty acids composition of guar seeds was studied and presented in Table 2. The *cis*-linolenic acid is the most predominant fatty acids in guar seeds (53.89 % ± 0.43), followed by *cis*-oleic acid (18.07 % ± 0.03) and palmitic (13.331 % ± 0.11). Both of linolenic (C18:3ω3) and stearic acid (C18:0) were in fairly similar ratio at 5.1%. Total unsaturated fatty acids are generally in a higher ratio (77.32%) than ratio of total saturated fatty acids (22.19 %). The metabolic conversion efficiency of C18:3 ω3 to n-3 polyunsaturated fatty acids (PUFA) in human is low and as a result n-3 PUFA are now regarded as dietary essential [43]. This n-3 PUFA has many roles in human health that are independent from its conversion to n-3 PUFA [44]. The ω-6/ ω-3 ratio was at level 10.55, this ratio is commonly used to assess the nutritional value and healthiness of food lipid material for human consumption [45]. It was recommended that ratio ω-6/ ω-3 below 4.0 in human diets to prevent the development of cardiovascular diseases and some chronic diseases including cancer [46].

Table 2: Fatty acids composition of guar seeds

Fatty acids	Common name	% of Fatty acids
C15:1	Pentadecanoic acid	0.26 ± 0.01
C16:0	Palmitic acid	13.33 ± 0.11
C18:0	Stearic acid	5.09 ± 0.04
C18:1ω9 c	*cis* -Oleic acid	18.07 ± 0.03
C18:2ω6 c	*cis* -Linoleic acid	53.89 ± 0.43
C20:0	Arachidic acid	1.28 ± 0.01
C18:3ω3	Linolenic acid	5.11 ± 0.04
C22:0	Behenic acid	0.82 ± 0.01
C23:0	Tricosanoic acid	0.27 ± 0.01
C24:0	Lignoceric acid	1.40 ± 0.01
Saturated fatty acids	-	22.19
Unsaturated fatty acids	-	77.32
PUFA	-	59.00
Mono unsaturated fatty acids		18.32
ω-6/ ω-3		10.55

Amino acids composition

In order to obtain more information about the chemical composition of guar seeds, the amino acids were determined and presented in Table 3. From the results, glutamic acid, arginine and aspartic acid were found to be the major free amino acid in seeds, with concentrations of 5.52 mg/100 g, 3.92 mg/100 g and 3.01 mg/100 g, respectively. These results are in agreement with those obtained by Kobeasy *et al* [40]. Following in the order is leucine, glycine, lysine, phenyl alanine and the other amino acids. The guar seeds are low in methionine (0.22 mg/100 g), like most legume seeds [47].

Table 3: Amino acids composition of guar seeds (mg/ 100 g) based on dry weight

Amino Acids	Results (mg/100 g)
Aspartic acid	3.01
Glutamic acid	5.52
Therionine	0.86
Serine	1.20
Proline	1.12
Glycine	1.69
Alanine	1.17
Valine	1.17
Isoleucine	0.94
Leucine	1.70
Tyrosine	1.18
Phenyl Alanine	1.31
Histidine	0.92
Lysine	1.32
Arginine	3.92
Methionine	0.22
Total Essential Amino Acids	9.62
Total Non-Essential Amino Acids	17.99

Carbohydrate profile of guar seeds

The sugar profile of guar seeds, based on dry weight**,** is shown in Table 4. Two sugars named D-glalactose (27.45%) and D-mannose (56.04%) are detected in the seeds. The mannose to galactose ratio is found to be 2:1 and that matches with literature values [48]. The high galactose and mannose contents of the guar seed make it a good source of food industries and other non-food industries. In case of GU1, there is a decrease in ratio of D-glalactose 14.79 %, and D-mannose 51.65%. The GU2 has a moderate ratio of D-glalactose 17.99%, and D-mannose 58.90%. The variation in sugars percent is due to the formation of galactomannan, which confirm the insertion of cationic moiety on guar backbone. This result is in a good agreement with McCleary *et al* [49].

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Table 4: Carbohydrate profile of guar seeds (%)

Sample	D-Galactose	D-Mannose
Guar seed powder	27.45	56.04
Extracted GU1	14.79	51.65
Extracted GU2	17.99	58.90

GU1: 1.0 g Guar gum was dissolved in 85 ml distilled water (1:85)
GU2: 2.0 gram was dissolved in 60 ml of distilled water (2:60).

Phenols of the guar seeds

The total phenolic compounds (2.47 mg/g) and tannins (2.85 mg/g) of guar seeds are shown in Table 5. The results are agreed with the literature values [40,50]. From our results, the toxicity of guar seeds extract may be due to the presence of phenolic compounds and tannins. The tannin content of guar seeds can be reduced by physical removal of testa, since most of tannins are found in the outer layer of the seed.

Table 5: Phenols of guar seeds (mg/g), based on dry weight

Constituent	Results
Total phenols (mg gallic acid/g)	2.47
Tannins (mg catechin/g)	2.85

Gas chromatography-mass spectrometry (GC–MS) analysis of guar seeds extract

Methanolic extract of guar seeds was analyzed by GC-MS and results are shown in Table 6 and Figure 2. Nine compounds were identified, where the most represented compounds were 3-hydroxymyristic acid, octadecanoic acid and linolelaidic acid methyl ester. It was reported that GC-MS analysis of ethanolic extract of *Cyamopsis tetragonoloba* seeds showed the presence of mome inositol, ethyl alpha-d-glucopyranoside and stigmasterol as a major phytochemical compounds [51]. Our results and the reported data are different due to the difference in extraction conditions.

Table 6: Chemical constitutions of guar seeds extract by GC-MS

No.	Retention time (R_t)	Compounds name
1	13.73	24,25-Dihydroxyvitamin D3
2	14.53	3-Hydroxymyristic acid
3	16.31	Octadecanoic acid
4	16.39	Retinal
5	17.43	3-(Adamantan-2-yliden-methoxymethyl)-phenol
6	17.64	Palmitic acid N-butyl ester
7	17.83	Eicosanoic acid-2-hydroxyethyl ester
8	18.89	Linolelaidic acid methyl ester
9	19.54	2,3,4,5-Tetramethoxyphenol

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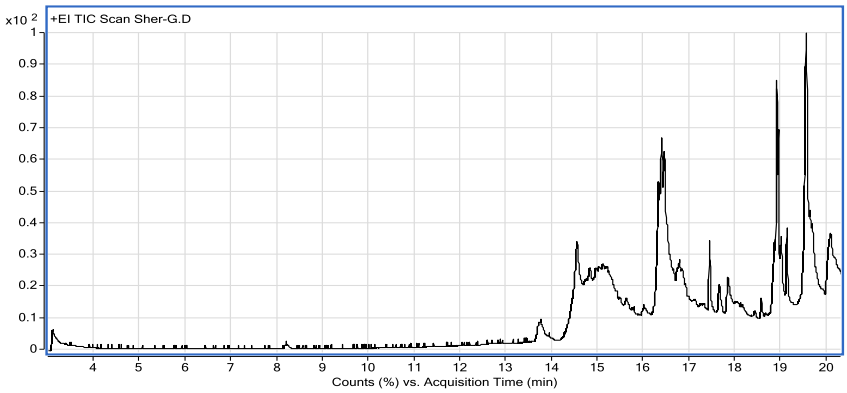

Figure 2: GC-MS Chromatogram of methanolic extract of *Cyamopsis tetragonoloba* seeds

CONCLUSION

In conclusion, guar seeds were investigated for their biological impacts and chemical composition. As observed, the methanolic extract of guar seeds has anticancer activities against intestine carcinoma (CACO-2) cell line, colon carcinoma cell line (HCT_{116}) and human prostate carcinoma cell line (PC3) with IC_{50} of 40.5, 41.0 and 101.0 µg/ml, respectively. Additionally, the extract showed antibacterial activity against *mycoplasma bovis*. The *Cyamopsis tetragonoloba* seeds (meal or crude extract) may become a valuable resource for antimicrobial agents on *mycoplasma bovis*. We will consider that point in details in our future studies. The potential of guar seeds as a source of proteins, minerals (e.g. iron, zinc and copper), fatty acids (c*is*-linolenic acid, *cis*-oleic acid and palmitic acid) and amino acids (e.g. glutamic acid, arginine and aspartic acid) is being realized. The sugar profile of guar seeds was D-glalactose (27.45%) and D-mannose (56.04%). The GC-MS demonstrated the types of some chemical constituents in guar seeds. We recommended more in *vivo* studies along with detailed phytochemical investigation using hyphenated techniques in natural products such as ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC–qTOF-MS/MS) to scientific all underpin the perspective use of guar seeds (crude extract, fractions, sub-fractions or pure compounds) for the prevention or therapy of diseases.

REFERENCES

[1] Pisani P, Parkin DM, Bray F, Ferlay J. Int J Cancer 1999; 83:18-29.
[2] World Health Organization. World Health Report 2001. Mental Health: New Understanding, New Hope. Geneva: WHO, 2001.
[3] Ho R, Nievergelt A, Pires CS, Cuendet M. Studies Natl Prod Chem 2012; 38: 247–267.
[4] Hong WK, Sporn MB. Recent Adv Chemopr Cancer Sci 1997; 278: 1073–1077.
[5] Ryan KJ, Ray CG (editors). Sherris Medical Microbiology (4th ed.). McGraw Hill. 2004; pp. 409–12.
[6] Ayling RD, Barker SE, Peek ML, Simon AJ, Nicholas RAJ. Vet Rec 2000; 146:745–747.
[7] Thomas A, Dizier BH, et al. Vet Rec 2002; 151:472–476.

[8] Jordan FTW. Avian Mycoplasmosis. 1996 Pages 81–93 in Poultry Diseases, 4th ed. Jordan FTW, Pattison M, ed. W. B. Saunders Company, London.
[9] Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, E.D. Janzen ED. J Veterinary Int Med 2011; 25: 772–783.
[10] Hannan P, Hanlon JO, Rogers NH. Res Vet Sci 1989; 46:202-211.
[11] Furneri PM, Piperno A, Sajia A, Bisignano G. Antimicrob Agents Chemother 2005; 48:4892–4894.
[12] Pająk P, Socha R, Gałkowska D, Rożnowski J, Fortuna T. Food Chem 2014; 15:300–306.
[13] Bouchenak M, Lamri-Senhadji M. J Med Food 2013; 16:185–98.
[14] Prabaharan M. Int J Biolog Macromol 2011; 49: 117–124.
[15] Khare CP. Indian medicinal plants. Springer publication. New York. 2007; 321.
[16] Curl CL, Price KR, ger Fenwick GR. Phytochem 1986; 25: 2657–2676.
[17] Coxon DT, Wallis JW, Levitt NS, Jakson WP. Phytochem 1980; 19: 1247–1248.
[18] Daniel M. Curr Sci 1989; 58: 1332–1333.
[19] Ali AM, Hussain N, Haq SA. Pakistan J Sci Industr Res 1977; 20: 279–281.
[20] Mukhtar HM, Ansari SH, Bhat ZA, Naved T. Pharm Biol 2006; 44: 10–13.
[21] Wang ML, Morris JB. Plant Genetic Resources: Characterization and Utilization. 2007; 5: 96–99.
[22] Badr SEA, Sakr DM, Mahfouz SA, Abdelfattah MS. Res J Pharm Biol Chem Sci. 2013; 4: 606-621.
[23] Vichai V, Kirtikara K. Nat Protoc 2006; 1:1112–1116.
[24] Frey ML, Hanson RP, Anderson DP. Am J Vet Res 1968; 29: 2163–2171.
[25] AOAC. Official Methods of Analysis of AOAC international 17th ed., Washington D.C. USA, 2000; 4: 969.3–991.39.
[26] AOAC. Official Methods of Analysis of AOAC international, 1995; Method No. 08-01.
[27] Iva J, Patrick B, Lse SJ. Anal At Spectrum 2003; 18: 54– 58.
[28] Morrison RW, Smith LM. The J Lipid Res 1964; 5: 600–608.
[29] AOAC. Official Methods of Analysis of AOAC international 18th Ed. Association of Official Analytical Chemists, Washington, D.C. 2006; 24–37.
[30] AOAC. Official Methods of Analysis. 19th ed. Gaithersburg, MD: AOAC International. 2012.
[31] Snell FD, Snell CT. Colorimetric methods. Toronto, New York, London: Organic, D. Van Nostrand Company. 1953; 3:606.
[32] Price ML, Socoyoc SV , Butter LG. Agric Food Chem 1978, 26: 1214–1218.
[33] Gamal-Eldeen AM, Amer H, Helmy WA. Chem Biol Inter 2006; 161: 229–240
[34] Shyale S, Chowdary K, Krishnaiah Y, Bhat NK. Drug Develop Res 2006; 67: 154–165.
[35] Sharma P, Dubey G, Kaushik S. J App Pharm Sci 2011; 01: 32–37.
[36] Soehnlen MK, Tran MA, Lysczek HR, Wolfgang DR, Jayarao BM. J Antimicrob Chemother 2011; 66: 574–577.
[37] Murwan KS, Abdalla AH. Nile Basin Res J 2008; 11: 48–54.
[38] Y S AI-Hafedh YS, Siddiqui AQ. Aquaculture Res 1998; 29: 703–708.
[39] Kobeasy MI, Abdel-Fatah OM, Abd El-Salam SM, Mohamed ZAM. Int J Biodiversity Conservation. 2011; 3: 83–91.
[40] Lönnerdal B. Am J Clin Nutr 2009; 89:1680S–1685S

[41] Isidoros I, Ioannis D, Stylianos MP. Mol Biol Int 2011; 2011:13.
[42] Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Circulation 2009; 119: 902–907.
[43] Hassan A, Ibrahim A, Mobdji K, Coëffier M, Ziegler F, Bounoure F, Chardigny JM, Skiba M, Savoye G, Déchelotte P, Marion-Letellier R. J Nutr 2010; 140: 1714–1721.
[44] Simopoulos AP. Food Rev Int 2004; 20: 77–90.
[45] Desmet RKS, Demeyer D. Animal Feed Sci Technol 2004; 113: 199–221
[46] Castillo-Israel K, Laurena A. Philippine J Crop Sci 2007; 32: 15–24.
[47] Murwan KS, Abdelwahab AH, Sulafa NH. ISCA J Biol Sci 2012; 1: 67–70.
[48] McCleary BV, Dea ICM, Clark AH, Rees DA. Carbohydrate Res 1985; 139: 237–260.
[49] Kaushal GP, Bhatia IS. J Sci Food Agric 1982; 33:, 461–470.
[50] Surendran S, Vijayalakshmi K. Int J Pharmacog Phytochem Res 2011; 3: 102–106
[51] Hanhineva K, Soininen P, Anttonen MJ, Kokko H, Rogachev I, Aharoni A, Laatikainen R, Kärenlampi S. Phytochem Anal 2009; 20: 353–364.