

THE POTENTIAL CYTOTOXICITY AND ANTIMICROBIAL ACTIVITIES FOR RIND AND SEEDS OIL EXTRACTS OF PUMPKIN (*Cucurbita pepo* L.)

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ABSTRACT

The main outcomes of this work were evaluation the bioactivities of some natural organic compounds extracted from rind and seeds of Pumpkin by using *in vitro* tests as well as, their chemical analysis. In one unique considers the first study carried out on the Pumpkin rind was found to be rich in β - carotene and minerals (Calcium, Iron, Zinc, Copper and Selenium) and a good source of protein; the potential cytotoxicity of rind extract obviously appears in its IC₅₀ "its dose which reduces survival to 50%" which equal to 0.54 and 0.60 μ g using breast and liver carcinoma cell lines respectively, besides it has moderate positive antibacterial effects on *Bacillus subtilis* and *Bacillus cereus*. Pumpkin seeds were found to be rich in fat and protein with an average yield of approximately 47.03 and 35.95% respectively, as well as they not only rich in essential fatty and amino acids but also in minerals. In addition, Pumpkin seeds oil has highly positive effects on both breast and liver tumor cell lines where, it has IC₅₀ 0.40 and 0.81 μ g respectively. The results show very clearly that the Pumpkin seeds oil has strong positive antimicrobial effect toward *Saccharomyces cerevisiae*, in the same words; neither rind extract nor seeds oil of Pumpkin has any antimicrobial positive effects on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Aspergillus niger*. The results obtained in this study indicated that, Rind extract and Pumpkin seeds oil are rich sources of nutrients. Therefore, they can be consumed as food or as supplementary ingredients especially in Egypt to alleviate the problems of health and nutrients/protein malnutrition.

Keywords: Pumpkin; Rind; Seeds oil; *in vitro*; Chemical analysis; Protein; Fat; Minerals; β - carotene; Essential fatty and amino acids; potential cytotoxicity; IC₅₀; Breast & Liver tumor cell lines; Antimicrobial effects.

INTRODUCTION

Pumpkin is the fruit of the species *Cucurbita pepo* or *Cucurbita mixta*. It can refer to a specific variety of the species *Cucurbita maxima* or *Cucurbita moschata*, which are all of the genus *Cucurbita* and the family *Cucurbitaceae*. Variation in nutrient content exists between different cultivars and varieties of pumpkin, Norrman *et al.* (1980). Number of fruits per plant varied from 1 to 3.0 and weighed from 1.25 to 9 kg of each. Polar circumference of fruit ranged from 43 to 85 cm. Similarly equatorial circumference ranged from 43.33 to 95 cm. Fruit shape varied from round, flat round oval and oblong type, Sudhakar *et al.* (2003).

The rind is smooth and variable in colour. Carotenes or Carotenoids are a yellow-orange pigment. Carotene, mostly as β -Carotene is used in many foods as a colouring additive. It can be converted into vitamin A by the body which is referred to as "provitamin A" which is known to exert antioxidant activity. β -Carotene content decreased at all temperatures but the decrease was more at 100°C than at 50°C and it followed zero order kinetics, Debjani *et al.* (2006). Lycopene and zeaxanthin are the two carotenoids with no pro vitamin A activity, though they play a vital role in human health, zeaxanthin and luteine (are structural isomers) accumulates in the eye where they play a very important role in the prevention of eye aging diseases such as cataracts, Bone *et al.* (2003). For lycopene, it serves as a very important antioxidant in the organism, that is, in the prevention of some forms of cancers and cardiovascular diseases, Argarwal and Rao (2000). Foods like pumpkin, solo papaya and water melon, which are the best sources of these carotenoids play a double role, first as a source of provitamin A and then as an antioxidant. The best sources of carotenoids provitamin A in fruits are mangoes, pumpkins and water melon, Gouado *et al.*(2007).

Pumpkin seed oil is a common salad oil in Austria. It is not only of interest because of its typical taste but also because of its potential in curing prostate disease. Besides the fatty acids, the micronutrients, which comprise vitamin E, phytosterols and lignans, are of special interest. Pumpkin seeds produce dark green yet excellent tasting oil. Pumpkin (*Cucurbita pepo*) seeds are used locally in Eritrea to treat tapeworm, Younis *et al.*, (2000). Fatty acids are major components of oils in many plants, foods and medicines, including pumpkin seeds (*Cucurbita pepo*). With the gas chromatography methods reported here, free fatty acids of this species can be quantified as their trimethylsilyl derivatives, Ganzera *et al.* (1999). The content of linoleic acid depends on temperature during ripening of the fruits. It increases significantly if the temperature is lower or the pumpkins are harvested later, Murkovic *et al.* (1999). New low-cost oil crops are needed to produce economical oils suitable for biodiesel production. One of the possible alternative oil crops for the Mediterranean area is pumpkin seed (*Cucurbita pepo* L.) Schinas *et al.* (2008). Pumpkin seed oils enjoy special and increasing popularity mainly due to their characteristic taste especially in the Central European region. These oils consist of approximately 70% unsaturated fatty acids; they also contain a number of hydrocarbons, tri-terpenoides, carotenoides, tocopheroles and phytosteroles, Murkovic *et al.* (1996),Younis *et al.* (2000) and Ernst *et al.* (2004). The oil is not contained in the fruit, as e.g., in case of olive oils, but in the seeds, which consist mainly of fat (50%) and proteins (40%). the oil quality also depends on the geographical origin, seasonal variations and climatic influences, Pinelli *et al.* (2003) and Ernst *et al.* (2004).

The Pharmacological studies of the pumpkin stated in Anthelmintic activity. Cucurbitin inhibits the growth of immature *Schistosoma japonicum* in vivo, and a patent has been granted for an effective aqueous extract of the seeds for use as a human anthelmintic, Mihranian (1968). In a randomized, 3-month, double-blind study, a preparation of pumpkin seeds oil improved certain parameters of benign prostatic hyperplasia including urinary flow, micturition time, residual urine, and urinary frequency vs placebo, Carbin

(1990). Severe toxicity has not been reported with the use of pumpkin seed oil. The animal data are summarized in one report, *C. maxima* in an oral preparation displayed strong antimalarial activity in mice, reducing the parasites by 50%, Amorim (1991). In another report, an extract of seeds demonstrated antitumor potential against *Neurospora crassa*, Rojas (1980). Characteristics, Basaran (1998) and nutritional aspects, Jaroniewska (1997) of pumpkin have been addressed. Studies on antilipolytic activity; also have been performed, Wong (1985). The influence of seeds on age-associated impairments has been reported, Wichtl (1992). Related species *C. ficifolia* exhibits hypoglycemic actions in rabbits, Roman (1992) and Roman (1995). In a 53-patient, randomized, double-blind trial, no side effects from oil seeds were noted, Carbin (1990). Ingestion of pumpkin seeds by rats and pigs over a 4-week period resulted in no changes in glucose, urea, creatinine, liver enzymes, blood counts, etc; Dequeiroz (1994). One report on pumpkin seeds describes dermatitis, Yue and Jansson (2001).

The chive aims of this research were to study the some different bio-activities (Anti- tumor and Anti- microbial effects) for both seeds oil and rind extracts of Pumpkin *Cucurbita pepo* especially the later, where it considers the first study carried out on the rind of the pumpkin to evaluate their chemical composition.

MATERIALS AND METHODS

1- Sampling:-

1-1- Pumpkin collection:

Pumpkin ripe fruits were purchased and collected from different markets of eleven governorates; Cairo, Giza, Kaluobia, Helwan and 6-October "Great Cairo", Alexandria, El-behaira, Dokkahlia, Marsa Matrouh, Beni-Swif and Assuit with selection of the full- colored mature pumpkin with fine texture.

1-2- Rind and Seeds Pretreatment:

Fruits were washed with tap water, dried and peeled to isolate the rind from the flesh according to Brian (2002) and Gouado *et al.* (2007) and remove the seeds from the flesh. Rind was air-dried for 6- days in Lab. temperature with daily stirring and handly crushed to give brain. As in Brian (2002), seeds were washed to remove the clinging fibrous pumpkin tissues, dried in the sun for three days with frequently stirring then, hulls were removed manually to obtain the cotyledons as the methodology of Sara *et al.* (2008) and the seeds were grounded to a fine powder and then dried for 2 hour at 100 °C as proceeded in Schinas *et al.* (2008).

2-Chemical analysis:-

2-1- Ingredients analysis:

Moisture content; crude protein; fiber and fat according to AOAC (2000); ash obtained by AOAC (1995) were estimated to rind and seeds powder of pumpkin. According to Sara *et al.* (2008) carbohydrate content was estimated by difference. Estimation of minerals (calcium, iron, zinc, copper

and Selenium) in rind and Pumpkin seeds powder by inductive coupled plasma ICP "optima 2000" according to AOAC (2002) and Iva *et al.* (2003).

2-2- Estimation of β - Carotene:

According to Leth and Jacobsen (1993) β - Carotene in both rind and seeds powder of Pumpkin was determined.

2-3- Determination of Amino acids:

Amino acids determination for both rind and Pumpkin seeds was performed according to method of the AOAC (2005). Oxidation with performic acid, to protect methionine and cystine from distraction during acid hydrolysis with (6 M HCL) were carried out in closed conical flask for determine all amino acids other than tryptophan. Sample of 20-30 mg weighted in conical flask and 5 ml of performic acid was added. The flask was closed and placed in ice water bath for 16 hr. Sodium metabisulfate and 25 ml HCL 6 N were added to the oxidized mixture. The flask was placed in an oven at 110 ° C for 24 hr. The flask was then opened and all removed by evaporating samples to dryness in rotary evaporator. A suitable volume of sodium citrate puffer (pH 2.20) was added to the dried film of hydrolyzed sample. After all soluble material completely dissolved, the samples analyzed for amino acids using Eppendorf LC 3000 (EZ Chrom, software used for data collection and processing). The results were calculated as percentage of total crude protein. Determinate tryptophan was carried out using method described by Miller (1967) after hydrolysis of samples with barium hydroxide.

2-4- Rind extract:

Organic compounds of rind were extracted via 100 g of the rind were air dried and grinded, and finally subjected to extensive extraction by methanol (1.2 L) during a soaking for several times. After a complete extraction and concentration under reduced pressure and low temperature (45 °C), the residue water extract was applied to extraction with chloroform. After a complete extraction, the chloroform layer was evaporated *in vacuo* to dryness, affording a pale oily orange extract (4.1g).

2-5- Extraction of pumpkin seeds oil:

According to AOAC (2000) and Schinas *et al.* (2008) continuously extraction of the Pumpkin seed oil was carried out . Soxhlet extraction apparatus was employed and hexane was used as solvent in the extraction process. Fatty acid composition of pumpkin seed oil methyl ester was also determined by gas chromatography analysis. Iodine number of test method EN 14111 and Acid value (mg of KOH/ g) of test method EN 14104 as well as Kinematics viscosity (40 °C) mm² / S were estimated of EN ISO 3104 test method.

3- Bio- activities of the rind extract and pumpkin seed oil:

3-1- Antitumor activities:

These estimations were carried out and documented in the Cancer Biology Department, Pharmacology Unit, National Cancer Institute, Cairo University. Potential cytotoxicity by Sulpho Rodamine B assay for the rind extract and pumpkin seed oil were tested using the method of SKeahan *et al.* (1990) on HEPG2 [liver carcinoma cell line] and MCF7 [breast carcinoma cell line].

Cells were plated in 96- multiwell plate (10^4 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentration of the two compounds under test (0- 10 $\mu\text{g/ml}$) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the two compounds for 48 hours at 37 °C and in atmosphere of 5 % CO_2 . After 48 hours, cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentrations is plotted to get the survival curve of each tumor cell line after the specified compound.

3-2- Antimicrobial activities.

These assays are reported and documented in Food safety and Biotechnology Lab, Regional Center for Food and Feed, Agriculture Research Center, Egypt.

Materials:-

Bacterial strains:

The bacterial strains "*Escherichia coli* and *Salmonella*" as Gram negative bacteria, "*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*" as Gram positive bacteria and "*Saccharomyces cerevisiae*" as yeast and finally, "*Aspergillus niger*" as fungal strain were used to evaluate the antimicrobial effects of the rind extract and pumpkin seed oil.

Brain Heat Infusion Broth.

Ingredients:- Formula (in grams per liter)- Calf Brain, infusion form - Beef Heart, infusion form- Peptocomplex- Glucose- Sodium Chloride- Di Sodium Phosphate. Method of preparation:- Suspend 52g of agar and 37g of broth in 1000 ml of cold distilled water. Heat to boiling and autoclave at 121 °C for 15 minutes, final pH 7.4 ± 0.2 .

Antibiotic Seed Agar A1.

Formula (in grams per litre):- Peptomeat- Peptone Bios D- Yeast Extract- Beef Extract- Glucose- Agar Bios LL- Final pH 6.5 ± 0.1 . Preparation and Description:- Suspend 30.5g in 1000 ml of cold distilled water; heat to boiling and autoclave at 121 °C for 15 minutes.

Oxytetracycline Yeast Extract agar.

Ingredients:-500gm makes 13.5 litres. Typical formula (g/l):- yeast extract 5.0; glucose 20.0, agar 12.0 pH 7.0 ± 0.2 at 25 °C. suspend 18.5 g in 500 ml of distilled water and bring to the boil to dissolve Completely. Sterilise by autoclaving at 115 °C for 10 minutes. Cool to 50 °C and aseptically add the contents of 1 vial of Oxytetracycline supplement or 1 vial of chloramphenicol supplement, reconstituted as directed. Mix thoroughly and pour into sterile Petri dishes. Adjust the pH as required.

Method:-

Preparation of bacterial and yeast suspension.

Each bacterial and yeast cultures were collected separately and inoculated in a sterile bottle 100 ml Brain Heart Infusion broth which then incubated at 25 °C, 30 °C and 37 °C for *Saccharomyces cerevisiae*, *Bacillus cereus*, and *Escherichia coli*, *Salmonella*, *Bacillus subtilis* and *Staphylococcus aureus* respectively for 24 hours.

Agar diffusion assay: According to **Taradon et al. (2007):-**

- 1- Each one hundred ml of sterile antibiotic medium, were inoculated by 1 ml from each single type of bacterial strains suspension (*Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) shaken well and then distributed into 6 sterile Petri dishes each contained about 10 ml.
- 2- One hundred ml of Oxytetracycline Yeast Extract agar were inoculated with one ml of *Saccharomyces cerevisiae*'s suspension, shaken well and then distributed into 9 sterile Petri dishes each contained about 10 ml.
- 3- Three wells pored in each plate with a sterile cork porer 0.8 mm.
- 4- From each type of microorganism wells in 2 plates were filled with 0.1 ml of rind extract and the last 3 plates were filled with Pumpkin seed oil.
- 5- *Saccharomyces cerevisiae* inoculated plated were incubated in an upright position at 25 °C for 5- 7 days, *Bacillus cereus* inoculated plated were incubated in an upright position at 30 °C for 24 hours and *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Bacillus subtilis* inoculated plated were incubated in an upright position at 37 °C for 24 hours, 48 hours and 72 hours, respectively.
- 6- All inhibition zones around the inoculated wells were measured in mm and record (table +) and all the plates were captured using Gel Documentation System (Alpha Imager).
- 7- Agar diffusion assays occurred according to **Cassandra et al. (2004)**, anti- *Aspergillus niger* assays carried out for Rind extract and pumpkin seed oil. Three sterile Whatmann No. 1 filter paper (+5m) placed on Potato Dextrose Agar plate inoculated with 100µl of a fungal spore solution. A potential antifungal substance (10µl) is then applied on these filter disks. For a fungal strain, a blank without filter disks was made. Plates were incubated aerobically at 25 °C and examined for inhibition zones around the filter disks during 10 days. The radius of the observed inhibition zones was measured as an average of three.

"RESULTS AND DISCUSSION"

1- Chemical analysis for rind and seeds powder of Pumpkin.

1-1- Ingredients analysis:

Table (1):-Ingredients estimations for rind and seeds powder of Pumpkin (g/100g sample):-

Ingredient	Rind	Seeds powder
Ash	010.65	003.55
Carbohydrates	019.45	006.37
Fat	006.57	047.03
Fiber	029.62	005.30
Moisture	009.76	001.80
Protein	023.95	035.95
Minerals (ppm) :-		

Ca⁺⁺	5571.00	0420.55
Fe⁺⁺	0247.30	0149.64
Zn⁺⁺	0042.92	0089.29
Cu⁺⁺	0012.91	0024.49
Se⁺⁺ (ppb)	0012.71	0012.40

Since the ash content of a sample is a reflection of the minerals it contains, **Sara et al. (2008)** therefore, high ash pumpkin rind and seeds powder are expected to be rich in the studied minerals (Calcium, Iron, Zinc, Copper and Selenium), and were found to be 10.65 and 03.55%, respectively as shown in table (1).

The results obtained in this study indicated that pumpkin rind and seed powder are rich sources of nutrients especially protein (23.95% and 35.95%) respectively, thus pumpkin rind and seeds powder could contribute significantly to the recommended human daily protein requirement which was reported to be ranged from 23% to 56%, **NRC (1980)**. In addition to; the current study is the first research carrying out on the rind of the Pumpkin which appears from table (1) that, rind is a good source for carbohydrates (19.45 %) and fiber (29.62 %), in the opposite, Pumpkin seeds powder is poor source of carbohydrate (6.37 %) and fiber (5.30 %).

Fats are essential in diets as they increase the palatability of foods by absorbing and retaining their flavors and help in the transport of nutritionally essential fat-soluble vitamins (**Omotoso, 2006**). The results of the proximate composition of the Pumpkin rind and seeds powder are shown in table (1), the results showed that Pumpkin seeds powder is excellent source of fat (47.03%) while, Pumpkin rind is poor (06.57%). Therefore, Pumpkin rind and seeds powder can be consumed as food or as supplementary ingredients to alleviate the problem of nutrient / protein malnutrition. Further work is needed to evaluate the nutritional value by using *in vivo* tests.

1-2- β- Carotene:

Carotenoids are a group of natural pigments responsible for yellow, orange or red

colour of many foods. Besides the well-known provitamin "A" activity of some of these pigments, they have also been associated with lowered risk of developing degenerative diseases such as cancer, cardiovascular diseases and macular degenerative (**Gouado et al., 2007**). At least 254 million pre-school aged children globally suffer from clinical and subclinical vitamin A deficiency, **WHO (2000)**. Thus, alleviation of vitamin A deficiency is a major objective particularly in poor target countries. Food based strategies are one of the best means that are used for combating VAD in developing countries, **Ruel (2001)**. β- Carotene, α-carotene and β-Cryptoxanthin are the major provitamin A carotenoids found in most foods. However its unceasing growing importance in public health, notably in the prevention of different forms of cancer like prostate cancers, **Gann and Khachik (2003)** and **Hardley et al. (2002)** and problems linked to aging of the eye, **Landrun and Bone (2001)**, lycopene and zeaxanthin respectively are becoming of much interest nowadays. Lycopene and zeaxanthin are the two carotenoids with no pro

vitamin A activity, though they play a vital role in human health: zeaxanthin and luteine (are structural isomers) accumulates in the eye where they play a very important role in the prevention of eye aging diseases such as cataracts, **Bone et al. (2003)**. For lycopene, it serves as a very important antioxidant in the organism, that is, in the prevention of some forms of cancers and cardiovascular diseases, **Agarwal and Rao (2000)**.

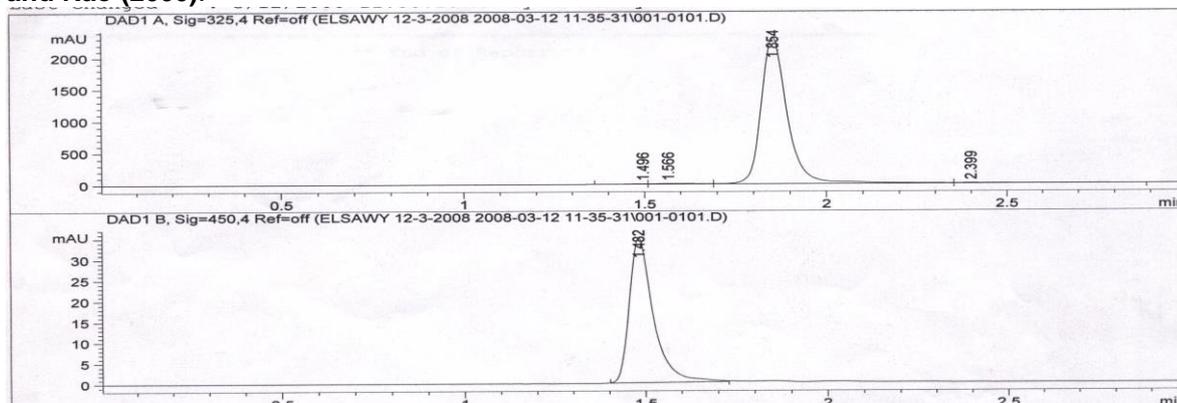


Fig (1) H.P.L.C. signals of β - Carotene present in the Pumpkin rind.

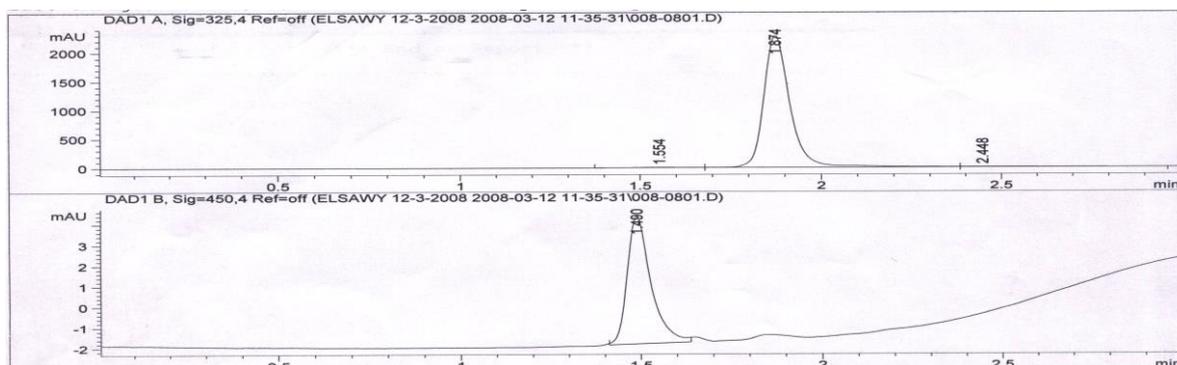


Fig (2) H.P.L.C. signals of β - Carotene present in the Pumpkin seed powder.

Figures (1) and (2) illustrate the concentrations of the β - Carotene in both Pumpkin rind and seeds powder which showed that; Pumpkin rind is a good source (751.99 μ g/100g) while Pumpkin seeds powder is a poor source (078.89 μ g/100g) of β - carotene. Variations between and within the same fruit are therefore an indication of the vast diversity of sources of carotenoids caused by seasonal and regional variations. Differences observed between carotenoids content of fruits harvested during different seasons are probably due to climatic characteristics. In fact, several studies on others fruits, **Markus et al. (1999)** and **Lima et al. (2005)** noticed that carotenoids content was higher in mature fruits harvested during rainy season (poor sunshine, high rainfall and low temperature) than in those harvested during the dry season. Taking into consideration the vitamin A from pumpkin and with high

bioavailability, meet the daily requirements of vitamin A for the population concerned.

1-3- Amino acids:

FAO/WHO (1973) and **Marian (1981)** stated that; amino acids are the building blocks for proteins, which provide the structure for all living things .They are linked together by peptide bonds and enable vitamins and minerals to perform their jobs properly, as well as are necessary for the brain to receive and send messages.

Table (2):- Amino acids composition of pumpkin rind and seeds powder (%).

Amino Acids	Rind	Seeds powder
Aspartic acid (Asp.)	2.64	05.59
Threonine (Thr.)	0.71	01.79
Serine (Ser.)	1.02	02.87
Glutamic acid (Glu.)	2.53	11.50
Proline (Pro.)	0.79	02.23
Glycine (Gly.)	0.96	02.85
Alanine (Ala.)	0.90	02.56
Valine (Val.)	0.87	02.61
Isoleucine (Iso.)	0.70	02.04
Leucine (Leu.)	1.21	04.15
Phenyl alanine (Phe.)	0.95	03.05
Histidine (His.)	0.62	02.00
Lysine (Lys.)	1.16	01.90
Arginine (Arg.)	1.03	05.42
Cystine (Cys.)	0.24	00.63
Methionine (Meth.)	0.23	01.18
Tryptophan (Tyr.)	0.85	00.40
Total	17.41 %	52.77 %

Human body must break protein down into its constituent amino acids to build the specific proteins it needs. Proteins are essential for living cells to exist; the enzymes and hormones that regulate all body processes; regulating the body's water balance and maintaining the proper pH (acid-base balance) and are exchanging nutrients between body fluids and the tissues, blood and lymph. As well as, they forming chromosomes and passing genetic information to offspring .Making muscles, ligaments, tendons, organs, glands, nails, hair, and many vital body fluids. On other hand, the body requires all of the 9 essential amino acids (histidine, isoleucine, leucine, lysine, treptophan, methionine, phenylalanine, threonine and valine) to make proteins the body needs. Even if we eat a balanced diet with sufficient protein, impaired absorption, stress, trauma, infection, age, drug use and imbalances of other nutrients can lead to inadequate amounts of the essential amino acids.

Where rind and seeds powder of Pumpkin are good sources of protein as showed in table

- (1) that is a strong outcome to know the profile of their peptide linkage and their amino

acids [AA's] patterns as shown in figures (3) and (4) respectively, for each protein of them. Table (2) illustrates the constituents of AA's for Pumpkin rind and powder seeds in which, the essential amino acids [EAA's] which previously listed represent 07.25% and 19.12% of the total AA's for rind 17.41% and seed powder 52.77% respectively. All the AA's present in the rind have got lower values than that in the seeds powder except tryptophan, which records the higher percentage than that in seeds powder. The pattern of seed powder AA's does not agree with **Zdunczyk et al. (1999)** but in convineint with the results of **USDA (1990)**, in the same site not exist any previous study on the AA's estimation of Pumpkin rind. According to **Zhu et al. (2006)** the AA's of both Pumpkin rind and seeds powder can be classified according to their chemical nature and behaviors as listed in table (3).

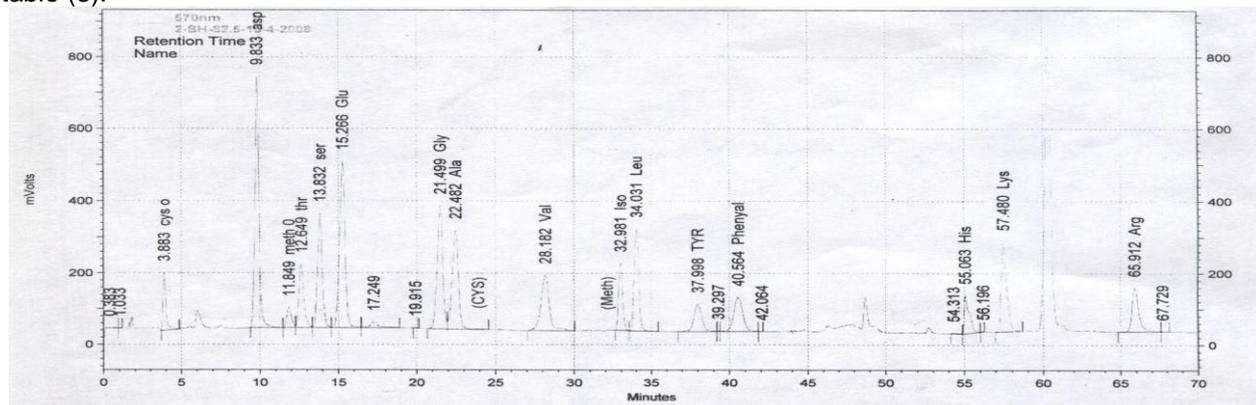


Fig (3) Amino acids profile for the crude protein of Pumpkin rind.

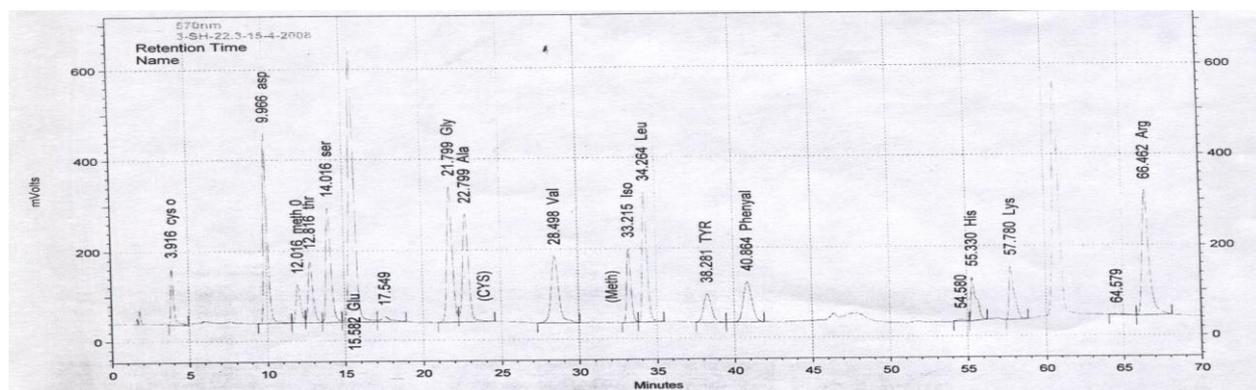


Fig (4) Amino acids profile for the crude protein of Pumpkin seeds powder.

Table (3):- Classification of Amino Acids present in Pumpkin rind and seeds powder.

Classification of AA's	Rind	Seeds Powder
Hydrophobic (nonpolar) ^a	07.46 %	21.07 %
Uncharged polar ^b	01.97 %	05.29 %

Basic ^c	02.81 %	09.32 %
Acidic ^d	05.17 %	17.09 %
Sulfur- containing ^e	00.47 %	01.81 %
Aromatic ^f	01.80 %	03.45 %

a: Gly., Ala., Val., Leu., Pro., Meth., Phe., Try. and Iso. b: Ser., Thr. and Cys.
c: Lys., Arg. and His. d: Asp. and Glu. e: Cys. and Meth. f: Phe. and Try.

As shown in table (3), the hydrophobic AA's recorded the high percentage in classified Pumpkin rind and seeds powder AA's while that opposite in case of sulfur-containing AA's. Pumpkin seeds powder is rich source of essential and non- essential AA's and this result agrees with many results as **Zdunczyk et al. (1999)** **El-Soukkary (2001)** and **Zhu et al. (2006)** while the AA's of rind not evaluate due to not exist another study and the current study consider the Pumpkin rind is a good source of AA's.

1-4- Pumpkin seeds oil characterization.

The oil content of pumpkin seeds is 47.03% on dry weight basis as showed in table (1) which is higher than that obtained results of **Schinas et al. (2008)** and in convenient with that obtained of **Michael et al. (2004)**. Pumpkin seeds oil is a dark green oil and from table (4); it showed that a high content of free fatty acids composition and acid value record 0.62 (KOH,mg /g), Pumpkin seed oil had a viscosity of 3.28mm²/ Sec. The iodine number is an indication of the number of double bonds in oil and therefore is a parameter that quantifies the degree of unsaturation of pumpkin seed oil and this property greatly influences oxidation stability and the polymerization of glycerides as well as at high saturation. The iodine number is directly correlated to pumpkin seed oil viscosity. In the current study, the iodine number of the produced oil was 109 as shown in table (4).

Table (4) Physicochemical properties and fatty acids composition of Pumpkin seeds oil:

Physicochemical Properties/Fatty Acids Composition.	Values	
Kinetic viscosity (40 °C) mm ² S ⁻¹ .	03.28	
Iodine number.	109	
Acid value, KOH, mg g ⁻¹ .	00.62	
Fatty Acids Composition:-	Relative Distributio	
1- Saturated Fatty Acids:-		
Myristic Acid, C14:0.	CH ₃ (CH ₂) ₁₂ COOH	00.1%
Palmitic Acid, C16:0.	CH ₃ (CH ₂) ₁₄ COOH	13.4%
Stearic Acid, C18:0.	CH ₃ (CH ₂) ₁₆ COOH	07.8%
Arachidic Acid, C20:0	CH ₃ (CH ₂) ₁₈ COOH	00.5%
2- Mono Unsaturated Fatty Acids:-		
Oleic Acid, C18:1ω-9	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	43.8%
Vaccenic Acid, C18:1ω-7	CH ₃ (CH ₂) ₆ CH=CH(CH ₂) ₇ COOH	01.8%
Gadolic Acid, C20:1ω-9	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₉ COOH	00.1%
3- Poly Unsaturated Fatty Acids:-		
Linoleic Acid C18:2ω-6	CH ₃ (CH ₂) ₃ (CH ₂ CH=CH) ₂ (CH ₂) ₇ COOH	31.1%
α-Linolenic Acid C18:3ω-3	CH ₃ (CH ₂ CH=CH) ₃ (CH ₂) ₇ COOH	00.1%

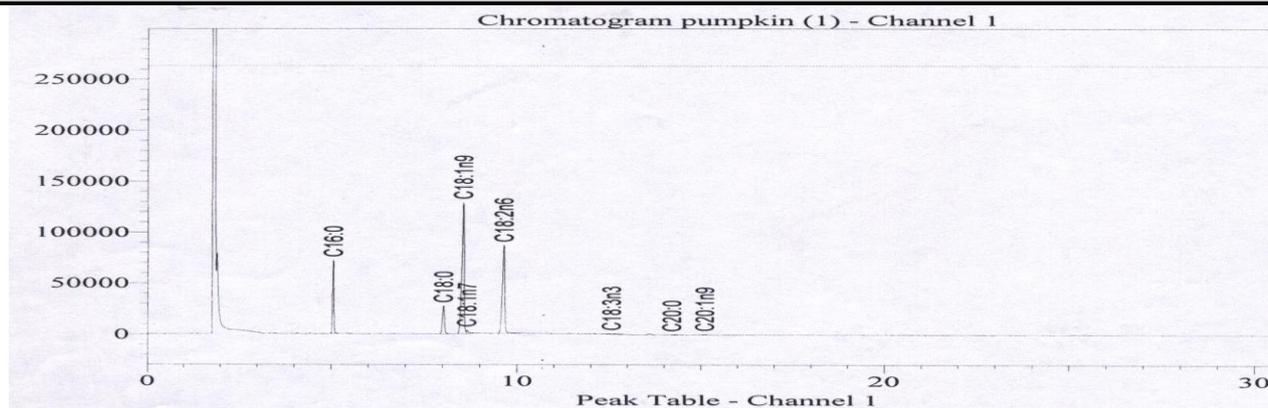


Fig (5) Fatty Acids analysis of the pumpkin seeds oil.

On the other hand, the fatty acids profile of Pumpkin seeds oil was identified and quantified as shown in table (4) and fig (5) and are illustrate that, the four dominant fatty acids are oleic, linoleic, palmitic and stearic acids with relative distribution of about 43.8, 33.1, 13.4 and 07.8% respectively, which make up $97 \pm 0.1\%$ of the total amount of fatty acids, others being found at levels well not exceed 1.8%. As resulted with **Younis et al. (2000)** and **Murkovic et al. (1996)**, the variability in the current oil content is very high resulting from a broad genetic diversity. As obviously in table (4) and fig (5), pumpkin seed oil provide the highest essential fatty acids (Omega 3 and Omega 6) and as examined by **Taylor et al. (2006)** they required for healthy mind and body functions as well as to prevent and alleviate bladder and prostate problems. The resulting highly unsaturated fatty acids of Pumpkin seeds oil are necessary for cell membrane function, the proper development and functioning of the brain and nervous system and according to **Brenda and Penny, (2003)** omega-3 (ω 3) and omega-6 (ω 6) fatty acids are unsaturated "Essential Fatty Acids EFAs" that need to be included in the diet because the human metabolism cannot create them from other fatty acids. Since these fatty acids are polyunsaturated, the terms n-3 PUFAs and n-6 PUFAs are applied to omega-3 and omega-6 fatty acids, respectively and The two EFAs, are showed in fig (6), both polyunsaturated fats; Linoleic (the parent n-6 fatty acid) and α - Linolenic (the parent n-3 fatty acid) and humans are able to convert Linoleic and Linolenic to more physiologically active fatty acids through a series of elongation and desaturation reactions. The ω - 6/ ω -3 ratio is 311 and this altered ratio is contributing to a lot of excess inflammation in the human body, which is probably the source of many of our chronic diseases, including arthritis, heart disease, and diabetes.

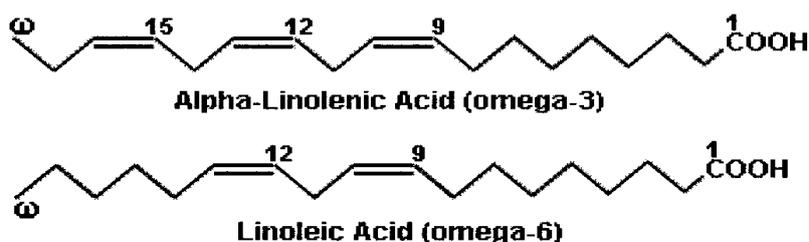


Fig (6) Chemical structure of α - Linolenic & Linoleic essential fatty acids.

3- Bio- activities of the pumpkin seed oil and rind extract:

3-1- Antitumor activities.

Table (5):- Level of cytotoxicity for the breast carcinoma cell line:

Concentration (μg)	MCF7-Pumpkin Seed Oil	MCF7-Pumpkin Rind Extract
0.0	1.00000	1.00000
1.0	0.11665	0.06219
2.5	0.07132	0.06676
5.0	0.05697	0.05968
10.0	0.03830	0.04196

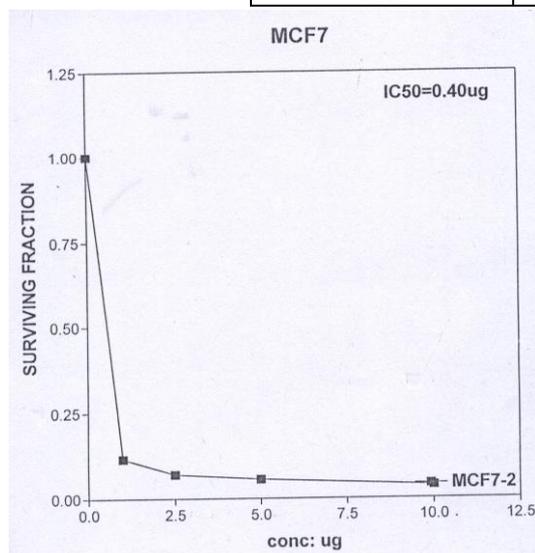


Fig (7) Potential Cytotoxicity of the Pumpkin seed oil using breast carcinoma cell line.

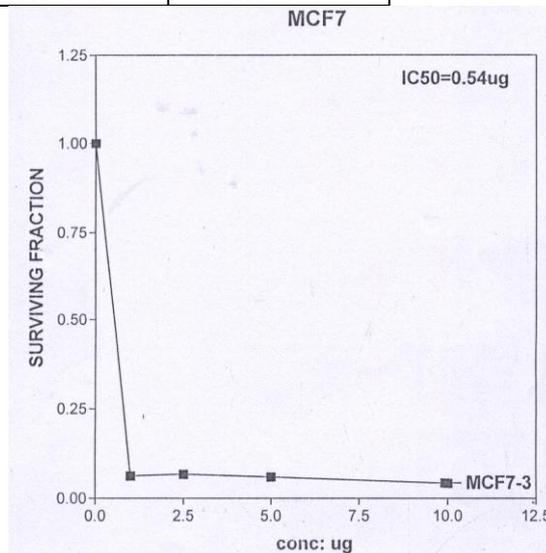


Fig (8) Potential Cytotoxicity of the Pumpkin rind extract cell line.

Table (6) illustrates Pumpkin seeds oil and rind extract concentrations between (1- 10 µg) using the Sulfo- Rhodamine B assays. Where the Pumpkin seeds oil is viscous and dark green oil, while the rind extract is oily and dark orange in their nature, the inhibition concentration "IC₅₀" which is the dose of the compound which reduces survival to 50% is varied from seeds oil and rind extract. On the other side, The relation between surviving fraction and drug concentrations were plotted to get the survival curve for breast tumor cell lines "MCF7" after the specified compounds to give figures (7) and (8) of Pumpkin seeds oil and rind extract respectively which are illustrated that, 0.40µg is the dose of pumpkin seeds oil "IC₅₀" and which has the cytotoxicity activity on the breast carcinoma cell lines and reduces survival to 50% as showed in fig (7), in the same site, 0.54µg is the corresponding dose of Pumpkin rind extract "IC₅₀" which has the cytotoxicity activity to reduces the breast carcinoma cell lines to 50% as obviously in fig (8).

On the other hand, table (7) showed the Pumpkin seeds oil and rind extract concentrations between (1- 10 µg) using the same last dye to evaluate their cytotoxicity activities using liver tumor cell lines "HEPG2". The relation between surviving fraction and Pumpkin seeds oil and rind extract concentrations which is obviously appears in figures (9) and (10) respectively. Potential cytotoxicity of the Pumpkin seeds oil was tested produced 0.81µg of this drug is equal to IC₅₀ which reduce survival to 50% of liver tumor cell line as shown in fig (9). In the same word; the potential cytotoxicity of Pumpkin rind Extract was examined result that, 0.60µg of that drug was the IC₅₀ of the Pumpkin rind extract to reduce the liver tumor cells to 50% as clear in fig (10). In the same hand, the recent studies **Simopoulos (2002); Morris (2003) and USDA (2005)** of Pumpkin seeds oil stated that; excessive amounts of omega-6 polyunsaturated fatty acids and a very high omega-6/omega-3 ratio have been linked with pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. The ratio of omega-6 to omega-3 in modern diets have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer.

Table (6) Level of cytotoxicity for liver

carcinoma cell line:

Concentration (µg)	HEPG2- Pumpkin Seed Oil	HEPG2- Pumpkin Rind Extract
0.0	1.00000	1.00000
1.0	0.44996	0.96320
2.5	0.28839	0.82079
5.0	0.24180	0.50468
10.0	0.16157	0.29634

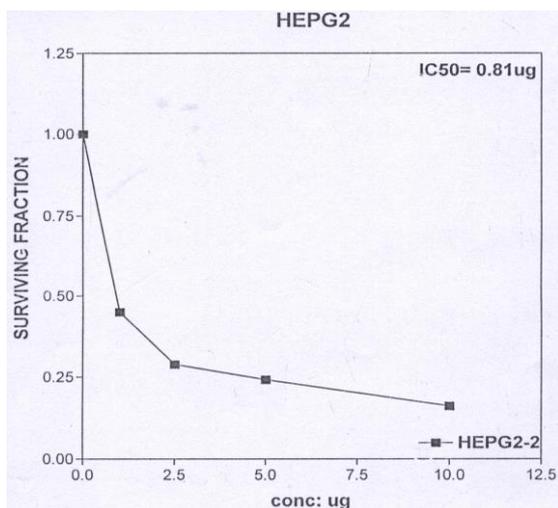


Fig (9) Potential cytotoxicity of the Pumpkin seed oil using liver carcinoma cell line.

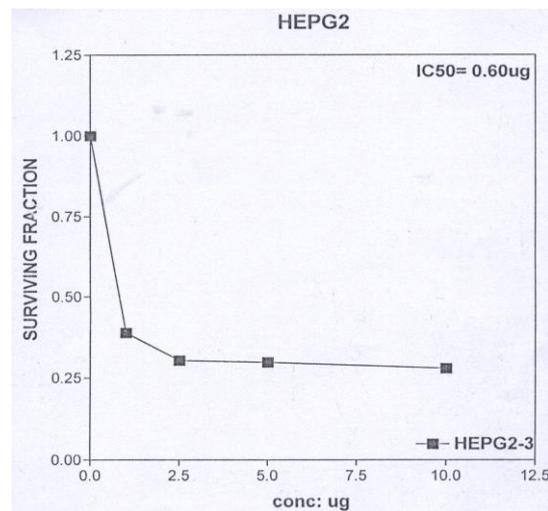


Fig (10) Potential cytotoxicity of the Pumpkin rind extract using liver carcinoma cell line.

These researchers have suggested that there is not very strong evidence for the benefits of these ratios, and that it may be better to increase the consumption of omega-3 fatty acids rather than decrease the consumption of omega-6 fatty acids because a reduction of polyunsaturated fats in the diet would increase the incidence of cardiovascular disease. While haven't any previous study belongs the cytotoxicity activity of the Pumpkin rind extract; the current study is the unique.

3-2- Antimicrobial Effects.

Table (7):- The antimicrobial activities of Pumpkin seeds oil and rind extracts toward different microbial strains.

Bacterial Strains	Pumpkin seeds oil	Pumpkin Rind extract
<i>Bacillus subtilis</i>	-ve	++
<i>Bacillus cereus</i>	-ve	++
<i>Staphylococcus aureus</i>	-ve	-ve
<i>Escherichia coli</i>	-ve	-ve
<i>Salmonella</i>	-ve	-ve
<i>Aspergillus niger</i>	-ve	-ve
<i>Saccharomyces cerevisiae</i>	++++	-ve

+ Weak positive effect. ++ Moderate positive effect. +++ Strong positive effect. -ve Negative effect.

Data in table (7) showed the antimicrobial effects of Pumpkin seeds oil and rind extract, it is clear from the obtained data that the Pumpkin seeds oil had no antimicrobial effects on all examined bacterial species (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* as well as *Aspergillus niger*) while strong effect was noticed on *Saccharomyces cerevisiae* as obviously showed in fig (11). In the other hand, the rind showed moderate effect on both *Bacillus subtilis* and *Bacillus cereus* as clearly illustrated in figures (12) and (13) respectively, while no effect was noticed on the other examined species (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Aspergillus niger* in addition to *Saccharomyces cerevisiae*)

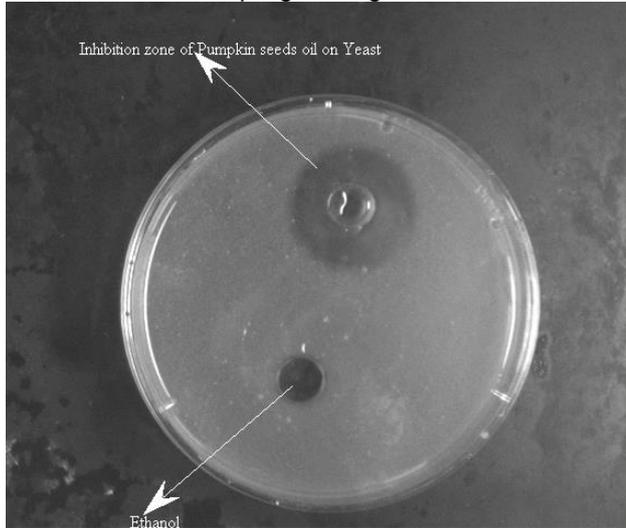


Fig (11): Antimicrobial effect of Pumpkin seeds oil on *Saccharomyces cerevisiae*

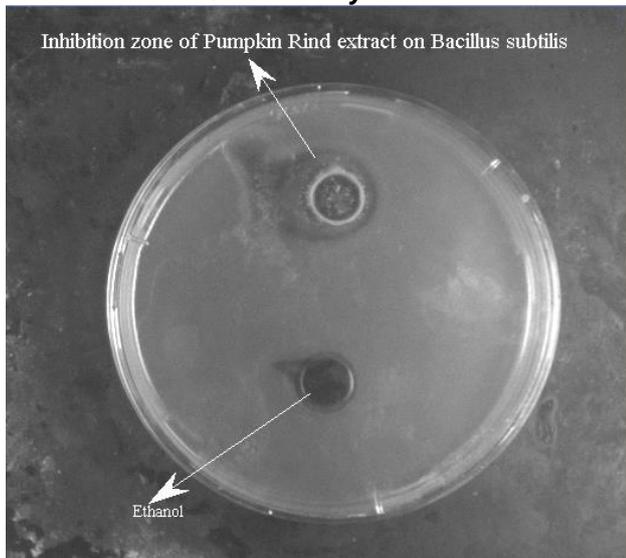


Fig (12): Antimicrobial effect of Pumpkin rind Extract on *Bacillus subtilis*.



Fig (13): Antimicrobial effect of Pumpkin rind Extract on *Bacillus cereus*.

"CONCLUSION"

The results of the current study clearly show Pumpkin seeds oil and rind extracts contain several major groups of active constituents of essential fatty acids, amino acids, minerals and β - Carotene especially the last for rind extract. The green oil of Pumpkin seeds are designed and implied to cure any disease (improves the function of the bladder and urethra via effectively reduces symptoms of Benign Prostatic Hyperplasia in addition to antiarthritic, antiparasitic, anticancer and antimicrobial effects) and illness as well as, can act as an aid dietary deficiency where one exists. The pumpkin seeds oil and rind extracts have highly potential cytotoxicity activity on the liver and breast tumor cell lines. Therefore, they can be consumed as food or as supplementary ingredients especially in Egypt to alleviate the problems of health and nutrients/protein malnutrition.

Further works are needed to evaluate not only the nutritional values for rind extract and Pumpkin seeds oil but also pharmacological investigations by using *in vivo* tests.

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التأثيرات المضادة لمستخلصات قشر و زيت بذور القرع العسلي (كوكريبتا بيبو. إل) على نشاطات الخلايا المسرطنة والميكروبات
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"الخلاصة"

الأهداف الرئيسية من هذا البحث كانت دراسة وتقييم بعض التأثيرات الحيوية (التأثيرات المضادة للأورام والميكروبات) لكل من مستخلصات زيت بذور و قشر القرع العسلي من النوع كوكربينا بيبو وخاصة الأخير حيث تعتبر هذه أول دراسة تجرى على قشر القرع العسلي بالإضافة إلى التحاليل الكيميائية الخاصة بهم.

النتائج الأساسية لهذا العمل كانت تقييم التأثيرات الحيوية لبعض المركبات العضوية المستخلصة من قشر وبذور القرع العسلي باستخدام خلايا سرطانية وسلالات بكتيرية في المعمل فضلا عن التحاليل الكيميائية لهم. في دراسة وحيدة تعتبر أول دراسة أجريت على قشر القرع العسلي وجد أنه غني بالبيتا كاروتين وبعض العناصر المعدنية (الكالسيوم- الحديد- الزنك- النحاس و السيلينيوم) ويعتبر مصدر جيد للبروتين، ووضح جليا تأثيره في القضاء على ٥٠% من الخلايا السرطنة للثدى والكبد عند استخدامه بتركيز ٠,٦٠ و ٠,٥٤ ميكروجرام نسبيا بجانب تأثيره الأيجابي بدرجة متوسطة كمضاد لنوعى البكتيريا *الباسيليس ستلس* و*الباسيليس سيربيوس*. بذور القرع العسلي وجد أنها غنية بالدهون والبروتين بمتوسط نتائج تقريبا ٤٧,٠٣ و ٣٥,٩٥% نسبيا فضلا عن إنها ليست غنية فقط بالأحماض الدهنية والأمينية الأساسية ولكن أيضا ببعض العناصر المعدنية، بالإضافة إلى التأثير الأيجابي لزيت بذور القرع العسلي على الخلايا السرطنة لكل من الثدى والكبد حيث استطاع القضاء على ٥٠% من الخلايا السرطنة للثدى والكبد عندما استخدم بتركيز ٠,٤٠ و ٠,٨١ ميكروجرام نسبيا، وبينت النتائج بوضوح جدا أن زيت بذور القرع العسلي له تأثير إيجابي قوى كمضاد للميكروب تجاه *سكاروميسيس سيريفيسيا*، لا مستخلص قشر القرع العسلي ولا زيت بذوره لهم أى تأثير إيجابي كمضاد للميكروب تجاه *ستافيلوكوكس اوربيوس*، *إيشريشيا كولاي*، *سالمونيلا* و*أسبيرجلاس نيجر*. النتائج التي حصل عليها في هذه الدراسة تدل على أن مستخلص قشر القرع العسلي وزيت بذوره مصادر غنية بالمغذيات. بناء عليه ممكن إستهلاكهم كغذاء أو كعلاج سريع خاصة في مصر لتخفيف وطأة المشاكل الصحية والمشاكل الناتجة من سوء التغذية كنقص المغذيات والبروتين. الحاجة لمزيد من الدراسات لتقييم ليس فقط القيم الغذائية لقشر القرع العسلي وزيت بذوره وإنما أيضا للفحوص الصيدلانية والدوائية باستخدام حيوانات التجارب.