



Diagnose the Bioactive Compounds in Flaxseed Extract and Its Oil and Use Their Mixture as an Antioxidant

Wasan K. A. Al-Temimi¹, Najla H.S. Al- Garory¹ & Ali A. Khalaf²

¹Department of Food Sciences, College of Agriculture, University of Basrah, Iraq

²General of Foodstuff Trading, Branch of Basrah Ministry of Trade, Iraq

Corresponding author: e-mail:dr.wasanaltemimi@gmail.com

Received 9 April 2020; Accepted 2 June 2020; Available online 27 June 2020

Abstract: The aim of this study is to extract the important antioxidants components of flaxseed in different ways. The Determination of total phenol content, antioxidant activity and percentage of linoleic acid inhibition by DPPH for the components (Lignan, oil, 80 and 100% ethanolic extract and the deionized water extract). The components of flaxseed oil were identified using GC/MS. The efficiency of the mixture (80% ethanolic extract and oil) was determined by inhibiting the process of oxidation of linoleic acid during storage periods (0, 7, 14 and 21 days) at laboratory temperature, and the following results were obtained: The yield of lignan, oil, ethanol extract (80 and 100)% and deionized water were (0.12,40.52,10.9,9.6 and 13)g.100g⁻¹ seed respectively, while the total phenolic content of the above components was (700,1165,3315,2098 and 483)mgGAE.100g⁻¹,respectively. Flaxseed oil gave the highest antioxidant activity 79.3% with an inhibition percentage 73.19%, compared with ethanolic extract, lignan and water extract. The diagnosis of GC/MS flaxseed oil showed that the oil was contained in compounds with antioxidant activity, including mono, di and tri-terpens such as Copaene, Monoterpene, β-Sesquiphellandrene, Squalene, diethyl phthalate, β-Sitosterol and Hexadecanoic acid, as well as, the presence of ascorbic acid and gamma tocopherol with good concentrations (8.68 and 2.62)% respectively. The mixture of 80% ethanol and oil extract showed an effect in decreasing of peroxide values with an increase of concentration of the added mixture (0.1, 0.4 and 0.10 mg.mg⁻¹). The maximum reduction of the peroxide value at the concentration was 0.6 mg. mg⁻¹, compared with the standard sample during the different durations of storage.

Key word: Flaxseed compounds, Bioactive compound, Total phenol, Antioxidant activity.

Introduction

The Kingdom Plantae has many phytochemicals with therapeutic or nutritional properties that can be an important source of human food and health. Rababah *et al.* (2004) indicated that plants and some oilseeds are a good source of natural antioxidants, which provide good protection against oxidation of

foods systems (Devie *et al.*, 2007; Elizabeth *et al.*, 2007).

Flaxseeds has been known since ancient times and has been used in human food thousands of years ago. It was planted in Mesopotamia about 4000-5200 BC. Flaxseed has become the focus of many researchers

because they believe it promotes consumer health and disease prevention due to it containing fibre, protein, lignan, unsaturated fatty acids, especially linolenic acid, as well as potassium, flaxseeds are considered nutraceutical as well as functional foods (Mentes *et al.*, 2008). The evolution of food sciences has provided an excellent opportunity to re-evaluate this important food. Food scientists have shown great interest in flaxseed after identifying its content of unsaturated fatty acids, especially linolenic acid, lignan, dietary fibre, etc., which benefit the health and nutrition of the consumer. Scientists at the National Cancer Institute of America showed that flaxseed was one of six sources that deserved special consideration (Basch *et al.*, 2007; Toure & Xueming, 2010).

Many studies have indicated that flaxseed contains lignan, unique proteins, phenolic acids, flavonoids and tocopheroles, these compounds are bioactive and have anti-oxidant properties. In addition to that they indicated that flaxseed is the richest plant source in the lignan compound, as it has anti-oxidant properties as well as its role in reducing the risk of cancerous tumours breast and prostate (Hosseinian *et al.*, 2006).

Food scientists and food producers have shown great interest in natural phytochemicals, which acts as an antioxidant, such as polyphenols, ascorbic acid and flavonoids.

Therefore, the current study aimed at determining the components of flaxseeds that have an antioxidant activity (Lignan, oil, water extract and alcohol extract with concentration of 80 and 100% ethanol) and study the effect of the mixture of the most effective ingredients to

prolonging duration of storage for sunflower oil.

Materials & Methods

Materials

Flaxseeds *Linum usitatissimum* L. were obtained from the local herbal shop of Basrah Governorate, Iraq.

Lignan extraction

Followed the method that was described by Richard *et al.* (1996) with some modification, 25 g of flaxseed was treated with a mixture of dioxin solvent and ethyl alcohol at a concentration of 1: 1 volume/ volume by mixing 1: 8 weight/volume of flaxseeds: solvent. The mixture was mixed for 4 hours on the magnetic stirrer and it was left for 24 hours with refrigeration. The mixture was filtered using filter paper No. 1. Evaporating solvent by using the rotary evaporator at 40 ° C to obtain the dry lignan compound.

Extraction of Lipids and Phenolic compounds

Oils were extracted from flaxseed in the manner described by Anwar & Przybylski (2012). Phenolic compounds were isolated from mixing 20 g of precipitation with 200 ml of ethyl alcohol (Sharlow Co., Spain) with concentrations of 80 and 100 ethanol using magnetic stirrer for 12 hours at 750 rpm under laboratory conditions. The process of the extraction was repeated twice, isolating the supernatant using filter paper No. 1. The supernatant was concentrated until dried using evaporator rotary at 45 ° C.

Water extraction

Bioactive compounds were extracted from flaxseed according to the method described by (Barthet *et al.*, 2014).

Yield Determination

The value of active compounds was calculated by the mathematical equation mentioned by Nur-Syukriah *et al.* (2014).

$$\text{Yield \%} = \frac{\text{Final weight (g)} \times 100}{\text{Initial weight (g)}}$$

Total Phenol

The total phenolic concentration of all extracts was estimated by the Folin-Ciocalteu method and described by Velioglu *et al.* (1998).

Standard Curve

The total phenols was calculated based on the correlation between the concentration of Gallic acid and the absorption at 725 nm wavelength using standard Gallic acid solution (0 - 500) $\mu\text{g.ml}^{-1}$ (Fig. 1). The standard solution of Gallic acid was prepared according to (Velioglu *et al.*, 1998).

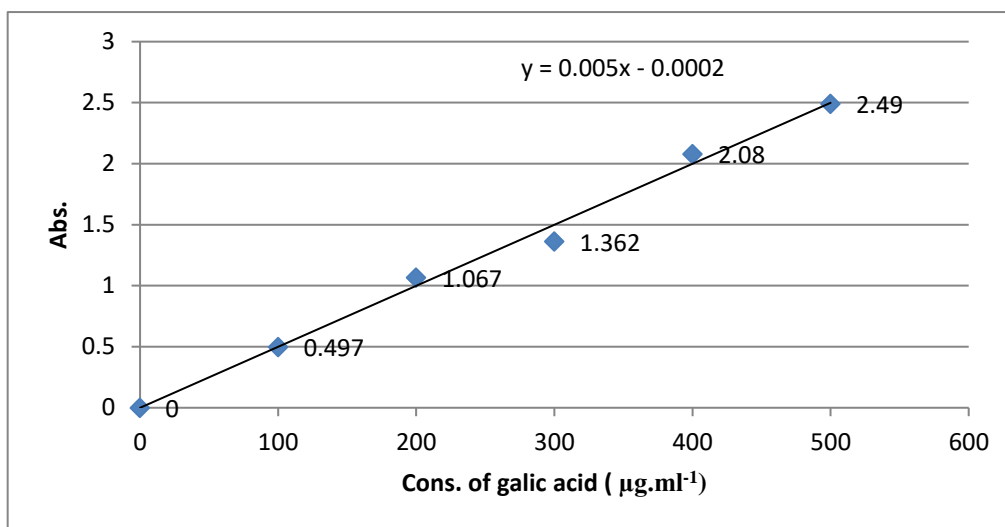


Fig. (1): Standard curve of Gallic acid

Determination of antioxidant activity

Antioxidant efficacy was measured by method (Amin & Tan, 2002). 1 ml of β -carotene (0.2 mg /ml chloroform) was transformed into a beaker containing 0.02 ml linoleic acid (supplied by Metrya-UK) and 0.2 ml of Tween-20. The mixture was preheated at 40 ° C to remove all chloroform. 100 ml of distilled water was immediately added and gradually stirred to form the emulsifier. Preparation of sample 1 mg. ml^{-1} dissolved in 70% ethanol. 0.2 ml of the sample was transferred to different

test tubes and added 5 ml of emulsion. The tubes were left in the water bath for two hours at 45°C. Absorption was measured at 470 nm every 15 minutes for 120 minutes. The control sample was prepared by mixing 0.2 ml of 70% ethanol with 5 ml of emulsion.

$$AA = 1 - \left(\frac{A_t - A_0}{A^{\circ}t - A^{\circ}0} \right) \times 100$$

AA =Antioxidant Activity

A_t , =Absorption of sample at zero time,

A₀= Absorption control sample at zero time.

A^o_t =Absorption of the sample at 120 minutes,

A^o₀ Absorption control sample at 120 minutes

The activity of free radicals DPPH

The free radicals scavenging activity was estimated by Diphenyl-1-picrylhydrazyl (DPPH) as described by Iqbal *et al.* (2005) with some modification, 5 ml of the DPPH (0.025 g.L⁻¹) was taken, 1 ml of the sample (prepared 25 µg.ml⁻¹ in ethanol) was added to the total of the liquid extract. Absorbance at 515nm was measured at wavelength for periods 0, 0.5 and 1, 2, 5 and 10 minutes. The DPPH percentage is calculated according to the equation which mentioned by Egan *et al.* (1988).

$$\% \text{ DPPH} = \left(\frac{A_t}{A_0} \right) \times 100$$

A₀ = Absorption at 0 min At = absorption at times (0, 0.5, 1, 2, 5 and 10) min.

Evaluation of the performance of antioxidants in food systems

Mixture was prepared from 80% of alcohol extract and flaxseed oil with concentration 1: 1. After that the mixture was added with concentrations 0, 0.1, 0.2 and 0.3 mg.g⁻¹ of sunflower oil free of antioxidants obtained from the State Oil Company, Baghdad, Republic of Iraq. The mixture was incubated at 45° C. The efficacy of the mixture (oil-alcohol) was measured as an antioxidant by estimating of the peroxide value during durations of storage (0, 7, 14 and 21 days) according to the method mentioned by Egan *et al.* (1981).

Statistical analysis

Results were statistically analysed using SPSS (SPSS, 2012), at the probability level P≤ 0.05.

Results & Discussion

Fig. (2) shown the yield of the lignan, oil and bioactive compounds extracted from flaxseed

with ethanol concentration (80 and 100%) and deionized water. The yield of components amount was 0.12, 40.52, 10.9, 9.6 and 13 g.100 g⁻¹ seeds respectively. It was noted that the oil is superior to the rest of the other ingredients used in the study. This increase was due to the flaxseed content of the oil. Ganorkar & Jain (2013) found that the proportion of oil in flaxseeds was 41 g.100 g⁻¹ seeds.

While a study of the chemical composition of flaxseed found that the seed content of oil was 46.22% (Ingale & Shrivastava, 2011). It was also noted that the yield of the water extract was superior to the alcohol extract for both concentrations (80 and 100)%. This increase of water extract yield may be was due to its ability to dissolve some polar compounds as well as to dissolve some carbohydrates (gums, pectin and sugars), and there was some other compound dissolved in water such as soluble fibre and proteins. A number of studies have indicated that flaxseeds contain a good proportion of carbohydrates, fibre and water-soluble proteins. Ganorkar & Jain (2013) explained that the flaxseed content of carbohydrates 29 g.100g⁻¹ seeds and that 10 g.100 g⁻¹ seeds were water soluble fibre.

Simbalista *et al.* (2012) pointed out that whole and defatted flaxseed content of soluble fibre ranged from 10.7 to 14.6 g.100 g⁻¹, respectively. Herchia *et al.* (2015) found through a study of chemical composition of flaxseed that the carbohydrate content was 34.1%, while the soluble fibre was 4.3 - 8.6%.

The reason for the increase in the amount of compounds extracted with ethanol at 80% concentration as compared to 100% may be due to increased polarity of the solvent by adding water to it or the chemical nature of the compounds and the solubility of these

compounds. Simbalista *et al.* (2012) pointed out that whole and defatted flaxseed content of soluble fibre ranged from 10.7 to 14.6 g. 100 g⁻¹, respectively. Herchia *et al.* (2015) found through a study of chemical composition of flaxseed that the carbohydrate content was 34.1%, while the soluble fibre was 4.3 - 8.6%.

The reason for the increase in the amount of compounds extracted with ethanol at 80% concentration compare with 100% may be due to increased polarity of the solvent by adding water to it or the chemical nature of the compounds and the solubility of these compounds.

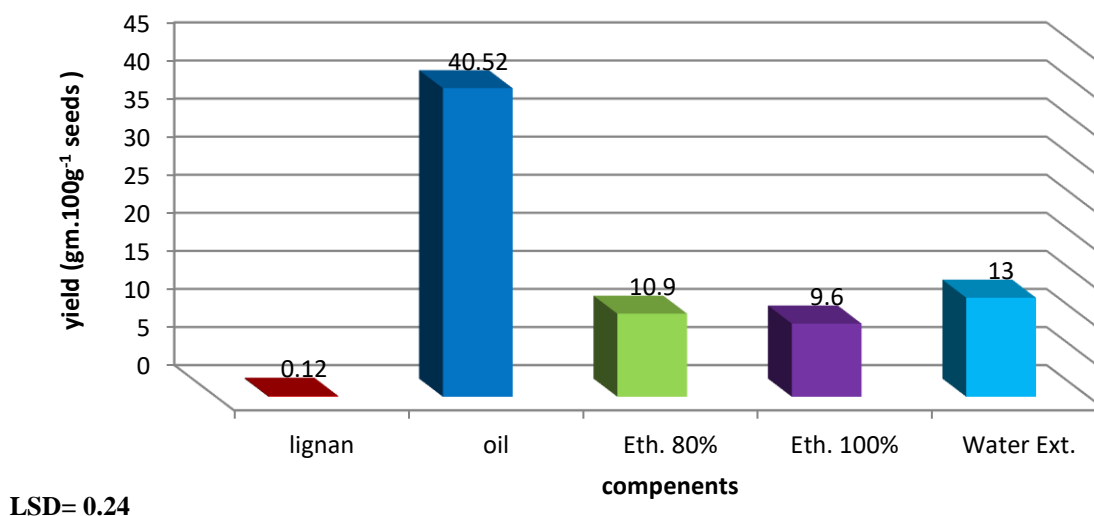


Fig. (2): Yield of lignan, oil and compounds extracted from flaxseeds using ethanol concentration (80 and 100%) and deionized water.

Many studies have indicated that the added water to ethanol caused an increase in the bioactive compounds, they found that the highest extract of bioactive compounds in flaxseed was at 80% methanol, while 80% ethanol gave higher amounts of flavonoids and better antioxidant capacity, they noted that polar solvents showed differences in phenol and flavonoid extraction, that was the strongest in its work as an antioxidant compared to non-polar solvents (Franco *et al.*, 2008; Anwar & Przybylski, 2012). It was confirmed by Sultana *et al.* (2009) that variations in active plant extracts were due to a number of reasons, including the polarity of the solvent, the

chemical nature of these compounds as well as their solubility, and that polar solvents such as methanol, ethanol and their mixtures are highly recommended for phenolic compounds. The reason for the decrease in the yield of lignan in flaxseed may be due to its low concentration of lignan in seeds. Thompson *et al.* (1997) indicated that flaxseeds are the richest in lignan, and the percentage of lignan in flaxseed depends on the type, location and date of cultivation. Hall *et al.* (2006) found that the content of lignan flaxseed ranged from (330000-370000) µg.100 g⁻¹ seeds. Ganorkar & Jain (2013) found that the flaxseed content of the lignan was 0.9-1.9%.

Determination of total phenols

Fig. (3) shown the total content of phenols mg.100g⁻¹ of flaxseeds for lignan, oil, alcohol extraction at 80% and 100% and deionized using the fallen reagent .The total phenolic content in the alcoholic extract (80%) was significantly higher at $P \leq 0.01$ than the other extracts, as it reached to 3315 mg GAE.100 g⁻¹ seeds. Followed by liquid extraction of flaxseeds using ethanol solvent 100% concentration of 2098 mg GAE.100 g⁻¹ seeds, while the total phenols in oil, lignan and deionized water extract of flaxseed were (1165, 700 and 483 mg) GAE .100 g⁻¹ seeds respectively.

The increase in total phenols in ethanol extracts may be due to the polarity of the solvent or to the chemical nature of the active compounds as well as the solubility of the phenolic compounds in the solvents used. And that the superiority of the concentration of total phenols in the extract treated with solvent 80% may be due to the addition of water that caused the increase in the polarity of the solvent, which positively affected the amount of phenols total, this is confirmed by Franco *et al.* (2008). That the addition of water to ethanol caused a significant increase in the value of bioactive compounds. Anwar & Przybylski (2012) noted that polar solvents such as ethanol and methanol showed significant differences in the extraction of total phenols and flavonoids, these compounds was strongest in their work as antioxidants compared to non-polar solvents, and they showed that total phenol extract from

defatted flaxseeds using n-butanol was very low. Also, some studies found that was a high concentration of total phenols in flaxseed oil extracted by using of hexane solvent, they explained that the reason for using hexane solvent in extraction was its chemical nature. The decrease in the total phenolic content in the lignan extract may be due to the decrease in the content of the lignan in the flaxseeds, which reached 0.12 g.100 g⁻¹ seeds, (Fig. 2). As well as showed decrease in total phenolic content in the deionized water extract for flaxseed, although the water acts as a polar solvent and caused increase in yield as shown in fig. (2), it may be due to dissolving a number of compounds, especially soluble fibre, proteins and sugars, which affected the total amount of phenols extracted. The results of this study varied with some studies. El-Beltagi *et al.* (2011) showed that the content of phenolic compounds from flaxseed was 85 mg.g⁻¹, these concentration increased to 96 mg.g⁻¹ after alkaline hydrolysis of flaxseed flour.

Anwar & Przybylski (2012) found that the total flaxseed content of phenolic compounds (2700, 2020, 1360 and 3260) was GAE.100 g⁻¹ when methanol and ethanol (80 and 100)% were used in the extraction, respectively. Differences in the amount of phenolic content between our study and other studies may be due to a number of reasons, including the seed class, the extraction methods used, the extraction temperature, the chemical nature of the phenolic and the solvent polarity, this is confirmed by a number of studies (Sultana *et al.*, 2009).

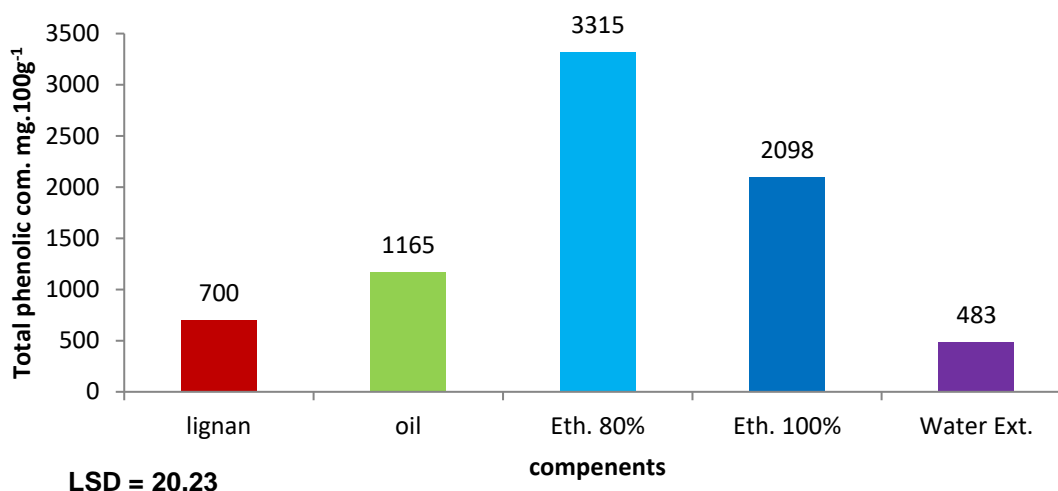


Fig. (3): Total phenolic content of lignan, oil and compounds extracted by using ethanol concentration (80 and 100%) and deionized water.

DPPH Test and percentage of linoleic acid inhibition

The antioxidant properties of the flaxseed and its alcoholic and water extracts (lignan, oil, alcohol extract at concentration of 80% and 100% and water extract) were measured in 1,1-Diphenyl 2-picrylhydrazyl (DPPH) as are shown in fig. (4). The DPPH values for flaxseed oil were significantly higher at $P \leq 0.01$, as it reached 79.3% and the rate of inhibition of linoleic acid oxidation was 73.19%. Followed by alcohol extracts at 80% then 100% with antioxidant activity amount to (71.24% and 52.11%), and with an inhibition ratio of 65.9% and 43.67% respectively, while both lignan and water extract gave a lower value from DPPH and an inhibition ratio (35.7 and 21.68%) and (30.29 and 16.21%) respectively. The results of antioxidant activity and the percentage of linoleic acid oxidation inhibition of flaxseed compounds and extracts were showed lower than those of BHT compound (95.4% and 90.2%) respectively.

The high antioxidant activity values may be attributed to flaxseed oil as it containing a good amount of phenolic compounds as are shown in fig. (3), as well as contain several compounds that will positively affect antioxidant activity values such as ascorbic acid and tocopherols. A number of studies have indicated that flaxseed contains ascorbic acid at a concentration of 0.5 mg.100g⁻¹ and tocopherols at a concentration of 569mg.100g⁻¹ included gamma tocopherol, alpha-tocopherol and delta tocopherol with concentrations (552, 7 and 10 mg.100 g⁻¹) respectively (Bernacchia *et al.*, 2014). Herchia *et al.* (2015) found the content of flaxseed from ascorbic acid was 1.35 mg.100 g⁻¹ seeds, noting the high acid values by increasing seed germination.

The increase in DPPH for alcohol extract (80%) compared to the alcohol extracted by (100%) may be due to increased polarity of the solvent when water was added, Which may help in dissolving more quantities of phenolic compounds, as is noted in fig. (3). A number

of studies have confirmed that the addition of water to ethanol has enhanced ethanol polarity and has helped dissolve phenolic compounds with antioxidant activity (Franco *et al.*, 2008).

While the lignan and water extract compounds showed a lower in DPPH and the percent inhibition of linoleic acid, that was may be due to the reduction of phenolic compounds total. Barthet *et al.* (2014) explanted there is a strong relationship between the total phenols

and DPPH values in the presence or disappearance of antioxidant activity; these results were confirmed by Slavova-Kazakova *et al.* (2016) pointed out that lignan extracted from flaxseed did not have the antioxidant effect, whereas hydrolyzed lignan showed low antioxidant activity. Hosseinian *et al.* (2006) reported that lignan compounds have the ability to give hydrogen and reduce or inhibit serial reactions to free radical formation.

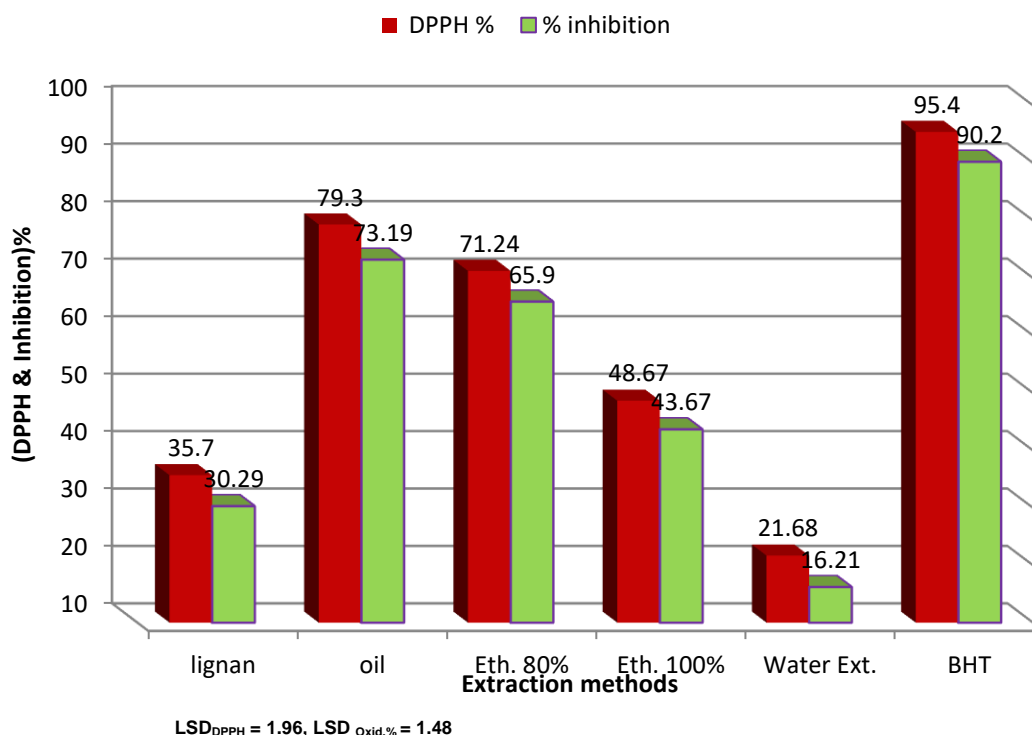


Fig. (4): Percentage of DPPH and oxidation inhibition for flaxseed components and extracts.

While the lignan and water extract compounds showed a lower in DPPH and the percent inhibition of linoleic acid, that was may be due to the reduction of phenolic compounds total. Barthet *et al.* (2014) explanted there is a strong relationship between the total phenols and DPPH values in the presence or disappearance of antioxidant activity; these results were confirmed by Slavova-Kazakova *et al.* (2016) pointed out that lignan extracted

from flaxseed did not have the antioxidant effect, whereas hydrolyzed lignan showed low antioxidant activity. Hosseinian *et al.* (2006) reported that lignan compounds have the ability to give hydrogen and reduce or inhibit serial reactions to free radical formation.

The our results of DPPH values and the percent of inhibition linoleic acid were close to a number of studies, Herchia *et al.* (2015) showed that DPPH values were ranged from

(40.14 - 52.48) % to non-germinated flaxseeds. Barthelet *et al.* (2014) found that the DPPH values were ranged from 35.6 to 63.5% with the superiority of acetone - derived compounds, while the water extract showed DPPH 37.2%.

Diagnosis of GC / MS flax seed oil

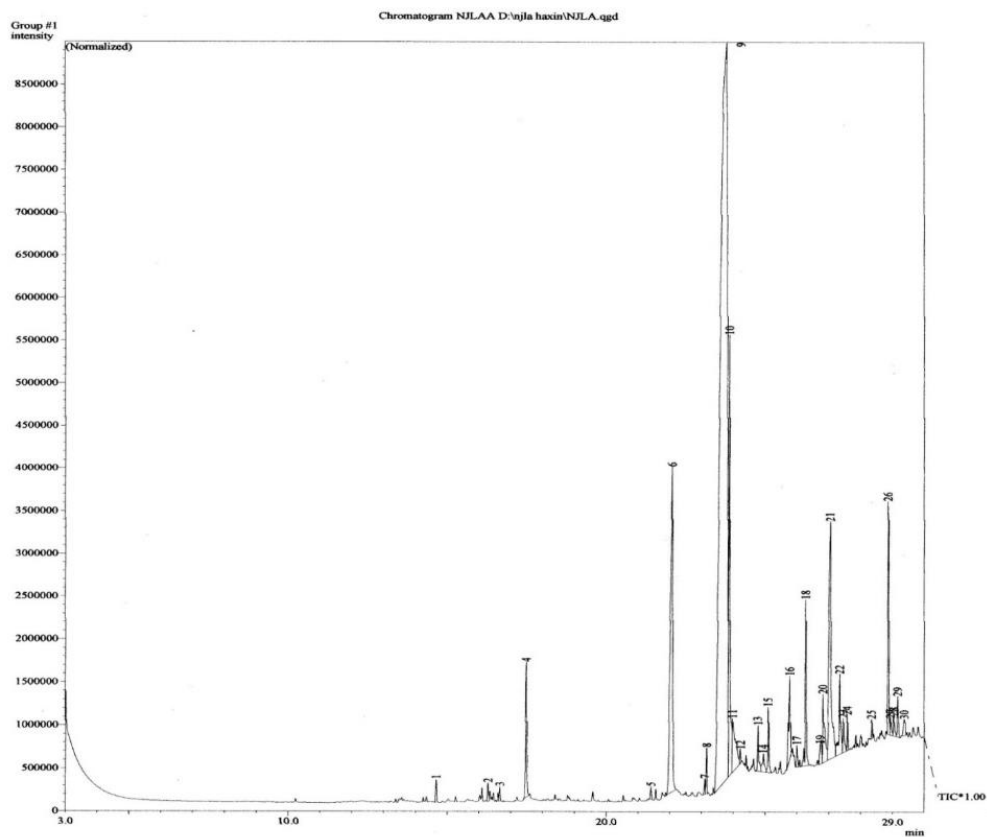
Fig. (5) and table (3) shown the results of the diagnosis of flaxseed oil that were extracted in the cold method by using the hexane solvent. The concentration of the oil components in the gas chromatography technique was estimated. It was observed 30 peaks, where unsaturated

fatty acids 69.14% was superior to linolenic acid by 65.77% (59.3% 9.12.15 octadecatrienoic acid and 6.47% Butyl 9, 12, 15 octadecatrienoic) as indicated at the peaks 9, 13 and 21 respectively. Saturated fatty acids were 11.47%, with octadecanoic acid C₁₈ superiority 6.47% as indicated in the peak 10. As it is noted from the table there is the presence of 1.11,13 heptadecatriene fatty acid, which consists of C₁₇. The results of the GC/MS flaxseed oil analysis showed that there were a number of compounds were particularly effective as antioxidants, as in table (2).

Table (2): Some bioactive components of flaxseed oil.

Name of compound	common name	Concentration%
Copaene	Sesquiterpenes	0.24
1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*,S*)]	Monoterpene	0.22
Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	β- Sesquiphellandrene	0.17
Diethyl Phthalate	Diethyl Phthalate	1.85
Ascorbic Acid	Ascorbic Acid	8.68
17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthr	β-Sitosterol	1.69
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(all-E)-	Squalene	0.32
Gamma.-Tocopherol	Gamma.-Tocopherol	2.62

UNIVERSITY OF BASRAH
 chromatogram of GC- MS



3 / 25

Fig.(5): Diagnosis results of GC / MS flax seed oil.

Table (3): Diagnosis results of GC / MS flax seed oil.

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	14.657	536792	0.24	Copaene
2	16.281	479714	0.22	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-
3	16.655	368113	0.17	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-
4	17.489	4121549	1.85	Diethyl Phthalate
5	21.408	305027	0.14	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene
6	22.073	19354337	8.68	l-(+)-Ascorbic acid 2,6-dihexadecanoate
7	23.100	340242	0.15	Methyl 12,15-octadecadienoate
8	23.155	1000913	0.45	8,11,14-Eicosatrienoic acid, methyl ester
9	23.796	130710879	58.65	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
10	23.872	14410537	6.47	Octadecanoic acid
11	23.975	4321858	1.94	1,E-11,Z-13-Heptadecatriene
12	24.217	356263	0.16	1,E-11,Z-13-Octadecatriene
13	24.764	1447631	0.65	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
14	24.942	980417	0.44	cis-13-Eicosenoic acid
15	25.093	1707461	0.77	Icosanoic anhydride
16	25.768	3764100	1.69	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthrene
17	25.990	591806	0.27	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
18	26.274	4505540	2.02	Dodecanoic acid, dodecyl ester
19	26.725	1109885	0.50	3-n-Butylthiophene-1,1-dioxide
20	26.819	3287629	1.48	Dodecanoic acid, undecyl ester
21	27.042	14412748	6.47	Butyl 9,12,15-octadecatrienoate
22	27.341	3448580	1.55	Dodecanoic acid, tetradecyl ester
23	27.450	1148083	0.52	13-Docosenamide, (Z)-
24	27.578	714643	0.32	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
25	28.340	408014	0.18	Hexadecanoic acid, dodecyl ester
26	28.861	5836577	2.62	gamma.-Tocopherol
27	28.942	542817	0.24	i-Propyl 9,12,15-octadecatrienoate
28	29.042	669431	0.30	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
29	29.167	1138190	0.51	Stigmast-5-en-3-ol, oleate
30	29.377	846117	0.38	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradeca-
		222865893	100.00	

The results of these study were close to that of some researchers. Popa *et al.* (2012) found that the GC/MS flaxseed oil content of saturated fatty acids was 11.01% distributed (6.58% palmitic acid and 4.43% stearic acid). The polyunsaturated fatty acids were 88.97% divided by 53.21% linolenic acid, 18.51% oleic acid and 17.25% linoleic acid respectively. Another study found the concentration of linolenic acid was 22.8 g.100g⁻¹ while octadecanoic was 1.3 g.100.g⁻¹, gamma tocopherol was 522 mg.100 g⁻¹ and ascorbic acid 0.5 mg. 100 g⁻¹ (Bernacchia *et al.*, 2014)

Evaluation of the performance of antioxidants in food systems

Fig. (5) shown the peroxide values of sunflower oil after adding different concentrations of mixture that contain (80% ethanol and flax seed oil) by 1: 1, at concentrations of mixture (0, 0.2, 0.4 and 0.6 mg.g⁻¹) were used. The change in the peroxide value was measured during the durations of storage (0, 7, 14 and 21) days at laboratory conditions. The obtained results were compared with the values of the peroxide of sunflower oil added to 0.05 mg. g⁻¹ BHT at the same durations. The peroxide value of the control sample was 1.21 meq.kg⁻¹ at 0 day of storage, rising to 27.25 meq.kg⁻¹ at the end of storage period (21 days), of storage period (21 days).

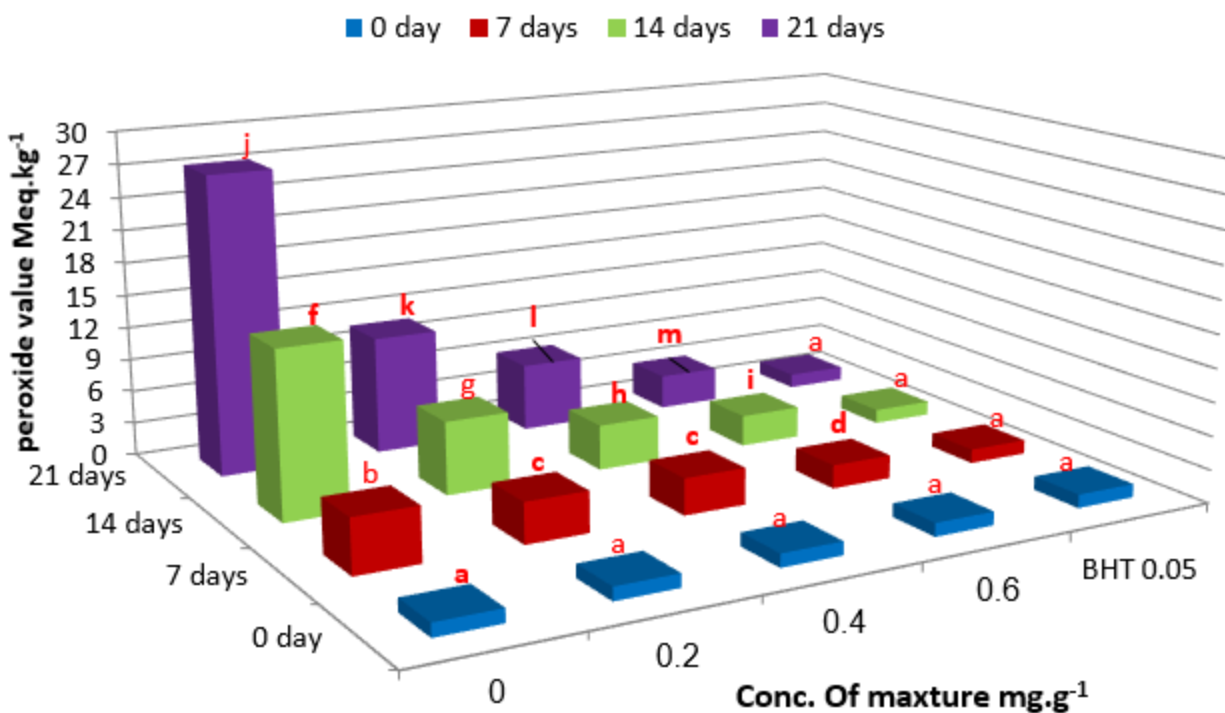


Fig. (5): Determination of the peroxide values of sunflower oil added to different concentrations of mixture (ethane and linseed oil) by 1: 1 and BHT during different storage periods. (The different letters refer to that there are significant differences between the rates at probability $P \leq 0.05$).

while the other concentrations showed a significant decrease $P \leq 0.05$ in the peroxide values for the other concentrations. A peroxide value for the concentration of 0.2 mg.g⁻¹ was 1.21 meq.kg⁻¹ at the 0 day of storage, reaching to 10.734 meq.kg⁻¹ at the end concentrations (0.4 and 0.6) mg. g⁻¹ showed the highest increase in peroxide values at the end of storage period (6.197 and 3.155) meq.kg⁻¹, respectively. The concentration of BHT added to the sunflower oil showed a slight change in the peroxide values during storage, as it was 1.25, 1.27, 1.28 and 1.3) for meq.kg⁻¹ for the mentioned periods.

The significant change in the peroxide values of sunflower oil, which has different concentrations of the mixture (alcohol extract: flaxseed oil), may be due to containment of mixture good concentrations of phenolic compounds that possessed bioactivity as shown in fig. (3) and fatty acid hexadecanoic acid ethyl ester, as well as the presence of ascorbic acid and gamma tocopherols with good concentrations (8.68 and 2.62%) respectively. Ascorbic acid and gamma tocopherols possessed antioxidant effect and their work as synergistic action, the tocopherols donate the hydrogen atom at the 6-Hydroxyl group to the chroman ring and single oxygen capture; this will produce radical of the tocopherol, with a strong reducing agent such as ascorbic acid, will act to reduce the tocopherol radical and convert it to tocopherol again. This process is called regenerated of tocopherol and thus the action of tocopherol continues as an antioxidant. Moreover, the ascorbic acid

played as scavenger or capture for free radicles, single oxygen and metals that caused oxidation in foods systems. Bernacchia *et al.* (2014) showed that the flaxseed content of gamma tocopherol was 522 mg.100g⁻¹ seeds and 0.5 mg.100g⁻¹ of ascorbic acid. Ivanova-Petropulos *et al.* (2015) noted that there were 41 terpenic compounds in sunflower oil, pumpkin seed oil and flaxseed oil. Flaxseed oil ranked third with 47.66% of total terpenes after sunflower oil pumpkin seed oil. Moreover, the results of the diagnosis showed a beta-sesquiphellandrene compound. Han *et al.* (2018) indicated that the effectiveness of ascorbic acid as an antioxidant in oils is higher than its presence in emulsifiers; the reason for that was attributed to the difference in polarization.

A number of studies have shown that copaene is a terpenic compound called sesquiterpenes, which is found naturally in the oils of some plants and has shown antioxidant activity, a study noted that copaene compound showed an inhibition activity for linoleic acid oxidation amounted 80% (Quintans *et al.*, 2013).

Ibraheim *et al.* (2012) explained that 1,3-cyclohexadiene, 5- (1,5-dimethyl-4-hexenyl)-2-methyl, [S- (R*, S*)] compound is monoterpene, and it is one of the compounds with antioxidant activity in tea tree oil. Zhao *et al.* (2010) pointed out that the compound of beta-sesquiphellandrene of antioxidants in turmeric. Arora *et al.* (2017) reported that cyclohexene, 1.5-dimethyl-4-hexenyl-6-methyl-[S- (R*, S*)] is called Beta-Sesquiphellandrene. It is one of the

sesquiterpene compounds that found in high concentrations in garlic oil and has antioxidant and cancer effects.

El-Sayed *et al.* (2015) found that the compound of diethyl hexyl phthalate obtained from *P. janthinellum* had an antioxidant activity amounted 77.99% at the concentration of 12 µg. ml⁻¹. A number of studies have also indicated that the beta-sitosterol compound is the common name of compound 17- (1, 5-Dimethylhexyl) -10,13-dimethyl-4 vinylhexadecahydrocyclopenta [a] phenanthr has an antioxidant effect (Gupta *et al.*, 2011). While Baskar *et al.* (2012) explained that the compound beta-sitosterol is a low to moderate antioxidant through its ability to reduce the level of reactive oxygen.

Tyagi & Agarwal (2017) found that hexadecanoic acid has an antioxidant effect. It was also noted that 2, 6, 10, 14, 18, 22-tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all-E) -the peak 24, which common name is squalene, were found in liver oil Shark and some seeds such as grape seeds and flaxseed. Arora *et al.* (2017) indicated that squalene is a tri-terpene and a natural antioxidant. Dessi *et al.* (2002) explained that the squalene has an ability to protect linolenic acid, linoleic, aracidone and decosahexanoic acid from oxidation during thermal treatment and ultraviolet radiation.

Conclusion

The results obtained by GC/MS for the extract of alcohol and flax seed oil have been observed to contain many important compounds that have a positive effect on the

preservation of human food and health. This benefits can be increased by combining these compounds (alcohol extract and oil) which lead to inhibition factors causing oxidation. It was concluded that the concentration of 0.6 mg.g⁻¹ at 1:1 mixing ratio showed a clear advantage in preserving the oxidation of sunflower oil compared to the standard sample.

Acknowledgements

Sincere thanks go to the Department of Food Science, College of Agriculture and University of Basrah for helping me complete our research.

Conflict interest

The authors declare that they have no conflict of interests.

References

- Amin, I. & Tan, S.H. (2002). Antioxidant activity of selected seaweeds. *Malays. J. Nutr.*, 8: 167-177. <http://nutriweb.org.my/mjn/publication/08-2/d.pdf>.
- Anwar, F. & Przybylski, R. (2012). Effect of solvents extraction on total phenols and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Sci. Pol., Technol. Aliment.*, 11(3): 293-301. PMID: 22744950.
- Arora, S.S. & Kumar, G. (2017). Screening and evaluation of bioactive compounds of *Cenchrus ciliaris* L. by GC-MS analysis. *Int. Res. J. Pharm.*, 8(6): 69-76. <https://doi.org/10.7897/2230-8407.08699>
- Barthet, V.J.; Klensporf-Pawlik, D. & Przybylski, R. (2014). Antioxidant activity of flaxseed meal components.

- Can. J. Plant Sci., 94(3): 593-602.
<https://doi.org/10.1139/cjps2013-018>
- Basch, E.; Bent, S.; Collins, J.; Dacey, C.; Hammerness, P.; Harrison, M.; Smith, M.; Szapary, P.; Ulbricht, C.; Vora, M. & Weissner, W. (2007). Flaxseed and flaxseed oil (*Linum usitatissimum*): A review by the natural standard research collaboration. J. Soc. Integr. Oncol., 5(3): 92-105.
<https://doi.org/10.2310/7200.2007.005>
- Baskar, A.A.; Al-Numair, K.S.; Gabriel, P.M.; Alsaif, M.A.; Muamar, M.A. & Ignacimuthu, S. (2012). β -sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer. J. Med. Food, 15(4): 335-343.
<https://doi.org/10.1089/jmf.2011.1780>.
- Bernacchia, R.; Preti, R. & Vinci, G. (2014). Chemical composition and health benefits of flaxseed. Austin J. Nutr. Food Sci., 2(8): 1045-1055.
https://www.researchgate.net/publication/292963467_Chemical_composition_and_health_benefits_of_flaxseed.
- Dessi, M.A.; Deiana, M.; Day, B.W.; Rosa, A.; Banni, S. & Corongiu, F.P. (2002). Oxidative stability of polyunsaturated fatty acids: effect of squalene. Eur. J. Lipid Sci. Techn., 104(8): 506-512.
[https://doi.org/10.1002/1438-9312\(200208\)104:8<506::AID-EJLT506>3.0.CO;2-1](https://doi.org/10.1002/1438-9312(200208)104:8<506::AID-EJLT506>3.0.CO;2-1)
- Devie, R.R.; Jayalekshmy A. & Arumghan C. (2007). Antioxidant efficacy of phytochemical extracts from defatted rice bran in the bulk oil system. Food Chem., 104(2): 658-664.
<https://doi.org/10.1016/j.foodchem.2006.12.014>
- Egan, H., Kirk, R.S. & Sawyer, R. (1981) Pearson's chemical analysis of foods. 8th Edn, Churchill Livingstone, London, New York
[https://www.scirp.org/\(S\(i43dyn45teexjx455qlt3d2q\)\)/reference/ReferencesPapers.aspx?ReferenceID=1749866](https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1749866)
- El-Beltagi, H.S.; Salama, Z.A. & El-Hariri, D.M. (2011). Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars, J. Gen. Appl. Plant Physiol., 33(3-4): 187-202.
http://www.bio21.bas.bg /ipp/gapbfiles/v-33/07_3-4_187-202.pdf.
- Elizabeth, N.R.G.; Annete, H.; Francisco, R. G.L.; Javier, F.I.P.; Graciela Z.G. & Alberto, J.G.I. (2007). Antioxidant and antimutagenic activity of phenolic compounds in three different color groups of common beans cultivars. Food Chem., 103, 521-527.
<https://doi.org/10.1016/j.foodchem.2006.08.021>.
- El-Sayed, O.H; Asker, M.M.S.; Shash, S.M. & Hamed, S.R. (2015). Isolation, structure elucidation and biological activity of Di- (2-ethylhexyl) phthalate produced by *Penicillium janthinellum* 62. Int. J. Chem. Tech. Res., 8(1): 58-66.
<https://www.researchgate.net/publication/281102694> .
- Franco, D.; Sineiroz, J.; Rubilar, M.; Sanchez, M.; Jerez, M.; Pinelo, M.; Costoya, N. & Nunez, M.J. (2008). Polyphenols from Plant Materials:

- Extraction and Antioxidant Power. *Elec. J. Environ. Agric. Food Chem.*, 7(8): 3210-3216.
https://www.researchgate.net/publication/228546690_Polyphenols_from_plant_materials_Extraction_and_antioxidant_power.
- Ganorkar, P.M. & Jain, R.K. (2013). Flaxseed- a nutritional punch. *Int. Food Res. J.*, 20(2): 519-525.
- Gupta, R.; Sharma, A.K.; Dobhal, M.P.; Sharma, M.C. & Gupta, R.S. (2011). Antidiabetic and antioxidant potential of β -sitosterol in streptozotocin-induced experimental hyperglycemia. *J. Diabetes*, 3(1): 29-37.
<https://doi.org/10.1111/j.1753-0407.2010.00107.x>.
- Hall, C.; Tulbek, M.C. & Xu, Y. (2006). Flaxseed. *Adv. Food Nut. Res.*, 51: 1-97.
[https://doi.org/10.1016/S1043-4526\(06\)51001-0](https://doi.org/10.1016/S1043-4526(06)51001-0).
- Han, H.; Yilmaz, H. & Gulcin, I. (2018). Antioxidant activity of flaxseed (*Linum usitatissimum* L.) shell and analysis of Its polyphenol contents by LC-MS/MS. *Rec. Nat. Prod.*, 12(4): 397-402.
<https://doi.org/10.25135/rnp.46.17.09.155>.
- Herchia, W.; Bahashwanb, S.; Sebeia, K.; Ben Salehc, H.; Kallela, H. & Boukhchina, H. (2015). Effects of germination on chemical composition and antioxidant activity of flaxseed (*Linum usitatissimum* L) oil. *Grasas y aceites*, 66(1): 1-8.
<https://doi.org/10.3989/gya.0463141>
- Hosseinian, F.S.; Muir, A.D.; Westcott, N.D. & Krol, E.S. (2006). Antioxidant capacity of flaxseed lignans in two model systems. *J. Am. Oil. Chem. Soc.*, 83: 835-840. <https://doi.org/10.1007/s11746-006-5034-x>
- Ibraheim, Z.Z.; Abdel-Mageed, W. M.; Dai, H.; Guo, H.; Zhang, L.; Jaspars, M. (2012). Antimicrobial antioxidant daucane sesquiterpenes from *Ferula hermonis* Boiss. *Phytother Res.*, 26(4): 579-86. <https://doi.org/10.1002/ptr.3609>.
- Ingale, E. & Shrivastava, S.K. (2011). Nutritional study of new variety of ground nut (*Arachis hypogaea* L.) JL-24 seeds. *African J. Food Sci.*, 5(8):490-498.
http://www.academicjournals.org/app/webroot/article/article1380017384_Ingale%20and%20Shrivastava.pdf.
- Iqbal, S.; Bhangar, M.I. & Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chem.*, 93: 265-272.
<https://doi.org/10.1016/j.foodchem.2004.09.024>.
- Ivanova-Petropulos, V.; Mitrev, S.; Stafilov, T.; Natalija Markova, N.; Leitner, E.; Lankmayr, E. & Siegmund, B. (2015). Characterisation of traditional Macedonian edible oils by their fatty acid composition and their volatile compounds. *Food Res. Int.*, 77(3): 506-514.
<https://doi.org/10.1016/j.foodres.2015.08.014>
- Mentes, O.; Bakkalbassi, E. & Ercan, R. (2008). Effect of the use of ground flaxseed on quality and chemical composition of bread. *Food Sci. Technol. Int.*, 19: 549-556.

- <https://doi.org/10.1177/1082013208097192>
- Nur -Syukriah, A.R.; Liza, M.S.; Harisun, Y. & Fadzillah, A.A.M. (2014). Effect of solvent extraction on antioxidant and antibacterial activities from *Quercus infectoria* (Manjakani). *Int. Food Res. J.*, 21(3).
- Popa, V.; Gruia, A.; Raba, D.; Dumbrava, D.; Moldovan, C.; Bordean, D. & Mateescu, C. (2012). Fatty acids composition and oil characteristics of linseed (*Linum usitatissimum* L.) from Romania. *J. Agroaliment. Proc. Technol.*, 18(2): 136-140. <https://www.researchgate.net/publication/268205116>.
- Quintans Jde, S.; Soares, B.M.; Ferraz, R.P.; Oliveira, A.C.; da Silva, T.B.; Menezes, L.R.; Sampaio, M.F.; Prata, A.P.; Moraes, M.O.; Pessoa, C.; Antonioli, A.R.; Costa, E.V. & Bezerra, D.P. (2013). Chemical constituents and anticancer effects of the essential oil from leaves of *Xylopija laevigata*. *Planta Med.*, 79: 123-130. <https://doi.org/10.1055/s-0032-1328091>
- Rababah, T.M.; Hettiarachy, N.S. & Horax, R. (2004). Total phenolics and antioxidant activities of feurgreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and ter-butylhrdroquinone. *J. Agric. Food Chem.*, 52: 5183-5186. <https://doi.org/10.1021/jf049645z>
- Richard, S.E.; Orcheson, L.J.; Seidl, M.M.; Luyengi, L.; Fong, H.H. & Thompson, L.U. (1996). Dose-dependent of mammalian lignans in rats and vitro from the purified precursor secoisolariciresinal diglucoside in flaxseed. *J. Nutr.*, 126: 2012-2019. <https://doi.org/10.1093/jn/126.8.2012>.
- Simbalista, R.L.; Frota, K.D.M.G.; Soares, R.A.M. & Areas, J.A.G. (2012). Effect of storage and processing of Brazilian flaxseed on lipid and lignan contents. *J. Ciênc. Technol. Aliment.*, 32(2): 122-143. <https://doi.org/10.1590/S0101-20612012005000037>.
- Slavova-Kazakova, A.; Karamac, M.; Kancheva, V. & Amarowicz, R. (2016). Antioxidant activity of flaxseed extracts in lipid systems. *Molecules*, 21: 17: 1-11. <https://doi.org/10.3390/molecules21010017>.
- SPSS. (2012) .Statistical packages of social sciences. Version (21). <https://spss.software.informer.com/21.0/>
- Sultana, B.; Anwar, F. & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14: 2167-2180. <https://doi.org/10.3390/molecules14062167>.
- Thompson, L.U.; Rickard, S.E.; Cheung, F.; Kenaschuk, E.O. & Obermeyer, W.R. (1997). Variability in anticancer lignan levels in flaxseed. *Nutr. Cancer*, 27: 26-30. <https://doi.org/10.1080/01635589709514497>.
- Toure, A. & Xueming, X. (2010). Flaxseed lignans: Sources, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefit.

