Egypt. J. Chem. **Vol. 65**, No. 4, pp. 287 – 299 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of Enzymatic Phenolic Extract from Garden Cress Seed Meal against Aflatoxigenic Fungi Isolated from Eggplant fruits

Marwa A. Younos[a], Engy M. Akl[b]

[a]*Food Toxicology and Contaminants Department, Food Industry and Nutrition Research instatue, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt.*

[b]*Fats and Oils department, Food Industry and Nutrition Research instatue, National Research Centre, Dokki, Postal Code 12622, Giza; Egypt.*

Abstract

Natural products, like phenolic compounds from plant sources, exhibited antifungal activity and other biological activities. The current work aimed at studying the effect of phenolic compounds produced via enzymatic extraction from garden cress seed meal on some aflatoxins producing fungi isolated from the Eggplant fruits. In this elucidation, the chemical composition, minerals content and protein isolate of garden cress meal were investigated. Various enzymes (protease, cellulase, amyloglucosidase and their mixture) were used in the extraction of phenolic compounds with different concentrations. The antioxidant activities of all phenolic extracts were assisted. The polyphenolic compounds were defined by HPLC. Amino acid profile of the prepared protein isolate was also determined. On the other hand, six samples of Eggplant (10 of each) from two different locations were analyzed for mycological examination and aflatoxins association. The antifungal activity of phenolic extracts was measured by linear growth method of aflatoxigenic fungi. The results showed that a mixture of protease, cellulase, and amyloglucosidase enzymes with equal ratios exhibited major phenolic compounds (15.97mg/g) and also high antioxidant activities. The enzymatic phenolic extracts with different concentrations (10 and 20%) had a significant antifungal activity against aflatoxigenic fungi and other tested fungi. The highest growth inhibition of aflatoxigenic fungi was detected with *A. parasiticus* (55.82% at 20% conc.), while *A. flavus* detected less growth inhibition which recorded 40.54% at 20% conc. It could be concluded that garden cress phenolic extract is effective against some aflatoxigenic fungi.

Keywords: garden cress seed meal, minerals, protein isolate, phenolic compounds, antioxidants, eggplant fruit, aflatoxigenic fungi, aflatoxins, antifungal activity

1. Introduction

Garden cress belongs to the Brassicaceae family, is an annual herb, which is wealthy in health promoting phytochemical compounds. One of the jaundice, gastrointestinal disorders, and spleen diseases. It had been additionally reportable to exhibited diuretic, antihypertensive, anti-asthmatic, anti-inflammatory, and antioxidant activities [1, 2]. Arial parts, leaves, and seed extracts of GCS found to have flavonoids, alkaloids, proteins, fats, carbohydrates, vitamins, minerals, glycosides, polyketides, which give the plant it's most vital groups of natural antioxidants is phenolic compounds. Garden cress seed GCS (*Lepidium Sativum* seed LSS) has been utilized in ancient medication for the treatment of liver problems, antihypertensive, diuretics, hepatoprotective, fracture healing, antimicrobial, milk production, antioxidant, anti-inflammation, respiratory disorder healing, chemoprotective, laxative, and many other therapeutic applications [3]. Mucilage of garden cress seeds may be useful in preventing enter-colitis [4]. Ethanolic extracts of the whole seed showed a reduction of the level of serum alanine transaminase

*Corresponding author e-mail: engy_akl@yahoo.com

Receive Date: 12 September 2021, Revise Date: 28 September 2021, Accept Date: 03 October 2021

DOI: 10.21608/EJCHEM.2021.95601.4487

©2022 National Information and Documentation Center (NIDOC)

(ALT), alkaline phosphatase (ALP) *aspartatetransaminase* (AST), and bilirubin (BIL) in the groups pretreated with LSS and silymarin [5].

Garden cress seeds extracts (petroleum ether, methanol, water, and ethanol) exhibited antifungal activity against different pathogenic microorganisms *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumonae, Pseudomonas aeruginosa,* and fungus *Fusarium equiseta, Aspergillus flavus Candida albican* and *Alternaria alternate,* so it can be exploited as an ideal treatment for future fungal diseases [6, 7].

The Eggplant, Aubergine (*Solanum melongena L.*), of the Solanaceae family, is grown in subtropical and tropical regions of the world. It is one of the most common vegetable crops grown in Egypt. It is as popular as the poor man's crop [8]. The nutritional content of the fruit is good; it is low in calories, sodium and fat. It also contains protein, starch, fibre, and additional nutrients like folic acid, potassium, magnesium, vitamin A, and B6.[9]. Eggplant fruits are attacked by fungal pathogens resulting in rot and post-harvest diseases [10]. Zacharia and Philip [11] isolated *Alternaria solani, Penicillium sp., Fusarium solani, Rhizopus nigricans, Colletotrichum, Mucor sp.,Botrytis cinerea, Curvularia lunata, Aspergillus niger* and *Rhizoctonia solani* from rotten fruits of eggplant. *A. flavus, Alternaria solani, A. alternata, M. Hiemalis* and *R. Stolonifer* were identified to be associated and responsible for eggplant spoilage [12]. The causative agents of eggplant fruit rot were *Collectotrichumme longenae* and *Phomopsis melongenae* [13]. These fungi cause significant economic losses not only in eggplant but in most fruits, and vegetables [10].

Aflatoxins are produced by certain types of moulds (*Aspergillus flavus and Aspergillus parasiticus*). Aflatoxins are among the most dangerous mycotoxins that have been studied today not only for scientific purposes but also within the scope of ongoing food safety control. There are four main types of aflatoxins, differing in chemical structure but similar in physiological effects - *B1, B2, G1, G2* and about 10 of their derivatives. Biosynthesis of aflatoxins occurs when fungi develop in different types of foods. *A. flavus* is a widespread microscopic mould that can grow and produce toxins in a several substrates [14]. Several factors influence on both the growth and the production of mycotoxins in different types of fungi, including temperature, pH, environment, humidity, nutrients, water activity, nature of the substrate, physiological state, level of inoculation, and microbial interactions [15].

Therefore, the main goal of this study is focused on studying two parts. First, evaluation the chemical composition, minerals content and protein isolate of garden cress seed meal. Various enzymes (protease, cellulase, and amyloglucosidase) used in the extraction of phenolic compounds with different concentrations single or their mixture, with assessment of its antioxidant activities. Second, isolation and identification of all mycoflora contaminated Eggplant fruits and determination of aflatoxins produced. Finally, estimation of the antifungal activity of the enzymatic phenolic extract of *Lepidium sativum* seed meal against aflatoxigenic fungi and other different fungi.

2. Material and Methods

2.1. Materials

2.1.1. Preparation of garden cress seed

Garden cress seed was purchased from a local market then subjecting to defatting by soxhlet apparatus and normal hexane as defatting solvent, then allowed to air-dry in a fume hood to remove residual hexane. The resulting defatted meal was ground in a coffee mill to obtain a finely divided material suitable for extraction studies.

All chemicals were obtained from Sigma Chemical Co.

2.1.2. Collection of samples:

Randomly 6 samples (10 of each) of Eggplant fruits were collected from two different locations. Each sample was collected in a sterile polyethene bags and conveyed to the microbiology laboratory for analysis within 24 hours of collection.

2.2. Methods

2.2.1. Preparations of GCSM phenolic extracts by Enzymatic Assisted Extraction

Enzymes used in this work were protease, cellulose, and amyloglucosidase. For each experiment, 2g of GCS meal was suspended in 200 ml distilled water (1:100, M: W ratio) was stirred by a magnetic stirrer with heating to the appropriate temperature for each enzyme. Each enzyme was added at concentrations 1, 2 and 3% (weight of enzyme: weight of meal). After adjusting the suitable pH for each enzyme, the mixture was transferred to a

shaking water bath for 3 hours. The incubating pH was fixed at the optimum range for each enzyme using 1N Na OH and 6N HCl. The mixture involving protease was incubated at a pH 7.5 and temperature 37° C, the mixture involving cellulose was incubated at a pH 4.2 and temperature 50°C, the mixture involving amyloglucosidas was incubated at a pH 4.5 and temperature 55°C. After the mixture was treated with a given enzyme at its optimum pH and temperature for a determined time, the pH was shifted to a value of (pH2) then the temperature was raised to 80°C for 5 minutes to assure complete inactivation of the enzyme. After enzyme inactivation, the mixture was centrifuged for 30min. at 3000xg and the supernatant was then taken for the determination of phenolic compounds.

A considerable amount of enzyme mixture phenolic extract with ratio (1:1:1) were prepared for determination of polyphenolic by HPLC and antifungal evaluation. The first hour was prepared according to enzyme cellulase at a pH 4.2 and temperature 50°C. Then second hour was prepared accordion to enzyme protease at a pH 7.5 and temperature 37° C. Then the third hour was prepared according to amyloglucosidasat a pH 4.5 and temperature 55°C. After that the enzymes were inactivated by lowering PH and raising temperature. The aqueous extract concentrated by a rotary evaporator and directly freeze dried by (Crest Alpha 1-4 LSC plus Germany). The freeze-dried phenolic extract kept in a refrigerator until used.

2.2.2. Preparation of garden cress seed meal protein isolate (GCSMPI).

Generally, the alkaline solution (0.02 N NaOH) was first added to the defatted garden cress meal and stirred for two hours to solubilize the proteins. The mixture was then centrifuged, and the pH of the supernatant was adjusted by dilute acid (6N HCl) to precipitate the protein at its isoelectric point (4.2- 4.8). Precipitated protein was then separated by centrifugation and the precipitate was washed several times with water followed by ether and acetone then dried and saved in the refrigerator until used.

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of GCSMPI.

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of GCSMPI were determined as described by Alfredo et al. [16] and expressed as g of water or oil held/g sample.

Determination of GCSM protein content.

The total protein content of GCSMPI was determined according to the method of A.O.A.C. [17].

2.2.3. Determination of amino acid profile of GCSMPI using HPLC.

The amino acid profile of GCSMPI was determined using HPLC-Pico-Tag method according to Millipore Cooperative (1987). The Pico-Tag method was described by Heinrikson and Meredith [18] and Cohen et al. [19]. The Pico-Tag method, which was developed commercially by Waters Associates, was an integrated technique for amino-acid analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamy (PTC) derivatives which were detected by their UV absorbance. The chromatographic analysis using HPLC was carried out using the following gradient of Pico-Tag solvent A and B (P/N 88108 and 88112). The sample was injected and loaded on Pico-Tag amino acids column (150 x 3.9 mm) stainless steel. Detection of the PTC derivatives is by ultraviolet absorption measurements using a fixed wavelength (254nm) Waters detector. Before injecting the sample, the illustrated was calibrated by two injections of the standards.

2.3. Methods of Analysis

Moisture, protein, oil, ash, fiber was determined according to A.O.C.S. [17] Standard methods of analysis. Nitrogen-free extract was determined by calculation.

2.3.1. Determination of Total Phenolic Compounds TPC

The content of phenolic compounds was determined according to the method of Fu et al. [20]. 200 µL of the sample was completed to 3ml distilled water. 2mL of 10% folin reagent was added then shake well for 5 minutes, 1ml of 7.5% sodium carbonate was added then shakes. The mixture was left for one hour in dark then the absorbance at 765 nm was measured using a spectrophotometer (T80 UV vis spectrophotometers).

2.3.2. Evaluation of the antioxidant activity of all soluble phase by two methods

a-DPPH radical-scavenging

The method described by De Ancose et al. [21] was utilized to determine the DPPH radical-scavenging. The reduction of the DPPH radical was measured at 517 nm.. Results were expressed as

percentage inhibition of the DPPH using the following equation:

Inhibition of DPPH (%) = absorbance control-absorbance sample/absorbance control × 100

Where the absorbance of control is the absorbance of DPPH solution without extract.

b- Ferric reducing antioxidant power (FRAP)

The reducing power of each extract was determined according to Zhao et al. [22]. The absorbance was measured spectrophotometrically at 700 nm. The measurement was compared to the standard curve of a prepared BHT solution. The final results were expressed as milligrams of BHT equivalents per gram based on the dry weight

.

2.3.3. Heavy metal analysis

Minerals contents in one gram meal were determined. A one g weight of each of the samples was placed in a clean crucible and placed in a furnace. The content was ashed at 650 °C for 10 h until a whitish-grey matter was obtained then cooled. 2M HCl was added then deionized water (completed to 20 mL) was added and filtered; the resultant filtrate was used for trace metal assay by using Flam Atomic absorption spectrometer (Agilent Technologies). The amounts of individual elements were calculated by the obtained values following their standard curves, and the measurements were made at 589.6 nm (Na), 766.5 nm (K), 239.9 nm (Ca), 202.6 nm (Mg), 248.3 nm (Fe), 279.5 nm (Mn), 324.7 nm (Cu) and 213.9 nm (Zn).

2.3.4. Analysis of Phenolic Compounds by HPLC

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programmed was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µmAcrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards [23].

2.3.5. Culturing Procedure:

Cut sections of naturally infected Eggplant fruits were surface sterilized with 70% sodium hypochlorite (bleach) solution for 1min and rinsed quickly in three successive changes of sterile distilled water, and blotted dry with sterile filter paper, and placed on Potato Dextrose Agar media (PDA) in Petri dishes. Four sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungal growth was noticed. After 5 days, the different isolates were sub-cultured on freshly prepared PDA to obtain their pure culture.

2.3.6. Identification of the Isolated Fungi:

All developing fungal colonies were examined morphologically and microscopically then identified according to Raper and Fennel, [24] Samson, [25] Domsch et al., [26]and Pitt and Hocking [27].

2.3.7. Aflatoxin analysis:

a-Aflatoxins production: All isolated aflatoxigenic fungi (*Aspergillus flavus* and *A. parasiticus*) were propagated as pure culture in 100 ml yeast extract sucrose (YES) to be tested for aflatoxins production according to Munimbazi and Bullerman [28]. Each flask was inoculated with 0.1 ml of spore suspension containing approximately 106 spores ml-1. Cultures were incubated at 26±2^{o}Cfor 14 days.

b- Extraction of aflatoxins from the culture media: The extraction was performed according to the procedure offered by Kumar et al. with some modifications as follows [29]. The cultures were filtered and mycelial mats were collected. Aflatoxins were extracted from culture filtrates with chloroform. A known volume of filtrate (25 ml) was added to 10 ml chloroform and was shaken for half hour. The chloroform contained aflatoxins were separated by separating funnel which was allowed to stand for some time until the two layers appeared. The upper aqueous layer was re-extracted many times with chloroform for complete separation. The lower chloroform layer was filtered over anhydrous sodium sulfate in 250 ml beaker, evaporated in a water bath (70-80°C) near dryness and the residue was washed

twice with chloroform (12 ml) into a glass vial which evaporated till dryness (dry film). The dried extract was kept in the refrigerator at -5°C for HPLC analysis [29].

c- High performance liquid chromatography (HPLC) analysis:

According to A.O.A.C., [30]. The HPLC system used was a water 600-pump system equipped with model 474-flourescence detector (water) set at 360 nm for excitation and 440 nm for emission wavelength. Water Nova-pack C18 column (150×3.9) was used for aflatoxins separation. The mobile phase (water: Acetonitrile: Methanol, 65: 5: 30) was isocratically flow at rate of 1.0 ml/min. Data were collected and integrated with a waters Millennium 32 chromatography Manager software program.

2.3.8. Antifungal activity (in vitro):

Determination of mycelial growth inhibition: The enzymatic phenolic extract of garden cress seed (*Lepidium sativum*) was mixed with sterilized Potato Dextrose Agar (PDA) medium at various concentrations (10 and 20% (v/v)) in separately sterilized Petri dishes. All Petri dishes were inoculated each alone at the centre with 5mm-disc inoculums 7-day old of each of the tested fungi (*Alternaria* sp., *Aspergillus flavus, A.niger, A.parasiticus, A.terrus and Penicillium sp*.) using sterilized cork borer. Three plates were used as replicates for each treatment then incubated at 28 ± 2°C. Colony diameter was measured after 5 d of incubation period [31]. Medium free of extract were used as a control. Growth inhibition percent was calculated according to Jabeen et al. [32] by using a formula:-

Growth inhibition (%) = (C - T) /C×100

Where C = growth in control, T = growth in treatment

3. Results and Discussions

3.1. Chemical composition of GCSM and minerals content

The information about the chemical composition of seeds is essential for several factors: it is the basic source for both animals and human food. Also, it contains various sources of drugs and anti-metabolites that affect nutrition of animals and human. **Table 1** showed the proximate chemical composition of both GCS and GCSM (percentage per gram dry weight). Chemical composition of GCS showed that it contains 23.2 ±0.2 crude oil, 26.3± 0.4 total proteins and low level of moisture 4.2± 0.3 which is an index of quality, stability, and increased shelf life of seeds. These results are in range reported by Zia-Ul-Haq et al., and Mohammed [33, 34]. While, GCSM contains high amount of protein (48.1± 0.5%) while, the defatting seed process doubled the protein content so, it considered as high food energy. Crude fibre content is about (10.4 ± 0.3), the results in range reported by Mohammed [34]. Also, GCS is a good source of minerals because of higher amount of ash contents. This result is in agreement with Singh, Paswan [35].

Mineral content of GCSM, **Table 2** showed that potassium, calcium, and magnesium constituted the major mineral 1239, 477.5and 374.7mg/100g respectively, while zinc, copper, and manganese contents are low. The same observation concluded by Pramod et al. [36]. So, GCSM can be used in improvement of some supplementary food.

Table 1 chemical composition of GCS and GCSM expressed as percentage

Chemical Composition %	GCS	GCSM
Moisture	4.2± 0.3	5.82± 0.2
Protein	26.3± 0.4	48.1± 0.5
Oil	23.2± 0.2	0.8± 0.1
Ash	6.1± 0.1	6.8± 0.2
Crude Fiber	10.8± 0.2	10.4± 0.3
Nitrogen free extract	29.4± 0.5	28.03± 0.4

Results are mean values of three replicates ± standard deviation

Table 2 Minerals content of GCSM expressed as mg per 100 gram dry weight

Minerals mg\100g	GCSM
Copper Cu	6±0.11
Iron Fe	5.5±0.2
Zinc Zn	2.8±0.1
Manganese Mn	2.1±0.01
Sodium Na	9.6±0.3
Magnesium Mg	374.7±2
Potassium K	1239.0±5
Calcium Ca	477.5±4

Results are mean values of three replicates ± standard deviation

2.4. Garden cress seed meal protein isolate (GCSMPI)

Amino acid profile of (GCSMPI) was illustrated in **Table 3**. As shown, it is rich in essential amino acids like arginine &histidine and nonessential amino acids as aspartic acid & glutamic acid which play a vital role in the metabolism of fats and sugars [37]. It is low in alanine, prolinne, and lysine. The total protein content of GCSMPI was 84% as determined by the Kjeldahl method. This is attributed to that it contains a large amount of mucilage which easily dissolves in water. Low purity garden cress protein isolate was due to unremoval of mucilage before extraction of protein from defatted garden cress. So, it was lower than the flaxseed protein isolate which was prepared via removal of mucilage before protein preparation [38].

Table (3) the amino acids profile of prepared GCSMPIg/100g

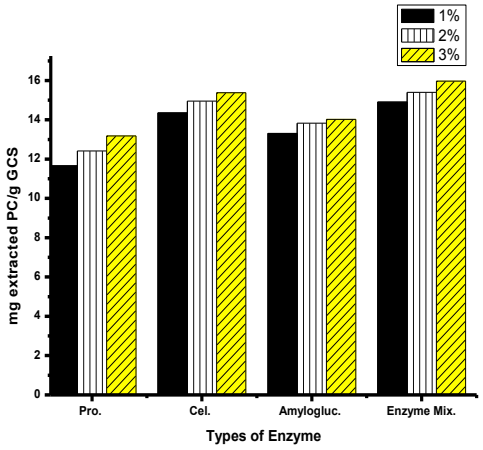
Amino acids of GCSMPI	g/100g sample
Non-essential amino acids	
Aspartic acid	11.11
Glutamic acid	7.34
Serine	6.45
Glycine	4.27
Alanine	1.07
Proline	0.90
Essential amino acids	
Cysteine	1.13
Tyrosine	1.93
Histidine	10.94
Arginine	19.74
Threonine	1.29
Valine	3.02
Methionine	1.42
Isoleucine	5.41
Leucine	3.82
Phenylalanine	2.69
Lysine	1.44

Hydroxyproline and tryptophan not determined.

Also, the functional properties of GCSMPI are promising like water holding capacity which recorded 15.95 g/g while oil holding capacity was 3.58 g/g. it is higher than garden cress protein isolate and flaxseed protein isolate observed by Mohamed et al. [38] and Mohammed [34] respectively. This is maybe due to the amount of mucilage dissolved with protein. So, the garden cress protein isolated may be a desirable food ingredient and can be used as supplementation or nutrient substitution [34].

2.5. Phenolic Compounds and Antioxidant Activity

In this study, and because of the important of phenolic compounds, the effect of various types of enzymes with different concentrations on the extraction yield of total phenolic compounds were investigated and its antioxidant activities. Garden cress meal consists of protein, fibre, polysaccharides and ash as illustrated in **Table 1**. So, various types of enzymes were used to improve the extraction of total phenolic compounds that release the insoluble-bound phenolics. The action of these enzymes causes cell structure degradation. The pretreatment of GCSM with protease, cellulase and amyloglucosidase enzymes were studied using different concentrations (1%, 2%, and 3%) single or (1:1:1) mixtures. The data showed that single enzyme cellulase with concentration 3% give high extraction yields of phenolic compounds 15.38±0.3 mg/g which was closed to the yield of phenolic compounds extracted by mixture enzyme followed by Amyloglucosidase14.02±0.4 mg/g and finally protease 13.18±0.2 mg/g. The results in **Figure 1** revealed that, in all enzymes used, with increasing the enzyme concentration the extraction of TPC increase. The utilization of mixture of three enzymes with concentration 3% was 15.97mg/g which was more effective than single enzyme; this is attributed to the release of cell components such as phenolic, protein and others. These results in agree with Akl et al.,; Taha and Hassanein [39,40] which studied the effect of enzymatic pretreatment on flaxseed meal on the yields of phenolic compounds, and cottonseed flakes on oil extractability respectively.



(Pro. Protease, Cel. Cellulase and Amylogluco. Amyloglucosidase, and Enzyme mixture)

Figure 1 Effect of different enzyme concentration on the extraction yield of total phenolic compounds (TPC) from GCSM expressed as mg/g

Antioxidant activity of the various extracts from garden cress meal by different enzymes showed that mixture of enzymes exhibited high free radical scavenging activity and also good ferric reducing power 95.64 % and 20.95mg/g than single enzyme respectively as presented in **Table 4**. There is no significant difference between mixture enzyme (2%and 3%) by both methods. There are significant differences between other concentrations of single enzymes. Garden cress seeds can act as in vivo and in vitro antioxidants because of a high content of phenolic compounds [41, 35]. It exhibited scavenging activity with IC_{50} 176.18 µg/ml by (DPPH) radical method. [42], and good Ferric Reducing Antioxidant Power (FRAP) as reported by Zia-Ul-Haq et al. [43]. Garden cress seeds may be used to reduce oxidative damage in the human body due to some factors such as aging, deterioration of physiological functions causing diseases like cirrhosis, cancer, various inflammatory diseases. Dandge et al. [41] showed that GCS possess antioxidant activity when measured by the same methods.

Table 5 illustrated the polyphenolic compounds determined by HPLC. It showed that gallic acid, chlorogenic acid, syringic acid, rutin, and ferulic acid are the major identified phenolic compounds, and appreciable amounts of Naringenin, Propyl Gallate, Querectin, and Cinnamic acid. The action of hydrolytic enzymes used leading to liberation of various phenolic compounds these results following Hur et al. [44].

Table 4 Antioxidant activity of all extracts from GCSM by different enzymes concentrations

GCSM	DPPH %	FRAP mg/g
Protease 1%	90.63±0.4^{d}	16.50±0.4^{f}
Cellulase 1%	94.1±0.3^{c}	18.2±0.3^{d}
Amyloglucosidase1%	94.05±0.3^{c}	17.38±0.5^{e}
Protease 2%	90.9±0.4^{d}	16.51±0.4^{f}
Cellulase 2%	94.15±0.2^{c}	19.2±0.6^{c}
Amyloglucosidase2%	94.23±0.3^{b}	18.5±0.5^{c}
Protease 3%	91.3±0.4^{d}	16.60±0.3^{e}
Cellulase 3%	94.2±0.5^{b}	20.97±0.6^{a}
Amyloglucosidase3%	94.42±0.6^{b}	19.19±0.3^{c}
Mix. of enzymes (1%)	94.70±0.8^{b}	20.1±0.2^{b}
Mix. of enzymes (2%)	95.19±0.9^{a}	20.63±0.3^{a}
Mix.of enzymes (3%)	95.6±0.8^{a}	20.95±0.6^{a}
LSD at the 5% level	.9341	.7379

Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences between enzymes at P<0.05 for each concentration

Table 5 HPLC of polyphenolic compounds determined in enzymatic extract of GCSM.

Polyphenolic compounds	Phenolic extract Conc. (µg / g)
Gallic acid	2895.95
Chlorogenic acid	10233.19
Catechin	0.00
Coffeic acid	0.00
Syringic acid	110.41
Rutin	123.15
Ellagic acid	107.67
Coumaric acid	0.00
Vanillin	0.00
Ferulic acid	963.91
Naringenin	119.91
Propyl Gallate	198.02
Querectin	59.71
Cinnamic acid	12.51

3.4. Total fungal count associated Eggplant fruits

Since most fruits and vegetables such as Eggplant contain high level of water and nutrients, so they serve as good substrates which support the growth of pathogenic microorganisms. Isolation of

mycoflora associated the Eggplant fruits on Potato dextrose agar (PDA) medium resulted that, 222 fungal isolates were isolated from two different locations as shown in **Table 6**. Out of them 124 fungal isolates equal 55.86 % were isolated from location A and 98 fungal isolates (44.14 %) were isolated from location B. On the other hand data show that, in location A, sample 1 had the highest fungal count (21.62 %) followed by sample 3 which recorded 18.92 % and sample 2 which resulted 15.32 %, while in loacation B, sample 3 had the highest fungal count (18.47 %) followed by samples 1 and 2 which recorded (16.67 & 9.01 % respectively). Similar results were obtained by Kwon and Jee [45] who reported that, a total of 50 fungal isolates were isolated from the diseased eggplant fruits which collected from the greenhouses in Korea. These variations in the fungal counts may be attributed to the geographical locations; the food analysis used techniques, media types and incubation temperature which have a significant effect on the fungal counts.

Table (6): Total fungal count associated Eggplant fruits

Samples	Locations				Total	%
	A		B			
	T.C	%	T.C	%		
1	48	21.62	37	16.67	85	38.29
2	34	15.32	20	9.01	54	24.32
3	42	18.92	41	18.47	83	37.39
Total	124	55.86	98	44.14	222	100

3.5. Fungal frequencies associated Eggplant fruits

Identification of fungal species associated Eggplant fruits indicated that, eleven fungal species belonging to eight fungal genera were identified and recorded as shown in Table 7. These are *Alternaria alternata, A. flavus, A. niger, A. parasiticus, A. terrus, Colletetrichum* sp., *Mucor* sp., *Pencillium* sp., *Rhizoctonia* sp., *Rhizopus* sp.,and *Stemphylium* sp. .On the other hand, *Pencillium* sp. had the highest fungal frequencies which record 27.48%, followed by *A. niger* (26.13%), *Rhizopus* sp. (9.91%), *Alternaria* sp. (8.56 %), each of *A. terrus, Mucor* sp.,and *Rhizoctonia* sp., which recorded 4.50%, *A.flavus* (4.05%) and both *Colletetrichum* sp. and *Stemphylium* sp. (3.60%). Less Fungal frequency was recorded with *A. parasiticus* fungus which gave 3.15%. The results agree with the findings of Gambari et al. [46], which isolated *Alternaria solani, Colletotrichum, Penicillium* sp. ,*Rhizopus nigricans, Mucor* sp., *Rhizoctonia solani* and *Aspergillus niger* from rotten fruits of eggplant. Tiwari et al., [47] isolated 14 different fungal species from eggplant fruits which identified as *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Trichoderma viride, Rhizopus* sp.,, *Rhizoctonia* sp.,, *Verticillium* sp.,, *Chaetomium globosum, Fusarium longipes, Fusarium oxysporum, Fusarium solani, Trichoderma hamatum, Penicillium citrinum,* and *Penicillium* spp. Yaji et al.,[48] isolated *Aspergillus flavus, Aspergillus terreus, Aspergellus fumigates* and *mucor* from eggplant fruits. The presence of fungi in large numbers in rotten eggplant varieties indicates poor handling of the fruits and poor storage conditions of the eggplant handlers.

Table (7): Fungal frequencies associated Eggplant fruits

Fungi	Locations				Total	%
	A		B			
	T.C	%	T.C	%		
Alternaria alternata	10	4.50	9	4.05	19	8.56
A.flavus	9	4.05	NF	-	9	4.05
A.niger	33	14.86	25	11.26	58	26.13
A.parasiticus	NF	-	7	3.15	7	3.15
A.terrus	10	4.50	NF	-	10	4.50
Colletetrichum sp.	NF	-	8	3.60	8	3.60
Mucor sp.	NF	-	10	4.50	10	4.50
Penicillium sp.	30	13.51	31	13.96	61	27.48
Rhizoctonia sp.	10	4.50	NF	-	10	4.50
Rhizopus sp.	22	9.91	NF	-	22	9.91
Stemphylium sp.	NF	-	8	3.60	8	3.60
Total	124	55.86	98	44.14	222	100

3.6. Aflatoxin Determination

Aflatoxins are secondary metabolites that are produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* under suitable conditions. Determination of aflatoxins produced by aflatoxigenic fungi isolated from Eggplant resulted that, four isolates were aflatoxins producers (one isolate of *A. flavus* from location A samples and three

isolates of *A.parasiticus* from loacation B samples). On the other hand, higher aflatoxin quantity was produced by *A.parasiticus* (isolate No 4) from location B samples which recorded 194.56 ng/g (162.86 AFB1, 22.22 AFB2 and 9.48 ng/g AFG2), followed by *A. parasiticus* (isolate No 5) isolated from location B samples which produced 77.19 ng/g (69.92, 1.28, 0.11 and 5.88 ng/g of AFB1, AFG1, AFB2 and AFG2 respectively), and *A. parasiticus* (isolate No 7) from location B samples produced 8.44 ng/g (3.36 AFB1, 0.76 AFB2 and 4.32 AFG2). Less aflatoxins quantity was produced by *A. flavus* (isolate No 2) from location A samples which gave 1.93 ng/g (AFB1), as shown in **Table. (8)** and **Figs. (2:6)**. In an extensive literature search, no studies were found on aflatoxin contamination produced by aflatoxigenic fungi isolated from fresh Eggplant fruits, only few studies were found on aflatoxin contamination in dried eggplants, in which Hacıbekiroğlu and Kolak [49] reported that, total aflatoxin levels in dried eggplant were 10 µg/kg. AFB1 was found in 64% of the dried eggplant samples [50], and aflatoxin G1, G2, B1, and B2 content in dried samples of eggplant were ranged between 0.82 and 2.58, 0.10 - 0.23, 0.32 - 1.35, 0.12 - 0.67, and 0.17 - 0.71 mg kg_1, respectively [51].

Table (8): Aflatoxin Determination

L	Fungi	No	The concentration of Aflatoxins (ng/g)				Total Aflatoxin (ng/g)
			B_1	G_1	B_2	G_2	
A	*A.flavus*	2	1.93	ND	ND	ND	1.93
B	*A.parasiticus*	4	162.8	ND	22.2	9.48	194.56
B	*A.parasiticus*	5	69.92	1.28	0.11	5.88	77.19
B	*A.parasiticus*	7	3.36	ND	0.76	4.32	8.44

ND = Not Detectd

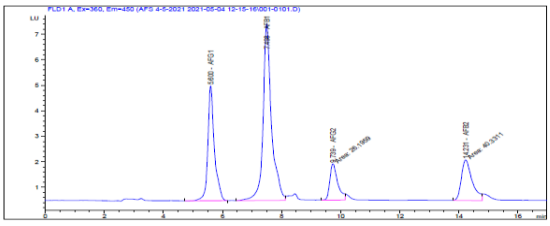


Fig. (2): Standard chromatogram of aflatoxins (B_1, B_2, G_1 and G_2)

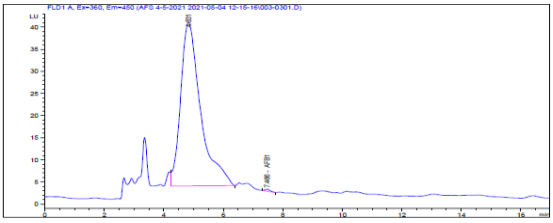


Fig. (3): Chromatogram of aflatoxins produced by *A.flavus* (isolate No 2) from location A samples

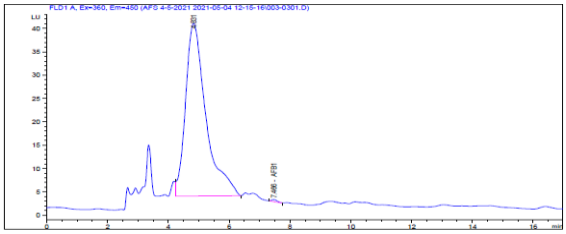


Fig. (4): Chromatogram of aflatoxins produced by *A.parasiticus* (isolate No 4) from location B sample

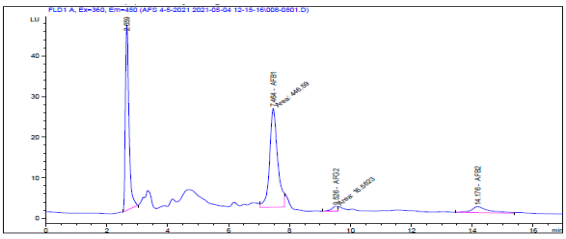


Fig. (5): Chromatogram of aflatoxins produced by *A.parasiticus* (isolate No 5) from location B samples

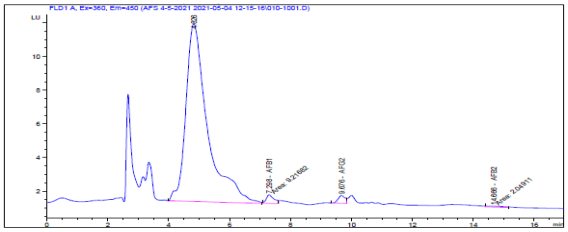


Fig. (6): Chromatogram of aflatoxins produced by A.parasiticus (isolate No 7) from location B samples

3.7. Antifungal activity of enzymatic phenolic extract of *Lepidium Sativum* seed meal

The investigation of of new natural products is considered to be a promising approach to discover new sources of antimicrobial activity. The antifungal activity of enzymatic phenolic extract of *Lepidium sativum* seeds was evaluated by using various concentrations (10 and 20%) against the aflatoxigenic fungi (*A. flavus, and A.parasiticus*) and other tested fungi (*Alternaria sp., A.niger, A.terrus*and *Penicillium sp.*) which isolated from the Eggplant samples as shown in **Table. (9)** .The results of the present study indicated that the enzymatic extract of *L. sativum* seeds showed a significant antifungal activity against all tested fungi at different concentrations, and the growth inhibition was increased with increasing the concentration used. In which the highest growth inhibition of aflatoxigenic fungi was detected with *A. parasiticus* which recorded 55.82 and 36.33 % at 20 & 10% conc. respectively, while *A. flavus* gave 40.54% at 20% conc. and 18.8 % at 10% conc. On the other hand, *A.terrus* recorded 82.00 % at 20% and 70.44% at

10% conc., followed by *Alternaria sp.* which gave 73.43% and 55.71% at 20 & 10 % conc. respectively, *Penicillium sp.* (56.82% at 20% and 31.41% at 10% conc.), and *A.niger* which recorded 50.96 and 36.33% at 20 & 10% conc. respectively. Similar results were obtained by many investigators, Sharma et al., [7] reported that, ethanolic extract of *L. sativum* showed antifungal activity against *Alternaria alternate, Aspergillus flavus* and *Fusarium equiseta* in different concentration, and shows maximum inhibition zone 16 mm, 13 mm and 12 mm in different dose respectively. *Penicillium sp., Penicillium marneffi, Aspergillus fumigatus, Candida albicans, Microsporum sp., and Fusarium sp.* were completely inhibited at a concentration of 90 mg/ml [52]. Baregama and Goyal [53] mentioned that, the methanolic extract completely inhibited the growth of *Aspergillus flavus* at a concentration of 30mg/ml, while Omer et al. [54] found that, the crude extract of *L. sativum* seeds showed effective inhibition activity against *A. niger* which were 18, 20 and 19 mm in concentration 50,100 and 200 mg/ml respectively. The presence of components such as flavonoids and tannins in the extract; gallic acid, chlorogenic acid, syringic acid, rutin, and ferulic acid which are the major identified phenolic compounds, and appreciable amounts of Naringenin, Propyl Gallate, Querectin, and Cinnamic acid as detected in **Table 5**); are responsible for its antimicrobial activity. So the antifungal activity of the crude extracts maybe due to the presence of some active secondary metabolite in the plant seeds.

4. Conclusion

The chemical composition of garden cress seed meal makes it eligible for entry into the manufacture of functional food and health drinks because of the high content of minerals and protein. As well as, the phenolic contents may act as *in-vivo* and *invitro* antioxidants. It could be considered that garden cress phenolic extract is effective against some aflatoxigenic fungi and other tested fungi isolated from eggplant fruits. As the results are promising, it appeals further studies on its medicinal and anticancer uses.

Data Analysis

All results were carried out in triplicates and values were expressed as Means ± Standard Deviation (SD). Significant statistical differences of investigated parameters were determined and analyzed using one-way analysis of variance (ANOVA PC-STAT, 1985 version IA copyright, University of Georgia) at a 5 % level of significance. Values with $p<0.05$ were considered statistically significant.

Conflicts of interest

"There are no conflicts to declare".

Formatting of funding sources

This research was funded by National Research Centre, grant number E120404.

Acknowledgments

The facilities offered to the authors by the Central Library at the National Research Centre, Cairo, Egypt, to collect the data of article are highly appreciated

Table (9): Antifungal activity of enzymatic phenolic extract of GCSM.

Conc.	Aflatoxigenic fungi						Other fungi						LSD 5%
	A. flavus		*A. parasiticus*		*Alternaria* sp.		*A.niger*		*A.terrus*		*Penicillium* sp.		
	linear growth (cm)	R %	linear growth (cm)	R %	linear growth (cm)	R %	linear growth (cm)	R %	linear growth (cm)	R %	linear growth (cm)	R %	
10%	7.17 ±0.16^{b}	18.8	5.52 ±0.13^{b}	36.33	2.25 ±0.28^{b}	55.71	5.08 ±0.22^{b}	36.33	1.28 ±0.13^{b}	70.44	5.83 ±0.58^{b}	31.41	1.303 A
20%	5.25 ±0.14^{a}	40.54	3.83 ±0.30^{a}	55.82	1.35 ±0.05^{a}	73.43	4.33 ±0.08^{a}	50.96	0.78 ±0.10^{a}	82.00	3.67 ±0.22^{a}	56.82	1.303 B
Control	8.83 ±0.16 ^{c}		8.67 ±0.16 ^{c}		5.08 ±0.22 ^{c}		8.83 ±0.16 ^{c}		4.33 ±0.16 ^{c}		8.50 ±00 ^{c}		1.303 C
LSD 5%	1.044 C		1.044 B		1.044 A		1.044 B		1.044 A		1.044 B		

Results are mean values of three replicates ± standard deviation. The different letters in each column indicate significant differences at P<

5. References

[1] Al-Asmari A. K., Al-Elaiwi A.M., Athar M.T., Tariq, M., Al Eid, A. and Al-Asmary S.M., A review of hepatoprotective plants used in saudi traditional medicine, *Evidence-based complementary and alternative medicine : eCAM* vol. **2014**: 890842. (2014) doi:10.1155/2014/890842

[2] Ahsan, S. Tariq, M. Ageel, M. Alyahya, M. and Shah, A., Studies on some herbal drugs used in fracture healing, International Journal of Crude Drug Research, **27**, 235–239(1989).

[3] Falana, H., Nofal W., and Nakhleh *H*., A review article *Lepidium sativum* (Garden cress)." Pharm-D Program, College of Nursing, Pharmacy and Health Professions, Birzeit University: 1-8. (2014).

[4] Akl E. M., Taha F.S., MOHAMED S.S., Mohamed R.S., Characterization of Garden Cress Mucilage and its Prophylactic Effect Against Indomethacin-Induced Enter-Colitis in Rats, Biointerface Research in Applied Chemistry **11**(6), 13911 – 13923(2021)

[5] Al-Asmari AK, Athar MT, Al-Shahrani HM, Al-Dakheel SI, Al-Ghamdi MA. Efficacy of *Lepidiumsativum* against carbon tetra chloride induced hepatotoxicity and determination of its bioactive compounds by GCMS. Toxicology Reports, **25**(2), 1319-1326. (2015) doi: 10.1016/j.toxrep.2015.09.006.

[6] Adam, S. I., Salih S. A., and Abdelgadir W. S. *In vitro* Antimicrobial Assessment of *Lepidiumsativum L.* Seeds Extracts. Asian Journal of Medical Sciences. **3**(6), 261-266(2011)

[7] Sharma R. K., Vyas K., and Manda H., Evaluation of antifungal effect on ethanolic extract of *lepidiumsativum L*. Seed. International Journal of Phytopharmacology. **3**(2):117-120(2012)

[8] Gargi C., and Kalita M.C., Biocontrol Potential of *Pseudomonas Fluorescens*Against Bacterial Wilt of Brinjal and Its Possible Plant Growth Promoting Effects. Annals of Biological Res., **3**(11), 5083-5094 (2012).

[9] Erica O., Eggplant. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa, (2011).

[10] Jidda M., and Musa A., Fungal Deterioration of Eggplant (*Solanummelongena*L.) Fruits in Maiduguri Metropolis, Borno State, Nigeria. African Journal of Basic & Applied Sciences, **8** (6), 309-313 (2016).

[11] Jidda M., and Musa A., Fungal Deterioration of Eggplant (*Solanummelongena*L.) Fruits in Maiduguri Metropolis, Borno State, Nigeria. African Journal of Basic & Applied Sciences, **8** (6), 309-313 (2016).

[12] Zacharia R.M., and Philip S., Biological Control of Postharvest Diseases of Brinjal. Academic Review. Journal of Microbiology Research, **9**(29), 1758-1763 (2010).

[13] Kuc'mierz J., and Sumera R., Fungi Isolated from Eggplant (*Solanummelongena*L.) Seeds, theirPathogenicity to Seedlings and Attempts of their Control. Progress in Plant Protection, **49**(1): 219-223(2009).

[14] Akwaji P.I., Okon E.I., Markson A.A., and Iso U.E., Fungi Associated With Pre-Harvest Deterioration of Egg Plant *Solanummelongena*L. And Their Control Using Fruit Extract of *TetrapleuraTetraptera*. Global Journal of Science Frontier Research. Biological Science, 16(2), 7-16 (2016).

[15] Enikova R. K., Stoynovska M.R., and Karcheva M. D., MycotoxinsIn Fruits and Vegetables. Journal of IMAB - Annual Proceeding (Scientific Papers), **26**(2), (2020).

[16] Aldars-garcía L., Berman M., Ortiz J., Ramos A.J., and Marín S., Probability Models For Growth And Aflatoxin B_1 Production As Affected By Interspecies Variability In *Aspergillusflavus*. Food Microbiology, 72, 166–175 (2018).

[17] Alfredo, V.O., Gabriel, R. R., G.Luis C. and David B. A. Physicochemical properties of a fibrous fraction from chia (*Salvia hispanica L.*). LWT - Food Science and Technolgy **42**: 168–173(2009)

[18] AOAC, Official Methods of Analysis, 15thEdn.Association of Official Analytical Chemists, Washington, DC. (2005)

[19] Heinrikson, R.L. Meredith, S.C. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate, Analatical Biochemistry **136**(1) (1984) 65–74, https://doi.org/10.1016/0003-2697(84)90307-5.

[20] Cohen, S.A., Mewyes, M., and Travin, T.L., The Pico-Tag Method: A Manual of Advanced Techniques for Amino Acid Analysis, Millipore Corporation, Milford, USA, p. 124(1989)

[21] Fu R , Zhang Y, Gue Y, Liu F, Chen F. Determination of phenolic contents and antioxidant activities of extracts of *Jatrophacurcas L*. seed shell, a by-product, a new source of natural antioxidant. *Industrial Crops* and *Products*,**58**, 265–270(2014).

[22] De Ancos B., Sgroppo S., Plaza L., and Cano M.,P., Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of* the *Science* of *Food* and *Agriculture***82**(8):790–796(2002)

[23] Zhao, H., W. Fan, J. Dong, J. Lu and Chen J., Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry, **107**, 296-304(2008)

[24] Kuyng H., K., Tsao R., Yang R., and CuiS W., Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry **95**, 466-473(2006)

[25] Raper K.B., and Fennel D.I., The Genus Aspergillus. Baltimore, Williams and Wilkins, Pp.686, (1965).

[26] Samson R. A., The Genus *Aspergillus* Described Since 1965. Studies In Mycology, **18**, 80 (1979).

[27] Domsch K.H., Gams W., and Anderson T.H., Compendium of Soil Fungi. New York, Academic Press., London (1980).

[28] Pitt J. I., and Hocking, A.D., Fungi and Food Spoilage. Blackic Academic and Professional , London, (1997).

[29] Munimbazi C., and Bullerman L., High-Performance Liquid Choromatographic Method ForThe Determination of Moniliformin in Corn. Journal of AOAC International, **81**, 999-10 (1998).

[30] Kumar A., Shukla R., Singh P., and Dubey N., Chemical Composition, Antifungal and Anti-Aflatoxigenic Activities of *Ocimum Sanctum* l. Essential Oil and Its Safety Assessment As Plant Based Antimicrobial. Food and Chemical Toxicology, **48**, 539-543(2010).

[31] A.O.A.C., Association of Official Analytical Chemists. Official Methods of Analysis. 16thed, revised, AOAC, Washington, DC., 319 (1995).

[32] Singh S., Srivastava S., Mishra J., Raaj R., and Sina A., Evaluation of Some Plant Extract Against Predominant Seed Mycoflora Of Mungbean*VignaRadiata* (L.) Wilczek Seed. Life Sciences Leaflets,(51) 83- 89 (2014).

[33] Jabeen N., Ahmed M., Shaukat S., and Salam I., Allelopathic Effects of Weeds On Wheat (*TriticumAestivum*L.) Germination and Growth. Pakistan Journal of Botany, **45**(3), 807-811 (2013).

[34] Zia-Ul-Haq M, Ahmad S, Calani L, Mazzeo T, Rio DD, Pellegrini N, Feo VD. Compositional study and antioxidant potential of Ipomoea Hederacea Jacq and *Lepidium sativum* L. seeds. Molecule.**17**:10306-10321(2012)

[35] Mohammed R.,F., Preparation and characterization of protein isolate and biodiesel from garden cress seed, European Journal of Chemistry, **4** (2), 85:91(2013)

[36] Singh C.S., and Paswan V.K., The Potential of Garden Cress (*Lepidiumsativum L.*) Seeds for Development of Functional Foods. Chapter 14 (2017) http://dx.doi.org/10.5772/intechopen.70355

[37] Pramod J K., Pranit N P., Dayanand D K., and Padma B D., Nutritional, elemental analysis and antioxidant activity of Garden cress (*Lepidium Sativum L.)* International Journal of Pharmacy and Pharmaceutical Sciences · **4**(3), 392-395(2012)

[38] Garattini S. Glutamic acid, twenty years later. The Journal of Nutrition, **130**,901S-909S (2000)

[39] Mohamed R.S., Fouda ., and Akl E.M.,. Hepatorenal protective effect of flaxseed protein isolate incorporated in lemon juice against lead toxicity in rats Toxicology Reports **7**, 30–35(2020).

[40] Akl E., M., Mohamed S., S., Hashim A.,I., and Taha FS Optimum Extraction of Phenolic Compounds from Flaxseed Meal. American Journal of Food Technology **12**, 152-169(2017)

[41] Taha F.S., Hassanein M. M. Pretreatment of cottonseed flakes with proteases and an amylase for higher oil yields. Grasas y Aceites, **58**(3) 186. (2007).

[42] Dandge P. B., Kasabe P. J., Patil P., N., and Kamble D., D., Nutritional, elemental analysis and antioxidant activity of garden cress seeds. International Journal of Pharmacy and Pharmaceutical Sciences, **4**(3):392-395(2012)

[43] Zia-Ul-Haq, M., Ahmad, S., Calani, L., Mazzeo,T., Rio,DD. PellegriniN.,andFeoV.D. *Molecules*, **17**, 10306-10321(2012)

[44] Kasabe P., J., PATIL P., N., KAMBLE D., D., and Dandge P., Nutritional, elemental analysis and antioxidant activity of Garden cress (LepidiumSativum L.) seeds International Journal of Pharmacy and Pharmaceutical Sciences **4**(3):392-395(2012)

[45] Hur, S. J., Lee, S.Y., Kim, Choi, I. and Kim, G.,B., Effect of fermentation on the antioxidant activity in plant based foods. Food Chemistry **16**0, 346– 356(2014)

[46] Kwon J., and Jee H., Soft Rot of Eggplant (*Solanum melongena*) Caused by *Choanephora*

Cucurbitarum in Korea. Mycobiology ,**33**(3), 163-165 (2005).

[47] Gambari U., Chiejina O., and Nneka V., Aetiology of Fungal Pathogens of Garden egg (*Solanummelongena*L. Juss) in Nsukka Area. International Journal of Applied and Natural Sciences, (4), 73-80 (2013).

[48] Tiwari V., Singh R., and Pandey A., Efficacy Of Some Antagonistic Fungi And Botanicals Against *Fusarium Solani* Causing Damping-Off Disease In Eggplant (*Solanum Melongena*L.). Journal of Applied Biosciences, **43** (1), (2017).

[49] Yaji M., Aernan P., and Sule M., Microorganisms associated with the spoilage Of cucumber, garden egg and pawpaw in makurdi metropolis, Benue Nigeria. International Journal Of Recent Research In Life Sciences, **3**(1), 11-18 (2016).

[50] Hacıbekiroğlu I.,and Kolak U., Aflatoxins In Various Food From Istanbul, Turkey. Food Additives & Contaminants: Part B, Vol. 6, No. **4**, 260–264 (2013).

[51] Norhayati A., Noor-Hasani H., and Nor-Shifa S., Natural Occurrence Of Aflatoxins and Ochratoxin A In Processed Spices Marketed In Malaysia. Food Additives Contaminants, A, **32**(4), 518-532 (2015).

[52] Çagindi O., and Gürhayta O.,Aflatoxins And Ochratoxin A In Dried Eggplant And Green Bell Pepper,Food Control, **70**, 216-220 (2016).

[53] George R.E., Thomas S.K., Kunjumon M., and Thankamani V., Analysis Of Phytoconstituents And *In Vitro* Antifungal Evaluation Of Methanolic Extract Of *LepidiumSativum*Linn. Seeds. International Journal of Pharma and Bio Sciences, **6**, 490-7 (2015).

[54] Baregama C., and Goyal A., phytoconstituents, pharmacological activity, and medicinal use of *Lepidium Sativum* LINN.: a review. Asian Journal of Pharmaceutical and Clinical Research, **12**(4) (2019).

[55] Omer A.B., Nour A.H., Ali M.M., Ishag O. A., Erwa I.Y., and Ali M.A., Phytochemical Screening, Antimicrobial and Antioxidant Activity of *Lepidium sativum* Seeds Extract. South Asian Research Journal of Natural Products, **3**(1), 10-17(2020).