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Bioactive Defensive Constituents against Lumpy Skin Disease and Levels of Natural Antioxidants in BWgarmon and Its Individual Components

Fathy M.M. Saleem[1], Mohammed A. Al-Shafei[1], Mohammed G.A. Mubark[2] and Gamal F. Abd Elnaem[1]

[1]Agricultural chemistry Dept., Faculty of Agriculture, Minia University, Minia-Egypt
[2]Chairman of Agricultural Research center (ARC), Professor of Animal Health Research Institute, Giza, Egypt.

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Corresponding author:
Fakhry, Gamal

Email:
gamalfakhry62540@yahoo.com

ABSTRACT

In the present article, five samples were subjected for analysis; the results could be summarized as follows. Five samples were examined for qualitative screening these are as follows: (1)-FPBCS= Fine powdered of black cumin seeds, (2)-PGCP = Peeled garlic clove paste, (3)-FPLP + LJ= Fine powered lemon peels + Lemon Juice, (4)-FPWB= Fine powdered of Wheat bran and (5)- BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB were examined for phytochemical screening which revealed the presence of following secondary metabolites in all samples: steroids, terpenoids, tannins, coumarins, alkaloids, phenolics, flavonoids and isoprenoids. BWgarmon contain 13 different groups. FPLP + LJ contain the highest concentrations of TPCs (552.0±5.62 mg Gallic acid equivalent GAE) /100g) followed by Fine powdered of Wheat bran (373.7±3.8 mg GAE) /100g) while the lowest concentrations (167.03±1.71) are in FPBCS. The concentrations of TPCs in the composed paste BWgarmon contain 395±4.1 mg GAE) /100g). TFs (mg Rutin Equivalents /100g) ranged from 69.8±0.75 for FPWB to 135.85 ± 1.68 for FPLP + LJ while BWgarmon reported an averaged value reached to be 115±1.48. The results show that scavenging activities (SA %) of TPCs (mg GAE /100g) assessed by $KMnO_4$ method are higher in FPLP + LJ (297.5±3.2) than those reported in BWgarmon (210.2±2.2). SA (%) of TFs (mg GAE)/100g) ranged from 37.4±0.4 from FPBCS to 72.82±0.7 for FPLP + LJ and the final paste (BWgarmon) reached to be 63.64±0.62. Scavenging activities (%) of total carotenoids (TCs µg/g) ranged from 1.42±0.01 for PGCP to 3.54±0.04 for FPBCS.

KEYWORDS: Antioxidant, black cumin, flavonoids, garlic, lemon, lumpy skin disease, phenolics and wheat bran.

1. INTRODUCTION

Lumpy skin disease (LSD) is a viral disease that was first identified in 1929 in Zambia and South Africa, and has since become endemic throughout Africa and the Middle East. The disease has been widespread since 2015 in the Russian Federation. Five years later, LSD virus

introductions were confirmed in several South Asian countries, including Bangladesh, China, India, Nepal, Taiwan, Bhutan, Vietnam, and Hong Kong (OIE, 2019).

The use of medicinal plants to treat many livestock diseases has become an urgent necessity because these plants are an important source of ethnoveterinary and the opportunity is available to developing and developed countries due to the discovery of some effective ethnoveterinary products. Ethnoveterinary practices are more common in developing countries due to various social and economic factors (Saleem *et al.*, 2024).

Egypt culture many medicinal plants which consider a good source for bioactive compounds belonging to secondary metabolites which are playing defensive roles.

Nowadays, the prices of livestock, feed, and veterinary medicines have increased, leading to a shortage in the supply of meat and a decrease in the quality of meat and some infectious diseases have spread, such as lumpy skin disease. In this article, we present a cheap alternative available in the local environment to resist one of the most dangerous diseases that cause economic losses to the world and to Egypt, and lead to human malnutrition and milk shortages.

Lumpy skin disease (LSD) belongs to the family Poxviridae. It is an infectious disease that affects cattle in many African and Asian countries and falls under the genus Capripoxvirus. This disease has become a serious disease and is characterized by the appearance of lumps in the skin (Chouhan *et al.*, 2022).

Wheat bran, a by-product of wheat grain produced by grinding grains, is used in livestock feed and the production of dark bread (Mao *et al.*, 2020). Wheat bran has a high concentration of phytic acid, which acts as a high-potential antioxidant and other immune-enhancing compounds, increases metabolic rate, protects normal cells from oxidative stress, and helps in combating aging and cell cancer due to its antioxidant activities (Yan *et* al., 2015).

Garlic is one of the oldest plants used by humans for food and medicine. Garlic is also used in many parts of the world to treat many diseases, such as high blood pressure, diabetes, inflammation, and toxins. Garlic is currently used as an anticholesterol and to reduce the risk of heart disease, and it also has antitumor and antimicrobial properties (Koch and Lawson, 1996).

Nigella sativa seeds, including saponins, flavonoids, cardiac glycosides, thymoquinone, thymol, limonene, carvacrol, *p*-cymene, α-pinene, 4-terpineol, longifolene, τ-anethole benzene, isoquinoline, and pyrazole alkaloids, as well as unsaturated fatty acid such as linoleic acid, oleic acid, and palmitic acid (Kooti *et al.*, 2016).

The functional group of the inorganic and organic compounds of the garlic powder identified by FTIR. FTIR spectroscopy is high sensitivity and capable of providing strong insight into the structural and functional alterations induced by various factors (Gorgulu *et al.*, 2007, Nagarajan, and Kumar. 2017, Abeyrathne *et al.*, 2021).

In a recent study carried out by Athanasiadis *et al.*, (2024) by using new cloud point extraction (CPE). This method employed in extraction process to obtain lemon peel in order to assess the recovery of bioactive compounds. As such, the optimal procedure can easily be used to obtain extracts rich in polyphenols, at the same time utilizing industrial waste and bestowing added value to them.

The main objectives of the present article are:

(1)-Screening for some plant extracts effective in reducing the incidence of lumpy skin disease (LSD). (2)-Preparation of a newly composed paste efficient for lowering the disease incidence. (3)-Treatment of animals infected by lumpy skin disease (4) - Providing farmers with a cheap and safe preparation available as an alternative to expensive veterinary medicines.

2. MATERIALS AND METHODS

2.1. Preparation of formulations and extracts:-

In the present article five samples were subjected for analysis; these are as follows: (1)-PGCP = Peeled garlic clove paste, (1)-FPBCS= Oil from fine powdered of black cumin seeds (3)-FPLP + LJ= Fine powered lemon peels + Lemon Juice, (4)-FPWB= Fine powdered of wheat bran and (5) - BWgarmon = Paste composed from FPBCS+PGCP + FPLP + LJ+ FPWB were examined for phytochemical screening.

2.2.Peeled garlic cloves paste (PGCP)

Using cold watery garlic extract we took a quantity of garlic cloves and removed their wrappings and then cut into small pieces with the scissors, after which the small pieces were homogenized with distilled water in a ratio of 1 gram garlic: 2 ml of distilled water using an electric mixer for one hour at room temperature and then filtered the mixture The product was obtained by extracting medical gauze to obtain the aqueous filtrate and the filtrate was distributed in test tubes and exposed to centrifugation at a speed of 3500 rpm (round per minute) and for a period of 20 minutes, the upper liquid was taken and kept in the refrigerator to make the experiment.

2.3.Preparation of oil extracted from oil from fine powered black cumin seeds (FPBCS):

It followed Harbone's modified method (Harbone, 1998)

2.4. Preparation of fine powered lemon peels + Lemon Juice (FPLP+LJ):

Preparation of lemon extract was prepared using the dried inner pulp + outer peels and using an electric mixer; it was homogenized and then put the extract in the refrigerator for use in the experiment.

2.5.Preparation of fine powdered of wheat bran (FPWB):-

Wheat bran (Rada) is prepared by removing the outer husks covering of the wheat grain and using it in the experiment. In the present Thesis it be used the wheat bran

2.6.Preparation of BWgarmon:-

The paste or ointment called BWgarmon composed from PGCP + FPBCS + FPLP+LJ + FPWB. Take 100 grams of peeled garlic cloves paste (PGCP) extracted by distilled water and add 30 grams of fine powdered wheat bran (FPWB) and mix well until homogeneous. Add 50 ml of black cumin seed oil (FPBCS) to the mixture and stir continuously. Then add FPLP+LJ and continue mixing until you get an oily preparation. This ointment is what we treat infected animals with after washing the area.

2.7.Qualitative chemical analysis:-

The qualitative analyses of five samples namely PGCP, FPBCS, FPLP+LJ, FPWB and BWgarmon were carried out according the following the methodology outlined by Harborne, (1973) and others as follows: For detection of steroids a method described by Gibbs, (1974) was applied. Detection of terpenoids was by Ayoola *et al.,* (2008). Detection of tannins was by Treare and Evans, (1985). Detection of saponins was done by Kumar *et al.,* (2009). Detection of anthocyanins in the extract was performed by method of Paris and Moyse, (1969).

Detection of glycosides according to Khandewal, (2008). Emodins were detected in the examined samples by Rizk, (1982). For detection of alkaloids a method of Gibbs, (1974) was used. Detection of phenolics was assayed by Gibbs, (1974). Detection of flavonoids was estimated by Khandewal, (2008).

2.8.Determination of chlorophylls and carotenoids:-

The quantification of chlorophyll and carotenoid was conducted following the methodology described by Holden (1965). The concentration of pigments in each sample solvent was measured at wavelengths of 663 nm, 645 nm, and A452 nm using a 1cm quartz cell. The following equations were employed to calculate the concentrations of chlorophylls in (mg/g) and carotenoids (Mazaheri *et al*. 2019).

$$\text{Chl. } a \text{ content} = (15.65 \times A_{666}) - (7.34 \times A_{653})$$

$$\text{Chl. } b \text{ content} = (27.05 \times A_{653}) - (11.21 \times A_{666})$$

$$\text{Total carotenoids content} = \frac{1000 \times A470 - (2.86 \times \text{Chl.}a + 129.2 \times \text{Chl.}b)}{245}$$

2.9.Estimation of total phenolic compounds (TPCs):

The concentrations of total amount of phenolic compounds in extracts of PGCP, FPBCS, FPLP+LJ, FPWB and BWgarmon were determined by the method of Mahrous *et al.,* (2023).

2.10. Estimation of total flavonoids (TFs):-

TFs content of each sample was determined according to the methods by Ebrahimzadeh *et al.,* (2008).

2.11. Spectral Analyses by spectroscopy (FTIR)

FTIR spectra of all samples were analyzed in Faculty of Science, Sohag University. Samples oil or powder of PGCP, FPBCS, FPLP+LJ, FPWB and BWgarmon was loaded in FTIR spectroscope with a scan range from 400 to 4000cm^{-1} with a resolution of 4.0 cm^{-1}. FTIR spectroscopy is an excellent tool for analysis as the intensity of the bands in the spectrum is proportional to the concentration. It is the most powerful tool for identifying the types of chemical bonds or functional groups present in phytochemicals. The wavelength of transmitted light and the prominent feature of chemical bonds can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in the compound can be identified. The dried powder was coated in 100 mg KBr granules, in order to prepare the transparent sample disk. The results were recorded with the help of a Fourier transform spectroscopy model (I-R Prestige 21 Shimadzu, Japan). The FTIR results were subjected to Microsoft software (ORIGEN-Prog) to calculate the transmission and/or absorption ratio of the apparent band, depending on the nature and composition of the sample (Jaggi and Vij, 2006, Guillén, and Goicoechea 2007 and Fan *et al.,* 2012).

2.12. Measurements of the antioxidant activities

In the present work, the antioxidant activities were measured by method of Gaber *et al.* (2021) by Potassium permanganate $KMnO_4$ method. The scavenging actions of the extracts of TPCs, TFs and TCs were measured. The antioxidant scavenging activity (ASA%) was calculated as the follows:

$$\%ASA = \frac{(A_{514}\text{ nm of control} - A_{514}\text{ nm sample})}{A_{514}\text{ nm of control}} \times 100$$

2.13. Statistical analysis

Results were expressed as mean ± standard deviations. Differences between LSDV-infected and healthy groups were calculated using a two-sample t test. $P < 0.05$ was considered statistically significant. All statistical analyzes were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Qualitative screening of bioactive secondary metabolites in the used preparations:-

A phytochemical screening was carried out by specific coloring and precipitation reactions. The phytochemical screening revealed the presence of various bioactive constituents. In this thesis, five samples were examined for qualitative screening these are as follows: (1)-FPBCS= Fine powdered of black cumin seeds, (2)-PGCP = Peeled garlic clove paste, (3)-FPLP + LJ= Fine powered lemon peels + Lemon Juice, (4)-FPWB= Fine powdered of Wheat bran and (5)- BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB were examined for phytochemical screening which revealed the presence of following secondary metabolites in all samples: steroids, terpenoids, tannins, coumarins, alkaloids, phenolics, flavonoids and isoprenoids. The results given in Table (1) indicated that BWgarmon contain 13 different groups. Allylthio derivatives were the most appearing in garlic whereas lemon and Wheat bran were free from allylthio derivatives. All studied samples contain coumarins and lemon ranked the high levels. The purposes of identification of these constituents are to evaluate antioxidant potentials. The lemon peels contain high level of coumarins reached to be 34.25 mg/kg and furanocumarins 85.43 mg/kg, these compounds are secondary metabolites having antioxidant activates and antiviral potentials (Dugrand *et al.,* 2013 and Li *et al.,* 2022).

Wheat bran extracts contain several phenolic acids, including vanillic acid, coumaric acid, and largely ferulic acid (Kahkonen *et al.,* 1999). These compounds, especially ferulic acid, are not evenly distributed in wheat; Most of them are found in bran (Bablis *et al.,* 2002). Wheat bran extract, which contains a high concentration of phenolic acids, has been shown

Table 1. Qualitative screening of bioactive secondary metabolites in garlic, lemon, black cumin oil, wheat bran and BWgarmon

Group	Garlic	Lemon	Black cumin	Wheat bran	BWgarmon
1. Steriods	+	+	+	+	++
2. Terpenoids	++	+++++	++++	+	+++
3. Tannins	+++	+	+++	++	+++
4. Saponins	+	-	+++	-	+
5. Anthocyanins	+	-	-	-	+
6. Coumarins	++	++++	+++	++	++
7. Emadins	-	-	-	+	+
8. Alkaloids	+	++	+++	++	++
9. Glycosides	+	++	+++	++	++
10. Phenolics	+++	+++	++++	+++	+++
11. Flavonoids	+++	+++	++++	++	+
12. Allyl-S derivatives	+++++	-	+++	-	+
13. Isoprenoids	+	+	+	+++	++

FPBCS= Fine powdered of black cumin seed oil; PGCP = Peeled garlic clove paste
FPLP + LJ= Fine powered lemon peels + Lemon Juice; FPWB= Fine powdered of Wheat bran; BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB

to have stronger antioxidant activity than other parts of wheat (Baublis *et al.,* 2000). Zhu *et al*., (2004) reported that wheat grains, bran, and fractions have different antioxidant activities and total phenolic contents (TPC).

The phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions were studied by Kim *et al.* (2006) who reported that wheat bran extracts are rich sources of phenolics and antioxidants that have important roles.

3.2.Levels of TPCs, TFs and Total carotenoids:-

Fine powered lemon peels + Lemon Juice contain the highest concentrations of TPCs (552.0±5.62 mg GAE) /100 g) followed by Fine powdered of Wheat bran (373.70±3.8 mg GAE) /100 g) while the lowest concentrations (167.03±1.71) are in Fine powdered of black cumin seeds (FPBCS). The concentrations of TPCs in the composed paste BWgarmon contain 395±4.1 mg GAE) /100 g) (Table 2 and Fig. 1).

Table 2. Levels of TPCs, TFs and Total carotenoids in BWgarmon and its individual constituents

Constituents	Bioactive constituents		
	TPCs (mg GAE) /100g	**TFs (mg RE/100g**	**Total Carotenoids (µg/g)**
1. FPBCS	167.03^{c}±1.71	108.50^{b}±1.09	6.35^{a} ± 0.391
2. PGCP	173.8^{c}+±1.84	119.55^{b}±1.23	2.65^{d}±0.064
3. FPLP + LJ	552.01^{a}±5.62	135.85^{a} ± 1.68	3.58^{c}±0.062
4. FPWB	373.7^{b}±3.8	69.80^{c}±0.75	4.65^{b}±0.07
5. BWgarmon	395.10^{b}±4.1	115.01^{b}±1.48	4.30^{b}±0.75

FPBCS= Fine powdered of black cumin seed oil
PGCP = Peeled garlic clove paste
FPLP + LJ= Fine powered lemon peels + Lemon Juice
FPWB= Fine powdered of Wheat bran
BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB

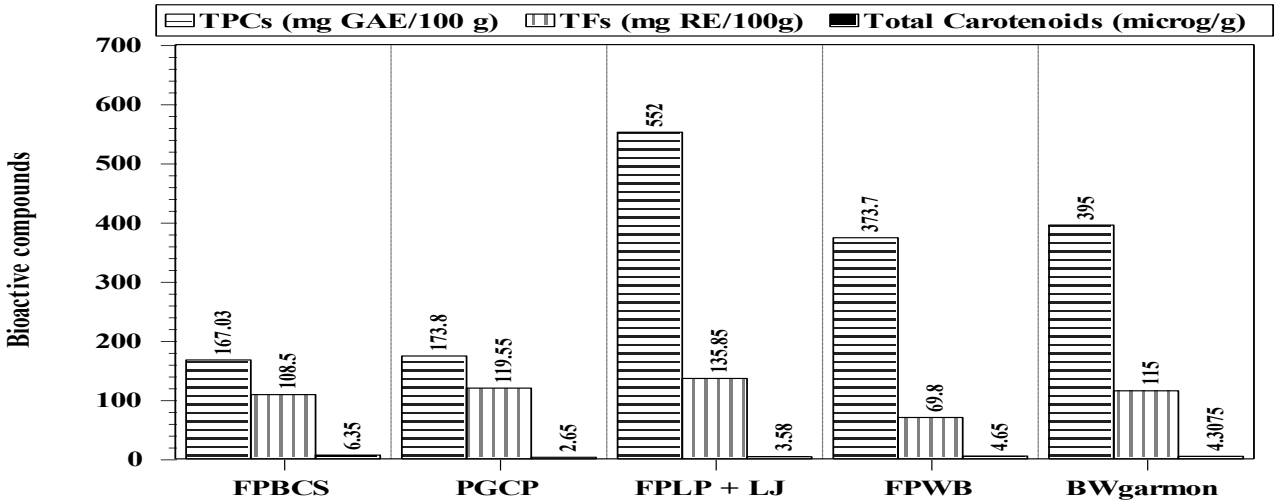

Figure 1. Levels of TPCs, TFs and Total carotenoids in BWgarmon and its individual constituents

Despite FPLP+LJ contain the highest concentration of TPCs when compared to the paste formed from BWgarmon, its cannot be used alone in treatments because FPLP+LJ is lack in saponins, anthocyanins emadins and Allyl-S derivatives, and all of these compounds play an important role in resistance as reported in the results of qualitative screening of bioactive secondary metabolites (Table 2).

Table (2) and Fig. (1) show that TFs (mg RE/100g) ranged from 69.8±0.75 for FPWB to 135.85 ± 1.68 for FPLP + LJ while BWgarmon reported a averaged value reached to be 115±1.48.

The study dealt with estimating the concentrations of total carotenoids in the five samples (µg/g), and the concentrations ranged from 2.65±0.064 in PGCP to 6.35±0.391 in FPBCS. We consider these compounds to be high-potency antioxidants, anti-cancer, and anti-inflammatory agents. Total polyphenols (mg gallic acid/kg oil) 310.26±6.82. The phenolic composition showed the presence of high amounts of epicatechin (1.88-2.37 mg/g) and rutin (0.96-1.21 mg/g) in black cumin seed extracts (Table 2).

Citrus peels and their extracts are rich in flavonoids that are beneficial to human health. The total flavonoid content of lemon was highest at 103.48±0.68 mg/g dry weight (DW) by $NaNO_2$-$Al(NO_3)_3$-NaOH spectrophotometry (Li *et al.*, 2022).

TFs mainly consist of flavanones and polymethoxysulfones (PMFs), including naringin, hesperidin, nariotin, nobiletin, and neohesperidin (Ma *et al.*, 2008). The most abundant flavonoids vary between different citrus fruits; For example, mandarins and hybrids contain more hesperidin, pomelo contains more naringin, and lemons contain the most eryocitrin (Khan *et al.,* 2010). The main flavonoid from citrus peel is quercetagtin (Yang *et al.,* 2011). Flavonoids in citrus peel are recognized as a good source of dietary antioxidants, and protect cells by transporting hydrogen, scavenging free radicals, and chelating divalent metal ions (Sarian *et al.*, 2017 and Li *et al.*, 2022).

3.3.FTIR spectroscopy of raw materials:-

Figure (2) shows the FTIR spectrum of BWgarmon (Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB). By checking the spectrum obtained from the FTIR technique, it becomes clear that there are 10 peaks, of which 7 peaks are in the area confined from 500 to 1748 cm^{-1}, and three peaks are in the area between 2859.19 and 3952.96 cm^{-1}.

The spectrum was characterized by strong asymmetric and symmetric stretching vibrations of the carboxyl group at 2852.19 m^{-1} and the aldehyde and ketone group (C=O) with the carboxyl group at 1748.00 cm^{-1}. The C-O group combines with the carboxl group at 1160.57 m^{-1}.C-H of alkanes are strongly stretched at 2919.26, 2852.19 and 1459.64 cm^{-1}.

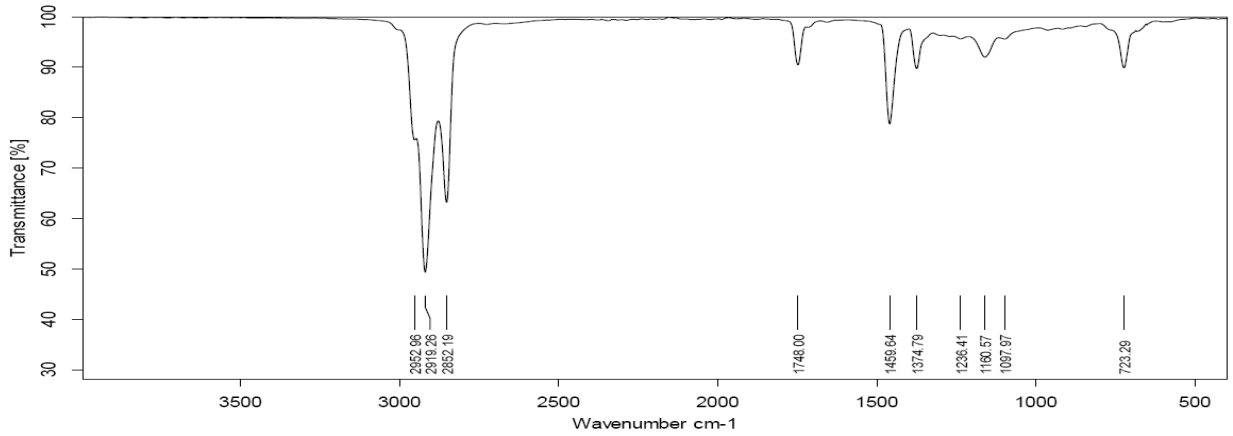

Figure 2. FTIR spectrum of BWgarmon (Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB).

Figure (3) shows the FTIR spectrum of oil black cumin seeds. By checking the spectrum obtained from the FTIR technique, it becomes clear that there are 16 peaks, of which 13 peaks are in the area confined from 500 to 1743.12 cm^{-1}, and three peaks are in the area between 2853.79 and 3007.92 cm^{-1}.

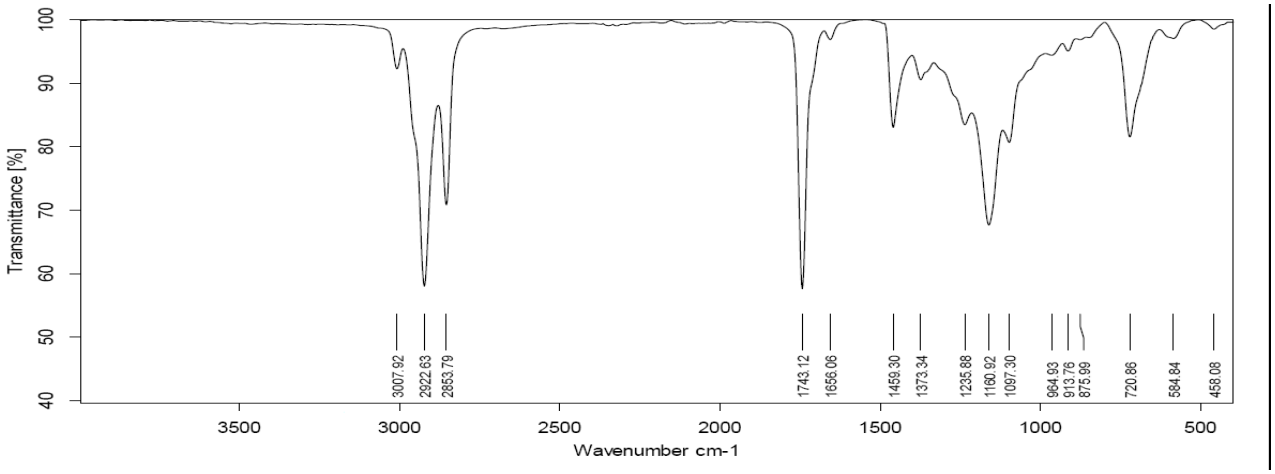

Figure 3. FTIR spectrum of fine oil of black cumin seeds

Figure (4) shows the FTIR spectrum of fine powdered of wheat bran. By checking the spectrum obtained from the FTIR technique, it becomes clear that there are 21 peaks, of which 17 peaks are in the area confined from 436.92 to 1741.49 cm^{-1}, and four peaks are in the area between 2854.84 and 3278.87 cm^{-1}.

Fourier transform infrared analysis of arabinoxylan showed characteristic spectra. The bands observed were the band around 1655 cm due to bound water (Kacurakova *et al.,* 1994), the band around 1430 cm due to –CH stretching modes, the bands around 3000-2800 cm^{-1} due to –CH stretching modes, the prominent band around 3327 cm^{-1} due to vibrations of Hydroxyl stretching of sugars and water involved in hydrogen bonding, the band is around 1046 cm^{-1} due to C-O, C-C and C-O-H bending vibrations (Fringant *et al.* 1995, Nandini and Salmath 2003).

The overlapping bands in the range of 1470-1370 cm^{-1} arose due to the combination of deformation patterns of methyl and methylene groups. Other strong peaks present at 1160 and 1098 cm^{-1} are that the first band represents a joint asymmetric stretching of (C–C(=O)–O) and (OC–C) bonds, while the band at 1099 cm^{-1} is associated with (C– O – C) Symmetric expansion of triglycerides and esters. The more intense band at the lower end of the spectra centered at 720 cm^{-1} is attributed to a combination of oscillatory vibration and out-of-plane methylene deformation in unsubstituted olefins (Rosas-Mendoza *et al.,* 2017).

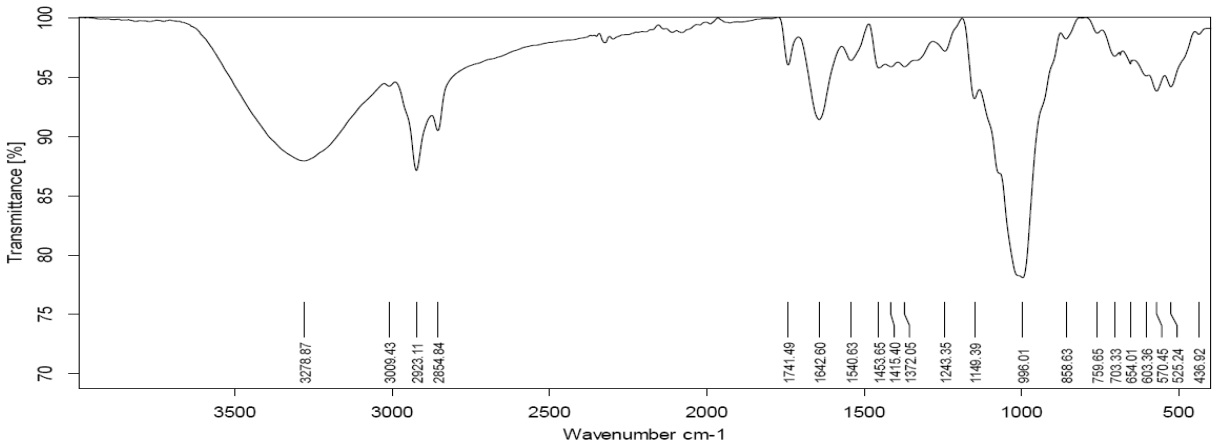

Figure 4. FTIR spectrum of fine powdered of wheat bran

Figure (5) shows the FTIR spectrum of FPLP + LJ. By checking the spectrum obtained from the FTIR technique, it becomes clear that there are 16 peaks, of which 13 peaks are in the area confined from 525.84 to 1714.82 cm^{-1}, and three peaks are in the area between 2856.02 and 3278.42 cm^{-1}.

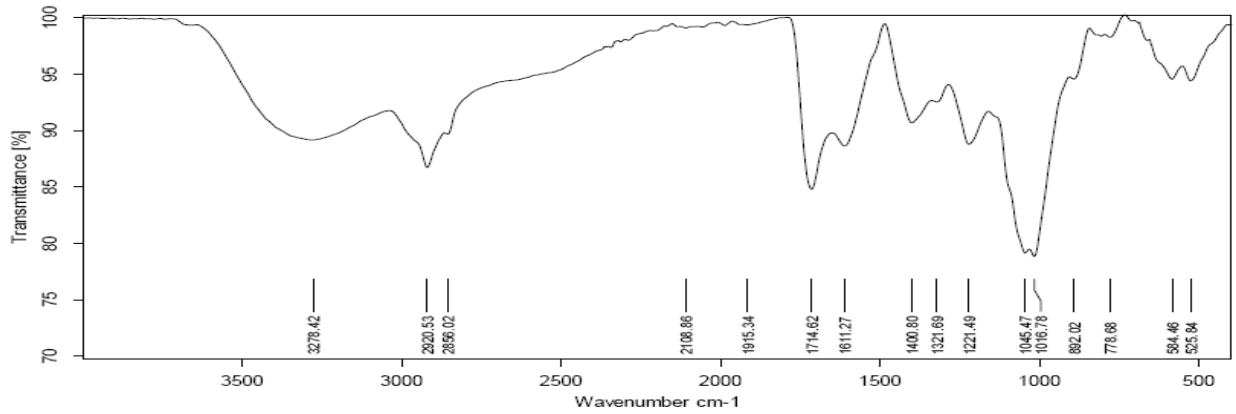

Figure 5. FTIR spectrum of fine powdered of lemon powdered +LJ.

Figure 6 shows the FTIR spectrum of a finely crushed peeled garlic clove. By examining the spectrum obtained from the FTIR device, it is found that there are 15 peaks, including 11 peaks in the region between 528.08 and 1624.66 cm^{-1}, and four peaks in the region between 2909.17 and 3605.45 cm^{-1}.

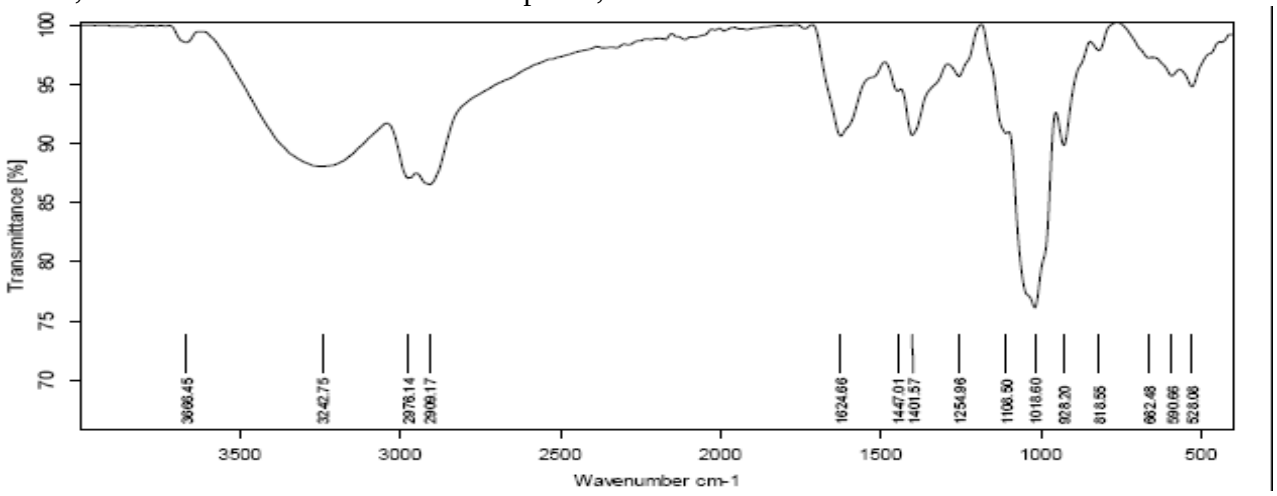

Figure 6. FTIR spectrum of fine powdered of peeled garlic clove powdered

FTIR technology has been used to evaluate the type of organic and inorganic complexes in plants. The present study FTIR spectroscopy showed the presence of plant components in garlic extract. Preliminary phytochemical screening of garlic powder (Table 2) showed the presence of investigated phytochemicals such as amino acids, alkaloids, carbohydrates, amines, carboxylic acids, alkenes, proteins, sulfur compounds and lipids.

Identification of the secondary metabolic fingerprint by chromatography and spectroscopic tools provides useful information about the quality, quantity, and pattern of its formation.

Biomolecules were studied by Nagarajan and Kumar. (2017). Recently, FTIR reveals phytochemical profiles containing overlapping signals from a wide range of compounds. *Allium sativum* samples extracted with the help of solvent ether were continuously subjected to FTIR to determine whether garlic could be differentiated on the basis of biochemical profiles. They identified the reactive functional groups present in garlic powder. They tentatively indicate that garlic (*Allium sativum*, L.) will be used as a precursor to new medicines for the benefit of humanity.

3.4.Analysis of FTIR spectra of TPN, LFBs and HFBs of all samples

Table (4) show the total peaks number (TPN) in the spectra of samples from the FTIR-spectra could be noticed that the peaks at frequencies from 2800 to 3300 cm^{-1} 3 or 4 peaks are observed.

Table 4. Analysis of FTIR spectra, total peaks number (TPN), lower frequencies bands (LFBs) and higher frequencies bands (HFBs) of BWgarmon **and its individual constituents.**

Samples	Total peaks number	Lower frequencies bands 500 to 1746 cm^{-1}	Higher frequencies bands 2800 to 3500 cm^{-1}
Black cumin seeds oil	16	13	3
PGCP	15	11	4
FPLP + LJ	16	13	3
Fine powdered of Wheat bran	21	17	4
BWgarmon	10	7	3

TPN = Total peak numbers
FPBCS= Fine powdered of black cumin seeds
PGCP = Peeled garlic clove paste
FPLP + LJ= Fine powered lemon peels + Lemon Juice
FPWB= Fine powdered of Wheat bran
BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB

We have resorted to using FTIR in analyzing the BWgarmon and its constituents to learn more about the functional and effective groups that are originally present in the newly composed paste or that are transferred to the BWgarmon paste during pressing and mixing in addition to knowing the changes that occur to the samples during storage, handling and circulation.

3.5.Antioxidants activities of bioactive compound extracts of BWgarmon and its individual constituents by KMnO4 method:-

Antioxidant activities of TPCs, TFs and TCs in BWgarmon and its individual constituents were assessed by Potassium permanganate method (Table 5). The results show that scavenging activities (%) of TPCs (mg GAE) /100g) are higher in FPLP + LJ (297.5±3.2) than those reported in BWgarmon (210.2±2.2). Fine powdered of black cumin seeds (FPBCS) reported the lowest levels of scavenging activities (%) (89.51±0.91) followed by Peeled garlic clove paste (93.15±0.95) (Table 5).

Scavenging activities (%) of TFs (mg GAE)/100g) ranged from 37.4±0.4 from FPBCS to 72.82±0.7 for FPLP + LJ and the final paste (BWgarmon) reached to be 63.64±0.62.

Scavenging activities (%) of total carotenoids (TCs $\mu g/g$) ranged from 1.42±0.01 for PGCP to 3.54±0.04 for FPBCS. The present

Table 5. Levels of scavenging activities (%) of the bioactive compounds of BWgarmon and its individual constituents assessed by $KMnO_4$ method.

Constituents	Scavenging activities (%) of the bioactive compounds assayed by $KMnO_4$		
	TPCs	**TFs**	**TCs**
FPBCS	89.51^{c}±0.91	58.20^{b}±0.60	3.54^{a}±0.04
PGCP	93.15^{b}±0.95	64.10^{a}±0.65	1.42^{c}±0.01
FPLP + LJ	297.50^{a}±3.2	72.82^{a}±0.70	1.91^{c}±0.02
FPWB	201.40^{a}±2.2	37.40^{c}±0.40	2.52^{b}±0.03
BWgarmon	210.20^{a}±2.2	63.64^{a}±0.62	2.31^{b}±0.03

FPBCS= Fine powdered of black cumin seeds
PGCP = Peeled garlic clove paste
FPLP + LJ= Fine powered lemon peels + Lemon Juice
FPWB= Fine powdered of Wheat bran
BWgarmon = Paste composed from FPBCS+PGCP +FPLP + LJ+ FPWB

results also indicated the differences in scavenging activities (%) are in close extent. The potentials of TPCs, TFs and TCs as antioxidants could be arranged in the following order: - TPCs> TFs> TCs and this trend consistent with final concentrations in the extracts. The present results are in agreement with those reported by El-ghfar *et al.,* (2016) and Garcia-Castello *et al.,* (2015) who stated that bioactive compounds in *Citrus sp.* are antioxidants.

Citruses are a highly abundant fruit worldwide, and their processing generates significant amounts of secondary metabolites (Imeneo *et al.,* 2022 and Russo *et al.,* 2021). The majority of these produced residues are either fed to animals or discarded into the environment, without appropriate processing (Gómez-Mejía *et al.,* 2019). The peels, pulps, and seeds of fruits usually contain beneficial substances that can be isolated and used as natural antioxidants (Putnik *et al.,* 2017). These antioxidants can prevent the oxidation of certain foods or be incorporated into functional food products (El-ghfar *et al.,* 2016 and Garcia-Castello *et al.,* 2015).

4. DISCUSSION

The present results indicated the differences in scavenging activities (%) are in close extent. The potentials of TPCs, TFs and TCs as antioxidants could be arranged in the following order: - TPCs> TFs> TCs and this trend consistent with final concentrations in the samples.

FTIR spectrum of oil black cumin seeds, by checking the spectrum obtained from the FTIR technique, it becomes clear that there are 16 peaks, of which 13 peaks are in the area confined from 500 to 1743.12 cm^{-1}, and three peaks are in the area between 2853.79 and 3007.92 cm^{-1}.

The newly formulated preparation BWgarmon is rich in natural antioxidants which play important role in the defence process. An improvement in the measured indicators and brought their levels close to those in the healthy cattle group. Unfortunately, lumpy cow skin disease has no direct antiviral treatment. Instead, the infected animals receive supportive care, which involves the use of antibiotics, painkillers, and wound care sprays to treat symptoms. As there's no treatment, vaccines are used to control disease transmission.

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الملخص العربي

المكونات الدفاعية النشطة بيولوجيًا ضد مرض الجلد العقدي ومستويات مضادات الأكسدة الطبيعية في مستحضر الـ BWgarmon ومكوناته الفردية

فتحي مصطفي محمد سليم[١]، محمد عبد العزيز الشافعي[١]، محمد جمال عبد الرازق مبارك[٢] و جمال فخرى عبد النعيم[١]

[١]قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنيا

[٢]معهد بحوث صحة الحيوان، الجيزة، مصر.

في هذا البحث، تم إخضاع خمس عينات للتحليل؛ ويمكن تلخيص النتائج على النحو التالي. تم فحص خمس عينات التحليل الوصفي وهي كما يلي: (١) –FPBCS = مسحوق ناعم من بذور حبة البركة السوداء، (٢) –PGCP = معجون فص ثوم مقشر، (٣) –FPLP + LJ = قشور ليمون مطحونة ناعمة + عصير ليمون، (٤)–FPWB = مسحوق ناعم من نخالة القمح و (٥)– BWgarmon = معجون مكون من FPWB + LJ + FPLP + PGCP + FPBCS تم فحصه للتحليل الوصفي للمركبات الفعالة والذي كشف عن وجود نواتج التمثيل الغذائي الثانوي التالية في جميع العينات: الستيرويدات، التربينويدات، التانينات، الكومارين، القلويدات، الفينولات، الفلافونويدات والإيزوبرينويدات. يحتوي BWgarmon على ١٣ مجموعة مختلفة.

تحتوي قشور الليمون المطحونة الناعمة + عصير الليمون على أعلى تركيزات من 5.62 ± 552.0) TPCs مجم 100 / (GAE جم) تليها نخالة القمح المطحونة الناعمة (٣٧٣.٧ ± ٣.٨ مجم 100 / (GAE جم) بينما أقل التركيزات (١٦٧.٠٣ ± ١.٧١) موجودة في مسحوق بذور حبة البركة السوداء (FPBCS). تحتوي تركيزات TPCs في المعجون المركب BWgarmon على ٣٩٥ ± ٤.١ مجم 100 / (GAE جم). تراوحت الفلافونيدات الكلية (مجم 100 / RE جم) من ٦٩.٨ ± ٠.٧٥ لـ FPWB إلى ١٣٥.٨٥ ± ١.٦٨ لـ FPLP + LJ بينما سجلت BWgarmon عن متوسط قيمة وصل إلى ١١٥ ± ١.٤٨.

تم تقييم الأنشطة المضادة للأكسدة لـ TPCs و TFs و TCs في BWgarmon ومكوناتها الفردية بطريقة برمنجنات البوتاسيوم. أظهرت النتائج أن أنشطة المضادة للاكسدة (%) لـ 100g / (TPCs (mg GAE أعلى في 3.2 ± 297.5) FPLP + LJ من تلك المذكورة في 2.2 ± 210.2) BWgarmon). تراوحت الأنشطة المضادة للاكسدة (%) لـ 100g / (TFs (mg GAE من ٣٧.٤ ± ٠.٤ من FPBCS إلى ٧٢.٨٢ ± ٠.٧ لـ FPLP + LJ ووصلت العجينة النهائية (BWgarmon) إلى ٦٣.٦٤ ± ٠.٠٦٢. تراوحت الأنشطة المضادة للاكسدة (%) من إجمالي الكاروتينات (TCs µg/g) من ١.٤٢±٠.٠١ لـ PGCP إلى ٣.٥٤±٠.٠٤ لـ FPBCS.