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Review article

A review on comparative pharmacognostic and pharmacological aspects of Neem

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ABSTRACT

Azadirachta indica, Murraya koenigii, Melia dubia are the culinary important plants of Indian origin, and also been a component of many formulations used in the Ayurvedic system of medicine since many centuries. A scrutiny of literature reveals some notable pharmacological activities of these plants. Different alkaloids which are abundantly present in the leaves, fruits, roots and bark of this plant, have been reported for their antidiabetic, anticancer, antibacterial, anti-nociceptive and antioxidant activities. Besides these activities, the plant is described to have a wide array of therapeutic activities. Phytochemistry and pharmacology of these plants necessitates a comprehensive review of its prospects as an important therapeutic agent for the management of numerous diseases commonly affecting humans. The current review provides a detailed comparative report of the pharmacological works carried out on this culinary plant and also throws light on thete therapeutic prospects.

Keywords: *Azadiracta Indica, Murraya Koenigii, Melia Dubia,* Pharma cological activity.

INTRODUCTION

Azadirachta indica commonly known as Neem, It is widely planted and naturalised in semiarid areas throughout Asia and Africa. Neem is an evergreen of the tropics and sub-tropics. It belongs to the family Meliaccae and is a cousin of the Chinaberry. Each part of the neem tree has some medicinal property and is thus commercially exploitable. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. The sanskrit name of the neem tree is 'Arishtha' meaning 'reliever of sickness' and hence is considered as 'Sarbaroganibarini'The tree is still regarded as 'village dispensary' in India [1].

Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae. It grows throughout India and the Andaman Islands. Its leaves are used in many dishes in India and neighbouring countries. Often used in curries, the leaves generally called by the name "curry leaves", though they are also translated as "sweet neem leaves" in most Indian languages (as opposed to ordinary neem leaves which are bitter). The leaves of *Murraya koenigii* are also used as a herb in Ayurvedic medicine.

Melia dubia is also called as a Maha neem or Forest neem. Which is fastest growing tree species, within 6-7 years the plantation is ready to harvest. Melia dubia originates from the Meliaceae family and is an indigenous species of tree to India, South East Asia and Australia, where it has been cultivated as a source of firewood. Melia azedarach linn (synonym: Melia dubia Cav, Indian lilac, Persian lilac) belonging to the family Meliaceae is a tree found in India. It is commonly found in the hills at elevations ranging from 600 – 1800m. M. dubia occurs in the tropical moist deciduous forests of the Sikkim, Himalayas, North Bengal and upperAssam, the Khasi hills of Orissa, N.Circars, Deccan and the Western Ghats, at altitudes of 1,500-1,800 meters. The wood is having good demand from ply wood industries.

Botanical Description

Azadirachta indica is a small to medium-sized tree, usually evergreen, up to 15 (30 max.) m tall, with a round, large crown up to 10 (20 max.) m in diameter; branches spreading; bole branchless for up to 7.5 m, up to 90 cm in diameter; bark moderately thick, with small, scattered tubercles, deeply fissured and flaking in old trees, dark grey outside and reddish inside, with colourless, sticky foetid sap. Leaves alternate, crowded near the end of branches, simply pinnate, light

green, with 2 pairs of glands at the base, otherwise glabrous; petiole 2-7 cm long, subglabrous; rachis channelled above; leaflets 8-19, very short petioluled, alternate proximally and more or less opposite distally, ovate to lanceolate, sometimes falcate (min. 2) 3.5-10 x 1.2-4 cm, glossy, serrate; apex acuminate; base unequal. Inflorescence an axillary White fragrant flowers in drooping panicles to 10" long bloom in spring. Trees are polygamous (both bisexual flowers and male flowers exist on the same plant). Edible fruit is a smooth olive-like drupe. Each fruit contains one (rarely 2-3) elongated seeds.

Curry leaf is a small, Each odd-pinnate leaf typically has 11 to 21, thin, ovate, shiny, dark green leaflets (1-2" long). Fragrant white flowers (each to 5/16" across) in many flowered panicles (terminal cymes) bloom irregularly throughout the year. Flowers are followed by 1-2 seeded, ovoid to oblong, bluish-black fruits (each to 2/3" diameter). Fruits are edible but the seeds are not. Curry leaves are highly aromatic when rubbed or bruised. They are best used fresh in cooking (dried leaves may be used but have significantly diminished flavour). *Melia dubia* tree up to 25 m tall. Bark dark brown, flakes large rectangular in shape. Youngparts scurfytomentose, branchlets terete, glabrous when mature.

Chief Chemical Constituents

Previous phytochemical investigations with *Azadirachta indica* led to the isolation of triterpenoid bitter principles (nimbidin, nimbin, nimbinine, 6-desacetylnimbinine, nimbidol, nimbolide and bakayanin), saponins, flavonoids, tannins and alkaloids. In addition to these, the leaves contain azadirachtin, salanin, meliantriol, margosopicrin, paraisine, azadinine, nimbinene, nimbolide, quercetin and its glycosides, beta-sitosterol, n-hexacosanol, nonacosane, ascorbic acid and amino acids. Barks contain nimbolins A, B, organic acids, tannin, margosin and azadarin. Flowers contain essential oil, kaempferol, kaempferol glucoside, nimbosterin and N-nonacosane. Fruits contain resins, tannins, triterpenoids, salanin and azadriachtin, melianone, oil and organic acids. Kernel contains triterpenoids, salanin, azadirachtin, oil and fatty acids. Seeds contain six tetranor-triterpenses and four new limonoids, 11-hydroxyazadirachtin- B, 1-tigloyl-3-cetyl-azadirachtin, 1, 2-diacetyl- 7-tigloyl-12-hydroxylvilasinin and 23-desmethyl-limocin-B. Neem oil contains margosic acid.

A number of chemical constituents from every part of the M. *koenigii* have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source fof carbazole alkaloids. Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed by Ito. *M. koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine.

The preliminary phytochemical screening of petroleum ether extract, ethyl acetate extract, chloroform extract, ethanol extract and aqueous extract was performed. The presence of alkaloids, flavonoids, carbohydrates, and sterol in various extracts were observed.

Leaves are aromatic and contain proteins, carbohydrates, fibbers, minerals, carotene, nicotinic acid and vitamin C. The leaves contain high amount of oxalic acid, leaves also contains crystalline glycosides, carbazole alkaloids, koenigin and resin. Fresh leaves contain yellow colored volatile oil conversely also rich in vitamin A and calcium. It also contains girinimbin, iso-mahanimbin, koenine, koenigine, koenidine and koenimbine. Mahanimbicine, bicyclomahanimbicine,phebalosin, coumarine as Murrayone imperatoxin etc are isolated from leaves. Triterpenoid alkaloids like cyclomahanimbine and tetrahydromahanmbine are also present in the leaves Bark mainly contains the carbazole alkaloids as murrayacine, murrayazolidine, murrayazoline, mahanimbine, girinimbine, koenioline and xynthyletin. The pulp of fruits generally contain 64.9% moisture, 9.76% total sugar, 9.58% reducing sugar, 0.17% non-reducing sugar and negligible amount of tannin and acids. It also contains 13.35% of vitamin C. The pulp of fruits contain trace amount of minerals 1.97% phosphorus, 0.082% potassium, 0.811% calcium, 0.166% magnesium and 0.007% iron. It also contain markable amount of protein.

The photochemistry and pharmacological studies conducted on this medicinal herb have successfully isolated several natural products including diterpenes, monoterenes, triterpenes, saponins, flavonoids hexoses, organic acids, rosmarinic acid, chromene and myoinositol. The phytochemical components of Melia dubia (Cav) leaf extract has been evaluated using GC-MS-MS. It represents the presence of unsaturated fatty acids, terpenoids (diterpenes and sesquiterpenes) antioxidants, phenolic derivatives and lipophylic organic compounds. Phytochemical compounds such as Linolenic acid, Palmitic acid, Caryophyllene, Humulene, Aromadendrene, Probucol, Germacrene-D, Phthalic acid 6-ethyl-3-octyl, Butylated hydroxyl toluene [2].

Comparative pharmacological activity

Antioxidant Activity

Azadirachta indica, ethanolic extract of leaves and ascorbic acid was utilized for DPPH (1, 1-diphenyl-2-picrylhydrazyl) and nitric oxide radical scavenging, iron chelating and reducing power activity evaluate the antioxidant activity. Here a dose dependent antioxidant activity of ethanolic extract of leaves comparable with standard ascorbic acid and leaves exhibit significant in vitro free radical scavenging properties [3].

Three morphotypes of *Murraya koenigii* L were evaluated by ABTS and phosphomolybdenum assays along with plant polyphenolic constituents and reference antioxidants. With the help of

spectrophotometer Total polyphenol content (111-532 mg/g), flavonoid (0.01-0.09%) contents in dried plant samples was determined. Here ABTS and phosphomolybdenum assays along with plant polyphenolic constituents and reference antioxidants were utilized. All examined methanolic extracts showed significant activity to scavenge ABTS and hydroxyl free radicals, reducing power, iron chelating ability and total antioxidant capacity in the order: Brown>Dwarf>Regular. It is a good source of antioxidants especially brown type [4].

With the help of nitric oxide radical scavenging method revealed that the ethanol fraction of activity of *Melia dubia* Leaves exhibit better radical scavenging with an IC_{50} value of 16.89(µg/ml).Among the five extracts tested for the leaves of plants, ethanol extract of *Melia dubia* shown high potent α- *amylase* inhibiting property with IC_{50} value of 24.82(µg/ml).

Antimicrobial Activities

In the present study we compared the antimicrobial efficacy of aqueous extracts of leaf, bark and seeds of *A. Indica* against human pathogenic bacteria (S*taphylococcus aureus, Enterococcus feacalis, Proteus mirabilis* and *Pseudomonas aeuroginosa*) and fungi (*Aspergillus fumigatus* and *Candida albicans*). Agar well diffusion method and micro-broth dilution methods [5].

Leaf extract exhibited strong antimicrobial activity against bacteria and fungi at all the concen-trations tested (500, 1000 and 2000µg/ml). Bark extract was found to be moderate on bacteria and fungi (effective at 1000 and 2000µg/ml).Whereas seed extract exhibited least antimicrobial activity. Here aqueous extracts of *Azadirachta Indica* leaf and bark exhibit high antimicrobial activity.

This is *in-vitro* method by using hexane, methanol, chloroform, Aqueous fraction isolated from root of *Murraya koenigii* L.was assessed by disc diffusion method against four bacterial stains (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus,*and *salmonella typhi*) and three fungal strains (*Aspergillus niger, Candida albicans and Trichophoton rubrum*). The methanolic extract showed maximum inhibitory effect against *S. aureus*(IZ=15.00 mm;AI=0.75) with MIC value of 0.078 mg/ml while hexane extract showed minimum inhibitory effect against *E. coli* (IZ=7.00 mm;AI=0.29) with MIC value of 0.625 mg/ml and Aqueous extract showed no inhibitory effect against (*B. subtilis, E. coli, S. aureus,*and *s. typhi*).In antifungal screening, The methanolic extract showed maximum inhibitory effect against *T. rubrum*(IZ=14.00mm;AI=0.70) with MIC value of 0.156 mg/ml hexane extract showed minimum inhibitory effect against *T. rubrum*(IZ=6.00 mm;AI=0.30) with MIC value of 0.625 mg/ml. Aqueous extract showed no inhibitory effect against (*A. niger, C. albicans and T. rubrum*) [6].

The chemical constituents of Melia dubia leaf volatile oil have been studied by GC-MS. The chemical compound "monoterpine camphene" shows the good antimicrobial activity that inhibits 78% of skin isolates at 250ml concentration, whereas the Melia dubia leaf volatile oil that contains 21.68% of camphene as a major constituent inhibits 88% of skin pathogens.

Hepatoprotective activity

Azadirachta indica, ethanolic and aqueous extracts of leaves was examined against carbon tetrachloride induced liver damage in mice using silymarin as control. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) Enzyme activities were analyzed. Both extracts of leaves exhibited moderate activity over carbon tetrachloride treated animals.

Murraya koenigii the aqueous extract of the leaves against lead induced oxidative damage in hepatic tissue. Lead caused alterations in all the parameters studied. All these changes were mitigated when the rats were pre-treated with CuLE. Concentration of lead in liver tissue was also decreased following pre-treatment with CuLE.

Ethnologic extract of *Melia* azedarach Leaves (300 mg/kg and 500 mg/kg) treated against simvastatin (20mg/kg.p.o) induced hepatotoxicity in rats using Standard drug Silymarin (25 mg/kg). There was a significant changes in biochemical parameters (increases in serum glutamate Pyruvate Transaminase (SGPT), Serum glutamate Oxaloacetate Transaminase (SGOT), alanine Phosphatase (ALP), serum bilirubin and decrease the total proteins content). The leaf showed significant hepatoprotective activity.

Antiulcer activity

The stem bark extract of Azadirachta indica gastric ulceration was studied in albino rats Azadirachta indica extract (100-800 mg/kg p.o., 100-25 mg/kg i.p.) significantly inhibited gastric ulceration induced by indomethacin (40 mg/kg). Administration of 800 mg/kg p.o. and 250 mg/kg i.p. caused 100% cytoprotection against indomethacin (40mg/kg, i.p.)-induced gastric ulceration. The effect of the extract alone and in combination with histamine (1mg/kg) and cimetidine (0.12 mg/kg) on gastric acid secretion in situ was studied. Azadirachta indica (250 mg/kg) significantly inhibited the basal and histamine-induced gastric acid secretion. Extract showed significant antiulcer agents, which probably act via histamine H receptor.

Pylorus ligation and NSAIDs induced ulcer model in albino rats was studied for the anti-ulcer effect of aqueous extract Murraya Koenigii. In this model the extract reduced ulcerative lesion, gastric volume, free and total acidity but raised the PH of gastric juice. The extract at dose of 200,400 mg/kg produced significant inhibition of gastric lesion induced by NSAIDs and Pylorus ligation induced ulcer. Aqueous extract showed significant anti-ulcer activity [7].

The aqueous and alcohol extracts of leaves of Melia azedarach Linn. (Meliaceae) were screened for antiulcer activity in

aspirin induced and pylorus – legated rats. Antiulcer effects were compared with standard drug Omeprazole (20 mg/kg b.w., p.o.). These observations helped us to conclude that aqueous extract (250 mg/kg b.w., p.o.) is endowed with antiulcer property. Aqueous extract and alcohol extract of M. azedarach leaves (250 mg/kg b.w., p.o.) were administered orally [8].

Analgesic Activity

In this analgesic activity neem seed oil was use on albino rats tested by tail flick method by using the analgesiometer (Techno). Neem seed oil in the doses of 0.25, 0.5, 1 and 2 ml/kg body weight was given intraperitoneally to different group of rats. Tail flick latency was measured in seconds before and after the drug injection. Neem seed oil showed significant analgesic effect in the dose of 1 and 2 ml/kg. Results were compared with morphine and statistically analysed.

Analgesic activity was tested by acetic acid-induced writhing method (for peripheral action) and radiant heat tail-flick test (for central action) on leaf extract of *Murraya koenigii* in Swiss-Albino mice. In the acetic acid-induced writhing test, the extract in doses of 200 and 400 mg/kg showed 41.96% ($p<0.001$) and 50.48% ($p<0.001$) inhibition of writhing respectively. In radiant heat tail-flick method the crude extract produced 29.75% and 38.88% elongation of tail flicking time 60 minutes after oral doses of 200 and 400 mg/kg body weight respectively.

Hydro alcoholic extract of Melia azedarach Linn roots were evaluated for analgesic activity In the doses of 100 and 200 mg/kg, extract inhibited 82.23 % and 88.94 % writhing induced by acetic acid.

Anti-inflammatory activity

Anti-inflammatory activity was tested in carrageenan induced rat paw oedema (for 24 hours) and cotton pellet granuloma (7 days) with 70% alcoholic extract of neem root. NRE in the dose of 400 and 800 mg/kg showed significant ($P<0.05$ and $p<0.01$) in cotton pellet granuloma. But in both models it was less significant than Aspirin. NRE in the dose of 800 mg/kg showed significant ($p<0.05$) in carrageenan induced rat paw oedema model.

Aqueous, methanolic, petroleum ether and hexane extracts of dried leaves of *Murraya koenigii* Linn was used for the anti-inflammatory activity by using carrageenan- induced hind paw oedema in albino rats. The methanol extract showed significant ($P < 0.001$) reduction comparison to aqueous extracts. Petroleum ether and hexane extracts showed no reduction in paw oedema. The methanol extract showed anti-inflammatory effect in dose dependent manner when compared with the control and standard drug, Aspirin (10mg/kg, p.o). These inhibitions were statistically significant ($p < 0.05$) (31). Hydro alcoholic extract of Melia azedarach Linn roots were evaluated anti-inflammatory activity In the doses of 100 and 200 mg/kg, extract reduces 15.08 % and 26.45 % paw volume in carageenan induced paw edema.

Anti-cancer activity

The present study was designed to evaluate the chemo preventive potential of the neem limonoids azadirachtin and nimbolide based on in vitro antioxidant assays and in vivo inhibitory effects on 7, 12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. On a comparative basis, nimbolide was found to be a more potent antioxidant and chemo preventive agent and offers promise as a candidate agent in multitargeted prevention and treatment of cancer. Hydro-methanolic extract of curry leaves (CLE) was prepared and its total phenolic content determined by, the Folin-Ciocalteau's method. CLE decreased cell viability and altered the growth kinetics in both the breast cancer cell lines in a dose-dependent manner. It showed a significant arrest of cells in the S phase albeit in cancer cells only. Annexin V binding data suggests that cell death was via the apoptotic pathway in both the cancer cell lines. CLE treatment significantly decreased the activity of the 26S proteasome in the cancer but not normal cells.

The present study was undertaken to investigate anticancer activity of these M. azedarach in comparison with A. indica on cancer cell lines and also to evaluate their safety in humans by testing them on normal cell line. In this study, the cytotoxic activity of crude extracts from M. azedarach and A. indica leaves, pulps and seeds as well as three main fractions of their leaf extracts were assayed against HT-29, A-549, MCF-7 and HepG-2 and MDBK cell lines. Seed kernel extract of M. azedarach had the highest cytotoxic activity and selectivity to cancer cell lines (IC50 range of 8.18- 60.10 μg mL-1). In contrast to crude seed extract of A. indica, crude pulp and crude leaf extracts of this plant showed remarkably stronger anti-proliferative activity (IC50 ranges of 83.45 - 212.16 μg mL-1 and 34.11- 95.51 μg mL-1 respectively) than those of M. azedarach (all IC50 values of both plants > 650 μg mL-1 [9].

Antidiabetic Activity

Aqueous extract of *Azadirachta indica* leaves was examined against anti-diabetic action. Treatment with the extract caused a significant ($p<0.05$) reduction in (NT) rats. The serum a- amylase activity was significantly lower ($p<0.05$) in the extract treated Diabetic rats (DT) when compared to the placebo treated Diabetic control (DC).However, there was no significant diffrence ($p<0.05$) in the serum a-amylase activity on normal treated (NT) rats when when compared to the normal control [10].

The present study was aimed to evaluate the anti-hyperglycaemic efficacy of Murraya koenigii leaves in STZ induced diabetic rats. Ethanolic extract of M. koenigii at a dose of 200 mg/kg/ b.w./day for a period of 30 days significantly decreased the levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine in diabetic treated group of animals. The results suggest that M.

koenigii possesses statistically significant hypoglycaemic potential in STZ-induced diabetic rats.

The present study was aimed the characteristics and Antidiabetic activity of "Melia Azedarach" (Meliaceae) Leaves. Extraction was carried out by using Ethanol and limonoids were separated from the ethanolic extract. Furthermore, the phytochemical investigations were performed by using different test and TLC method. An analysis of limonoid was made using column chromatographic, HPLC (High Performance Liquid Chromatography) and FT-IR. Laboratory bioassays were carried out on alloxan induced diabetic rate. Finally, limonoid, flavonoid and sterols containing leaf extracts of M. azedarach were effective in decreasing blood glucose level in alloxan induced diabetic rats [11].

Anti-malarial activity

An acetone-water neem leaf extract with anti-malarial activity was evaluated in vitro at 5 micro/ml for inhibition of adhesion of malaria parasite-infected erythrocytes and cancer cells. The extract showed antiretroviral activity with a mechanism of action that may involve inhibition of cytoadhesion. The results may help in the development of novel antiretroviral and anti-malarial drugs. Larvicidal, pupicidal, repellent and anti-vector activity of aqueous extract from Murraya koenigii was investigated against the larvae and pupae of malaria vector, Anopheles stephensi. Larvicidal and pupicidal activity of M. koenigii were exhibited in the first instar, second instar larvae and pupae of A. stephensi. The LC50 and LC90 values were 5.909% and 33.352% for the first instar, 8.929% and 33.323% for the second instar, respectively. During the pupal stage the plant extract showed that the LC50 and LC90 values were 15.255%and 36.546%, respectively. This method was considered as a new approach to control vectors and it gave a much effective results in mosquitocidal activity. This was a basic research result [12].

Seeds extracts of *Melia dubia* Methanolic, Ethanol, Petroleum ether, Ethyl acetate were used for antimalarial activity. Highest mortality of 93 %and 81 % was recorded for third and fourth instar larvae of *Culex quiquefaciatus* respectively at 4.5% concentration. The data were recorded and statistic data regarding LC 50, 95% confidence limit, LC 90 and chisquare values were calculated. The highest sensitivity of third instar larvae was evident by their lowest LC values (LC50 3.240 and LC 90 4.786 ppm) .Least susceptibility was shown by fourth instar larvae (LC50 4.073 and LC90 4.942 ppm) .No mortality was observed in control. The LC 50 values of ethyl acetate extract of Leucas aspera were 75.40, 93.09, 132.20 and 138.60 ppm against first, second, third and fourth larvae of *C. quinquefasciatus,* respectively.

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