GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

Effect of Shelf Stable Concentrates of Tender Coconut Water and Testa Phenolics on Lipid Profile and Liver Antioxidant Enzymes in High Fat Fed Rats

Geetha V, Mohan Kumar A S, Chetana R, Gopala Krishna A G & Suresh Kumar G*
Department of Traditional Foods and Sensory Science, Central Food Technological Research Institute, Mysore – 570 020, Karnataka, India
*Corresponding Author

Abstract
Concentrate from Tender coconut water (TCW) and Testa phenolic concentrate(PHE) were prepared from by-products of coconut processing industries. Hypolipidemic and antioxidant properties of concentrates were evaluated in rats fed with high fat diet (HFD) along with or without concentrates at dosage of 500mg and 1000mg/kg body weight for TCW group; 25mg and 50mg/kg body weight dose for PHE group. Results showed HFD group had hyperlipidemic condition. Animals treated with higher doses of TCW (1000mg/kg of body weight) and PHE (50mg/kg of body weight) showed reduced lipid profile. TG-triglycerides -1.7 and 1.4 fold; TC-total cholesterol 1.3 fold in both concentrates). Antioxidant enzymes activities were ameliorated at different levels with dose. Altered organ weights were observed in HFD group particularly in liver (7.8g), normalized with TCW (6.5g) and PHE (6.7g) concentrate. Since these concentrates retained all the bioactive nutrients for a prolonged time, they were considered as stable and can be incorporated into food formulation for health improvement.
***Key words*:** Phenolic concentrate, Hyperlipidemia, Lipid profile, Malondialdehyde, Superoxide Dismutase

1. Introduction
Processed foods, an integral part of present food industry caters food across the world with similarity to natural product. Besides, food processing industries are aiming at converting perishable food materials to stable form through food processing and also maintain their natural qualities like flavor, nutrients and taste. Currently, ready-to-eat foods are more popular and catering worldwide, these foods need to be enriched with health beneficial stable compounds to make desired food products for various ailments and for people of all age groups.

Coconut, *Cocos nucifera* is an important palm species which is cultivated mainly for the endosperm and oil (Aduja et al., 2012, Gopala Krishna, 2012). Coconut is grown in more than 93 countries in the world and India is the third largest after Indonesia and Philippines. Coconut processing industry is commercialized with its valuable oil, however the industry produces large amount of byproduct including tender water, coconut cake and testa during its processing.

Tender coconut water is basically liquid endosperm (Haseena et al., 2010) that contains essential vitamins and minerals hence it has been considered as "functional food" (Priya and Lalitha, 2014) and an excellent natural soft drink with 17.4 calorie/100g. It consists of vitamins namely nicotinic acid (B_3), pantothenic acid (B_5), biotin (B_7), riboflavin (B_2), folic acid (B_9), trace amounts of thiamine (B_1) and pyridoxine (B_6) apart from it sugars, sugar alcohols, vitamin C, free amino acids, phytohormones (auxin, diphenylurea and cytokinins), enzymes (Acid phosphatase, Catalase, Dehydrogenase, Diastase, Peroxidase, RNA polymerases) and growth promoting factors. However, perishability of coconut water is very high when exposed to air, it becomes sour, develops off flavor and taste. Its natural freshness is lost within 24 to 36 h even under cold, unless treated scientifically.

Testa, a brown outer part of the coconut is a by-product of the coconut industry. It is removed during the preparation of products like desiccated coconut, coconut milk and virgin coconut oil and used as fodder. However they are excellent source of phytonutrients which are underutilized (Prakruthi et al.,2014). It mainly contains flavonoids and phenolics which can be used as natural antioxidants and is rarely studied in the context of human health benefits.

Change in the diet pattern of current life style leads to life threatening illnesses including Coronary heart disease (CHD). Due to elevated levels of triacylglycerols, cholesterol, and low density lipoprotein (LDL), cholesterol along with generated free radicals increases risk for CHD. This has been attributed to the involvement of oxidative modification of low-density lipoprotein (LDL) (Paterson et al., 2006; Joshi and Joshi, 2007). Current status is over 59%, due to high levels of cholesterol even though drugs are being used for their control (Cornel, 2001). Statins and fibrates are general class of drugs for treatment; whose long term implications are harmful. Hence natural therapy using plant sources are more appropriate which contains antioxidants that have CHD protective effects (Halliwell and Gutteridge,1999;Wiseman et al.,1997;Frankel et al., 1995) .

Various plant extracts are known to have hypolipidemic and hypocholesterolemic effects (Luo et al., 2004; Eddouks et al.,2005; Patil et al.,2010).In the current study the prepared tender coconut water concentrate (TCW) from tender coconut water and phenolic concentrate (PHE) from coconut testa (Process from CFTRI, Mysore, Karnataka, INDIA) have been used to analyze their health beneficial effect in the hyperlipidemia induced experimental animals- high fat diet fed rats.

2. Materials and methods
2.1. Preparation of concentrates
The experimental concentrates; Tender coconut water concentrate (TCW) from tender coconut water and Phenolic

concentrate (PHE) from testa, were prepared by a process developed at CSIR-CFTRI, Mysore. Raw materials for animal diet preparation were procured from HI-MEDIA, Mumbai and casein from Nimish Corporation, Mumbai.

2.2. Experimental animals and diets

Animal experiments were conducted in weaning male wistar rats (40-50g) to evaluate hypolipidemic property of tender coconut water (TCW) and phenolic concentrates (PHE) from coconut processing industries. Animals with equal body weights were grouped into six, ND: Normal/ Control diet fed group, HFD: High fat fed group, TCW 500: Group treated with Tender coconut water concentrate 500 mg, TCW 1000: Group treated with Tender coconut water concentrate 1000 mg, PHE 25: Group treated with 25 mg phenolic concentrate, PHE 50: Group treated with phenolic concentrate 50 mg/kg body weight. The animal studies had the IAEC (296/2014) clearance from CSIR-CFTRI, Mysore. Total six rats were housed in polycarbonate cages (2 rats per cage) and maintained in 12 h light/ dark cycle. The animals were given free access to diet and water for 2 weeks for acclimatization. Animal diet was prepared according to **(Table 1)** Normal diet (AIN-76) was fed to normal diet group and other groups were fed with high fat diets (30%) for 45 days. Test concentrates were intubated daily to respective groups with doses 500 and 1000 mg/kg body weights for two TCW groups and 25 and 50 mg/kg body weights for two PHE groups. During the experimental period diet intake and body weights were measured. After sacrificing the animals, blood was collected for serum separation and the harvested organs were weighed and stored at – 80°C till further processes. Obtained serum was subjected to analysis of lipid profile such as TG, cholesterol, LDL and HDL. Blood glucose was estimated using kit (Agappe Diagonistics limited)

Table 1. Diet composition according to AIN -76 with slight modification for preparation of 1kg diet containing with and without TCW and PHE concentrates

Composition	Normal Diet (g)	High fat diet (30%) (g)
Starch	653	453
Casein	200	200
Vitamin mix	10	10
Mineral Mix	35	35
Choline chloride	2	2
DL Methionine	0.020	0.020
Lard	-	200
Ground nut oil	100	100
Bile Salt	-	50
Cholesterol	-	50

2.3 Evaluation of antioxidant enzyme activities and TBA reactant (MDA) from Liver tissue

2.3.1. Superoxide dismutase (SOD)

The enzyme activity of SOD was carried out by xanthine-xanthine oxidase ferricytochrome C (X/XOD/Cyt C3+) method (McCord and Fridovich, 1969). The reaction mixture containing 2.9 ml of solution A (5µmol xanthine in 0.001N sodium hydroxide and 2µmol Cyt C in 50mM potassium phosphate buffer containing 0.1mM EDTA) and 50µl sample. Reaction was started with 50µl solution B (Containing freshly prepared xanthine oxidase in 0.1mM EDTA phosphate buffer of pH 7.8) mixed well and absorbance of enzyme kinetics is measured at 580 nm and expressed as units/mg protein.

2.3.2. Glutathione peroxidase (GPx)

The enzyme activity was carried out according to the method by Flohe and Gunzler,(1984). Briefly, reaction mixture containing 0.4 ml GSH (0.1 mM), 0.2 ml TBS solution (Tris 50mM, NaCl 150 mM pH 7.4) and 0.2 ml sample. After 5 min incubation at 25°C, 0.2 ml of H_2O_2 (1.3mM) was added to the mixture. The content was incubated at 37°C for 10 min. The reaction was stopped by 1 ml of 1% trichloroacetic acid (TCA) and centrifuged. Absorbance was recorded at 412 nm and the activity was expressed as units/mg protein.

2.3.3. Catalase

The activity was followed according to the method by Aebi,(1984) and determined by monitoring the rate of H_2O_2 consumption in a mixture containing 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2 and sample for 3 min at 240 nm and expressed as units/mg protein.

2.3.4. Lipid peroxidation by TBARS method (TBA reactant/MDA)

Lipid metabolites were measured as MDA (TBA reactant) as an indicator of lipid peroxidation using TBARs method (Liu et al., 1997; Leonard et al. 2004, Esterbauer and Zollner,1989;Ohkawa et al.,1979).Tissue homogenized with KCl, centrifuged and supernatant was used as test sample, solutions were mixed with SDS (8%) and acetic acid (20%). Mixture was kept in boiling water bath along with thiobarbutric acid (0.8%) for 1h and butanol was added after cooling. Butanol fractions were separated by centrifugation and absorbance was measured at 532 nm for formation of TBA reactant (MDA) using TEP (1,1,3,3 tetraethoxy propane) as standard.

2.4. Statistical analysis

All data were expressed as mean ± S.E.M and the differences between the mean values were assessed using Analysis of Variance (ANOVA) by Duncan's multiple range test (DMRT). Statistical significance was considered at $p<0.05$.

3. Results

3.1. Effect of tender coconut water and phenolic concentrate on diet intake, body weight and serum lipid profile in high fat fed Rats

Tender Coconut Water (TCW) and Phenolic concentrates (PHE) were prepared from the byproducts of coconut processing industries. Hypolipidemic and hypocholesterolemic properties of the concentrates were analyzed in high fat fed rats. Animals were maintained for about 45 days with different diets, normal, high fat and with and without concentrates. The doses were fixed based on the previous reports as well as the trial experiments. All the presented results are obtained by comparing the treated group with high fat fed groups. Body weight and diet intake were recorded and presented in, (**Table 2)**. Diet intake was observed more (22.4 g) in control (ND) animals and least (6.3 g) in HFD group and slight increase in the diet intakes were observed in treated groups, (**Table 2)**. The average body weight of HFD group was increased (243 g) when compared to control (228 g) and were reduced with TCW and PHE concentrate in dose dependent manner but the highest doses of TCW 1000 & PHE 50 showed better reduction (221 g and 223 g) and are statistically significant when compared to HFD group, (**Table 2)**. Study clearly indicated that gain in body weights in high fat fed animals is due to consumption of high calorie diet and was reduced significantly after treating with both the concentrates. However, diet intake was more in ND groups which could be due to less calorie value (starch based diet) with that of HFD groups. The consumption of diet in treated groups was increased when compared to HFD group. After 45 days animals were sacrificed under over-anesthesia. The collected blood was subjected for the serum separation followed by blood glucose and lipid profile analysis. Blood glucose was found in border (127 mg/dL) line in HFD group when compared to ND (108 mg/dL) group and were reduced in their blood glucose after treating with concentrates (TCW: 125 and 119, PHE: 121 and 112 mg/dL). The optimum concentrations of TCW and PHE concentrate could be 1000 and 50 mg/ kg body weights respectively for better effect. Lipid profile, in which triglycerides (TG), total cholesterol (TC) and LDL levels were increased in HFD when compared to ND groups. However, after treatment with concentrates, levels of TG significantly reduced with dose dependent manner (TCW: 34 and 25 mg/dL, PHE: 39 and 30 mg/dL) whereas cholesterol and LDL also showed effect but were not significant during 45 days of experimental period between the two doses, (**Table 2).**

Table 2. Effect of tender coconut water and phenolic concentrates on lipid profile in control, high fat fed and treated group (TCW and PHE concentrate).

Groups	Diet intake (g)	Body weights (g)	Glucose (mg/dL)	TG (mg/dL)	Cholesterol (mg/dL)	LDL (mg/dL)
ND	22.40±2.1^{c}	228.1±16^{a}	108.3± 5.1^{a}	33.9 ± 2.1^{b}	42.9 ± 0.5^{a}	06.5 ± 0.4^{a}
HFD	06.26±1.8^{a}	243.5±18^{b}	127.3 ± 5.3^{c}	42.2 ± 2.5^{c}	54.4 ± 1.5^{c}	12.3 ± 1.2^{b}
TCW-500	07.66±1.5^{a}	229.2±19^{a}	125.4 ± 4.8^{c}	34.0 ± 1.3^{b}	46.0 ± 1.8^{b}	11.0 ± 0.9^{b}
TCW-1000	06.52±1.0^{a}	221.0±18^{a}	119.1 ± 4.1^{b}	25.1 ± 1.3^{a}	41.4 ± 1.3^{a}	07.8 ± 1.1^{a}
PHE-25	12.00 ± 2.0^{b}	226.3±17^{a}	121.0 ± 5.2^{c}	39.1 ± 2.3^{c}	38.0 ± 1.4^{a}	06.4 ± 0.5^{a}
PHE-50	09.64±1.5^{a}	223.2±18^{a}	112.7 ± 6.3^{b}	30.4 ± 3.0^{b}	41.3 ± 1.6^{a}	07.4 ± 0.4^{a}

Abbreviations: ND; Control diet group, HFD; High fat fed group, TCW-500; Tender water concentrate 500mg treated group, TCW-1000; Tender water concentrate 1000 mg treated group, PHE-25; Phenolic concentrate 25mg treated group, PHE-50; Phenolic concentrate 50mg treated group.

Values are means ± SE (n=6). Column not sharing a common alphabets are significantly different at $p < 0.05$.

3.2. Effect of coconut water and phenolic concentrate on organ weights in high fat fed rats

Change in the organ weights, (**Table 3)** could reflect the normal biological functions of the animal. In connection to weight of liver, HFD group had more weight than the ND group. After treating with concentrate (TCW and PHE) containing diets, the liver weight was reduced significantly when compared with HFD. The adipose tissue weight was more in HFD when compared to ND group. However, the weight reduced significantly after treatment with concentrates (TCW & PHE) and are significant at $p< 0.05$ levels. The difference in the weights of kidney between ND and HFD is highly significant ($p < 0.01$), and the weights reduced significantly ($p < 0.05$) after treatment with the concentrate when compared to HFD group. However, no such significant changes were observed in the weights of heart.

Table 3. Effect of coconut water and phenolic concentrate on organ weights (g/100g body weight) of high fat fed rats.

Groups	Kidney	Liver	Heart	Adipose
ND	1.2± 0.3^{a}	6.3 ± 0.9^{a}	0.6 ± 0.1^{a}	3.0 ± 0.5^{a}
HFD	1.6 ± 0.4^{b}	7.8 ± 0.3^{b}	0.7 ± 0.1^{b}	3.8 ± 0.8^{b}
TCW-500	1.3 ± 0.3^{a}	7.1 ± 1.2^{b}	0.6 ± 0.1^{a}	3.1 ± 0.8^{a}
TCW-1000	1.3 ± 0.4^{a}	6.5 ± 1.5^{a}	0.5 ± 0.1^{a}	2.8 ± 0.9^{a}
PHE-25	1.4 ± 0.3^{b}	6.4 ± 1.2^{a}	0.6 ± 0.1^{a}	2.9 ± 0.1^{a}
PHE-50	1.3 ± 0.2^{a}	6.7 ± 1.3^{a}	0.6 ± 0.1^{a}	2.7 ± 0.8^{a}

Abbreviations as in **Table 2.** Columns not sharing a common alphabets are significantly different at $p< 0.05$.

3.3. Effect of tender coconut water and phenolic concentrate on the liver antioxidant (AOX) enzyme activities and TBA reactant (MDA) levels in high fat fed rats

In HFD group, catalase activity was reduced whereas SOD and GPx were increased when compared to ND group. Treatment with TCW and PHE ameliorated the effect of high fat feeding on AOX enzymes in a dose dependent manner (**Table 4**). The highest dose of TCW 1000 and PHE 50 showed increased activities ~1.2 fold when compared to high fat fed animals, respectively whereas activity of SOD ameliorated dose dependently. However, not much significant effects were observed in the GPx activities. The level of MDA (TBA reactant) was reduced with increased concentrates of TCW and PHE and the level of MDA reached to normal in PHE 50 group, (**Table 4).** This indicates that AOXs is effectively involved in reducing lipid peroxidation.

Table 4. Effect of Tender coconut water and phenolics concentrates on the antioxidant enzymes of liver tissues of rats.

Groups	Catalase U/mg protein	SOD U/mg protein	GPX U/mg protein	MDA/nm/mg protein
ND	25.65 ± 0.96^{c}	0.125 ± 0.06^{a}	0.057 ± 0.005^{a}	0.08 ± 0.002^{a}
HFD	04.70 ± 0.36^{a}	0.188 ± 0.06^{c}	0.088 ± 0.006^{c}	0.11 ± 0.003^{b}
TCW-500	08.56 ± 0.56^{a}	0.169 ± 0.02^{b}	0.080 ± 0.002^{c}	0.10 ± 0.004^{b}
TCW-1000	15.07 ± 0.75^{b}	0.159 ± 0.04^{b}	0.072 ± 0.007^{b}	0.07 ± 0.005^{a}
PHE-25	05.10 ± 0.33^{a}	0.184 ± 0.06^{c}	0.078 ± 0.002^{b}	0.09 ± 0.003^{a}
PHE-50	25.59 ± 0.62^{c}	0.154 ± 0.02^{b}	0.076 ± 0.005^{b}	0.08 ± 0.004^{a}

Abbreviations as in **Table 2**. Column not sharing a common alphabets are significant

4. Discussion

The prepared concentrates of tender coconut water (TCW) and testa phenolic (PHE) were tested for their hypolipidemic effect. Several studies have shown health beneficial effect of phenolic extracts other than coconut. However, in the present investigation we made an attempt to convert the perishable form of extracts *viz*. tender coconut water and testa phenolic extract, to stable concentrates and were evaluated for health beneficial effects. Concentrates TCW (500 and 1000 mg/kg body weight) and PHE (25 and 50 mg/kg body weight) were intubated daily for 45 days and compared with HFD group intubated with saline. Body weight gain was observed in HFD group when compared to other groups. It has been shown that consumption of high calorie diet increases the body weight and elevates the blood glucose, which was attenuated by feeding the diet containing *Moringa oleifera* leaves in male Wistar rats (Bais et al.,2014). In another study tender coconut water was shown to improve insulin sensitivity (Bhagya et al.,2012). Salihu et al.,(2009) showed the orally intubated tender coconut water reduced the blood glucose in alloxan induced diabetes.

Treatment of high fat fed rats with coconut water showed reduced triglycerides, cholesterol and LDL+VLDL cholesterol in serum as well as in tissues (Sandhya and Rajamohan, 2006). Reports from separate studies have shown amelioration in the lipid profiles of alloxan induced diabetic and hypertensive animals after treating with coconut water (Preetha et al, 2013; Bhagya et al., 2010). Furthermore, nicotine induced reproductive dysfunction in rats that showed augmented level of lipid profile in serum was reduced by coconut water treatment (Nair and Rajamohan, 2014). Presence of L-arginine in TCW is an important amino acid shown to have hypolipidemic, antihypertensive and anti-atherogenic effect (Bhagya et al., 2010;Nair and Rajamohan , 2014;Salil and Rajamohan, 2001).Reduced fat accumulation was also observed in various organs analyzed through histological studies (Flohe and Gunzler,1984). The current study showed amelioration in abnormal blood glucose, lipid profile and body weight upon treatment with TCW and PHE concentrates from coconut.

High fat feeding to animals induces the generation of free radicals within the system through various mechanisms, their uncontrolled conditions lead to development of metabolic disorders like diabetes and their secondary complication (Li and Periwal, 2013; Reinke et al,1987). To control such incidences, biological system has adapted antioxidant defense mechanism within the tissues that includes non-enzymatic antioxidants (e.g., glutathione, uric acid, bilirubin, vitamins C and E) and enzymatic co-ordination of superoxide dismutase, catalase and glutathione peroxidase activities. Treatment with various bioactives such as phenolic compounds and phenolic extracts on such biochemical conditions either by *in-vitro* or *in-vivo* have been well studied (Christine and Joseph, 2010; Lee et al., 2013). Loki and Rajamohan, (2003) have shown the effect of TCW on hepatoprotective and antioxidant enzyme activities in the animal models with CCL_4 induced liver damage, in which SOD, Catalase and GPx activities were ameliorated significantly. SOD combining with protons to form hydrogen peroxide and oxygen and dismutates the superoxide anion. Catalase decomposes hydrogen peroxide into water and oxygen. Glutathione peroxidase removes hydrogen peroxide by converting reduced glutathione into oxidized glutathione (Szymonik et al,2003). Higher level of hydrogen peroxide is removed by catalase enzyme and a lower level is removed by reacting with reduced glutathione (Hemnani and Parihar,1998). Another study claims that the TCW treatment could reverse high blood glucose and improved insulin sensitivity through inhibition of lipid peroxidation and amelioration of antioxidant status (Ramalingum and Mahomoodally, 2014) .Our concentrates showed significant increase in liver catalase enzyme activity when compare to HFD group animals, which shows antioxidant potency of the prepared concentrates. The work also warrants that the phenolics or other bioactives may inhibit enzymes necessary for hydrolysis

of dietary macromolecules such as lipids and carbohydrates in GI tract which further reduce or delays absorption, thus lipid profile may be reduced.

5. Conclusion

The effect of tender coconut water on various biological activities has been well studied. However, prepared concentrates of Tender coconut water (TCW) and Testa phenolic concentrate (PHE) from coconut testa makes novel extracts. Health beneficial concentrates having hypolipidemic effect, ameliorating antioxidant enzymes and organ weights as evaluated with the present work. It is evident from the results that both the concentrates (TCW and PHE) retain bioactives in the active form. It can be incorporated in preparing various health food products, particularly TCW concentrates can be used as a health drink with natural bioactives.

Acknowledgement

The authors express their thanks to the Director, CSIR-Central Food Technological Research Institute, Mysore. The authors Geetha and Mohan thank the Coconut Developing Board, Kochi, Kerala for the financial assistance.

Conflict of interests

The authors declare that no conflict of interest exists regarding any material described in this manuscript.

References

Aduja,N., Raghavendra, S.N. and Raghavarao, K.S.M.S. (2012). Production of coconut protein powder from coconut wet processing waste and its characterization. *Applied Biochemistry and Biotechnology*, 167:1290-1302.

Aebi, H. (1984). Catalase *in vitro. Methods in enzymology*, 105, 121-126.

Bais, S, Singh, G.S. and Sharma, R. (2014). Antiobesity and hypolipidemic activity of *moringa oleifera* leaves against high fat diet-induced obesity in rats. *Advances in Biology*, 1-9. http://doi.org/10.1155/2014/162914

Bhagya , D., Prema, L. and Rajamohan, T. (2010) .Beneficial effects of tender coconut water on blood pressure and lipid levels in experimental hypertension. *Journal of Cell and Tissue Research*, 10, 2139–2144.

Bhagya, D., Prema, L. and Rajamohan, T. (2012). Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pacific Journal of Tropical Medicine*, 5, 270-276.

Christine, J.W. and Joseph, J.C. (2010). Measurement of superoxide dismutase, catalase and glutathione perioxidase in cultrured cells and tissues. *Nature Protocols*, 5, 51–66.

Cornel, P. (2001). The current status of primary prevention in coronary heart disease. *Current controlled trials cardiovascular medicine*, 2, 24–37.

Eddouks, M., Lemhadri,A. and Michel, J.B. (2005) . Hypolipidemic activity of aqueous extract of *Capparis spinosa L.* in normal and diabetic rats. *Journal of Ethnopharmacology*, 98, 345–350.

Esterbauer, H. and Zollner, H. (1989). Methods for determination of aldehydic lipid peroxidation products. *Free Radical Biology & Medicine*, 7,197-203.

Flohe, L., and Gunzler, W.A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology*, 105, 114-121.

Frankel, E.N., Waterhouse, A.L. and Teissedre, P.L.(1995) .Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low density lipoproteins. *Journal of Agricultural and Food Chemistry*, 43, 890–894.

Gopala Krishna A.G. , Coconut oil : Science, technology and applications. Inform: inform June 2012, Vol. 23 (6), p.395-399.

Halliwell, B., and Gutteridge, J.M.C. (1999). Free radicals in biology and medicine. Oxford University Press, Oxford.

Haseena, M., Kasturi Bai, K.V. and Padmanabhan, S. (2010). Post-harvest quality and shelf-life of tender coconut. *Journal of Food Science and Technology*, 47, 686–689.

Hemnani, T. and Parihar, M.S. (1998).Reactive oxygen species and oxidative damage. *Indian Journal of Physiology and Pharmacology*, 4, 440-452.

Joshi, S.C., and Joshi, V. (2007). Effect of *Ammomum subulatum* on oxidative stress and atherosclerosis in cholesterol fed rabbits. *Pharmacologyonline*, 1, 451-463.

Lee, L.S., Cho, C.W., Hong, H.D., Lee, Y.C., Choi, U.K. and Kim, Y.C. (2013). Hypolipidemic and antioxidant properties of phenolic compound-rich extracts from white ginseng (*Panax ginseng*) in cholesterol-fed rabbits. *Molecules*, 18, 12548-12560.

Leonard, T. R., Gregory, W. T.,Michael L. C., Gerald, C., Raphael, B-O. and David B-O.(2004). Lipid peroxidation and the thiobarbituric acid assay: Standardization of the assay when using saturated and unsaturated fatty acids. *Journal of Biochemistry and Molecular Biology*, 37,749-752.

Li, Y. and Periwal,V. (2013).Synergy in free radical generation is blunted by high fat diet induced alterations in skeletal muscle mitochondrial metabolism. *Biophysical Journal*, 104, 1127-1141.

Liu, J., Yeo, H. C., Doniger, S. J. and Ames, B. N. (1997).Assay of aldehydes from lipid peroxidation: gas chromatograph mass spectrometry compared to thiobarbituric acid. *Analytical Biochemistry*, 245, 161-166.

Loki, A.L. and Rajamohan, T. (2003). Hepatoprotective and antioxidant effect of tender coconut water on carbon tetrachloride induced liver injury in rats. *Indian Journal of Biochemistry and Biophysics*, 40, 354-357.

Luo, Q., Cai ,Y., Yan, J., Sun, M. and Corke, H. (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum. Life Sciences*, 76, 137–149.

McCord, J.M. and Fridovich, I. (1969). Superoxide dismutase. An enzymic function for erythrocuprein (*hemocuprein*). *The Journal of Biological Chemistry*, 244, 6049–6055.

Nair, S.V.G. and Rajamohan, T. (2014). The role of coconut water on nicotine-induced reproductive dysfunction in experimental male rat model. *Food and Nutrition Sciences*, 5, 1121-1130.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reactions. *Analytical Biochemistry*, 95, 451–458.

Paterson, E., Gordon, M. H., Niwat, C., George, T.W., Parr, L., Warronphan, S. and Lovegrove, J.A. (2006). Supplementation with fruit and vegetable soups and beverages increases plasma carotenoid concentrations but does not alter markers of oxidative stress or cardiovascular risk factors. *Journal of Nutrition*, 136, 2849-2855.

Patil, R.H., Prakash, K. and Maheshwari,V.L. (2010).Hypolipidemic Effect of *Celastrus paniculatus* in experimentally induced hypercholesterolemic Wistar Rats. *Indian Journal of Clinical Biochemistry*, 25, 405–410.

Prakruthi, A., Sunil, L., Prashant Kumar, P.K. and Gopalakrishna, A.G. (2014). Composition of coconut testa, coconut kernel and its oil. *Journal of the Americal Oil Chemists' Society*, 91, 917-924.

Preetha, P.P., Devi, V.G. and Rajamohan, T. (2013). Comparative effects of mature coconut water (*Cocos nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. *Brazilian Journal of Pharmacognosy*, 23, 481-487.

Priya, S.R. and Lalitha, R. (2014).Tender Coconut Water – Natures elixir to mankind. *International Journal of Recent Scientific Research*, 5, 1485-1490.

Ramalingum, N.and Mahomoodally, M.F. (2014). The therapeutic potential of medicinal foods. *Advances in Pharmacological Sciences*,1-18. http://doi.org/10.1155/2014/354264

Reinke, L.A., Lai, E. K., DuBose, C. M. and McCay, P.B. (1987). Reactive free radical generation *invivo* in heart and liver of ethanol fed rats: correlation with radical formation *invitro*. *Proceedings of the National Academy of Sciences*, USA, 84, 9223-9227.

Salihu, M.A., Luqman, A.O., Oshiba, O.J., Rabiu, O.J, Sikiru, A.J, Ayokunle, O. and Adesola, I.R.A. (2009). Comparative study of the hypoglycemic effects of coconut water extract of Picralimanitida seeds (*Apocynaceae*) and Daonil in alloxan-induced diabetic albino rats. *African Journal of Biotechnology*, 8, 574-576.

Salil, G. and Rajamohan, T. (2001). Hypolipidemic and antiperoxidative effect of coconut protein in hypercholesterolemic rats. *Indian Journal of Experimental Biology*, 39, 1028–1034.

Sandhya, V.G. and Rajamohan, T. (2006).Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. *Journal of Medicinal Food*, 9, 400-407.

Szymonik, L.S., Czechowska, G., Stryjecka, Z.M., Słomka, M., Madro, A., Celiński, K. and Wielosz, M. (2003) .Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *Journal of Hepato-Biliary-Pancreatic Surgery*, 10, 309-315.

Wiseman, S. A., Balentine, D. A. and Frei, B. (1997) . Antioxidants in tea. *Critical Reviews in Food Science and Nutrition*, 37, 705–718.