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Pharmacological Research on Noni *(Morinda citrifolia* L.*)*- A Review

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Key words : *Morinda citrifolia*, pharmacology, analgesic effect, anti-inflammatory activity, antioxidant activity, anticancer activity

Abstract : *Morinda citrifolia* L., commonly called as Noni has a long history as a medicinal plant. It is reported to have a wide range of pharmacological effects such as antinflammatory, antihypertensive, anticancer, immune, antihelmintic, antioxidant, antituberculous, antiprotozoal, antibacterial, antiviral, antifungal, antianxieity and hypnotic effects. These findings from experimental pharmacological studies are of great value in exploring the therapeutic potential and clinical effects of Noni in treating various diseases. There is no complete review available on these various pharmacological actions of Noni and hence an attempt was made for a comprehensive compilation of the same. Since Noni has a wide range of therapeutic benefits and in recent times it is recommended for treatment of a variety of diseases and the safety of Noni is also reviewed in conjucation. This review is aimed at providing necessary information on the profile of pharmacological effects of Noni already explored and hence further pharmacological research or improvements in the area can very well be undertaken.

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Introduction

Polynesians utilized the whole Noni plant in various combinations for herbal remedies. The fruit juice is in high demand in alternative medicine for different kinds of illnesses (Wang et. al ., 2002) like arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction. Scientific evidence on the benefits of Noni fruit Juice is limited. This review is aimed at providing necessary information on the pharmacological effects of Noni already studied and hence further pharmacological research or improvements in the area already studied can very well be undertaken.

Pharmacological activities of Noni

Analgesic effect

Noni has dose related central analgesic effect (Younos et al., 1990) and analgesic effect antagonized by Naloxone. Efficacy of Noni is 75% as that of morphine and no addiction nor side effects when compared to the latter.

Analgesic effect can be evaluated in mice by two methods

1. Twist method-Counting number of twists produced in mice by Potassium antimony tart rate estimated.

2. Hot plate method- Toleration time noted.

Dose dependent analgesic effect can be both methods and the active compound causing analgesia is Xeronine.

Anti-inflammatory activity

Anti-inflammatory activity of Noni can be evaluated by Carrageenan induced rat paw oedema method and acute injury induced by CC14 (McKoy et al., 2002). Potent anti-inflammatory activity is observed in both methods

Mechanism: The underlying mechanism is selective inhibition of COX-2.Two forms of COX are identified, COX-1 and COX-2. The over expression of Cox-2 leads to angiogenesis and inflammation. Inhibition of COX-2 and subsequent reduction in inflammation have cancer preventive effect. The COX-1 and COX-2 activities were determined based on PGE2 levels generated by incubation of human platelets. IC50 COX 2/ COX 1 ratio was 0.76 for Noni and 0.34 for Celecoxcib a known COX-2 inhibitor. This was the first scientific evidence for a strong Anti-inflammatory activity of Noni.

Antioxidant effect

Antioxidant effect of Noni is based on protection from free radicals and consequent lipid peroxidation (Su et. al.,2005). Antioxidant effect of Noni was analyzed by Tetrozolium nitro blue (TNB) assay and Leucomethylene blue (LMB) assay methods.

Three experimental models were used in these assays :

1. In vitro assay
2. CCl4 induced liver injury
3. Current smokers

1. *In vitro* studies

Noni produced dose dependent inhibition of Lipid Peroxides (LPO) and Superoxide Anion Radicals (SAR). SAR scavenging activity of Noni was compared to other Antioxidants. It is 2.8 times that of Vitamin C, 1.4 times that of Pyrenogenol and 1.1 times that of grape seed powder. Antioxidant effect of Noni is due to Selenium, Neolignan and Americanin A compounds.

2. CCl4 induced liver injury model

CCl4 is a liver hydroperoxidation inducer

10% Noni juice in water was administered in rats for 12 days. The following results were observed

i. Reduction in LPO and SAR level within 3 hours after CCl4 administration.

ii. LPO level reduced to 20%.

iii. SAR level reduced to 50%.

These results prove the protective effect of Noni on liver from oxidant damage.

3. Protective effect in current smokers

A one month double blind, randomized, placebo controlled clinical trial was conducted among smokers (Wang et. al., 2002). The results showed that Noni produced 23% reduction in Superoxide Anion Radicals (SAR) level and 27 % reduction in LPO level. This proves that Noni protects the body from oxidative damage caused by cigarette smoking.

Anticancer effect of Noni

Noni fruit extract showed cytotoxic effect in Breast carcinoma cell line and Colon carcinoma cell line studies (Mc Clatchey et. al., 2002).Study on cultured leukemia cell line showed that Noni induced apoptosis at lower doses and Cancer cell necrosis at higher doses.

Anticancer mechanism of Noni

1. Noni affects several genes of apoptic pathway and cell cycle. This inhibits cell growth.

2. Damnacanthol of Noni inhibits RAS oncogenes (which stimulate cell proliferation and inhibit apoptosis).

3. Noni stimulates immune system and releases immune mediators like TNF a, IL ß, IL-10, IL-12 and NO. An immunomodulatory polysaccharide was isolated from Noni (Furusawa et al., 2003)

4. Noni inhibits angiogenesis. It is proved in human breast cell tumours with placental vein explants (Hornick et al., 2003)

Anticancer effect in retinoblastoma

Retinoblastoma is an incurable intraocular tumour in children. Noni produced apoptosis in Retinoblastoma 79 cells (Surendiren, et al., 2007). It produced microscopic evidence of Cell shrinkage, Membrane blebbing and Granule formation. Cytotoxic effect of Noni was confirmed by MTT based cell variability assay. Apoptic cells initiated DNA fragmentation.

Cancer preventive effect of Noni

Cancer preventive effect of Noni was demonstrated in mammary breast carcinogenesis induced by DMBA (7,12-dimethyl benzanthracene) in rats (Akihisa et al., 2007). DMBA treated rats showed lesions like epithelial hyperplasia, benign tumours and in situ carcinomas. Noni administered rats showed normal histology or mild hyperplasia. This proves that Noni prevents mammary breast cancer.

Mechanism :

1. Prevention of DNA adduct formation. Chemical carcinogens bind to DNA and form DNA adducts. Carcinogen-DNA adduct induces DNA damage lead Cancer growth. Noni inhibits carcinogen-DNA adduct formation. This prevents cancer development.

2. Antioxidant effect - In in vitro studies, CCl4 induced lever toxicity in cigarette smokers. Antioxidant effect of Noni scavenges free radicals, quenches lipid peroxidation and reduces the risk of cancer.

Immunological activity

Noni stimulates immune mechanism (Hirazumi et al., 1999).

1. Noni inhibits production of TNF a. It is an endogenous tumour promoter.

So tumour growth is suppressed due to activation of immune system.

2. Noni stimulates thymus growth and size of thymus increased to 1.7 times. Thymus generates T cells. T cells are involved in ageing process and cellular immune functions. Thus Noni improves immune function by stimulating thymus growth.

Anti bacterial activity

Noni inhibits growth of infectious bacteria like *Pseudomonas aeruginosa, Proteus morgii, Staphylococcus aureus, E. coli, Salmonella* and *Shigella*. Antibacterial activity is due to L-.asperuloside and alizarin compounds (Zaidan et al.,

2005). Another compound scopoletin of Noni inhibits *E. coli*. Noni inhibits the growth of H. pylori which is responsible for gastric ulcer.

Anti tubercular effect

Noni kills *M. tuberculosis* and concentrated extract of Noni leaves kills 89% of bacteria. (Saludes et al., 2002). It is comparable to Rifampicin, a standard anti TB drug which kills 97% at the same concentration. Ethanol extract and Hexane fraction of Noni also showed anti TB activity. Active compounds which show anti TB activity are ephytol and epidioxysterol

Antiviral activity

Noni inhibits the replication of HIV-1 (III B) in MT-4 cells exhibiting a maximum protection of 18% .(Selvam et al., 2007). Noni also inhibits in vitro HCV subgenomic replication. 50% Effective concentration was 10 + 2 µg/ml. Anthraquinone from Noni roots suppressed cytopathic effect of HIV infected MT-4 cells.

Antifungal activity

Aqueous extract of Noni inhibits serum induced morphological conversion of *Candida albicans* to a filamentous form (which causes pathogenicity) (Siddiqui et al.,2003). Also it inhibits the germination of *Aspergillus nidulans* spores. Noni has anti fungal activity against *Candida* species in animal cell line and Vero cell line. Noni can control the growth and infection of *Candida* species. Noni has anti fungal activity against Fusarium semitectum which can cause corneal ulcer. The effect was equivalent to that of other commercially available antifungal agents.

Antimalarial activity

Chloroform extract of *M. citrifolia* has anti malarial activity (Tona et. al.,2004). IC 50 value is less than 25 µg/ml and effective against *P. falciparum* infection.

Antiamoebic effect

Aqueous and methanolic extracts of *M. citrifolia* showed antiamoebic effect (Cimanga et al., 2006). IC50 values are 3.1 and 1.7 µg/ml respectively. Active compounds having anti ameobic activity are Kaempferol, Apigenin and Luteolin.

Antiparasitic effect

Noni kills Ascaris lumbricoides (round worm) (Pawlus et al., 2007). It produces paralysis and death of worm within 24 hrs. Methanolic extract of stems is effective in cutaneous leishmaniasis A marked improvement occured in 50% of the above cases. Noni kills Filarial parasite. It has inhibitory effect on cattle parasite called Setaria digitata. Also it has insecticidal activity against Drosophila species.

Anxiolytic and sedative effect

These effects were evaluated by GABAa binding assay (Deng et al., 2007). Methanol extract of Noni fruit has affinity for GABAa inhibitory neurotransmitter receptors. This binding produces anxiolytic and sedative effects. Noni relieves anxiety and produces sleep.

Antihypertensive effect

Noni extract lowered blood pressure in anaesthetized dogs (DANG-VAN-HO 1955). A methanolic extract of stems inhibited spontaneously contracting rabbit jejunum and also it inhibits potassium induced contractions. These results indicate the calcium channel blocking activity which contributes to antihypertensive activity.

Antithrombotic and hypolypidemic effect

Noni juice produced antithrombotic effect on jugular vein thrombosis in rats induced by FeCl3 (Kamiya et al.,2004). It reduced serum cholesterol and triglyceride level in smokers. It inhibits LDL oxidation as evaluated by thiobarbituric acid reactive substance. This effect is important in preventing arteriosclerosis. Compounds responsible for the antithrombotic and hypolypidemic activity are americanin, americanol and americanoic acid.

Anti diabetic effect

Noni reduces blood sugar level in streptazocin induced Diabetic rats (Kamiya et al.,2008). Fasting blood sugar level is lowered by 29% compared to Diabetic controls. This lowering of blood sugar level hastens wound healing in Diabetic rats. A good correlation was observed between wound contraction and blood glucose values.

Wound healing activity

M. pubescens fruit extract on topical application in rats accelerated wound healing Complete wound contraction and complete hair growth were achieved within 15 days. (Mathivanan et al.,2006). *M. citrifolia* fruit extract reduced blood sugar level and hastened wound healing in diabetic rats (Nayak et al.,2007a,b). The wound size decreased (73%), weight of granulation tissue increased and the hydroxylproline content also increased. All these prove the wound healing effect of Noni.

Hepatoprotective effect

The hepatoprotective effect of Noni was studied in female SD rats against CCl4 induced liver injury (Wang et al., 2008). Noni prevented hepatotoxic lesions and necrosis of hepatocytes.It prevented free radical induced oxidative pathological

changes and also decreased the level of Serum Alanine Transferase (SAT) and Aspartate Amino Transferase (AST). Thus Noni is effective in protecting the liver from extrinsic toxin exposure.

Endodontic irrigant activity

The in vitro effectiveness of Noni to remove smear layer from canal walls of endodontically instrumented teeth was evaluated (Murray et al., 2008). Sixty premolar teeth with a single canal were chosen. They were inoculated with Enterococcus fecalis and incubated for 30 days. The irrigation with Noni juice effectively removed the smear layer from root canal. The effect was equal to that of NaOCl or EDTA. Noni is the first fruit juice to be used as an intracannal irrigant.

Erogenic potential

Noni has antifatigue and endurance promoting potential (Ma et al., 2007). This effect was evaluated in rats by: forced swim test and rota rod test. The results were compared with young and aged controls .Average time was longer by 36 to 45% in swim test, similar to young controls 59 to 128% in rota rod test. Noni prevents fatigue, improves endurance and also physical performance.

Estrogenic activity

The uterotropic bioassay was done in vivo in mice to assess the Estrogenic activity of Noni (Müller et al.,2009). Low dose of Noni increased the wet weight and blotted weight of mice uterus. Noni did not produce opening of vagina nor cornification of vaginal epithelium. These results prove a very low estrogenic potency of Noni. So, short or moderate consumption of Noni is unlikely to cause physical problems due to oestrogenic effect.

Pharmacokinetic studies

The pharmacokinetic studies were conducted on female SD rats (Wang et al., 2002). A dose of 1ml/kg body weight was administered orally and compound Scopolectin was chosen as a marker. Its concentration in plasma and different tissues was monitored. Absorption of Noni was rapid. A 50% peak concentration was reached in 30 min. Peak plasma level reached within 2 hrs. Plasma concentration after 12 hrs was 12% and at the end of 24 hrs after administration, level dipped to 2 %. In tissue , the peak concentration reached in 3 hrs. The compound Scopoletin approached its highest level in breast than any other tissue.

Safety of Noni

Safety of Noni has been evaluated thoroughly (West et al., 2006; Pawlus et al., 2007; Potterat et al., 2007). No adverse effect of Noni use has been reported.

Acute toxicity studies.

Noni was administered to SD rats at a dose of 1500 mg/kg. The animals were observed for 2 weeks. There was no sign of toxicity nor behavioural changes. The animals are healthy and gained weight at the end of the study. We could conclude LD50 is greater than 1500 ml/kg. There was no histological change in the 55 organs studied, and blood picture, liver enzymes, renal parameters remained normal. Noni showed no allergenic potential nor genotoxicity.

Summary

M. citrifolia is a versatile medicinal plant with excellent pharmacological activities and safe herbal medicine. Further pharmacological research on *M. citrifolia* will strengthen the scientific validation and standardization of therapeutic uses of Noni.

Reference

Akihisa T, Matsumoto K, Tokuda H, Yasukawa K, Seino K, Nakamoto K, Kuninaga H, Suzuki T. and Kimura Y 2007. Anti-inflammatory and potential cancer chemopreventive constituents of the fruits of *Morinda citrifolia* (Noni). J. Nat. Prod. 70:754-7

Cimanga K, Kambu K, Tona L, Hermans N, Apers S, Totté J, Pieters L, Vlietinck AJ 2006. .Antiamoebic activity of iridoids from *Morinda morindoides* leaves. Planta Med. 72:751-3.

Dang-Van-Ho 1955. Treatment and prevention of hypertension and its cerebral complications by total root extracts of *Morinda citrifolia*. Presse. Med. 63:1478-40.

Deng S, West BJ, Palu AK, Zhou BN and Jensen CJ 2007. Noni as an anxiolytic and sedative: a mechanism involving its gamma-aminobutyric acidergic effects..Phytomedicine. 14:517-22.

Furusawa E, Hirazumi A, Story S. and Jensen J. 2003. Antitumour potential of a polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (Noni) on sarcoma 180 ascites tumour in mice. Phytother. Res. 17:1158-64.

Hirazumi A. and Furusawa E. 1999. An immunomodulatory polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (Noni) with antitumour activity. Phytother. Res. 13:380-7.

Hornick CA, Myers A, Sadowska-Krowicka H, Anthony CT and Woltering EA.2003. Inhibition of angiogenic initiation and disruption of newly established human vascular networks by juice from *Morinda citrifolia*. Angiogenesis, 6:143-9.

Kamiya K, Hamabe W, Harada S, Murakami R, Tokuyama S. and Satake T 2008. Chemical constituents of *Morinda citrifolia* roots exhibit hypoglycemic effects in streptozotocin-induced diabetic mice. Biol. Pharm. Bull. 31:935-8.

Kamiya K, Tanaka Y, Endang H, Umar M. and Satake T. 2004. Chemical constituents of *Morinda citrifolia* fruits inhibit copper-induced low-density lipoprotein oxidation. J. Agric. Food. Chem.52:5843-8.

Ma DL, West BJ, Su CX, Gao JH, Liu TZ and Liu YW 2007. Evaluation of the ergogenic potential of Noni juice Phytother. Res. 21:1100-1

Mathivanan N, Surendiran G, Srinivasan K and Malarvizhi K 2006 *Morinda pubescens* JE Smith (*Morinda tinctoria* Roxb) fruit extract accelerates wound healing in rats.J. Med. Food. 9:591-3.

McClatchey W. 2002 From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae).Integr. Cancer. Ther. 1:110-20.

McKoy ML, Thomas EA. and Simon OR. 2002 .Preliminary investigation of the anti-inflammatory properties of an aqueous extract from *Morinda citrifolia* (Noni).Proc. West. Pharmacol. Soc. 45:76-8.

Müller JC, Botelho GG, Bufalo AC, Boareto AC, Rattmann YD, Martins ES, Cabrini DA, Otuki MF and Dalsenter PR 2009. *Morinda citrifolia* Linn (Noni): *In vivo* and *in vitro* reproductive toxicology. J. Ethnopharmacol. 121:229-33.

Murray PE, Farber RM, Namerow KN, Kuttler S. and Garcia-Godoy F 2008 Evaluation of *Morinda citrifolia* as an endodontic irrigant. J Endod. 34(1):66-70.

Nayak BS, Isitor GN, Maxwell A, Bhogadi V. and Ramdath DD 2007. Wound-healing activity of *Morinda citrifolia* fruit juice on diabetes-induced rats. J. Wound. Care. 16:83-6.

Nayak BS, Sandiford S. and Maxwell. A. 2007. Evaluation of the Wound-healing Activity of Ethanolic Extract of *Morinda citrifolia* L. Leaf.Evid. Based. Complement. Alternat. Med. 28:102-5

Pawlus AD. And Kinghorn DA. 2007. Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (Noni). J. Pharm. Pharmacol. 59:1587-609.

Potterat O and Hamburger M 2007. *Morinda citrifolia* (Noni) fruit--phytochemistry, pharmacology, safety. Planta. Med. 73:191-9.

Saludes JP, Garson MJ, Franzblau SG.and Aguinaldo AM 2002. Antitubercular constituents from the hexane fraction of *Morinda citrifolia* Linn. (Rubiaceae). Phytother. Res. 16:683-5.

Selvam P, Murugesh N and Myriam W 2007. Studies of comparative antiviral activity and cytotoxicity of *Morinda citrifolia* L Noni Clin. Res. J., 1: 22-24.

Siddiqui BS, Ismail FA, Gulzar T. and Begum S. 2003. Isolation and structure determination of a benzofuran and a bis-nor-isoprenoid from Aspergillus niger grown on the water soluble fraction of *Morinda citrifolia* Linn. leaves. Nat Prod Res. 17(5):355-60.

Su BN, Pawlus AD, Jung HA, Keller WJ, McLaughlin JL. And Kinghorn AD 2005. Chemical constituents of the fruits of *Morinda citrifolia* (Noni) and their antioxidant activity. J. Nat. Prod. 68:592-5.

Surendiren G., Seethalaksmhi T, Malikarjun K, Krishnakumar S. and Mathivanan N 2007 Cytotoxicity in Human Retinoblastoma Y79 cells by the fruit extract of Noni, Proceedings of Second National Symposium on Noni for health and wellness 224-231.

Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Van Miert S, Pieters L, Totté J. and Vlietinck AJ. 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 93:27-32.

Wang MY, Anderson G, Nowicki D, Jensen J 2008 Hepatic protection by Noni fruit juice against CCl(4)-induced chronic liver damage in female SD rats. Plant. Foods. Hum. Nutr. 63:141-5.

Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK. and Anderson G. 2002. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacol. Sin. 23:1127-41.

West BJ, Jensen CJ. and Westendorf J 2006 .Noni juice is not hepatotoxic. World. J. Gastroenterol. 12:3616-9.

Younos C, Rolland A, Fleurentin J, Lanhers MC, Misslin R. and Mortier F 1990 .Analgesic and behavioural effects of *Morinda citrifolia*. Planta. Med. 56:430-4.

Zaidan MR, Noor Rain A, Badrul AR, Adlin A, Norazah A. and Zakiah I . 2005. *In vitro* screening of five local medicinal plants for antibacterial activity. Trop. Biomed. 22:165-70.

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In vitro anticancer activity of fruit extract and small molecule from the fruits of *Morinda citrifolia* L. against retinoblastoma Y79 cell line

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Keywords : *Morinda citrifolia*, retinoblastoma, Y 79 cells, apoptosis, annexin V-FITC, scopoletin

Abstract : The present study evaluated anticancer activity of *Morinda citrifolia* L. against retinoblastoma Y79 cell line. The IC50 of *M. citrifolia* fruit extract as determined by MTT assay was 0.8 mg/ml at 72 h of incubation. *M. citrifolia* induced apoptosis in Y79 cells was visualized by light microscopy, which showed cell shrinkage, membrane blebbing and granule formation. Annexin V - FITC staining showed translocation of phosphatidyl serine (PS) from the inner surface of plasma membrane to the cell surface, which also confirmed the apoptosis induce by *M. citrifolia* fruit extract. The apoptosis was further confirmed by analysis of DNA fragmentation using agarose gel electrophoresis, which was dose dependent manner. Finally, it was revealed that the apoptotic bodies induced by fruit extract of *M. citrifolia* in Y79 cells were caspase - 3 dependent manner, which was confirmed by RT - PCR analysis, and there was no change in BNIP3 expression. The active principle responsible for the above mentioned activity was isolated from the fruits of *M. citrifolia* and purified by silica gel column chromatography. Among seven fractions, the fraction M2 was effective in inducing apoptosis in Y79 cells as determined by MTT method. Further, the IC50 of purified fraction was determined as 0.5 µg/ml at 48 h of incubation. The fraction, M2 was identified as scopoletin by various spectral analyses and its structure was elucidated. The mechanisms of action of scopoletin are under investigation using DNA microarray.

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Introduction

Morinda citrifolia L., popularly known as noni, is distributed worldwide mostly in the tropical countries. The fruit of *M. citrifolia* has been used by the Polynesians in a variety of medicinal preparations and as a famine food (Nelson, 2006; Mathivanan et al., 2005; Wang et al., 2002). *M. citrifolia* belongs to the family Rubiaceae has several common names, viz. Indian mulberry, Nuna, cheese fruit, mengkudu, etc. and it is also known as Pain killer bush, head ache tree due to its

medicinal properties. The fruit is widely used for the extraction of juice as health tonic, which has been exploited commercially in many countries. Not only the fruit even the leaves, bark and root of *M. citrifolia* have been commercially exploited for the preparation of dyes, facial creams, soaps, lotion, tea, etc. Our Indian ancestors have aware the medicinal properties of Morinda spp. and they used them in their traditional systems of medicine such as Siddha and Auyurveda.

In recent years focus on the nutritive and therapeutic properties of *M. citrifolia* is on the rise, which resulted in a wide range of products in the market. Notably, noni has been promoted to treat a vast array of diseases ranging from cancer to sexual dysfunction. The indigenous tribes of Australia use the ripe fruits of *M. citrifolia* for treatment of respiratory infections and the recent scientific studies demonstrated the pharmacological properties of *M. citrifolia*. The ethanolic extracts from the aerial parts of *M. citrifolia* have antibacterial (Surendiran et al., 2006) and analgesic activities and the root and fruit extracts have hypotensive and analgesic effects (Wang et al., 2002). It has been demonstrated that juice from M. citrifolia is effective in inhibiting new angiogenic growth in human placental vein explants (Hornick et al., 2003), reducing the rate of capillary proliferation and inducing apoptosis in newly formed angiogenic networks and suppressing both angiogenic incidence and vessel development in human breast cancer explants. However, no report is available yet on the effect of *M. citrifolia* on retinoblastoma, the common primary intraocular tumor in children. The incidence of RB is high in developing countries and in less affluent populations (Biswas, et al., 2003).

Angiogenesis plays an important role in tumor invasion and survival. Critical steps during tumor angiogenesis are the outgrowth of endothelial cells from pre-existing capillary vessels initiated by the migration of endothelial cells away from the parental vessels. Endothelial cells proliferate in response to vascular endothelial growth factor (VEGF). Proliferating endothelial cells subsequently remodel the extracellular matrix around neovasculature sites, align into tube-like structures, and eventually form new functional blood vessels. Extracellular matrix remodeling occurs continuously throughout the tumor angiogenic process in a well-orchestrated fashion involving numerous extracellular matrix-degrading enzymes. Among them, matrix metalloproteinases (MMPs) are believed to be a critical group of enzymes that affect tumor angiogenesis, tumor growth, local invasion, and subsequent distant metastasis2 (Moses, 1997; Nelson et al., 2000). In addition, VEGF is being influenced by the NOS enzymes in the tumor environment through the depletion of intracellular iron; the expression of VEGF is activated. NOS2 is known to function as an up-regulator of VEGF-regulated kinases and mitogen-activated protein kinases. Thus, novel therapies have emerged that use this phenomenon as a target for cancer therapies. To date, more than three dozen clinical trials have been approved that target tumor angiogenic and antiangiogenic

factors. However herbal drugs and their potential in antitumorigenic and antiangiogenic properties have not been much explored, which has got less toxicity and less morbidity to the cancer patients. With this background, the present study was aimed to investigate *M. citrifolia* induced antiproliferative effect on retinoblastoma Y79 cells.

Materials and Methods

Chemicals and reagents

The reagent for MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was obtained from sigma- Aldrich, St Louis, USA and other chemicals used in this study were of special grade.

Cell culture

The retinoblastoma Y79 cell line was obtained from the American Type Culture Collection (ATCC). It was originally derived from the primary tumor in the right eye of 2 years-old Caucasian girl. It was maintained in RPMI 1640 medium with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L bicarbonate supplemented with 15 % FBS throughout the experiment period.

Preparation of *M. citrifolia* fruit extract

M. citrifolia tree was identified using the keys of The Flora of Madras Presidency (Gamble and Fisher, 1957). The ripen fruits (6 kg) were collected from the trees located in the coast of Kasargod, Kerala State, India. They were washed thoroughly with distilled water, cut into small pieces, air dried and ground using pestle and mortar. Coarsely powdered fruit material was extracted with methanol for 48 h, three times each, at room temperature and filtered through Whatman No. 1 filter paper. The solvents were removed by a rotary evaporator under reduced pressure at 40°C, which yielded 130.0g of thick syrupy extract. This crude extract was tested for its antiproliferative effect against retinoblastoma Y79 cells.

Assay of cell viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) method, described by Mosmann (1983) was used to quantitatively detect live but not dead cells. In brief, approximately 5x103 cells/well was seeded onto 96 wells plate, 100 µl of RPMI 1640 medium was added and incubated at 37ºC for 24 h. After 24 h, medium was discarded and fresh medium was added with different concentrations of *M. citrifolia* fruit extract (100 - 2000 µg/ml). The plates were incubated for 1-3 days at 37ºC in CO2 incubator. After respective incubation period, medium was discarded and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml) and incubated at 37ºC in a CO2 incubator. After 4 h, medium was

discarded and 100 µl of DMSO was added to dissolve the formazon crystals. Then the absorbance was read in a spectrophotometer at 570 nm and cell survival was calculated by the following formula.

$$\text{Viable cell (\%)} = \frac{\text{Test OD}}{\text{Control OD}} \times 00$$

Assessment of apoptosis

Approximately 5x104 cells/well was seeded onto 24 wells plate, 100 µl of RPMI 1640 medium was added and incubated at 37ºC. After 24 h, medium was discarded and fresh medium was added with 100 - 400 µg/ml of M. citrifolia fruit extract. The cells were monitored under microscope, without fixation to assess the morphological changes (apoptosis) induced by the treatment of M. citrifolia alcoholic fruit extract, under the condition of normal illumination using inverted microscope (Vento et al., 1998). Apoptosis was also assessed by staining with annexin V - FITC. This assay is based on the affinity between annexin V - FITC towards phosphatidylserine (PS), which translocates from the inner surface of plasma membrane to the cell surface during apoptosis. Annexin V is not an absolute marker of apoptosis. Therefore in this study we used another vital dye, propidium iodide (PI) in conjunction with annexin V - FITC. The Y79 cells were collected, washed with phosphate buffered saline (PBS) once and centrifuged at 14,000 rpm for 5 min. The resultant pellet was resuspended in 100 µl of PBS. The cell suspension of 20 µl was added to a slide and allowed to dry. Cells were fixed with 4% p-formaldehyde for 5 min. The annexin reagent (20 µl of annexin V - FITC + 20 µl of PI) was mixed, applied to the slides and incubated for 20 min. The slides were mounted with mounting reagent and observered under fluorescent microscope.

DNA fragmentation assay

After incubation with *M. citrifolia* fruit extract, the Y79 cells were processed for DNA fragmentation analysis by agarose gel electrophoresis. The treated Y79 cells were washed twice with PBS and DNA extraction was performed using Quick Genomic DNA Extraction Kit (Qiagen, USA) according to the manufacturer instructions. The DNA pellet was resuspended in TE buffer (10 mmol/L Tris-HCI, 1 mmol/L EDTA, pH 8.0) prior to loading (10 µl) on to a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed at 35 V for 4 h, DNA fragments were visualized and photographed under UV illumination. DNA marker was used to estimate the size of DNA fragment.

Reverse Transcriptase-PCR (RT-PCR)

The total RNA was extracted by the Trizol method, TRI Reagent (Sigma, USA) (Kania et al., 2007). Cells were harvested from the cultures and collected in a vial. The harvested cells were centrifuged at 10,000 rpm for 5-10 min and the supernatant was discarded using a pipette. To the pellet, 1 ml Trizol reagent was added, vortexed and incubated at room temperature (28 ± 2°C) for 5 min. To this, 500 µl of CHCl3 was added and shaken well for 15 sec. Cells were centrifuged at 12,000 rpm for 15 min. Aqueous layer that had RNA was collected and transferred to new vial. To this 500 µl isopropanol was added and incubated at room temperature for 10 min. Cells were centrifuged at 12,000 rpm for 10 min and the supernatant was discarded. To the pellet, 75 % alcohol was added and mixed well. Cells were centrifuged at 12,000 rpm for 5 min and the supernatant was discarded. Pellet was air dried at room temperature for 2 min and 25 µl DEPC treated water was added. From the total RNA extracted, 5 µl was run in 2% agarose gel to see the quality of RNA and the rest was stored at -80°C until further use. RT-PCR was performed using sensiscript reverse transcriptase, which is a recombinant heterodimeric enzyme. The reaction mixture contained 2.0 µl of 10x buffer; 2.0 µl DNTPs; 2.0 µl Oligo dT; 1.0 µl RNase; 1 .0 µl sensiscript RT; 10.0 µl RNAse free water; 2.0 µl template RNA. The setup was incubated at 37oC for 60 min. PCR amplification of the first strand cDNA was performed using specific primer pairs, along with housekeeping gene, glyceraldehyde-3 phosphate dehydrogenase (GAPDH) as an internal control. The mRNA expression of respective gene was analyzed using Y79 and control cell lines. PCR products were fractionated by electrophoresis using 2% agarose gel containing 0.5% Ethidium bromide with molecular marker Hinf I X digest to confirm the size of the resultant products.

Isolation of active principle

Bioassay guided separation method was used to isolate bioactive compound responsible for the antiproliferative activity of the methanolic fruit extract of *M. citrifolia*. Column was packed with hexane using silica gel 100 - 200 mesh size as a matrix. About 15 g of methanolic syrupy extract were loaded as dried slurry of silica gel and the column was eluted with increasing concentrations of ethyl acetate and methanol to increase polarities. The fractions were analyzed by pre-coated TLC plates (E-Merck, Germany) and spots were visualized by exposure to iodine and observed under UV light. Seven fractions (M1 - M7) during the purification process and tested for its antiproliferative effect by MTT assay. The fraction M2 (Rf value: 6.7 at 10% methanol in chloroform) showed prominent antiproliferative activity against retinoblastoma Y79 cells. The fraction M2 was further purified by silica gel column chromatography (230-400 mash) to yield purified compound. The structure of purified compound was elucidated by spectral data's (IR, NMR,

EI-Mass, ESI-Mass, UV-Vis Spectrophotometer) and its structure was confirmed by CHEM DRAW 2004 software.

Results and discussion

In the present work we demonstrated the *M. citrifolia* L. fruit extract induced apoptosis in Y79 cells (Fig. 1a.). The fruit extract of *M. citrifolia* reduced the Y79 cell viability as determined by MTT based dye reduction method, which measured mitochondrial respiratory function and detected the onset of cell death. It was found from the study that the fruit extract reduced the viability of cells to 50% (IC50) at 800 µg/ml on incubation for 72 h at 37°C, after which the Y79 cells were degenerated progressively. It was inferred from the study that the loss of cell viability was time and dose dependent, which supported the previous study of Vento et al. (1998), where they obtained similar result when the retinoblastoma Y79 cells incubated with different time period with C2-ceramide.

Light microscopy examination of Y79 cells after treatment with fruit extract of *M. citrifolia* for 48 h showed prominent apoptotic morphology (Fig. 1b). The treatment of fruit extract induced cell shrinkage, membrane blebbing and membranous apoptotic bodies as described earlier (Vento et al., 1998). Annexin V and propidium iodide staining of the treated Y79 cells easily detected the PS externalization, which can be detected using fluorescent microscope. The progressive stages of apoptosis induced by different concentrations of fruit extract of *M. citrifolia* were also observed. The Y79 cells showed evident of early apoptosis, by increasing green fluorescent colour on the cell surface, at the concentration of 200 µg/ml after 48 h of incubation. The cell membrane became increasingly permeable at the concentration of 300 µg/ml after 72 h of incubation and at higher concentration (400 µg/ml), the propidium iodide move readily across the cell membrane, resulted a red florescent signal (data not shown).

It was observed that the apoptotic morphology induced by the fruit extract of *M. citrifolia* was accompanied by DNA fragmentation. The evidence of fragmentation started at the concentration of 300 µg/ml and the progressive DNA ladder was observed at 400 µg/ml. It is well known that the internucleosomal DNA fragmentation responsible for DNA fragmentation is usually maintained to be peculiar to apoptosis (Gerschenson and Rotello, 1992). Depending on the cell type, the same drug can either suppress or stimulate apoptosis, and in the same cell types DNA fragmentation can accompany apoptosis or not, depending on the drug employed (Falcieri et al., 1993). However, our present study clearly indicated the fragmentation of Y79 cells due to from *M. citrifolia* fruit extract stimulated apoptosis.

The downstream effector caspases degraded multiple cell protein and are responsible for the morphological changes in apoptosis. Another gene called

BNIP3, an important hypoxia-responsive protein with strong pro-apoptotic activity (Kania et al., 2007). With this back ground, the present study was designed to confirm the apoptosis induced by the fruit extract of *M. citrifolia* was caspase dependent. In this study, we investigated the Caspase 3 and BNIP3 mRNA expression in control and fruit extract treated Y79 cells. There was no change in the levels of pro-apoptotic gene BNIP3 mRNA in both control and treated cells as confirmed by densitometric analysis of band intensity in control and treated lanes (Fig. 2). However, there was activation of caspase 3 mRNA level in treated cells compared to control cells (Fig. 3). The activation of caspase 3 mRNA level was in dose dependent manner. There was no change in the levels of caspase 3 mRNA expression at lower concentration (100 µg/ml), but there was activation in the levels of caspase 3 mRNA level at 200 - 400 µg/ml, as confirmed by densitometer analysis of band intensity in control and treated lanes.

Seven different compounds (M1-M7) were separated from *M. citrifolia* fruit extract by bioassay guided purification method. All the seven fractions were tested for its antiproliferative effect by MTT assay. The fraction M2 showing the Rf value of 6.7 at 10% methanol in chloroform (Fig. 4) showed prominent antiproliferative against retinoblastoma Y79 cells. The fraction M2 was further purified by solvent fractionation using silica gel column chromatography. The purified compound was characterized by various spectral analyzes such as IR, NMR, EI - Mass, ESI-Mass, UV-Vis and identified as scopoletin. Further, its chemical structure and molecular formula were elucidated and confirmed by CHEM DRAW 2004 software. The IC50 of scopoletin against Y79 cells was determined as 0.5 µg/ml. The purified scopoletin showed potential antiproliferative activity and its mechanisms of action is under investigation using DNA microarray.

Acknowledgements

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References

Biswas, J., Das, D, Krishnakumar, S and Shanmugam, M.P. 2003. Histopathological analysis of 232 eyes with retinoblastoma conducted in an Indian tertiary-care ophthalmic center. J. Pediatr. Ophthalmol. Strabismus, 40: 265-267.

Falcieri, E., Martelli, A.M., Bareggi, R., Cataldi, A and Cocco, L. 1993. The protein kinase inhibitor staurosporine induces morphological changes typical of apoptosis

in MOLT-4 cells without concomitant DNA fragmentation. Biochem. Biophys. Res. Comm., 193: 19-25.

Gamble J.S and Fisher, L.E.F. 1957. The flora of Presidency of Madras - Vol-I - III, Botanical Survey of India, Calcutta.

Gerschenson, L.E. and Rotello, R.J. 1992. Apoptosis, a different type of cell death. FASEB J., 6: 2450-2455.

Hornick, C.A., Myers, A., Sadowska-Krowicka, H., Anthony, C.T. and Woltering E.A. 2003. Inhibition of angiogenic initiation and disruption of newly established human vascular networks by juice from *Morinda citrifolia* (noni). Angiogenesis, 6: 143-149.

Kania, K., Matlawska-Wasowska, K., Osiecka, R. and Jozwiak, Z. 2007. Analysis of aclarubin - induced cell death in human fibroblasts. Cell Biol. Int., 31: 1049-1056.

Mathivanan, N., Surendiran, G., Srinivasan, K., Sagadevan, E. and Malarvizhi, K. 2005. Review on the current scenario of Noni research: Taxonomy, distribution, chemistry, medicinal and therapeutic values of *Morinda citrifolia*. Int. J. Noni Res., 1: 1-16.

Moses, M.A. 1997. The regulation of neovascularization of matrix metalloproteinases and their inhibitors. Stem Cells, 15:180-189.

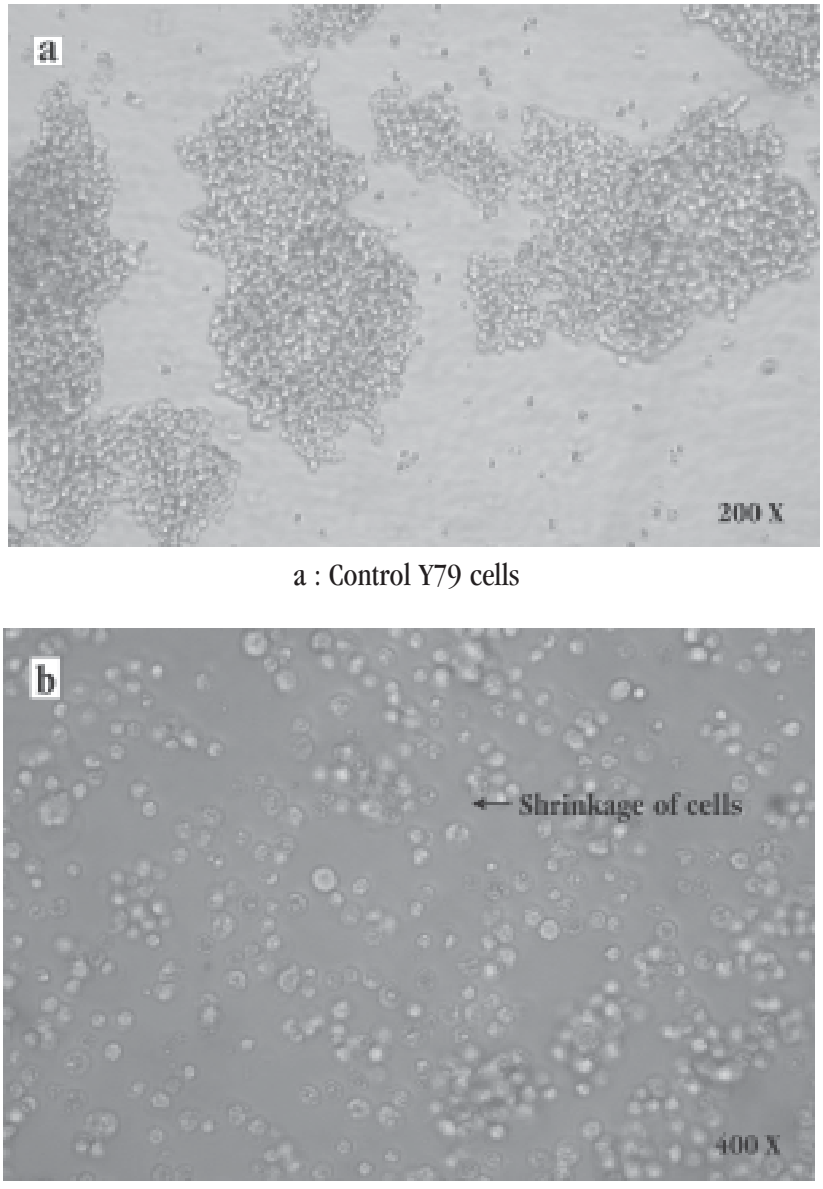
Nelson, A.R., Fingleton, B., Rothenberg, M.L. and Matrisian, L.M. 2000. Matrix metalloproteinases: Biological activity and clinical implications. J. Clin. Oncol.,18: 1135-1149.

Nelson, S.C. and Elevitch, C.R. 2006. The Complete Guide for Consumers and Growers. Permanent Agriculture Resource, University of Haiwaii, Hilo, Hawaii, USA. 102 p.

Surendiran, G., Sagadevan, E. and Mathivanan, N. 2006. Antifungal activity of *Morinda citrifolia* and *Morinda pubescens*. Int. J. Noni Res., 1: 4-9.

Vento, R., Giuliano, M., Lauricella, M., Carabillo, M., Di Liberto, D. and Tesoriere, G. 1998. Induction of programmed cell death in human retinoblastoma Y79 cells by C2 - ceramide. Mol. Cell. Biochem., 185: 7-15.

Wang, M.Y., West, B., Jensen, C.J., Norwicki, D., Su, C., Palu, A.K. and Anderson, G. 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacologica Sinica, 23: 1127-1141.



a : Control Y79 cells

b : Fruit extract at 400 µg/ml treated cell (after 72 h)

Fig. 1. *M. citrifolia* fruit extract induced apoptotic morphology in Y79 cells

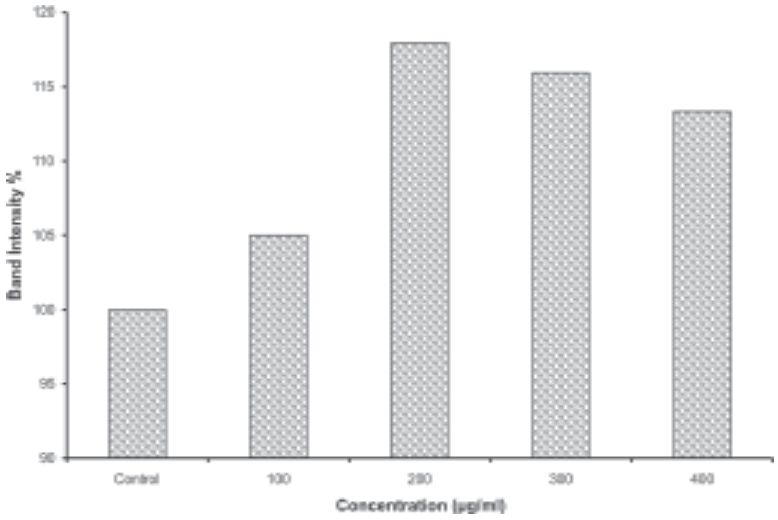


Fig. 2. Densitometer analysis of Caspase 3 levels in control and *M. citrifolia* fruit extract treated Y79 cells

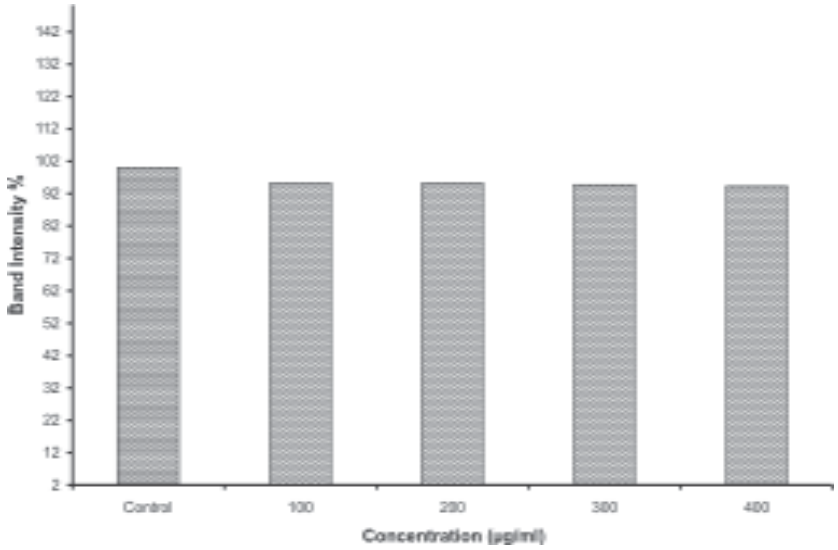


Fig. 3. Densitometer analysis of BNIP3, levels in control and *M. citrifolia* fruit extract treated Y79 cells

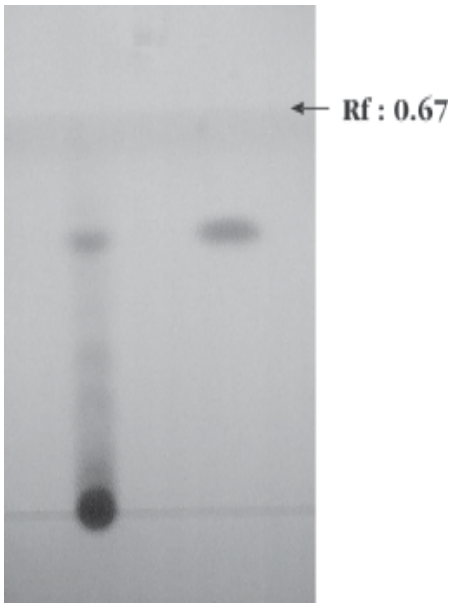


Fig. 4. Thin layer chromatography of methanolic fruit extract and purified scopoletin

Left : Crude fruit extract Right : Purified scopoletin

Rangadhar Satapathy

The microfilaricidal ability of noni juice (*Morinda citrifolia*) on the animal model of *Mastomys coucha* mouse - An *in vivo* study

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Keywords : Filariasis, Noni, microfilaricidal,

Abstract : Lymphatic filariasis caused by lymphatic dwelling nematodes is a serious public health problem in many tropical countries including India. It affects about 120 million people globally and listed as second leading known cause of disability. Diethyl carbamazine citrate (DEC) is the only drug available at present for the treatment of lymphatic filariasis which has been used for more than 50 years. Thus, there is a need for screening and/or developing a new compound to treat filarial infection. Noni juice (*Morinda citrifolia*) has been shown to have anti helminthic activity. An attempt is being made to evaluate the microfilaricidal ability of Noni juice in experimental filarial infection. The Setaria digitata - Mastomys model is standardized in order to study the clearance of microfilaria following oral administration. Intraperitoneal implantation of adult female worms of S. digitata in M. coucha could induce microfilaraemia lasting about 160 days in circulation. Microfilaria was detected in the peripheral blood on day four of post-implantation. The effect of oral administration of Noni juice (*Morinda citrifolia*) on appearance of microfilaria has been studied.

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Introduction

Filarias, a serious public health problem in many tropical countries including India affecting about 120 million people globally is the second leading known cause of disability. Diethyl carbamazine citrate (DEC) is the only drug available at present for the treatment of lymphatic filariasis which has been used for more than 50 years. Thus, there is a need for screening and/or developing a new compound to treat filarial infection. Noni juice (*Morinda citrifolia*) has been shown to have anti helminthic activity. An attempt is being made to evaluate the microfilaricidal ability of Noni juice in S. cervi implanted Mastomys rat model. The objective of the present study is to evaluate the microfilaricidal ability of Noni to clear microfilaria in experimentally infected animal

Material and Methods

Fresh gravid female *S. cervi* worms collected from cattle were used for implantation. Animals were anaesthetized and then three gravid female worms were implanted into the peritoneal cavity of each animal through a small slit. Slits were then stitched with surgical catgut after implantation. Induction of microfilaria was monitored by taking 20µl blood through the tail vain. Experiments were performed in two groups of three mastomys model.

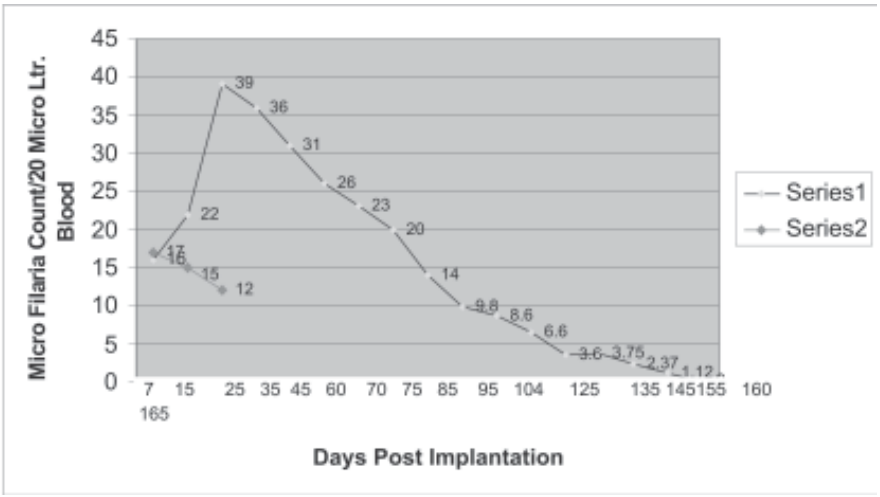
The first group is experimental group which consists of three Mastomys rats model. Each Mastomys rat model was implanted with S. cervi filarial parasite. Then each one was given Noni juice orally with a dose 25 µl per day for consecutive three days of 7th, 8th, 9th day of implantation.

The second group is called control group that also consist of three mastomys rats model. Each one was implanted with filarial parasite and was given saline water alone.

Results

The results are presented in Fig. 1

Fig. 1: Microfilarial count after implantation



The series-1 is control group and the series-2 is experimental group. After 7 days of implantation the mean microfilarial load in control group is 17 and experiment group is 16. After 15 days of implantation the mean microfilarial load is 22 in control group and 15 in experiment group. After 25 days of implantation the mean microfilarial load is 39 in control group and 12 in experiment group.

Conclusion

Preliminary study indicates that oral administration of Noni could reduce the microfilaria density in experimental filarial infection. However, a detailed clinical study need to be taken up for confirmation.

Reference

Wang M. Y., West, B.J., Jensen, C.J., Nowicki, D., Su, C., Palu, A. K., and Anderson, G. 2002. *Morinda citrifolia* L (Noni): A literature review and recent advances in Noni research. Acta Pharmacologica Sinica, 23 (12): 1127-1141.

Morton, J. F. 1992. The oceangoing Noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its `colorful' relatives. Economic Botany, 46: 241-256.

Raj, R. K. 1975. Screening of indigenous plants for antihelmintic action against human Ascaris lumbricoides: Part-II. Indian Journal of Physiology & Pharmacology, 19: 47-49.

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Studies on anti-HIV activity and cytotoxicity of leaf extract of *Morinda citrifolia* L.

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Keywords : *Morinda citrifolia*, anti-HIV activity, cytotoxicity, MTT assay

Abstract : Different leaf extracts of *Morinda citrifolia* L. were studied for antiviral activity against replication of HIV-1 (IIIB) and HIV-2(ROD) in MT-4 cells. Cytotoxicity of *M. citrifolia* against mock-infected MT-4 cells was also assessed by the MTT method. Ether extract (EMC) of leaf extract exhibited maximum protection of 58 % against replication of HIV-1 in MT-4 cells (EC50=38 µg /ml and CC 50= 66 µg/ml) Methanolic extract (MMC) displayed cytostatic activity in MT-4 cells (C-type Adult T cell leukemia cells) with CC50=51µg/ml.

Introduction

Morinda citrifolia L (Noni) is a versatile medicinal plant with a broad spectrum of pharmacological activities. *M. citrifolia* possesses hepatoprotective (Wang et al., 2008a,b) anticancer (Akihisa et al., 2008), immunomodulatory (Palu et al., 2008), anti-inflammatory (Palu et al., 2007), wound healing (Nayak et al., 2007), antioxidant(Su et al., 2005), anti-tubercular (Saludes et al., 2002) and wide spectrum of biological activity (Pawlus and Kinghorn 2007) and reported as a safe herbal drug (West et al.,2006). Recently much attention has been devoted for searching potential antimicrobial agents from natural products and anti-HIV activity of M. citrifolia. The present work is to study the anti-HIV activity of various extracts of the leaf powder of *M. citrifolia* against HIV 1 and 2 in MT-4 cells.

Materials and Methods

The leaves of *M. citrifolia* are dried under shade and powdered. The powder is extracted with different solvents (acetone, chloroform, methanol, ether and ethyl accetate) for five days by cold maceration. It is then filtered to get the extracts evaporated to dryness under vaccum. The dried extracts of acetone (AMC) chloroform (CMC), ether (EMC), ethylacetate (EAMC) and methanol (MMC) were used for anti-HIV activity and cytotoxicty studies.

Anti HIV Assay

The leaf extracts of *M. citrifolia* was tested for their inhibitory effects against replication of HIV-1 (IIIB) in MT-4 cells (De Clercq, 1988). The MT-4 cells were grown and maintained in RPMI 1640 DM medium supplemented with 10% (v/v) heat-inactivated Fetal Calf Serum (FCS), 2 mM-glutamine, 0.1% sodium bicarbonate and 20µg/ml gentamicin (culture medium). Inhibitory effect of *M. citrifolia* on HIV-1 replications was monitored by inhibition of virus-inducted cytopathic effect in MT-4 cells and was estimated by MTT assay. Briefly, 50 µl of HIV-1 (IIIB) (100-300 CCID50) were added to a flat-bottomed microtiter tray with 50 µl of medium containing various concentrations of extracts. MT-4 cells were added at a final concentration of 6 x 105 cells/ml. After 5 days of incubation at 37°C, the number of viable cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny1 tetrazolium bromide (MTT) method. Cytotoxicity of extraxt against mock-infected MT-4 cells was also assessed by the MTT method. The anti-HIV activity and cytotxicity data are presented in Table 1.

Results

Various extract of leaf powder of *M. citrifolia* .L were evaluated for its anti-HIV activity and cytotoxicity (Table 1) against HIV-1 and -2 replication in acutely infected MT-4 cells. The extracts exhibited a maximum protection of 8-58 against the replication HIV-1 and -2 in acutely infected MT-4 cells. Ether extract of *M. citrifolia* (EMC) exhibited antiviral activity with EC50=38 µg/ml and CC50=66 µg/ml and 58% maximum protection against the replication HIV-1 in acutely infected MT-4 cells. The methanolic extract (MMC) displayed cytostatic activity against MT-4 cells (Adult T Cell leukemia) with CC50= 51.20±15.74 µg/ml.

Table 1. Anti-HIV activity of and Cytotoxicity of leaf extracts of morinda citrifolia

Extract	Strain	EC50 (µgm/ml)[a]	CC50(µgm/ml)[b]	Maximum Protection (%)
AMC	IIIB	>138.75	138.75±2.06	10
	ROD	>138.75	138.75±2.06	8
CMC	IIIB	>119.28	119.28±18.35	11
	ROD	>119.28	119.28±18.35	9
EAMC	IIIB	>147	147±19.71	10
	ROD	>147	147±19.71	8

EMC	IIIB	38.50	66±0.06	58
	ROD	>49.70	49.70±14.59	18
MMC	IIIB	>51.20	51.20±15.74	10
	ROD	>51.20	51.20±15.74	15
AZT	IIIB	0.00012	65.90	126
	ROD	0.00062	65.90	148

a. 50% Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.

b. 50% Cytotoxic concentration of compound, required to reduce the viability of mock-infected MT-4 cells by 50%.

Discussion

The Polynesians utilized the whole Noni plant in various combinations for herbal remedies (Wang et al., 2002, McClatchey, 2002) such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction. Antiviral activity against HIV of Noni is relatively less explored. The present study showed that the leaf extracts of *M. citrifolia* exhibited cytotoxic activity in MT-4 cells and ether extract (EMC) inhibited replication of HIV 1 at concentration below the cytotoxic level. The studies are continued to isolate active principles from ether extract of leaf powder.

Reference

Akihisa T, Matsumoto K, Tokuda H, Yasukawa K, Seino K.I, Nakamoto K, H. Kuninaga H, Suzuki T and Kimura Y. 2008. Anti-Inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda citrifolia* (Noni), J. Nat. Prod., 71(7): 1322-25.

De Clercq E. 1988. Rapid and automated tetrazolium-based colorimeteric assay for detection of anti HIV compounds J. Virol. Methods. 20:309-321.

McClatchey W. 2002. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae). Integr Cancer Ther. 1:110-20.

Nayak B.S, Isitor G.N, Maxwell N.A, Bhogadi V, Ramdath D.D. 2007. Wound-Healing Activity of *Morinda citrifolia* Fruit Juice on Diabetes-Induced Rats. J. Wound Care., 16(2):83-7.

Palu A K,. Kim A.H, West B.J, Deng S, Jensen J, White L. 2007. Anti-Inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda citrifolia* (Noni), J. Nat. Prod., 70(5): 754-60.

Palu A K,. Kim A.H, West B.J, Deng S, Jensen J, White L. 2008. The Effects of *Morinda citrifolia* L. (Noni) on the Immune System, Its Molecular Mechanisms of Action, J. Ethnopharmacol., 115(3): 502-7.

Pawlus A.D and Kinghorn D.A. 2007. Review of the Ethnobotany, Chemistry, BiologicalActivity and Safety of the Botanical Dietary Supplement *Morinda citrifolia* (Noni). J. Pharm. Pharmacol., 59(12): 1587-92.

Saludes J.P, Garson M.J, Franzblau S.G, Aguinaldo A.M. 2002. Antitubercular Constituents from the Hexane Fraction of *Morinda citrifolia* Linn. (Rubiaceae), Phytother Res., 16(7):683-87..

Su B.N, Pawlus A.D, Jung H.A, Keller W J, Mclaughlin J.L, Kinghorn A.D. 2005. Chemical Constituents of the Fruits of *Morinda citrifolia* (Noni) and their antioxidant Activity, J. Nat. Prod., 68(4): 592-8..

Wang M.Y, Anderson G, Nowicki D, Jensen J. 2008a. Hepatic Protection by Noni Fruit Juice against CCl4 -Induced Chronic Liver Damage in Female SD Rats,. Plant Foods Hum. Nutr., 63(3), 141-47.

Wang M.Y, Nowicki D, Anderson G, Jensen J, West B. 2008b. Liver Protective Effects of *Morinda citrifolia* (Noni), Plant Foods Hum. Nutr., 63(2): 59-63.

Wang M.Y, West B.J, Jensen C.J, Nowicki D, Su C, Palu A.K, Anderson G. 2002. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacol Sin., 23:1127-41.

West B.J, Jensen C.J, Westendorf J. 2006. Noni Juice is Not Hepatotoxic, World J. Gastroenterol., 12(22): 3616-20.

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Noni as a functional food

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Keywords : Functional food, phenolics, dietary antioxidants, joint juice, carotenoids, Vitamins

Abstract : "Functional Foods" are foods or dietary components that may provide a health benefit beyond basic nutrition. Noni is categorized as one of the "Superfruits". The "Superfruits" define foods with outstanding health-promoting properties. These are used widely in functional foods and beverages and other nutraceutical sectors. The success of specific category of superfruits depends largely on nutrient density, antioxidant property, research intensity & marketing efficiency. The major functional micronutrients of Noni are phenolic compounds (anthrquinones, aucubin, asperuloside and scopoletin are the most important ones), organic acids (caproic and caprylic acids are the main ones) and alkaloids (xeronine and pro-xeronine). Phenolic compounds are a group of secondary metabolites widespread in plant kingdom. Phenolic compounds possess various important biological activities, including antioxidant activity, capillary protective effect & tumour inhibiting effect. Phenolics are able to scavange reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on their stability in different systems, as well as the number and location of hydroxyl groups. Flavanoids occur widely in the plant kingdom and are major source of colors of flowers, leaves, fruits etc. From ancient times, flavanoids have been utilized as natural colors in drugs & herbal medicines. Many flavanoids have a hydroxyl group in their structure and are called "polyphenols". Recently, polyphenols are in the spotlight for their various antioxidative properties and their ability to reduce blood cholesterol levels. Similarly condensed tannins are of great interest in nutrition and medicines because of their potent antioxidant capacity. It has recently been hypothesized that the free radical scavenging properties of condensed tannins may reduce risks of cardiovascular diseases, cancer, blood clotting, urinary tract infections. Dietary antioxidants such as water-soluble vitamin C, lipid-soluble vitamin E and carotenoids contribute to both the first and second line of defense against oxidative stress. Small amounts of toxic oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl free radical are formed as by-products of oxidative metabolism

in different type of cells. Oxygen radicals & peroxides are capable of damaging lipids in the membranes. Per oxidation of their fatty acid residues lower the membrane fluidity and can lead to cell lyses. The thyol groups of cysteine of proteins can also be oxidized, thereby cross-linking & inactivating proteins. Oxidative damage to DNA may induce mutations. Hydrogen peroxide is a strong oxidant to degrade nutrients, to denature enzymes and to damage membranes by oxidizing the glycerophospholipid of membrane components. Hydrogen peroxide is a free radical initiator to autooxidative chain reaction. In biological tissues hydrogen peroxide can be removed by catalase or glutathione peroxidase enzymes. Noni juice contributes a large number of phytochemicals that scavange free radicals and save body from oxidative stress.Leading trends in functional food industry are that health and wellness is becoming the new standard for the food industry. Every food and beverage must have some positive nutritional values. All foods are fast becoming functional. Companies market intrinsic healthfulness of foods they produce. Foods with personalized nutritional benefits are becoming popular. Foods with low GI, low fat foods for calorie management, low cholesterol for heart-health, high calcium for osteoporosis, low sugar for diabetics etc. "Joint juice" beverages offer per bottle some 1500mg of glucosamine-an ingredient scientifically established to reduce joint pain.

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Introduction

The Health India Laboratories (HIL), Chennai presently produces a large number of Noni based food products for sale in the market which include Noni Apple Twist; Noni Pineapple Twist; Noni Amla Twist; Noni Mango Twist; Noni Orange Twist; Noni Pomegranate Twist; Noni Guava Twist; Noni Kokum Twist; Noni Ginger Twist; Noni Aloe vera Twist; Noni Strawberry Twist; Noni Green Tea Twist; Noni Litchi Twist; Noni Papaya Twist; Noni Mix Fruit Twist; Noni Green Mango Twist; Chilli Sauce; Fruit & Vegetable Wash; Mixed fruit jam; Noni Tees; Pineapple Jam; Soya sauce; Tomato sauce, Noni protein powder etc. These products are sold as food supplements.

Consumer health awareness continues to rise and with this, the consumption of functional foods is growing fast. The interest in functional foods and drinks has been fuelled by a desire for health and convenience. However, success of such products in the market is increasingly dependent on establishing a relationship of trust with the consumer. Scientific support to testimonials on positive health effects of Noni is being attempted worldwide. Product range of Noni based foods is being

diversified through product development and awareness about nutritional benefits of noni based products as food supplements is being increasingly created through publications or web based advertisements/ write ups. Noni is defined as a superfruit because of its healing powers.

Indian Noni (*Morinda citrifolia* L.) is an underutilized fruit of Indian origin that grows in wild in tropical parts of India with enormous health benefits when consumed as a food supplement. It possessess a large number of bioactive compounds, which are extra nutritional constituents which typically occur in small quantities in foods. Different types of bioactive compounds are phytochemicals, omega-3-fatty acids and plant sterols. Phytochemicals are pronounced as "fight-o-chemicals" i.e. they fight to protect health. Phytochemicals occur naturally in fruits and vegetables that work together with vitamins, minerals and fibre to promote health in many ways. Phytonutrients are usually related to the color of fruits and vegetables like green, yellow, red and purple. Different classes of phytochemicals are Anthocyanins, Carotenes, Flavonoids, GLucosinolates, Phenolic acids, Lignans, etc. These are extremely essential and beneficial for human beings to strengthen their immune system and help fight cancer, diabetes, CHD etc. The omega-3-fatty acids and plantsterols also act as antioxidants. Since the food that we provide to our body is deficient in the essential nutrients and micronutrients, our body is incapable of protecting our cells, tissues and vessels. In this scenario, taking Natural Supplements which have the highest forms of whole vitamins, trace minerals, essential amino acids and all the necessary phytonutrients is one of the ways to ensure nutrients to body which are missing in the diet. It is a great way to support the body nutritionally, which could protect the body from so many diseases. Indian Noni contains all the vitamins, a lot of trace minerals, 17 out of 20 amino acids along with more than 150 phytochemicals and micronutrients and prevents many diseases. Another class of phytochemicals i.e. sterols is similar to cholesterol in structure but helps in lowering cholesterol. A combination of sterols with calcium, potassium or magnesium is effective during clinical trials in lowering elevated serum cholesterol and elevated blood pressure and can also help to maintain bone health. Phenolics and anti-oxidants in fruits bind free radicals which cause cancer.

Functional food or medicinal food is any fresh or processed food claimed to have a health-promoting and / or disease-preventing property beyond the basic nutritional function of supplying nutrients. whole, fortified, enriched or enhanced foods which have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels. Clearly, all foods are functional, as they provide taste, aroma, or nutritive value. Within the last decades, however, the term functional as it applies to food has adopted a different connotation -- that of providing an additional physiological benefit beyond that of meeting basic

nutritional needs. This is an emerging field in food science in which such foods are usually accompanied by health claims. The general category includes processed food or foods fortified with health-promoting additives, like "vitamin-enriched" products. They are thus specially fortified food for a specific purpose as well as nutrition. Fermented foods with live cultures are considered as functional foods with probiotic benefits (fruit juices with bifidus bacteria are new introduction in this category). The term was first used in Japan in the 1980s when there was a government approval process for functional foods called Foods for Specified Health Use (FOSHU). Canada and Sweden, have specific laws concerning the labeling of such products. As scientists discover more and more herbal compounds with healing effects, the functional foods industry is busy trying to use them in different kinds of foods that could help control chronic diseases. Obesity and diabetes are on top of the priority list, but functional foods are also being developed for cardiovascular diseases, osteoporosis and digestive disorders. The new generation of functional foods being developed in labs would go well beyond fibre foods and healthy oils and bacteria-rich yoghurt. They are almost medicine. In fact, they will be medicine. Noni is one of the candidates of this category. The major functional micronutrients of Noni are Phenolic compounds (anthrquinones, aucubin, asperuloside, serotonins, scopoletin, damnacanthal are the most important ones), Organic acids (caproic and caprylic acids are the main ones) and Alkaloids (xeronine and proxeronine). Phenolic compounds are a group of secondary metabolites widespread in plant kingdom. Phenolic compounds possess various important biological activities, including antioxidant activity, capillary protective effect and tumor inhibiting effect. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on their stability in different systems, as well as the number and location of hydroxyl groups. Flavanoids occur widely in the plant kingdom and are major source of colors of flowers, leaves, fruits etc. From ancient times, flavanoids are utilized as natural colors in drugs and herbal medicines. Many flavanoids have a hydroxyl group in their structure and are called "polyphenols". Recently, polyphenols are in the spotlight for their various antioxidative properties and their ability to reduce blood cholesterol levels. Similarly condensed tannins are of great interest in nutrition and medicines because of their potent antioxidant capacity. It has recently been hypothesized that the free radical scavenging properties of condensed tannins may reduce risks of cardiovascular diseases, cancer, blood clotting, urinary tract infections. Dietary antioxidants like as water-soluble vitamin C, lipid-soluble vitamin E and carotenoids contribute to both the first and second line of defense against oxidative stress. Small amounts of toxic oxygen species like superoxide anion, hydrogen peroxide and hydroxyl free radical are formed as by-products of oxidative metabolism in different type of cells. Oxygen radicals and peroxides are capable of damaging lipids in the membranes. Per oxidation of their fatty acid residues lowers the membrane fluidity and can lead to cell lyses.

The thyol groups of cysteine of proteins can also be oxidized, thereby cross-linking and inactivating proteins. Oxidative damage to DNA may induce mutations. Hydrogen peroxide is a strong oxidant to degrade nutrients, to denature enzymes and to damage membranes by oxidizing the glycerophospholipid of membrane components. Hydrogen peroxide is a free radical initiator to autooxidative chain reaction. In biological tissues hydrogen peroxide can be removed by catalase or glutathione peroxidase enzymes. Noni juice contributes a large number of phytochemicals which scavange free radicals and save body from oxidative stress.

Food As Medicine: Beyond nutrition, foods now gain a dimension of medical functionality. Functional foods labeled as "nutraceutical products" are a hybrid of nutrition and pharmaceuticals. Nutraceuticals refer to extracts of foods claimed to have a medicinal effect on human health. These extracts were added to the list of supplements under the US Dietary Supplement Health and Education Act (DSHEA). The nutraceutical is usually contained in a capsule, tablet or powder in a prescribed dose. Examples of claims made for nutraceuticals are resveratrol as an antioxidant, soluble dietary fiber products, such as psyllium seed husk for reducing hypercholesterolemia and soy or clover (isoflavonoids) to improve arterial health. Other nutraceutical examples are flavonoid as antioxidants, alpha-linolenic acid from flax seeds, beta-carotene from marigold petals, anthocyanins from berries, etc. Many botanical and herbal extracts like ginseng, garlic oil, etc. are developed as nutraceuticals. Nutraceuticals are often used in nutrient premixes or nutrient systems in the food and pharmaceutical industries. Noni capsules fall under this category. The opinion that "Let food be thy medicine and medicine be thy food", embraced by Hippocrates nearly 2,500 years ago, is receiving renewed interest. Most Indians know that curcumin in turmeric is anti-inflammatory, anti-malarial, anti-HIV and anti-tumour, anti-diabetic and inhibits reproduction of herpes simplex virus at even low concentrations. It also lowers production of glucose in the liver. However, curcumin is not being used as medicine traditionally but is a regular part of Indian culinary. It is shown that curcumin absorption improves with piperine, a molecule found in pepper and chillies. The traditional Indian way of cooking with turmeric and chillies makes perfect sense, according to modern science. Recent studies show that fruit juices can provide many health benefits. Scientists showed recently that orange juice is a healthy food even to diabetics because of presence of flavanoids, a broad category of compounds derived from plants. Drinking orange juice is, thus, healthy, but it is normal food. But it could be turned into a functional food through the addition of other ingredients, and turned into medicine, orange juice fortified with sterols, plant-based steroids can lower cholesterol, particularly low density lipids (LDL). Studies show that drinking one glass of beetroot juice a day can lower blood pressure significantly. Regular consumption of beetroot juice is thus beneficial for hypertension, but it may not be practical because one would then need to drink a different vegetable or fruit juice for each disease. In fact, the

art of combination is one of the principles of developing functional foods.

Food supplements are food products that supplement diets through nutrients or other substances they contain or influence the nutritional or physiological functions of people in some way or the other even though they may look like and be used in the same way as medicinal products. They must not have been approved for medicinal use. They are not meant to be used as substitutes for a varied diet.

Food supplement means a pre-packaged product sold in the form of a pellet, capsule, pastille, tablet, pill, powder, concentrate, extract, liquid or some other dosage form marketed as a food product. Food supplements are taken in small doses and the amount of energy obtained from them is not significant in terms of the diet. The energy obtained from a food supplement, according to its maximum dosage instruction, is not significant if it does not exceed 200 kJ (50 kcal) per day and provide at least 30% of the daily intake of reference value/ daily intake recommendation.

Food supplements include products for intake of vitamins, minerals, fiber and fatty acids and various herbal products. Food supplements are normally used for one of their nutritional characteristics, for instance as sources of vitamins, minerals or fatty acids to supplement the diet. They may also have a physiological effect, for instance on digestion, blood pressure or cholesterol level. People use products containing food supplements like iron, calcium, selenium or chromium, mostly on an occasional basis. The natural product business has categorized food supplements in the following way:

- Vitamins and minerals
- Products containing plants or plant extracts
- Fibre and weight control products
- Lecithin and fatty acid products
- Algae products
- Bee products
- Sports nutrients
- Other food supplements

Free radicals and health: A free radical is a compound with an odd number of unpaired electrons. In some cases they may add oxygen directly to the double bond to form a biradical essential for body immunity and to fight diseases. Millions of free radicals are formed every second in our body and excessess are neutralized by enzymes like catalase, glutathione peroxidase, etc. However, under stress conditions or polluting environment (Adulterated food, chemical residues and toxins) much more free radicals are produced in our body (Plate 1). Body enzymes

are unable to neutralize all of these and they start harming the vital systems of the body as well. It is under such situations that consumption of antioxidant laden foods becomes helpful (Plate 2). Investigations reveal that an inverse relation exists between consumption of antioxidant rich foods like Noni , fruits and vegetables and diseases. Relationship and mechanisms have been demonstrated by Steinmetz and Potter (1991.

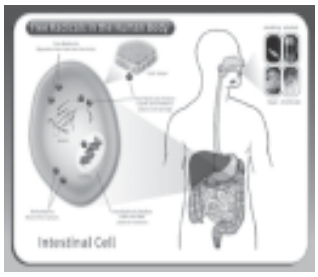
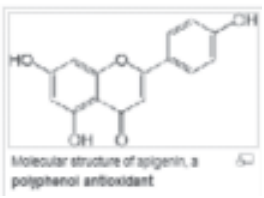


Plate 1

Source: www. Bmglabtech.com

"Super Fruits" and Fight-O-Chemicals: "Superfruits" are a new category of exotic fruits with exceptionally high antioxidant potential. The terminology of "Superfruits" was first coined in the USA in mid-1990's, to define foods with outstanding health-promoting properties. Several examples of the most popular superfruits are Noni (Plate 6), Pomegranate, Indian gooseberry (Plate 14), Jamun (Plate 11), Bael (Plate 7), blueberry and mangosteen. These are used widely in functional foods and beverages and other nutraceutical sectors. The success of specific category of superfruits depends largely on nutrient density, antioxidant property, research intensity and marketing efficiency. The enduring vibrancy of the superfruits markets rests largely on research-oriented supplies and marketing strategies. Amongst the superfruits, noni is one of the most potent fruits that helps body fight several diseases because of its antioxidants - phenols, organic acids, alkaloids, anthocyanins, carotenes, flavonoids, glucosinolates, phenolic acids, lignans, phenolic compounds (anthrquinones, aucubin, asperuloside and scopoletin are the most important ones), organic acids (caproic and caprylic acids are the main ones) and alkaloids (xeronine and proxeronine). These phytochemicals of noni play beneficial role in preventing several diseases.



Molecular structure of apigenin, a polyphenol antioxidant

Grapes contain certain polyphenol antioxidant compounds.

Plate 2 Plate 3 Plate 4

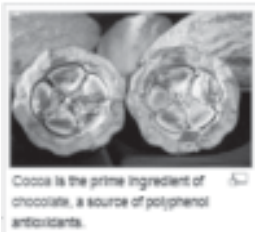


Plate 5

Plate 6

Plate 7

Source for photos: www. Iacmusic.com

Effects of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation was demonstrated by Day et al 1998 (Plate 3).

Noni Twists (Joint Juice Beverages): Joint juice beverages are a concept where benefits of several fruits (Plates 5, 8, 9) are availed by combining their juices. Aonla fruit is rich in phenol (290 mg/100g) and antioxidant activity (56.8 mM) B-carotene lineolate system (92%) and superoxide anion scavenging activity (85%). Vitamin C

Plate 8

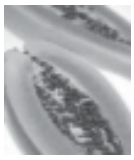


Plate 9

Plate 10

Plate 11

Plate 12

Plate 13

Plate 14

(500-1500mg/100g) (Plate 14). Pomegranate on the other hand is one such fruit in which the synergy of punicalagin and free ellagic acid exists (Plates 4, 12).

These two types of ellagitannins are found only in pomegranate which enhance the antioxidant effect of each other. Pomegranate can actually reduce existing plaque formations in arteries.

Strawberry contains moderate antioxidants with an activity of 12-64 mM FRAP. Pink Guava (Plate 10) contains lycopene and ellagic acid. Lycopene is the most efficient biological carotenoid singlet oxygen quencher as observed by Di Mascio et al. (1989). The potential role of lycopene for human health is also studied by Gerster (1997), whereas, Jamun contains Combolian glucoside that arrests conversion of starch to sugar and is hence useful for people with diabetese. Gerberesin, gallic acid and other coloured compounds in jamun are potent antioxidants. Bael with 2 furocoumarins-psoralen (>tolerance to sunlight) and marmelosin ($C_{13}H_{12}O_3$)-exhibit laxative and diuretic effects. Tannins (9%) in bael fruit are potent antioxidants. Hence, by combining juices of these fruits one can avail multiple benefits of these different super fruits. In citrus based blended beverages, biochemistry and beneficial biological functions of citrus limonoids are shown by Hasegawa and Miyake (1996).

Source for pictures: www.mixextrading.tradeindia.com

Plate 15

Noni juice concentrate, a functional food supplement contains

- 150+ Phyto-Chemicals
- Phenolic compounds :
- Organic acids :
- Alkaloids :
- Fibre
- Mood Enhancers
- Performance Enhancers
- Digestion Improvers (Plate 15)

The role of phytochemicals in cardiovascular diseases is illustrated for healthcare professionals from the American Heart Association by Howard and Kritchevsky (1997).

Noni tees: Ayurved tee, Sena tee, Morinda leaves tee, Gymnema tee and Green Tee are available in markets. Inhibition of carcinogenesis by tea was studied by Dreosti

et al (1997), with the evidence from experimental studies proving that skin glows and its health improves by tea consumption. There is improvement in digestion, gas problems, constipation, regenerative and antioxidant properties etc. Green Tea is added to smoothies, juice drinks and water for its vitamin E, weight management and brain health benefits. Each 500 ml RTD bottle contains 230 mg catechins, isoflavones, vitamin D and collagen said to be beneficial for skin health. Tea is second only to water as the most widely consumed beverage in the world. A great deal of attention is directed to polyphenolic that comprises up to 30% of the total dry weight of fresh tea leaves. The four major green tea catechins are epigallocatechin-3-gallate, epicatechin, epigallocatechin, epicatechin-3-gallate which are inversely associated with mortality from CHD (Heart Health) (Plate 16).

Plate 16

Noni wellness water: Wellness drops can change a bottle of water into enjoyable experience! One can experiment by mixing any of Noni's twelve flavours to create own unique wellness drops. These Near Water Drinks as available internationally are lightly flavoured bland drinks with a low calorie content targeted primarily at young women. "Active conditioning water" contains maltodextrins, vitamins B & C, reishi, seaweed extract and chamomile (Plate 17). Near water drink with aloe and non-alcoholic chardonnay wine extract is popular in japan. Similarly a canned, ready-to-drink "tea water" is marketed by Coca-cola company in Japan. Silk water that is non-carbonated anad low-calorie water with silk powder promote collagen regeneration for beautiful skin. Also the low calorie water with collagen, vitamin C, 1% grape juice/ peach juice with dietary fibre and vitamin B are becoming very popular.

Plate 17

Noni Smoothy: Smoothy is a semi-liquid diet containing all essential nutrients required for efficient functioning of body systems, taking care of dietary requirements in full (Plate 18). It contains all natural wholesome foods in the form of 7 vegetables, 7 fruits, 7 herbs, 7 nuts, 2 oils and Noni juice rich in nutraceuticals. These food materials are obtained in natural way by organic farming, without any pesticide. Preparation is neither coloured nor has any added

preservatives. Smoothy takes care of all the nutritional requirements, in addition to health promoting disease preventing activities. It prevents a number of diseases like cancer, inflammatory diseases (e.g. arthritis) and degenerative diseases which are caused due to improper diet. It is the real food for longevity and people can live a comfortable and disease free life for a long time. It is not a substitute or supplement but a complete diet full of nutrition & energy. Smoothie is thus rightly called a "natural superfood" that has witnessed sales to rocket from zero in 1999 to over $ 240 million by 2007. The combination of health, newness, rarity value and convenience enables such fruit and herb blends to command massive price premium of 400% in case of pomegranate and 800% in case of mangostein based smoothies compared to regular juices.

Plate 18

Chilli Soya and Tomato Sauce:- Provide a new world of taste and wellness (Plate 19). Soy proteins and tomatoes lycopene are the phyto-chemicals which provide health benefits in these products. The cholesterol-lowering effect of soy is the most well-documented physiological effect. Consumption of soy protein results in significant reductions in total cholesterol (9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%), with a small but insignificant increase (2.4%) in high density lipoprotein (HDL) cholesterol. Several classes of anticarcinogens are identified in soybeans, including protease inhibitors, phytosterols, saponins, phenolic acids, phytic acid, and isoflavones (Messina and Barnes, 1991). Of these, isoflavones (genistein and daidzein) are particularly noteworthy because soybeans are the only significant dietary source of these compounds. Isoflavones are heterocyclic phenols structurally similar to the estrogenic steroids. Because they are weak estrogens, isoflavones may act as antiestrogens by competing with the more potent, naturally-occurring endogenous estrogens (e.g., 17b-estradiol) for binding to the estrogen receptor. Soy may also benefit bone health (Anderson and Garner, 1997). The role of soy products in reducing risk of cancer was demonstrated by Messina and Barnes (1991), Erdman and Potter (1997).

Plate 19

Noni Jam's:- Noni pineapple and mixed fruit jams contain permitted food colours 102, 122 and 127 and added flavours, natural, nature identical, artificial flavouring substances and permitted class II preservatives (Plate 20, 21). Internationally in functional food category Jams with 360mg of polyphenols, high anthocyanin content from blueberries and jelly-dessert in bite size come in green tea flavours that contain isoflavones, vitamin D and collagen beneficial for skin and general health.

Plate 20

Plate 21

Noni Wellness Shake: Instant protein noni drink gives body a load of nutrients (Plate 22). This ideal meal alternative manages calorie intake while providing nutrition and hence is a healthy and complete diet for all age groups. It contains protein, carbohydrates, vitamins, minerals and many nutraceuticals. It has protein digestibility corrected amino acid score (PDCAAS) of 1.0, representing the highest possible score for a quality protein source. It provides all essential amino acids contributed either by soy protein isolate or Morinda citrifolia.

Plate 22

Future Vision :

Mood Foods: Serotonin (Noni) is an important brain neurotransmitter, and plays a significant role in temperature regulation, sleep, hunger and sexual behaviour. A powerful link exists between nutrition, mood and mental health. About 50 mg of L-theanin an aminoacid in green tea causes an increase in frequency of alpha brain waves that are associated with relaxation. Similarly beta brain waves associated with tension, anxiety and irritation are caused to decrease of 2-thanin. Also the ingredients such as GABA (an amino acid), PS (Phosphatidylserine) also stimulate brain relaxation (Plate 23, 24, 25).

Plate 23

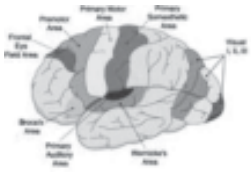


Plate 24

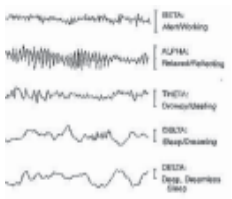


Plate 25

Source: www. Web us. com

Foods For Coronary Heart Diseases: India is becoming global capital for cardiovascular and diabetic diseases (Plate 26). Functional foods with ethnic culinary properties need to be developed for specific Indian markets. Noni if used in such food supplements would prove helpful in preventing or delaying occurrence of such problems. Scopoletin(C10H8O4) in Noni reduces Blood Pressure. Orange juice (Noni Twist) fortified with plant based steroids can lower cholesterol, particularly low density lipids (LDL). Studies show that drinking a glass of beetroot juice a day can lower blood pressure significantly. Regular consumption of beetroot juice is thus beneficial against hypertension.

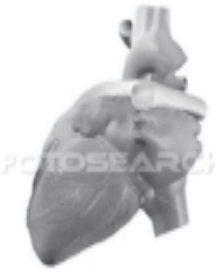


Plate 26

Performance Foods: The foods that impact physical and mental performance. The products based on Ginseng and Gingko biloba that contain high polyphenols and caffeines stimulate mental performance and mood and impact occurrence of Alzheimer's disease.

Foods for digestive health: Probiotic and Prebiotic juices "help enhance digestion and speed intestinal transit time". The digestive system is a hub of the entire body. (Plates 27, 28, 29. 30). If due to some reasons, overacids are produced in the body during digestion, a glycoprotein-mucoidplaque is formed to protect the mucous membranes from damage. When this condition continues for long time digestion becomes more and more dysfunctional and constipation also sets in. It is under such situations that anthraquinones (Noni) remove the plaque and restore body back to normal digestion. Probiotic and Prebiotic juices "help enhance digestion and speed intestinal transit time".

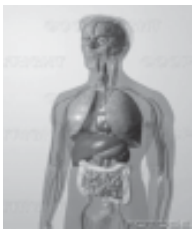


Plate27

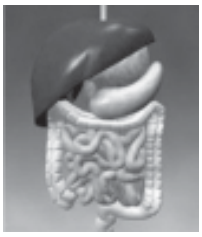


Plate 28


Plate 29


Plate 30

Sports and Energy Drinks: Though in "infancy," but are expected to post strong growth in future in the niche segment. The fortified juice market is experiencing credible "double digit growth," and is the fastest growing functional beverage item in the market. In the near future, the market is expected to grow at least 30-35% "due to a growing necessity of convenience packaged foods that serve the benefits of fresh fruits reinforced with added

vitamins and minerals." In fact, the fortified juice market is expanding so rapidly "that it is beginning to reach the mainstream beverage market."

References

Anderson, J.J.B. and Garner, S.C. 1997. The effects of phytoestrogens on bone. Nutr. Res. 17: 1617-1632.

Anonymous. 1998. U.S. topselling herbs and supplements. Nutraceuticals International, 3 (2): 9.

Day, A. P., Kemp, H.J., Bolton, C., Hartog, M. and Stansbie, D. 1998. Effects of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. Annals of Nutrition and Metabolism, 41: 353-357.

Di Mascio, P., Kaiser, S., and Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Archives of Biochemistry and Biophysics. 274: 532-538.

Dreosti, I.E., Wargovich, M.J., and Yang, C.S. 1997. Inhibition of carcinogenesis by tea: The evidence from experimental studies. Critical Reviews of Food Science and Nutrition, 37: 761- 770.

Erdman, J.W., Jr., and Potter, S.M. 1997. Soy and bone health. The Soy Connection 5 (2): 1, 4.

Gerster, H. 1997. The potential role of lycopene for human health. J. American Coll. Nutrition. 16: 109-126.

Hasegawa, S. and Miyake, M. 1996. Biochemistry and biological functions of citrus limonoids. Food Reviews International. 12: 413-435.

Howard, B.V. and Kritchevsky, D. 1997. Phytochemicals and cardiovascular disease -- A statement for healthcare professionals from the American Heart Association. Circulation 95: 2591-2593.

Messina, M. and Barnes, S. 1991. The role of soy products in reducing risk of cancer. Journal of National Cancer Institute. 83: 541-546.

Sanders, M.E. 1994. Lactic acid bacteria as promoters of human health. In: "Functional Foods -- Designer Foods, Pharmafoods, Nutraceuticals", ed. I. Goldberg, pp. 294-322. Chapman & Hall, N.Y.

Steinmetz, K.A. and Potter, J.D. 1991. Vegetables, fruit and cancer II. Mechanisms. Cancer Causes Control, 2: 427-442.

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Role of Noni in Nutrition Security

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Healthy human capital is a prerequisite of any country and sought after by all countries for sustainable human development which is important for socio-economic development of any country. The food and nutrition security of population is mainly dependant on plant foods grown in the region, although animal foods also contribute to nutrition security to a certain extent. After the country attained independence, due to various socio economic seasons population had no access to adequate quantity of even grains which contribute mainly cereals and proteins to Indian diet. Public health requirements were also not met in many regions of the country. This situation was prevalent in 1950-60 and the vulnerable sectors of population especially women and pre school children could not meet their protein and caloric requirements. This situation compromised their immune system resulting in their vulnerability to a number of communicable diseases such tuberculosis, malaria smallpox, chickenpox, diarrhea etc.

In 1960s there was tremendous pressure on all concerned including government and farmers to increase food production specially cereal grains. This led to green revolution which took care of calorie and protein gap. Simultaneously prophylaxis programmes such as distribution of iron, folic acid tablets, administration of vitamin A capsules and immunization programmes were initiated. Nation can't wait until all foods were produced to provide access (physical as well as financial access to all) to thwart certain nutrient deficiencies. Hence, public health programmes took care of certain vitamin and mineral deficiencies.

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While green revolution was pushed forward to augment protein and calorie deficiencies, little attention was paid to micro nutrient content of diets, as there was not adequate access on micro nutrient rich foods like fruits and vegetable. The white and yellow revolutions did take care of the economy but not so much of the micronutrient needs of population. Dairy development did increase milk production, but did not increase milk consumption of vulnerable sectors as envisaged at the beginning.

Micronutrient deficiency although known as hidden hunger is affecting large population. The UN millennium declaration adapted a series of goals to reduce extreme poverty and promote food and nutrition security.

The goals established are :

1. Eradicate extreme hunger and poverty
2. Achieve universal primary education
3. Reduce gender inequality and empower women
4. Reduce child mortality
5. Improve maternal health
6. Combat HIV/AIDS, malaria and other diseases
7. Ensure environment sustainability
8. Develop a global partnership for development

Most of the goals are related and can be achieved only with food and nutrition security.

Although calorie and protein deficiency has been overcome, the nation is caught up with micronutrient gaps. Micronutrients are recognized to be essential as micronutrient malnutrition not only compromises the immune system but also irreversibly retard brain development in uterus and for upto two years of age in infants. Physically and mentally handicapped child will not be able to achieve the genetic potential in cognitive ability. Such children may be of less fit to control their environment and provide for their own food and nutrition security in later life to compete with education and better opportunities. All this mean that micronutrient insufficiency if unchecked, will contribute to the degenerative cycle of poverty.

Having said that, what is the solution to make up micronutrient deficiency, certainly first priority is for production of well accepted popular foods such milk, animal foods, fruits and vegetable. Even today animal foods and fruits are beyond the reach of poorer sections of population. Among vegetables, the post harvest / past-production system is not geared as yet to prevent micronutrient losses. Of late even vegetables are competing with animal foods in terms of cost escalation. Home gardens/ back yard gardens are dwindling as the household space in urban areas are getting reduced. Situation demands us to look for some less known, uncommon fruits and vegetables to meet the micronutrient requirement of population.

Noni will be a fruit which can provide several important micronutrients to the diet. Although Noni is not new to Indians, it is new as an edible fruit. It is a Hawaian name for Morinda citrifolia. The Polynesians are known to be using Noni fruit for medicinal purpose for more than 1000 years. The fruit is claimed to cure several diseases and is primarily used to stimulate immune system. It is useful to control fungal, bacterial, viral and parasitic populations. It is claimed to be arresting

formation and proliferation of tumour including malignancy (Dixon 1999, Earle 2001).

Recently Noni juice has become popular because of commercial interest in Noni has increased in recent years as observed by increasing number of patents for Noni foods in USA and Europe. Nevertheless there are few scientific studies on the nutritional composition and availability of nutrients present in Noni.

Chemical composition of Noni

About 160 phytochemical compounds have been identified in the noni plant and the major micronutrients present in Noni are phenolic compounds, organic acids and alkaloids Of the phenolic compounds reported, the most important are anthraquinones, aucubin, asperuloside and scopoletin. The main organic acids are caprioc acid, caprylic acids (Dittmer, 1993) and the principal alkaloids is xeronine (Heinicke, 1985). Chemical composition is known to differ with variety and much information is not reported about this.

Minerals account for 8.4% of dry matter and are mainly potassium, sulphur, phosphorus and traces of selenium (Chunhieng, 2003). The minerals are not the ones that are deficient in normal Indian diet. Of the vitamins, ascorbic acid (24-15mg/100 g dry matters) and carotene are reported to be present (Morton 1992, Dixon et al., 1999; Shovic and Whistler, 2001).

The therapeutic benefits are largely attributed to these compounds present in micro quantities and traces. However, they are important and are used in biological function. Much work needs to be carried to have clear and in depth understanding. Some of the beneficial compounds and their activities are ad follows.

Glycosides

Noni contain many glycosides - Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule. The nonsugar component is known as the Aglycone. The sugar component is called the Glycone. If the glycone group of a glycoside is glucose, then the molecule is a glycoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide etc. In the body, toxic substances are often bonded to glucuronic acid to increase their water solubility; the resulting glucuronides are then excreted. Many plants store important chemicals in the form of inactive glycosides; if these chemicals are needed, the glycosides are brought in contact with water and an enzyme, and the sugar part is broken off, making the chemical available for use. Asperuloside is glycoside. Traditionally, this glycoside has been used for diurises (reducing water retention), treating inflammation and varicose veins and phlebitis. Research has indicated that it is anticlastogenic (that is,

prevents the breakage of chromosomes). As a result, it is anti-mutagenic or resists mutation within the cell's DNA.

Three new glycosides isolated from *Morinda citrifolia* (Noni) fruit. They are:

1. 6-O-(-D-glucopyranosy1)-1-O-ictanoy1- -D-glucopyranose,
2. 6-O (-D-glucopyranosg1-1)-1-O-nexanoyl- -glucopyranose
3. 3-methylbut-3-enyl-6-O- -glucopyranosyl- glucopyranoside

The phytochemicals present in noni

Rutin

Rutin, a phytochemicals has also the property to chelate metal ions, such as iron. Hence noni can be helpful in thalassemia condition.

Rutin also seems to stabilize vitamin C. If rutin is taken together with vitamin C, the activity of ascorbic will be intensified

Rutin is important because it strengthens capillaries and can help people who bruise or bleed easily. Rutin may help to prevent atherogenesis and reduce the cytotoxicity of oxidized LDL - cholesterol. Hence it helps to prevent formation of atherosclerotic plaques inside the arteries and thus has cardio protective function.

Studies have demonstrated that rutin can help to stop venous edema, which is an early sign of chronic venous disease of the leg. Hence it may help in varicosity of vein

Caprylic acid

Caprylic acid is a fatty acid that has antifungal properties

Scopoletin

Scopoletin exhibits a cholesterol lowering effect and vasodilating properties. Hence, it helps in hypertension, hypercholesterolemia and prevention of CAD. Cyclooxygenase and 5-lipoxygenae have been found to cause inflammation and pain. Scopoletin has been demonstrated to have inhibitory effects on these mediators. Hence, noni has anti inflammatory and analgesic activity. Scopoletin has shown to have anti-spasmolytic activity on the uterus and small intestine, therefore being benefical in menstrual cramping and irritable bowel conditions. Scopoletin has bacteriostatic activity against various species of bacteria, including Escherichia coli, Staphylococcus aureus, Streptococcus sp., Klebsiella pneumoniae and Pseudomonas aeruginosa

Ursolic acid

Ursolic acid has anti fungal property. It inhibits the growth of Candida albicans and Microsporium lenosum. Ursolic acid has anti-inflammatory properties and is used in ointments to treat burns.

Physico-chemical composition of Noni juice

Characteristics (2002)b	Shovic and Whistler (2001)a	European Commission
pH-value	-	3.4-3.6
Dry matter	-	10-11%
Total soluble solids (Brix)	-	-
Protein content	0.4 g/100 g	0.2-0.5%
Lipid	0.30 g/100g	0.1-0.2%
Glucose	-	3.0-4.0 g/100g
Fructose	-	3.0-4.0 g/100g
Potassium mg/100 g	188 mg/100 g	30-150g
Sodium	21 mg / 100 g	15-40 mg/100g
Magnesium	14.5 mg/100 g	3-12 mg / 100g
Calcium	41.7 mg / 100 g	20-25 mg / 100g
Vitamin C	155 mg / 100 g	3-25 mg / 100g

References

Dittmar, A., 1993. *Morinda citrifolia* L. - Use in indigenous Samoan medicine. Journal of Herbs, Spices and Medicine Plants, 1:77-92.

Dixon, A.R., McMillen, H., Etkin, N.L., 1999. Ferment this: the transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). Ecological Botony 53: 51-68.

Earle, J.E., 2001. Plantas Medicinales en el Tro' pico Hu' medo. Editorial Guayaca' n, San Jose'.

Heinicke, R.M., 1985. The pharmacologically active ingredient of Noni. Bulletin of the National Tropical Botanical Garden, 15: 10-14.

Morton, J.F, 1992. The ocean-going Noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colourful" relatives. Ecological Botony, 46: 241-256

Shovic, A.C., Whistler, W.A., 2001. Food sources of provitamin A and vitamin C in the American Pacific. Tropical Science, 41: 199-202.

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Noni Utilization and Value addition - A review

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Keywords :

Abstract : The noni (*M. citrifolia* L.) is becoming the most economically important plant species among the members of the genus Morinda. Its different potentials have already been realized and several developed products being marketed worldwide.The processing of noni fruits (*Morinda citrifolia* L.) for value added products depends on intended processing methods for pulp/juice extraction and their utilization in preparation of value added products which has commercial potential. Most of the noni juice processor prefers the hard white stage of fruit as harvesting indices for the extraction of pulp and juice production. The juice extraction efficiency by traditional drip extraction method accounted nearly 42-50% yield depending on original fruit weight. The great variation of traditional drip extraction method resulted in a non-fermented sweet juice. The extracted juice during storage have shown marked degradative changes in nutraceutical value and hence there is an urgent need of the hour to utilize noni fruit pulp as well as juice for the preparation of value added products like squash, flavoured beverage, diet noni, syrup and bar, which are having commercial importance in the market. Moreover, the noni fruit pulp could be exploited for the preparation of fruit powder, toffee and various noni powder enriched food products like cookies and fruit bar. The technologies should be standardized and assessed for the preparation of value added products which can be easily available at cheaper cost for commercialization. So the primary challenge to the food technologist is to effectively translate nutraceutical and medicinal benefits of this folk remedy into shelf stable food products. So that whatever technologies were developed must be assessed and scale up for their techno-economic feasibility which can be disseminated to the common masses to develop their entrepreneurship. This will helps in turn to open a new avenue for the farmers, unemployed youth and rural women to establish agri-processing units at their own village or region.

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Introduction

Noni (*Morinda citrifolia*) also known as Indian mulberry is highly gaining popularity as botanical remedy and food supplement traded on the international market. It is usually the fruit juice that is sold fresh or in the form of dry powder. All parts of the plant, leaves, break, root and fruits are useful as medicine. A number of diseases are cured by regular use of noni juice. These are pain, arthritis, diabetes, high blood pressure, skin and stomach ulcers, depression, senility, diarrhea, CHD, arteriosclerosis, cancer, tumor, AIDS, skin parasites, bad breath etc. Noni contains 150 nutraceuticals and other physiologically active compounds such as xeronin, anthraquinones, alkaloids, ursolic acid, cprylic acid, bet-sitosterols, etc which accounts for the medicinal effect of Noni (Wang et al. 2002; Mathivanan et al. 2005). Though originated in India and its medicinal uses are recorded in Ayurveda and Siddha systems of Indian medicine, but till today noni plant is not fully exploited in India except World Noni Research Foundation (WNRF), Chennai.

Consumption Trends

Urban consumers in India have become more exposed to western lifestyles, through overseas travel and presence of foreign media in India. For example, more than 5 million Indians travels abroad every year and this number is expected to increase by 15 per cent to 20 per cent per annum. Increase in the population of working women and increasing prevalence of nuclear double income families, especially in urban areas, are other trends shaping lifestyles. The food processing sector has been impacted by these trends as there has been an increase in the demand for processed, ready-to-cook, ready-to-eat food and awareness about food safety and nutraceutical. It has been assessed by Euromonitor International, a market research company, that the amount of money Indians spend on meals outside the home has more than doubled in the past decade to about US$ 5 billion a year, and is expected to further double in the next 5 years. These trends imply significant growth potential for the sector in future and add to its investment attractiveness (KPMG Report on Food Processing, 2006).

Value Added Products from Whole Noni Plant

Each and every part of the *Morinda citrifolia*.L plant can be utilized for the preparation of various value added food products depending upon the therapeutic values of respective parts. Zin et al. 2002. Studied the antioxidative activity of extracts from different parts of Morinda citrifolia L., including leaf, fruit and root. They reported that roots showed the highest activity of the parts tested. The results suggested that several compounds contributed to antioxidative activity of different

parts of noni. Activity in the roots may be due to both polar and non-polar compounds but, in the leaf and fruit, only to non-polar compounds.

Bark and Root

Noni bark or roots are employed in Hawaii's and elsewhere as a dye used on Broussonetia papyrifera Vent (Moraceae), felted clothing (so-called "bark cloth"). The inner bark of Mcitrifolia is scraped or "scratched" off of the tree and pounded in the dye production process. The final dye is then painted (or marked) on the clothing. The passage of the legend can be interpreted as referring to the process of preparing this dye and may have nothing to do with consumption of the fruit. M citrifolia bark (and other parts) contain a number of anthraquinones that are responsible for traditional use of the root and stem bark as a dye (Leister, 1975). Morton (1992) speculated that the anthraquinones are the reason that bark extracts are used to treat ringworm. Bark powder is used as an adulterant to sandal powder, got applications in facial creams, soaps and lotions.

Root contains carbonates, phosphates manganese, ferric ion, sodium, glucosides, resins, orindin, morinadadial, rubiatin, sterols etc. and its aqueous extract have analgesic effect, with no side effects & sedative at high doses. Root extract lower the blood pressure and has a hypotensive effect. It is also used to treat joint swelling and gout and act as an ideal therapeutic for nasal congestion, lung infections and hemorrhoids.

Flower

Noni contains three types of glucophyranosides. Eye drops can be made from flower decoctions for eye complaints.

Seed

Seeds or seed powder is especially used for the treatments of bowel disorders. In Philipines, seeds are eaten to expel intestinal worms.

Leaves

Leaves of Morinda Citrifolia are rich in amino acids (18), anthraquinones, glucosides, plant sterols, b-sitosterols, urosilicacid,resins, bioflavonoids and antioxidants. Chewing leaves used to help sooth sore throats. Leaf extract inhibit excessive blood or inhibit formation of blood clot .Leaves are used to treat gout, gingivitis, tuberculosis, ring worms, arthritis, joints, headache,cough, nausea and colic due to its anti inflammatory activity. Contains novel compounds called iridoids which can be used to treat cancer and other diseases.Besides these uses,it is also used for the skin to treat ulceration and minor infections and as general tonic.

Herbal Tea And Noni

Consumers are now replacing their regular tea with herbal teas. Herbal teas are infact infusion or decoction of flowers, leaves, roots, barks, fruits and seeds of herb/herbs.Consumers wants variety of herbal teas for their choice depending upon their needs such as slimming tea, stress relieving tea, mild care tea , diabetes tea, cardiac tea etc.Indian consumer products market is growing at fast rate and herbal products forms significant part of that market.

Noni Herbal Tea

Caffeine free tea can be made from nutrient rich noni leaves.Hot water releases therapeutic compounds found in noni leaf which after drinking quickly assimilated into the blood stream.Sensory properties of noni leaf tea can be improved by fortifying herbs, spices, medicinal and other aromatic plant parts.

The taste, aroma, flavor and therapeutic properties can be enhanced by using especially tulsi, cinnamon , cardamom , ginger, amla, mint, lemon grass, vanilla, chamomile etc, because of their specific inherent therapeutic properties, which are highlighted below. Color and appearance could be improved by using safflower petals, rosella calyx, beetroot powder, vanilla, corn silk , annatto etc.

- Tulsi : Restorative and stress relieving
- Ginger : Good for digestion, blood circulation , cough and cold
- Amla : Amriphal, rich source of vitamin C and antioxidant, effective against number of diseases, anti aging , protect free radical damage
- Chamomile Flowers : Soothing and calming effect
- Corn Silk : Rich in vitamin K , diuretic, blood clotting- a 700 year old recipe

Number of formulations can be developed by using noni leaf and fortifying with other herbs. Thus naturally noni fortified herbs is caffeine free tea that helps in eliminating toxins from body thereby maintaining proper digestion and healthy immune system as well as protect cells and tissues from free radical damage.

Noni Fruit

Noni fruit is the only natural resource to contain more than 150 isolated nutraceuticals. Major groups of nutraceuticals includes anthraquinones, terpenes,sterols, amino acids (17 out of 20), essential fatty acids, proxeronine, scopoletin, phyto nutrients, selenium, soluble and insoluble fibre, minerals, vitamins, phenolic compounds (Wang et al., 2001).

Therapeutic Properties of Noni Fruit

Yanine Chan-Blanco et al. (2006). reviewed the nutritional and therapeutic properties of noni fruit. *Morinda citrifolia* L., had been used in traditional Polynesian medicine for over 2000 years. Recent studies had shown that this fruit got antibiotic and antioxidant properties in vitro and also reported that in the future, the nutritional and medical values of the noni could be assessed, especially its anti-cancer activity, this fruit could play a noticeable economic role in producing countries.

M. citrifolia L. fruit has high amount of proxeronine and proxeronase, precursor of xeronine, an alkaloid, which plays a key role in encouraging proper cell function and growth in the human body.

Noni fruit is considered important because of its wide range of therapeutic potentials such as antibacterial, antiviral, antitumor, antihelminthic, analgesic, hypotensive, antinflammatory and immune enhancing effect or immuno-modulator, low glycemic index and antioxidents. Effective for muscular pain, arthritis, headache, skin burn, fever, cold, cough, pneumonia, constipation, depression, ageing, diabetes, hypertension, skin and stomach ulcers, diarrhea, CHD, arteriosclerosis, cancer, tumor etc. (Bratman and Girman, 2003).

Major Constraints in Commercial Utilization

The major constraints in commercial exploitation of noni fruits are as follows :

- Unpleasant Taste and flavour : When fully ripe, the fruit has a pronounced odor like rancid cheese. Consumer acceptability is low because it is not a table fruit like Aonla and others.
- Lack of technical know-how for processing.
- Limited research on active components, mostly patented.
- Medicinal evidences needs to be highlighted clearly.
- Limited clinical studies and lack of proper marketing.
- Highly expensive commercial products.

Value Added Products From Noni Fruit

The fruit has been exploited commercially world wide and several patents have been registered, which leads to the raise of several commercial products branded as Tahitian Noni, Indian Noni,Amazon Noni etc. The juice has been recently accepted in the European Union as a novel food. The matured but unripe fruits

can be utilized for Powder preparation whereas ripe fruits can be used for Pulp, Juice and Powder production as well.

Processing of Noni Fruit

Morinda citrifolia.L fruit is for all practical purposes inedible, therefore the fruit must be processed in order to make it palatable for human consumption and included in the nutraceutical. The processing of noni fruits (*Morinda citrifolia*.L) for value added products depends on intended processing methods for pulp/ juice extraction and their utilization in preparation of value added products which has commercial potential. Most of the noni juice processor prefers the hard white stage of fruit as harvesting indices for the extraction of pulp and juice production. The juice extraction efficiency by traditional drip extraction method accounted nearly 42-50% yield depending on original fruit weight. The great variation of traditional drip extraction method resulted in a non-fermented sweet juice. The extracted juice during storage have shown marked degradative changes in nutraceutical value.Therefore , need of the hour is to utilize noni fruit pulp as well as juice for the preparation of value added products like squash, flavoured beverage, diet noni, syrup and bar as well as in combination with other herbs and fruits, which are having commercial importance in the market.

Moreover, the noni fruit pulp could be exploited for the preparation of fruit powder; toffee and various noni powder enriched food products like cookies and fruit bar.

Value Added Products of Noni Juice

Noni Juice can be used as a base ingredient for the preparation of Ready to Serve (RTS)/ Ready to Drink (RTD) beverages which includes Squash, Cordial, Crush, Syrup, Nectar, Noni Punch. The other category of products which can be prepared from noni juice are concentrated juice, sweetened concentrated juice, sweetened concentrated reconstituted juice, flavoured beverage, fortified beverage, mixed/ blended juice in combination with aonla and ginger as herbal drink, diet Noni juice, wine etc.

Value Added Product of Noni Pulp

Noni pulp being more consistent in texture as compared to juice could be exploited for the preparation of various generic processed food products as given below (Satwadhar et al, 2007).

- Fruit Leather/ Bar
- Sweet Pulp Concentrate
- Puree, Sauce, Chutney
- Noni Ketchup
- Jam, Spread, Mixed Fruit Jam
- Noni Chhunda,
- Toffee, Sqeezy
- Noni Prash / Noni Herbal Prash
- Pulp Powder
- Noni Protein Supplements
- Noni Cheese

Noni Herbal Prash

Chyawanprash traditional Polyherbal formulation, which widely used as tonic, rejuvenator, anabolic, immunomodulator and memory enhancer. Chyawanprash manifests the entire human quest for immortality, freedom from disease and prevention of aging.The formulation as whole is an expression of a blessing from Rigveda. Perhaps it also represents the quest of mankind for a 'Panacea', which could address a wide array of health issue from aging to cough and common cold. Chyawanprash contains the pulp of Emblica officinalis as the prime ingredient, along with powders and extracts of several other herbs.Chywanprash is an ayurveda's best known and trusted health tonic, contains amla pulp,herbs , sugar and ghee. Its recipe was originated by Charak in 4th BC (Kasar Rahul et al., 2007).

Noni Herbal Prash could prepared as product similar to traditional Chywanprash that would contain noni fruit pulp, aonla, fig and dates which are having inherent therapeutic properties and other herbs possessing therapeutic, medicinal as well as nutritive value and energy.

Dates

- Food of high nutritive value and high energy
- Provides natural sugars, glucose
- Effective remedy for weak heart, intestinal disorders & constipation
- Improves sterility

Fig

- Excellent tonic for weak people
- Helps in quick recovery after prolonged illness
- Removes physical and mental exertion
- Beneficial in the treatment of number of diseases such as piles,asthma etc.

Aonla

- Amrut phal, Antioxidant
- Increase immune system, Anti aging
- Good brain tonic, Heart disorders, Diabetes
- Enhance digestion

Noni Herbal Prash formulation could be in the following combinations.

1. Noni-Aonla Prash
2. Noni-Fig Prash
3. Noni-Dates Prash
4. Noni- Aonla-Fig-Dates Prash

The above mentioned formulations would be rich in energy nutrients and health enhancing properties; useful for maintaining good health, body,mind and beauty. Such products with high antioxidant properties will strengthen body's internal defense mechanism , immune system.It may also reduce signs of aging and increase longevity and keeps and feels young. Instead of food supplements we could call different combinations of noni herbal prash as almost complete food/ balanced food, which is the requirement of consumers who works late hours or more than 12 hours, especially for Business Executives, CEOs, Computer professionals, Technocrats etc.

Noni Fruit Powder

Noni fruit pulp could be exploited for the preparation of fruit powder by different methods as per requirements of powder as a base ingredient for various value added products.Noni powder can find its utility in various forms such as follows.

- Novel food ingredient
- Reconstituted for beverage preparation
- Capsules
- Pills and Tablet (Vati)

Future Research Areas

The future research areas which needs to be given more stress for commercialization of value added noni food products are highligted below.

- Preparation of culinary products using Noni
- Low cost processing technology
- Packaging requirements
- Wide marketing and publicity
- Newer product invention based on consumer perception
- Phytochemical studies
- Mechanism of action of active components
- Clinical studies

Conclusion

- Primary challenge to the food technologist is to effectively translate nutraceutical and medicinal benefits of this folk remedy into shelf stable food products.
- The technologies should be standardized and assessed for the preparation of value added products which can be easily available at cheaper cost for commercialization.
- Developed technologies must be assessed and scale up for their techno-economic feasibility which can be disseminated to the common masses to develop their entrepreneurship.
- This will helps in turn to open a new avenue for the farmers, unemployed youth and rural women to establish primary processing units at their own village or region.

References

Bratman, S. and Girman, A. 2003 Handbook of Herbs and Supplements and their Therapeutic Uses. St. Louis: Mosby; 2003; pp. 769-771.

Kasar Rahul P., Laddha K.S., Chaudhary Jayesh and Shukla Anil. 2007.:'Chyawanprash - Truth or Mythy'. Pharmacognosy Reviews, 1 (1) 185-190.

Leister, 1975. Isolation, identification, and biosynthesis of anthraquinones on cell suspension cultures of *Morinda citrifolia*. Planta Medica, 27:214-224.

Mathivanan, N., Surendiran, G., Srinivasan, K., Sagadeva E. and Malarvizi, K. 2005. Review on the current scenario of noni research: Tomanomy, distribution, chemistry medicinal and therapeutic values of *Morinda citrifolia* L. Intl. J. Noni Res., (1):1-9.

Morton J. 1992. The ocean-going noni, or Indian Mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorful" relatives. Econ Bot, 46:241-256.

Satwadhar P.N., Siddiqui A. N. and Deshpande H.W. 2007. Development of Noni (*Morinda citrifolia*, L.) based Nutraceuticals for Health Security. Proceedings of the Second National Symposium on Noni for Health and Wellness. pp:167-176.

Wang, M.Y. and Su, C. 2001. Cancer Preventive Effect of *Morinda citrifolia* (Noni) Annals of New York Academy of Science, 952:161-168.

Wang, M.Y., West, B., Jensen, J., Nowicki, D., Su, C., Palu, A., Anderson, G. 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacol Sin, 23(12):1127.

Yanine Chan-Blanco, Fabrice Vaillant, Ana Mercedes Perez, Max Reynes, Jean-Marc Brollouet and Pierre Brat. 2006. The noni fruit (*Morinda cirtifolia* L.): A review of agricultural research, nutritional and therapeutic properties. Journal of Food composition and Analysis, 19(6-7): 645-654.

Zin, Z. M., Abdul-Hamid, A., Osman, A. (2002). Antioxidative Activity of Extracts from Mengkudu (*Morinda citrifolia* L.) Root, Fruit and Leaf. J. Food Chemistry, 78(2):227-231.www.ibef.org (KPMG Report on Food Processing, 2006)

World Noni Research Foundation

With the mission of educating the people, the World Noni Research Foundation, a non-profit organisation dedicates itself to love and care for *Morinda citrifolia.,* through research and development. Learning from the wisdom of the simple people, WNRF aims at working with everyone to conserve and improve Noni towards sustainable human and ecological health. It will share the Noni's past glory, ethnobotany, history, science, benefits and its multiple uses with all. The WNRF also serves as a facilitatory body for all Noni farmers, industries and consumers to establish a sustainable Noni economy network. The WNRF collectively represents the interests of all people in the Noni research and industry. It is an independent body and committed to exclusive Noni research and development. The WNRF website, journals and news letters are established to provide a non-biased forum for the researchers, consumers and industries to publicise their research findings and experiences with *Morinda* species.

WNRF believes that this synergistic effort of scientists and people of 'Noni Solidarity' would empower millions of ordinary masses to find their dignity and economic freedom, more naturally. This will lead to the realization of our vision "Healthy people, Healthy nation" in India and rest of the world.

Our Programmes Focus on

- Conserving the *Morinda* species in India and rest of the world from its degradation.
- Organising "Noni Biodiversity Action Network" (NBAN) to save endangered (Red listed) *Morinda* species in the above regions.
- Developing Bioinformatics database on *Morinda* species existing in India and rest of the world and record all Indigenous Technical Knowledge about it.
- Supporting the research and development programmes on discovering the multiple potential of *Morinda* species in fields like pharmaceutical, nutraceutical, cosmetology, dye, agriculture, etc.
- Sharing the cutting edge action-programmes and research findings with researchers, farmers, consumers, food industry leaders, health - drug industry leaders, students and masses.
- Connecting the *Morinda* species researchers in India and rest of the world.
- Promoting the Indian Noni for health regenerative systems and processes through clinical studies & biotechnological research.
- Developing "Noni Villages" for Noni based socio-economic development of people at the grass-root level.
- Monitoring and encouraging quality *Morinda* products in the Market.
- Regenerating the glory of Indian Noni

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