European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

NON-ANTIOXIDANT ENZYMES AND *IN VITRO* ANTIOXIDANT ACTIVITY OF ROASTED SOYBEAN AND FLAXSEED

Dharshini[1] Dr.Sumayaa. S[2] Dr.A.Julius[3], Dr.M.V.Dass Prakash[4]*

[1]Dharshini HOD, Professor, Department of Home Science, Karur Velalar College of Arts and Science for Women, Karur.
[2]Dr.Sumayaa. S Principal/Head, Department of Home Science, ThassimBeevi Abdul Kader College of Arts and Science for Women, Kilakarai.
[3]Dr.A.Julius Professor & Head , Department of Biochemistry, Balaji Dental College and Hospital, Chennai. Bharath Institute of Higher Education and Research (BIHER)
*[4]Dr.M.V.Dass Prakash*Assistant Professor, Department of Biochemistry, Sri Sankara Arts and Science College, Kanchipuram.*
Corresponding Author
Dr.M.V. Dass Prakash
Assistant Professor
Department of Biochemistry
Sri Sankara Arts and Science College
Enathur, Kanchipuram-6310561
Mobile: 9944467306
Email: dassbms@gmail.com

Abstract: The roasted forms of flaxseed and soybean were chosen for study as they are found to be enriched with phenolic components. They are also found to possess medicinal benefits particularly against non-communicable diseases like cancer. Hence the investigator analyzed the non-antioxidant enzymes and in vitro antioxidant activity of roasted soybean and flaxseed. After a deeper analysis, it was observed that flaxseed possessed greater anti-oxidant activity than soybean comparatively.

Keywords: FLAX SEED, ANTIOXIDANT, in vitro.

1. INTRODUCTION

Flaxseed ***(Linum usitatissimum L***.) and Soybean (***Glycine Max)*** are found to possess a lot of bioactive phenolics that can demonstrate biological activities like antiradical, antioxidant, antimicrobial and anticancer effects (1). The compounds that are capable of delaying some types of cell damage and quenching reactive radical species formed during oxidative reactions in

metabolism are called antioxidants. They mainly include the main groups of phytochemicals like phenols, polyphenols, carotenoids, anthocyanins and tocopherols (2). The basic structure of phenol has a hydroxyl group (-OH) linked to an aromatic ring. Depending on the position and the number of phenolic goups and their location, the biological activities of the phenolic compounds differ (3).

Vegetables and fruits are the main naturally occurring sources of antioxidants and they reduce the risks of certain diseases like cancer. For this reason they are preferred by the consumers (4). Hence, the identification and the importance of natural antioxidants should be put under research from the perspective of health-improving properties (5). Report also says that the usage of natural antioxidant additives decreases the oxidative stress level and chronic disease (6) and that the synthetic antioxidant additives results in side effects such as mutagenic, carcinogenic and toxic impacts. So, the usage of synthetic antioxidants is restricted (5,6) and there is an increasing demand for safer and natural antioxidants for food, biological and pharmaceutical systems. Since plant constituents are found to have antioxidant activity and free radicals scavenging effects, the consumers are preferably choosing natural and safer anti-oxidants from plant origin.

One of the factors responsible for the primary onset and/or secondary pathology of variety of human disorders are oxidative stress. So far, antioxidant therapy had only limited success in treating many diseases but cancer treatment is believed to be an exception (Halliwell 2012).

2. MATERIALS AND METHODS

To protect against oxidative stress, plants have developed diverse mechanisms to scavenge the level of ROS by interacting network of antioxidant systems, including enzymes and non-enzymatic antioxidants, such as superoxide dismutase (SOD), ascorbic acid (AsA) and glutathione (GSH) (Nagalakshmi and Prasad, 2001). The AsA-GSH cycle is essential in removing H2O2, especially in the plastids (Alscher, 1989; Halliwell and Foyer, 1976; Noctor *et al.*, 1998). The AsA is the most abundant antioxidant in plant cells, which is ubiquitous and found in most of sub-cellular compartments, including the apoplast (Pignocchi and Foyer, 2003; Smirnoff, 2000). It helps to detoxify H2O2 production under the abiotic stress conditions and can be regenerated via the AsA-GSH cycle (Foyer and Noctor, 2000; Halliwell and Foyer, 1976) that is important for protection against adversity. Antioxidant enzymes were analyzed only for the processed supplements, namely roasted soybean and flaxseed.

Table: 1 Determination of Non-Antioxidant Enzymes

S.NO	Non-Antioxidant enzymes	Methods Followed
1.	Vitamin-A (BetaCarotene)	Spectrophotometry (Bayfiedl and Cole (1980)

4.	Vitamin – E (Tochopherol)	Spectrophotometry (Rosenberg, 1992)
3.	Vitamin-C	Tirymetric method (Sadasivam and Theymoli, 1987)

Table: 1 Determination of *in vitro* Antioxidant Activities

S.NO	Antioxidant enzymic activity	Methods Followed
1.	DPPH radical scavenging assay	Spectrophotometry (Blois and Zhao, 1958)
2.	FRAP Assay Reagents	Spectrophotometry (Benzie and Strain, 1996)
3.	Hydroxyl radical scavenging activity	Spectrophotometry (Chung *et al*., 1997)
4.	Superoxide radical activity	Spectrophotometry (Liu et al., 1997)

3. RESULT AND DISCUSSION

Non-Antioxidant Enzymes, Vitamin A, E and Vitamin C Content of Roasted Flaxseed: Vitamin A, E and vitamin C are regarded as antioxidants and play a vital role in overcoming oxidation and degenerative diseases. The presence of these vitamins in foods indicates that they can help in prevention of many diseases. Non-Antioxidant Enzymes, Vitamin A, E and Vitamin C Content of Roasted flaxseeds antioxidants are compounds that will quickly react with oxygen to form new substances. Antioxidants will react with the oxygen before lipids do. Important dietary antioxidants are vitamin A, E and vitamin C. The results of the non-antioxidant enzymes, vitamin A, E and C content in roasted flaxseed and soybean are presented in figure-1.

It is clear from the figure-1 that considerable amount of non-antioxidant enzymes Vitamin A, E and vitamin C are present in roasted flaxseed powder. It was found that roasted flaxseed has 0.71 μg of vitamin A, 0.97 μg of Vitamin – E and 0. 32 mg of Vitamin – C .Flaxseed is rich beta-carotene, vitamin E and vitamin C. Devahi. *et al*., (2009) reported that supplementation of flaxseed based mid-morning snacks on school children increased brain development and activities, due to the presence of these vitamins. Stankovic. *et al*., (2011) stated that natural antioxidants such as a-tochopherol, b- carotene and vitamin C found in higher plants are being used in the food industry to inhibit lipid per oxidation and they can protect the human body from free radical and retard progress of many chronic diseases. Antioxidants in food contribute to disease prevention, which are essential nutrients or precursors of nutrients. Each of these antioxidant nutrients has specific activities and they often work synergistically to enhance the antioxidant capability of the body. It is also clear that roasting has increased the vitamin contents of the flaxseed. The synergistic action of wide spectrum of antioxidant is better than the activity of a single antioxidant and that antioxidants from natural source have a higher bioavailability and therefore higher protective

efficacy against oxidative stress than synthetic antioxidants. Thus the mixtures of all three antioxidant enzymes are present in roasted flaxseed may be believed to play a vital role in human body.

Determination of *In-Vitro* Antioxidant Activity

DPPH radical scavenging activity

Antioxidants through their scavenging power are useful for the management of various diseases. The antioxidant activities of the aqueous extracts of flax seeds were measured generally using antioxidant assay, such as DPPH free radical scavenging assay. DPPH has been used extensively as a free radical to evaluate the reducing substances (Cotelle *et al*., 1996) and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan *et al*., 2006). The DPPH radical scavenging activity of the extract has been attributed to the ability of the extract in pairing with the odd electron of DPPH radical (Park *et al*., 2004).

DPPH radical scavenging activities of aqueous extract of (Roasted) flax seeds are presented in **Figure 2**. A dose dependent increase the DPPH radical scavenging activity was observed in the aqueous extract of flax seeds (IC50 value = 232 μg/ml) which is in par with standard ascorbic acid. In the present study, the elevated DPPH radical scavenging ability of the flax seeds might be due to the presence of high contents of flavonoids. These results are also supported by the results of Duffy and Power, (2001) who reported different samples in different solvents to give different antioxidant potentials. Previously plants have been investigated for antioxidant activity. Evacuasiany *et al*., (2014) reported Cytotoxic and antioxidant activities of catechins in inhibiting the malignancy of breast cancer. Catechin epimers have good free radical scavenger activity against peroxyl radicals, singlet oxygen, peroxynitrite and hypochlorous acid have high antioxidant efficacy.It has been reported that the antioxidant activity of many botanicals were proportional to their flavonoids content. (Perumal *et al*., 2010).

Ferric reducing antioxidant power (FRAP) activity

The antioxidant activities of natural components might have a reciprocal correlation with their reducing powers. Reducing power of a compound serves as a significant indicator of its potential antioxidant activity. The antioxidant power was measured by FRAP method at 595 nm which was based on comparison of total amount of antioxidant with the reducing capacity of the samples.

The FRAP assay of the aqueous extract of flax seeds was determined and is shown in **Figure 3**. The ascorbic acid and aqueous extracts of flax seeds at higher concentration demonstrated best ferrous chelating efficacy than standard used. Reducing powers of the extracts from different concentrations of seed extract and standards were almost statistically comparable. The aqueous extracts of flax seeds showed significant effects on the reducing capacity at all amount. Reducing power of a compound might serve as a significant indicator of its potential antioxidant activity (Gulcin *et al*., 2003).

The FRAP assay is fast and easy to perform, and the response is reproducible and linearly related to the molar concentration of the antioxidants (Naidu *et al*., 2008). Flax seeds extract possess a

good reducing power capacity and the activity was dependent on concentration. The study conducted by Toth and Pavia, (2000) showed that other compounds such as polysaccharides were more effective than tannins for the detoxification and resistance to copper accumulation in A. *nodosum*. Andjelkovic *et al*. (2006) reported that the ability of phenolic compounds to chelate iron were far lower than that of EDTA. In addition, some peptides as well as proteins have also been reported to possess the abilities to chelate metal ions (Saiga *et al*., 2003).

The antioxidant activity has been reported to be concomitant with the development of reducing ability. Therefore, the antioxidant activity of the extracts might partially be a result of its reducing ability (Okuda *et al*., 1983).

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity of the aqueous extract from flax seed was investigated by measuring its ability to prevent oxidative degradation of deoxyribose substrates. The extract exhibited dose-dependent hydroxyl radical scavenging activities at concentrations ranging from 100 to 500 μg/mL. A similar trend to the DPPH radical scavenging results was observed in the hydroxyl radical scavenging activity with the highest activity was found in 500 μg/mL concentration. On the other hand, the other concentrations exhibited relatively lowest hydroxyl radical scavenging capacities. This result also suggested that compounds with the strongest hydroxyl radical scavenging capacity were rich in flax seeds than the level of hydroxyl radical scavenging activity of the extract was also similar to those of the standard **(Figure 4).**

Super oxide radical scavenging activity

The superoxide radical scavenging activities of the aqueous extract of flax seeds was determined and results obtained are shown in **Figure 5**. The aqueous flax seed extract showed the maximum inhibition (95.3 and 98.2 ± 0.6 %) at a concentration of 500 μg/mL which showed a significant radical scavenging activity than all other concentrations, the IC50 value of (106 and 110 μg/mL) exhibited an elevated level of superoxide radical scavenging activity which is similar to the standard BHT (130 μg/mL) Similarly, several nutritional foods have been reported for superoxide radical scavenging activity (Yasantha *et al*., 2006).

The scavenging effect of the extracts for superoxide radicals drastically increased with increasing concentration of the flax seed and soybean extract. The extract exhibited an enhanced superoxide anion radical scavenging activity at varying concentrations and also flax seeds extract have maximum scavenging activity and also it had similar radical scavenging activity to that of the standard BHT.

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Miller, 1993). Superoxide radicals could promote oxidative reactions due to their ability to reduce transition metals, release protein-bound metals and form per hydroxyl radicals which initiate lipid oxidation (Aikens and Dix, 1991; Elias *et al*., 2008).

4. CONCLUSION

Different *in vitro* antioxidant assays and non- enzymatic assays were carried out and all the assays showed maximum inhibition activity against the free radicals. The non-antioxidant enzymes Vitamin A, E and vitamin C are present maximum in roasted flaxseed than compared to soybean powder. A dose dependent increase the DPPH radical scavenging activity was observed in the aqueous extract soybean and flax seeds extract (IC50 value = 232 µg/ml) which is in par with standard ascorbic acid. FRAP method also revealed the antioxidant capacity of the samples. The ascorbic acid and aqueous extracts of soybeans and flaxseed at different concentration demonstrated comparable reducing activities. The aqueous extracts of soy beans and flax seeds showed significant effects on the reducing capacity at different amounts. Hydroxyl radical scavenging capacity was found to be greater in flaxseed than in soybean. The superoxide radical scavenging activities of the aqueous extract of soybean and flaxseed were found to be the maximum at a concentration of 500 µg/ml than at other concentrations.

Figure 1 Non –antioxidant Enzymes in roasted seeds

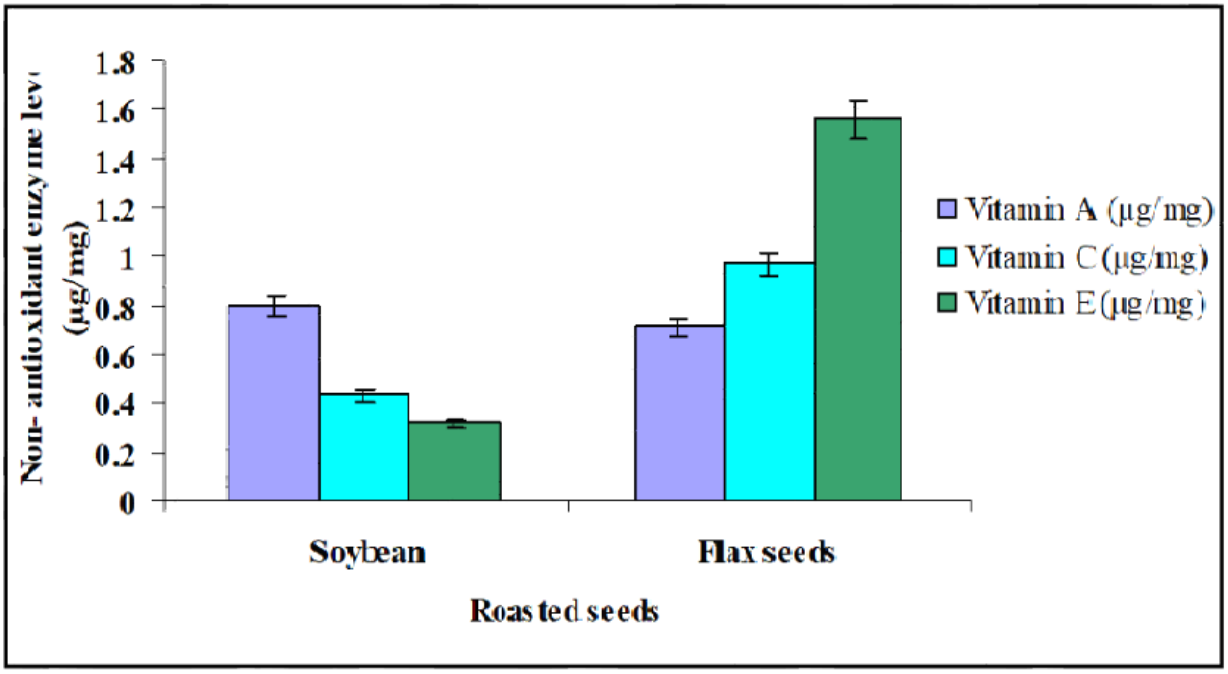
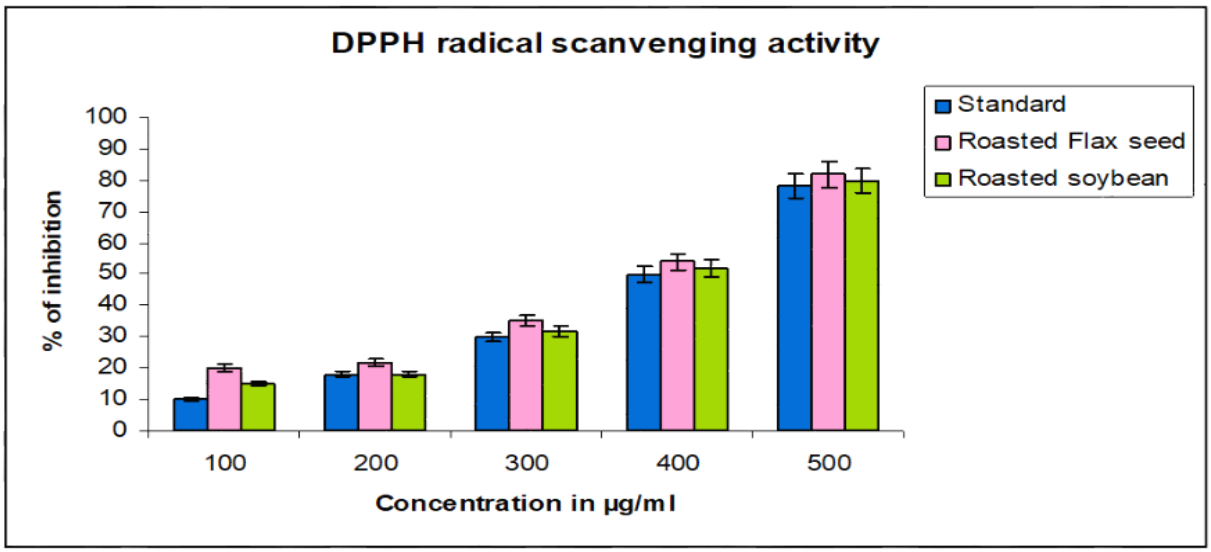


Figure 2.

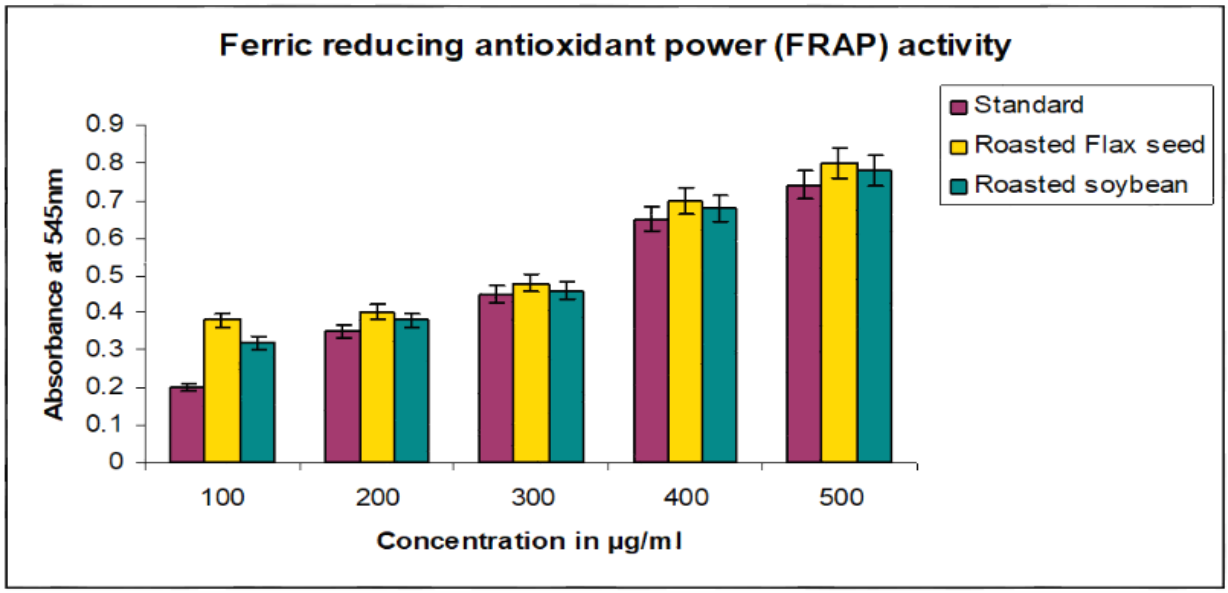
Concentration dependent percent inhibition of DPPH radicals by aqueous extract of roasted seeds (Soybean and flax seeds) *in vitro*.



Each value is expressed as mean ± SD (standard- ascorbic acid; n = 3).

Figure .3

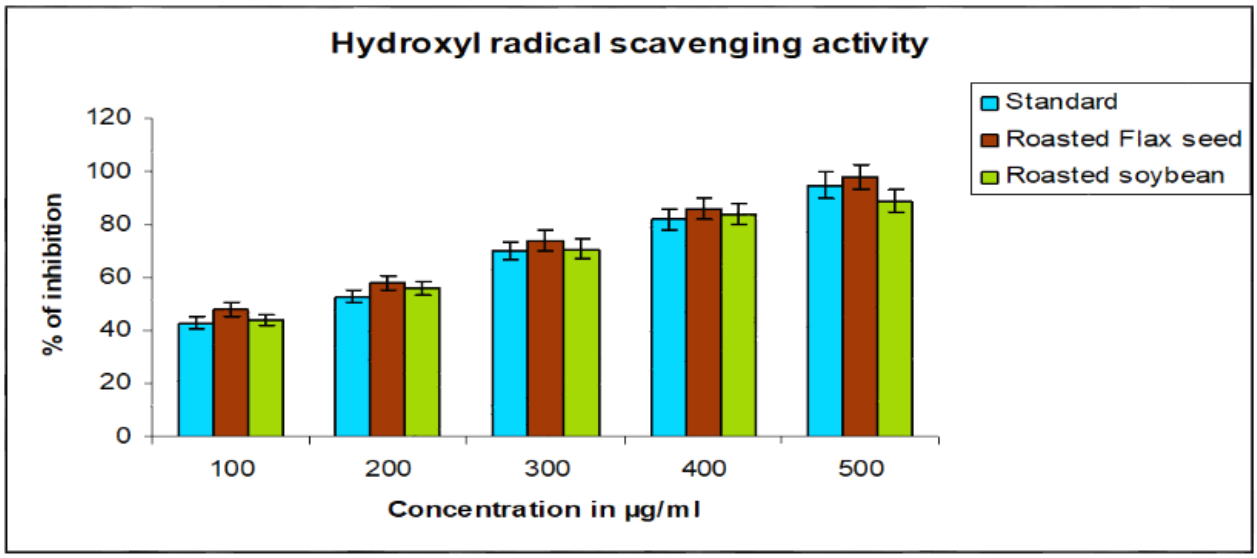
Concentration dependent ferric reducing antioxidant power by aqueous extract roasted seeds (Soy bean and flax seeds) *in vitro*.



Each value is expressed as mean ± SD (standard- ascorbic acid; n = 3).

Figure .4

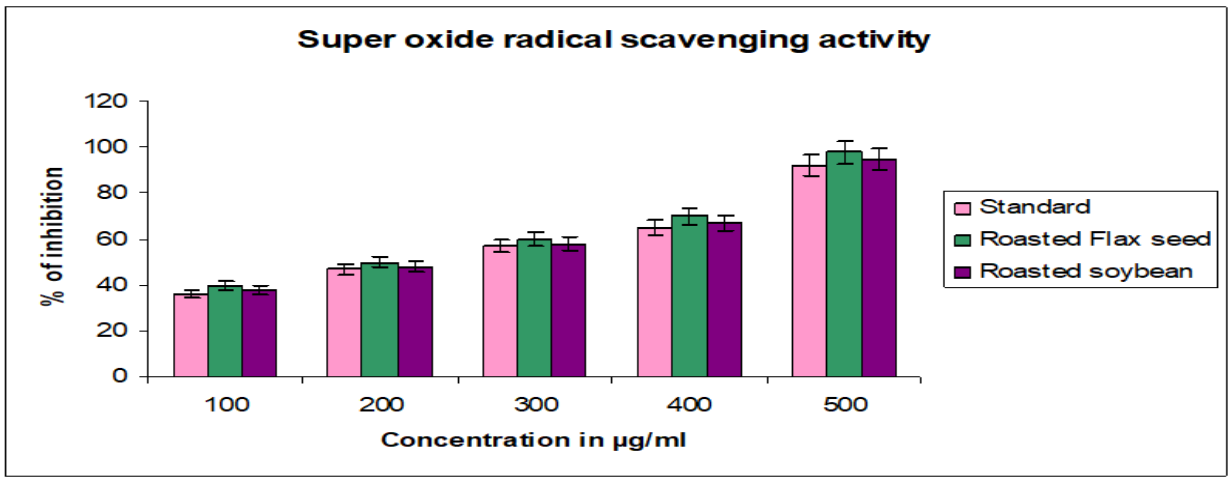
Concentration dependent percent inhibition of hydroxyl radicals by aqueous extract roasted seeds (Soy bean and flax seeds) *in vitro*.



Each value is expressed as mean ± SD (standard BHT- butylated hydroxy toluene; n=3).

Figure .5

Super oxide radical scavenging activity of roasted seeds (aqueous extract of Soy bean and flax seeds) *in vitro*.



Each value is expressed as mean ± SD (standard – Ascorbic acid; n=3).

5. REFERENCES

1. H. Tohma, E. Köksal, Ö. Kılıç, Y. Alan, M.A. Yılmaz, İ. Gülçin, E. Bursal and S.H. Alwasel (2016). RPHPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of Salvia L. species, Antioxidants, 5, 38.
2. İ. Gülçin (2012). Antioxidant activity of food constituents-An overview, Archiv. Toxicol. 86, 345-396.
3. M. Topal, H. Gocer, F. Topal, P. Kalin, P. Polat Köse, İ. Gülçin, K. Cetin Çakmak, M. Küçük, L. Durmaz, A.C. Gören and S.H. Alwasel (2016). Antioxidant, antiradical and anticholinergic properties of cynarin purified from the illyrian thistle (Onopordum illyricum L.), J. Enzyme Inhib. Med. Chem. 31, 266-275.
4. L. Polat Köse, İ. Gülçin, A.C. Gören, J. Namiesnik, A.L. Martinez-Ayala and S. Gorinstein (2015). LCMS/MS analysis, antioxidant and anticholinergic properties of galanga (Alpinia officinarum Hance) rhizomes, Ind. Crops Prod. 74, 712-721.
5. P. Kalın, İ. Gülçin and A.C. Gören (2015). Antioxidant activity and polyphenol content of Vaccinium macrocarpon, Rec. Nat. Prod. 9, 496-502.
6. M. ık, M. Korkmaz, E. Bursal, İ. Gülçin, E. Köksal and H. Tohma (2015). Determination of antioxidant properties of Gypsophila bitlisensis, Int. J. Pharmacol., 11, 366-371.
7. H. Yılmaz, S. Çarıkçı, T. Kılıç, T. Dirmenci, T. Arabacı and A.C. Gören (2017). Screening of chemical composition, antioxidant and anticholinesterase activity of section Brevifilamentum of Origanum (L.) species, Rec. Nat. Prod. 11(5), 439-455.
8. Nagalakshmi, N. and Prasad M.N.V (2001) Response of glutathione cycle enzymes and glutathione metabolism to copper stress in Scenedesmus bjugatus. Plant science., 160: 291-299 Pp.
9. Foyer, C.H. and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133, 21-25 Pp.
10. Bayfield RF and Cole ER (1980) Colorimetric estimation of vitamin A with trichloroacetic acid. Methods in Enzymology 67, 189-203 Pp.
11. Rosenberg, H.R. (1992) Chemistry and Physiology of Vitamins. Inter Science Publishers Inc., New York. 452 – 3 Pp.
12. Sadasivam, S. and Manickam, A. (1996) Biochemical Methods for Agricultural Sciences, New Age International (P) Ltd., New Delhi.
13. Blois, M.S. (1958) Antioxidant determinations by the use of a stable free radical. Nature. 29:1199-1200 Pp.
14. Benzie, I.F. and Strain J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 15:239(1):70-6 Pp.

15. Chung, S.K., T. Osawa and S. Kawakishi. (1997) Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (Brassica nigra). Biosci. Biotechnol. Biochem., 61: 118-123 Pp.
16. C.A., Khaled H., Liu M.C., Martin M., Namer M., O'Shaughnessy J.A., Shen Z.Z. and Albain K.S. (2005) The global breast cancer burden: variations in epidemiology and survival. Clin. Breast Cancer, 6 (5): 391-401 Pp.