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Communication Address:
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World Noni Research Foundation
12, Rajiv Gandhi Road, Sreenivasa Nagar, Perungudi, Chennai - 600 096.
Phone : 044-2454 5401-04   Fax : 044-2454 5406
E-mail : mail@worldnoni.net   Website : www.worldnoni.net

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Phytochemicals from *Morinda citrifolia* L. (Noni) and their bioactivities: A review

**Abstract**: The purpose of this paper was to review the literatures of *Morinda citrifolia* Linn (noni) study to underline the known major chemical constituents and their corresponding bioactivities. We summarized the major phytochemicals or secondary metabolites, including their biological activities, identified from noni, as reported in journal articles found on PubMed database and other sources. Noni is a tropical plant grown in the south Pacific islands and has been used as a folk medicine and edible plant by Polynesians for over 2,000 years. It has been claimed to have a variety of health benefits in Polynesian tradition and modern medical clinical surveys. Noni fruit juice has been approved as a novel food by the European Commission in 2003, 2008, and 2010 respectively. It has also been approved as a new botanical food resource by the Ministry of Health, People’s Republic of China in 2007. The reported number of health benefits of noni juice is continuously increasing, such as enhancing the immune system and eliminating symptoms of various degenerative diseases. Our laboratory and others have demonstrated that noni juice has anticancer and cancer preventive activities *in vitro and in vivo*. Thus, isolating and screening for major anticancer components from noni fruit will become our new research endeavor. The discovery and development of a novel alternative product derived from noni fruits aimed at cancer prevention or treatment are our ultimate goals.

**Introduction**

Commercial noni fruit juice became quite popular as a liquid dietary supplement in the US market starting in the mid 1990s. Noni fruit juice and its related products have been distributed in over 80 countries around the world as a novel food, folk medicine, and nutritional supplements. Noni is one of the most important and ancient traditional folk medicines and edible plants used by Polynesians for over 2,000 years (Whistler, 1991; 1992). The genus *Morinda* of the *Rubiaceae* family, consisting of more than 80 species, is distributed globally in tropical regions, especially in the south Pacific islands. The most well known species of this genus
is *Morinda citrifolia* Linn (noni) (Abbott, 1992; Morton, 1992; McClatchey, 2002). It was believed that noni was one of the “canoe plants” carried by the ancestors of Polynesians leaving Southeast Asia in search of new migration across the sea. The precious noni plant was prized as their secret to good health. Ancient Ayurvedic texts call it shyuka, which is Sanskrit for ‘longevity’. These texts explain that noni balances the body, stabilizing it in a state of perfect harmony. Other common names of the noni plant include Indian Mulberry, Hog Apple, Mengkoedoe, Mora de la India, Ruibarbo Caribe, Wild Pine, and Cheesefruit, etc in different origins. Noni fruit, bark, leaves, and seed have been used in herbal remedies in Polynesian folk medicine (Bruggenecate, 1992; McClatchey, 1993). The traditional Polynesian healers (Kahunas) have used the plant for a number of ailments including colds, conjunctivitis, cough, diarrhea, elephantiasis, eye infection, female problems, insect bites, intestinal parasites, jaundice, pleurisy, rheumatism, sore throat, thrush, toothache, tuberculosis, vomiting, skin infection, abscesses, and wound healing (antiseptic and analgesic). In a modern clinical survey, data has been collected from more than 1,300 health professionals representing over 27,000 noni juice users in eighty countries. Results indicated that noni juice has a broad spectrum for functional body balance. For example, users claimed that it aided in symptoms of allergies, arthritis, asthma, fibromyalgia, lumbago, obesity, depression, stress, stroke and athletic injury, bone fractures, cancer, diabetes (type 1 and 2), digestion problems, respiratory problems, skin and hair problems, heart and kidney disease, HIV, immune disorders, sleep disorders, menstruation, Parkinson’s Disease, high blood pressure, pain, smoking addiction, mental dullness, multiple sclerosis, muscle atrophy, energy loss, decreased sexual libido, while increasing general overall well-being (Wang et al., 2002; Palu et al., 2008). Millions of people using noni juice with a claim of “cure all”, numerous scientists have endeavored to discover the chemical composition of the noni plant. Scientific studies have reported that noni possesses antibacterial, anti-viral, anti-fungal, anti-inflammatory, anti-histamine, anti-tubercular, anti-tumor, antiseptic, analgesic, hypotensive, anti-helmintic, and immuno-modulating activities (Wang, 2002; Bruggeneate, 1992).

Noni research conducted in our laboratory over the last twelve years has shown that noni juice made from Tahitian noni fruit indeed possesses strong anti-oxidative, anti-inflammatory, anti-mutagenic, anti-angiogenic, and anti-carcinogenic activities. Noni reduced the cancer risk at the initiation stage in current smokers and experimental breast carcinogenic models by scavenging oxygen reactive free radicals, quenching lipid hydroperoxides, and blocking carcinogen-induced DNA adduct formation (Wang, et al., 2003; 2004; 2005;2008; 2009; Wang and Chen, 2002; Jensen et al., 2006). Our clinical trial demonstrated that noni juice was able to reduce smoking-related aromatic DNA adduct levels and oxidative status by drinking 1 to 4 oz of noni juice per day for one month. We also demonstrated that noni juice was able to improve lipoprotein profiles in current smokers by increasing HDL and reducing
total cholesterol, triglyceride, and LDL levels as well as reducing the inflammatory status by suppressing high C-reactive protein levels. The second clinical trial demonstrated that noni juice was able to improve the hearing ability and the quality of life in menopausal women. The latest clinical trial demonstrated that noni juice improved the quality of life and reduced the symptoms of osteoarthritis in more than 74% patients. No adverse side effects from noni juice were observed in the clinical trials. Therefore, scientific and clinical studies on noni juice confirmed some of the Polynesian claims of multiple health benefits.

Noni Juice as New Resource of Nutraceuticals

Although we do not have enough scientific evidence to support the Polynesians’ claims of noni health benefits, noni juice has become a leading “flagship” dietary supplement among modern nutritional supplements and novel health foods. As a recognized dietary supplement, noni has been sold in various forms including juice, extract, powder, capsules, and tea. The fruit juice is the predominant product in the market. Noni juice is usually blended with other juices and flavors to overcome its unpleasant taste. It is even combined with other herbs such as Goqizi (wolfberry) and Ba Ji Tian (Morinda officinales). Noni has become widely available as a juice or encapsulated powder in health food stores.

Functional foods have become a primary focus for advancement in alternative health care. More and more people prefer to take care of their health by using foods that have a specific health benefit instead of taking synthetic drugs with known potential serious toxicities. The public has become increasingly intrigued with natural functional foods that contain major bioactive components, supported by strong scientific evidence aimed at a specific health condition. A functional food has been defined as a food or dietary supplement that provides a health benefit beyond basic nutrients. Noni has been accepted as a liquid dietary supplement in the USA, a Novel Food by the European Commission, and a new botanical food resource by the Ministry of Health, People's Republic of China (European comm., 2003; Ministry of Health, 2007). According to the US Government Food Advisory Committee, functional foods are ordinary foods that have components or ingredients incorporated into them and give a specific medical or physiological benefit, other than a purely nutritional effect (Benford, 1995).

All foods have a function when consumed in the proper balance as part of an overall healthy diet. There is a difference between Western functional foods and the Eastern perspective. In the Orient, the concept of functional foods has been a part of their eating culture for centuries. In Traditional Chinese Medicine (TCM), foods have medicinal properties which have been documented since 1000 BCE. The Chinese believe that foods have preventive and therapeutic effects. Eating good food as an integral part of health is now being increasingly recognized around the world (Wang, 2009, 3(1-2)).

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and Lu, 1992; Weng, 1996). In the Western countries, the health function of food is viewed as a revolution, resulting in a fast growing segment of the food industry (McClatchey, 2002; Europ. Comm., 2002). Scientific data that reveals any new health benefit of a functional food is being published in scientific journals and appears as front page news. Consumers are eager to take action from these findings and eat healthier to prevent disease and improve their overall well-being. Dietitians continue to recommend a diet high in fruits and vegetables to public. Further studies of specific foods and their components should focus on both treatment and prevention of particular diseases.

Noni juice, made from Tahitian noni fruit, is known to contain bountiful high energy botanical nutrients, necessary for building strength and vigor that could prove to underline its multiple preventative and therapeutic qualities. Noni fruit juice made from the ancient noni plant contains various amounts of phytochemicals that may provide health benefits beyond nutritional value such as vitamin C and trace minerals. Plants are reemerging as a major source of new pharmaceuticals. Nutraceuticals is a term popularized by the Foundation for Innovation in Medicine and refers to “any substance that may be considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease” (Zhu and Woerdenbag, 1995). Consequently phytochemicals and phytoneutrients refer to edible plant, fruit, and vegetable components that have health-promoting properties that protect against disease. Currently, the term is used in a much broader sense and applies to any plant component that has any health-enhancing benefits. We want to emphasize that noni juice, based on its history as a folk medicine for over 2,000 years, may have a great pharmaceutical value. For example, noni juice possesses antioxidant activity and appears to be the fruit juice to be identified as a possible alternative to the use in intracanal irrigation (Murray et al., 2008). Evaluation of preliminary medicinal studies of the nitric oxide scavenging activity of noni juice is found to be useful in treatment of inflammatory diseases (Delhi, 2003; Jagetia and Baliga, 2004; Jan, 1975; Basu and Hazava, 2006). It is also reported that Noni juice can be used as an anxiolytic and sedative through a mechanism involving its gamma-aminobutyric acidergic effects (Deng et al., 2007).

A few case reports have suggested a correlation of noni juice in the development of chemical-induced hepatitis in Europe. However, no causal role has been established in any of these cases. An official European investigation of these cases determined that no relationship between noni juice and hepatitis was evident and the consumption of noni juice is unlikely to induce adverse human liver effects (EFSA, 2006). Animal and human studies have revealed that noni juice is not hepatoxic, even at very high doses (West et al., 2006; 2006a), but it protects liver (Issell and Gotay, 2005).
Currently known Components of Noni

While searching for food, the ancients found that some foods had specific properties of relieving or eliminating certain disease and maintaining good health. This was the beginning of herbal medicine (Zhu and Woerdenbag, 1995; Palu et al., 2008). Can we discover scientific evidence that supports the health benefits of noni fruit juice and furthermore, identify the major bioactive components in noni fruits that are responsible for any specific health benefit? This question leads scientists toward a renewed interest in reviewing the current knowledge of noni in terms of its phytochemistry, pharmacology, medicinal use, and safety. All the parts of noni plant have been used in over 40 recorded herbal remedies and concoctions (Nayak et al., 2007, Pawlus and Kinghorn, 2007). Noni fruit is mostly used in food products and other parts of the noni plant, such as root, bark, stems, flowers, leaves, and seeds are mainly used in traditional medicine. Based on chemical analysis, noni fruit is a good source of vitamin C, Vitamin A, amino acids, linoleic acid, sterols, sugars, sulfur-containing compounds, polysaccharides, and fatty acids. The major nutritional components of noni include glucose, fructose, protein, and lipids. Secondly, the positive medical effects of the minerals in noni juice are the relatively high potassium level, followed by calcium, sodium, magnesium, iron, selenium, zinc, copper, and sulfur (Shovic and Whistler, 2001; Potterat and Hamburger, 2007). In this review, we summarize the major phytochemicals or secondary metabolites in Table 1, which include the biological activity of isolated compounds from Morinda citrifolia L. (noni) over the last 20 years.

Table 1. Chemical constituents of Morinda citrifolia Linn (noni) and corresponding bioactivities

<table>
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<th>Noni plant organ(s) or parts : Fruit and Fruit Juice</th>
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<td>Polysaccharides (or noni-ppt) (Hirazumi and Furusaw, 1999; Anh et al., 2006)</td>
<td>The polysaccharide fraction consists predominantly of Galp (53.6 mol%), Araf (13.6 mol%), Galp (17.9 mol%) and Rhap (9.5 mol%). Glycosyl linkage analysis : 1) Pectic polysaccharides: HG(1,4-linked) RGI (3,5-linked arabinan and 4,6-or 3,6-linked galactans- arab). 2) AGPs (3,6-or3,6-linked) with lower amount of 3,6-2,4,3,4- and 3,6-linked. Galp1, 4-and Glcp1,4,1,2-or 4,6-linked.</td>
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### Polysaccharides (or noni-ppt)
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**Glycosyl linkage analysis:**
1) Pectic polysaccharides: HG(1,4-linked) RGI (3,5-linked arabinan and 4,6-or 3,6-linked galactans-arab).
2) AGPs (3-,6-or 3,6-linked) with lower amount of 3-, 2,4-, 3,4- and 3,6-linked. Galp1,4- and Glcp1,4,1,2-or 4,6-linked.

### Oligosaccharide compounds
(Dalsgaard et al., 2006; Samoylenko et al., 2006) (Wang et al., 1999; Akohisa et al., 2007)

1) 2,6-di-O-(α-D-glucopyranosyl)-1-O-hexanoyl-α-D-glucopyranose, white powder. [M + H]+ 603.2512 (C_{24}H_{42}O_{17}).

2) 2,6-di-O-(α-D-glucopyranosyl)-1-O-Decanoyl-α-D-glucopyranose. Colorless oil. [M + H]+ 659.3130 (C_{28}H_{50}O_{17}).

3) 2-O-(6-O-octanoyl-α-D-glucopyranosyl)-6-O-(β-D-glucopyranosyl)-I-O-octanoyl-α-D-glucopyranose. Oil. [M + H]+ 757.3382 (C_{24}H_{60}O_{18}).

4) 2-O-(6-O-hexanoyl-β-D-glucopyranosyl)-6-O-(β-D-glucopyranosyl)-1-O-octanoyl-β-D-glucopyranose or 2-O-(6-O-octanoyl-β-D-glucopyranosyl)-6-O-(β-D-glucopyranosyl)-1-O-hexanoyl-β-D-glucopyranose. Oil. M + H]+ 729.3601 (C_{22}H_{56}O_{18}).

5) 2,6-di-O-(α-D-glucopyranosyl)-1-O-octanoyl-α-D-glucopyranose. White powder. FAB [M + Na]+, 653 (C_{26}H_{46}O_{17}).

### Oligosaccharide fatty acid ester
(Li et al., 2001; Akohisa et al., 2007)
1) 2-O-(α-D-glucopyranosyl)-1-O-hexanoyl-α-D-glucopyranose. Colorless gum. [M + Na]+ 463.1788. (C_{14}H_{23}O_{7}).

These compounds were proposed or documented to have immunostimulatory, immunomodulatory, anti-bacterial, and anti-cancer activities. For example, compound 5) exhibited a potent anti-inflammatory activity and against the (EBV-EY) virus [44].

### Disaccharide fatty acid ester
(Chemical characterization from BuOH-insoluble or methanol-insoluble fractions.)

1) 2-O-(α-D-glucopyranosyl)-1-O-hexanoyl-α-D-glucopyranose. White powder. Exhibited a potent inhibitory activity in the mouse ear edema model and inhibited AP-1 transactivation and cell transformation in the mouse epidermal JB6 cell line [44-46].
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2) 2-O-(α-D-glucopyranosyl)-1-O-octanoyl-α-D-glucopyranose. Colorless gum. [M+Na]⁺ at m/z 491.2110. (C₂₀H₃₆O₁₂).

3) 6-O-(α-D-glucopyranosyl)-1-O-hexanoyl-α-D-glucopyranose. White powder. APCIMS 458. [M + NH₄]+ (C₁₈H₃₂O₁₂).


5) 3-methylbut-3-enyl-6-O-α-D-glucopyranosyl-α-D-glucopyranose. White powder. APCIMS: 428 [M+NH₄]+ + (C₁₇H₃₀O₁₂).

Compounds 2) and 4) exhibited potent anti-inflammatory activities with ID₅₀ values of 0.46-0.79 mg per ear. They also inhibit the (EBV-EV) virus [44].

### Monosaccharides

(Samoylenko *et al.*, 2006; Akihisa *et al.*, 2007)

3-methylbut-3-enyl-α-D-glucopyranose. 1-O-(3’-methylbut-3’-enyl)-β-D-glucopyranose, Colorless powder. HRMS (ESI), [M-H]-247.1193; (C₁₁H₁₉O₆).

Glucuronic acid;

Galactose;

Arabinose;

Rhamose;

Methyl-α-D-fructofuranoside,

Methyl-α-D-fructofuranoside, nicotifloroside,

α –sitosterol- 3-O-α –D-glucopyranoside.

Monosaccharides compounds partially inhibit AP-1 transactivation and cell trans- formation in the mouse epidermal JB6 cell line. For example, Compound 1) against 12-O-tetradecanoylphorbol-13-acetae (TPA) induced inflammation in mice[44].

### Anthraquinones (damnacanthal)

(Kamiya *et al.*, 2005; Akihisa *et al.*, 2007; Lin *et al.*, 2007)


2) 1,5,15-tri-O-methylmorindol[. Yellow brown fine needles. [M]- 327.0861. (C₁₈H₁₆O₆).

3) 5,15-O-dimethylmorindol. Yellow amorphous powder. HR-EIMS: [M]+314. 0797. (C₁₇H₁₄O₆)

4) 1,8-dihydroxy-2-hydroxymethyl-5-methoxy-an-thraquinone.

5) 1,3-dihydroxy-2-methoxyanthraquinone.

The anthraquinones from the noni plant possess potent antibacterial effects in digestive tract (Staphylococcus, Shinge-la, Salmonella) and have anti-inflamma-tory as well as potential cancer chemo-preventive activities[44-48].

### Chemical characterization

Chemical characterization from an BuOH-insoluble and methanol-insoluble fractions

Chemical bond characterization: 1?2 Or 1?6, 1-O-fatty acid.
6) 1,6-dihydroxy-5-methoxy-2-methylanthraquinone.
7) 2-hydroxy-1-methoxyanthraquinone.
8) Alizarin-1-methylether.
9) Anthragallol-1,3-dimethylether.
10) Anthragallol-2-methylether.
11) 6-hydroxy-anthragallol-1,3-dimethylether.
12) Morindone-5-methylether.
13) Morinaphthalenone (7-hydroxy-10-hydroxymethyl-4a,5a-dimethyl-6-((6′-methylheptyloxy)5,6,7,8,9,10-hexahydro-2(HH)-naphthalenone.
Amorphous powder. [M]+ 352.2613. (C_{21}H_{36}O_{4}).
14) 1,3-dihydroxy-anthraquinone.
15) 1,2-dihydroxy-anthraquinone.
16) 9-hydroxy-2-methoxy-4-methyl-3,10-anthracene dione (new).
17) 4-methoxy-3-heptadecylxanthone (new).
18) 1-hydroxy-2-methylanthraquinone.
19) 2-hydroxymethylanthraquinone.
20) 1,5,7-trihydroxy-6-methoxy-2-methoxymethyl anthraquinone.

For example, compound 1) has a potent quinone reductase-inducing activity which is about 40 times higher than a positive control (L-sulforaphane). Furthermore, no discernible cytotoxicity at the highest dose tested[45]. Compound 2) has a moderate anti-Epstein-Barr virus early antigen (EBV-EA) activity[44-46].

Iridoid glycosides
(Kamiya et al., 2006; Su et al., 2005; Samoylenko et al., 2006; Deng et al., 2011; Dussossoy et al., 2001)
1) Morindacin 1,4-bis(hydroxymethyl)-3-hydroxy-3,4,6,7,3a,7a-hexahydro-6-oxainden-5-one. A colorless syrup. [M+H]+215.05091. (C_{10}H_{14}O_{5}) hydroxyadoxoside. Colorless gum. [M + Na]+ 429.1378. (C_{17}H_{26}O_{11}.)
2) 6α, 7α-epoxy-8-epi-splendoside. Colorless gum. [M]+ 443.1147. (C_{17}H_{24}O_{12}.)
3) Asperulosidic acid. Colorless oil, APCI MS, [M+NH4]+450. (C_{28}H_{24}O_{12}.)
4) Borreriagenin.
5) 4-epi-borreriagenin (a white powder) HRMS (ESI), [M + Na]+, 237.0726 (C_{10}H_{14}O_{5}).

Iridoid glycosides were found to have moderate antioxidant activities in a free-radical (DPPH) scavenging bioassay [53]. Compound 4) inhibits phorbol ester- and EGF-induced AP-1 transactivation and cell transformation in mouse epidermal JB6 cells [58] [46].

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Flavonol glycosides

(Su et al., 2007; Anh et al., 2006; Akihisa et al., 2007; Deng et al., 2007; Pawlus and Kinghorn 2007)

1) Rutin. Yellow powder, [M + 1] + 611 (C_{27}H_{30}O_{16}).
2) Narcissoside.
3) Flavonol glycoside.
4) Quercetin.
5) Kaempferol.

Lignans

(Wang et al., 1999; Jensen et al., 2006; Lin et al., 2007)

1) Americanoic acid A. Pale yellow amorphous powder. [M]+ 344.0923 (C_{18}H_{16}O_{7}).
2) Morindolin. Pale yellow amorphous powder. [M]+ 344.0869. (C_{18}H_{16}O_{7}).
3) (-)-3,3'-bisdemethylpinoresinol. Pale yellow amorphous powder. [M]+ 330.1071. (C_{18}H_{18}O_{6}).
4) Americanol A. Pale yellow amorphous powder. [M]+ 350.1113. (C_{18}H_{18}O_{6}).
5) Americanin A. Pale yellow amorphous powder. [M+H]+ 329.0996 (C_{18}H_{18}O_{6}).
6) Isoprincepin. Pale yellow amorphous powder. [M - H]- 493.1476. (C_{25}H_{27}O_{9}).
7) (+)-3,4,3',4'-tetrahydroxy-9,7[[* epoxylignano-7[[* ,9'-lactone. Light brown resinous semisolid. [M]+ 345.0954 (C_{18}H_{16}O_{7}).
8) (+)-3,3'-bisdemethylnanegool. Greenish brown resinous semisolid. [M]+ 349.1265 (C_{20}H_{22}O_{6}).
9) (-)-pinoresinol. [M]+ 358. (C_{20}H_{22}O_{6}).
10) Isoamericanic acid A.
strong antioxidant activities, which equal or better than a known antioxidant 2,6-di-tert-butyl-p-cresol[43].

In both of DPPH and ONOO(-) bioassays, compound 5 americanin A is a potent antioxidant[52]. Compounds 7), 8), and 9) inhibited 5- and/or 15-lipoxygenase activity.

### Coumarin compounds

(Su et al., 2005; Samoylenko et al., 2006; Deng et al., 2007)

2. Isoscopoletin (6-Hydroxy-7-methoxy-coumarin). (C10H8O4).

### Succinate derivative compounds

(Samoylenko et al., 2006)

1. 1-n-butyl-4-(5'-formyl-2'-furanyl) methylsuccinate. Gum. HRMS (ESI), [M + Na]+ 305.0989. (C14H18O6)
2. 1-n-butyl-4-methyl-2-hydroxy-succinate. Oil.
3. 1-n-butyl-4-methyl-3-hydroxy-succinate Oil.

### Vitamins and Minerals:

(Shovic and whistler, 2001; Potterat and Hamburger, 2007)

Relatively high potassium (about 30-150 ppm), calcium, sodium, magnesium, selenium, zinc, copper; sulfur, etc. Content of ascorbic acid (vitamin C) in noni juice is 30-155mg/kg.

### Nutritional composition

(Pottevat and Hamburger, 2007)

- Glucose (3-4%)
- Fructose (3-4%)
- Protein (0.2-0.5%)
- Lipids (0.1-0.2%)

The vitamins and minerals in noni juice may be associated with the health benefits.

The compounds were used as marker constituents for a commercial noni juice.

Chemical characterization from n-ButOH-insoluble fraction.

Chemical characterization from EtoAc-insoluble fraction or BuOH-insoluble fraction of EtOH extracts.
### Anthraquinones

(Takashima et al., 2007)

1,5,15-trimethylmorindol. Yellow amorphous powder. \([M+]+328.0945, (C_{18}H_{16}O_6)\).

### Iridoid glycosides

(Saludes et al., 2002)

1) Citrifoside. Colorless amorphous solid. \([M+Na]-397.1115 (C_{16}H_{22}O_{10}Na)\)
2) Citrifolinoside A. Amorphous solid. \([M-H]-563. (C_{28}H_{36}O_{14})\)
3) Citrifolinoside. \([M-H]-625. (C_{25}H_{30}O_{17})\)
4) Citrifolinin A-1: iridoid dimer glycoside (based on the cyclopenta [c] Pyranoid skeleton represented by iridane (cis-2-oxabicyclo [4.3.0] nonane). \([M-H]-817. (C_{34}H_{42}O_{23})\)
5) Citrifolinin A. Yellow powder. APCI-MS \([M-H]-609. (C_{27}H_{30}O_{16})\)
6) Citrifolinin B. Amorphous solid. APCI-MS, \([M-H]\)- at 417. \((C_{17}H_{22}O_{12})\)

### Flavonol glycosides

(Sang et al., 2001)

1) Quercetin-3-O-â-D-glucopyranoside. Yellow powder. APCI-MS \([M-H]-463\).
2) Kaempferol-3-O-L-rhamnopyranosyl-â-D-glucopyranoside. Yellow powder. APCI-MS, \([M-H]-593\).
3) â-D-glucopyranoside. Yellow powder. APCI-MS, \([M-H]-609(C_{27}H_{30}O_{16})\).
4) Quercetin-3-O-â-D-glucopyranosyl-(1L-â-D-galacopyranoside. Yellow powder. APCI-MS, \([M-H]-771\).
5) Kaempferol-3-O-â-D-glucopyranosyl-(1L-â-D-galacopyranoside. Yellow powder. APCI-MS, \([M-H]-755\).

### Lipids fraction:

(Saludes et al., 2002)

1) E-phytol.
2) Cycloartenol.
3) Stigmasterol.

| Compound | 
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 3) Citrifolinin B-1 | obtained from alkaline EtOAc-soluble fraction of MeOH extracts | Compound 3) has an inhibitory effect on AP-1 activity in cell cultures\[^{[67]}\]. |
| 4) Citrifolinin B | from the dried noni leaves. | Compound 4) is a novel iridoid dimer. |
| 5) Citrifolinin B-1 | from the dried noni leaves. | Compounds 4), 5) citrifolinin A, and compound 3) citrifolinoside showed significant inhibition on AP-1 activity in cell cultures. \[^{[68-72]}\]. |
| 6) Citrifolinin B-1 | from the dried noni leaves. | Compound 6) showed an activity of free radical scavenging\[^{[63]}\]. |

All of these compounds showed DPPH free radical scavenging activity at the concentration of 30 M \[^{[63]}\]. n-BuOH insoluble fraction from the leaves of Morinda citrifolia.
4) Beta-sitosterol.
5) Campesta-5,7,22-trien-3beta-ol.
6) Ketosteroids stigmasta-4-en-3-one.
7) Stigmasta-4-22-dien-3-one. E-Phytol.

   Others[65].
1) 5-benzofuran carboxylic acid-6-formyl methyl ester.
2) 4-(3'(R)-hydroxybutyl)-3,5,5, trimethylcyclohex-2-en-1-one.

This fraction showed a pronounced anti-tubercular activity. The leaves and bark are made into a liquid tonic for urinary complaints as well as muscle and joint pain. A tea made from noni leaves is used as a remedy for tuberculosis, arthritis, rheumatism, and anti-aging[64].

Chemical characterization from hexane fraction which a mixture of Diterpine, Triterpine and lipids from a crude ethanol extracts of Morinda citrifolia leaves.

<table>
<thead>
<tr>
<th>Stems, Roots and Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthraquinones</strong></td>
</tr>
<tr>
<td>(Siddiqui et al., 2006; Lin et al., 2007)</td>
</tr>
</tbody>
</table>
| 1) Morindicinone: 2-hydroxy-1,8-dimethoxy-7-methoxymethylanthraquinone. Light yellow needles (MeOH), HREIMS: [M]+ 328.0937. (C18H16O6).
*3) Morindicone: 9-hydroxy-2-methoxy-4-methyl-3,10-anthracenedione.
*4) Morinthone: 4-methoxy-3-heptadecy laxanone.
*5) 1-hydroxy-2-methylanthraquinone.
*6) 2-hydroxymethylanthraquinone.
*7) 2-hydroxyanthraquinone.
*8) 2-methoxyanthraquinone. |

Anthraquinones isolated from stems of noni tree have quinone reductase-inducing activity[52, 71].

These two new compounds are considered as seco-anthraquinones from a biogenetic point of view[71].

**Benzophenones**
(Siddiqui et al., 2006)
1) Morinditrifolin A. Colorless gum. [M+H]+ 335. 0794. (C17H16O7)
2) Morinditrifolin B.,Colorless gum. [M+H]+ 385. 0913. (C18H18O8)

Morindin and Morindone

Dyes, yellow, and red colorants are used for tapa cloth and antibacterial purpose.

Chemical characterization from CHCl3 fraction of MeOH-H2O insoluble of noni roots.

### a. All compound data are from the literatures reported. MW of some compounds is unavailable and marked with an asterisk (*).

### b. Classification of all compounds is based on the molecular skeleton.
Early studies have found anthraquinones, damnacanthal, 7-hydroxy-8-methoxy-2-methyl-anthraquinone, morenone 1, and morenone 2 in the roots of noni (Sang et al., 2001; Rusia and Srivastava, 1989; Jain et al., 1992). Ricinoleic acid has been extracted from the seeds of noni (Srivastava and Singh, 1993). Two known anthraquinones (morindone and physcion) and one new anthraquinone glycoside have been isolated from the heartwood (Tiwari and Singh, 1977). Studies on the chemical components of the noni flowers have resulted in the identification of one anthraquinone glycoside and two flavone glycosides (Singh and Tiwari, 1976). In the ripe fruit, volatile compounds are characterized by a large amount of carboxylic acids, especially octanoic and hexanoic acids (Farime et al., 1996). Several nonvolatile compounds, including acetyl derivatives of asperuloside and glucose, have been identified in the fruits (Siddiqui et al., 2006). Although many compounds have been isolated and identified, characteristic new skeleton compounds have not yet been found in the noni plant.

So far, an alkaloid called xeronine is a nitrogen-containing secondary metabolite compound has been detected in the noni plant. Heinicke, a retired biochemist, proposed a “xeronine system”, a new cellular mechanism that explains the health promoting action of noni and bromelian (Heinicke, 2001; Mcclathey, 2002). He believed that a major active ingredient in noni fruit is xeronine (proposed formulas are $C_{11}H_{17}O_8N_3$, MW = 319.28 or $C_{13}H_{17}O_7N_3$, MW = 329.29) and it is able to enhance the enzyme activities by modifying protein structures and their functions. He also stated that only a trace amount of xeronine exists in the noni fruit. Unfortunately, this hypothesis has not been confirmed yet by other scientists.

**Target Specific Health Product**

“Let food be thy medicine and medicine be thy food”, was taught by Hippocrates, the father of medicine. Although people are convinced by the significant health benefits of botanical food, many conventional doctors do not subscribe to this way of thinking. Western doctors treat their patients with drugs rather than with foods or diet. Thus, “let food be thy medicine” has been largely neglected. It may change quickly with time as there is growing interest and research in functional foods, increasing their medicinal possibilities (Palu et al., 2008).

From ancient to modern times, plants have been the best resource of new drug development. Phytochemical studies have led to many new drug developments such as aspirin, originating from the white willow, and digitalis from foxglove. Natural source also plays a dominant role in the evolution of anticancer drugs. It is known that phytochemicals have potent antitumor properties and have provided multiple active compounds in the past, such as paclitaxel (Taxol), vincristine (Oncovin), vinorelbine (Navelbine), teniposide (Vumon) and various water-soluble analogs of

*Weiping Yin and Wang, Phytochemicals from *Morinda citrifolia* L. (Noni) and their bioactivities: a review*
camptothecin (Hycamtin). Although anticancer drug development has experienced a significant transition from the empirical drug screening of cytotoxic agents to the ‘designer’ target-orientated drug screening, the broad spectrum anticancer activity of natural products across multiple signaling pathways is the significant advantage and warrants further exploration. Natural products with their chemical and structural complexity, affordability, lack of substantial toxicity, and inherent biological activity makes them ideal candidates for new anticancer drug development. Natural products disrupt multiple abnormal signal transduction pathways leading to cancer (i.e., hyperproliferation, deregulation of apoptosis, angiogenesis, invasion, and metastasis) as well as act synergistically with chemotherapy and radiotherapy. Between 1981 and 2002, about 74% of the drugs approved as anticancer agents were natural products, had natural product base structure, or mimicked natural products. These natural products are believed to suppress the transformative, hyperproliferative, and inflammatory processes that initiate carcinogenesis, but are rather cytotoxic drugs. However, natural products can be used alone or as adjuncts to conventional chemotherapeutic agents to enhance the efficacy of the anticancer drugs and minimize chemotherapeutic agents-induced toxicities. Since cancer is primarily a disease of the more fragile and lower socioeconomic aging population, finding less toxic and inexpensive therapies is the major priority. It is estimated that more than 80% of the world’s population cannot afford modern medicines. In addition to cost, current cancer therapies are minimally effective and exhibit toxicities that are intolerable in most cases. Therefore, the advantages of alternative anticancer agents are natural, safe, effective, and affordable.

The characteristic phytochemical and pharmacological studies of the noni plant from the different research groups around the world have been involved in three fields of life science: cancer, inflammatory and metabolic diseases. No pharmaceutical product has been developed thus far on isolated compounds from the noni plant. This review serves to stimulate future research in the great unexplored potential of alternative medicine in this functional food.

If Noni is proven to have a number of potential biological activities, of which specific phytochemical constituents are responsible, then scientists can definitively answer why noni can be so beneficial to human health. Therefore, this review lays a foundation role for the direction of future research. We will focus on the study and development of new cancer preventive products and/or new anticancer drugs from noni. Our proposed project is fully supported by the results of a NIH-sponsored phase-1 clinical study in Hawaii University (Issell and Gotay, 2005). It indicated that noni is able to relieve pain and improve the quality of life in advanced cancer patients. In order to provide more evidence that noni contains superior nutritional phytochemicals for cancer patients when compared to other natural products, we must demonstrate that: 1) noni juice is effective and safe in shrinking tumors,
improving symptoms and the quality of life in cancer patients; 2) noni is target specific for certain tumors or cancers and has stronger anticancer activities with less side effect compared with conventional anticancer drugs; 3) there are specific anticancer mechanisms in noni and new anticancer drugs developed from this plant. The advantages of a noni anticancer drug would be cost effectiveness and safety. We predict that noni will bring a new hope for cancer patients either as an alternative medicine and/or a new anticancer drug.

Further phytochemical studies are necessary to discover specific bioactive ingredients responsible for anticancer and other properties observed in noni. The study will focus on the following points: 1) the fingerprinting techniques of noni as an alternative product will be a comprehensive phytochemical profiling analysis and set up standards for quality control; 2) screening of the major components for the spectrum of health benefits by using experimental models to find out specific targets; and 3) identifying marker compounds for monitoring any discovered bioactivity. These goals are essential for new noni product development (Pawlus and Kinghorn, 2007). Specific target products should be developed and used for specific health conditions to maximize health benefits for preventive and/or therapeutic purposes of noni.

Conclusions

Based on the literature review, various compounds have been successfully isolated from noni fruit, leaves, bark, and roots. Although most of them possess certain bioactivities in vitro test systems, there is insufficient scientific evidence for human use. Since noni has been reported to have versatile and traditional uses in humans for over 2000 years, we will further explore the major anticancer components in the noni. Our research will be focused on the isolation of major anticancer components and other bioactive components from noni. We found the potential of the noni fruit for the novel anticancer constituents reported in this review and we hope that this review will stimulate research on this remarkable plant, *Morinda citrifolia* L. (noni). Future studies will focus on the discovery of major bioactive components from noni and their action mechanisms.

References


Some observations on Noni as a Rasayan in the perspective of Ayurveda

Abstract: Noni (Morinda citrifolia L.) is one of the large numbers of Rasayam drugs described in Ayurvedic texts. Majority of the wide range of therapeutic effects claimed in Noni seem to be the secondary attributes of its primary Rasayam effects. It is desirable to undertake comparative studies on Noni and Ginseng with classical Ayurvedic Rasayan drugs like Amla, Ashwagandha, Brahmi etc.

Introduction

Noni (Morinda citrifolia L) is an extremely popular medicinal and food plant which identified in the folklore by Europeans in 1700 who noticed its natural folk use in South East Asia. During world war-2 the US soldiers used Noni as food supplement. In view of its unfolding folklore claims as a health plant worldwide investigations were carried out to validate its efficacy and safety on scientific parameters. Since the establishment of World Noni Research Foundation, extensive research has been carried out and a large number of scientific publications have appeared in many journals and research reports mostly focusing on following aspects: 1. Identification descriptors and pharmacognosy; 2. Conservation, cultivation and plant protection, 3. Pharmacological and safety studies, 4. Pharmaceutical aspects and dosage forms.

A critical review of such publications reveals that claims of its efficacy in divergent medical applications are still not fully validated. Most of the biomedical studies have been conducted in experimental settings. There is hardly any good clinical trial on this important medicinal plant in human settings. However, the available reports would show some lead evidence for anti-inflammatory, antihypertensive, antidiabetic, hypolipidemic, immunoenhancing, anti-oxidant, anticancer, anthelmintic, antitubercular, anxiolytic, antibacterial, antifungal and antiviral effect. It has also been reported by certain investigators that it is safe for human use and it has no major adverse effect.
However most of these studies warrant confirmation by further studies and clinical evaluations. The author of this presentation feels that Noni, Ginseng and Indian Ashwagandha are three most researched medicinal plants and thousands of research papers have appeared on the medicinal use of these plants but exact mode of action, long term safety and real therapeutic efficacy is still to be established. However there is enough lead for further study.

The Perspective

It is amazing that Noni (Morinda citrifolia L) as a single drug has been found to show such a wide range of efficacy. It seems to be a panacea for everything. Such an observation prompts me to examine this drug in the perspective of Rasayan therapy of Ayurveda and I am tempted to consider Noni as a Rasayan in the language of Ayurveda.

Noni as such is a traditional folk name of the plant Morinda citrifolia L. Its exact Ayurvedic name has not been determined but some scholars (Singh and Chunekar, 1980) consider Ayurvedic drug Akshi (Akshiki phala) described by Caraka Samhita and certain other texts (CS.Su.27:163,183 ; SS.Su.45:179) in their Ahar varga comparable to this plant. Akshi is described as a fruit plant with Pitta and Kapha curing effect and sweet and sour taste and Rasayan effect. It is really not a popular Ayurvedic drug in its classical tradition.

However the wide range of the therapeutic attributes claimed for this plant drug can only be explained in the frame of a Rasayan remedy. Ayurvedic Rasayanas are a category of holistic remedies which produce broad based holistic beneficial effect in the organism acting mainly through the nutrition dynamics leading to a fundamental bio-balancing effect improving the biological qualities and functions of body cells and tissues. Rasayan therapies do not fall into the category of a pharmacological drug or a therapeutic agent alone. Rasayanas are more nutraceuticals than pharmaceutical agents. In view of the therapeutic attributes reported in Noni plant and its traditional use, it is imperative to label Noni as a Rasayan remedy for its main stream usage. Rasayan Therapy is one of the eight branches of classical Astanga Ayurved.

What is Ayurved ?

Ayurved is the most ancient science of life and health care in the world, its antiquity going back to the sacred Vedas. Ayurved has its own unique pro nature holistic principles, its own materia medica, diagnostics and therapeutic procedures. Because of its safe cost-effective green pharmacy and pro nature holistic approach, Ayurveda has remained a popular system of traditional health care down the ages. However, due to the fast emergence of European medicine during 19th and 20th Century
Ayurved as any other traditional wisdom shrank into the background although it continued to be in an unbroken practice in India and several other South East Asian countries and is still responsible for health coverage of over two thirds of Indian population. Now with the changing scenario of health problems and the inadequacies of the conventional modern medicine the current interest in Ayurved is resurging fast world over. It is believed that Ayurveda and similar other traditional systems of medicine have a great contemporary role to play in the promotive and preventive health care of the masses. As also admitted by WHO in its Alma Ata declaration “Health for All” is not possible without the utilization of time tested readily available natural remedies and local resources of different traditional systems of medicine prevalent in different countries.

The classical wisdom of ancient Ayurved has descended to the present generation through its two sets of three classic texts each popularly known as *Brihattrayi* (1.Caraka samhita, 2.Susruta samhita,3.Vaghbhatta samhitas) and *Laghutrayi* (1.Madhav nidan,2.Sharangdhar Samhita,Bhay prakash) claimed to have been compiled during pre-Christian era and medieval period, respectively.

Ayurveda even in its classical period was practiced through its eight clinical specialties hence known as *Astanga Ayurved* namely:

1. Kayachikitsa (internal medicine)
2. Shalya tantra (surgery)
3. Shalakya tantra (ENT and Ophthalmology)
4. Kaumarbhritya (Pediatrics, Obst. and Gynecology)
5. Agad Tantra (Toxicology)
6. Bhutavidya (demonology and psychiatry)
7. Rasayan (nutrition, rejuvenation and geriatrics)
8. Bajikaran (sexology and reproduction)

It suggests that Ayurveda even several thousand years earlier was already a well developed system of medicine with specialization programs at par to modern medicine. Ayurveda largely uses whole herbs and minerals drawn from the nature as its materia medica available in a wide range of dosage forms. Its emphasis is more on promotion of health and prevention of diseases besides holistic management of different types of chronic life style related diseases. Ayurved largely prescribes life style management, healthy holistic dietetics and green pharmaceuticals in its professional practice.

Besides the written codified tradition of Ayurved many therapies and remedies are prevalent in India and other parts of the world in the folklore which forms the oral tradition of Ayurveda. Noni seems to be a member of folk tradition where as there are many such rejuvenative herbs described in Ayurvedic texts which are in use of the Ayurvedic practitioners in *Shastriya tradition*. *Ashwagandha, Amala* and *Brahmi* are some examples.
Ayurved is a full science of life, health care and medicine. It has its own biology, pathology, diagnostics, pharmacology and therapeutics. Ayurvedic biology attempts to explain the entire function of body-mind-spirit system through its doctrine of Loka-Prush Sanyā (macrocosm-microcosm continuum) governed by the law of Samanya and Visheshb (homology vs heterology) and the theory of Swabho-paramavada (self healing). The structure and function of the body is described in terms of the five basic elements ie. Pancamahabhuta, tridosha (vata-pitta-kapha), Triguna (sattva-raja-tama), Saptadhatus ie seven primary tissues of the body, Agni (biofire system), Ojas (vital force and immune strength) etc. This alternative model of Ayurvedic biology seems to have been developed to suit the eternal holistic approach in contrast to conventional cell-tissue-organ-system approach which has been designed in recent times on reductionist principles of conventional life science. Ayurveda considers life as integrated assembly of body-mind-soul.

Thus Ayurveda is a holistic body-mind-spirit integrative medicine where self healing power of the organism is the principal healing factor. The medicinal medications are used merely to assist the spontaneous natural healing process already going on in the body through bio balancing. All Rasayanas including Noni, Brahmi, Amla and Ashwagandha have nonspecific holistic healing attribute more through the nutrition dynamics of the body and much less through pharmacological effect.

The Rasayan approach

Classically a Rasayan is not merely a drug, it is a regimen incorporating healthy lifestyle (Acarā Rasayan), healthy diet (Ajasrik Rasayan) and nutraceutical rejuvenative drugs (Ausadhi Rasayan). All Rasayanas are claimed to be the means of procuring the best qualities of Dhatus (cells and tissues) in the body leading to healthy aging and longevity, immunity against disease and better mental faculties. It is postulated that most Rasayan remedies (drug or non drug) produce the above mentioned positive health effect through improved molecular nutrition through their impact on three important bio factors in the living body:

1. **Rasa** by directly enriching the nutrient pool of circulating plasma.
2. **Agni** by promoting the bio fire system of the body optimizing the digestion of food and metabolism at different levels promoting in turn the nutritional status of the organism.
3. **Srotas** optimizing microcirculation and tissue perfusion affording better molecular nutrition. Such Rasayanas have ability to cleanse the micro channels of the body so that the nutrients, medicaments, energies and impulses have free access to the molecular level of the body.

The Rasayan effect of all Rasayan drugs is greatly enhanced if the body is cleansed and purified by Panchbākarma therapy. Hence Samsodhan or biopurification with
the help of *Panchakarma* therapy is considered an important pre requisite for any kind of *Rasayan* therapy in Ayurved.

**Some Recent Observations**

Similar to Noni many *Rasayan* remedies of Ayurveda have been scientifically studied in recent years showing evidence of their rejuvenative effect on body-mind system. If the body mind system is in a positive state under influence of *Rasayan* therapy, their remedies may also exhibit a wide range of therapeutic attribute in a holistic fashion although such attributes may not be of specific nature. They are the effect components of the holistic canvas of the *Rasayan*/Rejuvenative effect. For instance Noni is said to be antibacterial, anticancer, anti-diabetic, hypolipidemic, anti-oxidant, immunoenhancer as well as antiolytic. These divergent attributes appear to be largely non-specific and are secondary to the improved nutritional status and generalized rejuvenative effect in the body at molecular level. Hence Noni like any Ayurvedic *Rasayan* should be promoted more as a health supplement for promotive and preventive health care rather than the ambition of developing specific drug therapy for any given specific ailment. Similar is the case of other Ayurvedic *Rasayanas*. *Rasayan* effect in itself is a fundamental therapeutic effect which improves the physiology and life process irrespective of pathology.

**Table 1 : Impact of 60 days treatment with Noni in Diabetes Mellitus (NIDDM)**

<table>
<thead>
<tr>
<th>Observations</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar Fasting 1</td>
<td>65.71 ± 21.66</td>
<td>120.25 ± 19.83</td>
<td>8.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sugar PP</td>
<td>269.25 ± 28.01</td>
<td>171.13 ± 21.81</td>
<td>22.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycylated Hb</td>
<td>8.14 ± 0.86</td>
<td>7.17 ± 0.65</td>
<td>7.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>285.75 ± 48.85</td>
<td>190.73 ± 23.27</td>
<td>6.67</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

(Venkatasubramanian and Priya, 2008)

**Table 2 : Anti Aging effect of Aswagandha (Withenia somnifera) in elderly persons**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before Treat. Mean ± S.D</th>
<th>After Treat. Mean ± S.D</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAS Total Score</td>
<td>19.33 ± 4.57</td>
<td>14.95 ± 4.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Immediate Memory Score</td>
<td>4.55 ± 0.86</td>
<td>4.85 ± 1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BPRS Total Score</td>
<td>32.40 ± 5.22</td>
<td>22.93 ± 2.86</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

(Dwivedi and Singh, 1997)
Table 3: Antistress Effect in some Rasayana remedies

<table>
<thead>
<tr>
<th>Plant Drugs</th>
<th>Prevention of Increase of Adrenal Ascorbic Acid wt.</th>
<th>Prevention of Increase of Adrenal Cortisol</th>
<th>Prevention of Restraint Induced Ulcer Activity as Ed. 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tulasi (O.sanctum)</td>
<td>12.0 ± 1.6</td>
<td>13.0 ± 1.5</td>
<td>13.4 ± 2.0</td>
</tr>
<tr>
<td>2. Ashwagandha (W.somnifera)</td>
<td>13.0 ± 1.4</td>
<td>14.5 ± 1.5</td>
<td>16.0 ± 1.8</td>
</tr>
<tr>
<td>3. P. ginseng</td>
<td>15.0 ± 1.8</td>
<td>24.1 ± 2.1</td>
<td>24.7 ± 2.2</td>
</tr>
</tbody>
</table>

(Singh et al., 1987)

Conclusion

Rasayan therapy is one of the Eight branches of ancient classical Ayurved. It deals with medicinal nutrition, rejuvenation and immunoenhancing designed to achieve healthy longevity and promotive and preventive health care with the help of healthy life style, positive dietetics and a range of rejuvenative medications. This is the core approach of Ayurved. Large number herbomineral Rasayan drugs are described in Ayurvedic texts besides many such remedies prevalent in the folklore. Noni (Morinda citrifolia) is one of such plant drugs. Most of the wide range of therapeutic effects claimed in Noni seem to be the secondary attributes of its primary Rasayan effect. It is worthwhile to undertake comparative studies on Noni and Ginseng with classical Ayurvedic Rasayan drugs like Amla, Ashwagandha, Brahmi etc.

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Noni (*Morinda citrifolia* L.) alters the expression of PI3K, Akt and COX-2 in experimentally induced glioma in rats.

**Authors' affiliation:**
A. J. Vanisree
D. Sabarinathan
Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, Tamilnadu, India
E-mail: vanielango@gmail.com, drajvuom@gmail.com

**Keywords:** Fruit extract of Noni, PI3K, Akt, Cox-2

**Abstract:** Gliomas, comprised of a significant percentage of intrinsic neoplasms of the central nervous system in both adults and children. In tumor condition, Akt phosphorylation is increased leading to activation of survival pathway and the phosphorylated Akt is involved in the regulation of certain key enzymes like PI3K, COX-2 (via the production of prostaglandin E2 (PGE2)) which play key role in tumor progression. The present study was undertaken to determine the involvement of PI3-K/Akt pathway in the regulation of COX-2 expression and PGE2 synthesis in experimentally induced glioma. Animals were divided into six groups with six animals in each group. Group I (Control): Animals were injected with 10µ1 of MEM supplemented with 10 % FBS. Group II: Animals were injected with cell suspension of C6 glioma cells (10µ1 of MEM supplemented with 10 % FBS containing 10^5 cells) under a controlled pressure. Group III: Animals served as drug control (500 mg of Noni extract administrated orally / kg bw for 30 days). Group IV: Rats were induced glioma as mentioned in group II and treated with Noni extract as in group III (from the second day of tumor implantation). Group Va: Rats were induced glioma as mentioned in group II and treated with celecoxib (COX-2 reference drug) 20 mg administered orally / kg of BW for 30 days (for the comparison of COX-2 expression). Group Vb: Rats were induced glioma as mentioned in group II and treated with 5 µl wortmannin (PI3K reference drug) 20 µmol/L administered intracranially at single dose. After the experimental period, the animals were sacrificed and brain tissues subjected to RT-PCR and western blot analysis of pAkt, PI3k and Cox-2. Akt was highly expressed in glioma induced rat brain with simultaneously up-regulated COX-2, PI3K expressions. The levels of PGE2, which indirectly promote tumor angiogenesis, were higher in group II. The observed changes on tumor induction were altered on Noni extract treatment. Inhibition of PI3-K by Noni extract was comparable to that of Wortmannin inhibition which blocked Akt phosphorylation and inhibited expression of COX-2. Administration of Noni extract targets Akt phosphorylation, causes down-regulation of COX-2 and thus combats against the aggressiveness of C6 glioma cells implanted in the rat brain. It can be concluded that the Noni...
extract acts against PI 3-K/Akt survival pathway, involved in the regulation of COX-2 and PGE2 synthesis in C6 induced glioma, the finer investigation of which will be helpful in targeted drug discovery.

Introduction

Cyclooxygenase (COX) is expressed as two isoforms, COX-1 (chromosome 9 (9q32-9q33.3)) and COX-2 (chromosome 1 (1q25.2-25.3)). COX-2 contains 10 exons and is approximately 8.3 kb with a 4.5 kb transcript. IL-1B is a major inducer of COX-2 up-regulation in the CNS (Kurzel et al., 2002). COX-2 is undetectable in most normal tissues (Pamela, 2004). Normal astrocytes alone do not express COX-1, whereas COX-2 expression is minimal when compared with astrocytoma cell lines (Deininger and Schluesener, 1999). COX-1 and -2 expressions are present in all grades of astrocytoma, with COX-2 more often expressed than COX-1. It was found out that COX-2 levels serve as the most reliable indicator of aggressive gliomas (Inoue et al., 1995). There is also compelling evidence that this enzyme may have a role in carcinogenesis in that COX-2-derived prostaglandins may modulate production of angiogenic factors in colon cancer cells.

Protein kinase B (PKB/Akt), a 57-kDa protein–serine/threonine kinase serves a key role in mediating anti-apoptotic actions of growth factors on cell. Mammalian genomes contain three genes encoding Akts (termed Aktα/Akt1, Aktβ/Akt2 and Aktγ/Akt3). Akt3 is highly expressed in brain (Zinda et al., 2001) and plays an important role in neuronal protection (Hui et al., 2005). Stimulation of tyrosine kinase growth factor receptors activates PI3K, which leads to Akt activation. Akt activation is correlated with phosphorylation of Thr-308 at its catalytic domain and of Ser-473 at the C terminus. Over activation of Akt is reported in a variety of cancer including glioma (Haas-Kogan et al., 1998; Holland et al., 2000). Activated Akt can phosphorylate a variety of substrates and thereby regulates important cellular processes, including cell-cycle progression, cell growth, cell survival, cell motility and adhesion, translation of mRNA into protein, glucose metabolism, and angiogenesis. (Knobbe et al., 2002)

Phosphatidylinositol 3-kinase (PI3K), a ubiquitous lipid kinase, is composed of a regulatory subunit (p85) and catalytic subunit (p110) (Jiang and Liu, 2008). PI3K catalyzes the phosphorylation of phosphoinositol-4,5 phosphate (PIP2) at the D3 position to form phosphatidylinositol-(3,4,5)-tri phosphate (PIP3) and activates various downstream elements including Akt. PI3K regulates a number of important cellular processes like cellular growth and transformation, membrane ruffling, actin rearrangement, vesicular trafficking and cell survival. Promotion of cell survival by the activation of PI3K/Akt occurs by the inhibition of proapoptotic signals and the induction of survival signals (Jones and Howell, 1997; Vanhaesebroeck et al., 2001), which may contribute to malignant transformation. Inhibition of PI3K /Akt
results in cell cycle arrest and differentiation in certain cell types, such as the human colon cancer cell lines HT29, Caco-2 (Wang et al., 2001) and in glioma cells (Joy et al., 2003).

COX-2, an inducible enzyme involved in mitogenesis, cellular adhesion, invasion by its metabolite (Kurzel et al., 2002) such as PGE2 in human cancer cells is inappropriately induced, up-regulated in a number of malignant cancer cells including C6 cells (Hwang et al., 2004) and regulated via PI3K/Akt pathway (St-Germain et al., 2004). Glial cells are assumed to be an important source of PGE2 in the CNS (Minghetti et al., 1998). PGE2 is the major PG involved in this process and plays a role in the cytoprotection (Smith, 1989).

The current study assesses the effect of methanolic fruit extract of *Morinda citrifolia* on the expression of COX-2, PI3K and Akt at the level of mRNA as well as protein by RT-PCR and western blot along with the levels of PGE2 by HPLC analysis

**Materials and Methods**

**Chemicals**

pAkt (Mouse monoclonal) and PI3K (Rabbit polyclonal) were purchased from Calbiochem, USA. COX-2 (mouse polyclonal), and â-actin (mouse monoclonal) and TRI reagent from M/s Sigma chemical company, USA. Standard PGE2 were obtained from Genei, Bangalore.

**Animal model**

Male Wistar rats, weighing between 250 - 300 g, were purchased from Kings Institute, Guindy, Chennai, India and maintained under controlled environmental conditions. The animals were provided with pellet food (Gold Mohor rat feed, M/ s. Hindustan Lever Ltd., Mumbai) and water ad libitum. This study was conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and by Animal Ethics Committee Guidelines of our Institution (IAEC No. 01/012/08).

**Experimental protocol**

Animals were divided into six groups with six animals in each group.

Group I (Control): Animals were injected with 10 µ1 of MEM supplemented with 10 % FBS.

Group II: Animals were injected with cell suspension of C6 glioma cells (10 µ1 of MEM supplemented with 10 % FBS containing 105 cells) under a controlled pressure.
Group III: Animals served as drug control (500 mg of Noni fruit extract administrated orally / kg of bw for 30 days).

Group IV: Rats were induced glioma as mentioned in group II and treated with Noni fruit extract as in group III (from the second day of tumor implantation).

Group Va: Rats were induced glioma as mentioned in group II and treated with celecoxib (COX-2 reference drug) 20 mg administered orally / kg of BW for 30 days (for the comparison of COX-2 expression).

Group Vb: Rats were induced glioma as mentioned in group II and treated with 5 µl wortmannin (PI3K reference drug) 20 µmol/L administered intracranially at single dose. (for the comparison of PI3K expression).

Reverse Transcription–Polymerase Chain Reaction (RT–PCR) Analysis of COX-2, Akt and PI3K.

The mRNA expression of COX-2, Akt and PI3K were analysed using RT -PCR. Total RNA extraction from brain tissue was performed using TRIZOL reagent which is based on the acidic phenol-chloroform method and about 5µg of total RNA was taken for the synthesis of cDNA. The reaction mixture for the synthesis of cDNA contains 5 µg of total RNA, 1 µl of random hexamer (0.2 mg/µl) and 12.5 µl of nuclease free water. Then the contents were mixed for 3-5 seconds and spun at 70ºC for a minutes and snap frozen on ice. To the mixture, 4.0 µl 5X reaction buffer-Reverse transcriptase, 0.5 µl Ribonuclease inhibitor (20units/µl), 6.0 µl DEPC treated water and 4.0 µl MgCl₂ (25mM) were added. Then the reaction mixture was kept at 25ºC for 5 min. To the reaction mixture, 5 µl of Reverse transcriptase (40 units/ µl) was added and maintained at 25 ºC for 10 min and suddenly transferred to 42 ºC for 60 min and the reaction was stopped by heating the mixture to 70ºC for 30 min and snap frozen. The resulting mixture contains the cDNA product which was used for PCR analysis. The reaction mixture consist of Millipore water - 4.95 µl, 10X PCR -1.0 µl 10mM, dNTPs -0.2 µl, Primer forward-1.0 µl (5 pmoles/ µl), Primer Reverse-1.0 µl (5 pmoles/ µl), Taq polymerase-0.05 µl, Template-1.0 µl. PCR was performed using the following profile: Initial denaturation temperature - 95ºC -5 min, Cyclic denaturation temperature - 95ºC -30 sec, Annealing temperature - varies for different primers- 45 sec, Extension temperature -72ºC -30 sec, Stage 2 was repeated for 29 cycles and Final extension temperature -72ºC-3min. PCR product were visualized under UV light.
Nucleotide sequences of sense and antisense primers designed for RT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size (bp)</th>
<th>Source</th>
<th>Tm(°C)</th>
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<tr>
<td>COX-2</td>
<td>5’-TGGTGCCGGGTCTGATGATG-3’</td>
<td>253</td>
<td>Gustafson-Svärd et al., 1996</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>5’-GCAATGCCGTTCTGATACCT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td>5’-CACCTGGACTTTGGAACCT-3’</td>
<td>233</td>
<td>Primer-3 software</td>
<td>60</td>
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<tr>
<td></td>
<td>5’-GAATCAGAATCTCCGGAAC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Akt</td>
<td>5’-TGGTTCGAGAGGCAAGT-3’</td>
<td>213</td>
<td>Primer-3 software</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>5’-AAAAACAGCTCTCCCATATT-3’</td>
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<tr>
<td>â-Actin</td>
<td>5’-ACCACACGCTGAGGAAAAATCG-3’</td>
<td>276</td>
<td>Gong et al., 2002</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>5’-AGAGGTCTTTAGGATGCAAGG-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Western blot analysis of pAkt, PI3K and COX-2

COX-2, pAkt and PI3K protein expressions were analyzed using a protocol, adapted from Fiddo et al. (1995). 50 mg of total protein containing tissue homogenate was mixed with 2x sample buffer and boiled for 5 min. The sample mixture was run on 12% SDS-PAGE gels in 1x running gel buffer at 100 V for 2.5 h and electro transferred to a PVDF membrane at 30 V for 1 h 30 min. The membrane was blocked in blocking buffer containing 5% BSA for overnight. After overnight, the blocked membranes were incubated with primary antibodies diluted in TBS-T: pAkt (1:1000), PI3K (1:1000), COX-2 (1:2000) and â-actin (1:5000) overnight at 4°C, with gentle shaking. The membranes were washed with TBS-T gently and subsequently incubated with appropriate secondary antibodies (anti-rabbit or anti-goat IgG) linked to HRP at a 1:4000 dilution for 1 h. â-actin served as internal control to check for equal loading of protein. The bands were visualized using luminol reagent and intensity of each band was determined using an image analyzer (quantity none software from Bio Rad).

Estimation of PGE₂

The tissue PGE₂ was extracted by the method of Bligh and Dyer, (1959) with the final proportions of 0.13 g of tissue, 1.6 ml of H₂O, 2 ml of methanol, and 2 ml of CHCl₃. After removal of the first CHCl₃ extract, the H₂O:methanol phase and solid interface were acidified with HCl (final concentration 0.01 M) and re-extracted with 2 ml of CHCl₃. An additional CHCl₃ extraction of the acidified H₂O:methanol phase was performed in order to maximize the recovery of PGs. The combined CHCl₃ extracts which contained butylated hydroxytoluene were back-extracted with H₂O (1 volume of H₂O/3 volumes of CHCl₃) until the pH of the H₂O was neutral. The CHCl₃ extract was concentrated under a stream of N₂ and aliquots were taken for HPLC. The recovery of standard PGE₂ was 85%. The HPLC system was normal phase using a
25 cm x 4.6 mm Zorbax C18 column with 5-µm particles. The solvent system was 0.1% TFA in water and acetonitrile (v/v/v) at a flow rate of 0.7 ml/min.

Statistical analysis

All the grouped data were evaluated with SPSS/15 software. Testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of < 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for six animals in each group.

Results

Effects of Noni extract on mRNA expression of COX-2, PI3K and Akt.

RT-PCR analyses were performed to determine the mRNA expression of COX-2, PI3K and Akt in control and experimental groups. mRNA of COX-2 (Fig. 1a), PI3K (Fig. 1b) and Akt (Fig. 1c) showed high levels of expression in glioma induced rats (group II).

![Figure 1. representing mRNA Expression of COX-2, PI3K and Akt in brain tissue from the control and experimental rats. Both actin served as internal control. Lane 1: control (Group I); Lane 2: glia induced (Group II); Lane 3: drug control (Group III); Lane 4: Tumor induced + Noni treated (Group IV); Lane 5: Tumor induced + saline drug (Group V). See Fig. 2a and 2b.](image-url)
when compared to that of control rats. In Noni extract treated group, there was a low level of mRNA expression of these enzymes when compared to that of glioma induced group. α- actin mRNA expression served as a internal control.

Effects of Noni extract on protein expression of COX-2, PI3K and pAkt

Western blotting analyses were performed to determine the protein expression COX-2, PI3K and pAkt in control and experimental groups. The protein expression of COX-2 (Fig.2a) PI3K (Fig.2b) and pAkt (Fig. 2c) were also showed significantly high level of expression in glioma induced rats (group II) when compared to that of control rats. In Noni extract treated group, there was a low levels of expression of these proteins when compared to that of glioma induced group. α- actin expression served as a internal control.

Figure 2. representing protein expression of COX-2, PI3K and pAkt respectively, in brain tissues from the control and experimental rats. Beta actin served as internal control. Lane 1: control (Group 1); Lane 2: glioma induced (Group 2); Lane 3: drug control (Group 3); Lane 4: Tumor induced + Noni treated (Group 4), Lane 5: Tumor induced + reference drug (Group 5a for Fig. 2a, group 5a for Fig. 2b and Fig. 2c). Values are expressed as mean ± S.D. (n = 6). *p symbol represents statistical significance at p < 0.05. Comparisons are made as (a) Group 1 vs. Group 2, (b) Group 2 vs. Group 4, (c) Group 1 vs. Group 4, (d) Group 4 vs. Group 5 and † non significant Group 1 vs. Group 3.
A. J. Vanisree and D. Sabarinathan, *Noni alters the expression of PI3K, Akt and COX-2 in experimentally induced glioma in rats.*

The observation of reduced PI3K expression was comparable to and close to that of wortmanin (a PI3K inhibitor, reference drug) treated group and reduced COX-2 expression was comparable and close to that of celecoxib (COX-2 inhibitor, reference drug) treated group.

**Effects of Noni extract on PGE2**

The HPLC analysis of PGE2 revealed that the production was increased in glioma induced group when compared to that of control rats. The administration of Noni extract to glioma induced group markedly reduced the production of PGE2 (Fig. 3).

![HPLC analysis of PGE2](image)

**Figure 3** Effect of Noni on PGE2 in brain tissues of the control and experimental groups.

1. Standard PGE2
2. Control (Group 1)
3. Glioma induced (Group 2)
4. Tumor induced + Noni treated (Group 4)

Area under the peaks (PGE2) was determined to represent the concentration of PGE2. * symbol represents statistical significance at p < 0.05. Comparisons are made as (a) Group 1 vs. Group 2 and (b) Group 2 vs. Group 4.
Discussion

Badie et al. (2003) suggested that intracranial tumours express more COX-2 than models of subcutaneous C6 glioma which is consistence with current study showing an over expression of COX-2 (mRNA and enzyme) as well as high level of PGE\textsubscript{2} production in glioma induced group. This elevation can influence the mechanisms involved in carcinogenesis, such as angiogenesis, inhibition of apoptosis, stimulation of cell growth as well as the invasiveness of C6 glioma cells (Lapulescu, 1996) in group II where expression were altered on Noni treatment in the study.

Recent evidence indicates that COX-2 is an important molecular target for anticancer therapies (Pamela, 2004). In the Noni treated rats there was a marked decrease in the expression of COX-2 and in the production of PGE\textsubscript{2}. The effect of Noni was comparable and close to celecoxib induced COX-2 inhibition.

The activation of PI3K/Akt pathway which has a key role in regulating cell cycle processes (Thaler et al., 2009), angiogenesis (Dimmeler and Zeiher, 2000), anti-apoptotic functions (Nicholson and Anderson, 2002) has been reported. It has also been reported that increased PI3K activity due to gene amplification and mutation of catalytic and regulatory subunits of PI3K is common in high grade glioma, (Mizoguchi et al., 2004) and that inhibition of PI3K is cytotoxic in several human glioma cell lines in vitro (Guillard et al., 2009). Obviously, glioma implanted rat brain registered an increased expression of PI3K/Akt in group II rats leading to a positive influence on the cell survival mechanism. When the expression in group IV rats was observed, it is prompting to interpret the results on the basis of reduction in the number of proliferating C6 glioma cell.

Noni extract was reported as dual inhibitors of both COX-2 and 5-LOX enzymes which are potential candidate for neuroprotection, anticancer and anti-inflammation. (Wang et al., 2002). Recently SC236, a COX-2 inhibitor is shown to induce apoptosis of gastric cell line through MAPK and Akt pathway (Fan et al., 2004). pAkt is said to increase the expression of COX-2 (St-Germain et al., 2004). As Akt is said to influence COX-2 expression, the observed reduced expression of pAkt in group IV is thought to be involved in COX-2 regulation. Thus, the block of PI3K/Akt and perhaps the Akt regulated expression of COX-2, were putatively seem to be involved in the effect of Noni against experimental glioma.
References


Fan XM, Jiang XH, Gu Q, Ching YP, He H, Xia HHX, Mi Lin MC, Chan AOO, Yuen MF, Kung HF and Wong BCY. 2006. Inhibition of Akt/PKB by a COX-2 Inhibitor Induces Apoptosis in Gastric Cancer Cells. Digestion., 73: 75-83.


Studies on anticancer activity of ethanolic extract of Noni fruit (*Morinda citrifolia* L.)

**Authors’ affiliation:**
Periyasamy Selvam1*
T. Paul Pandi1
P.Vasanth Raj2
1. Devaki Amma Memorial College of Pharmacy, Malapuram 676364, Kerala, India,
2. Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India.
Email: periyasamy_selvam@yahoo.co.in

**Abstract:** Noni (*Morinda citrifolia* L.) is used in Indian system of medicine for treatment of a variety of diseases. This plant is enriched with flavanoids, anthroquinone and glycosides. The present work is to study the effect of ethanolic extract of *Morinda citrifolia* fruits (MCF-ET) on HepG2 (human liver cancer) cell culture and Hep2 (Human laryngeal epithelial carcinoma) cell culture respectively. Noni (*Morinda citrifolia* L.) fruits were collected from WNRF, Chennai were shade dried and extracted using ethanol to study its *in vitro* cytotoxicity activity against HepG2 (human liver cancer) cells and Hep2 (Human laryngeal epithelial carcinoma) cells using methods like MTT and SRB assay. Ethanolic extract of Noni fruits *Morinda citrifolia* L (MCF-ET) showed very potent cytotoxicity against HepG2 and Hep2 cells with CTC50 (cytotoxicity 50 %) values of 171 µg/ml and 181 µg/ml respectively. MCF-ET showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. Hence this extract merits further investigation to screen its anti cancer activity using *in vitro* and *in vivo* models.

**Keywords:** *Morinda citrifolia*, Hep2 cells and HepG2 cells, MTT, SRB.

**Introduction**

Noni (*Morinda citrifolia* L) is a versatile medicinal plant with a broad spectrum of pharmacological activities. *Morinda citrifolia* possesses hepatoprotective (Wang et al., 2008a, b), anticancer (Akihisa et al., 2008), immunomodulatory (Palu et al., 2007), anti-inflammatory (Palu et al., 2007), wound healing (Nayak et al., 2007), antioxidant (Su et al., 2005), anti-tubercular (Saludes et al., 2002), wide spectrum of biological activities (Pawlus and Kinghorn., 2007) and anti-HIV (Umezawa et al., 1992; Masakazu et al., 2006; Bina et al., 2007). Recently much attention was devoted for searching potential safe herbal medicines from natural products for the treatment of various diseases and *Morinda citrifolia* used for the treatment of a variety of diseases and safe herbal drug (West et al., 2006). The present work is to study the inhibitory activity of ethanolic extract of the fruit powder of *Morinda citrifolia* against Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells.
Materials and Methods

**Preparation of Extracts:** The fruit powder of *Morinda citrifolia* is dried under shade and further powdered. The powder is extracted with ethanol for five days by cold maceration. It is then filtered to get the extracts evaporated to dryness under vacuum. The dried ethanolic extract (MCF-ET) is used for cytotoxicity studies in Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells.

**Preparation of suspensions**

The ethanolic extract of Noni fruits (*Morinda citrifolia* L) was dissolved in DMSO and the volume was made up to 10ml with DMEM/MEM to obtain a stock solution of 1mg/ml concentration and stored at -20 °C prior to use. Further dilutions were made to obtain different concentrations ranging from 1000–62.5µg/ml with respective media and used for *in vitro* investigations.

**Cell lines and growth media**

Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (Human liver cancer) cells were cultured in MEM (minimum essential medium) and DMEM (Dulbecco's modified eagles medium) medium respectively. The medium also contains 10% fetal calf serum, penicillin (100 U) and streptomycin (100 µg).

**In vitro cytotoxicity screening**

The ability of the cells to survive a toxic insult is the basis of most cytotoxicity assays. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0x10^5 cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100ml of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and MTT (Francis D and Rita L., 1986) and SRB (Philip et al., 1990) assays performed.

**Morphological observation by acridine orange staining**

Staining cells with fluorescent dyes, such as acridine orange is used in evaluating the nuclear morphology of apoptotic cells. To confirm that apoptosis have been induced by *Morinda citrifolia* (MCF-ET) plant extract, HepG2 cells were analysed in the presence of acridine orange (AO). Acridine orange (AO) is a vital dye that will stain both live and dead cells (Javadev et al., 2004). Two different concentrations were chosen based on the IC₅₀ values determined by MTT assay, which were 100 and 200 ig/ml. As a control, HepG2 cells were cultured in complete media and stained with AO. Cells stained green
represent viable cells, whereas yellow staining represented early apoptotic cells, and reddish or orange staining represents late apoptotic cells. As shown in Figure 1 HepG2 cells treated with 100 and 200 μg/ml of MCF-ET showed changes in cellular morphology, including chromatin condensation, membrane blebbing and fragmented nuclei. Our result clearly shows that the Morinda citrifolia (MCF-ET) induced apoptosis after 48 hours incubation at both the concentration of plant extract tested.

Results

Ethanolic extract of Noni fruits (Morinda citrifolia L) (MCF-ET) showed very potent cytotoxicity against HepG2 and Hep2 cells (Table 1&2) with CTC 50 (cytotoxicity 50 %) values of 171 μg/ml and 180 μg/ml respectively. MCF-ET showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. Hence this extract merits further investigation to screen its anti cancer activity using in vitro models. It was evident from nuclear morphology studies that Morinda citrifolia (MCF-ET) showed nuclear morphology changes (Fig. 1) similar to that of apoptotic cell morphology in cancerous cell culture HepG2 (Human liver cancer cells). In normal cell culture tested, here was no such nuclear morphological change. This in vitro study has proved the selective toxicity Morinda citrifolia (MCF-ET) against cancer cells. Hence this work can be taken up for in vivo and pre clinical studies.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>CTC 50 in (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-ET</td>
<td>163 ± 1.15</td>
</tr>
</tbody>
</table>

Table 1 Determination of CTC 50 by using MTT and SRB assay in HepG2 (human liver cancer) cell cultures

<table>
<thead>
<tr>
<th>Extracts</th>
<th>CTC 50 in (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-ET</td>
<td>180.5 ± 2.12</td>
</tr>
</tbody>
</table>

Table 2 Determination of CTC 50 by using MTT and SRB assay in Hep2 (Human laryngeal epithelial carcinoma) cell cultures

Average of six determinations, values are mean ± SER
Discussion

The Polynesians utilized the whole Noni plant in various combinations for herbal remedies (Wang et al., 2002, McClatchey W. 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. Noni possessed wide spectrum of anticancer activity. From there studies, ethanolic extract of noni fruit was screened for anticancer activity. Ethanolic extract of noni fruit showed potent toxicity against two different human cancer cells from liver and laryngeal origin respectively. This extract merits further investigation to screen its anti-cancer activity using in vivo models and further studies regarding isolation of active constituents from ethanolic extract under way. Our result clearly shows that the *Morinda citrifolia* (MCF-ET) induced apoptosis after 48 hours incubation at both the concentration of plant extract tested.

Figure 1: Nuclear staining using acridine orange. 1) Normal HepG2 cells; 2) HepG2 cells + MCF-ET (100 µg/ml) treated. 3) HepG2 cells + MCF-ET (200 µg/ml) treated. Arrows indicate membrane blebbing.
Periyasamy Selvam et al., Studies on anticancer activity of ethanolic extract of Noni fruit (Morinda citrifolia L.)

References


Periyasamy Selvam et al., Studies on anticancer activity of ethanolic extract of Noni fruit (*Morinda citrifolia* L.)


Studies on anti-HIV activity and cytotoxicity of stem extracts of *Morinda citrifolia* L Noni

Authors' affiliation:
Periyasamy Selvam
Christophe Pannecouque
Erik De Clercq
1. Devaki Amma Memorial College of Pharmacy, Chelembra, Kerala, India. E-mail: periyasamy_selvam@yahoo.co.in
2. Rega Institute for Medical Research, Katholieke Universiteit-Leuven B-3000, Leuven, Flanders, Belgium.

Abstract: Stem extracts of *Morinda citrifolia* L studied for antiviral activity against HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells. Cytotoxicity of the extracts for mock-infected MT-4 (Adult C type T leukemia cell) cells was assessed by the MTT method. Chloroform extract of *Morinda citrifolia* exhibited a 21% maximum protection of against replication of HIV-2 in acutely infected MT-4 cells at subtoxic concentration. Ethanol and methanol extracts displayed marked cytostatic activity in MT-4 cells with (CC50 = 0.15 and 0.17µg/ml).

Keywords:

Introduction

*Morinda citrifolia* L (Noni) is a versatile medicinal plant with a broad spectrum of pharmacological activities. *Morinda citrifolia* is reported to possess 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), antioxidand(Su et al., 2005), anti-tubercular (Saludes et al., 2002) and wide spectrum of biological activity ( Pawlus and Kinghorn 2007) and 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 *morinda citrifolia* is relatively less explored.

We have previously prepared different extracts of leaf and fruit of *Morinda citrifolia* and screened for their anti-HIV activity and cytotoxicity, some of these extracts exhibited anti-HIV activity against HIV-1 and marked cytotoxicity in MT-4 cells (Selvam et al., 2007; 2008). The present work is to study the anti-HIV activity of various extracts of the stem powder of *Morinda citrifolia* against HIV 1 and 2 in MT-4 cells. Cytotoxicity of the extracts for mock-infected MT-4 (Adult C type T leukemia cell) cells was assessed by the MTT method

Experimental

The stem portion of *Morinda citrifolia* is collected from the Calicut area, Kerala and dried in shade. The dried stem powder of *Morinda citrifolia* was extracted (cold maceration method) with acetone, chloroform, methanol and ethanol for five days. It...
was then filtered to get the crude extracts, which was then evaporated to dryness under vacuum and the dried extracts of acetone (AMC), chloroform (CMC), ethanol (EMC) and methanol (MMC) were used for anti-HIV activity and cytotoxicity studies.

Anti-HIV Assay:

The dried extracts were tested for anti-HIV activity against the replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells (Selvam et al., 2008). The cells were grown and maintained in RPMI 1640 Medium supplemented with 10% heat-inactivated Fetal Calf Serum (FCS), 2 mM-glutamine, 0.1% Sodium bicarbonate and 20 µg/ml gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain and HIV-2 (LAV-2ROD) strain were used in the experiment. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at -70°C until used.

Inhibitory effects of the compounds on HIV-1 and HIV-2 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 µl of HIV-1 and HIV-2 (100-300 CCID50) were added to a flat-bottomed MT-4 cells (6x10^5 cells/ml). After 5 days of incubation, at 37°C the number of viable cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock-infected MT-4 cells was assessed by the MTT method. The anti-HIV activity and cytotoxicity data of Morinda citrifolia are presented in Table 1.

Table 1. Anti-HIV Activity of Stem Extracts of Morinda citrifolia L

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Strain</th>
<th>EC50 a(µgm/ml)</th>
<th>CC50 b(µgm/ml)</th>
<th>Maximum Protection (%)</th>
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<td>AMC</td>
<td>IIIB</td>
<td>&gt;0.81</td>
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<tr>
<td></td>
<td>ROD</td>
<td>&gt;0.81</td>
<td>0.81±0.03</td>
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<td>CMC</td>
<td>IIIB</td>
<td>&gt;0.15</td>
<td>0.15±0.01</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;0.15</td>
<td>0.15±0.01</td>
<td>21</td>
</tr>
<tr>
<td>EMC</td>
<td>IIIB</td>
<td>&gt;0.17</td>
<td>0.17±0.04</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;0.17</td>
<td>0.17±0.04</td>
<td>18</td>
</tr>
<tr>
<td>MMC</td>
<td>IIIB</td>
<td>&gt;0.77</td>
<td>0.77±0.06</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;0.77</td>
<td>0.77±0.06</td>
<td>12</td>
</tr>
<tr>
<td>AZT</td>
<td>IIIB</td>
<td>0.00012</td>
<td>65.90</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>0.00062</td>
<td>65.90</td>
<td>148</td>
</tr>
</tbody>
</table>

a50% Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.
b50% Cytotoxic concentration of compound, required to reduce the viability of mock-infected MT-4 cells by 50%.
Results and Discussion

*Morinda citrifolia* L stem extracts has been evaluated for its anti-HIV activity and cytotoxicity (Table 1) against HIV-1 and 2 replication in acutely infected MT-4 cells. Various extracts of *Morinda citrifolia* exhibited a 8-21% maximum protection against the replication of HIV-1 and HIV-2 in acutely infected MT-4 cells. Chloroform extract of *Morinda citrifolia* (CMC) exhibited a maximum protection of 21% against replication of HIV-2 (ROD) in MT-4 cells at subtoxic concentration. Ethanol (EMC) and methanol (MMC) extracts displayed marked cytostatic activity in MT-4 cells with (CC<sub>50</sub> = 0.15 and 0.17ig/ml).

The Polynesians utilized the whole Noni plant in various combinations for herbal remedies (Wang et al., 2002, McClatchey W. 2002) such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastritis, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction. Review of literature revealed that only two studies were available for anti-HIV activity of Noni. 1) The compound isolated from Noni roots named 1-methoxy-2-formyl-3-hydroxyanthraquinone suppressed the cytopathic effect of HIV infected MT-4 cells, without inhibiting cell growth (Umezawa et al., 1992). 2) Viral protein R (Vpr), one of the human immunodeficiency virus type 1 (HIV-1) accessory proteins, contributes to multiple cytopathic effects, G2 cell cycle arrest and apoptosis. The mechanisms of Vpr have been intensely studied because it is believed that they underlie HIV-1 pathogenesis. Damnacanth (Dam), a component of noni fruit, as an inhibitor of Vpr induced cell death (Masakazu et al., 2006). Antiviral activity against HIV of stem portion of *Morinda citrifolia* is relatively less studied and from this studies different extracts of stem portion of *Morinda citrifolia* L noni exhibits marked cytotoxic activity in less than 1 ug/ml in Adult C type leukemic cells (MT-4 cell).

References


Analgesic and anti-pyretic activities of aqueous and alcoholic extracts of Noni
(Morinda citrifolia L.)

Abstract: One up-coming botanical fruit of Morinda citrifolia, has Xeronine, the key constituent of Noni also acts as body supreme pain killer because it works with endorphins in the body to numb pain and produce feeling of euphoria. Alcoholic and aqueous extracts of Noni at graded doses showed significant analgesic action. The alcoholic extracts of Noni with 1000 mg/kg, however has better analgesic action which persisted for four hours as compared to aqueous extract at the same dose rate. The percent analgesic score of both extracts persisted up to 3 h of administration. Both the aqueous and alcoholic extracts of Noni significantly reduced the yeast induced pyrexia starting from 1 h post administration and it was completely brought back to normal within 4 h post administration. The alcoholic extract @ 1000 mg/kg produced better antipyretic action as compared to aqueous extract at the same dose up to 3 h of administration.

Introduction

Over the past a few years, natural products have become increasingly popular and the field of natural herbal remedies has flourished to a great extent. One up-coming botanical fruit of Morinda citrifolia, whose Polynesian name is Noni, is currently the subject of much science, myth and marketing hype. Noni, as called in the Hawaiian language, is also called the great morinda and Indian mulberry (Samy, 2005) analgesic and anti-pyretic. The present study is concentrated to find out the effects of Morinda citrifolia.

A phyto-therapeutic approach to modern drug development provides many invaluable drugs from traditional medicinal plants. Numerous plants and polyherbal formulations are used for the treatment of various infections, to numb pain and to reduce body temperature.

Materials and Methods

The experiment was conducted on 36 healthy adult albino rats of either sex, weighing between 120-150g. The rats were procured from small animal section of Veterinary College Hissar and maintained in polypropylene cages and housed in the
lab animal section of the department under standard managemental conditions. Rats were fed standard ration ad libitum and had free access to clean water. De-worming was done before the start of experiment by praziquantel @ 1 tablet per 10 kg body weight, single dose. Before experimentation, rats were fasted overnight but water was given ad libitum. The experiment was conducted in six groups of rats consisting of six rats in each group. The rats were administered the aqueous and alcoholic extracts of Noni orally in graded doses of 250, 500, 1000 mg/kg body weight.

The analgesic activity of Noni was studied by the method of Curzen et al. (1980) using the hot plate analgesiometer. The licking of hind paw in hot plate was taken as the index of analgesia. The time between putting the rat on hot plate and start of licking the paw was the latent period or reaction time for analgesic activity of drug.

Percent analgesic scope (PAS) = $1 - \frac{T_2}{T_1} \times 100$.

Where $T_1$ = reaction time (in sec.) before drug administration, $T_2$ = reaction time (in sec.) after drug administration

The rats were individually caged in a room maintained at a constant temperature of 25 °C. Normal rectal temperature was recorded by a Telethermometer and its hourly variation was noted over a period of 4 hours at the beginning of the experiment.

In above rats, pyrexia was produced according to the method of Gujral et al. (1956). A suspension of 15 percent dried Brewers yeast and 2 percent gum acacia in normal saline was injected subcutaneously. The volume injected was 1.0 ml of suspension per 100 g of body weight. A stabilized temperature was recorded in 18 hours. In pyretic rats, the rectal temperature was recorded at hourly interval for a period of 4 hours after administration of the drug.

The model of experiment on analgesic and antipyretic was as follows-

- **Group I** - Aqueous extract of Noni (250 mg /kg bwt. orally)
- **Group II** - Aqueous extract of Noni (500 mg/kg bwt. orally)
- **Group III** - Aqueous extract of Noni (1000 mg/kg bwt. orally)
- **Group IV** - Alcoholic extract of Noni (250 mg /kg bwt. orally)
- **Group V** - Alcoholic extract of Noni (500 mg/kg bwt. orally)
- **Group VI** - Alcoholic extract of Noni (1000 mg/kg bwt. orally)

**Results and Discussion**

Aqueous extracts of Noni in doses of 250 mg/kg body weight did not show any significant increase in the reaction time except after 2nd and 3rd hours, where the increase being from 3.26 ±0.17 to 4.31 ± 0.31 and 4.85 ± 0.31 seconds. The
aqueous extracts of Noni in doses of 500 mg/kg body weight showed significant increase in the reaction time, the increase being from 3.08 ± 0.24 to 4.84 ± 0.31 and 5.0 ± 0.30 respectively at 2nd and 3rd hour of treatment. The dose of 1000 mg/kg body weight of aqueous extracts of Noni significantly increased the reaction time from 3.14 ± 0.11 to 5.21 ± 0.40, 6.48 ± 0.30 and 6.51 ± 0.33 in 1st, 2nd and 3rd hours of treatment (Table 1).

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Table 1: Analgesic activity of orally administered aqueous and alcoholic extracts of Noni by hot plate method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) oral</th>
<th>Reaction time in sec. (Mean and SE)</th>
<th>Percent analgesic score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before drug</td>
<td>After drug administration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hr</td>
<td>2 hr</td>
</tr>
<tr>
<td>Aqu. ext. of Noni</td>
<td>250</td>
<td>3.66 ± 0.17</td>
<td>4.31 ± 0.31</td>
</tr>
<tr>
<td>Aqu. ext. of Noni</td>
<td>500</td>
<td>3.08 ± 0.24</td>
<td>4.12 ± 0.33</td>
</tr>
<tr>
<td>Aqu. ext. of Noni</td>
<td>1000</td>
<td>3.14 ± 0.11</td>
<td>5.21 ± 0.40</td>
</tr>
<tr>
<td>Alc. ext. of Noni</td>
<td>250</td>
<td>3.15 ± 0.37</td>
<td>4.42 ± 0.40</td>
</tr>
<tr>
<td>Alc. ext. of Noni</td>
<td>500</td>
<td>3.22 ± 0.13</td>
<td>5.0 ± 0.30</td>
</tr>
<tr>
<td>Alc. ext. of Noni</td>
<td>1000</td>
<td>3.28 ± 0.17</td>
<td>5.5 ± 0.12</td>
</tr>
</tbody>
</table>

Each treatment consists of six rats.
Means in a particular class (row) with different superscripts differ significantly from each other.
Means of a particular class (row) with at least one alphabet as common superscripts do not differ significantly from each other.
Alcoholic extracts of Noni in doses of 250 mg/kg body weight did not show any significant increase in the reaction time except after 3rd and 4th hours, where the increase being from 3.15 ± 0.37 to 4.71 ± 0.38 and 4.74 ± 0.36 seconds. The alcoholic extracts of Noni in doses of 500 mg/kg body weight showed significant increase in the reaction time, the increase being from 3.22 ± 0.13 to 5.61 ± 0.14 and 5.82 ± 0.20 respectively at 2nd and 3rd hour of treatment respectively. The dose of 1000 mg/kg body weight of alcoholic extracts of Noni significantly increased the reaction time from 3.28 ± 0.17 to 5.5 ± 0.12, 6.90 ± 0.10 and 7.20 ± 0.10 in 1st, 2nd and 3rd hour of treatment respectively. (Table 1)

The peak analgesic effect of alcoholic and aqueous extracts of Noni after its oral administration was observed after 2nd hour of its administration and both the extracts showed the dose dependent analgesic activity. Percent analgesic score for aqueous and alcoholic extracts of Noni were 75.47 and 89.02 percent respectively at 3rd hour of treatment.

Prostaglandins of E series are known to stimulate the pain receptors directly or indirectly by sensitizing them to other algogenic agents like bradykinin or 5-HT (Collier and Schneider, 1972). The analgesic effect of Noni in the present study is probably due to its ability to inhibit the biosynthesis of various prostaglandins.

Heinicke (1985) reported that Noni fruit contains a natural precursor for Xeronine called Proxeronine. Proxeronine is converted to the alkaloid, Xeronine in the body by an enzyme called Pro xeroninase. Xeronine is the key constituent of Noni and acts as body supreme pain killer because it works with endorphins in the body to numb pain and produces feeling of euphoria. According to Younos et al. (1990) the analgesic efficacy of the Noni extract is 75 percentages as strong as morphine.

In the present study aqueous and alcoholic extracts of Noni was administered in graded doses of 250, 500 and 1000 mg/kg body weight and the results indicated that both the extracts exhibited dose related analgesic activity. Percent analgesic score for aqueous and alcoholic extracts of Noni were 75.47 and 89.02 percent respectively at 3rd hour of treatment. The alcoholic extracts of Noni with 1000 mg/kg has better analgesic action which persisted for four hours.

Aqueous extracts of Noni at doses 500 mg / kg showed a significant decrease in pyretic temperature, the decrease being from 102.73°C F ± 0.09 to 101.21°C F ± 0.02, 100.15°C F ± 0.01 and 100.06°C F ± 0.02 in 2nd, 3rd and 4th hours of treatment respectively. Aqueous extracts of Noni at doses 1000 mg / kg also showed a significant decrease in pyretic temperature, the decrease being from 102.82 °C F ±0.05 to 100.47°C F ± 0.09 and 100.07°C F ± 0.01 in 2nd and 3rd hours of treatment respectively. (Table 2)
Alcoholic extracts of Noni at doses 500 mg / kg also showed a significant decrease in pyretic temperature, the decrease being from 102.70°C F ± 0.09 to 100.38°C F ± 0.04 and 100.03°C F ± 0.01 in 2nd and 3rd hours of treatment respectively. (Table 2)

Alcoholic extracts of Noni at doses 1000 mg / kg showed a significant decrease in pyretic temperature from first hour onwards, the decrease being from 102.76°C F ± 0.09 to 101.14°C F ± 0.02, 100.06°C F ± 0.02 and 100.01°C F ± 0.25 in 2nd, 3rd and 4th hours of treatment respectively. (Table 2)

### Table 2: Antipyretic activity of orally administered aqueous and alcoholic extracts of Noni on yeast induced pyrexia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) oral</th>
<th>Pyretic temperature in Fahrenheit (Mean and SE)</th>
<th>Temperature after drug in Fahrenheit (Mean and SE)</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aequorine</td>
<td>250</td>
<td>102.62 ± 0.13</td>
<td>102.15 ± 0.09</td>
<td>101.34 ± 0.06</td>
<td>100.40 ± 0.08</td>
<td>100.20 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Aequorine</td>
<td>500</td>
<td>102.75 ± 0.09</td>
<td>102.09 ± 0.01</td>
<td>101.21 ± 0.02</td>
<td>100.13 ± 0.01</td>
<td>100.06 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Aequorine</td>
<td>1000</td>
<td>102.82 ± 0.05</td>
<td>101.57 ± 0.06</td>
<td>100.47 ± 0.09</td>
<td>100.07 ± 0.01</td>
<td>100.01 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>250</td>
<td>102.89 ± 0.06</td>
<td>102.08 ± 0.01</td>
<td>101.15 ± 0.05</td>
<td>100.27 ± 0.01</td>
<td>100.14 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>500</td>
<td>102.70 ± 0.09</td>
<td>101.54 ± 0.04</td>
<td>100.36 ± 0.04</td>
<td>100.05 ± 0.01</td>
<td>100.01 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>1000</td>
<td>102.76 ± 0.09</td>
<td>101.14 ± 0.02</td>
<td>100.14 ± 0.02</td>
<td>100.06 ± 0.02</td>
<td>100.01 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

Each treatment consists of six rats.

Means in a particular class (row) with different superscripts differ significantly from each other.

Means of a particular class (row) with at least one alphabet as common superscripts do not differ significantly from each other.
Pyrexia occurs due to the imbalance between the heat production and loss. The bacterial endotoxins and other pyrogens, like yeast, act by inducing the enzyme cyclooxygenase which ultimately leads to the biosynthesis and release of endogenous pyrogens by neutrophils and other cells. The endogenous pyrogen, which is known to be prostaglandin E, acts upon the hypothalamic thermoregulatory centre to set the thermostat at a higher point, with the result the rate of heat production increases and heat loss decreases leading to pyrexia. The role of prostaglandins in pyrexia was reported by Feldberg and Myres (1964).

Salawu et al. (2008) reported that the methanolic extract of *C. febrifuga* caused a dose-dependent decrease in rectal temperature (25, 50, 100 mg/kg). The effect became significant at 30 and 60 min at the highest dose of 100 mg/kg which was 38.38 in 0 hour to 37.3 in 30 min and 37.6 in 60 min respectively.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary Science and Animal husbandry, Mhow for providing research facilities and WNRF, Chennai for financial support and free supply of fruits.

References


Anxiolytic activity of *Morinda tinctoria* L.

**Abstract**: A study was conducted to characterize the putative anxiolytic-like activity of an ethanolic extract prepared from the leaves of *Morinda tinctoria* (MT) using the elevated plus maze (EPM) and light-dark exploration test in mice. Male mice were either treated orally with the MT extract or the positive control diazepam, respectively, 1 hour before behavioral evaluation. Oral administration of 200 and 400 mg/kg of MT extract significantly (*P* > 0.01) increased the percentage time spent on and the number of entries into the open arms of the EPM. The effect was comparable to that of the benzodiazepine diazepam (2 mg/kg p.o.). In light-dark exploration test, diazepam-treated rats significantly increased the time spent in light arena and decreased the duration of immobility, while MT-treated rats also showed a significant (*P* >0.01) increase in the time spent (200 and 400 mg/kg) in light arena. Diazepam and the MT extracts do not produce any overt motor dysfunction. These results indicate that MT is an effective anxiolytic agent.

**Keywords**: *Morinda tinctoria*, Anxiolytic activity

**Introduction**

Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger (Barar., 2005). It may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade (Eisenberg *et al.*, 1998). There are various ways of explaining the mechanisms of action of anti-anxiety agents because of the involvement of many CNS chemical mediators. The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. GABAA receptors were involved in anxiety and their direct activation would have an anxiolytic effect (Vogel and Vogel, 2002). Anti-anxiety drugs have also been shown to act on limbic system, hypothalamus, and the brain stem reticular system (Satoskar *et al.*, 2005).
Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs (Griffiths and Sannerud, 1987). However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects (Griffiths et al., 1987).

In recent years, the development of new anxiolytics has been an area of interest. It has been established that there are lot of secondary plant metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system such as noradrenaline, serotonin, gamma-aminobutyric acid (GABA), and benzodiazepine (BDZ) neurotransmitters’ activities. Various types of herbal medicines have been used as anxiolytic agents in different parts of the world (Heinrich and Gibbons, 2001). Drugs derived from traditional herbs may have possible therapeutic relevance in the treatment of anxiety (Beaubrum, 2000). Research has been conducted to investigate natural anxiolytic agents in the search for an alternative, more specific, and perhaps cost-free therapy. Various types of herbal medicines have been used as anxiolytics in different parts of the world.

*Morinda tinctoria* (MTR) belongs to the family of *Rubiaceae* that grows wild and is distributed throughout Southeast Asia. It is commercially known as *Nunaa* and is indigenous to tropical countries. In the traditional system of medicine, leaves and roots of *Morinda tinctoria* are used as an astringent, deobstremengogue and to relieve pain in the gout (Nadkarni, 1998).

On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic-like activity of an ethanolic extract prepared from the leaves of *Morinda tinctoria* Linn.

### Materials and Methods

#### Plant Material

*M. tinctoria* Roxb. (*Rubiaceae*) leaves were collected from Krishnankoil, Tamil Nadu, India in August 2002. The plants were authenticated by the taxonomist, Dr. V. Ganaesan of Anja College of Arts and Science, Sivakasi. A voucher specimen is preserved in Arulmigu Kalasalingam college of Pharmacy, Krishnankoil, India.

#### Preparation of Extract

The leaves were dried and coarsely powdered. The dried powdered material was then exhaustively extracted with 95% ethanol, concentrated under controlled temperature, and was used for the pharmacological investigation.
Drugs

Diazepam ampoule (10 mg/2 ml; Watson Pharmaceuticals, India) was used as reference drugs. Diazepam was diluted to 1.5 mg/10 ml with distilled water. Two different concentrations (200 and 400 mg/kg) of the Ethanolic extract of MT were prepared by dissolving the extracts in distilled water. All solutions were prepared freshly on test days and administered orally (p.o.) in a volume of 0.1 ml/10 g body weight of mice.

Animals

Swiss albino male mice (22-25 g) were used to study the anxiolytic effect. The animals were housed in groups of six mice per cage and maintained at 24°C ± 1°C, with relative humidity of 45-55% and 12:12 hours dark/light cycle. The experiment was carried out between 10:00 and 17:00 h. The animals had free access to food (Standard chew pellets, Chakan Oil Mills, Sangli) and water ad libitum. Food, not water, was withdrawn three hours before and during the experiment. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute Toxicity Studies

MT ethanolic extract at different doses (50-2000 mg/kg) was administered orally to mice. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any for seven days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, and mortality were observed and doses selected were 200 mg/kg and 400 mg/kg, body weight. LD50 dose of MT leaf extract was reported to be 9.89 g/kg, body weight in the previous reported study (Tandon and Gupta, 2006).

Assessment of Anxiolytic Activity

Treatment schedule

The anxiolytic activity of MT was examined using the elevated plus maze (EPM) and the light and dark model in mice. The animals were divided into four groups, consisting of six mice per group. Group one received vehicle (distilled water); Groups two and three received MT 200 and 400 mg/kg, respectively; Group four received diazepam 2 mg/kg.

Elevated plus maze

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, with the plus maze elevated (25 cm) from the floor used to observe anxiolytic behavior in mice (Kulkarni and Reddy, 1996; Vogel and Vogel, 1997). Each mouse was placed at the center of the elevated plus maze with its head facing the open arm. During the 5-min experiment, the behavior of the mouse was recorded as: (i) preference of the mouse for its first entry into the open or closed arms, (ii) the number of entries into the open or closed arms, and (iii) time
spent by the mouse in each of the arms. Ethanol extracts of MT (200 and 400 mg/kg) were administered orally using a tuberculin syringe fitted with oral canula. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus maze could invoke maze anxiety.

**Light and dark exploration test**

The apparatus consisted of an open top wooden box. Two distinct chambers, a black chamber (20 x 30 x 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 x 30 x 35 cm) painted white and brightly illuminated with 100-W white light source, were located 17 cm above the box. The two chambers were connected through a small open doorway (7.5 x 5 cm) situated on the floor level at the centre of the partition (Costall et al., 1988). Each mouse was placed individually in the center of the light compartment and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the light and dark compartments. Diazepam dose of 2 mg/kg, i.p. was used as a reference standard (Crawley and Goodwin, 1981; Amborgi and Giachetti, 1986).

**Measurement of locomotor activity**

Since the plus maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity. Separately from the experiment with the elevated plus-maze, spontaneous locomotor activity was measured using activity cage. Each mouse was placed in the activity cage and after an adaptation period of 10 min, the test compound administration protocol was implemented. Diazepam was administered orally 30 minutes prior to the experiment.

MT extract was administered orally 60 minutes prior to the experiment. Ambulatory activity was measured for 10 minutes after oral administration of the agents. Percentage change in motor activity was measured. (Kulkarni, 2005).

**Statistical analysis**

All data are presented as mean ± SEM and analyzed by one-way ANOVA, followed by Dunnett's test. The groups treated with extracts were compared with the respective vehicle group. The diazepam-treated group was compared with vehicle. \( P\) values <0.01 were considered statistically significant.
Results

Locomotor Activity

Locomotor activity was significantly decreased by diazepam (2 mg/kg). Locomotor activity was also decreased in animals pretreated with extract of MT (200 and 400 mg/kg) compared with that in the vehicle group. MT inhibited locomotor activity to a lesser extent than diazepam and thus had a better profile for anxiolytic agents.

Elevated Plus Maze

Oral administration of 200 and 400 mg/kg of MT produced a significant ($P < 0.01$, ANOVA followed by Dunnet's test) increase in permanence in the open arms of the maze [Table 1], suggesting an anxiolytic effect of this extract. Animals treated with diazepam (2 mg/kg, i.p.) spent more time in the open arms of the maze.

Table 1. Effects of diazepam and Morinda tinctoria leaves extract on behaviour of mice in elevated plus maze paradigm

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Number of entries</th>
<th>Time spent in open arm (Sec)</th>
<th>Motor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arm</td>
<td>Closed arm</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>3.4±0.23</td>
<td>18.4±0.57</td>
<td>24.3±1.23</td>
</tr>
<tr>
<td>MTEE 200</td>
<td>8.5±0.54</td>
<td>10.6±0.45</td>
<td>31.4±1.45**</td>
<td>104.3±2.56**</td>
</tr>
<tr>
<td>MTEE 400</td>
<td>9.4±0.45</td>
<td>11.4±0.52</td>
<td>52.5±1.72**</td>
<td>94.65±2.46**</td>
</tr>
<tr>
<td>Diazepam</td>
<td>9.6±0.56</td>
<td>12.3±0.61</td>
<td>131.4±1.53**</td>
<td>72.5±1.23**</td>
</tr>
</tbody>
</table>

Data are expressed as mean values± SEM (n=6)** $P<0.05$ compared with control (ANOVA followed by Dunnett's test)

Light and Dark Box

Diazepam-treated rats significantly ($P < 0.01$) increased the time spent in light arena and decreased the duration of immobility. MT-treated rats also showed a significant ($P < 0.01$) increase in the time spent (200 and 400 mg/kg) in light arena. The test drug reduced the duration of immobility at the highest dose (200 mg/kg). An increase in the number of entries into light chamber was not significant [Table 2].
Table 2. Effect of diazepam and MTEE on behaviour of mice in light and dark exploration test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>No of entries in the light compartment</th>
<th>Time spent in the light compartment (seconds)</th>
<th>Motor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>7.4±0.92</td>
<td>27.4±1.32</td>
<td>163.34±2.08</td>
</tr>
<tr>
<td>MTEE 200</td>
<td>10.86±0.26</td>
<td>58.72±1.71**</td>
<td>113.65±2.01**</td>
<td></td>
</tr>
<tr>
<td>MTEE 400</td>
<td>18.92±0.32</td>
<td>69.94±1.84**</td>
<td>101.24±2.11**</td>
<td></td>
</tr>
<tr>
<td>DIAZEPAM</td>
<td>2</td>
<td>16.32±0.51</td>
<td>158.31±1.83**</td>
<td>98.13±1.09**</td>
</tr>
</tbody>
</table>

Data are expressed as mean values± SEM (n=6)**P<0.05 compared with control (ANOVA followed by Dunnett's test)

Discussion

The elevated plus-maze is a well-established animal model for testing anxiolytic drugs (Dawson and Tricklebank, 1995) and (Soderpalm et al., 1989). Diazepam, a standard anxiolytic used clinically, is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic-like effects, even when the compound being screened does not act via benzodiazepine receptors (Treit, 1985).

In the present study, we found that the extract of MT increased the percentage of open arm entries and time spent in open arms and thus showed anxiolytic effects in this model. The anxiolytic effects of drugs such as benzodiazepines are accompanied by decreased locomotor activity and sedation (Lewis, 1980). The extract inhibited locomotor activity in this experiment. However, MT inhibited locomotor activity to a lesser extent than diazepam, and thus has a better profile for an anxiolytic agent. There is considerable interest in the development of new anxiolytics that do not induce sedative effects and do not inhibit locomotion.

The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. GABA receptors were involved in anxiety and their direct activation would have an anxiolytic effect (Vogel and Vogel, 2002). It is well documented that pentylenetetrazole-induced convulsions are produced due to diminution of GABA level in brain. A recent study showed that MT possesses anticonvulsant activity particularly against pentylenetetrazole-induced convulsion. Therefore, it is likely that MT might possibly be producing anticonvulsant action by increasing the level of GABA, an inhibitory neurotransmitter in the central nervous system. This is in accord with the pharmacological effects of benzodiazepine...
and highlights the relevance of the putative anxiolytic effect of MT. In conclusion, the action of extract upon the anxiety models tested are in accord with the traditional use of MT L. and could be useful in primary medical care. In the same way, identification of compound(s) responsible for biological activity could be used as prototype(s) to design new substances with anxiolytic activity. Further major active components and precise anxiolytic mechanisms need to be identified.

References


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