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RESEARCH ARTICLE

LIPOXYGENASE INHIBITORY AND ANTIOXIDANT PROPERTIES OF THE DEVELOPED FUNCTIONAL FOOD SUPPLEMENTS (FFS)

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ABSTRACT

The evolution of the human diet over the past 10,000 years to our current modern pattern of intake has resulted in profound changes in the dietary behavior leading to lifestyle diseases reaching an epidemic proportion in industrialized countries, with similar trend being observed in developing countries. Foods contain a wide range of bioactive compounds with multiple physiological properties. Because of the importance of a healthy diet on lifestyle disease prevention and management, potential health benefits of naturally occurring bioactive compounds in foods are of greater interest. This research area has recently led to a wide variety of functional foods available in the world wide market. Locally available food substances are found to be healthy alternatives to medicines as they are rich in antioxidants and other inhibitory properties. In this context, a study was conducted to develop two Functional Food Supplement (FFS) using locally available food items like Barley, Ragi, Banana, Defatted Soy Flour, Drumstick leaves and Mushrooms. Dehydration and Fermentation were the processing techniques applied to standardize the FFSs. In depth study on antioxidant and lipoxygenase (LOX) inhibitory properties were carried on and the results showed higher activities in FFS developed with dehydration technique. The lipoxygenase (LOX) inhibitory activity of fermented FFS reduced to 40 per cent when compared to 55 per cent of dehydrated FFS.

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INTRODUCTION

Since the start of the industrial age, lifestyles of human beings have dramatically changed andhave pushed people into various fast-eating cultures with more instant and tasty meals, but decreased quantity and quality in nutrients (Bagchi *et al.,* 2004). The largest contributor to mortality and morbidity worldwide is non communicable disease, including cancer, CVD, neurodegenerative diseases and diabetes (Benzie and Wachtel-Galor, 2012). Even though theseare multi-factorial diseases with many pathophysiological mechanisms,a common finding is oxidation-induced damage throughoxidative stress (Finkel and Holbrook, 2000; Benzie and Wachtel-Galor, 2010). Appropriate antioxidant intake has beenproposed as a solution to counteract the deleterious effects of reactiveoxygen species (ROS), with substantial evidenceupholding the contention that: a diet rich in natural antioxidantssupports health (Holt *et al.,* 2009; Benzie and Wachtel-Galor, 2010), is associated with lower oxidative stressand inflammation (Hou *et al.,* 2011), and is therefore associated withlower risk of cancer, CVD, Alzheimer's disease and cataracts (Liu, 2003).

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Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties (Ali *et al.,* 2008). Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Numerous vegetables, crops, spices and medicinal herbs have been tested in an effort to identify new and potentially useful antioxidants (Zheng and Wang, 2001). More recently, plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk and oxidative stress by indirect antioxidant actionthrough a number of different mechanisms. A purified phytochemical may not have the same health benefit as that phytochemical present in whole foods or a mixture of foods (Norman, 2006). Berger and Shenkin (2006) viewed that functional foods play positive roles in maintaining well being, enhancing health, and modulating immune function to prevent specific diseases and has significant advantages in reducing the health care cost. People can optimize the health-promoting capabilities of their diet by way of supplementation and by consuming foods that have been formulated or fortified to include health-promoting factors (Wildman and Kelley, 2007). In the above context a study was conducted as an approach to develop two functional

food supplements from natural resources as an attempt to accomplish desirable therapeutic outcomes and to validate their health benefits through antioxidant and inhibitory assays.

MATERIALS AND METHODS

Selection of ingredients

Food substances containing bioactive compounds, which has got greater health benefits like hypoglycemic, hypocholestremic and hypotensive effects,but are less utilized or consumed in the daily diet, were selected for the formulation of FFS. Thus based on the support from the previous scientific investigations, the constituents for the FFS was chosen to contain Barley, Ragi and Banana (Rasakadhali/ Njalipoovan - mature, unripe), Defatted Soy Flour, Drumstick leaves powder and Mushroom powder in different proportions. The materials for the study was procured locally and processed in the laboratory.

Standardization of FFS

In the first processing technique (I), the constituents were dried individually in a cabinet drier, powdered mechanically, sieved thrice to obtain a fine powder and then blended into different proportions to formulate the FFS (I). In the second processing technique (II), fermentation followed by dehydrationwas the processing techniques. Ragi was cleaned, washed, allowed to soak in triple the amount of water for 12 hrs, drained excess water, kept covered in a muslin cloth, allowed to germinate for 24hrs, cabinet dried for four hours, then powdered, sieved and packed. The other constituents were dried individually, powdered mechanically and then packed separately to formulate the FFS II. Approximate proportion of ingredients for formulating the supplement is as follows: Barley – 20 to 40 per cent, Ragi – 20 to 30 per cent, Banana powder – 20 to 40 per cent, Defatted Soy Flour - 15 to 20 per cent, Drumstick leaves powder – 0 to 10 per cent, Mushroom powder -0 to10 per cent.The best identified FFSs were furtherinvestigated in depth for their antioxidant properties and anti lipoxygenase.

DiphenylPicrylHydrazyl (DPPH) Free Radical Scavenging Activity

The most commonly used DPPH assay is simple and highly sensitive. DPPH is commercialized in the radical form due to its stability. This radical shows a strong absorption maximum at 517 nm (purple). In the presence of antioxidants, the color turns from purple to yellow. Therefore the sole equipment needed for the assay is a UV-Vis spectrophotometer. Initially, DPPH radical was thought to be reduced to the corresponding hydrazine when it reacted with the donating hydrogen substances. However, more recent studies have shown that what occurs is mainly a fast electron transfer from the sample to DPPH radical. The abstraction of hydrogen from the sample by DPPH radical is marginal, because it occurs very slowly and depends on the hydrogen-bond accepting solvent. Methanol and ethanol, solvents generally used for antioxidant ability assays, are strongly hydrogen bond-accepting; therefore the hydrogen-abstracting reaction occurs very slowly (Miguel, 2010). Due to its simplicity and sensitivity, DPPH method alone for evaluating the antioxidant activities is helpful. DPPH free radical scavenging assay was measured using DPPH free radical test, by employing the method of Wong *et al.* (2000). The different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in the dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid served as the standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of the control
A_1 is the absorbance of test samples.

All the tests were performed in triplicates and the results are reported as IC_{50}, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 per cent.

In-Vitro Enzyme Inhibition Assay

Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes that play an important role in several inflammationrelated diseases (Rackova *et al.,* 2007). The lipoxygenase LOX inhibition assays (Anthon and Barrett, 2001) were performed with crude extracts of FFS I and II. The inhibitory activity was measured by a modified spectrophotometric method. LOX (EC 1.13.11.12) type I-B (Soybean) and linoleic acid were purchased from Sigma (Sigma-Aldrich, UK) and used without further purification. Enzyme solution of 1.03 µM was prepared in 0.2 mM borate buffer of pH 9.0. The substrate, linoleic acid solution of 0.32 mM was also prepared in the borate buffer at same pH. Crude extracts (10mg/100µl) were prepared in DMSO. The assay mixture was made of 50 µl of LOX, 50µl of test solution and 360 µl of the substrate. The final volume was made up to 2 ml with 1.54 ml borate buffer. The activity of LOX was measured on the formation of hydroperoxy-octadecadienoic acid which was monitored at 234 nm on a spectrophotometer, HITACHI U 2900. The same procedure was repeated with different concentrations of inhibitor for the confirmation of LOX inhibitory activity. Nordihydroguaretic acid (NDGA), a known inhibitor of Soybean Lipoxygenase, was used as positive control. 2gm of the FFSs mixture were taken and extracted with 60ml of methanol. The extract was then concentrated to 10ml and from that 500µl was pipette out and kept for dryness. Then the extract dissolved in 1ml DMSO from that and analyzed for enzyme inhibition assay. Graph showing the time-dependent activity of the enzyme was plotted using Origin Pro Software. Lipoxygenase inhibitory activity was expressed as percentage-inhibition of lipoxygenase, calculated by following the equation:

Inhibition (%) = (1-B/A)×100

Where, A is the change in absorbance without test sample and
B is the change in absorbance with the test solution.

RESULTS AND DISCUSSION

The best combination of the constituents for FFS I and II was identified based on their computed nutritional qualities, amino acid scores, phytochemical contents and sensory attributes. The identified combination from the dehydration technique was denoted as FFS I and that from the fermentation technique was denoted as FFS II.

Table 1. DPPH free radical scavenging activityof FFS I & II in comparison with standard

Concentration (µg/ml)	FFS I		FFS II		Standard (Ascorbic acid)	
	% inhibition	IC_{50} value (µg/ml)	% inhibition	IC_{50} value (µg/ml)	% inhibition	IC_{50} value (µg/ml)
100	11.67	585.35	10.67	639.00	15	477.86
200	21.67		19.67		27.5	
400	33.0		31.33		48.5	
600	53.67		49.33		61.5	
800	68.33		64.67		77	
1000	80.0		72.33		89.5	
F value: 394.645**						
CD-values (i=2 to n); (j=1 to i-1)						
13.2434						
14.8065 14.8065						

*Each value is presented as mean; **The $IC_{50}$was obtained by linear regression equations

Table 2. Observed and expected DPPH scavenging activityof FFS I & II

DPPH	FFS I (% inhibition)		FFS II (% inhibition)		Standard (Ascorbic acid) (% inhibition)	
Concentration (µg/ml)	Observed value	Expected value	Observed value	Expected value	Observed value	Expected value
100	11.67	12.58	10.67	11.84	15	19.21
200	21.67	20.29	19.67	18.92	27.5	27.36
400	33	35.71	31.33	33.08	48.5	43.66
600	53.67	51.13	49.33	47.24	61.5	59.96
800	68.33	66.55	64.67	61.40	77	76.26
1000	80	81.97	72.33	75.56	89.5	92.56

DPPH Scavenging Activity of Functional Food Supplements I & II

During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to the bio molecules. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. From the table 01, it was evident that the IC_{50} value of FFS I (585.35) and II (639.00) was closer to the IC_{50} values of standard ascorbic acid (477.86) which is a potent antioxidant though there is significant variation at 1 per cent. Though it was evident that there was variation in the % inhibition of FFS I & II, when compared to standard, their potency of inhibition is still higher. Thus it could be concluded that, both FFS I & II possesses greater antioxidant properties through their inhibitory effects. The values for IC 50 were obtained by fitting the regression equation; Y = 4.870 + 0.077x, Y = 4.759 + 0.077x and Y = 11.055 + 0.082x for FFS I, II and standard ascorbic acid respectively. From the above figures it could be noted that the observed values and the expected values are almost on the same line when fitted a linear regression equation. In all the three observations, it could be noted that the R^2 values of FFS I, II & standard (0.994, 0.990 and 0.987 respectively) are almost 1. This shows the validity of the data analyzed.

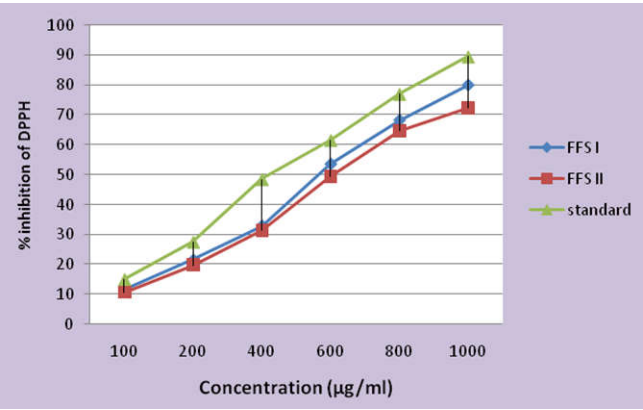

Fig. 1. Comparison of dose inhibition curve and IC_{50} values FFS I & II with standard by fitting regression equation

Flavonoids are most commonly known for their antioxidant activity and show anti-allergic, anti-inflammatory, antimicrobical and anticancer activity (Ekam and Ebong, 2007). Yang *et al.* (2001) reviewed the inhibition of carcinogenesis by dietary polyphenolic compounds.Tannins (tannic acids) and saponins are responsible for the antibacterial activity of the plant seed extracts (Gloor, 1997). People suffering from coronary heart disease are encouraged to consume moderately oxalate rich foods as it helps to reduce blood cholesterol (Savage, 2000). Literature data showed that DPPHscavenging activity differs depending on used solvent and food matrix. Researchers studied selected tropical fruits from Malaysia and stated that DPPHscavenging activity of pineapple, banana and guava varied significantlyas per the variation in the solvent used for extraction (Alothman *et al.,* 2009). The antioxidant potency of the FFSs might be the net reaction of the high amounts of phytochemicals like flavonoids, phytates, tannins and saponinsetc present in the various constituents of the FFSs. Since processing techniques like fermentation is found to bring about drastic changes in the phytochemical contents of the food, the variation in antioxidant property of FFS II in comparison with FFS I can be substantiated.

In-Vitro Enzyme Inhibition Assay

The establishment of new in-vitro test systems has stimulated the screening of plants, aiming to find leads for the development of new drugs. The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals. For this reason, the in vitro inhibition of lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential. LOXs are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy- radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. Enzyme assay were carried out and the crude extract showed 55 per cent for FFS I and 42 per cent for FFS II. The graph depicting LOX inhibitory activity of the extract is shown in Figures.

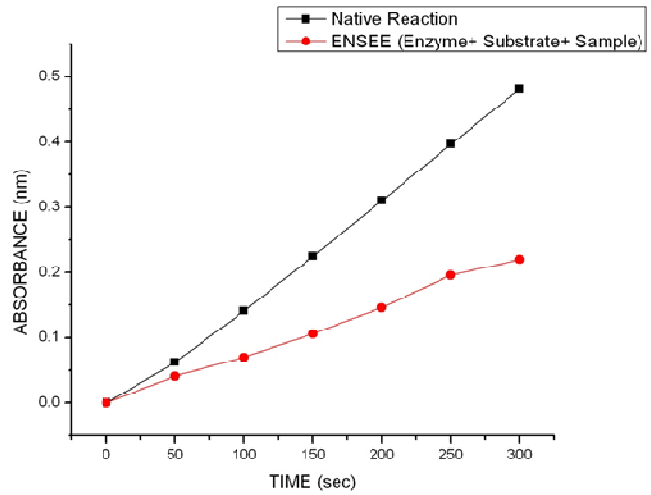

Fig. 2. Progression curve showing the 5-LOX inhibitory activity of the extract of FFS I

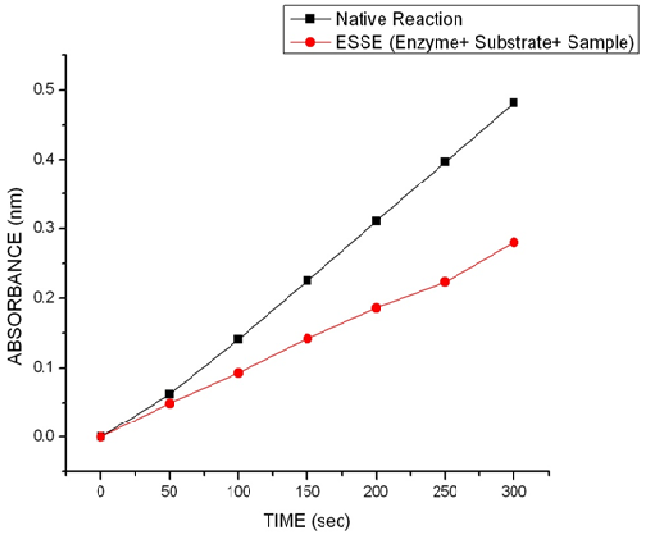

Fig. 3. Progression curve showing the 5-LOX inhibitory activity of the extract of FFS II

Presence of favorable amounts of antioxidants like vitamins, minerals and other phytochemicals are responsible for the high scavenging activities of both FFS I & II. The observed differential scavenging activities of the extracts against various systems may be referred to the different mechanisms of the radical antioxidant reactions in the different assays (Khan *et al.,* 2012). Anwar *et al*. (2013) reported that Cauliflower extracts obtained from air-dried, sun-dried, and oven-dried samples using different extraction solvents exhibited appreciable but varied scavenging activity in relation to both the extracting solvents and drying processes. Most of these compounds in plants could be removed by several processing methods such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods (Soetan, 2008). The developed functional food supplements with favorable sources of proximate compositions, vitamins, minerals, phytochemicals and antioxidant properties proves its role as a functional product in prevention and management of various chronic and lifestyle diseases like diabetes, CVD, hypertension, cancers etc. Their higher scavenging and inhibitory effects are also notable from their ability in immune modulation and anti-oxidation. Thus foods with functional properties can become a healthy alternative for drugs when properly consumed.

Summary and Conclusion

The above study justifies the importance of locally available food substances being evident as a source of packed nutrients and phytochemicals.The higher antioxidant properties and lipoxygenase (LOX) inhibitory properties of the FFS I and II stresses the need of choosing foods with nutritional and therapeutical functions for the management of lifestyle diseases. In due course, maintaining a proper dietary pattern rich in antioxidants throughout life will help to reduce the use of medications and the financial burden brought by it.

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