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Biological activities of phenolic compounds extracted from flaxseed meal

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Abstract

Background: There is a worldwide demand for phenolic compounds (PC) because they exhibit several biological activities. The present investigation deals with a comprehensive study on the biological activities of phenolic compounds extracted from flaxseed meal (FM) with the aid of ultrasonic waves.

Results: The antioxidant activity of the PC extract of FM is considerably high when measuring it by the three methods (the β-carotene coupled oxidation method, the DPPH free radical scavenging activity method, and measuring the reducing antioxidant power). The toxicity test revealed that the PC extract was nontoxic on normal retina cell line. Also, it has no anticoagulating activity. Evaluation of antimicrobial activity showed that it is effective towards four strains only from seven. FM phenolic extract has been evaluated as chemo-preventive agents by testing the product for any cytotoxic activity against human tumor cell lines. The highest inhibitory effect was achieved on cell lines of colon carcinoma and lung carcinoma with IC_{50} = 22.3 and 22.6 μ/ml respectively.

Conclusion: The PC extracted from FM showed high antioxidant activity, nontoxic on normal retina cell line, no anticoagulating activities, and an antimicrobial effect on some pathogenic bacteria, so the phenolic compounds extracted from flaxseed meal showed significant biological activities.

Keywords: Flaxseed meal, Phenolic compounds, Antioxidant, Antimicrobial, Anticoagulant, Cytotoxicity, Anticancer

Introduction

The components of flaxseed meal were found to have a wide spectrum of biological activity. Utilization of flaxseed has been shown to exhibit a huge number of advantages to health including the diminishing rate of tumor development, lowering serum cholesterol level, and diminishing rate of breast, prostrate, and colon diseases (Muir et al. 2003; Hemmings et al. 2004; Hosseinian et al. 2006; Choo et al. 2007).

Early research showed that flaxseed possesses a very powerful antioxidant system; ground and whole flaxseeds were stable at room temperature for numerous months [Chen et al. 1994; Malcolmson et al. 2000]. Ground/milled and whole flaxseeds stored showed negligible oxidative deterioration at room temperature, which indicates the presence of an efficient antioxidant system, where compounds other than tocopherols may play an important function [Chen et al. 1994); Malcolmson et al. (2000); Hosseinian et al. (2006)].

Flaxseed is particularly rich in lignans, e.g., secoisolariciresinol diglucoside (SDG), which are also present in flaxseed oil. These components have some antioxidant activity (Hosseinian et al. 2006). The proved functions of lignans are related to health benefits and the protection from some types of cancer and heart diseases [Kim and Ilich 2011; Park and Velasquez 2012; Ayella et al. 2010; Rodriguez-Leyva et al. 2010]. Besides lignans, flaxseed contains 8:10 gkg^{-1} of phenolic acids, mainly p-coumaric, vanillic, sinapic, and ferulic, which are present in the seed as glycosides with ester and ether bonds [Oomah et al. 1995); Johnsson (2004)]. Phenolic acids have antioxidant activities, related to the positions of hydroxyl groups on the ring [Przybylski and Daun 2005); Tuberoso et al. (2007)].

Previous studies demonstrated that the antioxidant compounds enhance human health, for example, restraint of tumor cells, enhancing the state of cardiovascular sicknesses and diabetes [Żuk et al. 2011], curing human chronic ulceration [Skórkowska-Telichowska et al. (2010)], against allergenic, hostile to atherogenic, mitigating against microbial, antioxidant, against

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thrombotic, cardioprotective, and vasodilatory impacts [Benavente-García et al. 1997].

Khouya et al. (2015) evaluated several biological activities including anticoagulant, anti-inflammatory, and antioxidant from aqueous extracts of thyme varieties. Wee et al. (2010) extracted active compounds from Korean red ginseng which were identified as phenolic compounds (namely vanillic, caffeic, ferulic, and p-coumaric acid). These products possess anticoagulant activities. Daud et al. (2013) stated that the ethanolic extracts from *Averrhoa bilimbi* even from fruits or leaves showed a considerable anticoagulant effect on rats.

Plants produce a large number of organic compounds that possess antimicrobial activity. The compounds are found in various parts of plant such as leaves, bark, stems, roots, fruits, flowers, or and seeds. These compounds include alliin/allicins, isothiocyanates, plant pigments [Cutter 2000], proteins, hydrolytic enzymes, essential oils [Smid and LGM 1999], and phytoalexins or phenolic compounds [Cutter 2000; Smid and LGM 1999].

A huge number of chemical compounds are present in seeds or seed coats, including alkaloids, lectins, and phenolic compounds such as lactones, tannins, and flavonoids. These compounds play a role in the seed protection from microbial attack until conditions are improved for germination [Cai et al. 2004; Komutarin et al. 2004].

Dietary use of seedcake is effective; it is utilized against number of diseases, as skin diseases, respiratory tract diseases, and gastrointestinal tract diseases. Secoisolariciresinol diglucoside (SDG) is the richest components of flaxseed cake, the precursor of lignans, which have many advantages on human health. SDG is known to possess anticancer properties by reducing cell proliferation and growth, especially in breast and prostate cancer [Wang et al. 2005]. SDG, which has also antimicrobial effect including anti-viral, antibacterial, and anti-fungal properties, is an antioxidant, and it has been shown to improve immune system functioning [Adolphe et al. 2010]. Besides that flaxseed cake includes phenolic acids, flavonoids, and other phenylpropanoids, it is known to have antioxidative properties and thus having helpful actions on human health [Korkina 2007].

Intact flaxseed is generally considered as a healthy food that has anticancer action. Controlled trial diets have exhibited various valuable impacts of flaxseed utilization [Clark et al. 1995; Cunnane et al. 1993; Jenkins et al. 1999]. Dietary flaxseed cake decreases epithelial cell generation and nuclear deviation in mammary glands of female rats. This finding showed that flaxseed may lessen the development rate of mammary tumor (Serraino and Thompson 1991). Furthermore, it has been noticed that flaxseed lignan diminishes mammary tumor development in the later phases of carcinogenesis (Thompson et al. 1996). Essentially, it has been demonstrated that the substitution of corn meal, with flaxseed meal (15%) or corn oil with flaxseed oil (15%) in a basal eating routine, diminished tumor generation and size in the small digestive system and colon of Fischer 344 male rats. The creators presumed that flaxseed meal and oil are successful chemo-preventive operators (Bommareddy et al. 2009).

In a recent publication (Akl et al. 2017), the same authors reported on the extraction of phenolic compounds (PC) from flaxseed meal (FM) using a variety of extraction techniques, and then evaluated its phenolic contents of each method. The present investigation aims to deal with a comprehensive study on the biological activities of optimum condition obtained by ultrasonic assisted extraction. Some biological activities of these phenolic extracts (antioxidant, antimicrobial, anticoagulation, and anticancer effects) were evaluated.

Materials and methods

Materials

One hundred grams of flaxseed meal were added to 3 l of distilled water and stirred well then transferred to ultrasonic bath for 2 h at 35 °C then centrifuged to give the supernatant which contains the phenolic compounds. This crude extract was purified then freeze dried by Crest Alpha 1-4 LSC plus, Germany, and reserved in refrigerator until use.

Methods

β-Carotene coupled oxidation method

Determination of the AOA of the extracts was based on the coupled oxidation of β-carotene and linoleic acid according to the method of (Taga et al. 1984), with some modifications. β-Carotene (0.1 g) was dissolved in 20 ml of $CHCl_3$ solution. Three milliliters of aliquots of the former solution was added to a round bottom flask along with 40 mg of linoleic acid and 400 mg Tween 20. Chloroform was evaporated until dryness under reduced pressure at low temperature (less than 30 °C). One hundred milliliters distilled water were added to the dried mixture and mixed well (β-carotene emulsion. Aliquots from the PC extracts to be examined (500 μl each) were added to 5 ml β-carotene emulsion and incubated in a water bath at 50 °C for (60 min). Absorbance of the sample was measured against blank at 470 nm after 60 min. A blank sample formerly prepared (40 mg linoleic acid and 400 mg Tween 20 in distilled water). Also, an aliquot (500 μl PC extract added to 5 ml β-carotene emulsion) was shaken well and measured immediately (zero time).

The degradation (Dr) of β-carotene was calculated using the first order kinetic equation:

Dr of sample = ln $(A°/\mathrm{At})/T$

where $A°$ = absorbance of sample (PC extract)—absorbance of blank at zero time

At = absorbance of sample—absorbance of blank at t time T = 60 min of incubation at 50 °C

Dr of control sample = ln ($A°$/At)/T

where $A°$ = absorbance of control sample at zero time

At = absorbance of control sample at time t (60 min) at 50 °C

Antioxidant activity was expressed as:

AOA% = Dr of control sample − Dr of sample × 100/Dr control sample.

Determination of antioxidant activity by the DPPH free radical scavenging activity method

The scavenging activity of DPPH free radicals were measured according to (Zhao et al. 2008). DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml methanol (it has a violet color). Four milliliters of DPPH solution and 0.5 ml of PC extract were mixed. The mixture was shaken vigorously and left in the dark to stand at 30° C for 30 min.

A blank for each sample was prepared: 0.5 ml of sample with 4 ml methanol. Four milliliters of methanolic DPPH solution against methanol served as control.

Decolorization of the methanolic DPPH solution was determined by measuring the decrease in absorbance at 517 nm using a spectrophotometer model (UV-VIS Spectrophotometer PG Instruments, UK), and DPPH was calculated according to the following equation:

Scavenging rate = (1− (A1 − A2)) × 100

where A1 represents the absorption of the sample PC extract and A2 represents the absorbance of control.

Measuring the reducing antioxidant power

The reducing antioxidant power of the PC extracts was measured according to the method of (Oyaizu 1986). Different concentrations of the PC extract (500 ppm) completed to 1 ml of distilled water were mixed with sodium phosphate buffer (2.5 ml, 1%), then 2.5 ml of 1% potassium ferricyanide (K_3 Fe $(CN)_6$) was added to the mixture. The mixture was incubated at 50° C for 20 min then 2.5 ml of trichloroacetic acid (10%) were added, then centrifuged for 15 min at 3000 rpm, 2.5 ml of the upper layer of solution of the mixture were mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and were mixed well. The absorbance was measured at 700 nm a blank using a spectrophotometer model (UV-VIS Spectrophotometer PG Instruments, UK). The increased absorbance of the reaction mixture indicates increase in reducing power.

Determination of antimicrobial activity

The antimicrobial activity of FM phenolic extracts of different concentrations was determined by the agar well diffusion method [Con et al. (2001)]. The seven pathogenic indicator bacteria strains were obtained from the stock cultures of the Dairy Microbiological Lab, National Research Centre: *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 7644, and *Yersinia enterocolitica* ATCC 9610. Each strain was activated in tryptone soy broth by fermentation at 37° C for 24 h. One milliliter culture of the activated indicator strain (105 cells/ml) was inoculated into 20 ml of Mueller-Hinton agar (Becton Dickinson, USA) and poured in petri dishes. After solidification of the agar, wells of 5 mm in diameter were cut from the agar with a sterile borer, and 50 μl of phenolic extract were introduced in each well. Dishes were incubated for 24 h at 37 °C.

The zone diameter of wells cut in Mueller-Hinton agar was 5.0 mm, and the diameter of inhibition zone (DIZ) of negative a control for each bacterium was also 5.0 mm. If the DIZ value is 5.0 mm (*), it means the sample has no inhibitory activity against that bacterium. The diameters of the inhibition zones were measured (Rammelsberg and Radler 1990).

Cytotoxic effect on human normal retina cell line (RPE1)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT 3-(4,5-dimethylthiazol tetrazilium bromide) to purple formazan (Bassyouni et al. 2014).

Determination of anticarcinogenic effect

Cell line Carcinomas

Liver carcinoma cell line (HEPG2), larynx carcinoma cell line (HEP2), colon carcinoma cell line (HCT), cervical carcinoma cell line (HELA), breast carcinoma cell line (MCF7), intestinal carcinoma cell line (CACO), and normal melanocytes (HFB4) were supplied and used in The National Cancer Institute, Biology Department, Cairo, Egypt, and the evaluation was done by the Sulfo-Rhodamine-B stain (SRB) assay, according to the method of Skehan et al. (1990).

Anticoagulation assay

The clotting times were measured using a blood coagulation analyzer (Behnk Elektronik Norderstedt, Germany). To measure the thrombin time (TT), 50 μl of 0.02 $MCaCl_2$, 50 μl of thrombin, and 50 μl of sample were pre-incubated at 37 °C for 3 min. The coagulation reaction was started by the addition of 100 μl of citrated human plasma.

Table 1 Antioxidant activity of FM phenolic extracts as measured by three different methods

PC extract flaxseed meal	β-Carotene method, %	DPPH method, %	Reducing antioxidant power, μg/g
	73.52	55.28	4240

Results

The antioxidant activity of the PC extract of FM is considerably high when measured by the three methods. The antioxidant activity of PC extract (Table 1) prepared by UAE from FM was determined by three methods: (1) β-carotene coupled oxidation method, (2) DPPH free radical scavenging activity method, and (3) the reducing antioxidant power. The results of the three experiments proved that FM PC extract possessed antioxidant power. β-Carotene, DPPH method, and reducing antioxidant power were: 73.52, 55.28 and 4240, respectively, for FM extract. BHT as a control measured 82.91% by β-carotene method, 61.72% by DPPH method, and 5745 mg/g.

The results in Table 2 reveal that FM phenolic extract was nontoxic according to the conditions of the experiment stated in the experimental part.

The phenolic extracts of FM showed various degrees of inhibition against the seven bacterial strains using the agar well diffusion method as represented in Table 3. FM phenolic extract inhibited the growth of 4 out of 7 of the tested bacteria. FM extract had nil effect on the following three strains, *Yersinia enterocolitica*, *Bacillus cereus*, and *Staphylococcus aureus*. *Listeria monocytogenes* was inhibited by FM extract by 26 mm inhibition zone diameter, *Salmonella typhimurium* inhibition zone diameter 25 mm, *Escherichia coli* inhibition zone diameter 22 mm, and *Pseudomonas aeruginosa* inhibition zone diameter 10 mm.

The results of coagulant activity of the phenolic extract are listed in Table 4, indicates that conc. 2.0 and 1.0 mg/ml of FM phenolic extract presented no clotting activity, while at 5.0 mg/ml clotting happened after 19.0 s, so the FM extract showed almost no anticoagulating activities.

FM phenolic extract have been evaluated as chemopreventive agents. This was established by testing the product for any cytotoxic activity against the following human tumor cell lines (Table 5).

Normal fibroblasts (BHK), intestinal carcinoma cell line (CACO), breast carcinoma cell line (MFC7), colon carcinoma cell line (HTC), lung carcinoma sell line (A549), liver carcinoma cell line (HEPG2) FM phenolic extract had no effect on normal fibroblasts. The highest inhibitory effect was achieved on cell lines of colon carcinoma and lung carcinoma with IC_{50} = 22.3 and 22.6 μ/ml. The next inhibited is the liver cell line carcinoma with IC_{50} = 39.4 μ/ml, this followed by intestinal carcinoma then breast carcinoma cell lines with IC_{50} = 46.9 and 49 μ/ml.

Table 2 Cytotoxic activity of FM phenolic extracts

Sample code	Remarks
Flaxseed extract	27.3% at 100 ppm
DMSO	5% at 100 ppm
Negative control	0%

Table 3 The antimicrobial activity of FM phenolic extracts determined by the agar well diffusion method on seven pathogenic indicator bacteria strains

Species of pathogenic bacteria	Flaxseed extract
B.C, *Bacillus cereus* ATCC 33018	Nil
List *Listeria monocytogenes* ATCC 7644	26
Staph *Staphylococcus aureus* ATCC 20231	Nil
sal *Salmonella typhimurium* ATCC 14028,	25
E.C *Escherichia coli* 0157: H7 ATCC 6933	22
Psed *Pseudomonas aeruginosa* ATCC 9027	10
Yersinin *Yersinia enterocolitica* ATCC 9610	Nil

Discussion

Previously, the same authors reported on the extraction of phenolic compounds (PC) from flaxseed meal using a variety of extraction techniques (Akl et al. 2017). The wide range of biological activities of the components of the flaxseed meal (e.g., the introduction part), initiated our interest to evaluate the biological activities of these phenolic extracts (antioxidant, antimicrobial, anticoagulation, and anticancer effects).

Antioxidant activity of PC extracts from FM

All living organisms utilize oxygen to metabolize and use the dietary nutrients to produce energy for survival. Oxygen thus is a vital component for living. Oxygen meditates chemical reactions that metabolize fats, proteins, and carbohydrates to produce energy. While oxygen is one of the most essential components for living, it is also a double-edged sword. Oxygen is a highly reactive atom that can become part of potentially damaging molecules commonly called “free radicals,” which can attack the healthy cells of the body. This may lead to damage, disease, and severe disorders. Cell damage caused by free radicals appears to be a major contributor to aging and

Table 4 Effect of FM phenolic extracts on thrombin clotting time

	Thrombin time (s)		
	Final conc. mg/ml		
PC extractof FM	5.0	2.0	1.0
	19.0	–	–

Table 5 Anticarcinogenic effect of PC from FM, measured as μg/ml

IC_{50} μg/ml	
Cell line	PC extract
BHK	–
CACO	46.5
MCF7	49
HCT	22.3
A549	22.6
HEPG2	39.4

LC_{50} is the lethal concentration of sample which causes the death of 50% of the cells in 48 h

diseases like cancer, heart diseases, decline in brain function, and decline in immune system. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases.

Phenolic compounds are phytochemicals which play a major role in the protection of oxidation processes. The antioxidant properties of phenolic compounds can act as free radical scavengers, hydrogen donators, metal chelators, and singlet oxygen quenchers. It must be emphasized that most of the information on phenolic antioxidant potential comes from studies in vitro. There are several nutrients in food that contain antioxidants. Vitamin C, vitamin E, and β-carotene are among the most commonly studied dietary antioxidants [Chen et al. 1994; Malcolmson et al. 2000; Hosseinian et al. 2006].

Starting from the antioxidant activity and the following biological evaluations were all done on the semi-pilot scale prepared phenolic extract from FM.

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potent antioxidant activity. Fe^{+3}/Fe^{+2} reducing power method is usually used for the determination of reducing power. In the reaction mixture, the presence of an antioxidant will reduce Fe^{+3} ferricyanide to Fe^{+2} ferrocyanide. The amount of Fe^{+2} can be determined by measuring the generation of Perl's Prussian blue at 700 nm. Fu et al. (2014) concluded that all of three extracts of *Jatropha curcas* seed shell contained high contents of phenolic compounds and exhibited relatively strong antioxidant activities.

Slavova-Kazakova et al. (2016) studied antioxidant activity of flaxseed extracts in lipid systems. They reported that flaxseed extract, its alkaline hydrolysate, and SDG are not able to inhibit effectively lipid autoxidation in TGSO model. Both extracts act as natural antioxidants in a β-carotene-linoleate emulsion system. SECO exhibited a stronger activity than SDG.

Due to antioxidant properties in emulsion system, flaxseed extract and its hydrolysate can be used as natural antioxidants for meat, mayonnaise, and dressing, thus prolonging shelf life.

Toxicity test

Toxicology test is known as a safety assessment and is conducted to determine the degree to which a substance can damage living or non-living organisms. The concentration of the chemical in air that kills 50% of the test animals during the observation period is the LC_{50} value. Other durations of exposure (versus the traditional 4 h) may apply depending on specific laws.

Phenolic antioxidants function as free radical terminators. Phenolic compounds and some of their derivatives are very efficient in preventing autoxidation; however, only a few phenolic compounds are currently permissible by law as food antioxidants. The major concerns for acceptability of such antioxidants are their activity and potential toxicity.

The antimicrobial activity of PC extract from FM

Phenolic compounds are produced by plants mainly for protection against stress and predators. The functions of phenolic compounds in plant physiology and interactions with biotic and abiotic environments are difficult to overestimate. Phenolics play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and support to plants. Importantly, phenolic phytoalexins, secreted by wounded or otherwise perturbed plants, repel or kill many microorganisms, and some pathogens can counteract or nullify these defenses or even disrupt them to their own advantage.

Thus, FM phenolic extract can be considered to have as antimicrobial activity. Gamal et al. (2011) working with guava seeds found the methanolic phenolic extract to exhibit antimicrobial activity. Taha et al. (2011) found that working with sunflower meal isolates chlorogenic acid (a phenolic compound) and that it had an antimicrobial activity and inhibited the growth of some food pathogens. Fernández-Lópeza et al. (2005) investigated the antioxidant and antibacterial effect of rosemary, orange, and lemon extracts in cooked Swedish-style meatballs. Activity in a lard system was established for all the extracts.

Anticoagulation activity

Flavanols have antioxidant, vasodilatory, anticoagulant, and anti-inflammatory properties. Flavanols have antidepressant effects on laboratory animals. Intake of flavanols enhances human cognitive performance and cerebral blood flow. Intake of flavanols such as dark chocolate counteracts negative emotions. Flavanol-rich cocoa-derived products may complement traditional antidepressant regimes.

Antihemorrhagic action of plant phenolic compounds is primarily due to compaction, increasing the strength of vascular tissue barriers. But there is a certain importance and influence of phenols on coagulation of blood. Hesperidin, rutin, and ellagic acid reduced the duration of bleeding. It is well known that the citrine, rutin, and other drugs increase the amount of calcium in the blood involved in the coagulation process. Finally, the acceleration of coagulation can be achieved indirectly—adrenaline promotes the formation of blood clots and phenolic compounds to protect it from inactivation in the bloodstream.

Wee et al. (2010), in conclusion, stated that the phenolic compounds in KRG have potent anticoagulant activity, whereas the saponin fractions, which were previously shown to possess antiplatelet aggregation activity, do not. Taken together, these results suggest that both saponins and phenolic compounds contribute to the cardiovascular effects of KRG through their antiplatelet aggregation and anticoagulant activities, respectively. Additionally, in vivo studies of the anticoagulant activities of phenolic compounds will be useful to better understand the pharmacology of these compounds.

The results of coagulant activity of the phenolic extract mean that PC caused hardly any clotting perhaps because the phenolics known to delay clotting time are not present according to HPLC analysis reported by Akl et al. (2017). Only p-coumaric and not coumaric was present as 9.3 μg/g meals in FM. Hesperidin, rutin, and ellagic acid reduced the duration of bleeding, and coumarin anticoagulants are bound to serum proteins.

Anticarcinogenic activity of PC from FM

Saenglee et al. (2016) stated that phenolic compounds present in our diet play an important role in colon cancer chemoprevention. Previous results demonstrated that peanut testa extract inhibited both histone deacetylase (HDAC) activity and the growth of colon cancer cells.

Flaxseed is extensively consumed in three ways: whole seed, powder, and flaxseed oil. In the last decade, flaxseed has gained attention due to its reported health benefits. Studies have shown the benefits of flaxseed alpha-linolenic acid (ALA), lignans, and fiber. The presence of these bioactive compounds helps in the prevention of cardiovascular diseases, diabetes, memory loss, and constipation. The phenolic compounds of flaxseed help in reduction of the fasting plasma glucose levels. Flaxseed contains biologically active estrogenic compounds called phytoestrogens which help in decreasing cell proliferation and prevents cancer. Higher levels of flaxseed are associated with prevention of memory loss and constipation. Flaxseed also contains several non-nutritional compounds such as cyanogenic glycosides, cadmium, trypsin inhibitors, and phytic acid that negatively influence health and well-being (Mishra and Verma 2013). Flaxseeds contain phenolic compounds called lignans, and secoisolariciresinol-diglucoside (SDG) is a major lignan with putative health benefits such as antioxidant and anticancer effects. The role of SDG and its metabolites such as enterolignans is gaining attention due to their mitigating effects against cancers especially prostate and breast cancer. Several epidemiological, in vitro and animal studies add evidence to this anticancer benefit of SDG. However, more research activities, especially clinical and pharmacokinetic studies in humans, are required to corroborate this evidence. The focus on the roles of SDG and its metabolites in preventing breast tumors, including an evaluation of potential mechanisms of action, was reviewed by Alphonse and Aluko (2015).

Conclusion

The biological activities of PC extracted from FM using the more effective extraction procedure (ultrasonic assisted extraction) showed high antioxidant activity when measured by the three methods. Also, toxicity test proved that the PC extract from FM was nontoxic on normal retina cell line. The FM extract showed almost no anticoagulating activities. The FM extract has antimicrobial effect on some pathogenic bacteria four out of seven. FM phenolic extract had no effect on normal fibroblasts. The highest inhibitory effect was achieved on cell lines of colon carcinoma and lung carcinoma followed by liver cell line carcinoma, then intestinal carcinoma, and then breast carcinoma cell. Therefore, the phenolic compounds extracted from flaxseed meal showed significant biological activities.

Abbreviations

°C: Celsius; AA: Antioxidant activities; ALA: Alpha linolenic acid; ANOVA: Analysis of variance; BHT: Butylated hydroxyl toluene; CaAG: Caffeic acid glucoside; DIZ: Diameter of inhibition zone; DMSO: Dimethylsulphoxide; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FM: Flaxseed meal; FRAP: Ferric reducing antioxidant power; HPLC: High-performance liquid chromatography; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetraazolium bromide; PC: Phenolic compounds; ROS: Reactive oxygen species; RT: Retention time; SDG: Secoisolariciresinol diglucoside; SDS: Sodium dodecyl sulfate; SE: Soxhlet extraction; SECO: Secoisolariciresinol; TGSO: Triacylglycerols of sunflower oil; TPC: Total phenolic compounds; UAE: Ultrasonic-assisted extraction

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Authors' contributions

EA worked the experimented part and collected the data. FT, SM, and AH wrote, read, and approved the final manuscript.

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