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In vivo and *in vitro* antioxidant and hypolipidemic activity of methanol extract of pineapple peels in wistar rats

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Abstract

The medicinal values of pineapple has been well documented in literature. However, pineapple peels constitute the major waste of the pineapple fruit. Recent interest has been on harnessing plants waste materials for economic and industrial values. The study investigated the in *vivo*, in *vitro* and hypolipidaemic activity of pineapple peel extract on wistar albino rats. Twenty wistar albino rats grouped into four groups were used for the study. Group A served as control group, while group C-D received varying doses of the crude extract (200, 300 and 500mg/kg body weight). Experimental period lasted for twenty eight days, after which animals were fasted overnight and blood collected via ocular puncture for lipid profile analysis and liver obtained for antioxidant analysis. The in *vitro* antioxidant activity was determined using 2, 2- diphenyl-1-picrylhydrazine photometric assay and ferric reducing ability assay. Result indicated a concentration dependent (25- 400 μg/mL) for the extract in 2, 2- diphenyl-1-picrylhydrazine photometric assay and ferric reducing ability assay. In *vivo* antioxidant assay showed no significant *($P<0.05$)* difference in the extract administered group when compared to the control group. Serum HDL estimation showed a significant *($P<0.05$)* increase in the extract administered group (200 and 500mg/kg) when compared to the control group. Result for in *vivo* showed that the extract did not induce oxidative stress and can be a positive agent in preventing lipid peroxidation while increase in functional HDL indicated that pineapple peels may be useful in the management of hyperlipidaemia and other associated diseases.

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Introduction

Pineapple scientifically known as *Ananas comosus* has been identified the most important plant of the *Bromeliaceae* family. Pineapple is profound in polyphenolic compounds and vitamins, fibers as well as nutritional minerals such as calcium, phosphorous, and iron. The pharmacological activity of the fruit is attributed to its high amount of compounds such as polyphenols and ascorbic acid, which fund the antioxidant activity of the fruit (Hossain and Rahman, 2010). Juice derive from pineapple has traditionally been used to alleviate sore throats and also assist in wound healing. Pineapple contains an important digestive enzyme "Bromelain" (Bartholomew *et al.,* 2003).

In traditional medicine, root of pineapple is used to treat inflammatory and digestive disorders. Bromelain found in pineapple has been reported to have anti-bacteria effect (Bartholomew *et al.,* 2003). Review by Prasenjit *et al.* (2012) reported consumption of pineapple can lead to a reduction of cholesterol, speed healing of tissue injury, improves male fertility, reduces joint and muscle pain, strengthen bones, treatment of cough.

Characterization of pineapple peels showed that it contains cellulose and hemicellulose in high proportion (Bardiya *et al.,* 1996). Waste derived from pineapple processing have been used as by-products for production of bromelain, ethanol, sugar and this serves as sources of vitamins and growth factors (Dacera *et al.,* 2009). Guo *et al.,* (2003) reported pineapple peels consist 2.01mmol FRAP/100g wet weight. Animal studies by Ochuko *et al.,* (2011) showed a modulatory effect of pineapple peel on lipid peroxidation and catalase activity in alcohol induced toxicity indicating it can be used to manage the toxic condition.

The link between hyperlipidaemia and oxidative stress in humans has been discussed in studies by Rui-Li *et al.,* (2008). Increased generation of reactive oxygen species (ROS) has been associated with many chronic diseases such as inflammation, cancer, obesity and atherosclerosis (Chisolm, 2001). Antioxidant plays defense role in the fight against oxidative stress and diseases. They achieve this by mopping up free radicals. ROS are generated in aerobic respiration carried out by the mitochondria. Li *et al.* (2014) identified and quantified major phenolics in pineapple peels and showed that they possess antioxidant activities. Antioxidants also play a strong role in the fight against hyperlipidemia.

Discarded plant waste materials causes burden to the environment if not properly handled. However, the search for under-utilized plant materials as source of antioxidants and for other medicinal component has increased over time. Considering the high phenolic content possessed by the pineapple fruit, the study will determine if pineapple peel possess antioxidant and hypolipidemic properties in normal rats model.

The study was aimed to investigate if pineapple peels a potential waste of the pineapple fruit possess antioxidant and hypolipidemic ability in rat model. This will promote harnessing the plant waste material rather than discard it in the environment.

Materials and methods

Plant sampling

Pineapple was bought from Ndioru Market in Ikwuano Local Government Area of Abia State Nigeria, and was identified as *Ananas comosus* by Dr. Garuba Omosun, a taxonomist in the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike.

Plant material preparation

The pineapple fruit was washed with running water from a tap to remove sands and debris on the fruit. The back of the fruit was carefully peeled with a kitchen knife, to obtain the peel. The peel was air-dried for four days under shade and pulverized using an electronic blender. The dried and pulverized peels were extracted by cold maceration method for 72 hours at room temperature using methanol (Sigma-Aldrich) in a winchetser bottle (Sukhdev *et al.,* 2008).

The mixture was filtered with Whatman No. 1 filter paper. The filtrate was concentrated using vacuum rotary evaporator at 40°C to give residue. The extract yield was calculated and the extract stored in a refrigerator at 4°C.

Animals

Twenty male wistar albino rats (95-110g) were obtained from the Veterinary Department of the University of Nigeria, Nsukka, Enugu State and were transported in stainless steel cages to the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State. The animals were allowed two weeks acclimatization period and were fed ad libitum with standard commercial pelleted grower feed (Vital Feed, Nigeria) and free access to clean drinking water. They were maintained in accordance with the recommendation of the Guide for the care and use of laboratory animals (DHHS, 1985). The protocol was approved by the Animal Ethical Committee of the University and was allocated reference number MOUAU/COLNAS/BCM/2015/312.

Experimental Procedures

In *vitro* antioxidant activity was achieved using *2, 2-Diphenyl-1-picrylhydrazyl (DPPH)* photometric assay as described by Mensor *et al*., (2001). The ferric reducing antioxidant power was carried out as described by Benzie and Strain (1993).

Experimental Design

The animals were randomly distributed into four groups (A-D) of five animals each. Group A, served as control and received only distilled water, Group B received 200mg/kg body weight of the extract, Group C received 300mg/kg body weight of the extract and Group D received 500mg/kg body weight of the extract. The animals were dosed daily for 27days. The animals were fasted overnight on the last day and blood obtained through ocular puncture for serum lipid estimation. Blood was spun using a centrifuge at 895 x *g* for 10mins and obtained was transferred into a serum bottle. The animals were further euthanized by cervical dislocation and the liver obtained was homogenized for antioxidant assay.

Liver tissue preparation

Phosphate buffer (9ml) was added to one gram of liver tissue. The mixture was homogenized and centrifuged at 1000 *x g* for 5mins. The supernatant was separated from the pellet and labelled the aliquot. This was used for antioxidant assay.

In vivo antioxidant

Estimation of catalase activity

Catalase activity was determined using the modified method described by Atawodi (2011). Aliquot was added to test-tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of fresh 30mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240nm for 5min on a spectrophotometer. A molar extinction coefficient of 0.041mM^{-1} cm^{-1} was used to calculate catalase activity.

Estimation of SOD activity

Superoxide dimutase activity was determined by the method described by Sun *et al.,* (1988). Xanthine-Xanthine oxidase was used to generate a superoxide flux and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was measured by the degree of inhibition of reaction unit of enzyme providing 50% inhibition of NBT reduction.

Determination of lipid peroxidation

The level of thiobarbituric acid reactive substance (TBARs) and malondialdehyde (MDA) production was determined by the method described by Draper and Hadley (1990). Aliquot was deproteinized by add 1mL of 14% TCA and 1mL of 0.6% TBA.

The mixture was heated in water bath for 30min to complete the reaction and then cooled on ice for 5min. After centrifugation at 2000g for 10mins, the absorbance of the colored product (TBARs) was measured at 535nm with a UV spectrophotometer. The concentration of TBARs was calculated using the

molar extinction coefficient of malonaldehyde (1.56 x 10^5 mol/L/cm).

Lipid profile estimation

Serum HDL estimation

Serum HDL was determined by the method described by Assman *et al.,* (1983).

Serum cholesterol estimation

Serum cholesterol was determined by the method described by Allain *et al.,* (1974).

Serum LDL estimation

Serum LDL was determined by the method described by Assman *et al*., (1983).

Serum triacylglycerol estimation

Serum triacylglycerol was determined by the method described by Tietz (1995).

Serum VLDL estimation

Serum VLDL was determined using the formula, VLDL= total cholesterol – (HDL+LDL).

Statistical analysis

Data obtained were statistically analyzed using one way analysis of variance (SPSS software). The variant mean were separated by least significance difference. Significance was accepted at 95% confidence level. The result was reported as Mean± SD using tables.

Results

Percentage yield = weight of extract x 100
Weight of plant material

42 x 100 = 7%
600

Discussion

Antioxidants which can be natural, man-made or enzymatic play important role in biological systems, they prevent cellular damage by countering effects of free radicals that affect membranes Diplock *et al.,* (1998). Free radicals are constantly occurring in living systems that require oxygen for cellular respiration.

Result for *in vitro* antioxidant showed that pineapple peels exhibited a dose dependent effect. DPPH is a stable free radical with an unpaired valence electron at one atom of Nitrogen Bridge (Eklund *et al.,* 2005) is widely used to determine the antioxidant power of any compound. Result of this study showed that pineapple peel extract had a strong inhibition of DPPH as compared with ascorbic acid which was used as standard agent.

The highest inhibition (40%) occurred at a concentration of 400 *µg*/mL of extract. The DPPH inhibiting property of pineapple peels extract is strongly dependent on the high amount of antioxidants present in the peels. Critical review by Atul *et al.,* (2010) reported high amount of polyphenolic compounds in pineapple peels.

Table 1. *In vitro* antioxidant activity of pineapple peel extract.

DPPH (%)		FRAP (µM		(125 *µg*/mL)
Extract	Ascorbic Acid	Extract	Ascorbic Acid	
25 *µg*/mL	24.89±0.00*	95.52±0.32	0.028±0.00	
50 *µg*/mL	27.86±1.65*	95.67±0.04	0.072±0.01	
100 *µg*/mL	22.51±0.49*	95.74±0.31	0.144±0.02*	2.000±0.00
200 *µg*/mL	30.14±1.04*	95.17±0.27	0.318±0.02	
400 *µg*/mL	40.20±0.23*	94.96±0.23	0.579±0.00	

*Significantly (P<0.05) different when compared to Ascorbic Acid

Result for DPPH and FRAP showed a dose dependent activity.

FRAP which is the ferric reducing antioxidant power is another important tool for assessing the antioxidant ability of a compound in laboratories. This is achieved by the reduction of ferric to ferrous ion (Iris and Strain, 1996). Highest FRAP activity was also achieved at 400 *µg*/mL of extract. FRAP activity of the extract was also shown to be dose dependent. Studies by Guo *et al.,* (2003) also showed that

pineapple peels had FRAP activity.

Result for in vivo antioxidant assay (MDA, SOD and Catalase) showed no significant *(P<0.05)* difference in the test and control group. This indicated that extract of pineapple peels did not induce oxidative stress and can be useful in the fight against free radicals. During oxidative stress, level of enzymatic antioxidant are low in tissue and serum.

SOD is an antioxidant enzyme that catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. Superoxide is derived from metabolism requiring oxygen and it is widely known to react with nitric oxide forming reactive peroxynitrite (Maritim *et al.,* 2003). SOD concentration in the test group revealed no significant *(P<0.05)* difference when compared to control at the different doses administered, although SOD activity was high. The increased SOD activity in this study indicates that the extract possess antioxidant activity in biological system and can be useful in defense against ROS. Dietary source of polyphenol possess scavenging ability for superoxide anion in biological system (Emmanuel *et al.,* 2015). This was seen in the study as reflected by SOD activity which was kept at its maximum since superoxide anion might not be present in the cell or be reduced by polyphenol from the peel extract.

Table 2. *In vivo* antioxidant activity of pineapple peel extract.

	MDA (nMole/g protein)	SOD (Unit/g protein)	CATALASE (µMole/g protein)
Control	5892.01±227.8	9.93±2.85	7537.07±291.4
200mg/kg	7883.96±2928.7	12.90±2.41	10085.16±3746.4
300mg/kg	8967.38±4484.4	13.55±4.48	11471.08±5736.5
500mg/kg	6664.56±2580.2	11.61±4.44	8525.30±3300.6

Result for in *vivo* antioxidant showed no significant *(P<0.05)* difference when the test group was compared with the control group. Also, comparison with the test group showed no significant *(P<0.05)* difference.

However, hydrogen peroxide a breakdown product of superoxide is known to be a good agent of oxidative stress acting as an oxidizing agent since it is uncharged and a freely diffusible reactive oxygen species (Milton, 2004). Studies by Wijeratne (2005) reported that increase in hydrogen peroxide concentration can lead to cell membrane leakage and DNA damage. Catalase which is also an antioxidant enzyme catalyzes the degradation of hydrogen peroxide to water and oxygen (Chelikani *et al.,* 2004) thereby protecting cells and biological system from oxidative stress induced by hydrogen peroxide. Result of this study also showed no significant *(P<0.05)* difference in the test groups when compared with the control group and it correlates that the extract could be useful in fight against ROS. During oxidative stress, an increase in SOD activity leads to production of hydrogen peroxide in high concentration, which is further handled by catalase, requiring that both enzymes must work hand in hand to achieve their desired result (Kaushik and Aryadeep, 2014). However, apart from hydrogen peroxide generated from reaction catalyzed by SOD, hydrogen peroxide can also be introduced into biological system through the peroxisomal pathway (Halliwell and Gutteridge, 1999), diet and from the environment.

ROS reacts strongly with lipids which is a strong component of cell membrane and if affected adversely can lead to lipid peroxidation (LPO) and cell death (Bakirel *et al.,* 2008). Lipid peroxidation induces a cascade of reactions that empowers oxidative stress creating lipid radicals that damages proteins and DNA. Generally, LPO affects membrane fluidity, damages membrane proteins and deactivating membrane receptors (Arulselvan and Subramanian, 2007). Malondialdehyde (MDA) serve as a biomarker for LPO. Increase in MDA concentration indicates increase in LPO which means an increase in ROS generation and oxidative stress. In our study, MDA

concentration showed no significant *(P<0.05)* difference in the test groups when compared with the control group, indicating that the extract has potentials to inhibit LPO and oxidative stress.

Result for in vivo antioxidants assay of the extract showed that they don't increase ROS generation or concentration and also lipid peroxidation. This indicates that pineapple peels might have little toxic potential and justifies it use in improving antioxidant defense system. Generally, the antioxidant ability of pineapple peels can be attributed to the high phenolic, flavonoid and vitamin C content of the peels.

Table 3. Concentration of serum lipids in test and control animals.

	CHO(mg/dL)	TAG(mg/dL)	HDL(mg/dL)	VLDL(mg/dL)	LDL(mg/dL)
Control	43.72±11.16	74.13±38.39	9.39±1.46	14.82±7.67	19.50±4.80
200mg/kg	47.80±10.33	50.18±16.49	13.67±3.13*	10.03±3.29	24.09±8.38
300mg/kg	47.03±18.96	49.04±22.14	12.76±2.87	9.80±4.42	24.45±18.76
500mg/kg	40.81±2.74	46.57±19.54	13.37±2.02*	9.31±3.90	18.14±2.86

* Higher than control group at 95% confidence level.

Result for HDL was significantly *(P<0.05)* higher in the 200mg/kg and 500mg/kg group when compared to the control group.

Serum lipid profile assay result showed that the extract significantly *(P<0.05)* increased HDL in the test groups (200mg/kg and 500mg/kg) when compared to the control group. This indicates that the extract might have potential in lowering risk to lipid associated diseases. Increased concentration of HDL has been shown to reduce exposure risk to risk to atherosclerosis, high blood pressure, obesity, diabetes, stroke, cardiovascular diseases and other related diseases. HDL achieve its goal by transporting fat molecules (cholesterol) out from artery walls (Gerd *et al.,* 2004). The increase in HDL in this study can be attributed to the soluble fiber present in pineapple peels. Studies by Hemalatha and Anbuselvi (Hemalatha and Anbuselvi, 2013) reported high amount of fiber in pineapple peels. Hermansen *et al.,* (2003) reported that HDL fraction can be increased by supplementation of fiber in diet.

Result indicated no significant *(P<0.05)* difference in LDL, cholesterol, VLDL and triglycerides concentration in the test groups when compared with the control group. LDL are known to transport fat to be circulated round the body. Cholesterol is synthesized in the liver and serve as precursor for many biologic compounds (Hanukoglu, 1992). From result of our study, the normal level of LDL and increase in HDL reflected in the concentration of cholesterol. Excessive level of cholesterol leads to fatty deposition in blood vessels a condition called plague. Increase in triglycerides level can also lead to atherosclerosis and other associated ailments and it increase is also associated with low HDL and high LDL. VLDL also transport triglycerides, and high VLDL has been associated with plague, blocking of artery impairing blood flow.

The hypolipidemic property of pineapple peels can be attributed to the rich phytochemicals present in the peels such as bromelain, saponins, tannis and alkaloids in high concentration. Studies by Jasmine and Estherlydia (2014) reported high amount of phytochemicals in pineapple peels. The role played by saponins in combating hyperlipidemia has been well reported by Ijeh and Ejike (2011) and pineapple peels propose a cheap source of saponins.

In conclusion, pineapple peels are discarded as waste with a lot of underlying potentials that can be used in medicine. From the antioxidant and hypolipidaemic activity of the peels, it can be concluded that the peels may have potentials to manage and prevent oxidative stress and also hyperlipidaemia.

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Conflict of interest

Authors have no conflict of interest regarding this study.

References

Allian C, Lucy S, Cicely S, Chan W, Paul C. 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 470- 475.

Arulselvan P, Subramanian S. 2007. Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra-structural changes of pancreatic beta cells in experimental diabetes in rats. Chemico- Biological Interactions **165(2),** 155- 164.

Assmann G, Schriewer H, Schmitz G, Hadge E. 1983. Quantification of HDL-C by precipitation with phosphotungstic acid and $MgCl_2$. Clinical Chemistry. **29,** 2026- 2030.

Atawodi S. 2011. Evaluation of the hypoglycaemic, hypolipidemic and antioxidant effects of methanolic extract of "Ata-Ofa" polyherbal tea (A- polyherbal) in alloxan- induced diabetic rats. Drug Invention Today **3,** 270- 276.

Atul U, Jeewan P, Shinkichi T. 2010. Utilization of pineapple waste: a review. Journal of Food Science and Technology **6,** 10- 18.

Bakirel T, Bakirel U, Keles O, Ulgen S, Yardibi H. 2008. *In- vivo* assessment of antidiabetic and antioxidant activities of Rosemary (*Rosmarinus officinalis*) in alloxan- diabetic rabbits. Journal of Ethnopharmacology **116(1),** 39- 42.

Bardiya N, Somayaji D, Khanna S. 1996. Biomethanation of banana peel and pineapple waste. Bioresource Technology **58,** 73-76.

Bartholomew D, Paull R, Rohrbach K. 2003. The Pineapple: Botany, Production and Uses. CABI Publishing, Wallingford, UK. 21.

Benzie F, Strain J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology **299,** 15-23.

Chelikani P, Fita I, Loewen P. 2004. Diversity of structures and properties among catalase. Cellular and Molecular Life Sciences **61(2),** 192- 208.

Chisolm G. 2001. The oxidative modification hypothesis of atherogenesis: an overview. Free Radical Biol. Med. **28,** 1815–1826.

Dacera D, Babel S, Parkpian P. 2009. Potential for land application of contaminated sewage sludge treated with fermentaed liquid from pineapple wastes. Journal of Hazardous Materials. **167,** 866- 872.

DHHS. 1985. Guide for the care and use of laboratory animals, institute of laboratory animal resources commission on life sciences, National Research Council. Washington DC, National academy press.

Diplock A, Charleux J, Crozier-Willi G. 1998. Functional food science and defense against reactive oxidative. British Journal of Nutrition. **80(1),** 77- 112.

Draper H, Hadley M. 1990. Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology **186,** 421- 431.

Eklund P, Langvik O, Warna J, Salmi T, Willfor S. 2005. Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans. Organic and Bimolecular Chemistry **21,** 3336–3347.

Emmanuel UE, Ebhohon OS, Omeh YN. 2015. Effect of fermented and unfermented cocoa bean on some liver enzymes, creatinine and antioxidant in wistar albino rats. Carpathian Journal of Food Science and Technology **7(4),** 132-138.

Gerd A, Antonio M, Gotto J. 2004. HDL cholesterol and protective factors in atherosclerosis. Circulation. **109,** 8- 14.

Guo C, Yang J, Wei J, Li Y, Xu J. 2003. Antioxidant activities of peel, pulp, and seed fractions of common fruits as determined by FRAP assay. Nutrition Research **23,** 1719-1726.

Halliwell B, Gutteridge J. 1999. Free radicals in biology and medicine, Oxford University press UK.

Hanukoglu I. 1992. Steroidogenic enzymes: structure, function and role in regulation of steroid hormone biosynthesis. Journal of Steroid Biochem. Mol. Biol. **102(2),** 306- 310.

Hemalatha R, Anbuselvi S. 2013. Physicochemical constituent of pineapple pulp and waste. Journal of Chemical and Pharmaceutical Research **5(2),** 240- 242.

Hermansen K, Dinesen B, Hoie L, Morgenstern E, Gruenwald J. 2003. Effects of soy and other natural products on LDL: HDL ratio and other lipid parameters: A literature review. Advances in Therapy **20(1),** 50- 78.

Hossain A, Rahman S. 2010. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Research International, 1–7.

Ijeh I, Ejike C. 2011. Current perspectives on the medicinal potentials of Vernonia amygdalina Del. Journal of Plant Medicinal Research **5(7),** 1051- 1061.

Iris F, Strain J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Analytical Biochemistry **239,** 70–76.

Jasmine P, Estherlydia D. 2014. Comparative study of phytochemical screening and antioxidant capacities of vinegar made from peel and fruit of pineapple (*Ananas comosus L.*). International Journal of Pharma and Bio Sciences **5(4),** 394- 403.

Kaushik D, Aryadeep R. 2014. Reactive oxygen species (ROS) and response of antioxidnats as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science **2,** 53.

Maritim A, Sanders R, Watkins J. 2003. Diabetes, oxidative stress and antioxidants: A review. Journal of Biochem. and Mol. Toxicology **17(1),** 24-38.

Mensor L, Fabio S, Gilda G, Alexandre S, Tereza C. 2001. Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. Phytother. Res. **15,** 127-130.

Milton N. 2004. Role of hydrogen peroxide in the aetiology of Alzheimer's disease: implications for treatment. Drugs Aging **21(2),** 81- 100.

Ochuko E, John A, Rachael A, Oluwatoyin O, Sunday A. 2011. Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. Asian Pacific Journal of Tropical Disease **11,** 2-9.

Prasenjit D, Prasanta D, Abhijit C, Tejendra B. 2012. A survey on pineapple and its medicinal value. Scholars Academic Journal of Pharmacy **1(1),** 24-29.

Rui-Li Y, Yong-Hui S, Gang H, Wu L, Guo-Wei L. 2008. Increasing oxidative stress with progressive hyperlipidemia in human: relation between malondialdehyde and atherogenic index. Journal of Clinical Biochemistry and Nutrition **48(3),** 154-158.

Sukhdev S, Suman P, Gennaro L, Dev R. 2008. Extraction techniques for medicinal and aromatic plants. International center for science and high

technology. http://dx.doi.org/10.1080/10942912.2012.732168

Sun Y, Oberley W, Li Y. 1988. A simple method for clinical assay of superoxide dismutase. Clinical Chemistry **34(3),** 497- 500.

Tietz N. 1995. Clinical guide to laboratory tests, 3rd ed, W B Sanders Company Philadelphia U.S.A, 578-580.

Ti L, Peiyi S, Wei L, Chengmei L, Ruihong L, Na Y, Jun C. 2014. Major polyphenolics in pineapple peels and their antioxidant interactions. International Journal of Food Properties **17(8),** 1805-1817.

Wijeratne S. 2005. Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco- 2 human colon cells. Journal of Agricultural and Food Chemistry **53(22),** 8768-8774.