RESEARCH PAPER | Zoology | Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

Antioxidant and Anticholesterol Activities of *Morinda Citrifolia* L. Linn in Rats

KEYWORDS

Dr. Shailesh Tawade
Department of Zoology, Patkar College, Goregaon (west) Mumbai -400 062, India

Dr. Rohini Sivabalan
Department of Zoology, Ramnarain Ruia College, Matunga, Mumbai -400 019. India

ABSTRACT *Plants are potential sources of natural antioxidants. Herbal and natural products have been used for centuries, throughout the world, in every culture. Natural antioxidants are widely used because they are safe and cause less adverse reactions. Morinda citrifolia L. commonly known as Noni from the family Rubiaceae is a plant typically found in Hawaii, Tahiti, tropical Asia, and southern Taiwan. It can be used as a raw material for nutraceutical and functional food products. Various parts of the plant, including the roots, stems, leaves and fruit have been consumed solely on the basis of the assumption that it possesses healing properties. However, it has never been scientifically proven that Morinda citrifolia L has the ability to cure these illnesses. Many In vitro and In vivo antioxidant studies of Morinda citrifolia L leaf showed that it possess antioxidant property. In vitro antioxidant study of fruit extract showed very low antioxidant potential. In the present study we have evaluated anti oxidant and antihyperlipidemic activity of Morinda citrifolia L fruit extract in rat. For antioxidant study fruit extract was administered at the dose of 500 mg/kg and 1000 mg/kg by oral route in rats for 8 weeks. Result of this study showed that there is non significant increased in SOD in male rats whereas significant increased in SOD value in female rats treated with 1000 mg/kg of fruit extract as compared to control. There is significant decreased in lipid peroxidation activity in both male and female groups treated with 500 mg/kg and 1000 mg/kg of fruit extract as compared to control. Significant increased in SOD and reduction in lipid peroxidation suggests that extract has antioxidant property. In the antihyperlipidemic study Morinda citrifolia L fruit extract was administered in triton WR – 1339 induced hyperlipidemic rats at the dose of 1000 mg/kg at 24 hrs treatment and lipid profile results were compared with hyperlipidemic rats treated with standerd drug Atorvastatin. Hyperlipidemic rats treated with fruit extract did not show any lipid lowering effect. Cholesterol, triglycerides and LDL levels are significantly more than positive control group. Results of above experiment shows that Morinda citrifolia L fruit extract has strong antioxidant property whereas anticholesterol experiment did not contribute substantially to explicate the favorable outcome of fruit extract on positive control Since herbal medicines are needed to be used in higher doses and for relatively longer periods for permanent effects, hence further study is to be carried out on prolonged exposure of fruit extract to evaluate its beneficial effect against hyperlipidemia and hypercholesterolemia.*

INTRODUCTION

Hyperlipidemia and Hyper-cholesterolemia

Both diabetes and obesity are emerging as leading health problems in India. [1]Hyperlipidemia and Hyper-cholesterolemia are not only secondary metabolic disregulations associated with diabetes but also represent increased risk factors for development of diabetes. [2, 3, 4] Besides the cause effect relationship with diabetes, elevated serum levels of triglycerides, cholesterol and low density lipoproteins are major risk factors in the premature development of cardiovascular diseases like atherosclerosis, hypertension, coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease [5, 6, 7] Since these risk factors play an important role in determining atherogenesis and the subsequent pace of atherosclerosis. Evidence from clinicopathological and epidemiological studies overwhelmingly confirms that hyperlipidemia is the primary prerequisite for atherosclerosis manifested in premature cardiovascular disability and death. Hyperlipidemia is caused by a diet high in fat, especially saturated fat and cholesterol. The International Atherosclerosis Project, found that the degree of atherosclerosis was directly proportional to the prevalence of CHD and stroke, and that lipid levels were directly related to plaque damage. The higher the serum cholesterol level the greater the plaques buildup. [8]

Reduction in serum cholesterol levels reduces the risk for CHD. [9] With the etiological preeminence of hyperlipidemia in CHD, various drugs have been utilized to lower the blood lipids, such as clofibrate, niacin, cholestyramine, statin and gemfibrozil. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [10]

Although these drugs were successful in reducing serum cholesterol levels, they produced unpleasant and distressing side effects. [8] The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. [11]

Oxidative stress

Different epidemiological studies have shown that the oxidative stress is one of the major contributors of the development of hyperlipidemia and atherosclerosis. Oxidation is one of the destructive processes, wherein it breaks down and damages various molecules. Molecular oxygen is an essential component for all living organisms, but all aerobic species suffer from injury if exposed to concentration more than 21 %.[13)

Free radicals are chemical species that have a single unpaired electron in an outer orbit. They attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA. [13, 14] Lipids are one of the most susceptible targets of free radicals. [14]

Antioxidants- Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and thereby eliminate the threat of pathological processes. [12] They block the initiation of free radicals and terminate radical damage and inhibit lipid peroxidation which was the factors of atherosclerosis. The body possesses several defense systems comprising enzymes and radical scavengers. [15]

Nowadays, natural medicine is constantly expanding, which has various kinds of herbal medicine. Plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metab-

Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

olites of photosynthesis. Natural antioxidants are widely used because they are safe and cause less adverse reactions [16, 17].

Morinda citrifolia L. (Noni)- Morinda citrifolia L. commonly known as Noni from the family Rubiaceae, is a plant typically found in Hawaii, Tahiti, tropical Asia, and southern Taiwan. Dried fruits or leaves powder boiled with water produces a tea, which is used to relief blood pressure, muscle pains and vomiting. It can be used as a raw material for nutraceutical and functional food products. Recently 'Noni' juice extract has been commercially processed and distributed internationally as a dietary supplement. Relatively unheard of 5 years ago, M. citrifolia has now exploded in to nearly a billion-dollar industry. [18] It has been reported to have a broad range of therapeutic and nutitional value. There are more than 120 nutraceutical compounds identified in Noni. [19] Noni juice extract which is obtained from fermented Noni fruits is the most effective product that has helped relieved people (n ≥ 10,000) from the suffering of about 22 conditions, such as arthritis, heart disease, diabetes, headache and muscle pain, high blood pressure, cancer, etc. [19]

Morinda citrifolia L. leaves may possess anti-oxidant activity against hyperlipidemia and atherosclerosis. Flavonol glycosides from the Noni leaf have a scavenging activity for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals [16]. In addition, the work of Zin et al. [20] demonstrated that a methanol extract from the Noni root and ethyl acetate extracts from the Noni leaf, fruit, or root exhibit significant antioxidant activity as determined by the ferric thiocyanate method or thiobarbituric acid test. [21] Subsequently, Zin et al. [22] reported that crude extracts of the Noni root, leaf, and fruit fractionated on a Sephadex LH-20 column with an ethanol eluate exhibit high antioxidant activity [23] Whereas work of Chin-hui chen et al. demonstrated that Noni fruit extracts, regardless of the near SF-CO2 extraction method consistently showed very poor antioxidant activity when comparing to the stem and leaf extracts. [24]

Objectives of the study

As far as our literature survey could ascertain, there are lot of in vitro and in vivo studies were carried out on Morinda citrifolia L. leaf which suggest strong antioxidant antihyperlipidemic potential of leaf extract whereas very little is known about fruit extract. There was no study report available on In vivo antioxidant and anticholesterol activity of Morinda citrifolia L. fruit extract in rat. Hence we decide to investigate the antioxidative property of Morinda citrifolia L. fruit extract In vivo, in order to ascertain the mode of the pharmacological action of the plant. Furthermore, another study was also carried out to evaluate anticholesterol activity of Morinda citrifolia L. fruit extract in triton induced hyperlipidemic rats.

MATERIALS AND METHODS

The Morinda citrifolia raw fruit extract was obtained from Brihans Pharmaceuticals Pvt. Ltd. Mulund, Mumbai, India.

ANIMAL PROCUREMENT AND MANAGEMENT- Albino male Wistar rats weighing between 200 to 250 g were procured from Haffkine Biopharmaceuticals limited, (Haffkine BPCL), CPCSEA Registered breeder & Supplier No: 200/2000/CPCSEA, Parel, Mumbai. All the animals were weighed and their health was verified. Animals were allowed acclimatization period of 7 days to the laboratory conditions prior to initiation of study. Animals were assigned to cages and groups, two or three per cage, sex-wise and the individual animal was fur marked. The females were nulliparous and non pregnant.

The rats were housed gender-wise; two or three per cage in polypropylene cages with stainless steel grill tops facilities for food and water bottle, and bedding of clean paddy husk which was changed everyday. A standard 'Amrut' brand Pelleted rat feed was provided to the test animals manufactured by M/s. Pranav Agro Industries Ltd., Sangli. Water was provided ad-libitum in glass amber coloured bottles.

The temperature was maintained at 24°C± 1°C and relative humidity between 50 to 70%; 12 hours each of dark and light cycle was maintained. Groups of animal at each dose level were identified by cage number and were individually colour coded, stained with diluted picric acid.

DOSE PREPARATION- Stock solution of different concentration of Morinda citrifolia L. plant raw fruit extract was freshly prepared as mg/ml, on the day of dosing by dissolving in the vehicle sample according to doses to be administered, the volume to be administered, either of the extract or vehicle was estimated to attain 1 ml/100g body weight.

Antioxidant studies- To investigate the effects of daily intake of medicinal plants on antioxidant enzymes, 36 rats 18 male and 18 female were randomly divided into three groups 6 animals of each gender in each group. The test solutions were administered to rats at the dose levels of 500 mg /kg (Group I) and 1000 mg / kg (Group II) for 8 weeks. The animals in the Control group (Group III) received the vehicle alone in a similar way. The dilutions were made in such concentrations to allow the administration of each dose in a constant volume of 1 ml / 100g bodyweight. The animals were administered orally by orogastric catheter once daily for 56 days. On 56th day, the animals were fasted for overnight and blood samples were collected through cardiac puncture from all animals used in the study. The blood was centrifuged at 1500 rpm for 5 min; and serum was separated for enzymes assay.

Estimation of Lipid peroxidation (Malondialdehyde (MDA) and Superoxide dismutase (SOD) was carried out by Malondialdehyde formation was estimated by the method of, Dr. M. M. Misro, Ms. Rekha, Ms. Ramya and Mr. Ibrahim.

(NOTE – Protein estimation was carried out by Bradford method)

Anticholesterol activity- Experiments were conducted only at the preliminary level for only one day. To investigate the anticholesrol effects of Morinda citrifolia L.fruit extract, 24 male rats were randomly divided into four groups 6 animals in each group. Triton WR 1339 (Tyloxapol, Sigma – Aldrich, USA) was administered intraperitoneally to the rats at the dose of 200 mg/kg B.W in order to develop oxidative stress in animals.

Experimental design: The groups were as follows.

Group I – Normal control group which Received normal diet.

Group II –Triton control –Triton WR 1339 200mg/kg induced hyperlipidemic rats at the dose of 200 mg/kg via intraperitoneal route.

Group III – In this group Triton WR 1339 200mg/kg induced hyperlipidemic rats treated with Morinda citrifolia L.fruit extract at the dose of 1000 mg/kg by oral route to evaluate anticholesterol activity

Group IV – positive control group. In this group Triton WR 1339 200mg/kg, induced hyperlipidemic rat treated with standard lipid lowering drug Atorvastatin at the dose of 30µg/kg. After 24 hours all the rats from four groups were killed by cervical dislocation and blood was collected by cardiac puncture. The blood was centrifuged at 1500 rpm for 5 min; and serum was separated for lipid profile. Serum was used for measuring the total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride level.

The total plasma cholesterol, triglyceride, High density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol were measured according to the instruction manual accompanying the diagnostic kits from AGAPPE diagnostic kits (AGAPPE diagnostic Ltd.) Total cholesterol was estimated by CHOD-PAP methodology, Triglycerides by GPO-PAP methodology, and HDL by the precipitation method using phosphotungstate magnesium acetate reagent.

Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

Calculations

$$VLDL = \frac{\text{Triglycerides}}{5}$$

$$LDL = \text{total cholesterol} - HDL - \frac{\text{Triglycerides}}{5}$$

RESULTS AND DISCUSSION

Plants are generally believed to be rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins. Of these metabolites, plant antioxidants such as the numerous phenolic compounds have received increased attention as useful nutraceutical in management of diseases

In the present antioxidant study fruit extract of Morinda citrifolia L. fruit extract was administered at the dose of 500 mg/kg and 1000 mg/kg by oral route in rats for 8 weeks. Result of this study showed that there was non significant increased in SOD in male rats whereas significant increased in SOD value in female rats treated with 1000 mg/kg of Morinda citrifolia L. fruit extract as compared to control. There was significant decreased in lipid peroxidation activity in both male and female groups treated with 500 mg/kg and 1000 mg/kg of Morinda citrifolia L. fruit extract as compared to control.

Treatment with test drug reduces the level of lipid peroxides indicating the effective anti-oxidant property of the test drug. Test drug extract produced higher but non significant levels of the superoxide dismutase for both doses in male and significant levels in females treated with 1000 mg/kg when compared with control. This may be due to decrease in the conversion of super oxide anions to hydrogen peroxide (H_2O_2) which is caused due to the catalysis produced by the SOD, Which is an enzyme prevents the further generation of free radical [40] hence it was assumed that extract has significant antioxidant property.

In pertinent study, tritonised animals have been used to test the antitriglyceridemic and anticholesterolemic efficacy of Morinda citrifolia L.fruit extract as, such a model has been used for the induction of acute hyperlipidemia [46] as well as for testing the potential of natural/chemical hypolipidemic drugs. [47, 48, 49, 50, 51, 52, 53] triton induced hyperlipidemic rats were treated with Morinda citrifolia L.fruit extract at the dose of 1000 mg/kg by oral route and the result were compared with positive control tritonised rats treated with standard drug Atorvastatin. Significant elevation is seen in the increased levels of plasma total cholesterol, triglyceride, and LDL in Triton WR 1339 induced hyperlipidemic rats within 24 hrs treatment. In hyperlipidemic Atrovastatin treated rats significant decrease was seen in the levels of plasma total cholesterol at 24 hour treatment, also the levels of triglyceride show significant decline in this group. Whereas hyperlipidemic rats treated with Morinda citrifolia L.fruit extract did not show any lipid lowering effect. Increased levels of cholesterol, triglycerides and LDL due to tritonisation did not show any declination in this group cholesterol, triglycerides and LDL levels are significantly more than positive control group. VLDL levels were also as compared to positive control group.

Findings of such research work did not contribute substantially to explicate the favorable outcome of Morinda citrifolia L.extract on positive control Since Ayurvedic/herbal medicines are needed to be used in higher doses and for relatively longer periods for permanent effects, hence further study is to be carried out on prolonged exposure of Morinda citrifolia L.fruit extract to evaluate its beneficial effect against hyperlipidemia and hypercholesterolemia.

We have observed that the reduction in lipid peroxidation levels and increase in SOD level. Significant differences in above stated anti-oxidant parameters indicating that this herb may possesses antioxidant activity and contain products that lowers the oxidative stress and might be beneficial in treatment of various oxidative stress- related diseases. Results of anticholesterol activity study of Morinda citrifolia L.fruit extract did not contribute substantially to explicate the favorable outcome on positive control may be due to short duration of extract consumption (24 hrs) for its beneficial effects on the body to become clinically manifest. Further study is to be carried out on prolonged exposure of Morinda citrifolia L.fruit extract to evaluate its beneficial effect against hyperlipidemia and hypercholesterolemia. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for its clinical use.

TABLES AND GRAPHS

Antioxidant activity of Morinda citrifolia L. fruit extract

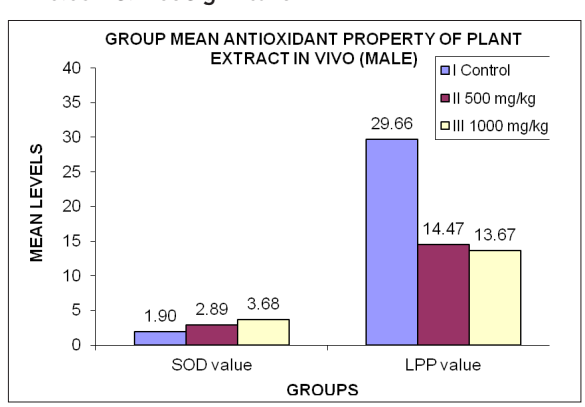
Sex – male

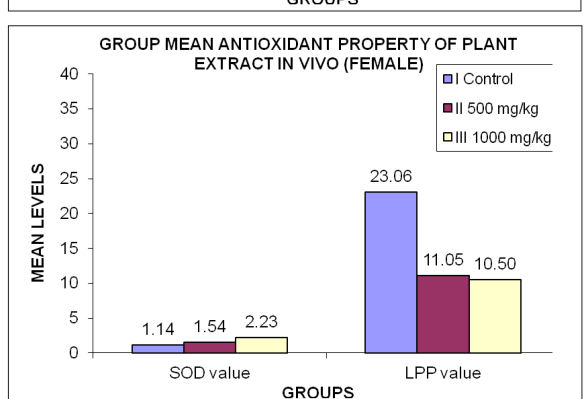
Dose group	SOD activity units/ml (mean ± sd)	LPP activity (mean ± sd)
Control	1.90 ±0.51	29.66 ± 4.11
500 mg/kg	2.89 ±1.34	14.47 ± 5.52
1000 mg/kg	3.68 ± 3.11	13.67 ± 4.16

Sex – female

Dose group	SOD activity units/ml (mean ± sd)	LPP activity (mean ± sd)
Control	1.14 ± 0.36	23.06 ± 3.30
500 mg/kg	1.54 ± 0.47	11.05 ± 1.33
1000 mg/kg	2.23 ± 0.32	10.5 ± 0.86

By Student t Test *P < 0.05 Significant
P > 0.05 NS: Not Significant





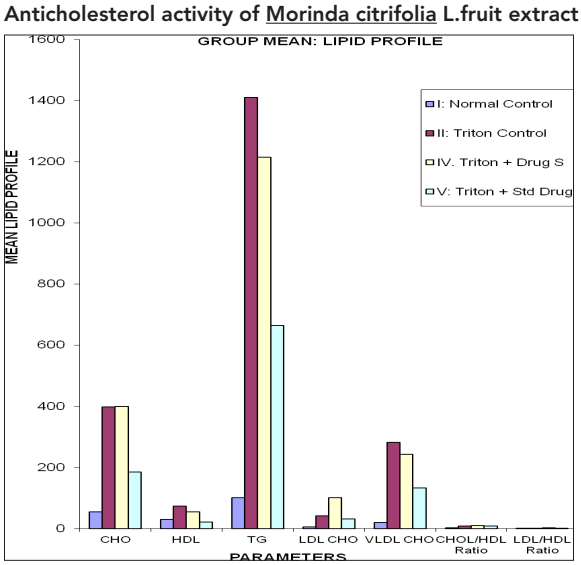
Volume : 4 | Issue : 7 | July 2014 | ISSN - 2249-555X

Anticholesterol activity of Morinda citrifolia L.fruit extract

GROUP	CHO (mg/dl) MEAN ± SD	HDL (mg/dl) MEAN ± SD	TG (mg/dl) MEAN ± SD	LDL CHO MEAN ± SD	VLDL CHO MEAN ± SD	CHOL/HDL RATIO MEAN ± SD	LDL/HDL RATIO MEAN ± SD
I:Normal Control	55.17 ±9.02	29.35 ±4.82	101.00 ±12.10	5.62 ±7.04	20.20 ±2.42	1.88 ±0.17	0.19 ±0.22
II:Triton Control	398.67 ±102.56	74.07 ±65.88	1411.17 ±213.21	42.37 ±99.55	282.23 ± 42.64	8.80 ± 5.58	1.54 ±2.31
III :Triton + Drug S	399.17 ±143.54	54.72 ±44.07	1215.33 ±203.97	101.38 ± 90.78	243.07 ± 40.79	9.28 ± 3.91	1.97 ± 1.73
V :Triton + Std Drug	185.17 ±41.11	21.10 ±2.97	665.33 ±158.92	31.00 ±22.74	133.07 ± 31.78	8.89 ± 2.07	1.57 ±1.31
Comparison I Vs V	* P<0.05	* P<0.05	* P<0.05	* P<0.05	* P< 0.05	* P< 0.05	* P< 0.05
Comparison II Vs V	* P<0.05	* P<0.05	* P<0.05	P> 0.05 NS	* P< 0.05	P> 0.05 NS	P> 0.05 NS
Comparison III Vs V	* P<0.05	P> 0.05 NS	* P< 0.05	* P< 0.05	P> 0.05 NS	P> 0.05 NS	P> 0.05 NS

By Student t Test ***P < 0.05 Significant** **P > 0.05 NS: Not Significant**

Anticholesterol activity of Morinda citrifolia L.fruit extract



REFERENCE

1. Ramachandran A. Diabetes and Obesity - the Indian angle. Indian J Med Res 2004; 120:437-9. | 2. Still WJ, Martin JM, Gregor WH. The effect of alloxan diabetes on experimental atherosclerosis in the rat. Exp Mol Pathol 1964; 3:141-7. | 3. Miller RA, Wilson RB. Atherosclerosis and Myocardial ischemic lesions in alloxan diabetic rabbits fed a low cholesterol diet. Arteriosclerosis 1984; 4:586-91. | 4. Chattopadhyay RR, Bandhopadhyay M. Effect of Azadirecta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African j Biomed Res 2005;8:101-4 | 5. Hardman, J.G. and L.E. Limbird, 2001. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th Edn., McGraw-Hill Publishers, USA | 6. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, et al . Implications of recent clinical trials for the national cholesterol education programme adult treatment panel III guidelines. Circulation 2004; 110:227-39. | 7. Massing MW, Sueta CA, Chowdhury M, Biggs DP, Simpson RJ Jr. Lipid management among coronary heart disease patients in diabetes mellitus or advanced age. Am J Cardiol 2001; 87:646-9. | 8. Temple, N.J. & Burkitt, D.P. 1994. Western Diseases: Their Dietary Prevention and Reversibility. Totowa, New Jersey: Humana Press 170-172. | 9. Jain, K.S., M.K. Kathiravan, R.S. Somani and C.J. Shishoo, 2007. The biology and chemistry of hyperlipidemia. Bio. Med. Chem., 15: 4674-4699. | 10. Davey Smith, G. and J. Pekkanen, 1992. Should there be a moratorium on the use of cholesterol lowering drugs? Br. Med. J., 304: 431-740. | 11. Kumar, A.S., A. Mazumder and V.S. Saravanan, 2008. Antihyperlipidemic activity of camellia sinensis leaves in triton wr-1339 induced albino rats. Phcog. Mag., 4: 60-64. | 12. Nasik SR. Antioxidants and their role in biological functions: An overview. Indian Drugs. 2003; 40:501–15. | 13. Shetgiri PP, D'Mello PM. Antioxidant activity of flavanoids-A comparitive study. Indian Drugs. 2003; 40:567–9. | 14. Gopinathan N, Srinivasan KK, Mathew JE. Free radical scavenging properties of ethanol extract of Saccharum spontaneum. Indian Drugs. 2004; 41:633–5. | 15. G.P. Rajani and Purnima Ashok, In vitro antioxidant and antihyperlipidemic activities of Bauhinia variegata Linn Indian J Pharmacol. 2009 October; 41(5): 227–232. | 16. Boik J. 1996. Cancer and Natural Medicine. USA: Oregon Medical Press. 150-156. | 17. Kumar V., Abbas A.K. & Fausto N. 2005. Robbins and Cotran Pathologic Basis of Disease, 7th ed. Philadelphia: Elsevier Saunders 16-18 | 18. Nandhasri, P., et al., Nutraceutical properties of Thai "Yor", Morinda citrifolia L.and "Noni" juice extract Songklanakarin J. Sci. Technol. Vol. 27 (Suppl. 2), 2005 : Thai Herbs | 19. Solomon, N. 1999. The Noni Phenomenon. Direct Source Publishing. USA, 43-47. | 20. Yang, J. H., Lin, H. C. and Mau, J. L. 2002. Antioxidant properties of several commercial mushrooms. Food Chem. 77: 229-235. | 21. Zin, Z. M., Hamid, A. A. and Osman, A. 2002. Antioxidative activity of extracts from Mengkudu (Morinda citrifolia L.L.) root, fruit and leaf. Food Chem. 78:227-231. | 22. Chung, Y. L., Wang, J. S. and Chen, S. Y. 2004. Comparison of antioxidative activity of crude extracts from leaves, stems and fruits of Morinda citrifolia L.(Noni). pp. 76-77. Master Thesis, Chia-Nan University of Pharmacy and Science. Tainan, Taiwan | 23. Zin, Z. M., Hamid, A. A., Osman, A. and Saari, N. 2006. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (Morinda citrifolia L.). Food Chem. 94: 169-178. | 24. Chin-Hui Chen, et al. determination of antioxidative properties of Morinda citrifolia L. using near supercritical fluid extraction, Journal of Food and Drug Analysis, Vol. 17, No. 5, 2009, Pages 333-341 |