

CHEMICAL COMPOSITION FOR RIND, FLESH AND SEEDS OF PUMPKIN (*Cucurbita pepo* L.) AND THEIR ANTIVIRAL ACTIVITIES

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ABSTRACT

Rind Extract, flesh extract, seeds oil and defatted seeds meal are four special natural products of Pumpkin (*Cucurbita pepo* L.). In unique study on the rind; its chemical analysis outcomes were; fiber 29.62%, protein 23.95% of non polar amino acids 07.46% as well as β - carotene (751.99 μ g/100g). While, flesh was contained fiber 11.25%, protein 15.50% of acidic amino acids 04.35%, carbohydrates 48.40% and β - carotene (3934.02 μ g/100g). In the same words, defatted seeds meal was composed of 07.12% fiber and 70.15% protein of 21.07% non polar amino acids. In addition, the studied three products of Pumpkin were rich in minerals (Calcium, Iron, Zinc, Copper and Selenium). On the other hand, the four dominant fatty acids of Pumpkin seeds oil profile 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 $98 \pm 0.1\%$ of the total amount of fatty acids as well as; its ω - 6/ ω - 3 ratio, was 311. *In vitro* antiviral activities recorded the obviously highly positive effects of rind extract, flesh extract and defatted seeds meal of Pumpkin toward Live NewCastle Disease Virus (NDV) vaccine strains, Komarov & La Sota; & Live infectious Bursitis Viruses (IBDV) vaccine strain D₇₈ while they did not gave any positive effects with the oil of Pumpkin seeds. Furthermore studies must be doing on the four natural products of Pumpkin to evaluate their nutritional and pharmacological values by using *in vivo* tests, and to demonstrate the applicable values of these compounds for the prophylactic treatment of NDV and IBDV – infection and other viral studies.

Keywords: Pumpkin (*Cucurbita pepo* L.); Chemical analysis; Rind, Flesh, Defatted Seeds Meal; Seeds Oil; Protein; Fiber; Minerals; New Castle Disease Virus (NDV); infectious Bursitis Virus (IBDV).

INTRODUCTION

There are three common types of pumpkin world- wide; *Curcubita pepo* L., *C. maxima* and *C. moschata*, but differences between varieties are not great with Whang, et al. (1999), all can be similar in size, shape and color. Although miniature pumpkins tend to be *C. pepo* and giant pumpkins tend to be *C. moschata* are the most commonly eaten varieties of pumpkin in both Asia and the United States, Jung et al. (2005). Fruit shape varied from round, flat round oval and oblong type. Pumpkins should have a hard rind and strangless, mature pulp "flesh" of ideal quality for cooking fresh after removing fibers and seeds from pumpkin flesh, Brian (2002). The rind is

smooth and variabfückule in color and has not any related previous study. Flesh is a low acid vegetable and requires special attention to preparation and processing, Brian (2002). At the fully matured stage, flesh varied in color as several shades of yellow, deep yellow to orange with 2-5 cm thick flesh and total carotenoids ranged from 2.34 mg to 14.85 mg with a population mean of 9.29 mg/100g of fresh weight, Sudhakar et al. (2003). The major carotenoide in freeze dried pumpkin flesh is β - Carotene "which can be converted into vitamin A by the body and is referred to as provitamin A which is known to exert antioxidant activity" with small amounts of α - carotene, lutein, lycopene and trace amounts of cryptoxanthin and *cis* β - Carotene, Jung et al. (2005). A t 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). Pumpkin seeds were milled into flours in the laboratory, samples of the seed flours were defatted using n-hexane, dried at 50 °C and sieved to pass through sieves, full fat and defatted seed flours were evaluated for functional properties, Fagbemi et al. (2006). Also; pumpkin "*C. pepo L*" seeds are used locally in Eritrea to treat tapeworm, they were found to be rich in oil (~35%), protein (38%), α -tocoferols (3 mg/100 g) and carbohydrate content (~37%). The physico-chemical properties and fatty acid composition of the seed oil were examined. Four dominant fatty acids are found: palmitic C16:0 (13.3%), stearic C18:0 (8.0%), oleic C18:1 (29.0%) and linoleic C18:2 (47.0%). The oil contains an appreciable amount of unsaturated fatty acids (78.0%) and found to be a rich source of linoleic acid (47.0%), within the three localities of the study, variations exist in seed properties and the fatty acids composition of the oil, Younis et al. (2000).

On the other hand; New Castle disease (ND) is the most important viral disease of chickens & other avian species, caused by an enveloped helical virus of 100-500 nm in diameter, of non segmented SsRNA-genome of negative polarity, a prototype Paramyxovirus (Pm V.1) of the nine known avian Paramyxovirus serotypes, of the Paramyxovirus genus of the Paramyxoviridae family, Melnick (1982). Also, infection bursitis disease (IBD) or Gumboro disease is an important disease in chickens caused by naked icosohedral virus of 55 nm diameter, of two segments of ds RNA-genome, number of the Birnavirus genus, family Birnaviridae, Brown (1986). In spite of the availability of the currently improved vaccines of NDV and IBDV, the two diseases still attack the chickens causing devastating epidemics & serious economic losses. Mostly, this problem is due to continuous mutation of NDV & IBD which led to the vaccination failure. A synthetic guanosine analog has been evaluated as potential therapeutic antiviral drugs for several human viral diseases, Sidwell et al. (1979). During the last decode, ribavirin was approved by United States Food & Drug Administration for the treatment of viral infection & since few years, national regularity authorities in Egypt has been issued for using many extract as a therapeutic antiviral drug in humans under different trade name. This work aimed to declare the potentiality of the antiviral activity of the pumpkin rind and flesh extracts beside to Pumpkin

seeds oil and defatted seeds powder on NDV & IBDV in VERO cells & Specific pathogen free (S P F) chickens embryos.

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, Flesh and Seeds Pre- treatment:

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 Yusuf et al. (2007) and Sara et al. (2008). The seeds were ground to a fine powder and then dried for 2 hour at 100 °C and defatted by extraction the pumpkin seeds oil and the defatted meal dried in oven at 90 °C. According to Mohamed et al. (2003), flesh of Pumpkin was sliced into 2 mm thickness and dried in the indirect type solar drying system was using forced circulation in the Solar Energy Department of National Research Center.

2-Chemical analysis:-

2-1- Ingredients analysis:

Moisture content; crude protein; fiber and fat according to AOAC (2000); as well as ash obtained by AOAC (1995) were estimated for rind, flesh, seeds powder and defatted seeds meal 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, flesh, seeds powder and defatted seeds meal of pumpkin 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 seed 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 acid 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 buffer (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 were extracted via 100 g of air dried rind, 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 (45C), the methanol layer was evaporated *in vacuo* to dryness, affording a pale oily orange extract (4.1g). The methanol crude extract of Rind was applied to detailed biological and chemical studies.

2-5- Extraction of Felsh:

The dried interior part of pumpkin (flesh) was applied to grinding. Such dried flesh powder (250 g) was applied to exhaustive extraction by methanol (2 L) during its soaking for several times. After full extraction, the methanol extract was concentrated *in vacuo* at 45C, and the methanol layer was evaporated *in vacuo* to dryness, affording oily orange extract (7.3g). The methanol crude extract of flesh was applied to detailed biological and chemical studies.

2-6- Extraction of pumpkin seeds oil:

Continuously extraction of the Pumpkin seed oil as was carried out as method of Schinas et al. (2008). Soxhlet extraction apparatus was employed and hexane was used as solvent in the extraction process. The Soxhlet device temperature was kept at 65–70 °C and the overall process lasted 24 h. At the end of the process, the oil was separated from the organic solvent using a rotary vacuum evaporator, dried at 60 °C and weighed. Yield was calculated on dry weight basis. Fatty acids composition of pumpkin seed oil methyl ester was also determined by gas chromatography analysis.

The fatty acids profile of the methyl ester was identified and quantified as proceeded with AOAC (2000) and Schinas et al. (2008). The oil is saponified with sodium hydroxide in methanol. The fatty acids are methylated with boron tri- fluoride in methanol, extracted with heptane and determined on a gas chromatograph with FID detector (PE Auto System XL) with auto sampler and Ezchrome integration system.

Carries gas (He); ca. 25 psi- air 450 ml / min- Hydrogen 45 ml – split 100 ml /min. Oven temperature 200 °C injector and detector 250 °C. According to AOAC (2000) and Schinas et al. (2008), 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- Antiviral activities for the rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin:

3-1-Viruses:

Live New Castle Disease Virus (NDV) vaccine strains, Komarov & La Sota; & Live infectious Bursitis Virus (IBDV) vaccine strain D₇₈ were obtained from commercially available vaccines. Strain Komarov & D₇₈ were adapted on VERO cells (a cell line derived from African green monkey kidney). Throughout seven successive passages by which the viruses showed distant cytopathic effect (degeneration & floatation of the infected cells) on the 3rd day after infection.

3-2-Evaluation of rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin as on inhibitory agents against NDV, strain Komarov & IBDV, strain D₇₈ replication in VERO cell cultures & its cytotoxicity:

These assays were performed in 24 well tissue culture plates following the roles of Cox et al., 1996. Confluent monolayer of VERO cells were infected with 50000 tissue culture infective dose fifty (TCID₅₀) / 0.2ml / well of NDV or 5000 TCID₅₀ of IBDV & incubated for two hours (for virus adsorption) then, inoculums was decanted, followed by addition of ten fold concentration of each compound separately (From 4µg to 500 µg / ml / wells / each conc.). Virus infectivity control & each compound cytotoxicity control were done. The plates under incubated at 37°C & 5% CO₂ for 4 days.

The cytotoxic concentration fifty (CC₅₀) of each compound was determined as the concentration of compound that induced any deviation of the morphology than the normal control cells in 50% of VERO cell monolayer. Antiviral inhibitory concentration fifty (IC₅₀) of each compound was assayed as the concentration of compound that fully inhibited virus-cytopathic effect (100 TCID₅₀) in 50% of monolayer. Also, the therapeutic index (TI) of each compound was expressed as CC₅₀ / IC₅₀. CC₅₀ & TCID₅₀ were calculated by the method of Reed and Muench (1938).

3-3-Evaluation of rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin in chicken embryos:

Groups of 9-11 day old specific pathogen free (SPF) embryonated chicken eggs (ECE), were inoculated with 500 embryo infective dose fifty (EID₅₀) / 0.2 / egg of NDV. Strain Lasota or 50 EID₅₀ of IBDV. Strain D₇₈, immediately followed by infection of ten fold concentration of each compound (4 µL to 500 µL / 0.2ml / egg) separately virus infectivity control & each compound controls were also conducted. The eggs were inoculated via the chorio-allantoic cavity. Test eggs were incubated for 5 days.

CC₅₀, IC₅₀ & T₁ values were determined as monitored before NDV, strain Lasota infectivity in ECE was detected by haemagglutinating activity of the allantoic fluids of the inoculated eggs as measured by microtechnique of Haemagglutination (HA) Test, Takatsy (1956), while IBDV, strain D₇₈-infectivity was determined by the criterion of distension of the abdominal region, mottled necrotic or Haemorrhagic liver & mortality scores in embryos.

RESULTS AND DISCUSION

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

1-1-Ingredients analysis:

Table (1):-Ingredients estimations for rind, flesh, seeds Powder and defatted seeds meal of Pumpkin (g/100g dry sample):-

| Ingredient | Pumpkin Rind | Pumpkin Flesh | Pumpkin Seeds powder | Defatted Seeds Meal |
|--------------------------|--------------|---------------|----------------------|---------------------|
| Ash | 10.65 | 06.64 | 03.55 | 09.16 |
| Fat | 06.57 | 00.18 | 47.03 | 01.41 |
| Fiber | 29.62 | 11.25 | 05.30 | 07.12 |
| Moisture | 09.76 | 18.03 | 01.80 | 02.63 |
| Protein | 23.95 | 15.50 | 35.95 | 70.15 |
| Carbohydrates | 19.45 | 48.40 | 06.37 | 09.53 |
| Minerals (ppm) :- | | | | |
| Ca ⁺⁺ | 5571.00 | 3662.00 | 0420.55 | 0617.40 |
| Fe ⁺⁺ | 0247.30 | 0091.33 | 0149.64 | 0219.67 |
| Zn ⁺⁺ | 0042.92 | 0320.50 | 0089.29 | 0131.12 |
| Cu ⁺⁺ | 0012.91 | 0016.25 | 0024.49 | 0035.77 |
| Se ⁺⁺ (ppb) | 0012.71 | 0014.00 | 0012.40 | 0018.04 |

The main goal of the data pre-treatment was to reduce the number of column and/or rows of the table (1) and design much smaller data contained table where; the moisture contents in the fresh rind, flesh and hulled seeds of Pumpkin were $84.18\% \pm 1.42$, $92.93\% \pm 1.01$ and $43.29\% \pm 4.38$ respectively. The results obtained in this study indicated that; rind and defatted seeds meal "DSM" as well as flesh have highly values of ash as shown in table (1) and 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 DSM are expected to be rich in the studied minerals (Calcium, Iron, Zinc, Copper and Selenium), and were found to be 10.65 and 09.16% flowed by flesh and seeds powder of Pumpkin 06.64 and 03.55% respectively, as shown in table (1). Flesh and rind of the Pumpkin were poor in there fat contents 0.18 and 06.57% respectively. In the same words, seeds powder contained higher amount of fat 47.03% and expected to interfere with it's other parameters determination so, after defatting process for seeds powder, the remaining oil content in the DSM was found to be 1.41%, these results reported for pumpkin seeds were higher than that obtained by Giami et al. (2005) but in convenient with Sara et al. (2008). Therefore, its protein content increased from 35.95 in seeds powder to 70.15% in the DSM and became not only excellent source of protein but also the studied minerals, while rind and flesh of Pumpkin have protein content equal to 23.95 and 15.50% respectively, as shown in table (1). Flesh has highly value of carbohydrate 48.40% and rind is a good source of carbohydrate 19.45% but DSM and seeds powder were lower in carbohydrates 9.53 and 6.37% respectively as appear in table (1). DSM, rind, flesh and seeds powder of Pumpkin are excellent sources of the last studied minerals. The current study is the first which carried out on the rind of Pumpkin.

1-2-β- Carotene:

Most of the carotenoids in pumpkin have not been identified or measured, although preliminary data is available from a few spectrophotometric and chromatographic studies.

Table (2):- Values of β- Carotene contents in rind, flesh and seeds powder of Pumpkin.

| Pumpkin items | Rind | Flesh | Seeds powder |
|------------------------------|---------|---------|--------------|
| β- Carotene Values (µg/100g) | 0751.99 | 3934.02 | 0078.89 |

Foods like pumpkin which is the best sources of this carotenoid play a double role, first as a source of provitamin A and then as an antioxidant as reported in Gouado et al. (2007). The vitamin A levels in different fruits were calculated taking into consideration that 1 µg of vitamin A is supplied by 12 µg of β-Carotene, West *et al.* (2002). On the other hand, table (2) shows the values of β-Carotene present in the all constituents of Pumpkin where; it's flesh was recorded the highest value and an excellent source (3934.02µg/100g of dry weight) of β-Carotene as showed in fig (1) and this is higher result than that obtained with gouado et al. (2007), while it's rind is a lower (0751.99µg/100g) and a good source of β-Carotene as obviously clear in fig (2) but it's seeds powder is the lowest value and a poor source of β-Carotene (0078.89µg/100g) as illustrated in fig(3).

Taking into consideration the contribution of vitamin A from Pumpkin and with high bioavailability, this food can therefore meets the daily requirements of vitamin A for the population concerned.

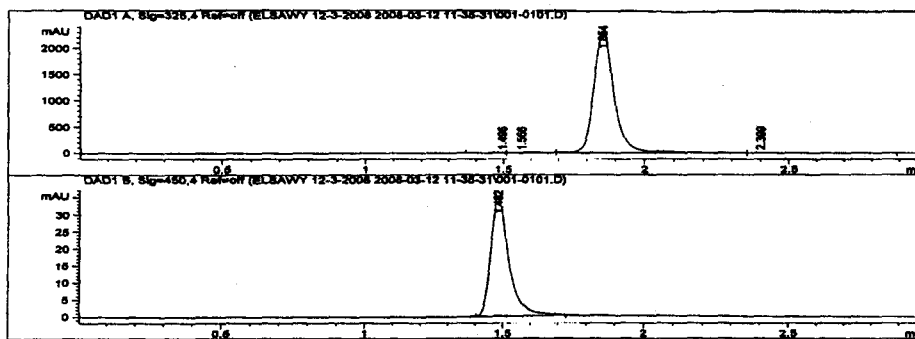


Fig (1): β- Carotene Peaks of Pumpkin rind.

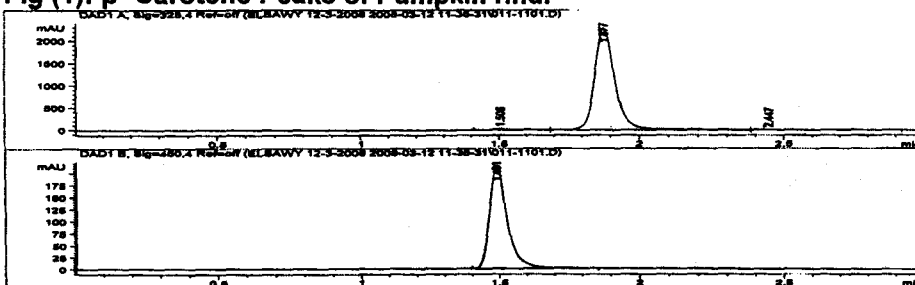


Fig (2): β- Carotene Peaks of Pumpkin flesh.

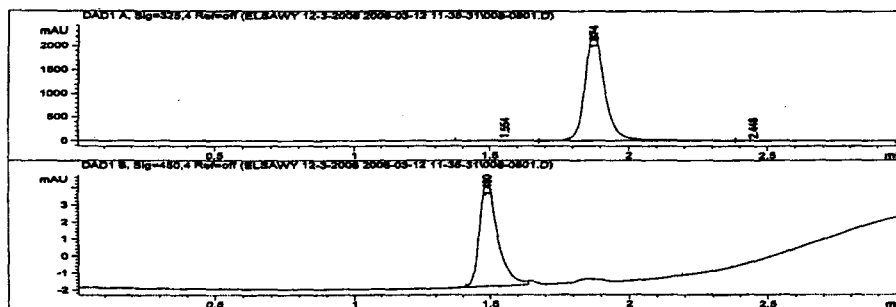


Fig (3): β - Carotene Peaks of Pumpkin seeds powder.

1-3-Amino acids of rind, flesh and defatted seeds powder of Pumpkin:

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.

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, flesh and defatted seeds powder of Pumpkin are sources of protein in varied ratios as showed in table (1) that is a strong outcome to recognize the profile of their peptide linkage and their amino acids [AA's] patterns as listed in table (3) and shown in figures (4), (5) and (6) respectively. Table (3) showed that the content of total amino acids of rind, flesh and DSM were 17.41, 08.26 and 52.77g/100g respectively. Fig (4) illustrates that the 17- amino acids of the rind were identified to be components of the polymer where aspartic acid was the main amino acid (2.64%), followed by glutamic acid (2.53%) and leucine (1.21%). Fig (5) shows that, the 17- amino acids of the flesh were identified to be components of the polymer where aspartic acid was the main amino acid (2.65%), followed by glutamic acid (1.70%) and arginine (0.49%). Fig (6) clearly appears the 17- amino acids of the DSM were identified to be components of the polymer where glutamic acid was the main amino acid (11.50%), followed by aspartic acid (05.59%) and arginine (05.42%).

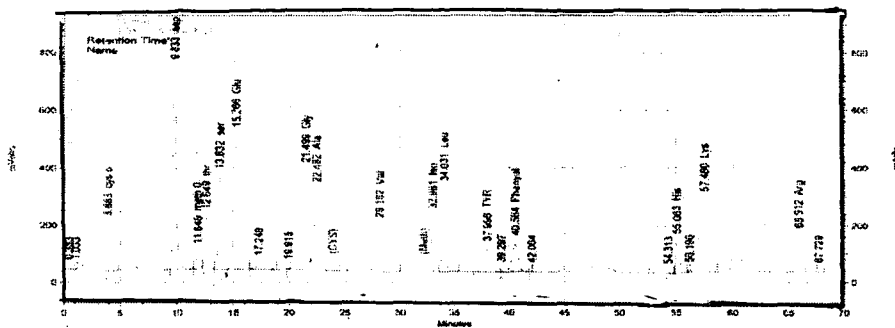


Fig (4): Amino acids profile for the crude protein of Pumpkin rind.



Fig (5): Amino acids profile for the crude protein of Pumpkin flesh.

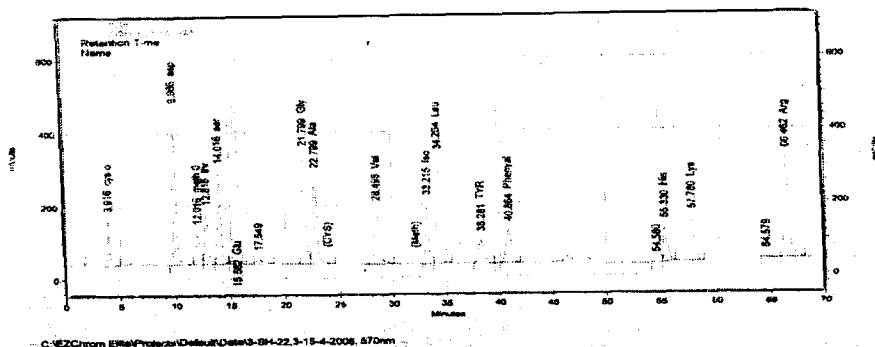


Fig (6): Amino acids profile for the crude protein of Pumpkin defatted seeds meal "DMS".

During Fu Caili et al. (2007) investigations of the hypoglycemic substances in pumpkin, they found that proteins of the fresh pumpkin seeds do not reduce the blood glucose levels of alloxan-diabetic rats, but those of germinant pumpkin seeds, in which the contents of arginine, glutamic acid, leucine and aspartic acid increase, but the relationship between the contents of amino acids and hypoglycemic activity of acidic protein-bound

polysaccharide from the fruit "Flesh" of Pumpkin is not clear. Preliminary investigations were shown that a pumpkin-rich dietary could reduce blood glucose and polysaccharides from pumpkin had hypoglycemic activity, Chen et al. (1994) and Cai et al. (2003) and also reported that protein-bound polysaccharides from the flesh of pumpkin could obviously increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose and hence could be developed as new antidiabetic agent, Li et al. (2005). Table (3) illustrates the constituents of AA's for Pumpkin rind, flesh and DSM in which, the essential amino acids [EAA's] which previously listed represent 07.25%, 01.97% and 19.12% of the total AA's for rind 17.41%, flesh 08.26% and seed powder 52.77% respectively.

Table (3):- Amino acids composition for rind, flesh and defatted seeds meal (%):

| Amino Acids | Rind | Flesh | Defatted Seeds Meal (DSM) |
|-----------------------|----------------|----------------|---------------------------|
| Aspartic acid (Asp.) | 2.64 | 2.65 | 05.59 |
| Threonine (Thr.) | 0.71 | 0.21 | 01.79 |
| Serine (Ser.) | 1.02 | 0.34 | 02.87 |
| Glutamic acid (Glu.) | 2.53 | 1.70 | 11.50 |
| Proline (Pro.) | 0.79 | 0.27 | 02.23 |
| Glycine (Gly.) | 0.96 | 0.21 | 02.85 |
| Alanine (Ala.) | 0.90 | 0.31 | 02.56 |
| Valine (Val.) | 0.87 | 0.28 | 02.61 |
| Isoleucine (Iso.) | 0.70 | 0.26 | 02.04 |
| Leucine (Leu.) | 1.21 | 0.33 | 04.15 |
| Phenyl alanine (Phe.) | 0.95 | 0.23 | 03.05 |
| Histidine (His.) | 0.62 | 0.23 | 02.00 |
| Lysine (Lys.) | 1.16 | 0.25 | 01.90 |
| Arginine (Arg.) | 1.03 | 0.49 | 05.42 |
| Cystine (Cys.) | 0.24 | 0.07 | 00.63 |
| Methionine (Meth.) | 0.23 | 0.09 | 01.18 |
| Tryptophan (Tyr.) | 0.85 | 0.34 | 00.40 |
| Total AA's | 17.41 % | 08.26% | 52.77 % |
| Sum of EAA's | 07.25 % | 01.97 % | 19.12 % |

The total AA's present in the flesh have got lower values than that in the DSM and rind and this may be due to it has got the highest moisture content as previously reported in table (1). In the same words; total AA's of rind are lower values than that of DSM except tryptophan, which records the highest percentage than that in DSM and flesh of pumpkin. The AA's pattern of flesh does not agree with that obtained in Fu Caili et al. (2007) while DSM AA's pattern does not agree with Zdunczyk et al. (1999) but in convenient 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 such Pumpkin flesh, rind and DSM can be classified according to their chemical nature and behaviors as listed in table (4) which shown; the hydrophobic AA's class recorded the highest percentage of AA's classes for DSM and rind of Pumpkin while, the acidic AA's class were the more predominant AA's in the flesh of pumpkin.

Table (4):- Classification of AA's present in Pumpkin rind, flesh and defatted seeds meal.

| Classification of AA's | Rind | Flesh | Defatted Seeds Meal (DSM) |
|-------------------------------------|---------|---------|---------------------------|
| Hydrophobic (nonpolar) ^a | 07.46 % | 02.32 % | 21.07 % |
| Uncharged polar | 01.97 % | 00.62 % | 05.29 % |
| Basic ^c | 02.81 % | 00.97 % | 09.32 % |
| Acidic ^a | 05.17 % | 04.35 % | 17.09 % |
| Sulfur-containing ^a | 00.47 % | 00.16 % | 01.81 % |
| Aromatic ^f | 01.80 % | 00.57 % | 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.

The opposite was in case of sulfur-containing AA's, they were recorded the lowest class in the AA's classifications for Pumpkin flesh, rind and defatted seeds meal. Pumpkin defatted seeds meal is rich source of essential and non-essential AA's and this result agrees with many results as Zdunczyk et al. (1999); El-Soukary (2001) and Zhu et al. (2006) while flesh was poor as compared with that obtained in case of DSM. The AA's of rind not previously evaluated due to not exists another study analyzed the AA's of Pumpkin rind where the current study considers that, 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 (5); 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 un saturation of pumpkin seeds 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 (5).

Table (5) Physicochemical properties and fatty acid composition of Pumpkin seeds oil:

| Physicochemical Properties/Fatty Acids Composition. | Value | |
|---|---|--------------|
| Kinetic viscosity (40 °C) mm ² S ⁻¹ . | 03.28 | |
| Iodine number. | 109 | |
| Acid value, KOH, mg g ⁻¹ . | 00.62 | |
| Fatty Acids Profile:- | Relative Distribution. | |
| 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% |
| 4- Non Identified Fatty Acids. | | 01.3% |
| 5- ω-6 /ω-3 ratio. | | 311 |

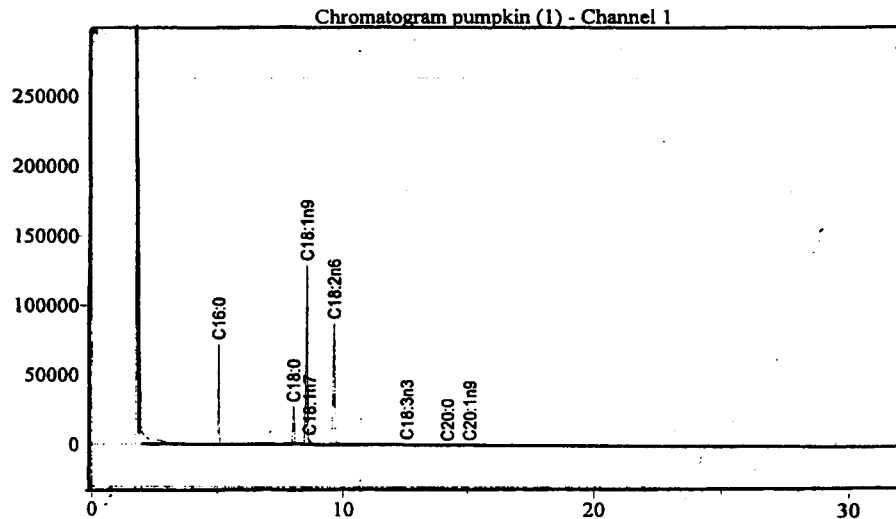


Fig (7): Chromatogram of fatty acids Profile for the pumpkin seeds oil.

On the other hand, the fatty acids profile of Pumpkin seeds oil was identified and quantified as shown in table (5) and fig (7) 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 (5) and fig (7), 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 ($\omega 3$) and omega-6 ($\omega 6$) 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 (8), 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.

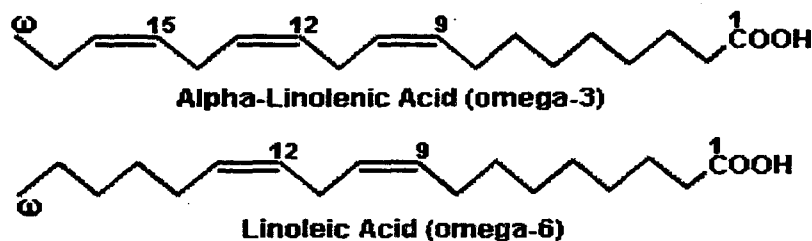


Fig (8): Chemical structure of α - Linolenic& Linoleic essential fatty acids.

2- Antiviral activities for the rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin:

Table (6): Susceptibility of NDV, strain Komarov and IBDV, strain D₇₈ to rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin and their cytotoxicity in VERO cells.

| Pumpkin Items | NDV-50000 TCID ₅₀ | | | IBDV 5000 TCID ₅₀ | | |
|---------------------|---|---|----------------|---|---|----------------|
| | CC ₅₀ ($\mu\text{g} / \text{ml}$) | IC ₅₀ ($\mu\text{g} / \text{ml}$) | T _i | CC ₅₀ ($\mu\text{g} / \text{ml}$) | IC ₅₀ ($\mu\text{g} / \text{ml}$) | T _i |
| Rind Extract | > 400 | ≤ 4 | > 100 | > 400 | ≤ 4 | >100 |
| Flesh Extract | > 400 | ≤ 5 | > 80 | > 300 | ≤ 6 | > 50 |
| Seeds Oil | negative | Negative | negative | negative | negative | negative |
| Defatted Seeds Meal | > 300 | ≤ 6 | > 50 | > 200 | ≤ 5 | > 40 |

CC₅₀: Toxic concentration 50 IC₅₀: Inhibiting concentration 50 T_i: Therapeutic index

Table (7): Susceptibility of NDV, strain Lasota and IBDV, strain D₇₈ to rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin and their toxicity in chicken embryos.

| Pumpkin Items | NDV-500 EID ₅₀ | | | IBDV 50 EID ₅₀ | | |
|---------------------|---|---|----------------|---|---|----------------|
| | CC ₅₀ ($\mu\text{g} / \text{ml}$) | IC ₅₀ ($\mu\text{g} / \text{ml}$) | T _i | CC ₅₀ ($\mu\text{g} / \text{ml}$) | IC ₅₀ ($\mu\text{g} / \text{ml}$) | T _i |
| Rind Extract | 500 | ≤ 5 | > 100 | 500 | ≤ 6 | >83 |
| Flesh Extract | 500 | ≤ 7 | >71 | 500 | ≤ 7 | >71 |
| Seeds Oil | - | - | - | - | - | - |
| Defatted Seeds Meal | 400 | ≤ 8 | >50 | 400 | ≤ 9 | >44 |

CC₅₀: Toxic concentration 50 IC₅₀: Inhibiting concentration 50 T_i: Therapeutic index

Table (8): Haemagglutinating activity of NDV, strain Lasota, inoculated alone or with rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin separately in ECE.

| ECE-inoculated with different dilution of NDV | | | | | | |
|---|-------|------|------|------|-----|-----------|
| Virus Dilution | 1 | 2 | 3 | 4 | 5 | Inf/total |
| 10 ⁻⁷ | 4096* | 4096 | 4096 | 4096 | - | 4/4 |
| 10 ⁻⁸ | 4096 | 4096 | 1024 | 512 | - | 4/4 |
| 10 ⁻⁹ | 1024 | 512 | 512 | 256 | 256 | 5/5 |
| 10 ⁻¹⁰ | 32 | 32 | 16 | 4 | 4 | 5/5 |
| 10 ⁻¹¹ | 0 | 0 | 0 | 0 | 0 | 0/5 |

Continued table (8):

| ECE inoculated with NDV dilution 10 ⁻⁷ with different extract compound | | | | | | | |
|---|--------|---|---|---|---|---|-----------|
| Pumpkin Item concentration / egg | | 1 | 2 | 3 | 4 | 5 | Inf/total |
| Rind Extract | 005 µg | 8 | 8 | 8 | 0 | 0 | 3/5 |
| | 500 µg | 0 | 0 | 0 | 0 | 0 | 0/5 |
| Flesh Extract | 005 µg | 8 | 8 | 8 | 0 | 0 | 3/5 |
| | 500 µg | 0 | 0 | 0 | 0 | 0 | 0/5 |
| Seeds Oil | 005 µg | 8 | 8 | 8 | 8 | 8 | 5/5 |
| | 500 µg | 8 | 8 | 8 | 8 | 8 | 5/5 |
| Defatted Seeds Meal | 005 µg | 8 | 8 | 0 | 0 | 0 | 2/5 |
| | 500 µg | 0 | 0 | 0 | 0 | 0 | 0/5 |

ECE: Embryonating chicken embryo

* Reciprocal of allantoic fluid dilution 0: < 2.

In VERO cell cultures, anti-NDV, strain Komarov and IBDV, strain D₇₈ activity of compounds rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin as determined by virus-cytopathic effect inhibition assay, demonstrated that compounds rind extract, flesh extract and defatted seeds meal of Pumpkin were completely inhibits 50000 TCID₅₀ and 5000 TCID₅₀ of IBDV-infectivity in the range of 4 to 6 µg/ml table (6), with substantial therapeutic indices of > 100, > 80, and 50 respectively. Cytotoxicity assay indicated that the CC₅₀ for compound rind extract, flesh extract and defatted seeds meal of Pumpkin were greater than 400 µg, 400 µg and 300 µg/ml respectively. Table (6) rind extract showed significant inhibition of NDV, and IBDV in Monolayer VERO cell culture as determined by evaluation cytopathic effect.

In chicken embryos, anti-NDV, strain Lasota and IBDV, strain D₇₈. The activity of compounds rind extract, flesh extract, seeds oil and defatted seeds meal of Pumpkin as determined by NDV, hemagglutinating activity in allantoic fluids, and IBDV, infectivity criterion in embryos, indicated that 5, 7 and 8 µg / 0.2 ml / egg of compound rind extract, flesh extract and defatted seeds meal of Pumpkin respectively were fully reduced the infectivity of 5 EID₅₀ of 6 IBDV & 500 EID₅₀ of NDV as obviously illustrated in table (7). In the same hand; rind extract, flesh extract and defatted seeds meal of Pumpkin anti-NDV & IBDV IC₅₀ were 5, 6, 7, 8 and 9µg/egg respectively. The toxicity assays of compounds rind extract, flesh extract and defatted seeds meal of Pumpkin in chicken embryos indicated that at concentrations of 5, 6, 7, 8 & 9 µg/egg, mottled ectric livers were showed by 100% of inoculated embryos without deaths on the 4th – 5th days after inoculation. Thus, the recorded therapeutic indices were 100, 83, 71, 50 & 44 respectively in NDV & IBDV.

Mitochondria toxicity in the cells has been proposed as a mechanism for several of the adverse events observed with many nucleoside analogs including bone marrow suppression, liver failure, neuropathy, myopathy and lactic acidosis (Styrt et al., 1996).

Contrasting this study results table (8); embryonated eggs inoculated with mixture of virus and compounds rind extract, flesh extract and defatted seeds meal of Pumpkin did not retard NDV replication and pooled alantoic fluid from each group of eggs receiving varying concentration of compounds with the same concentration of virus showed 1:8 to 1:16 HA titre.

CONCLUSION

The results obtained in this study indicated that, rind, flesh, seeds oil and defatted seeds meal of Pumpkin (*Cucurbita pepo L.*) are four natural and rich sources in different nutrients as minerals (Calcium, Iron, Zinc, Copper and Selenium), carbohydrates, proteins, β - carotene and amino acids as well as fatty acids of the Pumpkin seeds. Therefore, they can be consumed as food or as supplementary ingredients especially in Egypt to alleviate the problems of health and nutrients/protein malnutrition. With the unique study of the Pumpkin Rind, it proves that desire furthermore studies even in the pharmacological evaluation levels.

Compounds rind extract, flesh extract and defatted seeds meal of Pumpkin were proven to be effective for inhibiting NDV and IBDV replication in VERO cells and in chicken embryos. In spite of the presence of the essential fatty acids in the Pumpkin seeds oil; as well as its physicochemical characteristics and highly nutritional value, it has not any positive effects on NDV and IBDV. Interestingly, *in vivo* studies should be taken on rind extract, flesh extract and defatted seeds meal in addition to seeds oil of Pumpkin not only to evaluate their nutritional values and pharmacological investigations by using *in vivo* tests but also to demonstrate the applicable values of these compounds for the prophylactic treatment of NDV and IBDV – infection.

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التركيب الكيميائي لقشر ولحمة وبذور القرع العسلى (كوكريبيتا بيبو. إل) وتأثيراتهم المضادة للفيروسات
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مستخلص القشر ومستخلص اللحمه وزيت البذور والبذور منزوعة الدهون أربعة منتجات طبيعية خلسة بالقرع العسلى (كوكريبيتا بيبو. إل). فى دراسة وحيدة من نوعها على القشر كانت نتائج التحليل الكيمايى كالتالى:- الألياف ٢٩,٦٢% وبروتين ٢٣,٩٥% ذو أحماض أمينية غير قطبية ٠,٤٦% فضلا عن بيتا كاروتين (٧٥١,٩٩ ميكروجرام / ١٠٠ جرام) بينما اللحمه كانت تحتوى على ألياف ١١,٢٥% وبروتين ١٥,٥% ذو أحماض أمينية حامضية ٠,٣٥% وكربوهيدرات ٤٨,٤٠% وبيتا كاروتين (٣٩٣٤,٠٢ ميكروجرام/ ١٠٠ جم), وفى نفس سياق النتائج كانت البذور المنزوعة الدهون تحتوى على ٠,٧١٢% ألياف , ٧٠,١٥% بروتين ذو أحماض أمينية غير قطبية ٢١,٠٧% بالإضافة إلى أن هؤلاء الثلاثة النواتج الأساسية السابقة للقرع العسلى كانت غنية بالعناصر المعدنية التى تم تحليلها وهى (الكالسيوم والحديد والزنك والنحاس والسيلينيوم). من ناحية أخرى فإن الأربع أحماض الدهنية الأكثر تواجدا فى بذور القرع العسلى كانت أحماض الأوليك, اللينوليك, البالمتيك والأستياريك بنسبة توزيع ٤٣,٨٠ و ٣٣,١٠ و ١٣,٤٠ و ٠,٧,٨٠ نسبيا والذين يكونون ٩٨ ± ٠,١% من نسبة كمية الأحماض الدهنية الكلية. فضلا عن نسبة الأوميغا ٦ إلى الأوميغا ٣ كانت ٣١١.

معمليا تم دراسة التأثير المضاد لفيروسات النيوكاسل و الجمبورو لمستخلص القشر ومستخلص اللحمه والبذور المنزوعة الدهون للقرع العسلى وزيت بذور القرع العسلى على خلايا الفيرو و أجنة الدواجن .وقد ثبت من الدراسة أن مستخلص القشر ومستخلص اللحمه والبذور المنزوعة الدهون للقرع العسلى لها تأثير مضاد لعترات النيوكاسل (لاسوتا و الكوماروف) وكذلك عترة الجمبورو (د ٧٨) فى حين أن زيت بذور القرع لم تعطى اى تأثير مضاد لهذه العترات الفيروسية الحية.
دراسات كثيرة يجب إجراؤها على حيوانات التجارب مستخدما الأربعة نواتج الطبيعية للقرع العسلى لتقييم قيمتها التغذوية والصيدلانية باستخدام حيوانات التجارب وكذلك لاستخدام هذه المركبات الطبيعية فى العلاج الوقائى من الإصابة ببعض الفيروسات.