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In vitro Anti-Cholesterol and Antioxidant Activity of Methanolic Extracts from Flax Seeds (*Linum usitatissimum* L.)

Geetika Pant, Chirag Simaria, Riyaz A.H. Varsi, Pooja Bhan and G. Sibi
Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bangalore, India

Corresponding Author: Geetika Pant, Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bangalore, India

ABSTRACT

Methanolic extract from flaxseeds was investigated for its effect on anti-cholesterol and antioxidant activity. *In vitro* anti-cholesterol activity was measured by cholesterol enzymatic endpoint method using simvastatin as positive control. The total amount of phenolic compounds was determined spectrophotometrically and the results were expressed as Gallic Acid Equivalent (GAE g^{-1}). Antioxidant activity of flaxseeds in vitro was measured in terms of DPPH free radical scavenging and total antioxidant potential assay. Increasing anti-cholesterol activity by flaxseeds was observed up to 20 min and a maximum inhibition was found as 93.04%, which was comparable to the anti-hyperlipidemic drug simvastatin (95.1%). Phenolic compound content of flaxseeds was found as 0.059 mg GAE g^{-1} and antioxidant potential was 1.037 mg mL^{-1}. Lower DPPH free radical scavenging activity was observed after 30 min of incubation. The results indicated that flaxseed might reduce or control the cholesterol levels and oxidative damage and it is apparent that flaxseeds could contribute to new formulations with potential anti-cholesterol and antioxidant effects.

Key words: Flaxseeds, anti-cholesterol, antioxidant, hypolipidemic

INTRODUCTION

Hyperlipidemia is considered as one of the leading causes of death in the world. It is characterized by elevated levels of lipids circulating in the blood is linked to the development of cardiovascular and metabolic syndrome diseases (Adisakwattana *et al*., 2010). Free radicals are chemically active atoms created when cells use oxygen to generate energy in the form of ATP (Valko *et al*., 2004). They have excess or deficient number of electrons which damage cell membranes and DNA (Cerutti, 1991; Harman, 1994; Finkel and Holbrook, 2000). Scavengers of free radicals are of prime importance for protecting human heath from cancer, degenerative and other diseases by counteracting free radical formation. Antioxidants are helpful in preventing the harmful effects of free radicals and many synthetic antioxidants are used to reduce oxidative damages. A number of anti-hyperlipidemic agents and synthetic oxidants are used but the undesirable side effects associated with those drugs stimulate the search of alternative safe medicine with improved efficacy. Plants are rich sources of biologically active compounds and finding new lead molecules with anti-hyperlipidemic and antioxidant properties could be a useful alternative strategy. A number of medicinal plants were reported to have hypolipidemic

activity (Hor *et al*., 2011; Zhang *et al*., 2013; Ferreira *et al*., 2011; Machaba *et al*., 2014; Baskaran *et al*., 2015) and almost all medicinal plants have antioxidant potential (Krishnaiah *et al*., 2011).

Linum usitatissimum L. (family: Linaceae), commonly known as 'Flax' is an annual plant contains biologically active components and flax seeds have been the focus of interest in the field of diet and disease research. The seeds are flat and oval with a pointed, varies in colour from dark brown to yellow (Freeman, 1995). Flax seeds are rich in omega-3 fatty acid, alpha-linolenic acid, dietary fiber and natural antioxidants (Prasad *et al*., 1998; Makni *et al*., 2008). Lignan complex isolated from flaxseed reduced the extent of hypercholesterolemic atherosclerosis and reducing blood level of cholesterol (Prasad, 2005; Fukumitsu *et al*., 2010). Flax seeds decreased plasma total and LDL cholesterol and fat excretion (Kristensen *et al*., 2012). Flax seeds have been reported to lower total and LDL cholesterol concentrations in humans (Pan *et al*., 2010; Edel *et al*., 2015). Different types of phenolics are present in flaxseeds (Kasote, 2013), which have antioxidant properties and significantly reduce the effects of free radicals (Brodowska *et al*., 2014). Extensive studies has been undertaken to demonstrate antioxidant potential of flaxseed (Velioglu *et al*., 1998; Zanwar *et al*., 2010; Amarowicz *et al*., 1994) thus, can be used in nutraceutical and pharmaceutical industries. This study has carried out to determine the *in vitro* hypolipidemic and antioxidant activities of flax seeds.

MATERIALS AND METHODS

Sample preparation and extraction: Flax seeds were collected from local market of Bangalore, air dried, ground into powder and sieved (60 mesh). About 100 g of dried powder was extracted in methanol (1:7 w/v) at 25°C for 24 h. The extract was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered using Whatman No.1 filter paper and concentrated to dryness under reduced pressure in rotary vacuum evaporator. The final extract was stored in air tight containers at 4°C until used.

***In vitro* anti-cholesterol assay:** The anti-cholesterol assay was carried out as described by Iswantini *et al*. (2005) and cholesterol enzymatic endpoint method (Randox Laboratories, 2009). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL^{-1}. Ten microliter of the flax seeds extract was pipetted into micro titre plate followed by the addition of 2000 µL of Randox reagent and 10 µL of cholesterol as sample. Twenty microliter of distilled water and 2000 µL of Randox reagent were used as blank. Negative control comprised of 20 µL cholesterol and 2000 mL Randox reagent; standard comprised of 20 µL simvastatin and 2000 mL Randox reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control-Sample}}{\text{Negative control}} \times 100$$

Determination of total phenolics content: The amount of total phenolics in the extract was determined with Folin-Ciocalteu (FC) reagent (Ainsworth and Gillespie, 2007). To 200 mL of sample (3 replicates), 1 mL of 1:2 dilution of FC reagent and 800 mL of sodium carbonate (7.5% w/v) were added and the resulting mixture was incubated at room temperature for 30 min. The absorbance of the sample was measured at 765 nm using a spectrophotometer and the results were expressed as milligram of gallic acid equivalent per gram of dry weight.

DPPH assay: The capacity of the extract to scavenge the stable 2, 2'-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method reported by Gyamfi *et al*. (1999). In brief, 50 μL of the methanolic extract (100 μg mL^{-1}) was mixed with 1.8 mL of 0.5 mM DPPH in methanol solution. Methanol (50 μL) alone was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured at 517 nm. The percent inhibition was calculated from the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control}_{A517} - \text{Sample}_{A517}}{\text{Control}_{A517}} \times 100$$

Antioxidant potential assay: The Antioxidant potential assay was carried out by phosphomolybdenum reduction assay (Prieto *et al*., 1999). To 200 mL of the extract, 1 mL of the reagent containing 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid were added and the resulting mixture was incubated at 37°C for 60 min. The absorbance was measured at 695 nm using a spectrophotometer against blank using methanol and the antioxidant potential activity is expressed as milligram of ascorbic acid equivalent per gram of dry weight.

RESULTS

Methanolic extracts of flax seeds were prepared and analyzed for anti-cholesterol and antioxidant activity. Anti-cholesterol assay was performed and the results revealed the potential activity of flaxseeds. Increasing activity was observed up to 20 min and a maximum inhibition was found as 93.04%. Simvastatin was used as positive control and 95.1% inhibition was observed after 20 min of incubation. At the end of incubation period, 89.3 and 94.1% inhibitory activity was found for flaxseeds and simvastatin respectively (Fig. 1).

Antioxidant activity of flaxseeds was determined by performing DPPH free radical scavenging assay and total antioxidant potential assay. Total phenolics content of the extract was 0.059 mg mL^{-1} and 28% DPPH free radical scavenging activity was observed. Antioxidant potential

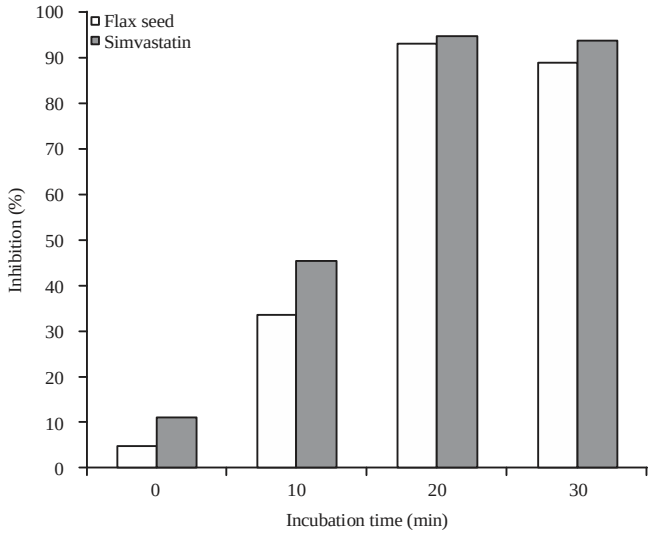

Fig. 1: Hypolipidemic activity of flax seeds

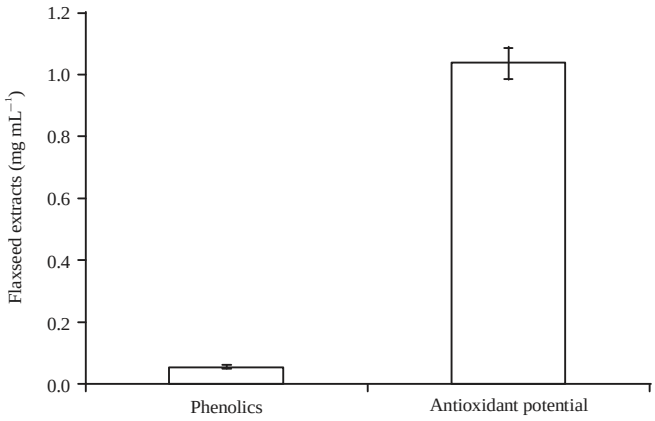

Fig. 2: Antioxidant activity of flaxseed extracts

determination using phosphomolybdenum reduction assay revealed the significant antioxidant potential of flax seeds (Fig. 2). Varying concentrations of ascorbic acid was used to calibrate the standard curve and the results revealed 1.037 mg mL^{-1} activity under *in vitro* conditions.

DISCUSSION

Free radicals are molecules possessing an unpaired electron emerging in oxidative stress and phenolic compounds have great importance in radical scavenging activities. Flax hosts PUFA omega-3 family, dietary fibers and phytoestrogen lignans which determine hypolipidemic activities (Martinchik *et al*., 2014). Further the presence phenolic acids, flavonoids and lignans are believed to exhibit antioxidant and anticancer effects in humans (Barbary *et al*., 2010). A number of phenols such as lignans, phenolic acids, flavonoids and phenylpropanoid glucosides were present in flaxseeds (Oomah *et al*., 1996; Luyengi *et al*., 1993). Higher amount of phenolic acids are present in flaxseeds and the content is attributed by seasonal effects (Oomah *et al*., 1995). Higher concentrations of ferulic and p-coumaric acid glucosides were present in flaxseeds (Beejmohun *et al*., 2007). Higher polyphenol contents were reported in flax seeds (Perez-Jimenez *et al*., 2010) and 0.059 mg GAE g^{-1} phenolics content was observed in this study. The DPPH, a stable organic free radical, is very often applied for the evaluation of the antioxidant activity of compounds and the method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant (Pyrzynska and Pekal, 2013; Anwar and Przybylski, 2012). The effect of antioxidants on DPPH free radical scavenging was considered to be due to their hydrogen donating ability. The DPPH radical scavenging activity of flax seed hull was found in the range of 52.74-69.32% (Herchi *et al*., 2014) whereas relatively low activity (28%) of whole flaxseed was observed in our findings. Flaxseed extraction using 80% methanol has produced highest yield but ethanol extracts exhibited highest antioxidant capacity (Kitts *et al*., 1999; Anwar and Przybylski, 2012). Antioxidant activity of flax seeds varies among the cultivars Gaafar *et al*. (2013) and in this study 1.037 mg AEAE g^{-1} was found in methanolic extract.

Flaxseed fibre added to bread was found to lower cholesterol in diabetics (Thakur *et al*., 2009) and dietary flaxseed supplementation was associated with adverse changes in lipid profile of pediatric hypercholesterolemia (Wong *et al*., 2013). Further, the levels of plasma cholesterol level and plaque formation induced by ovarian hormone deficiency were reduced by flaxseeds

(Lucas *et al*., 2004). In the present work, *in vitro* anti-cholesterol activity of flaxseed extracts were performed and compared with simvastatin. Total incubation time of 30 min was considered and the activity was increased with time and maximum anti-cholesterol activity was observed at 20 min of incubation. Simvastatin is a potential drug used to control cholesterol levels and is used as positive control to compare the potential anti-cholesterol activity of flaxseeds. From the results, significantly higher activities were exhibited by flaxseeds (93.04%) and simvastatin (95.1%) revealing the potential of flaxseed in controlling cholesterol levels. It was also noted that increasing incubation time above 20 min has reduced the inhibitory activity of both flaxseed and simvastatin.

CONCLUSION

The results obtained in this study show that flaxseeds has a significant anti-cholesterol and antioxidant activities. This is evidenced by the higher inhibitory activity which is comparable to anti-hyperlipidemic drug simvastatin. Higher phenolic compounds and antioxidant potential reveals flaxseeds be a source of natural antioxidants along with anti-cholesterol activity. It is apparent that flaxseeds could contribute to new formulation and *in vivo* studies are needed to ascertain its potential health effects in the prevention of oxidative damage and cholesterol induced degenerative diseases.

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