



Original Research Article

A Comparative Study on Proximate Composition, Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Linum usitatissimum* L. (flaxseeds)

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A B S T R A C T

Keywords

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antioxidant.

The present study was carried out to assess the proximate composition, phytochemical screening, antimicrobial and antioxidant activities of ethanol and chloroform extracts of *Linum usitatissimum* L. The antioxidant activity of extracts was determined by free radical scavenging activity using DPPH and hydrogen peroxide method. The median inhibitory concentration (IC₅₀) was calculated both DPPH and hydrogen peroxide method. According to DPPH method, the IC₅₀ value of ethanol extract of flaxseeds is 256.313 µg/ml while it is 33.718 µg/ml according to hydrogen peroxide method. An *in vitro* antimicrobial assay was carried out by disc diffusion method. The results showed that chloroform extracts are more effective than ethanol extract of seeds against various test microorganisms.

Introduction

Seeds are the eggs that contain nutrients to nourish the growth of a new plant. Therefore, their high nutrient content shouldn't come as a surprise. Seeds are an excellent 'nutrition package' with their gold mine of healthy minerals and their niacin and folic acid contents. Seeds are considered to be the best sources of iron and zinc. In fact, one ounce of pumpkin seeds contains almost twice as much iron as three ounces of skinless chicken breast and they provide more fiber per ounce than nuts. They are also good sources of

protein (Hornick & Yarnell, 2013). Since prehistoric times, plants have been used as a source of treatment for diseases. The demand for herbal medicines has got increased due to their natural origin and lesser side effects. Several plants or their parts have been exploited for this purpose. The medicinal value of these plants is attributed to the presence of some chemical substances (bioactive non-nutrient plant compounds) which produce a definite physiological action on human body (Edeoga et al., 2005). These

chemical substances are called phytochemicals (Sood et al., 2012). The word phyto is a Greek word 'phyto' meaning plant. Their function is to provide odor to the plant (terpenoids), pigmentation (tannins and quinines) and flavor (capsacin) (Mallikharjuna et al., 2007). They also help in natural defense mechanism of plants. These bioactive compounds are responsible for antimicrobial activity of plant extracts *in vitro*. Some common examples of phytochemicals are flavonoids, alkaloids, saponins, glycosides, terpenoids, tannins, sterols and carbohydrates (Sood et al., 2012).

Antioxidants are the compounds that inhibit or delay the oxidation of molecules by inhibiting the initiation or propagation of oxidizing chain reaction (Velioglu et al., 1998; Shahidi & Ho, 2005; Badarinath et al., 2010). Many natural antioxidants are present in plant materials in combinations involving a number of different compounds (Kosińska & Karamać, 2006). Depending on the type and source of the material used, the mode of action of natural antioxidants involves multiple mechanisms of action (Shahidi, 2000). Phenolics present in seeds provide protection against harmful free radicals and have been known to reduce the risk of certain types of diseases such as cancer, coronary heart diseases (CHDs), cardiovascular diseases (CVDs), stroke, atherosclerosis, osteoporosis, inflammation and other neurodegenerative diseases associated with oxidative stress (Hertog et al., 1993; Ness & Powles, 1997; Joseph et al., 1999; Mazza et al., 1999; Surh, 2003). It is believed that the regular consumption of dietary antioxidants may reduce the risk of several serious diseases. The addition of synthetic antioxidants (butylated hydroxyanisole, BHA and

butylated hydroxytoluene, BHT) in lipid food is often limited due to their safety and perceived carcinogenicity. On the other hand, the use of plant-based compounds in foods and preventive medicines are gaining a great deal of interest because of their potential health benefits (Ghiselli et al., 2000; Hussain et al., 2008). It is well accepted that plants are the richest source of antioxidants. Phenolic acids occurring in plants, primarily in bound form as conjugates, with sugars, fatty acids, or proteins act as effective natural antioxidants (Shahidi & Wanasundara, 1997; Wyen et al., 2000).

Plant phenolics have multiple biological functions such as antioxidant, anti-inflammatory, anti-cancer and antimicrobial activities (John & Grohmann, 2001). In view of the beneficial effects and vital role that natural antioxidants can play in human health, a greater demand currently exists for their isolation from more and more plants and agro-waste materials using some effective extracting techniques (Anwar et al., 2010). For many thousand years, plant extracts have been used for a wide variety of purposes (Jones, 1996) and researchers have aimed at identifying and validating plant-derived substances for the treatment of various diseases. It has been estimated that more than 25% of modern medicines are directly or indirectly derived from the plants. From the past few years, there has been a revival of interest in plant derived drugs from plants which is due mainly to the current widespread belief that 'green medicine' is safe and more dependable than much costlier synthetic drugs, most of which have side effects (Maity et al., 2009; Gheisari et al., 2011). The authentic effects of any plant extract on a particular human disorder prompt us to screen indigenous plants for their antioxidant and

antimicrobial activity (Agrawal & Srivastava, 2008).

A large number of secondary metabolites are produced by plants which represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. The major global health problem is that microorganisms are developing resistance against antibiotics and this compels the researchers and scientists to develop new drugs of plant origin (Khoobchandani et al., 2010). Antimicrobial compounds derived from plants might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes (Stein et al., 2005). Thus, there is a need to evaluate the herbs scientifically for their antimicrobial activity against the antibiotic resistant microorganisms in order to develop new drug of plant origin (Simoes et al., 2009; Gulfraz et al., 2011). In many studies, leaves, roots, flowers, whole plants and stems have been examined for useful phytochemicals, while few reports refer to seeds as source for pharmaceuticals. A large number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins and flavonoids. These chemical compounds probably protect the seed from microbial degradation until the conditions are favourable for germination (Cai et al., 2004; Komutarin et al., 2004). Given the reactivity of these compounds, it is surprising that seeds and their extracts are not frequently utilized in medicinal applications (Borchardt et al., 2008).

Flaxseeds, scientifically known as *Linum usitatissimum* L. (Amin and Thakur, 2014) belong to Linaceae family (Ganorkar & Jain, 2013). In the field of functional

foods, flaxseed is emerging as one of the key sources of phytochemicals (Shahzad et al., 2006). These phytochemicals (phenolic acids, cinnamic acids, flavonoids and lignins) are antioxidants and affect the cell growth and viability (Amin and Thakur, 2014). Flaxseed is an essential source of high quality protein and soluble fiber and has considerable potential as a source of phenolic compounds (Oomah, 2001; Shahzad et al., 2006; Amin and Thakur, 2014). Both the soluble as well as insoluble fiber is present in flaxseed with insoluble fiber contributing to about one-third of the total fiber content.

Czemplik et al. (2011) suggest that the extracts derived from new genetically modified (GM) flax types might be the effective source of antibacterial compounds and the promising alternative to antibiotic therapy. Czemplik et al. (2011) evaluated the activity of GM seedcake extract against the bacteria of clinical relevance- *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* which are known to be the cause of antibiotic-resistant infections. They also determined the minimal inhibitory concentration (MIC) and indicated the bacteriostatic or bactericidal action. The flavonoids (kaempferol and quercetin) present in flaxseeds inhibit DNA synthesis in *Proteus vulgaris* or RNA synthesis in *Staphylococcus aureus* (Mori et al., 1987). This mixture of flavonoids, phenolic acids and lignans is proposed to be more effective antibacterial agent than pure, single compound. The extracts of GM seedcakes may be more beneficial than isolated constituents since a bioactive individual component can change its properties in the presence of other compounds in the extracts (Borchers et al., 2004). The synergy of phenolic compounds might be one of the

explanations of that phenomenon. According to the experiments including antibacterial activity of propolis, the mixture of phenolic compounds was proved to be effective against many bacterial strains (Kosalec et al., 2005). Czemplik et al. (2011) in their study modified the flaxseeds genetically so that the composition and proportion of phenylpropanoids in seedcake extracts could be modified to make them effective anti-infectious agents.

Al-Bayati (2007) demonstrated antibacterial activity of four different extracts of flaxseeds against Gram positive and Gram negative bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* using agar-well diffusion method and comparing their antibacterial activities with the antibiotics Ampicillin, Cefalexin, Chloramphenicol and Tetracycline. Petroleum ether extract demonstrated significant inhibitory effects against all tested bacteria using all extract concentrations compared with used antibiotics except chloramphenicol, the clearest activity was seen against *K. pneumoniae* using the extract concentration of 50 mg/cm³. Ethanol extract possessed considerable antibacterial activities against the pathogenic bacteria, the highest inhibitory effect was observed against *B. cereus* using the extract concentration of 200 mg/cm³ followed by aqueous extract which revealed good inhibitory action against *Ps. aeruginosa* using the same concentration. The weakest extract used was the chloroform extract which was only active against *S. aureus*. Thin-Layer Chromatography (TLC) of petroleum ether extract indicated the presence of active fatty acids. Petroleum ether extract activity can be due to its content of palmitic acid,

linoleic acid and oleic acid as indicated by the thin-layer chromatography (TLC) techniques. These acids are the main essential fatty acids in linseed oil and are reported to contain antibacterial properties (Dilika et al., 2000; Mokbel & Hashinaga, 2005; Al-Bayati, 2007).

The present study was carried out to comparatively study the proximate composition, phytochemical screening, antioxidant and antimicrobial activities of *Linum usitatissimum* L. (flaxseeds).

Materials and Methods

Materials

Sample.

Flaxseeds were obtained from the local market of Srinagar, Jammu & Kashmir India (34°5'31" N/ 74° 48' 7" E). The seeds were washed, cleaned of extraneous matter and shade dried completely.

Microbial cultures

Microorganisms were obtained from ARS Culture Collection, Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, USA. The following microorganisms were used for the study: *Salmonella typhi*, *Enterococcus*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus subtilis*.

Media.

Nutrient agar was procured from Hi-Media.

Methods

Moisture content was determined by AOAC method 930.04 (AOAC, 1995).

Ash content was determined at 550°C (Elinge et al., 2012). Crude fat was determined by BIOSOX using petroleum as a solvent. Crude nitrogen was determined by Kjeldahl method (Elinge et al., 2012) and crude protein determined by using the formula (1) (AOAC, 1995; Alfawaz, 2004).

$$\text{Crude protein} = \text{Crude nitrogen} \times 6.25 \quad (1)$$

Available carbohydrate (CHO) and energy value were determined by using the formulae (2) and (3) as given below;

$$\text{CHO} = 100 - (\% \text{ash} + \% \text{crude protein} + \% \text{crude lipid} + \% \text{fiber}) \quad (2)$$

$$\text{Energy (kcal)} = [(\% \text{CHO} \times 4) + (\% \text{CP} \times 4) + (\% \text{CL} \times 9)] \quad (3)$$

Where, CHO, CP and CL are the carbohydrate, crude protein and crude lipid, respectively (Hassan et al., 2008; Elinge et al., 2012). Crude fiber was determined by following a gravimetric procedure of AOAC (AOAC Method, 920.86, 1985). Ash content was determined by incineration at 550°C in a muffle furnace for 6 hours (AOAC Method, 920.86, 1985). All of the samples were run in triplicates.

Preparation of extracts

The flaxseeds were ground and briefly, 10 g ground sample was mixed with six times ethanol and chloroform and extraction was performed at 4°C for 24 hours. The process was repeated three times and the supernatant collected and filtered through a Whatman No. 1 filter paper. The solvent from the extracts was then evaporated under reduced pressure using a rotary vacuum evaporator (BUCHI ROTARY EVAPORATOR, MODEL R-124) (Song et al., 2010).

Phytochemical screening

Qualitative phytochemical screening was done for evaluation of major phytochemical constituents such as tannins, saponins, flavonoids, sterols, terpenoids, carbohydrates, phenols, proteins and amino acids, alkaloids and glycosides using standard procedure of analysis (Olayinka & Anthony, 2011; Kaur & Arora, 2011; Sharma et al., 2011).

Estimation of total phenol contents

The total phenolic content in ethanol extract was estimated by Folin-Ciocalteu reagent (FC reagent) as a method described by Singleton & Rossi (1965). Gallic acid stock solution (1000µg/ml) was prepared by dissolving 100 mg of Gallic acid in 100 ml of ethanol. Various dilutions of standard Gallic acid were prepared from this stock solution. Folin-Ciocalteu reagent (FC reagent) was prepared by mixing Folin's reagent with phenol reagent (1:1) and diluted 1:1 in distilled water, before use.

Calibration curve (Figure 1) was plotted by mixing 1 ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml of Gallic acid solutions with 5.0 ml of Folin-Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75g/l). The absorbance was measured after 30 minutes at 20°C at 765 nm. One ml of ethanol extract (1.0 g/100 ml) was mixed with the same reagents as did in construction of calibration curve, and after 1 hour, the absorbance was measured for the determination of total phenolic compound in both the extracts separated by using formula:

$$C = C1 \times V/m$$

Where, C= Total content of phenolic

compounds in mg/g, in GAE (Gallic acid equivalent); C_1 = the concentration of Gallic acid established from the calibration curve in mg/ml; V = the volume of extract in ml; m = weight of plant extract in grams.

Evaluation of antioxidant activity

Free radical scavenging activity using DPPH method

The free radical scavenging activity of different extracts was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method (Kaur et al., 2008). Briefly, 0.1 mM solution of DPPH in ethanol and 1.5 ml of this solution was added to 0.5 ml of extract solution in ethanol at different concentrations (50-300 μ l/ml). The mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer. A blank without DPPH was used to remove the influence of color of the extracts and an ethanolic solution of DPPH was used as a negative control. Ascorbic acid was used as a reference. All of the measures were carried out in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$DPPH \text{ scavenging effect (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

Where, A_0 is the absorbance of negative control and A_s is the absorbance of sample, respectively.

Free radical scavenging activity using hydrogen peroxide

The free radical scavenging activity of different extracts was determined by using hydrogen peroxide method (Mohamad et

al., 2010). An aliquot of 0.6 ml hydrogen peroxide (43mM) and 1.0 ml of various concentrations of extracts prepared using phosphate buffer (200-400 μ g/ml) were mixed followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4). The resulting solution was kept for 10 minutes and the absorbance was recorded at 230 nm. All measures were repeated triplicate. For each concentration, mixture without sample was taken as a control and a mixture without hydrogen peroxide was taken as a blank. Ascorbic acid was used as a standard compound. The percentage scavenging activity of hydrogen peroxide was calculated as:

$$Scavenging \text{ activity (\%)} = \frac{A_0 - A_s}{A_s} \times 100$$

Where, A_0 is the absorbance of negative control, A_s is the absorbance of sample respectively.

Antimicrobial assay

The *in vitro* antimicrobial activity was carried out by disc diffusion method (Amin et al., 2013). The microbial suspension of different dilutions (10^0 , 10^{-1} , 10^{-2} and 10^{-3}) was prepared in 0.85% saline and 100 μ l of 10^0 and 10^{-3} dilutions were spread evenly on separate Nutrient Agar (NA) Plates (Amin et al., 2013). Then the blank sterile discs containing 20 μ l of the essential oil were placed on these PDA plates. The experiment was carried out in triplicates for each of the samples. The plates were then incubated in an incubator at 37°C for about 24 hours and the plates were observed for zone of inhibition, and the diameter of the zone of inhibition was calculated for all the plates. Antimicrobial activity was evaluated by measuring the diameter of the zone of

inhibition around the extract (Amin et al., 2013; Duyilemi & Lawal, 2009). The assay was repeated in three replicates and the results were recorded as mean \pm SD (Bhat et al., 2008; Nayik et al., 2013).

Statistical analysis

All of the measurements were performed in triplicates. The data is given either as mean \pm SD or mean \pm SEM. Correlation analysis was performed between the antioxidant activity values. P values less than 0.05 were considered to be statistically significant. One-way ANOVA diagnosis of the data was done using SPSS software version 20 (Bhat et al., 2008; Amin and Blessing, 2014; Nayik et al., 2013).

Results and Discussion

The proximate composition of flaxseeds is presented in Table 1. The moisture content of flaxseeds was found to be 7.82% and is in the range as reported by Hettiarachy et al. (1990) and is close to 7.7% as reported by Canadian Grain Commission (2009) and 7.2% reported by Eggie (2010) and is less than that reported by USDA Database (1998). Ash content of flaxseeds was $3.8 \pm 0.12151\%$ and is close to the value of 3.4%, 3.3% and 3.5% reported by Canadian Grain Commission (2009), Eggie (2010) and USDA Database (1998), respectively. Protein content was found to be $28.86 \pm 0.02646\%$ which is in the range of 26.9-31.6% reported by Hettiarachy et al. (1990) and much higher than the values of 22.9%, 23.1%, 19.5% and 10% as reported by Canadian Grain Commission (2009), Eggie (2010), USDA Database (1998) and Amin and Thakur (2014),

respectively. Crude fat content of flaxseeds was 33.43 ± 0.01528 which is found to be in the range of 31.9-37.8% as reported by Hettiarachy et al. (1990) and close to the value of 34% as reported by USDA Database (1998) and much lower than 45.2% reported by Canadian Grain Commission (2009). The crude fat content of flaxseeds was found to be much higher than 27.3% as reported by Eggie (2010). Crude fiber (5.610 ± 0.5373) obtained is close to the value reported by Srivastava (2012).

Phytochemical analysis of samples is presented in Table 2. It was contended from the results that tannins, flavonoids, terpenoids, phenols and proteins and amino acids were present in ethanol extracts of flaxseeds while as it did not contain saponins, sterols, and glycosides. Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku & Akumefula, 2007). Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo, 2009). Flavonoids are most commonly known for their antioxidant activity and act as transformers which modify the body's reactions to carcinogens, viruses and allergens. They possess anti-cancerous, anti-inflammatory, anti-microbial and anti-allergic activity (Balch & Balch, 2000) and may, therefore be useful in therapeutic roles (Jisik et al., 1992). Terpenoids are antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Cichewicz & Thorpe, 1996). Rao & Sung (1995) has reported the hemolytic property and induced toxicity effect of saponins while some others have reported the

Table.1 Proximate composition of flaxseeds

Parameter	Amount
Moisture content	<i>Wet Basis</i> = 6.89%
	<i>Dry Basis</i> = 7.82%
Ash	3.800 \pm 0.12151%
Crude protein	28.86 \pm 0.02646%
Crude fat	33.43 \pm 0.01528%
Crude fiber	5.610 \pm 0.53731%
Carbohydrate	67.050%
Energy Value	667.510 Kcal

Values are represented as Mean \pm SD

Table.2 Results of phytochemical screening of ethanol extracts of seeds

S. No.	Phytochemicals	
1.	Tannins	+
2.	Saponins	-
3.	Flavonoids	+
4.	Sterols	-
5.	Terpenoids	+
6.	Carbohydrates	N
7.	Phenols	+
8.	Proteins & Amino acids	+
9.	Alkaoids	N
10.	Glycosides	-

(+) confirms presence and (-) confirms absence of above shown category of phytoconstituents

Table.3 Total phenolic content of ethanol extracts of seeds

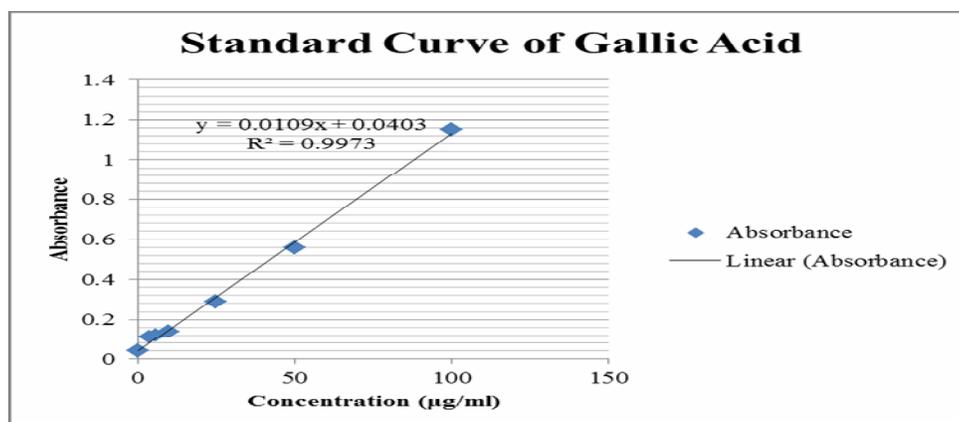


Figure.1 Calibration curve of Gallic acid

Sample	Total phenolics (%)
Flaxseeds	21.52 ± 0.13968

Table.4 Percentage scavenging of 1,1-Diphenyl-2-Picrylhydrazyl radical

Concentration of extract (µg/ml)	Percentage scavenging of DPPH radical	
	Ascorbic acid	Flaxseeds
100	48.08 ± 0.13667	32.33 ± 0.31424
200	58.59 ± 0.24230	32.95 ± 0.43075
300	75.45 ± 0.31533	56.88 ± 0.31424
400	76.89 ± 0.35796	76.89 ± 0.43075
500	87.89 ± 0.37248	82.53 ± 0.17954

Values are the average of triplicate experiments and represented as mean ± S.E.M

Table.5 Percentage scavenging of Hydrogen Peroxide

Concentration of extract (µg/ml)	Percentage scavenging of H ₂ O ₂ radical	
	Ascorbic acid	Flaxseeds
100	51.56 ± 0.14530	55.95 ± 0.02333
200	61.19 ± 0.50083	60.23 ± 0.07265
300	77.15 ± 0.01453	74.42 ± 0.01764
400	78.80 ± 0.1155	77.72 ± 0.02404
500	88.46 ± 0.2082	86.61 ± 0.02404

Values are the average of triplicate experiments and represented as mean ± S.E.M

Figure.2 Graph showing DPPH scavenging activity

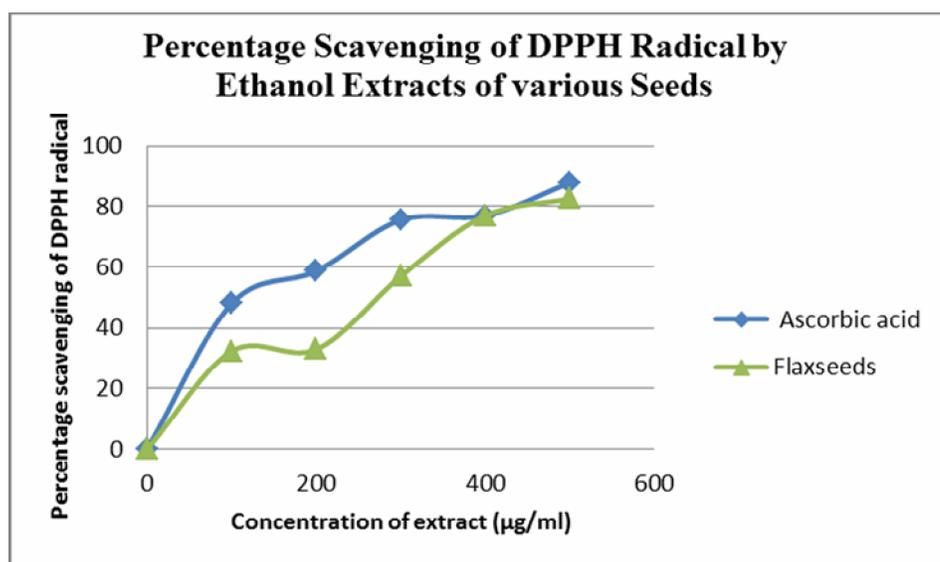


Figure.3 Graph showing Hydrogen Peroxide Scavenging Activity

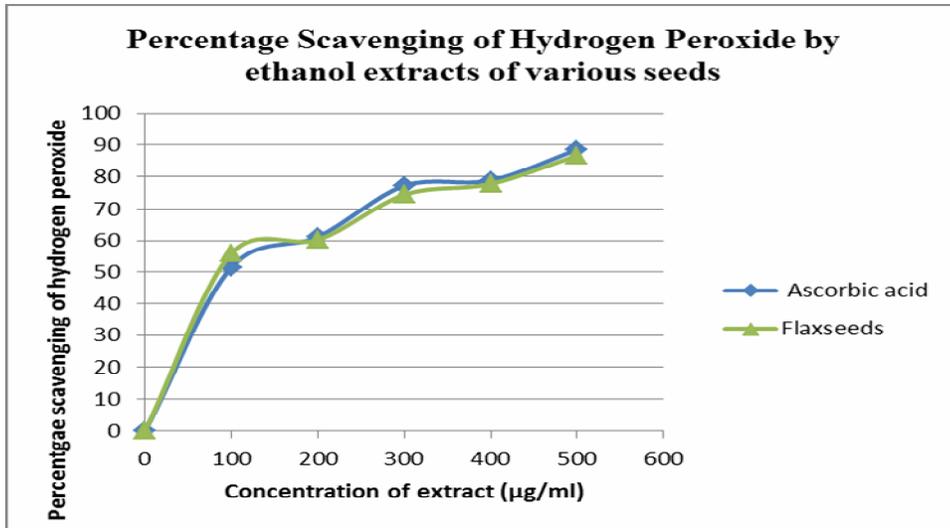


Figure.4 Median Inhibitory Concentration (IC₅₀) of ethanol extracts of flaxseeds based on DPPH method

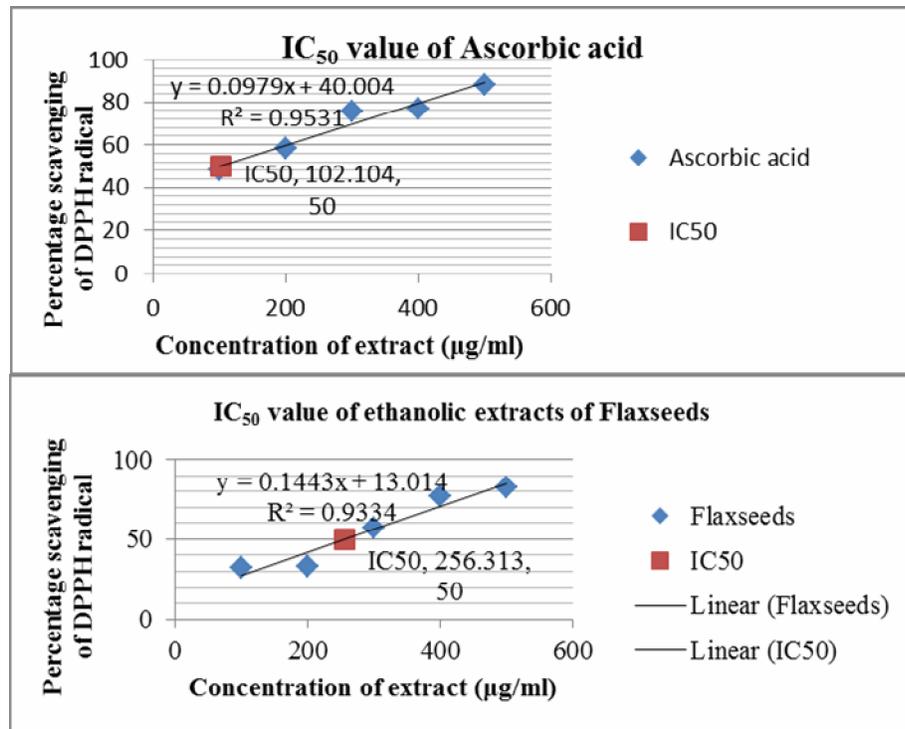


Figure.5 Median Inhibitory Concentration value (IC₅₀) of ethanol extracts of seeds based on H₂O₂ method)

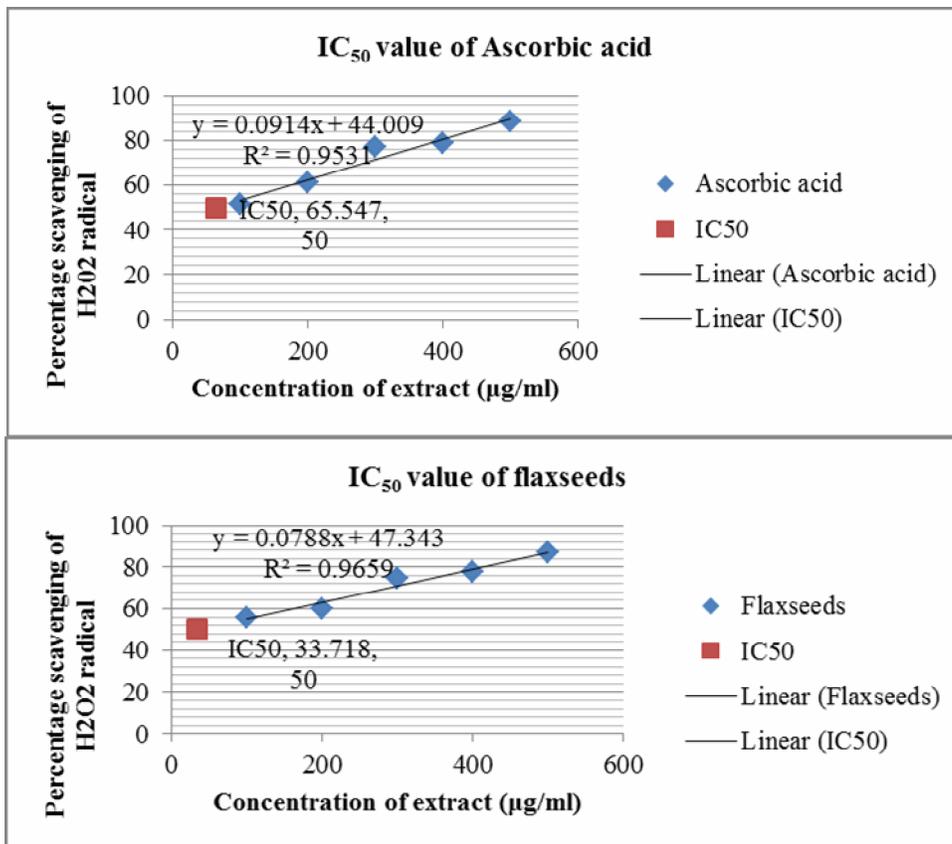


Table.6 Table showing zones of inhibition of ethanolic extracts of flaxseeds

Microorganism	Zone of inhibition (mm)
<i>Salmonella typhi</i>	34.58 ± 1.6782
<i>Enterococcus</i>	26.24 ± 1.3280
<i>Escherichia coli</i>	-
<i>Bacillus subtilis</i>	6.72 ± 0.2082
<i>Staphylococcus aureus</i>	7.34 ± 1.42156

Table.7 Table showing zones of inhibition of chloroform extracts of flaxseeds

Microorganism	Zone of inhibition (mm)
<i>Salmonella typhi</i>	29 ± 4.58258
<i>Enterococcus</i>	23.34 ± 0.57735
<i>Escherichia coli</i>	7.2 ± 0.00082
<i>Bacillus subtilis</i>	7.17 ± 0.28868
<i>Staphylococcus aureus</i>	17.67 ± 1.52753

expectorant action (Ayoola & Adeyeye, 2010), anti-mutagenic and anti-tumor activities and can lower the risk of human cancer by preventing the proliferation of cancer cells (Nafiu et al., 2011). Plants containing saponins are used to heal wounds (Okwu & Josiah, 2006) because saponins have the ability to precipitate and coagulate red blood cells (RBCs) (Sood et al., 2012). Glycosides were present only in ethanol extracts of quince seeds, musk melon seeds and bottle gourd seeds indicating that the ethanol extracts of these seeds may prove beneficial in reducing inflammation, protecting against endotoxemia and may be used in cardiac treatment of congestive heart failure since glycosides possess such properties (Matsumori et al., 1997; Sood et al., 2012). Alkaloids were absent indicating the seeds are safe to consume. Alkaloids are toxic as they are stimulatory in nature thereby leading to excitation of cells and neurological dysfunction (Nayak, 2012). Since the seeds under study contain phytochemicals, it may, therefore be suggested that the seeds may be used for treatment of congestive heart failure, cancer, lowering blood cholesterol in blood, healing of wounds, endotoxemia and reduction of inflammation.

Total phenolic content of the samples was calculated using standard calibration curve of Gallic acid curve (standard curve equation: $y = 0.0109x + 0.0403$ and $R^2 = 0.9973$) (Figure 1) and is presented in Table 3. The phenolic content in ethanol extract of flaxseeds was found to be $21.52 \pm 0.13968\%$. The values obtained are expressed as GAE/g of extract (GAE = Gallic Acid Equivalent). Total polyphenol contents were estimated with Folin-Ciocalteu colorimetric method. Folin-Ciocalteu reagent is a mixture of

phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$) and is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum. The color produced, the absorption of which is maximum in the wavelength range of 700 nm to 750 nm, is proportional to the amount of polyphenols present in plant extracts (EI-Haci et al., 2009).

Antioxidant activity of ethanol extracts of seeds were determined by DPPH method and hydrogen peroxide method. The DPPH is a stable organic free radical with an absorption maximum band around 515-528 nm (Stanković, 2011) and is widely used for evaluation of antioxidant potential of compounds. The results obtained for antioxidant assay by both the methods are shown in Tables 4 & 5 and Figures 2 & 3. The absorbance decreases as a result of a color change from yellow to purple as the radical is scavenged by antioxidants through donation of hydrogen to form stable DPPH-H molecule (Conforti et al., 2009).

The free radical scavenging activity is also expressed by the antioxidant concentration required for a 50% DPPH reduction (IC_{50}). The IC_{50} calculated by both the methods are shown in Figures 4 and Figure 5. According to DPPH method, the IC_{50} value of ethanolic extract of flaxseeds is 256.313 $\mu\text{g/ml}$ which would mean that 256.313 $\mu\text{g/ml}$ of ethanolic extract of flaxseeds is required to cause 50% scavenging activity of DPPH radical. While as according to hydrogen peroxide method, the IC_{50} value of ethanol extract of flaxseeds is 33.718 $\mu\text{g/ml}$. This means 33.718 $\mu\text{g/ml}$ of ethanolic extract of flaxseeds is required to cause 50% scavenging activity of H_2O_2 radical.

The zones of inhibition shown by ethanolic and chloroform extracts of seeds are presented in Tables 6 & 7, respectively. From the tables, it is quite evident that ethanolic extract of flaxseeds is effective against *Salmonella typhii*, *Enterococcus*, *Bacillus subtilis* and *Staphylococcus aureus* but did not show any antimicrobial activity against *Escherichia coli*. It can be seen from Table 7 that chloroform extracts are more effective than ethanoic extract of seeds against microorganisms. Chloroform extracts of flaxseeds showed antimicrobial activity against all the five microorganisms (*Salmonella typhii*, *Enterococcus*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*).

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