IJPSR (2023), Volume 14, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 November 2022; received in revised form, 24 December 2022; accepted 01 May 2023; published 01 July 2023

A COMPARATIVE EVALUATION OF BIOACTIVE PROPERTIES OF RAW, FERMENTED, AND GERMINATED DEOILED FLAXSEED FLOUR EXTRACTS BY DIFFERENT SOLVENTS

S. Ghosal, S. Sengupta and J. Bhowal *

School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah - 711103, West Bengal, India.

Keywords:

Deoiled flaxseed flour, Phytochemicals, Antioxidant, HPLC, FT-IR, Antibacterial activity, Antiarthritic activity

Correspondence to Author:
J. Bhowal

Assistant Professor,
School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah - 711103, West Bengal, India.

E-mail: jayatibhowal@gmail.com

ABSTRACT: The importance of flaxseeds in the food sector has been highly appreciated due to the presence of phytochemicals and antioxidants. The aim of the present study was to focus on the effect of different solvents used for the extraction of bioactive phytochemicals from raw, fermented, and germinated deoiled flaxseed flours and also evaluated their antioxidant activities. The phenolic content and flavonoid content of 80% ethanolic extract of germinated deoiled flaxseed flour were greater than the raw and fermented deoiled flaxseed flour. This study also focused on the identification of polyphenol compounds present in flaxseed flour extract which varies due to fermentation and germination processes and showed that germinated deoiled flaxseed flour extracts contained the highest number of polyphenol compounds. The germinated flaxseed flour extract was found to be exhibited at the highest zone of inhibition against *S. aureus*, *B. substilis* and *E. coli*. In our results, it was found that germinated deoiled flaxseed flour extract has higher antiarthritic activities. The study established that both the fermentation and germination processes help in the improvement of the phenolic content, flavonoid content, and antioxidant activities. This information can be adopted for the fortification of food in the food sector.

INTRODUCTION: Linseed (*Linum usitatissimum L.*) belongs to the family *Linaceae* and has recently gained popularity as a potential food additive due to its nutritional value [1]. Flaxseed is a source of phenolic compounds and flavonoids which are excellent natural antioxidants [2]. Germination of seed is a technological application widely used for its ability to improve the nutritional value of seed by causing desirable changes in nutrients, textures and secondary metabolite concentrations, which are considered beneficial as antioxidants [3, 4].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(7).3374-91

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(7).3374-91

The germination of flaxseed can be utilized in food technology and the nutraceutical industry [1]. Natural fermentation or proteolytic fermentation can occur naturally without starter culture under alkaline conditions in oil seeds or legumes, mainly due to species of the *Bacillus* genus or *E. coli* or *S. aureus*, or *Aspergillus* species [5]. The micro-organisms involved in this fermentation are not originated from the seeds; they are introduced through the air, water, and leaves used in wrapping [6].

Different seeds are also enhanced with phenol, flavonoid, and antioxidant properties through natural fermentation [7]. In Africa, fermented African seeds were used as a condiment to soup to increase their nutritional values [6]. Recently, interest has increased in to use of naturally occurring antioxidants in foods to replace synthetic

antioxidants [8]. In early studies, flaxseed was shown to possess a powerful antioxidant system [9]. Elderly people often have arthritis. Several drugs are used to treat arthritis disorders, including nonsteroidal anti-inflammatory drugs (NSAIDs) that have gastrointestinal side effects [10]. Researchers have found flaxseed oil to be effective for treating inflammatory conditions like rheumatoid arthritis [11].

Solvent extraction is the most often used technique for the isolation of antioxidant compounds from flaxseeds [12]. The best solvents for extracting polyphenols from flaxseeds are aqueous mixtures containing ethanol, methanol, and acetone [12].

The phytochemical analysis of the ethanol extract of flaxseeds found the presence of tannins, flavonoids, terpenoids, phenols, proteins, and amino acids; however, no saponins, sterols, or glycosides [13]. The researcher found that fermentation and germination improved the phytochemical composition of *M. oleifera* seed flour methanolic extract [14].

Many publications have been reported on the antioxidant and antimicrobial activities of flaxseed and deoiled flaxseeds. No reports on the phytochemical analysis and bioactive properties of the different solvent extracts of fermented and germinated deoiled flaxseed flour are available. Therefore, the study aimed to determine the best solvents for extracting phytochemicals and quantitative analysis of total phenols, flavonoids, and antioxidant activities of raw, fermented and germinated deoiled flaxseed flour. This study also aimed to determine the polyphenol and flavonoid compounds present in raw, fermented and germinated deoiled flaxseed flour extracts by HPLC and FT-IR methods.

MATERIALS AND METHODS: Flaxseeds were collected from the local market of Kolkata, West Bengal, India. All chemicals and solvents were procured from Sigma-Aldrich (MA, USA) and Merck (India). Standards like ascorbic acid, gallic acid, catechin, butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), α-tocopherol and diclofenac sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO, USA). For antioxidant assay ferrozine, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 3, 5-triphenyl-1, 3, 4-triaza-2-azoniacyclopenta-1, 4-diene chloride (TPTZ), and 2, 2'-azino-bis-3 ethylbenzothiazoline-6-sulfonic acids (ABTS) were purchased from Sigma (St. Louis, MO).

Standards of phenolic acids like lignan, gallic acid, Protocatechuic acid, vanillic acid, catechin, quercetin, rutin, ferulic acid, kaempferol and anthocyanin were procured from Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade water was purchased from Lichrosolv, Merck (Germany) (Batch# DL4DF41569), acetonitrile was purchased from Spectrochem (Lot# 070101) and trifluoroacetic acid was purchased from SRL (Batch# 65417(2029282)76-05-1, HS CODE-29153990), Phosphoric acid was purchased from Fluka (mol. wt: 98, Lot# BC BD2404, peak code=101053351 from Fluka).

Three pathogenic bacterial strains were used for the assay of antimicrobial activity. Gram-positive bacterial strains, *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), and Gram-negative bacterial strains, *Escherichia coli* (MTCC 443) were collected from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Each bacterial culture was maintained on an individual nutrient agar slant at 4˚C until further use.

Preparation of Raw Deoiled Flaxseed Flour: Raw flaxseeds were cleaned and dried in a hot air oven (Lasin, LSI 002Ds, Labsoul, India) at 50 °C for 1 h. After drying, the flaxseeds were ground to fine flour using a mixer grinder and oil extraction was administered by the solvent extraction method [15].

Raw flaxseed flour (20 g) was soaked in n-hexane (200 ml) for 24 h to extract the oil, filtered by Whatman No.1 paper. After filtration, the residue was collected from the Whatman No.1 paper onto a Petri plate. Then the residue was subjected to solvent evaporation duringa hot air oven (Lasin, LSI 002Ds, Labsoul, India) at 30 °C for 24 h. The flour thus obtained was packed in an airtight container and was labeled as raw deoiled flaxseed flour (RDFF) and stored at a desiccator (Photron-PHKJ-50S).

Preparation of Fermented Deoiled Flaxseed Flour: Raw flaxseeds were rinsed with distilled water and crushed by mortar pastel. The crushed flaxseeds were boiled for 1 h and cooled at room temperature. Then these flaxseeds are wrapped in blanched banana leaves and kept in an airtight plastic container at room temperature for 96 h for fermentation [14]. The fermented flaxseeds were taken at 24 h, 48 h, 72h, and 96 h. After each interval, the fermented flaxseeds were dried in a hot air oven (Lasin, LSI 002Ds) at 50 °C for 24 h, and ground to fine flour employing a mixer grinder. Fermented deoiled flaxseed flour was prepared using the same method described in Materials and Methods (section 2.1). Fermented deoiled flaxseed flours were designated as

24h FDFF - 24 h fermented deoiled flaxseed flour.

48h FDFF - 48 h fermented deoiled flaxseed flour.

72h FDFF - 72 h fermented deoiled flaxseed flour.

96h FDFF - 96 h fermented deoiled flaxseed flour.

Preparation of Germinated Deoiled Flaxseed Powder: Raw flaxseeds were rinsed with distilled water and spread on a clay tray. A fine cotton cloth covered the tray. Water was provided daily to the flaxseeds, and they were allowed to germinate at room temperature. The germinated flaxseeds were taken at 72h, 96h, and 120h. After each interval, the germinated seeds were dried in a hot air oven (Lasin, LSI 002Ds) at 50 °C for 6 h, and ground to fine flour employing a mixer grinder. The preparation of germinated deoiled flaxseed flour followed the procedure described in Materials and Methods (section 2.1). Germinated deoiled flaxseed flours were labeled as

72h GDFF - 72 h germinated deoiled flaxseed flour.

96h GDFF - 96 h germinated deoiled flaxseed flour.

120h GDFF - 120 h germinated deoiled flaxseed flour.

Preparation of RDFF, each FDFF (24h, 48h, 72h, and 96h) and each GDFF (72h, 96h, and 120h) Extracts: The extracts of RDFF, each FDFF, and each GDFF were prepared in different solvents as described by Sultana *et al*.[16]. 5g of RDFF, each FDFF, and each GDFF were individually taken in the separate Erlenmeyer flask and individually extracted with 50 ml of different solvents (80% and 100% methanol, 80% and 100% ethanol, 80% and 100% isopropanol). The extraction was performed by 24 h continuous mixing with a magnetic stirrer set at 300 rpm at room temperature. After extraction, the supernatant and sediment were filtrated using What-man No. 1 filter paper. The solvent from each extract was then evaporated under vacuum at 45 °C, using a rotary evaporator (BUCHI, Vacuum module V-801, Vacuum pump V-700 and Rota vapor R-3) and each residual extract was stored in an individual airtight glass vial at 4°C for further use. Each residual extract designate as

80% ME: 80% methanolic extract.

100% ME: 100% methanolic extract.

80% EE: 80% ethanolic extract.

100% EE: 100% ethanolic extract.

80% IE: 80% isopropanolic extract.

100% IE: 100% isopropanolic extract.

Determination of Total Phenolic Content (TPC) of 80% ME, 100% ME, 80%EE, 100% EE, 80% IE and 100% IE of RDFF, each FDFF (24h, 48h, 72h, 96h) and each GDFF (72h, 96h, 120h): The total phenolic content of each residual extract was done by the Folin-Ciocalteu procedure [17]. 0.2 ml (20mg/ml) of each residual extract was taken into separate test tubes containing 0.8 ml of methanol. 2.5 ml of Folin-Ciocalteu reagent (Folin: water 1:9 v/v) and 2 ml of Na_2CO_3 (7.5%) were added to each test tube and mixed thoroughly. The mixture was left to stand for 30 min in the dark at room temperature and the absorbance of the sample was measured against a reagent blank at 760 nm by using a spectrophotometer (JASCO V 630, Maryland, USA). Gallic acid was used as a standard. The results were expressed as mg of gallic acid equivalents per 100 g of extracts (mg GAE/100g). The total phenolic content was calculated by the following equation:

$$T = (C \times V) / M$$

Where, T= Total phenolic content in mg GAE/g of each extract, C= Concentration of gallic acid established from the standard curve as mg/ml, V= Volume of each extract in ml, M= Weight of each extract.

Determination of Total Flavonoid Content (TFC) of 80% ME, 100% ME, 80% EE, 100% EE, 80% IE and 100%IE of RDFF, each FDFF (24h, 48h, 72h, 96h) and each GDFF (72h, 96h, 120h): The amount of total flavonoid of each residual extract was determined by the procedure described by Sultana *et al.,* [18] with little modification using catechin as standard. 0.2 ml (20mg/ml) of each residual extract was taken into a separate 10 ml volumetric flask containing 5 ml distilled water and 0.3 ml of 5% $NaNO_2$ solution. After 5 min 0.6 ml of 10% $AlCl_3$ solution was added and the mixture was incubated for 6 min at room temperature. Finally, 1 ml of 1M NaOH was added and the volume was 10 ml with distilled water. The solution was mixed thoroughly, and absorbance was recorded at 510 nm using a spectrophotometer (JASCO V 630, Maryland, USA). The total flavonoid content was calculated based on the calibration curve of catechin. The results were expressed as mg of catechin equivalents per 100 g of extracts (mg CE/100 g).

Optimization of FDFF and GDFF for Antioxidant Activities: TPC and TFC content are correlated with antioxidant activities, if TPC and TFC increased, antioxidant activities increased as well [19]. So, FDFF and GDFF were optimized according to their TPC and TFC content to analyze antioxidant activities.

Antioxidant Activities of 80% ME, 100% ME, 80% EE, 100% EE, 80% IE and 100% IE of RDFF, Optimized FDFF and Optimized GDFF: Evaluations of the antioxidant potentiality of different solvent extracts (80% ME, 100% ME, 80% EE, 100% EE, 80% IE, and 100% IE) of RDFF, optimized FDFF and optimized GDFF were carried out using various antioxidant assays.

Free Radical Scavenging Activity using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) Method: The free radical scavenging activity of each extract was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) according to the procedure reported by Kaur *et al.*[20]. Briefly, 1 ml of 0.1 mM DPPH solution (in methanol) was added to 1 ml of each extract ranging from 0.05-0.25mg/ml. The mixture was vortexed and stood in dark at room temperature for 30 min and absorbance was measured against a blank at 517 nm using a spectrophotometer (JASCO V 630). A methanolic solution of DPPH was used as a control. Ascorbic acid was used as a positive control. The DPPH radical scavenging activity of each extract was calculated using the following equation;

$$\text{Scavenging activity (\%)} = (Ac - As) / Ac \times 100$$

Where Ac was the absorbance of the control solution and As was the absorbance of the sample solution. The IC_{50} value was also measured to calculate the sample concentration required to scavenge 50% of DPPH radicals. A low IC_{50} value indicates high scavenging activity.

Ferric Reducing Antioxidant Power Assay (FRAP): Ferric reducing antioxidant power assay was estimated according to Benzie and Strain [21]. The working FRAP reagent was freshly prepared by mixing three solutions; 300 mM Acetate buffer pH 3.6, 10 mM Tripyridyltriazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3,6H_2O$ in the ratio of 10:1:1. 3 ml of FRAP reagent was mixed thoroughly to 100 µl of each extract. The absorbance was measured at 593 nm using a spectrophotometer (JASCO V 630) after 4 min of the addition of the FRAP reagent. The FRAP values were determined from the standard curve of Fe^{2+} ($FeSO_4.7H_2O$). Each extract's ferric-reducing capacity was expressed as µmol $FeSO_4$ equivalents per ml of sample based on the ability to reduce ferric ions of the sample.

Fe^{2+} Chelating Activity: The metal chelating activity was measured by using Hsu et al. method with some modifications [22]. 1 ml of each extract (0.1 mg/ml – 1.5 mg/ml) was taken into a separate test tube containing 3.7 ml methanol. 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine solution were added to each test tube. All the mixtures were shaken well and incubated for 10 min, at room temperature. The absorbance was measured spectrophotometrically (JASCO V 630) at 562 nm. EDTA was used as a positive control.

A mixture without flaxseed extract was used as the control. The percentage of ferrous ion chelating ability was evaluated using the following equation;

Chelating activity (%) = (Ac – As / Ac × 100

Where, Ac = absorbance of the control, As = absorbance of the sample solution. The IC_{50} value was measured to calculate the sample concentration required to chelate 50% Fe^{2+} and ferrozine complex.

Reducing Power Assay: The reductive potential of each extract was done according to the method of Oyaizu with some modifications [23]. Each extract (1 ml) was mixed with 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Each mixture was incubated at 50 °C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added to each mixture and the mixture was centrifuged at 1000 rpm for 10 min. Each supernatant (2.5 ml) was mixed with deionized water (2.5 ml) and 0.5 ml of 0.1% of ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer (JASCO V 630). The reducing power of each sample was calculated based on the calibration curve of butylated hydroxytoluene (BHT).

ABTS Radical Scavenging Activity Assay: The ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity of each extract was assessed by the method described by Re *et al.,* with little modification [24]. The ABTS radical cation (ABTS•+) was chemically prepared by mixing 5.0 ml of ABTS solution (7 mM) with 88 µl of 140 mM potassium persulfate. The mixture was incubated in the dark for 16 h at room temperature. To prepare the standard working solution of ABTS radical, an aliquot of ABTS solution was diluted with 80% ethanol to get an absorbance of 0.7-0.75 at 743nm. Different concentrations of each extract (1 mg/ml-20 mg/ml) were mixed with 190 µl of ABTS radical working solution and incubated for 15 min at room temperature in dark. The absorbance was measured at 734 nm using a spectrophotometer (JASCO V 630). α-tocopherol was used as the positive control. The percentage of scavenging activity on the ABTS of each extract was calculated according to the following equation;

ABTS radical scavenging effect (%) = [1 – (Ac –As / Ac] × 100

Where, Ac = absorbance of the control, A_S = absorbance of the sample. The IC_{50} value was measured to calculate the sample concentration required to scavenge 50% ABTS radicals.

Identification of Phenolic and Flavonoid Compounds for 80%EE of RDFF, Optimized FDFF and Optimized GDFF by HPLC: Identification of phenolic compounds and flavonoid compounds was carried out by high-performance liquid chromatography (HPLC). HPLC analyses were performed with MDLC model #2695 (Multidimensional liquid chromatography, Waters) using EMPOWER#2 software with a 2487 dual λ absorbance detector. Individual compounds were separated on a Symmetry reverse phase C_{18} column (5 µm, 250 mm, and 4.6 mm i.d.).

Preparation of Standard Solutions: Several phenolic acids and flavonoids were used as standard. 1 mg/ml concentrated stock solution was prepared for each of these standards. 1 mg of each standard was dissolved in 0.5 ml HPLC grade methanol and then the volume was made up to 1 ml with the mobile phase solvent (0.1% Phosphoric acid in water and 90% Acetonitrile). Before injection, all the standard and the test sample solutions were filtered through a 0.22 µM Polyvinyl Difluoride syringe filter.

HPLC Analysis: The solvent system consisted of solvent (A) 0.1% phosphoric acid in the water, and solvent (B) 90% acetonitrile in water was used in the HPLC analysis of each extract. The column was thermostated at 25ºC. The injection volume of each sample was kept at 100µl and the flow rate was adjusted to 1ml/min.

A gradient elution method was performed by varying the solvent B to A proportion. The gradient elution was changed from 0 to 20% B and 100 to 80% of A within 0-5 min, from 20 to 30% of B and 80 to 70% of A in 5-15 min, from 30 to 20% of B and 70 to 80% of A in the next 14-20 min. In the last 5 min of the total 25 min run time, the mobile phase composition is back to the initial condition (0% B and 100% A).

HPLC chromatograms were detected using a photodiode array UV detector at three different wavelengths (280, 210 and 370 nm) according to the absorption maxima of the analyzed compounds. Each compound was identified by comparing its retention time and absorption with those of standard materials under the same conditions.Test samples were quantified using integrated peak areas, and content was calculated using a calibration curve based on peak area about the concentration of a standard sample.

FT-IR Spectroscopic Analysis of 80%EE of RDFF, Optimized FDFF and Optimized GDFF: The Fourier-transform infrared spectroscopy (FTIR) is a phenotypic method, which measures the infrared absorption of molecules, such as fats, carbohydrates, nucleic acids, and proteins, resulting in an FTIR spectrum that provides information on the sample's overall composition [25].

The chemical bonds in a compound can be determined by interpreting the infrared absorption spectrum. FT-IR of 80% EE of RDFF, optimized FDFF, and optimized GDFF were determined by the method described by Rohman and Che man [26]. One drop of each extract was placed on the surface of the ATR (Attenuated total reflection) crystal in FTIR spectroscope (Bruker-55model), with a scan range from 4000 to 450 cm^{-1} at a nominal resolution of 4 cm^{-1} for detection of the functional group of the samples with the help of FT-IR correlation chart.

Antibacterial Activity of 80% EE of RDFF, Optimized FDFF and Optimized GDFF: The antibacterial activity was assessed by the agar well diffusion method against *Staphylococcus aureus*, *Bacillus substilis* and *E. coli* culture [27]. These cultures were inoculated separately in nutrient broth and were incubated overnight at 37°C. Each of the bacterial cultures (20µl) was spread separately on separate Muller Hinton agar plates. The wells were cut into the Muller Hinton agar plates with the help of a sterile cork borer. Then each well of the agar plate was filled with 100µl of 80%EE of RDFF, optimized FDFF, and optimized GDFFalong with control (distilled water). The plates were incubated in an upright position at 37°C for 24 h. After the incubation period, the diameter of the inhibition zones was calculated for all plates.

***In-vitro* Antiarthritic Activity of 80% EE of RDFF, Optimized FDFF and Optimized GDFF:** The *in-vitro* antiarthritic activity of 80% EE of RDFF optimized FDFF and optimized GDFF were tested using the bovine serum protein denaturation method [28].

Percentage inhibition = 100 – (Absorbance of test solution – Absorbance product control / Absorbanec of test control / 100

Statistical Analysis: Each experiment was conducted at least in triplicate. All values were means ± standard deviation of three samples. The one-way analysis of variance (ANOVA) was used to compute a statistical decision and the means were compared across groups by the Tukey HSD test. $P \leq 0.05$ and $P \leq 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION:

Determination of Total Phenolic Content (TPC) of 80% ME, 100% ME, 80% EE, 100% EE, 80% IE and 100% IE of RDFF, each FDFF (24h, 48h, 72h, 96h) and each GDFF (72h, 96h, 120h): The TPC method is used to determine the phenolic content of plant-based samples. Phenolic compounds found in plants have redox properties, and their properties allow them to act as antioxidants [29]. The TPC of each extract of RDFF, FDFF (24h, 48h, 72h, and 96h) and GDFF (72h, 96h, 120h) was calculated by using the Gallic acid standard curve. TPC of each extract was presented in **Table 1**, which indicated that different solvent extracts of RDFF contained 1628 ± 8.19^{a} to 3103 ± 8.19^{a} mg GAE/100g, FDFF contained 1920 ± 8.54^{b} to 3250 ± 8.00^{d} mg GAE/100g and GDFF contained 2028 ± 7.02^{e} to 3813 ± 9.00^{f} mg GAE/100g of TPC. The highest phenolic compound concentration was exhibited by 96h GDFF extracts, followed by 72h FDFF extracts. Whereas, RDFF extracts were found to contain the least concentrations of phenol. **Table 1** also indicated that 80% ethanol extract of RDFF, all FDFF, and all GDFF contained higher TPC than those present in other solvents extracts of RDFF, FDFF, and GDFF. These results were consistent with previous research results in [15] that 80% ethanol flaxseed extract had higher TPC than 100% ethanol, 80% methanol, and 100% methanol flaxseed extracts. Olawoye and Gbadamosi determined the TPCs of raw, fermented and

germinated amaranth seed flour methanolic extracts and observed that the TPCs of fermented and germinated amaranth seed flour methanolic extracts were higher than raw amaranth seed flour which was similar to the present study [7]. GDFF had a higher TPC than RDFF, perhaps because of the enzyme hydrolysis during germination that causes the biosynthesis of phenolic compounds [7]. TPC of FDFF was also higher than RDFF, which may result from proteolytic enzymes from the microorganism hydrolyzing phenolic compounds into simpler and more biologically active compounds readily absorbed during fermentation [30]. The phenolic compounds in seeds contribute most significantly to their antioxidant properties and play a key role in preventing and controlling degenerative diseases [7].

TABLE 1: TOTAL PHENOLIC CONTENT (MG GAE/ 100GM) OF 80%ME, 100%ME, 80%EE, 100%EE, 80%IE, AND 100%IE OF RDFF, EACH FDFF (24H, 48H, 72H, 96H) AND EACH GDFF (72H, 96H, 120H)

Samples	80% ME	100% ME	80% EE	100% EE	80% IE	100% IE
RDFF	2047±5.57^{a}	2408±8.74^{a}	3103±8.19^{a}	2087±12.49^{a}	1915±7.00^{a}	1628±8.19^{a}
24h FDFF	2844±7^{b}	2957±10.07^{b}	3126±8.02^{b}	2797±7^{b}	2089±10.07^{b}	1920±8.54^{b}
48h FDFF	2967±8.54^{c}	3025±12.49^{c}	3167±8.19^{c}	2873±8.62^{c}	2130±8.00^{c}	1986±7.57^{c}
72h FDFF	3064±8.89^{d}	3100±11.14^{d}	3250±8.00^{d}	2948±9.85^{d}	2144±9.64^{c}	2078±7.55^{d}
96h FDFF	3003±9.50^{e}	2996±9.02^{e}	3152±9.17^{c}	2831±7.94^{e}	2107±8.08^{bc}	2007±8.19^{c}
72h GDFF	2700±7.51^{f}	3569±7.09^{f}	3698±7.02^{e}	2909±6.00^{f}	2151±5.51^{c}	2028±7.02^{e}
96h GDFF	2894±8.19^{g}	3700±11.14^{g}	3813±9.00^{f}	3035±8.00^{g}	2278±9.17^{d}	2183±7.00^{f}
120h GDFF	2795±7.51^{h}	3596±6.24^{f}	3719±6.03^{e}	2966±7.00^{d}	2208±6.51^{e}	2085±5.69^{d}

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript letters within the same column indicated significant ($p<0.05$) differences.

Determination of Total Flavonoid Content (TFC) of 80%ME, 100%ME, 80%EE, 100%EE, 80%IE, and 100%IE of RDFF, each FDFF (24h, 48h, 72h, 96h) and each GDFF (72h, 96h, 120h): Flavonoids are structural derivatives of flavones, containing conjugated aromatic systems, which are bound to sugar(s) as glycosides [31]. Flavonoids are polyphenolic plant compounds and they have been shown to exhibit anti-inflammatory, anti-thrombogenic, anti-diabetic and neuroprotective properties through diverse mechanisms [32]. The total flavonoid content of each extract was calculated using the standard calibration curve of catechin. The TFCs of different solvent extracts of RDFF ranged from 228.87±1.42^{a} to 410.00±2.38^{ab} mg CE/100 g, FDFF ranged from 220.20±1.71^{b} to 421.00±1.98^{d} mg CE/100 g, and GDFF ranged from 295.20±1.38^{e} to 456.00±2.01^{e} mg CE/100 g of extract **Table 2**. The highest flavonoid content was found in 96h GDFF extracts, followed by 72h FDFF extracts, and a lower level was found in RDFF extracts. TFCs of RDFF, all FDFF and all GDFF extracts were high when using 80% ethanol as extracted solvent. Anwar and Przybylski, reported that the TFC of flaxseed extract was high when 100% methanol was used as the extraction solvent, contrary to the present study [15]. Uchegbu and Amulu also identified that germination improved the flavonoid content of African yam beans [33]. Researchers speculate that some secondary plant metabolites such as anthocyanins and flavonoids may be produced during the germination process of seeds [33]. According to Adetuyi and Ibrahim, fermented okara seeds contained more TFC than nonfermented okara seeds [30]. This increase may be due to increased acidic value during fermentation, which liberates bound flavonoid components and makes them more bioavailable. Flavonoids are potent water-soluble antioxidants and free radical scavengers that prevent oxidative cell damage, have strong anticancer activity, and protect against the different levels of carcinogenesis. Flavonoids help to lower the risk of heart disease [31].

TABLE 2: TOTAL FLAVONOID CONTENT (MG GAE/ 100GM) OF 80%ME, 100%ME, 80%EE, 100%EE, 80%IE AND 100%IE OF RDFF, EACH FDFF (24H, 48H, 72H, 96H) AND EACH GDFF (72H, 96H, 120H)

Samples	80% ME	100% ME	80% EE	100% EE	80% IE	100% IE
RDFF	282.40±2.07^{a}	354.00±1.93^{a}	410.00±2.38^{ab}	273.00±1.88^{a}	248.24±2.48^{a}	228.87±1.42^{a}
24h FDFF	271.86±1.33^{b}	335.84±2.20^{b}	401.04±1.47^{a}	275.80±2.20^{a}	220.20±1.71^{b}	230.52±1.56^{a}
48h FDFF	285.76±2.27^{a}	350.81±2.29^{a}	412.13±1.56^{c}	289.86±1.97^{b}	234.95±1.29^{c}	237.15±1.84^{b}
72h FDFF	307.97±2.57^{c}	364.00±2.99^{c}	421.00±1.98^{d}	298.00±1.51^{c}	277.00±2.07^{d}	252.95±2.79^{c}

96h FDFF	280.75±1.61[a]	340.97±2.57[b]	406.30±1.89[b]	285.79±1.28[b]	229.82±1.56[e]	245.47±1.93[d]
72h GDFF	386.28±1.28[d]	394.28±1.47[d]	419.55±1.30[d]	411.55±1.10[d]	316.41±1.10[f]	295.20±1.38[e]
96h GDFF	409.04±1.56[e]	422.00±1.56[e]	456.00±2.01[e]	434.00±1.08[e]	355.82±1.10[g]	314.15±1.61[f]
120h GDFF	391.23±1.24[d]	403.11±0.84[f]	426.80±1.05[f]	415.40±1.38[d]	319.40±1.53[f]	300.36±0.96[g]

All values were presented as mean ± standard deviation for triplicate experiments; Mean values with different superscript letters within the same column indicated significant (P< 0.05) differences.

Antioxidant Activities of 80%ME, 100%ME, 80%EE, 100%EE, 80%IE, and 100%IE of RDFF, 72h FDFF and 96h GDFF: Antioxidant activities were done with each extract of RDFF, 72h FDFF, and 96h GDFF. 72h FDFF and 96h GDFF extracts were optimized for antioxidant activity because maximum TPC and TFC were achieved on those days.

Free Radical Scavenging Activity using the DPPH: DPPH assay is one of the most common and simple methods used to evaluate antioxidant activity. The decolorization of methanol solution of DPPH determined the hydrogen atom donating ability of the antioxidant molecule. DPPH in methanol produces purple color and fades to shades of yellow color in the presence of antioxidants [28]. In the present study, DPPH radical scavenging activity of the different solvent extracts of RDFF, 72h FDFF and 96h GDFF were evaluated and presented in **Table 3**. The maximum DPPH radical scavenging activity of RDFF extract was obtained from 80% EE of RDFF at a concentration of 0.25 mg/ml (57.16±0.15[c] %) and the IC_{50} value was found to be 0.197±0.002[d] mg/ml. These findings were lower than the earlier studies of the antioxidant activity of 80% ethanolic flaxseed extract (87.5% at 25 µg/ml) [15]. According to **Table 3**, the highest DPPH radical scavenging activity was obtained from 80% EE of 96h GDFF at a concentration of 0.25 mg/ml (70.11±0.18[c] %), and IC_{50} value was determined as 0.113±0.001 mg/ml. The 72h FDFF 80% EE also exhibited good DPPH radical scavenging activity (68.19±0.18[c] % at 0.25 mg/ml). These findings correlated well with Olawoye and Gbadamosi, who reported that fermentation and germination increased the DPPH radical-scavenging ability of amaranth seed flour methanolic extracts [7].

Ascorbic acid was used as positive control and significantly higher scavenging activity (88.13±0.11[c] % at 0.25 mg/ml) than RDFF, 72h FDFF, and 96h GDFF extracts. The high scavenging property of 96h GDFF extracts may be due to the presence of hydroxyl groups within the chemical structure of phenolic compounds, which are capable of providing the necessary component for radical scavenging [33]. It has also been observed that fermentation caused a significant ($p < 0.05$) increase in the DPPH radical-scavenging ability of okra seeds which was similar to the present study [30]. This may be due to the release of phenolic and isoflavone aglycones during fermentation by the catalytic action of β-glucosidase and the formation of reductones during fermentation [30]. This study observed that the DPPH radical-scavenging activity of RDFF, 72h FDFF and 96h GDFF extracts increased in correlation with TPC and TFC, so there was a positive correlation between TPC, TFC and DPPH radical-scavenging activity of those extracts.

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY (%) AND IC_{50} VALUE OF 80%ME, 100%ME, 80%EE, 100%EE, 80%IE, AND 100%IE OF RDFF, 72H FDFF, AND 96H GDFF AND ASCORBIC ACID

	Sample extract/ Conc. (mg/ml)	0.05	0.15	0.25	IC_{50} value (mg/ml)
RDFF	80% ME	25.23±0.21[a]	40.26±0.24[b]	55.21±0.21[c]	0.215±0.002[d]
	100% ME	28.24±0.22[a]	40.17±0.17[b]	54.21±0.21[c]	0.210±0.002[d]
	80% EE	31.18±0.16[a]	43.16±0.14[b]	57.16±0.15[c]	0.197±0.002[d]
	100% EE	26.13±0.12[a]	40.06±0.12[b]	54.18±0.16[c]	0.220±0.001[d]
	80% IE	22.07±0.13[a]	36.10±0.17[b]	50.14±0.24[c]	0.249±0.001[d]
	100% IE	20.20±0.18[a]	32.11±0.18[b]	45.18±0.16[c]	0.290±0.001[d]
72h FDFF	80% ME	32.23±0.21[a]	50.25±0.22[b]	65.18±0.18[c]	0.155±0.001[d]
	100% ME	36.22±0.20[a]	50.21±0.20[b]	67.23±0.20[c]	0.142±0.002[d]
	80% EE	36.88±0.09[a]	55.52±0.46[b]	68.19±0.18[c]	0.127±0.002[d]
	100% EE	32.07±0.12[a]	47.94±0.11[b]	65.03±0.14[c]	0.160±0.001[d]
	80% IE	30.09±0.15[a]	44.14±0.12[b]	60.11±0.19[c]	0.185±0.001[d]
	100% IE	28.09±0.16[a]	41.23±0.20[b]	54.19±0.19[c]	0.217±0.001[d]
	80% ME	35.21±0.21[a]	46.24±0.22[b]	66.23±0.21[c]	0.162±0.002[d]

	100% ME	34.22±0.20 [a]	46.24±0.23 [b]	64.26±0.24 [c]	0.154±0.001 [d]
96h GDFF	80% EE	40.19±0.18 [a]	56.22±0.20 [b]	70.11±0.18 [c]	0.113±0.001 [d]
	100% EE	31.14±0.13 [a]	47.17±0.16 [b]	63.14±0.14 [c]	0.168±0.001 [d]
	80% IE	26.10±0.10 [a]	40.11±0.20 [b]	54.27±0.24 [c]	0.220±0.001 [d]
	100% IE	25.11±0.18 [a]	40.19±0.18 [b]	52.12±0.21 [c]	0.230±0.001 [d]
Ascorbic acid		50.15±0.13 [a]	72.08±0.14 [b]	88.13±0.11 [c]	0.044±0.001 [d]

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript ([a, b, c, d]) letters within the same row indicated significant ($p < 0.01$) differences.

Ferric Reducing Antioxidant Power Assay (FRAP): FRAP assay is used to measure the antioxidant power reacting with a ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and producing a blue color ferrous tripyridyltriazine [Fe^{2+}-TPTZ] with an absorption maximum at 593 nm[21]. The FRAP values of RDFF, 72h FDFF and 96h GDFF extracts were presented in **Table 4**. Among all the extracts the highest reduction potential was shown by 80% EE of 96h GDFF (13.26±0.04[c] µM/ml) followed by 80% EE of 72h FDFF and RDFF. The lowest reduction potential was observed in 100% IE of RDFF 8.37±0.05[a]µM/ml. In another study, the reducing potential of the raw, fermented, and germinated amaranth seed flour methanolic extracts was estimated based on FRAP assay and reported that fermentation and germination increased the reducing power of amaranth seed flour methanolic extracts [7]. The increase in reducing the power of fermented samples is probably due to bioactive compounds' synthesis or enzymatic transformation during fermentation [7].

TABLE 4: FERRIC REDUCING ANTIOXIDANT POWER ASSAY OF 80% ME, 100%ME, 80% EE, 100% EE, 80% IE, AND 100% IE OF RDFF, 72H FDFF AND 96H GDFF

Sample	RDFF (µM/ml)	72h FDFF (µM/ml)	96h GDFF (µM/ml)
80% ME	10.97±0.05[a]	11.44±0.10[b]	12.50±0.04[c]
100% ME	11.08±0.05[a]	11.58±0.08[b]	12.75±0.04[c]
80% EE	11.29±0.06[a]	11.82±0.11[b]	13.26±0.04[c]
100% EE	10.31±0.05[a]	11.25±0.06[b]	11.81±0.05[c]
80% IE	9.18±0.06[a]	9.07±0.07[a]	10.01±0.04[c]
100% IE	8.37±0.05[a]	8.47±0.09[a]	9.64±0.07[c]

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript ([a, b, c]) letters within the same row indicated significant ($p < 0.01$) differences.

Fe^{2+}-chelating Antioxidant Activity: Metal chelating is usually regarded as the most potent and common antioxidant method. It has been reported that antioxidants have an effective Fe-binding ability due to their functional groups that act as metal binders. Interactions between Fe ions and antioxidant compounds may also alter their biological effects, including their antioxidant properties [34]. A ferrous ion chelating assay is conducted with chelating agent ferrozine (a secondary antioxidant), which interferes with the formation of the ferrozine-Fe2+ complex, reducing color. The reduction of color measured at 550 nm evaluates the antioxidant potential of the chelating agents [34]. The metal chelating activity reduces the redox potential by reducing the concentration of the transition metal ion (Fe^{2+}) which acts as a catalyst in lipid peroxidation and hydrogen peroxide degradation through Fenton reactions [35]. In the present study, as a standard, the ferrous ion chelating activity of RDFF, 72h FDFF, and 96h GDFF extracts and Ethylene diamine tetraacetic acid (EDTA) were estimated and were presented in **Table 5**. As shown in **Table 5**, metal chelating activities of different solvent extracts of RDFF, 72h FDFF, and 96h GDFF were within the range of 52.07±0.12[d] % to 68.85±0.14[d] % at 1.5mg/ml. The metal chelating activity of standard EDTA was found to be 83.06±0.12[d] % at 1.5 mg/ml. The maximum Fe^{2+} chelating activity was found in 96h GDFF extract (IC_{50} value. 0.61±0.005 [e] mg/ml) followed by 72h FDFF (IC_{50} value. 0.65±0.003[e] mg/ml) and RDFF (IC_{50} value. 0.83±0.005[e] mg/ml) extracts when extracted with 80% ethanol. Due to germination, phenolic compounds are degraded and their structure is altered to different products exhibiting higher antioxidant and metal chelating activity [36]. In another study, Gaffar *et al*. reported that Fe^{2+} chelating activities of five flaxseed cultivars namely (Amon, Blanka, Lithuania,

Sakha1 and Teka) ranged from 36.03 ± 0.49 % to 50.74 ± 0.27 % [37]. According to Olawoye and Gbadamosi, Fe^{2+} chelating activity of fermented amaranth seed flour methanolic extract was higher than germinated amaranth seed flour methanolic extract contradictory to the present study [7].

TABLE 5: Fe^{2+} - CHELATING ANTIOXIDANT ACTIVITY AND IC_{50} VALUE OF 80%ME, 100%ME, 80%EE, 100%EE, 80%IE AND 100%IE OF RDFF, 72H FDFF, AND 96H GDFF AND EDTA

	Sample extract/ Conc. (mg/ml)	0.1	0.5	1.0	1.5	IC_{50} value (mg/ml)
RDFF	80% ME	34.14±0.13[a]	43.00±0.09[b]	53.00±0.11[c]	60.07±0.08[d]	0.91±0.006[e]
	100% ME	38.68±0.12[a]	42.66±0.08[b]	50.03±0.05[c]	61.08±0.08[d]	0.87±0.003[e]
	80% EE	34.06±0.12[a]	44.13±0.12[b]	52.12±0.12[c]	63.07±0.12[d]	0.83±0.005[e]
	100% EE	34.54±0.07[a]	40.56±0.05[b]	48.05±0.09[c]	64.13±0.13[d]	0.93±0.003[e]
	80% IE	28.07±0.12[a]	35.52±0.13[b]	45.64±0.13[c]	56.07±0.12[d]	1.21±0.006[e]
	100% IE	25.07±0.13[a]	33.63±0.13[b]	40.61±0.11[c]	52.07±0.12[d]	1.42±0.006[e]
72h FDFF	80% ME	37.13±0.11[a]	45.10±0.11[b]	53.63±0.13[c]	67.06±0.10[d]	0.74±0.004[e]
	100% ME	39.55±0.09[a]	44.63±0.12[b]	54.64±0.13[c]	66.61±0.11[d]	0.71±0.006[e]
	80% EE	40.62±0.13[a]	45.07±0.12[b]	57.14±0.13[c]	67.07±0.12[d]	0.65±0.003[e]
	100% EE	38.11±0.11[a]	43.14±0.13[b]	53.08±0.10[c]	64.12±0.11[d]	0.80±0.004[e]
	80% IE	31.08±0.14[a]	40.07±0.13[b]	48.56±0.10[c]	60.14±0.12[d]	1.02±0.001[e]
	100% IE	30.06±0.10[a]	38.15±0.13[b]	48.22±0.21[c]	59.08±0.14[d]	1.07±0.01[e]
96h GDFF	80% ME	38.14±0.13[a]	45.36±0.07[b]	53.61±0.10[c]	65.61±0.12[d]	0.74±0.003[e]
	100% ME	38.05±0.09[a]	46.14±0.12[b]	58.07±0.13[c]	68.85±0.14[d]	0.65±0.002[e]
	80% EE	40.14±0.13[a]	47.07±0.13[b]	58.08±0.10[c]	67.57±0.13[d]	0.61±0.005[e]
	100% EE	37.57±0.13[a]	43.25±0.09[b]	55.12±0.12[c]	65.56±0.10[d]	0.76±0.005[e]
	80% IE	33.07±0.13[a]	40.83±0.11[b]	51.07±0.12[c]	60.76±0.10[d]	0.96±0.005[e]
	100% IE	34.15±0.13[a]	40.33±0.12[b]	50.65±0.09[c]	60.37±0.16[d]	0.97±0.004[e]
EDTA		51.17±0.14[a]	59.15±0.13[b]	70.05±0.09[c]	83.06±0.12[d]	0.076±0.004[e]

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript (a, b, c, d, e) letters within the same row indicated significant ($p< 0.01$) differences.

Reducing Power Assay: Reducing power assays work on the principle that substances with reduced potential react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which reacts with ferric chloride to form ferric–ferrous complexes with maximum absorption at 700 nm[38].

The reducing power activity of RDFF, 72h FDFF and 96h GDFF extracts were shown in **Table 6**. The higher reducing power activity was recorded by 80% EE of 96h GDFF (4.92 ±0.03[c] mg/ml) and the lower activity was recorded in 100% IE of RDFF (2.34±0.03[a] mg/ml). Germination and fermentation increased the reducing power activity of deoiled flaxseeds. James *et al.* indicated that germination increased the reducing power activity of African yam bean seed, which was similar to our results [39]. Hassan *et al.* reported after fermentation reducing power activity was increased in "Nabag" (*Ziziphus spina-Christi* L.) seeds [40].

It may occur because fermentation induces a structural breakdown of plant cell walls, releasing various antioxidant compounds [40]. Reducing power activity is closely related to phenolic content [16]. This study found that 80% ethanol extract of 96h GDFF contained the highest amount of total phenolics, hence, this may account for its highest reducing power activity than RDFF and 72h FDFF.

TABLE 6: REDUCING POWER ASSAY OF 80% ME, 100% ME, 80% EE, 100% EE, 80% IE, AND 100% IE OF RDFF, 72H FDFF, AND 96H GDFF

Solvent	RDFF (mg/ml)	72h FDFF (mg/ml)	96h GDFF (mg/ml)
80% ME	3.03±0.02[a]	3.11±0.02[b]	3.35±0.03[c]
100% ME	3.06±0.01[a]	3.15±0.02[b]	3.78±0.03[c]
80% EE	3.13±0.03[a]	3.21±0.01[b]	4.92±0.03[c]
100% EE	2.92±0.02[a]	3.03±0.01[b]	3.06±0.02[c]
80% IE	2.46±0.03[a]	2.97±0.03[b]	2.99±0.02[b]
100% IE	2.34±0.03[a]	2.85±0.03[b]	2.89±0.02[b]

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript ([a, b, c]) letters within the same row indicated significant ($p< 0.05$) differences.

ABTS Radical Scavenging Activity Assay of RDFF, 72h FDFF and 96h GDFF Extracts: ABTS assay measures how several antioxidants are able to scavenge ABTS. The ABTS is produced by combining an oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical by hydrogen-donating antioxidants is determined by suppressing its long-wave absorption spectrum [41]. The ABTS radical assay is a widely used method of screening for antioxidant activity and is reported as a decolorization assay applicable to RDFF, 72h FDFF and 96h GDFF extracts. A reduction of the color indicated a reduction of ABTS radical. In **Table 7** RDFF, 72h FDFF and 96h GDFF extracts were effective in scavenging radicals. **Table 7** revealed that in the ABTS assay, the scavenging potential (%) of different solvent extracts of RDFF were observed in the range of 42.32±0.12^{c} % to 74.05±0.01^{c} % at 20 mg/ml and IC_{50} values ranged within 5.06±0.02^{d} mg/ml to 26.17±0.11^{d} mg/ml. The highest ABTS radical scavenging activity (83.45±0.09^{c} % at 20 mg/ml) was shown in 80% EE of 96h GDFF (IC_{50} value- 2.18±0.06 mg/ml), whereas the least radical scavenging activity (42.32±0.12^{c} % at 20 mg/ml) was exhibited in 100% IE of RDFF. In a similar study, Carciochi *et al.* assayed the antioxidant potentials of raw, fermented and germinated quinoa seed against ABTS radical and reported that the germinated quinoa seed was found to exhibit the highest radical scavenging activity followed by fermented and raw quinoa seed [42]. The results exhibited the same trend of antioxidant potential of RDFF, 72h FDFF, and 96h GDFF extracts in DPPH and FRAP assays.

TABLE 7: ABTS RADICAL SCAVENGING ACTIVITY (%) AND IC_{50} VALUE OF 80%ME, 100%ME, 80%EE, 100% EE, 80% IE AND 100% IE OF RDFF, 72H FDFF AND 96H GDFF AND A-TOCOPHEROL

	Sample extract/ Conc. (mg/ml)	1	10	20	IC_{50} value (mg/ml)
RDFF	80% ME	30.35±0.14 ^{a}	42.57±0.06 ^{b}	62.48±0.09 ^{c}	13.21±0.02 ^{d}
	100% ME	36.20±0.09 ^{a}	48.57±0.13 ^{b}	69.37±0.14 ^{c}	9.55±0.04 ^{d}
	80% EE	42.53±0.05 ^{a}	59.6±0.01 ^{b}	74.05±0.01 ^{c}	5.06±0.02 ^{d}
	100% EE	31.46±0.07 ^{a}	45.19±0.06 ^{b}	64.30±0.12 ^{c}	12.08±0.03 ^{d}
	80% IE	14.13±0.12 ^{a}	26.10±0.09 ^{b}	44.45±0.09 ^{c}	23.95±0.03 ^{d}
	100% IE	15.19±0.17 ^{a}	24.40±0.17 ^{b}	42.32±0.12 ^{c}	26.17±0.11 ^{d}
72h FDFF	80% ME	36.47±0.05 ^{a}	47.16±0.15 ^{b}	67.47±0.05 ^{c}	10.11±0.02 ^{d}
	100% ME	45.42±0.07 ^{a}	61.13±0.12 ^{b}	76.48±0.03 ^{c}	3.60±0.01 ^{d}
	80% EE	45.47±0.05 ^{a}	58.46±0.07 ^{b}	70.17±0.05 ^{c}	4.15±0.02 ^{d}
	100% EE	40.04±0.07 ^{a}	54.13±0.12 ^{b}	72.03±0.05 ^{c}	7.14±0.05 ^{d}
	80% IE	22.05±0.08 ^{a}	34.12±0.11 ^{b}	52.11±0.13 ^{c}	19.11±0.07 ^{d}
	100% IE	15.46±0.07 ^{a}	28.07±0.12 ^{b}	45.45±0.09 ^{c}	23.20±0.07 ^{d}
96h GDFF	80% ME	30.54±0.07 ^{a}	44.07±0.12 ^{b}	62.10±0.09 ^{c}	13.00±0.04 ^{d}
	100% ME	44.05±0.09 ^{a}	56.05±0.09 ^{b}	74.04±0.07 ^{c}	5.25±0.05 ^{d}
	80% EE	47.13±0.12 ^{a}	66.10±0.09 ^{b}	83.45±0.09 ^{c}	2.18±0.06 ^{d}
	100% EE	42.46±0.07 ^{a}	57.10±0.09 ^{b}	72.07±0.12 ^{c}	5.71±0.02 ^{d}
	80% IE	22.55±0.09 ^{a}	44.54±0.07 ^{b}	53.11±0.10 ^{c}	16.57±0.04 ^{d}
	100% IE	20.05±0.09 ^{a}	31.13±0.12 ^{b}	49.05±0.09 ^{c}	21.18±0.06 ^{d}
α-tocopherol		54.20±0.06 ^{a}	70.22±0.10 ^{b}	99.68±0.09 ^{c}	0.057±0.003 ^{d}

All values were presented as mean ± standard deviation for triplicate experiments; mean values with different superscript ($^{a, b, c, d}$) letters within the same row indicated significant ($p< 0.01$) differences.

Identification of Phenolic and Compounds for 80% EE of RDFF, 72h FDFF and 96h GDFF by HPLC: The individual profile of phenolic and flavonoid compounds of 80%EE of RDFF, 72h FDFF and 96h GDFF were identified by HPLC coupled to a photodiode array detector. The HPLC chromatograms of RDFF, 72h FDFF and 96h GDFF extracts were represented in **Fig. 1, 2**, and **3** respectively and the phenolic and flavonoid compounds of the extracts were depicted in **Table 8**. Lignan is a well-known antioxidant that reacts with lipid radicals and converts them into stable products, which can lower the oxidation rate by different mechanisms [43]. Lignan also prevents cancer and diabetes [44]. The highest amount of lignan was present in RDFF extract (25.61%) followed by 72h FDFF (22.30%) and 96h GDFF (14.985%) extracts. Lignan was found in RDFF, 72h FDFF and 96h GDFF extracts at 6.939, 7.013, and 6.850 min RT respectively. Mukkker *et al.* also

reported that flaxseed contains lignan, corroborating this study [45].

Gallic acid is a colorless or slightly yellow crystalline phenolic compound that has antioxidant, antimicrobial, anti-inflammatory, anticancer, gastro-protective, cardio and neuroprotective properties [46]. The present study showed that 80% EE of RDFF 72h FDFF, and 96h GDFF contained gallic acid at 8.464, 8.435, and 8.663 min RT respectively. The highest amount of gallic acid 10.81% was found in 96h GDFF extract. Gallic acid was identified in defatted flaxseed by Kajla *et al.* [47].

Protocatechuic acid is a widely distributed naturally occurring phenolic acid. It has important bioactive properties and chemopreventive activity, including inhibition of generation and scavenging free radicals and upregulating antioxidant enzymes [48]. The highest amount of protocatechuic was identified in 80%EE of 96h GDFF (10.80%), followed by 72h FDFF and RDFF extracts at 7.369, 7.419, and 7.893 min RT respectively. Azad et al. reported that the super olein fraction of flaxseed oil contains protocatechuic acid [49].

Vanillic acid is an oxidized vanillin form found in edible plants and fruits. The anti-inflammatory mechanisms of vanillic acid involved the inhibition of oxidative stress and pro-inflammatory cytokine production [50]. The present study showed that 80% EE of RDFF and 96h GDFF contained vanillic acid at 10.250 min and 10.333 min RT, respectively. Vanillic acid was not found in 72h FDFF extract. Vanillic acid was found in sesame seed at 10.28 min RT, similar to the present study [51].

Catechin is a polyphenolic benzopyran compound and is important for its bioactive properties. Catechin reduces the risk of cardiovascular disease by lowering cholesterol and triglyceride levels [52]. Catechin was present in 96h GDFF extract (9.82%) at 12.602 min RT which was absent in RDFF and 72h FDFF extracts. Li *et al.* reported that catechin was present in grape seed extract [53].

Quercetin is a polyphenolic flavonoid compound that prevents free radicals damage and enhances the body's antioxidant activity with reduced oxidative stress, including the production of nicotine-induced ROS for treating diseases such as nicotine addiction [54]. Quercetin was present in 80% EE of 72h FDFF (13.815%) at 13.815 min RT which was absent in RDFF and 96h GDFF extracts. In another study, Doshi and Hemant identified quercetin by HPLC in *Benincasa hispida* seed (34%) [55].

Rutin (3, 30, 40, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is a flavonol, that helps to prevent neuro-inflammation, stroke. It has anticonvulsant, anti-Alzheimer, antiarthritic, antidiabetic, antihypercholesterolemic, antiulcer, antiasthmatic, antiosteoporotic, anticancer, antimicrobial and anti-cataract effects [56]. The highest amount of rutin was found in 80% EE of 72h FDFF (13.63%) at 12.821 min RT followed by 96h GDFF and RDFF extracts. Doshi and Hemantidentified rutin in *Benincasa hispida* seed (21.99%) [55].

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a phenolic compound that has a wide range of therapeutic effects such as anti-inflammatory, antimicrobial, anti-cancer, anti-cholesterolemic, and antidiabetic effects [57]. It also has a hepatoprotective effect against toxicity induced *in-vivo* by carbon tetrachloride, as reported by Srinivasan *et al.* [58]. The highest amount of ferulic acid was observed in RDFF extract (53.81%) followed by 72h FDFF and 96h GDFF extracts at 13.212, 12.949 and 12.932 min RT respectively. Ferulic acid was also found in deoiled flaxseed cake by Teh *et al.* [59].

Kamferol reduces the risk of developing some types of cancer including liver, colon, and skin cancer [60]. 80% EE of 72h FDFF exhibited the maximum amount of kaempferol (16.88 %) at 14.334 min RT followed by 80% EE of 96h GDFF (7.14%). A little amount of kaempferol was present in 80% EE of RDFF (0.004%). By using the HPLC method, Shalavadi et al. investigated the gallic acid and kaempferol content of *Cassia hirsuta* seeds extracts and noted that kaemferol is more abundant in ethanol extract of *Cassia hirsuta* seeds (0.65%) [61].

The germination process showed a progressive increase of phenolic compounds, which was more noticeable for gallic acid and protocatechuic acid **Table 8**. Catechin was present in 96h GDFF extract which was absent in RDFF and 72h FDFF extracts.

72h FDFF extract had the highest amounts of rutin and kaempferol. Quercetin was found in 72h FDFF extract which was absent in RDFF and 96h GDFF extracts.

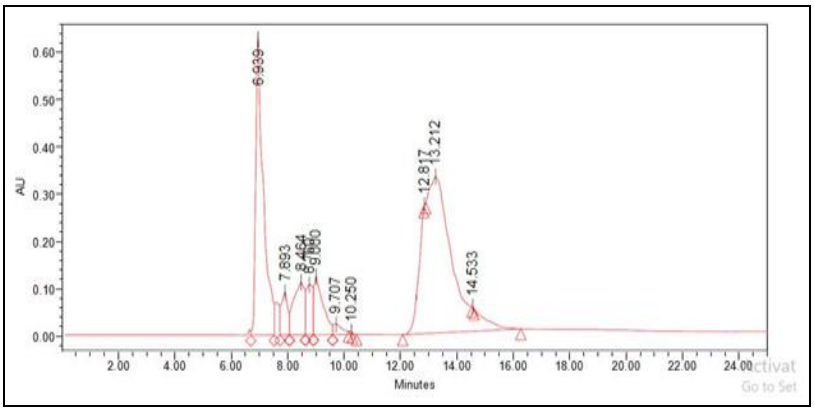


FIG. 1: THE HPLC CHROMATOGRAM OF 80% EE OF RDFF

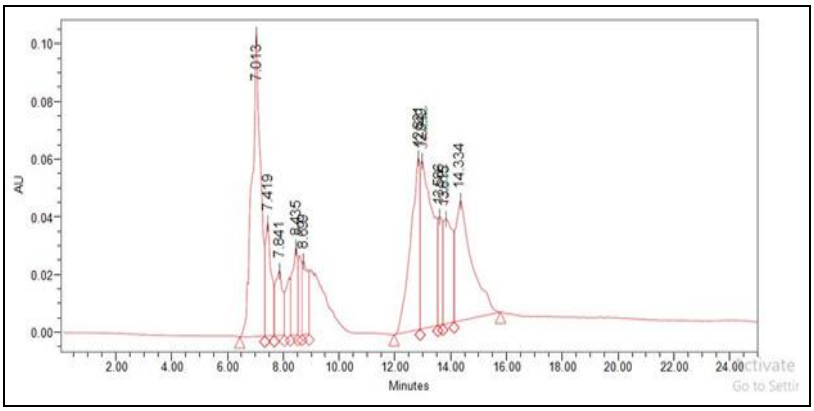


FIG. 2: THE HPLC CHROMATOGRAM OF 80% EE OF 72H FDFF

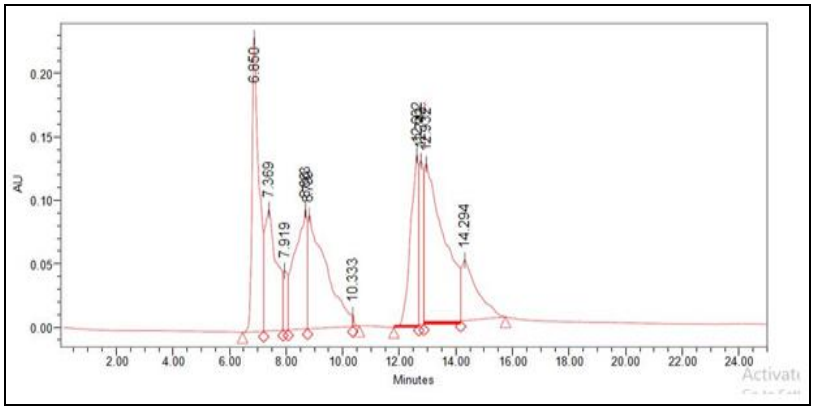


FIG. 3: THE HPLC CHROMATOGRAM OF 80% EE OF 96H GDFF

TABLE 8: HPLC IDENTIFICATION OF PHENOLIC AND FLAVONOID COMPOUNDS PRESENTS IN 80% EE OF RDFF, 72H FDFF AND 96H GDFF

Phenolic compound	80% EE of RDFF (%)	80% EE of 72h FDFF (%)	80% EE of 96h GDFF (%)
Lignan	25.61	22.30	14.985
Gallic acid	6.49	3.99	10.81
Protocatechuic acid	3.07	5.65	10.80
Vanillic acid	0.006	ND	0.11
Catechin	ND	ND	9.82
Quercetin	ND	8.24	ND
Rutin	0.006	13.63	5.36
Ferulic acid	53.81	17.60	23.42
Kaempferol	0.004	16.88	7.14

FTIR Spectroscopic Analysis of 80% EE of RDFF, 72h FDFF and 96h GDFF: The FTIR spectrum of 80% EE of RDFF, 72h FDFF and 96h GDFF was presented in **Fig. 4**. FTIR identified the functional groups and chemical bonds present in the extracts. The functional group of the active components was based on the peak values of IR radiation.

When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of their peak. 80% EE of RDFF, 72h FDFF and 96h GDFF had the characteristic IR absorptions associated with the region 3400-3200 $cm^{-1}$with a very broad band indicating the O-H stretch.

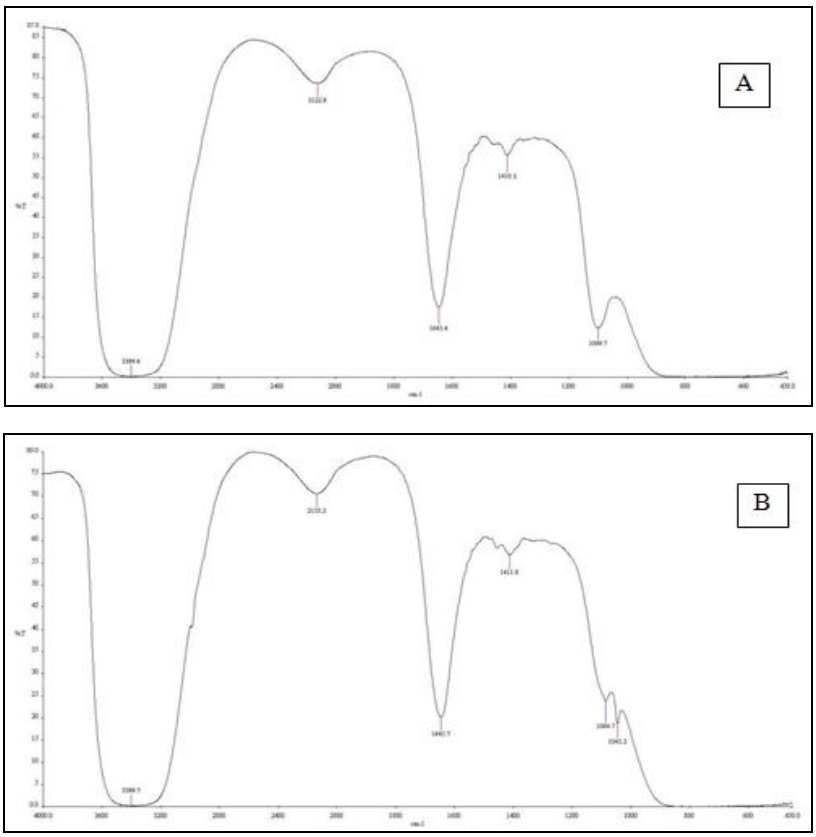
Similarly, Izza *et al.* reported that the broad absorption band located at 3321.96 cm^{-1} was assigned to the OH phenolic present in moringaseed ethanolic extract [62].

The bands in 2122.8 cm−1, 2135.2 cm−1, and 2138.6 cm−1 indicated C≡C groups in RDFF, 72h FDFF, and 96h GDFF extracts, respectively. Garnica-Romo *et al.* reported that the wave number at 2133 cm^{-1} corresponds to C≡C links in flaxseed mucilage [63]. The region of 1650-1600 cm^{-1} confirmed the C=O groups present in 80% EE of RDFF, 72h FDFF, and 96h GDFF. Similarly, the band at 1604 cm^{-1} can be assigned to the C=O bond asymmetric stretching vibration in the flaxseed mucilage [63].

80% EE of 96h GDFF showed the characteristic absorption band at 1424 cm^{-1} which indicated the CH band. This band was absent in RDFF and 72h FDFF extracts. A study by Garnica-Romo *et al.* revealed that the peak in 1425 cm^{-1} represents the -CH bending of flaxseed mucilage [63].

The bands in 1410 cm−1 to 1310 cm−1 indicated the $-CH_3$ group present in 80% EE of RDFF, 72h FDFF and 96 h GDFF. Tri Prasetya *et al.* reported that the peak at 1372.68 cm^{-1}showing $-CH_3$ group in papaya seed [64].

In the region, 1100–1000 cm^{-1} indicated the C-OH group present in 80% EE of RDFF and 72h FDFF, which was absent in 80% EE of 96h GDFF. In another study, Izza *et al.,* reported that the peak present in 1057.68 cm^{-1}, is for the C-OH group present in moringaseed ethanolic extract [62].



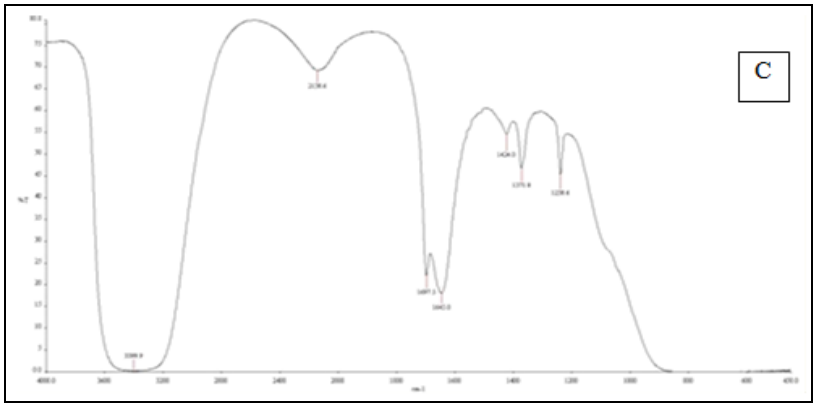


FIG. 4: FTIR OF 80% EE OF RDFF [A], 80% EE OF 72H FDFF [B] AND 80%EE OF 96H GDFF [C]

Antibacterial Activity of 80% EEs of RDFF, 72h FDFF and 96h GDFF: From the above study, it was observed that 80% EEs of RDFF, 72h FDFF, and 96h GDFF were the most promising extracts to scavenge free radicals. Therefore these three extracts were taken for the study of antibacterial activity.

The antibacterial activity of these three extracts was tested against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli*) bacteria. The results were shown in **Table 9**. 80% EE of 96h GDFF showed the highest zone of inhibition against Gram-positive bacteria, *B. subtilis* (12.33±0.58 mm), and Gram-negative bacteria, *E. coli* (14.33±0.29 mm). Whereas 80% EE of 72h FDFF was developed in the highest inhibitory zone against Gram-positive bacteria *S. aureus* (12.33±0.24 mm). According to Amin and Thakur, the antibacterial activity of ethanol and chloroform extracts of flaxseed was 7.34 mm and 17.67 mm, respectively, against *Staphylococcus aureus* [13].

Similarly, ethanol and chloroform flaxseed extracts exhibited antibacterial activity against *Bacillus substilis* 6.72 mm and 7.17 mm respectively [13]. Chloroform extract of flaxseed also showed antibacterial activity against *E. coli* (7.2 mm) [13].

TABLE 9: ANTIBACTERIAL ACTIVITY OF 80% EES OF RDFF, 72H FDFF AND 96H GDFF

	Zone of inhibition (mm)		
Sample	***B. subtilis***	***St. Aureus***	***E. coli***
80% EE of RDFF	10.67 ± 0.29^{a}	10.67 ± 0.24^{a}	12.17 ± 0.29^{a}
80% EE of 72h FDFF	11.37 ± 0.15^{b}	12.33 ± 0.24^{b}	13.33 ± 0.29^{b}
80% EE of 96h GDFF	12.33 ± 0.29^{c}	11.67 ± 0.12^{c}	14.33 ± 0.29^{c}

Results were expressed as mean ± SD (n=3). Mean Values having different superscript letters in columns were significantly different ($p\leq0.05$).

Antiarthritic Activity of 80% EE of RDFF, 72h FDFF and 96h GDFF: BSA was used in the anti-denaturation study to investigate the antiarthritic activity.

In response to heat, BSA undergoes denaturation and antigens are expressed that cause type-III hypersensitivity reactions, which, in turn, lead to diseases like serum sickness, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus [65].

Changes in electrostatic, hydrogen, hydrophobic, and disulphide bonds 65 most likely cause denaturation. The antiarthritic activity of 80% EEs of RDFF, 72h FDFF and 96h GDFF were presented in **Table 10**. Maximum inhibition of bovine serum denaturation was observed in 80% EE of GDFF (39.30 ± 0.16^{c} % at 0.1 mg/ml) followed by 72h FDFF and RDFF. Diclofenac sodium used as standard showed 83.03 ± 0.37^{d} % inhibition of denaturation of bovine serum at 0.1mg/ml.

To the best of our knowledge, the present study reported for the first time the antiarthritic activity of RDFF, 72h FDFF and 96h GDFF extracts. Singh *et al*. reported that flaxseed oil has antiarthritic activity [66]. The promising activities of the 80% EE of 96h GDFF support their traditional use in treating arthritis, rheumatism and other chronic inflammatory diseases.

TABLE 10: ANTIARTHRITIC ACTIVITY OF 80% EES OF RDFF, 72H FDFF AND 96H GDFF

Sample	Antiarthritic activity (%)	
	0.05 mg/ml	0.1 mg/ml
80% EE of RDFF	7.26 ± 0.14^{a}	21.64 ± 0.09^{a}
80% EE of 72h FDFF	10.18 ± 0.27^{b}	26.64 ± 0.16^{b}
80% EE of 96h GDFF	23.02 ± 0.12^{c}	39.30 ± 0.16^{c}
Diclofenac sodium	75.10 ± 0.26^{d}	83.03 ± 0.37^{d}

Results were expressed as mean ± SD (n=3). Mean Values having different superscript letters in columns were significantly different ($p \leq 0.05$).

CONCLUSIONS: The total phenolic content, flavonoid content, and antioxidant activities were more in 96h GDFF extracts than RDFF and 72h FDFF extracts. RDFF, 72h FDFF and 96h GDFF extracts have the highest TPC, TFC and antioxidant activities when extracted with 80% ethanol, compared to other solvents. The germination and fermentation processes enhanced some phenolic and flavonoid compounds in flaxseed. Both fermentation and germination processes improved antibacterial activity and antiarthritic activity. From this study, it was concluded that the presence of polyphenol compounds in the 80% EE of RDFF, 72h FDFF and 96h GDFF had antioxidant, antibacterial and antiarthritic

ACKNOWLEDGMENT: The authors gratefully acknowledged the Indian Institute of Engineering Science and Technology, Shibpur, for offering research facilities. I would also like to acknowledge the Indian Institute of Chemical Biology, Kolkata, for HPLC analysis during this work.

CONFLICTS OF INTEREST: Authors declare no conflict of interest.

REFERENCES:

1. Kajla PS, Sharma A and Sood DR: Effect of germination on proximate principles, minerals and antinutrients of flaxseeds. Asian J Dairy Food Res 2017; 36(1).
2. Kasote DM: Flaxseed phenolics as natural antioxidants. Int Food Res J 2013; 20: 27-34.
3. Xu JG, Tian CR, Hu QP, Luo JY, Wang XD and Tian XD: Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. J Agric Food Chem 2009; 57(21): 10392-10398.
4. Cevallos-Casals BA and Cisneros-Zevallos L: Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chem 2010; 119(4): 1485-1490. DOI: 10.1016/j.foodchem.2009.09.030.
5. Ugbogu EA, Nwoku CD, Ude VC and Emmanuel O: Evaluating bioactive constituents and toxicological effect of aqueous extract of fermented *Pentaclethra macrophylla* seeds in rats. Avicenna J Phytomed 2020; 10(1): 101-113.
6. Ogueke CC, Nwosu JN, Owuamanam CI and Iwouno JN: Ugba, the fermented African oil bean seeds; its production, chemical composition, preservation, safety and health benefits. Pak J Biol Sci 2010; 13(10): 489-496.
7. Olawoye BT and Gbadamosi SO: Effect of different treatments on in vitro protein digestibility, antinutrients, antioxidant properties and mineral composition of *Amaranthus viridis* seed. Cogent Food Agric 2017; 3(1): 1296402. DOI:10.1080/23311932.2017.1296402.
8. Lourenço SC, Moldão-Martins M and Alves VD: Antioxidants of natural plant origins: from sources to food industry applications. Molecules 2019; 24(22): 4132.
9. Barthet VJ, Klensporf-Pawlik D and Przybylski R: Antioxidant activity of flaxseed meal components. Can J Plant Sci 2014; 94(3): 593-602.
10. Powanda MC, Whitehouse MW and Rainsford KD: Celery seed and related extracts with antiarthritic, antiulcer, and antimicrobial activities. Prog Drug Res 2015; 70: 133-153.
11. Kaithwas G and Majumdar DK: Therapeutic effect of *Linum usitatissimum* (Linseed Flaxseed) fixed oil on acute and chronic arthritic models in albino rats. Inflammopharmacology 2010; 18(3): 127-136. DOI: 10.1007/s10787-010-0033-9.
12. Hanaa MH, Ismail HA, Mahmoud ME and Ibrahim HM: Antioxidant activity and phytochemical analysis of flaxseeds (*Linum usitatisimum* l). Minia J. of Agric Res Dev 2017; 37(1): 129-140.
13. Amin T and Thakur MA: Comparative Study on Proximate Composition, Phytochemical Screening, Antioxidant and antimicrobial Activities of (*Linum usitatisimuml*.) flaxseeds. Int J Curr Microbiol Appl Sci 2014; 3(4): 465-481. http://www.ijcmas.com.
14. Ijarotimi OS, Adeoti OA and Ariyo O: Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated and fermented *Moringa oleifera* seed flour. Food Sci Nutr 2013; 1(6): 452-463. DOI: 10.1002/fsn3.70.
15. Anwar F and Przybylski R: Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). Acta Sci Pol Technol Aliment 2012;11(3): 293-301.
16. Sultana B, Anwar F and Ashraf M: Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009; 14(6): 2167-2180. DOI: 10.3390/molecules14062167.
17. Škerget M, Kotnik P, Hadolin M, Hraš AR, Simonič M and Knez Ž: Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 2005; 89(2): 191-198. DOI: 10.1016/j.foodchem.2004.02.025.
18. Sultana B, Anwar F and Przybylski R: Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica* and *Eugenia jambolana* Lam Trees. Food Chem 2007; 104(3): 1106-1114. DOI: 10.1016/j.foodchem.2007.01.019.
19. Kim JS and Lee JH: Correlation between solid content and antioxidant activities in Umbelliferae salad plants. Prev

Nutr Food Sci 2020; 25(1): 84-92. DOI: 10.3746/pnf.2020.25.1.84.
20. Kaur P, Singh B, Kumar S and Kaur S: *In-vitro* evaluation of free radical scavenging activity of *Rubia cordifolia* L. J Chin Clin Med 2008; 3(5): 278-284.
21. Benzie IF and Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239(1): 70-76. DOI: 10.1006/abio.1996.0292.
22. Hsu C, Chen W, Weng Y and Tseng C: Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem 2003; 83(1): 85-92. DOI: 10.1016/S0308-8146(03)00053-0.
23. Oyaizu M: Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet 1986; 44(6): 307-315. DOI: 10.5264/eiyogakuzashi.44.307.
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26(9-10): 1231-1237. DOI: 10.1016/S0891-5849(98)00315-3.
25. Vogt S, Löffler K and Dinkelacker AG: Fourier-transform infrared (FTIR) spectroscopy for typing of clinical Enterobacter cloacae complex isolates, frontiers in. Microbiology 2019; 10. https://www.frontiersin.org/article/10.3389/fmicb.2019.02582.
26. Rohman A and Man YBC: Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. Food Res Int 2010; 43(3): 886-892. DOI: 10.1016/j.foodres.2009.12.006.
27. Collins CH, Lynes PM and Grange JM: Microbiological methods. (7thEdn.) Butterwort-Heinemann Ltd. Britain 1995; 175-190.
28. Rahman MM, Islam MB, Biswas M and Khurshid Alam AH: *In-vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Res Notes 2015; 8: 621. DOI: 10.1186/s13104-015-1618-6.
29. Johari MA and Khong HY: Total phenolic content and antioxidant and antibacterial activities of *Pereskia bleo*. Advances in Pharmacological and Pharmaceutical Sciences, Adv Pharmacol Sci 2019; 2019: 7428593. DOI: 10.1155/2019/7428593.
30. Adetuyi FO and Ibrahim TA: Effect of fermentation time on the phenolic, flavonoid and vitamin C contents and antioxidant activities of okra (*Abelmoschus esculentus*) seeds. Niger Food J 2014; 32(2): 128-137. DOI: 10.1016/S0189-7241(15)30128-4.
31. Panche AN, Diwan AD and Chandra SR: Flavonoids: an overview. J Nutr Sci 2016; 5(47): 47.
32. Uchegbu NN and Amulu NF: Effect of germination on proximate, available phenol and flavonoid content, and antioxidant activities of African yam bean (*Sphenostylis stenocarpa*). Int J Nutr Food Eng 2015; 9(1): 106-109. DOI: 10.17758/iaast.a0115004.
33. Gulcin İ, Alwasel SHI and Alwasel Metal Ions, Metal Chelators and Metal Chelating Assay as Antioxidant Method. Processes 2022; 10(1): 132. DOI: 10.3390/pr10010132.
34. Repetto MG, Ferrarotti NF and Boveris A: The involvement of transition metal ions on iron-dependent lipid peroxidation. Arch Toxicol 2010, 84(4): 255-262.
35. Singh A, Sharma S, Singh B and Kaur G: *In-vitro* nutrient digestibility and antioxidative properties of flour prepared from sorghum germinated at different conditions. J Food Sci Technol 2019; 56(6): 3077-3089.
36. Gaafar AA, Salama ZA, Askar MS, El-Hariri DM and Bakry BA: *In-vitro* antioxidant and antimicrobial activities of lignan flax seed extract (*Linum usitatissimum*, L.). Int J Pharm Sci Rev Res 2013; 23(2): 291-297.
37. Bhalodia NR, Nariya PB, Acharya RN and Shukla VJ: In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. Ayu 2013; 34(2): 209-214. DOI: 10.4103/0974-8520.119684.
38. James S, Nwabueze TU, Ndife J, Onwuka GI and Ata'Anda Usman M: Influence of fermentation and germination on some bioactive components of selected lesser legumes indigenous to Nigeria. Journal of Agriculture and Food Research 2020; 2. DOI: 10.1016/j.jafr.2020.100086.
39. Hassan AB, Al Maiman SA and Mohammed MA: Effect of natural fermentation on the chemical composition, mineral content, phytochemical compounds and antioxidant activity of *Ziziphus spina-christi* (L.) "Nabag" seeds. Processes 2021; 9(7): 1228.
40. Ratnavathi CV and Komala VV: Chapter 1. Sorghum grain quality. In: Ratnavathi CV, Patil JV, Chavan UD, eds. Sorghum Biochemistry 2016; 1-61. DOI: 10.1016/B978-0-12-803157-5.00001-0.
41. Carciochi RA, Galván-D'Alessandro L, Vandendriessche P and Chollet S: Effect of germination and fermentation process on the antioxidant compounds of quinoa seeds. Plant Foods Hum Nutr 2016; 71(4): 361-367.
42. Decker EA, Warner K, Richards MP and Shahidi F: Measuring antioxidant effectiveness in food. J Agric Food Chem 2005; 53(10): 4303-4310.
43. Touré A and Xueming X: Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. Compr Rev Food Sci Food Saf 2010; 9(3): 261-269.
44. Mukker JK, Kotlyarova V, Singh RS and Alcorn J: HPLC method with fluorescence detection for the quantitative determination of flaxseed lignans. J Chromatogr B Analyt Technol Biomed Life Sci 2010; 878(30): 3076-3082.
45. Kahkeshani N, Farzaei F and Fotouhi M: Pharmacological effects of gallic acid in health and disease: A mechanistic review. Iran J Basic Med Sci 2019; 22: 225-237.
46. Kajla P, Sharma A and Sood DR: Flaxseed-a potential functional food source. J Food Sci Technol 2015; 52(4): 1857-1871. DOI: 10.1007/s13197-014-1293-y.
47. Kakkar S and Bais S: A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol 2014; 2014: 952943.
48. Azad M, Nadeem M, Gulzar N and Imran M: Impact of fractionation on fatty acids composition, phenolic compounds, antioxidant characteristics of olein and super olein fractions of flaxseed oil. J Food Process Preserv 2021; 45(4): 15369.
49. Calixto-Campos C, Carvalho TT and Hohmann MS: Vanillic acid inhibits inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine production, and NFκB activation in mice. J Nat Prod 2015; 78(8): 1799-1808.
50. Mekky RH, Abdel-Sattar E and Segura-Carretero A and Contreras MDM: Phenolic compounds from sesame cake and antioxidant activity: A new insight for Agri-food residues' significance for sustainable development. Foods 2019; 8(10): 432. DOI: 10.3390/foods8100432.
51. Coşarcă S, Tanase C and Muntean DL: Therapeutic aspects of catechin and its derivatives – an update. Acta Biologica Marisiensis 2019; 2(1): 21-29.

52. Li W, Fong HHS, Singletary KW and Fitzloff JF: Determination of catechins in commercial grape seed extract. J Liq Chromatogr Relat Technol 2002; 25(3): 397-407.
53. Yarahmadi A, Moradi Sarabi M, Sayahi A and Zal F: Protective effects of quercetin against hyperglycemia-induced oxidative stress in hepatic HepG2 cell line. Avicenna J Phytomed 2021, 11(3): 269-280.
54. Doshi GM and Une HD: Quantification of quercetin and Rutin from *Benincasa hispida* Seeds and *Carissa congesta* Roots by high-performance thin layer chromatography and high-performance liquid chromatography. Pharmacogn Res 2016; 8(1): 37-42.
55. Ganeshpurkar A and Saluja AK: The pharmacological potential of Rutin. Saudi Pharm J 2017; 25(2): 149-164. DOI: 10.1016/j.jsps.2016.04.025.
56. Gohil KJ, Kshirsagar SB and Sahane RS: Ferulic acid – a comprehensive pharmacology of an important bioflavonoid. Int J Pharm Sci Res 2012; 3(1): 700-710.
57. Srinivasan M, Sudheer AR and Menon VP. Ferulic Acid: therapeutic potential through its antioxidant property. J Clin Biochem Nutr 2007; 40(2): 92-100. DOI: 10.3164/jcbn.40.92.
58. Teh SS and Birch J: Physiochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. J Food Compos Anal 2013; 30(1): 26-31. DOI: 10.1016/j.jfca.2013.01.004.
59. Wang J, Fang X and Ge L: Antitumor, antioxidant and anti-inflammatory activities of kaempferol and its corresponding glycosides and the enzymatic preparation of kaempferol. PLOS ONE 2018; 13(5): e0197563.
60. Shalavadi M, Chandrashekhar VM and Muchchandi I: High-performance liquid chromatography analysis of gallic acid and kaempferol in chloroform and ethanol extract of Cassia hirsuta seeds. Int J Green Pharm 2019; 13: 236.
61. Izza N, Dewi SR and Setyanda A: Microwave-assisted extraction of phenolic compounds from *Moringa oleifera* seed as anti-biofouling agents in membrane processes. MATEC Web Conf 2018; 204: 03003. DOI: 10.1051/matecconf/201820403003.
62. Garnica-Romo MG, Coria-Caballero V, Tranquilino-Rodríguez E, Dasgupta-Schubert N, Villicaña-Méndez M, Agarwal V and Martínez-Flores HE: Ecological Method for the Synthesis, Characterization, and Antimicrobial Effect of Silver Nanoparticles Produced and Stabilized with a Mixture of Mucilage/Proteins Extracted from Flaxseed. Journal of Inorganic and Organometallic Polymers and Materials 2021; 31(8): 3406–3415.
63. Prasetya AT, Mursiti S, Maryan S and Jati NK: Isolation and Identification of Active Compounds from Papaya Plants and Activities as Antimicrobial. IOP Conf S Mater Sci Eng 2018; 349. DOI: 10.1088/1757-899X/349/1/012007.
64. Elisha IL, Dzoyem JP, McGaw LJ, Botha FS and Eloff JN: The anti- arthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. BMC Complement Altern Med 2016; 16(1): 307.
65. Singh S, Nair V and Gupta YK: Linseed Oil: An Investigation of its Antiarthritic Activity in Experimental Models. Phytother Res 2012; 26(2): 246-252.

How to cite this article:
Ghosal S, Sengupta S and Bhowal J: A comparative evaluation of bioactive properties of raw, fermented, and germinated deoiled flaxseed flour extracts by different solvents. Int J Pharm Sci & Res 2023; 14(7): 3374-91. doi: 10.13040/IJPSR.0975-8232.14(7).3374-91.