

Research Article



***In Vitro* antioxidant and antimicrobial activities of Lignan flax seed extract (*Linum usitatissimum*, L.)**

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ABSTRACT

Flaxseed is one of dietary sources contain considerable amount of phenolics namely lignan. The aim of this study was to evaluate the potential activities of lignan extracts as a potential source of antioxidant and antimicrobial agents. Five flaxseed cultivars namely (Amon, Blanka, Lithuania, Sakha1 and Teka) were studied. DPPH scavenging activity and Fe²⁺-chelating were studied at the different concentrations of lignan extract from 5 µg/ml to 50 µg/ml. Lithuania cultivar revealed the highest inhibition % against the stable free radical DPPH (9.05 to 88.56 %) compared with butylated hydroxy toluene (BHT) as standard agent (40.31 to 93.94 %). In addition, lignan extracts of all flaxseed cultivars also showed highly Fe²⁺-chelating antioxidant power especially Lithuania and Teka cultivars. Identification of lignan extract by HPLC showed the presence of many phenolic compounds (*p*-coumaric acid (CouA), 5-hydroxymethyl-2-furfural (HMF), ferulic acid (FerA) and secoisolariciresinoldiglucoside (SDG) in variables levels. SDG was found as the most abundant constituent of lignan extract in all cultivars of flaxseed. Maximum antimicrobial activity was observed with ethanolic extract of Lithuania against Gram negative bacteria, whereas, ethanolic extract of Teka had antibacterial effect against Gram positive bacteria. All extracts were inactive against *A. niger*, and *C. albicans*. The MIC values were ranged from (224 to 488 µg/ml). Amon and Blanka cultivars displayed a highest MIC value (366 and 348 µg/ml) against Gram negative and (488 and 464 µg/ml) against Gram positive, respectively. While, *Lithuania* cultivar showed the lowest MIC (224 µg/ml) for each microorganism. These results are considered as most important and promising finding in pharmaceutical properties of lignans.

Keywords: Antioxidants and antimicrobial, Flaxseed, Lignans.

INTRODUCTION

Lignans, secondary plant metabolites, are widely distributed in edible plants.¹ Lignans are becoming increasingly important for their possible application in the fields of pharmacy and nutrition, and have been found to possess a variety of biological properties. Lignans belong to a group of plant phenols which are characterized by coupling of two phenyl propanoid units.² Lignans are found in most fiber rich plants, including grains such as wheat, barley and oats; legumes such as beans, lentils and soybeans; and vegetables such as garlic, asparagus, broccoli and carrots.^{3,4}

Flaxseed lignan such as secoisolariciresinol (SDG), the mammalian lignans such as enterodiol and enterolactone act as antioxidants.⁵ Flaxseed (*Linum usitatissimum*, L.) contains the largest amount of lignan, secoisolariciresinoldiglucoside (SDG) among all the grains, legumes, fruits, and vegetables⁶, and is the richest dietary source of the plant-based SDG⁷, which can be metabolized to the mammalian lignans, enterodiol and enterolactone by human intestinal micro flora.^{8,9} Secoisolariciresinol (SECO) and SDG are known to have a number of potential health benefits, including reduction of serum cholesterol levels, delay in the onset of type II diabetes, and decreased formation of breast, prostate and colon cancers^{10,11}, which may be partially attributed to their antioxidant properties.^{5,12} Other phenolic compounds such as *p*-coumaric acid and ferulic acid are

also present in glucosidic forms as a part of oligomers.¹³⁻¹⁵ Flaxseed derivatives such as defatted flaxseed meal or flax hulls have higher SDG concentrations; they were found to contain about 2.3 and 4% SDG. The flaxseed lignan is secoisolariciresinoldiglucoside (SDG); its concentration varies with the cultivated variety. SDG concentrations ranging from 1 to 1.9% on a fresh weight basis as was reported by.¹⁶ Flax hulls can be obtained from dry flaxseed by abrasive dehulling and separation of hulls from embryos by sieving or aspiration. The SDG content of flax hulls free of oil and mucilage was 2–10 times greater than that of the seed material used for dehulling.¹⁷ An inverse linear relationship between the SDG and the oil contents of the hull, the intact seeds and the embryos fractions resulted from a dehulling process.¹⁸ The lignans possess a wide spectrum of biological activities; including antimicrobial was investigation.¹⁹ The potential of some natural and semi-synthetic lignans against mycobacteria and oral pathogens.^{20,21} The authors reported that the hulls contained 46 times more SDG than the embryos. Therefore, defatted flaxseed meal and flax hulls are preferred to whole flaxseed meal as raw materials for SDG extraction, concentration and purification.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and secoisolariciresinoldiglucoside (SDG) were purchased



from Sigma–Aldrich from Sigma–Aldrich (St. Louis, MO). 3-(2-Pyridyl) -5, 6-diphenyl-1, 2, 4-triazine-4', 4''-disulfonic acid monosodium salt (ferrozine) were purchased from Fluka (Buchs, Switzerland). All other chemicals and solvents were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Plant materials and Cultivation Conditions

Five flax cultivars were obtained from (Field Crops Department, National Research Centre, Egypt and were grown under field conditions). These five flax cultivars of Egyptian origins were grown at the Agricultural Experimental station of NRC, Noharia district, West Delta during one successive winter growing season 2011. Flax cultivars were, namely, Amon, Blanka, Lithuania, Sakha1 and Teka were grown in Complete Randomized Block system in four replications. Each plot consisted of 10 rows (3.0 m in length and 20 cm in width, with an area of 6.0 sq.m). These cultivars were grown with 120 gm of flaxseeds (equivalent to 199.92/ha). The normal cultural practices of growing flax were followed as recommended by Ministry of Agriculture and land reclamation (MALR) information after seed broadcast until symptoms of ripening and appearance of full seed maturity, then harvested. Only irrigation was followed using sprinkler irrigation system.

Representative random samples of flax seed were taken for analysis for the one growing season, and hence the results followed a similar trend to.²² For comparison among different mean values, LSD test at 5% level was practiced. After seed maturity, the plants were immediately harvested and seed samples were collected. Clean parts of the harvested seeds were used for the extraction, determination of lignin compounds, and for the determination of their antioxidant and antimicrobial activities.

Lignans extraction

Preparation of Defatted Flaxseed Powder

Flaxseeds were ground in a grinder to obtain a fine powder (110–120 mesh). The powder was defatted with *n*-hexane (1: 6 w/v) at room temperature for 16 h. The defatted powder was air dried for 18 h and stored in deepfreeze (– 8 °C) for further use.

Lignans extraction procedure

The defatted powder of flaxseed cultivars (200 g) was blended with ethanol 70% (1.2 L) at 30 °C for 24 hours. The extract was filtered using a filter paper Whatman No. 1 then concentrated at 40°C using a rotary evaporator at 90 rpm. Light yellow syrup was obtained. The syrup was hydrolyzed with 1M NaOH at room temperature for 16 h. The hydrolyzed syrup was acidified with 0.5M HCl to pH 6. The solution was cooled down to 15°C then centrifuged at 2000 rpm for 10 min to precipitate and remove water-soluble polysaccharides and proteins.²³ After drying process for lignin extract, the weight of lignans was

determined and the acquired ratio of lignans was calculated using the following equation:

$$\text{Acquired ratio of lignans (\%)} = \left(\frac{\text{Weight of dried lignans}}{\text{Weight of defatted flaxseed powder}} \right) \times 100$$

Antioxidant activity

DPPH scavenging activity

The DPPH radical scavenging activity of lignan flaxseed extract was described by using, 0.1 mM of DPPH in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of lignan extracts at different concentrations (5, 10, 20, 35, 50 µg/ml). Methanol was used as blank. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Butyl hydroxyl toluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction reagent except the extracts. Then the absorbance was measured at 515 nm against blank (methanol pure). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.²⁴

The capacity to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (Inhibition \%)} = \left[\frac{A_c - A_s}{A_c} \times 100 \right]$$

Where A_c was the absorbance of the control reaction and A_s the absorbance in the presence of the lignan extracts.

Fe²⁺- chelating activity

Metal Chelating Effects on Ferrous Ions was measured by using, one ml of lignan flaxseed extract, and/or EDTA solution as a positive control at different concentrations (5, 10, 20, 35, 50 µg/ml) were mixed with 0.1 ml of 2 mM FeCl₂- 4H₂O and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min, at room temperature, then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity.²⁵

The percentage of ferrous ion chelating ability was calculated using the following equation:

$$\text{Iron chelating activity (Inhibition \%)} = \left[\frac{A_c - A_s}{A_c} \times 100 \right]$$

Where A_c was the absorbance of the control reaction and A_s the absorbance in the presence of the lignan extract.

Antimicrobial activity

The antimicrobial activities were carried out according to the conventional disk diffusion test, using cultures of *Bacillus subtilis* NRRL B-94, *Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspergillus niger* NRRL313, and *Candida albicans* NRRL477. The bacterial strains were cultured on nutrient medium, while the fungi & yeast strains were cultured on malt medium & yeast medium, respectively. Broth media included the same contents except for agar. For bacteria



and yeast, the broth media were incubated for 24 h. As fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation. For preparation of plate inoculation, 0.5 ml of inocula were added to 50 ml of agar media (50 °C) and mixed by simple inversion. The agar was poured into 120 mm Petri dishes and allowed to cool to room temperature. The sterile filter paper disk (2mm in diameter) was saturated by sample. The saturated filter paper steel to evaporate solvent and fixed on the surface of agar. The microbial growth inhibition zone was measured after incubation at 30°C at the appearance of the clear microbial free inhibition zones, beginning within 24 h for yeast, 24-48 h for bacteria and 48-72 h for fungus. Both antimicrobial activities could be calculated as a mean of three replicates.²⁶

Minimal inhibitory concentration (MIC)

The culture medium (25 ml) was poured into Petri dishes (9 cm in diameter) and maintained at 45°C until the samples were incorporated into the agar. The samples were added as 50, 100, 200, 300, 400, 500 and 600 µg/ml. The different microbial strains were layered by using an automatic micropipette to place 30 µL over the surface of the solidified culture medium containing a sample. After the microorganisms were absorbed into the agar, the plates were incubated at 30°C for 24–48 h. MIC was determined as the lowest concentration of lignan extracts inhibiting the visible growth of each organism on the agar plate.

Identification of phenolics (lignan) by HPLC

Identification of lignans and other phenolics of five flaxseed cultivars were carried out on a HPLC system (Agilent 1100 series) coupled with UV-Vis detector (G1315B) and (G1322A) DEGASSER. Sample injections of 5 µl were made from an Agilent 1100 series auto-sampler; the chromatographic separations were performed on ZORBAX-EclipseXDB-C₁₈ column (4.6×250 mm, particle size 5 µm).

A constant flow rate of 1 ml /min was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 280 nm.²⁷

lignan compounds of each sample was identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to µg phenolic / g dry weight. All chemicals and solvents used were HPLC spectral grade, and obtained from Sigma (St. louis, USA) from Merck – Shcuchrdt (Munich, Germany).

Statistical Analysis

Data were statistically analyzed using Costat Statistical package.²⁸

RESULTS AND DISCUSSION

Extraction ratio of lignan yields

Data presented in Table 1 showed the extraction ratio of lignan yields for five flaxseed cultivars. Blanka and Sakha cultivars showed similar values for the extraction yields of lignin (69.88 mg/g DW). Whereas, Amon cultivar showed the highest value of lignin (73.49 mg/g DW) followed by Teka (72.29) and Lithuania (71.08) cultivars. No significant association could be found between the extraction yields of all cultivars and the results from the different antioxidant assays.

Table 1: Extraction Ratio of lignan yield of five flaxseed cultivars

Cultivars	Amon	Blanka	Lithuania	Sakha1	Teka	LSD at 0.05
Extraction ratio mg/g	73.49	69.88	71.08	69.88	72.29	N.S

Antioxidant activity

DPPH scavenging activity

DPPH has been used widely for the determination of antioxidant activity of different plants, vegetables, fruits extracts.^{29,30}

DPPH free radical scavenging activity (Inhibition %) of lignan extracts in all flaxseed cultivars and BHT increased by increasing the concentration of lignan and BHT (Table 2). However, the lignan extract exhibited concentration dependence across the range tested. Nevertheless, BHT was more effective than lignan extract, especially at the lower concentration. The inhibition % was 40.31 and 84.34% at concentrations of 5 and 20 µg/ml for BHT as compared to 9.05 and 34.92 % for lignan extracts of Lithuania cultivars (as the highest inhibition against DPPH.) at the same concentrations. At the higher concentrations (35 µg/ml to 50 µg/ml), however, lignan extracts of all cultivars were as approximately effective as BHT and all exhibited strong free radical scavenging activity against the stable free radical DPPH. The inhibition % was 64.10 and 88.56% at concentrations of 35 and 50 µM of Lithuania lignan extract compared to 88.13 and 93.94% for BHT at the same concentrations. It was also concluded that SDG is considered as strong capability for scavenging free radicals. The free radical scavenging activity of lignin extract may be attributed to the presence of the content and the structures of complex phenols such as lignan especially SDG, *p*-coumaric acid, Ferulic and other derivatives, which differ in their electron transfer and hydrogen donating abilities.^{12, 31}

Fe²⁺- chelating antioxidant activity

The main values of ferrous ion chelating activity of five flaxseed cultivars and Ethylene diamine tetra acetic acid (EDTA), as synthetic chelating agent) are shown in Table (3). Lignan extract of Lithuania and Teka cultivars exhibited highest potential activity of ferrous ion chelating at all concentration. In addition, the lowest chelating power was observed of Sakha1 cultivars compared with EDTA chelating agent.

Antimicrobial activity

Different microbial species were used to screen the possible antimicrobial activity of ethanolic extracts of flax. Of the species used, *St. aureus* one of the most common gram-positive bacteria causing food poisoning. Its source is not the food itself, but the humans who contaminate

foods after they have been processed. Gram-negative bacteria are represented by *E. coli*, which belong to the normal flora of humans. However, enter hemorrhagic strain of *E. coli* has caused serious cases of food poisoning, and preservatives to eliminate its growth are needed. A clearly visible spoiling agent of bakery products is the mold that forms black-centered spots on the surface of products, *A. niger*.

To determine antimicrobial activity, flax extracts were tested against gram-positive (*B. subtilis* and *St. aureus*) and gram negative (*E. coli* and *P. aeruginosa*) bacteria. This was assumed to be sufficient for the antimicrobial screening. Very clear differences were found between the effects of different extracts in the study. The results of the antimicrobial screening assay of the flax extracts are shown in Table 4.

Table 2: Antioxidant scavenging activity of lignan extracts of five flaxseed cultivars against DPPH stable free radical

Cultivars	Inhibition %				
	5 µg/ml	10 µg/ml	20 µg/ml	35 µg/ml	50 µg/ml
Amon	5.57 ^a ± 0.28	10.76 ^a ± 0.28	21.90 ^a ± 0.28	38.59 ^a ± 0.46	55.17 ^a ± 0.56
Blanka	6.91 ^{bc} ± 0.28	13.52 ^c ± 0.46	27.58 ^c ± 0.56	46.61 ^b ± 0.55	64.95 ^c ± 0.55
Lithuania	9.05 ^d ± 0.76	20.18 ^d ± 0.66	34.92 ^e ± 0.64	64.10 ^d ± 0.76	88.56 ^e ± 0.28
Sakha1	6.36 ^b ± 0.38	12.42 ^b ± 0.38	24.59 ^b ± 0.37	43.98 ^b ± 0.56	63.18 ^b ± 0.59
Teka	7.52 ^c ± 0.55	14.43 ^c ± 0.46	30.21 ^d ± 0.74	52.17 ^c ± 0.46	71.74 ^d ± 0.80
BHT standard	40.31 ^e ± 0.56	51.62 ^e ± 0.69	84.34 ^f ± 0.56	88.13 ^e ± 1.73	93.94 ^f ± 0.49
LSD at 0.05	0.65	0.93	0.99	3.09	1.11

All values are means of three replicates and are significantly different at $p \geq 0.05 \pm$ standard deviation.

Table 3: Fe²⁺- chelating activity by different concentration of lignan extract in five flaxseed cultivars

Cultivars	Inhibition %				
	5 µg/ml	10 µg/ml	20 µg/ml	35 µg/ml	50 µg/ml
Amon	9.18 ^b ± 0.27	16.60 ^c ± 0.40	22.94 ^c ± 0.49	30.99 ^c ± 0.34	42.78 ^c ± 0.36
Blanka	6.75 ^a ± 0.49	14.04 ^b ± 0.59	20.11 ^b ± 0.40	28.57 ^b ± 0.43	41.52 ^b ± 0.34
Lithuania	15.52 ^d ± 0.54	23.62 ^e ± 0.59	31.85 ^e ± 0.49	38.73 ^e ± 0.49	50.74 ^e ± 0.27
Sakha1	5.94 ^a ± 0.71	12.01 ^a ± 0.49	18.62 ^a ± 0.49	23.75 ^a ± 0.49	36.03 ^a ± 0.49
Teka	13.81 ^c ± 0.28	21.05 ^d ± 0.49	29.55 ^d ± 0.62	33.47 ^d ± 0.36	48.36 ^d ± 0.27
EDTA standard	23.80 ^e ± 0.41	45.39 ^f ± 0.47	61.58 ^f ± 0.28	74.94 ^f ± 0.34	82.86 ^f ± 0.27
LSD at 0.05	0.90	0.99	0.89	0.81	0.76

All values are means of three replicates and are significantly different at $p \geq 0.05 \pm$ standard deviation.

Table 4: Antimicrobial activity of lignan extract in five flaxseed cultivars

Sample	Diameter of inhibition zone (mm)					
	Bacteria				Fungus	
	<i>E. Coli</i>	<i>P. aeruginosa</i>	<i>St. aureus</i>	<i>B. subtilis</i>	<i>A. nige</i>	<i>C. abbicans</i>
Amon	9.11	5.75	6.85	8.15	0.00	0.00
Blanka	8.10	6.40	7.54	6.45	0.00	0.00
Lithuania	18.26	16.60	15.84	13.35	0.00	0.00
Sakha1	12.53	8.55	6.45	12.86	0.00	0.00
Teka	12.80	11.50	14.35	15.65	0.00	0.00

Maximum antimicrobial activity was observed with ethanolic extract of Lithuania against Gram negative bacteria, followed by ethanolic extract of Teka which showed antibacterial effect against Gram positive bacteria. All extracts were inactive against *A. niger* and *C. albicans*. These different resistant patterns are likely to be related to differences in yeast, fungus and bacteria cell wall structures and protein synthesis. The antibacterial activity profile of Lithuania extracts against tested bacteria indicated that *E. coli* and *P. aeruginosa* was the most susceptible bacterium. These results are in agreement with.³²

The presence of lignans may bind both Ca^{+2} and Mg^{+2} , thereby reducing the Ca^{+2} and Mg^{+2} from lipopolysaccharide of the outer membrane causing a release of lipopolysaccharide, thereby destabilizing the membrane, which may increase the activity of lignans.³³

The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve a sufficiently long shelf life of foods and a high degree of safety with respect to food borne pathogenic microorganisms. In nature, there are a large number of different types of antimicrobial compounds that play an important role in the natural defense of all kinds of living organisms. As dietary compounds, flavonoids are widely known antioxidants and reduce thrombotic tendencies.³⁴ Attention has also been paid to their antimicrobial activity, but no dramatic evidence of their effectiveness has been reported.^{35, 36}

Minimum inhibition concentration (MIC)

The MIC of different extracts toward gram-positive and gram-negative bacteria was examined, and the results are summarized in Table 5. The tested extracts showed some variation in their antibacterial activity. The MIC values ranged from 224 to 488 $\mu\text{g}/\text{ml}$ of different strains and flaxseed cultivars. Lignan extracts was found to be the most effective antibacterial against the Gram negative bacteria such as (*E. coli* and *P. aeruginosa*) compared to Gram positive bacteria such as (*B. subtilis* and *St. aureus*). The MIC for Gram negative bacteria was ranged from 224 to 366 $\mu\text{g}/\text{ml}$, whereas, the MIC for Gram positive bacteria was ranged from 224 to 488 $\mu\text{g}/\text{ml}$ Table (5). The testing antibacterial activity of lignan extracts exhibited that *E. coli* was the most sensitive bacteria to the lignan extract (MIC = 224 $\mu\text{g}/\text{ml}$, Lithuania cultivars). The variation in the effectiveness of different extracts against different strains may depend on the differences in the cell permeability of these microbes.³⁷

Identification of lignin phenolic compounds in five flaxseed cultivars

The HPLC profile of lignan extracts is illustrated in Table 6. The alkaline and acid hydrolyses procedures were used to

separate SDG and other phenolic compounds. Lignans all absorb UV light, and UV detection may offer sufficient selectivity and sensitivity for the determination of lignans.²

Table 5: Minimal inhibitory concentration (MIC) of lignan extract in five flaxseed cultivars

Sample	MIC ($\mu\text{g}/\text{ml}$)			
	Bacteria			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>St. aureus</i>	<i>B. subtilis</i>
Amon	366	366	488	488
Blanka	348	348	464	464
Lithuania	224	224	224	224
Sakha1	232	232	232	232
Teka	240	240	240	240

Table 6: Identification profile of lignan compounds of five flax seed cultivars by HPLC

Cultivars	Lignan compounds mg/ 100 g DW			
	CouA	HMF	FerA	SDG
Amon	5.03	6.62	1.89	23.82
Blanka	61.02	-	29.56	47.59
Lithuania	38.42	10.16	7.22	103.55
Sakha1	22.53	3.57	4.37	24.90
Teka	34.48	14.08	16.64	85.71

SDG present maximum absorptions at ~ 280 nm, which is attributed to the aromatic chromophore and the substituents of $-\text{OH}$ and $-\text{OCH}_3$ on aromatic ring.⁶ SDG and *p*-coumaric were the major phenolic compounds in all flaxseed cultivars. Lithuania cultivars followed by Teka Cultivar showed the highest amount of SDG (103.55 and 85.71 mg/100 g DW). While, HMF exhibited the lowest concentration of phenolic compounds of all flaxseed cultivars. It could be observed phenolic compounds varied between plants according to their genus species, varieties, cultivars, types of fertilizer and agricultural conditions.³⁸ Secoisolariciresinoldigluconide (SDG) is the most abundant lignan in flaxseed (11.9–25.9 mg/g DW).³⁹ Other phenolic compounds have also been identified and quantified, and they include *p*-coumaric acid glucoside (1.2– 8.5 mg/g DW) and ferulic acid glucoside (1.6–5.0 mg/g DW).¹⁴

CONCLUSION

Various biological activities of the isolated lignans from five cultivars of flax growing in Egypt are summarized in this study. These lignans exerted diverse biological activities against tested methods to some extents. They were found to possess a high activity especially as antioxidant and antibacterial. These lignans which should be further evaluated to develop safe agents to introduce in modern therapy. Further studies should be made to reveal the mode action of lignans which might be helpful

in understanding the possible roles as anti-bacterial agents.

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REFERENCES

- Saarinen NM, Warri A, Airio M, Smeds A, Makela S, Role of dietary lignans in the reduction of breast cancer risk, *Mol Nutr Food Res*, 51, 2007, 857 - 866.
- Willfor SM, Smeds AI and Holmbom BR, Chromatographic analysis of lignans, *J Chromatogr A*, 1112, 2006, 64 –77.
- Chen H, Teng L, Li J, Park R, Mold D, Gnabre J, Hwu R, Tseng W, Huang R, Antiviral activities of methylated nordihydroguaiaretic acid. 2. Targeting herpes simplex virus replication by the mutation insensitive transcription inhibitor tetra *O*-methyl- NDGA, *J Med Chem*, 41, 1998, 3001–3007.
- Adlercreutz H, Fotsis T, Heikkinen R, Dwyer JT, Goldin BR, Gorbach SL, Lawson AM, Setchell KD, Diet and urinary excretion of lignans in female subjects, *Med Biol*, 59, 1981, 259–261.
- Kitts DD, Yuan YV, Wijewickreme AN, Thompson LU, Antioxidant activity of the flaxseed lignin secoisolariciresinoldiglycoside and its mammalian lignin metabolites enterodiol and enterolactone, *Mol Cell Biochem*, 202, 1999, 91–100.
- Zhang WB, Xu SY, Microwave-assisted extraction of secoisolariciresinoldiglycoside from flaxseed hull, *J Sci Food Agric*, 87, 2007, 1455–1462.
- Liggins J, Grimwood R, Bingham SA, Extraction and quantification of lignan phytoestrogens in food and human samples, *Anal Biochem*, 287, 2000, 102-109.
- Hallund J, Ravn-Haren G, Bugel S, Tholstrup T, Tetens I, A lignan complex isolated from flaxseed does not affect plasma lipid concentrations or antioxidant capacity in healthy postmenopausal women, *J Nutr*, 136, 2006, 112–116.
- Clavel T, Lippman R, Gavini F, Doré J, Blaut M, *Clostridium saccharogumia* sp. nov. and *Lactoni factor long oviformis* gen. nov., sp. nov., two novel human faecal bacteria involved in the conversion of the dietary phytoestrogen secoisolariciresinoldiglycoside, *Syst Appl Microbiol*, 30, 2007, 16–26.
- Hosseinian FS, Muir AD, Westcott ND, Krol ES, AAPH-mediated antioxidant reactions of secoisolariciresinol and SDG, *Org Biomol Chem*, 5, 2007, 644–654.
- McCann SE, Muti P, Vito D, Edge SB, Trevisan M, Freudenheim JL, Dietary lignan intakes and risk of pre- and postmenopausal breast cancer, *Int. J. Cancer*, 111, 2004, 440– 443.
- Hu C, Yuan YV, Kitts DD, Antioxidant activities of the flaxseed lignin secoisolariciresinoldiglycoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone *in-vitro*, *Food Chem Toxicol*, 45, 2007, 2219–2227.
- Johnsson P, Peerlkamp N, Kamal-Eldin A, Andersson RE, Andersson R, Lundgren LN, man PA, Polymeric fractions containing phenol glucosides in flaxseed, *Food Chem*, 76, 2002, 207 – 212.
- Eliasson C, Kamal-Eldin A, Andersson R, Aman P, High performance liquid chromatographic analysis of secoisolariciresinoldiglycoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction, *J Chromatogr A*, 1012, 2003, 151–159.
- Beejmohun V, Fliniaux O, Grand E, Lamblin F, Bensaddek L, Christen P, Kovensky J, Fliniaux MA, Mesnard F, Microwave-assisted extraction of the main phenolic compounds in flaxseed, *Phytochem Anal*, 18, 2007, 275–282.
- Nemes SM, Orsat V, Evaluation of a microwave-assisted extraction method for lignan quantification in flaxseed cultivars and selected oil seeds. *Food Anal Method*, 5, 2011, 551-563.
- Cui W, Han NF, Process and Apparatus for Flaxseed Component Separation, Unites States Patent and Trademark Office Granted Patent, 2006.
- Wiesenborn D, Tostenson K, Kangas N, Continuous abrasive method for mechanically fractionating flaxseed, *J Am Oil Chem Soc*, 80, 2003, 295–300.
- Saleem M, Kim HJ, Ali MS, Lee YS, An update on bioactive plant lignans, *Nat. Prod. Rep*, 22, 6, 2005, 696–716.
- Silva ML, Coimbra HS, Pereira AC, Almeida VA, Lima TC, Costa ES, Vinholis AH, Royo VA, Silva R, Filho AA, Cunha WR, Furtado NA, Martins CH, Carvalho TC, Bastos JK, Evaluation of Piper cubeba extract, (-)-cubebin and its semi-synthetic derivatives against oral pathogens, *Phytother. Res*, 21, 2007, 420-422.
- Silva ML, Martins C H, Lucarini R, Sato DN, Pavanb FR, Freitas NH, Andrade LN, Pereira AC, Bianco TN, Vinholis AH, Cunha WR, Bastos JK, Silva R, Da Silva Filho AA, Antimycobacterial activity of natural and semi-synthetic lignans, *Zeitschrift Fur Naturforschung Section C-a Journal of Biosciences*, 64, 2009, 779–784.
- Snedecor GW, Cochran WG, Statistical methods. d., 9th printing. Ames, IA: Iowa State Univ, 1989, Press.
- Zhang Zhen-Shan, Dong Li, Li-Jun Wang, Necati Ozkanc, Xiao Dong Chena, Zhi-Huai Mao, Hong-Zhi Yang. Optimization of ethanol-water extraction of lignans from flaxseed, *Sep Purif Technol*, 57, 2007, 17 – 24.
- Chu YH, Chang CL, Hsu HF, Flavonoids content of several vegetables and their antioxidant activity, *J Sci Food Agric*, 80, 2000, 561–566.
- Hsu C, Chen W, Weng Y, Tseng C, Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods, *Food Chem*, 83, 2003, 85–92.
- Greenwood D, Antimicrobial chemotherapy, Part II. In *Laboratory Aspects of Antimicrobial Therapy*. (D. Greenwood, Ed.) Bailliere, Tindall, London, 1983, 71.
- Ben-Hammouda M, Kremer RJ, minor HC, Sarwar MA, chemical basis for differential allelopathic potential of sorghum hybrids on wheat, *J Chem Ecol*, 21, 1995, 775–786.
- Anonymous A. Cohort Soft Ware Crop, Costat user manual version, 3.03, 1989, Berkeley CA, USA.
- Goupy P, Hugues M, Boivin P, Amiot J, Antioxidant composition an activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds, *J Sci Food Agric*, 79, 1999, 1625–1634.
- Yu L, Zhou K, Antioxidant properties of bran extract from 'platte' wheat grown at different locations, *Food Chem*, 90, 2004, 311–316.
- Hu C, Kitts DD, Studies on the antioxidant activity of Echinacea root extract, *J Agric Food Chem*, 48, 2000, 1466 – 1472.



32. Gezer K, Duru ME, Kivrak I, Turkoglu A, Mercan N, Turkoglu H, Gulcan S, Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey, *Afr J Biotechnol*, 5, 2005, 1924–1928.
33. Cowan MM, Plant products as antimicrobial agents. *ClinMicrobiol Rev*, 12, 1999, 564–582.
34. Hertog MGL, Feskens EJM, Hollman PC, Katan MB, Kromhout D, Dietary antioxidant flavonoids and risk of coronary heart disease, The Zutphen elderly study, *Lancet*, 342, 1993, 1007–1011.
35. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M, Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*, *J Ethnopharmacol*, 50, 1996, 27–34.
36. Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P, Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds, *Int J Food Microbiol*, 56, 2000, 3–12.
37. Dorman DJH, Deans GS, Chemical composition, antimicrobial and *in-vitro* antioxidant-properties of *Monardacitriodora* var. *citriodora*, *Mynsticafragrans*, *Origanumvulgare* ssp., *Hirtum pelargonium* sp. and *Thymus zygisoils*, *J Essent Oil Res*, 16, 2004, 145–150.
38. Nakatani N, Antioxidants from spices and herbs. In: *NaturalAntioxidants: Chemistry, Health and Applications*. (F. Shahidi, Ed.), Champaign, IL: AOCS Press, 1997, 64–75.
39. Muir AD, Westcott ND, Quantification of the lignansecoisolariciresinoldiglucoside in baked goods containing flax seed or flax meal, *Journal of Agricultural and Food Chemistry* 48, 2000, 4048-4052.

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