WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 4, 1544-1559. Review Article ISSN 2277–7105

A REVIEW ON IN VITRO ANTIFUNGAL ACTIVITY OF MORINDA TINCTORIA ROXB AGAINST SELECTED FUNGAL PATHOGENS

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Article Received on 20 Jan. 2026,
Article Revised on 10 Feb. 2026,
Article Published on 16 Feb 2026,

https://doi.org/10.5281/zenodo.18757121

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How to cite this Article: Dr. N. Saranya, Pharm D., K.Divya, V. Ramesh, S. Soniya. (2026). A Review on In Vitro Antifungal Activity of Morinda Tinctoria Roxb Against Selected Fungal Pathogens. World Journal of Pharmaceutical Research, 15(4), 1544–1559.

ABSTRACT

Morinda tinctoria Roxb. (noni / Indian mulberry) belongs to the Rubiaceae family and is an important medicinal plant used in Indian medical systems. The study aims to collect and evaluate the pharmacognostical, phytochemical, and pharmacological properties of *Morinda tinctoria* leaves. The plant has significant ethnomedical value and is traditionally used to treat inflammation, diabetes, infections, wounds, gastrointestinal issues, and skin illnesses. Leaf extracts were prepared using solvents of increasing polarity: aqueous, ethanolic, acetone, n-hexane, benzene, and ethyl acetate. Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, phenolic compounds, saponins, and anthraquinones. The results support traditional claims and indicate promising therapeutic potential, warranting further investigation for pharmaceutical use. In-vitro studies demonstrated antimicrobial and antifungal activities; literature reports antioxidant, antidiabetic, anti-inflammatory, anticancer, hepatoprotective, anticonvulsant, and wound-healing properties.

KEYWORDS: Morinda tinctoria, Rubiaceae, In vitro antifungal activity, Medicinal plant, Candida albicans.

INTRODUCTION

Morinda is the largest genus of the Rubiaceae family, with 11 species found in India, native to southern Asia. *Morinda tinctoria*, commonly known as aal or Indian mulberry, is a flowering plant also called great Morinda, noni, beach mulberry, and cheese fruit. It is an

evergreen shrub or small tree growing 5–10 m tall. Leaves are 15– 25 cm long, oblong to lanceolate. Flowers are tubular, white, scented, and about 2 cm long. The fruit is a green syncarp with a diameter of 2–2.5 cm. The plant is extensively cultivated in India for the production of morindone dye sold as "Suranji." Various Morinda products such as capsules, tablets, skin products, and fruit juices are available in the market for the treatment of several health complaints. Morinda species contain phytochemicals including iridoids, flavonoids, flavonoid glycosides, anthraquinones, coumarins, lignans, noniosides, phenolics, and triterpenoids. Traditional medicinal plants rich in phytochemical components are commonly used for wound healing and various skin-related diseases. Morindone is used for dyeing cotton, silk, and wool in red, chocolate, or purple shades. The colouring matter is mainly present in the root bark and is collected when plants are three to four years old. Mature trees contain very little colouring substance. Small roots yield the most dye, while roots above 1 cm diameter are discarded. The active substance is extracted as the glucoside morindin, which produces the dye upon hydrolysis. Morindone acts as a mordant dye producing yellowish-red with aluminium, chocolate with chromium, and dull purple to black with iron mordants.

LITREATURE REVIEW

PLANT DESCRIPTION

BOTANICAL CLASSIFICATION

Rank	Name
kingdom	Plantae
phylum	Spermatophyta
class	Magnoliopsida
subclass	Asteridae
order	Rubiales
family	Rubiaceae
genus	Morinda
species	Morinda tinctoria

VERNACULAR NAME

- English : Indian Mulberry, Morinda tree.
- Tamil : Manchanari.
- Hindi : Aal, Auch, Togar.
- Malayalam : Mannappavitta, Kadappilavu, Manganathi.
- Telugu : Maddi, Togari, Bandamaddi.
- Kannada : Haladipavette, Maddi, Tagatemara, Aineshe
- Marathi : Dhaula, Aseti, Baratondi.

- Marathi : Dhaula, Aseti, Baratondi.
- Oriya : Achu, Pindra, Aachhu.
- Sanskrit : Paphanah.
- Bengali : Hurdi, Nani.

Morinda tinctoria, or Indian Mulberry, is an evergreen shrub/small tree (5- 10m) from Southern Asia, known for its rough bark, large oblong leaves, and fragrant white flowers, producing green, syncarp fruits, but most famous for its root bark yielding morindone dye for textiles (reds/purples) and possessing traditional medicinal uses like anti-inflammatory, antioxidant, and antibacterial properties, with extracts showing potential in various health areas.

Habit: Evergreen shrub or small tree, reaching 5-10 meters.

Stem: Short, crooked, with rough bark and deep longitudinal cuts.

Leaves: Opposite, 15-25 cm long, oblong to lance-like, with distinct cross- veins.

Flower: White, tubular, scented, about 2 cm long, in spherical heads.

Fruit: Green syncarp (a fleshy fruit from multiple flowers), 2-2.5 cm.

ETHANO BOTONICAL INFORMATION

LEAVES

The leaves are dark green, oppositely arranged, oval-shaped (10–20 cm long) with pointed tips and a smooth, waxy texture. Leaf extracts exhibit antioxidant, antimicrobial, antifungal, wound-healing, hepatoprotective, and anti-inflammatory activities, and the leaf juice is used for digestive disorders. The leaves contain phenolic compounds, flavonoids, saponins, and antioxidants.

FLOWERS

The flowers are white, scented, tubular, bisexual, and arranged in terminal globose heads. The calyx limb is truncate, and the corolla is about 2 cm long with a short tube, villous

internally, and four oblong, recurved lobes. Four stamens are attached to the corolla throat with exerted anthers. The ovary is inferior, 1.5 mm long, two-celled or incompletely four-celled, with a 4 mm style and a bilobed stigma. Although flowers are less commonly used medicinally, they are occasionally employed in folk remedies for skin conditions and contain flavonoids and volatile oils.

FRUITS

The fruits of Morinda tinctoria are globose syncarps (15–18 mm across), containing four oblong pyrenes, and ripen to a yellowish-brown colour. Although the fruit is rarely used medicinally due to its unpleasant odour, fermented fruit beverages are rich in potassium and may inhibit enteropathogenic bacteria. Traditionally, the fruit is used for dye extraction, and in some regions, unripe fruits are employed in folk remedies for fever and digestive disorders. The fruit contains anthraquinone derivatives (morindone), sugars, and organic acids.

SEEDS

The seeds are small and hard, located within the syncarp fruit. They are rarely used medicinally but are sometimes utilized in experimental formulations or for propagation of dye-yielding plants. The seeds contain fatty acids and trace amounts of anthraquinones.

COMMON USES

Anti-bacterial, Antidiabetic, analgesic, anti-oxidant, anti-inflammatory, Astringent, Laxative, Sedative, Hypotensive potentials, Hypoglycaemic potentials, Acetylcholinesterase inhibitors activity for many ailments from medicinal plants in Ayurveda medicines. It also used to make the indone dye. Morindone is used for the dyeing of cotton, silk and wool in shades of Red, chocolate or purple.

ETHANOCLAIM USES

LEAF

The leaves are traditionally used for digestive disorders in children and for treating infantile diarrhoea, gout discomfort, fever, and as a general tonic (Kritikar and Basu, 1935; Muthu et al., 2006). Experimental studies have shown that n-hexane, dichloromethane, methanol, and petroleum ether extracts of the leaves possess antibacterial, antifungal, and anticonvulsant activities (Jayasinghe et al., 2002; Kumaresan and Saravanan, 2009).

BARK AND HEARTWOODS OF MORINDA TINCTORIA

Morinda tinctoria yields the dye morindone, primarily obtained from the root bark of three–four-year-old plants, which is used to dye cotton, silk, and wool in red to purple shades. The dye occurs as morindin and morindonin, glucosides that hydrolyse to release morindone, producing different colours depending on the mordant used (Mell, 1928; Balakrishna et al., 1960). Additionally, stem and bark extracts exhibit antibacterial and antifungal activities, and the plant is recognized for its good fuelwood properties (Jayasinghe et al., 2002; Jain and Singh, 1998).

FLOWERS AND FRUITS OF MORINDA TINCTORIA

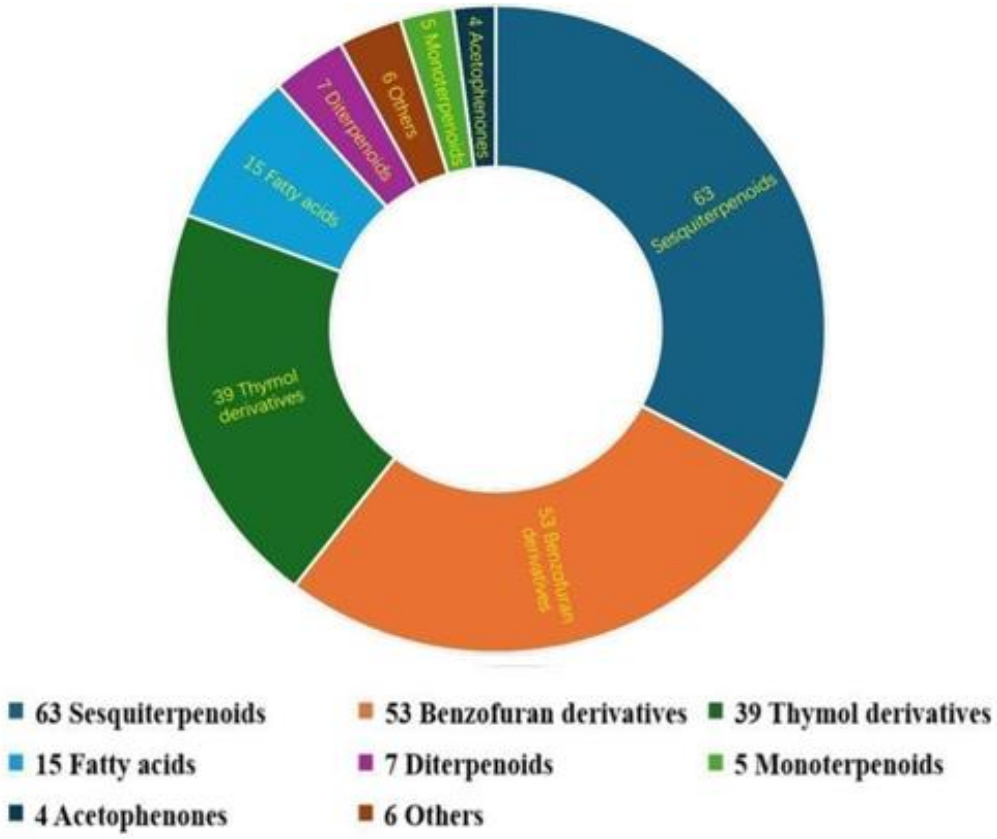
(Jain and Pal, 1998) Morinda tinctoria is traditionally used externally for rheumatism, gout, and boils, and orally as an astringent for cholera and diarrhoea. Fruit extracts of M. tinctoria and M. citrifolia exhibited significant antidiabetic activity in alloxan-induced mice, with higher efficacy observed in M. tinctoria (Mathivanan and Surendiran, 2006). Additionally, extracts from both species showed plant growth promoting effects in rice and green gram.

A. Whole Plant B. Leaves C. Flower D. Fruit.

ROOTS OF MORINDA TINCTORIA

Morinda tinctoria roots are traditionally used to treat inflammation, pain, rheumatism, gastrointestinal disorders, skin diseases, fever, and urinary ailments. Phytochemical studies have identified flavonoids, anthraquinones, and phenolic compounds in the roots, which are associated with their anti-inflammatory, antioxidant, and antimicrobial activities.

CHEMICAL CONSTITUENTS



PHARMACOLOGICAL ACTIVITY

1. Antidiabetic and Antioxidant Activity

Oral administration of Morinda tinctoria fresh fruit aqueous extract significantly reduced blood glucose, lipid levels, and lipid peroxidation while enhancing plasma insulin and antioxidant enzymes (CAT, SOD, GSH, GPx) in streptozotocin-induced diabetic rats. The extract effectively improved glycaemic control and antioxidant status, indicating its potential in preventing diabetic complications (Pattabiraman et al., 2011).

2. Anti-biofilm Activity

Methanolic fruit extracts of M. tinctoria at different maturity stages significantly inhibited biofilm formation of AmpC β-lactamase-producing Klebsiella pneumoniae at low BIC values (0.06 mg/mL). The study highlights M. tinctoria fruits as a promising source of anti-biofilm agents against multidrug-resistant pathogens (Satishkumar et al., 2014).

3. Anticancer Activity

Methanolic leaf extract of M. tinctoria showed strong in vitro cytotoxicity against multiple human cancer cell lines and significant in vivo anticancer activity in EAC-bearing mice. The

extract reduced tumour volume, enhanced antioxidant defence, protected haematological parameters, and increased survival, validating its ethnomedicinal anticancer use (Raju et al., 2017).

4. Antihyperglycemic Activity

Methanolic fruit extract of M. tinctoria significantly decreased fasting blood glucose and increased glutathione levels in streptozotocin-induced diabetic rats, particularly at higher doses. The findings suggest the presence of bioactive compounds that improve glucose tolerance (Muralidharan et al., 2009).

5. Analgesic and Anti-inflammatory Activity

Methanolic leaf extract of *Morinda tinctoria* exhibited significant central and peripheral analgesic as well as anti-inflammatory activity in standard experimental models. The effects were comparable to standard drugs, supporting its strong antinociceptive and anti-inflammatory potential (Srikanth et al., 2009).

6. Hepatoprotective Activity

Aqueous and methanolic leaf extracts of M. tinctoria significantly protected against paracetamol-induced hepatotoxicity by normalizing liver enzyme markers and restoring hepatic architecture. The hepatoprotective effect is attributed to its rich phytochemical composition (Subramanian et al., 2013).

7. Anticonvulsant Activity

Morinda tinctoria extract demonstrated significant anticonvulsant activity against chemically induced seizures and maximal electroshock models in mice, indicating dose- dependent neuroprotective effects (Thirupathy et al., 2009).

8. Wound-healing Activity

Jeya Rajkumar et al., reported the topical application of aqueous leaf extract of *Morinda tinctoria* significantly enhanced wound contraction and epithelialization in rats compared to oral and control treatments, confirming its effectiveness as a wound healing agent.

9. Antimicrobial Activity

Methanolic leaf extract of *Morinda tinctoria* showed broad-spectrum antibacterial activity against human pathogens, with highest sensitivity observed in Proteus vulgaris. The activity is linked to the presence of diverse secondary metabolites, highlighting its potential as a

source of antimicrobial leads (Sampath Kumar et al., 2021).

10. Antigenotoxic Activity

Kantha Deivi Arunachalam et al. reported the preventive effect of *Morinda tinctoria Roxb*. methanolic leaf extract (MEMT, 200 mg/kg), administered with the standard hepatoprotective agent silymarin (400 mg/kg). M. tinctoria, an evergreen Rubiaceae shrub native to tropical regions, is traditionally valued for its antiinflammatory and antioxidant properties. Antioxidant (SOD, CAT, lipid peroxidation) and genotoxicity assays (micronucleus, binucleus, and comet assays) demonstrated significant improvement in antioxidant defence and antigenotoxic status in various tissues of cadmium-intoxicated Pangasius sutchi (Deepti et al., 2012).

11. Antimalarial Activity

Morinda tinctoria leaf and bark ethanol extracts that were investigated for antiplasmodial activity against P. falciparum showed significant effectiveness. Conversely, it was found that the larvicidal activity of C. Quinquefasciatus was not affected by the methanol extract of *Morinda tinctoria* leaves.

12. Antifungal activity

The antifungal activity of *Morinda tinctoria* (leaf ethanol extract with DMSO) was tested in vitro using the agar cup method against two clinical fungal isolates, Candida albicans and Aspergillus niger, in order to determine the biological importance and capacity of the plant extract. The antifungal activity of the plant species is displayed in the table. The leaf extract's antifungal activity against Candida albicans showed no activity at concentrations of 25µl and 50µl, but at concentrations of 100µl, it showed an inhibitory activity of 14 mm. However, at all the different doses, no inhibitory efficacy against A. niger was discovered (Table 3).

Table 1: Phytochemical analysis of *Morinda tinctoria* leaf extracts

Phytochemicals	Glycosides	Phytosterols	Alkaloids	Oils	Sapponins	Phenols	Flavanoids
Water	+	+	-	+	+	+	+
Acetone	+	+	+	+	+	+	+
Chloroform	+	-	+	+	+	+	-

+: Present, - : Absent

Table 2: Zone diameter of inhibition of ethanol leaf extract of *Morinda tinctoria* (L)

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
E.Coli	-	-	18	20
P.aeroginosa	-	-	16	20

Table 3: Zone diameter of inhibition of ethanol leaf extract of *Morinda tinctoria* (L).

Test organisms	Zone of inhibition in mm			Positive Control
	Concentration of leaf extracts			
	25	50	100	
C. albicans	-	-	14	25
A. niger	-	-	-	25

According to the current investigation, *Morinda tinctoria* leaf extracts in ethanol were more effective against the clinical bacterial infections P. aeroginosa and E. coli. In comparison to bacterial activity, antifungal activity was found to be modest. According to reports in the literature, the leaf extract's antibacterial activity is caused by a variety of chemical agents, such as terpenoids, flavonoids, essential oils, and other substances that are categorized as active antimicrobial compounds. The study's findings support the idea of using an ethnobotanical method to screen plants for potential sources of bioactive chemicals and, to some extent, the use of traditional medicinal plants in the treatment of human and animal diseases.

MATERIALS AND METHODS

PLANT MATERIAL

The green leaves of *Morinda tinctoria* were collected, shade-dried, and then crushed into a powder using a crusher**.**

EXTRACTION PROCEDURE

In an aspirator container, powdered *Morinda tinctoria* leaves were thoroughly extracted using n-hexane, benzene, ethyl acetate, and methanol in that order using the cold percolation method (48 hours). Distillation on a water bath at atmospheric pressure eliminated about half of the solvent from the extract. Distillation under low pressure was used to extract the residual solvent. These four extracts' antimicrobial and antifungal properties were evaluated using the dilution method.

TEST ORGANISM

The study's goal is to examine leaves in vitro antibacterial and antifungal properties. The bacterial and fungal subcultures were collected.

ANTIFUNGAL ACTIVITY

The following four types of culture plates were prepared.

1. Culture plates having S.D.A without extract (Control group)
2. Culture plates having S.D.A with extract (Experimental groups)
3. Culture plates having nutrient agar without extract (Control groups)
4. Culture plates having nutrient agar with extract (Experimental groups)

CULTURE MEDIA

Fungi were grown using SDA (sabouraud Dextrose Agar) M086, which contained 10 gm/l of peptones special, 20 gm/l of dextrose, 17 gm/l of agar, and a final pH adjustment of 7.0. The culture medium was stored in sterile petri dishes. For 20 minutes, sterilization was carried out under 15 pounds of pressure in an autoclave.

PREPARATION OF CONTROL

In a round-bottom conical flask, 2.48 g of sabouraud Dextrose Agar (SDA) was dissolved in 40 ml of double-distilled and sterile water. A spirit lamp was used to gently shake the flask's contents and allow them to warm. Distilled water was used to fully dissolve the material. Non-absorbent cotton was used to plug the conical flask, and a thick piece of white paper was wrapped over the flask's mouth to secure the cotton plug. The conical flask was autoclaved at 15 pounds of pressure for 20 minutes. After being placed within the inoculation chamber, the medium was allowed to cool. Two sterile Petri dishes were filled with 19 ml of the medium and 1 ml of blank DMF (dimethylformamide).

PREPARATION OF SAMPLE

Antifungal properties are examined using Petri plates and the agar diffusion method, 14 distinct solvent leaf extracts of *Morinda tinctoria* were measured. The plates were preincubated at room temperature for one hour. The antifungal activity was measured using Sabouraud Dextrose as a control. The sterile petri dishes were filled with 1 ml of blank DMF and 19 ml of the SDA medium. For 20 minutes, sterilization was carried out under 15 pounds of pressure in an autoclave. One millilitre of DMF was used to dissolve the sample extract using the solvents n-hexane, benzene, ethyl acetate, and methanol. This was mixed with 19

mL of medium SDA suspension. The solidified medium was covered with petri plates impregnated with extracts (25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL in dimethyl sulfide). The inoculation process uses all of the control and extract-incorporated nutrient agar petri dishes. At particular intervals, the following organisms were added to the media: Aspergillus flavus, Candida albicans, Mucor sp., Trichophyton mentagraphytes, and Microsporum gypsium. The concentrations of the organisms employed to inoculate the control and extract-containing media were comparable. Every inoculated fungal petri plate, both experimental and control, was incubated at 37 C for 24 hours. The growth of microorganisms in control petri plates was closely examined in relation to the growth of organisms at various concentrations15 of extract media.

Antifungal studies of the leaf extract (obtained by different solvents ethyl acetate, methanol, benzene, n-hexane) were confined to these opportunistic fungi and two dermatophytic fungi.

Oppurtunistic fungi

1. Aspergillus flavus
2. Mucor sp
3. Candida albicans

Dermatophytic fungi

1. Trichophyton mentagraphytes
2. Microsporum gypseum

The leaf extract from each solvent was mixed into SDA medium to get medium with leaf extract conc. of 25 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/ml. The fungi were inoculated into the medium and incubated at 30 C for 3-7 days.

PURIFICATION OF SOLVENTS

ETHANOL

A calcium chloride guard tube and a double surface condenser were installed in a dry round-bottom flask 50–75 milliliters of commercial pure alcohol were added to the flask after dry magnesium turnings (5 grams) and iodine (0.5 grams) were added. After warming the combination until the magnesium was transformed into ethanolate, 900 milliliters of commercial absolute alcohol were added, and the mixture was refluxed for thirty minutes. The ethanol is utilized after it has been directly distilled into a vessel.

DISTILLED WATER

Water obtained by distillation is used aqueous extraction of powdered drug material.

PREPARATION OF EXTRACTS:

Prepration of the extract of powdered leaves of Morinda Tinctoria is done by using following solvents

i. Aqueous extract
ii. Ethanolic extract
iii. Acetone extract

AQUEOUS EXTRACT

The coarse leaf powder(100mg) that had been shade-dried was carefully packed in a soxhlet apparatus and hot extracted continuously for 24 hours using 500 milliliters of distilled water. To totally eliminate the solvent, the extract was distilled under vacuum and pressure. Before being used in experiments, it was dried and stored in a decicator. Weighing the extracted material allowed us to calculate the yield percentage in terms of air-dried powdered crude material.

ETHANOLIC EXTRACT

A soxhlet apparatus was filled with 100 mg of shade-dried coarse leaf powder, which was then continuously hot extracted for 24 hours using 500 ml of 100% alcohol. To entirely eliminate the solvent, the extract was distilled under pressure and vacuum. After drying, it was stored in a decicator until further testing. The extracted material was weighed, and the percentage yield was computed using air-dried powdered crude material.

ACETONE EXTRACT

The shade-dried coarse leaf powder (100mg) was carefully packed in a soxhlet apparatus and continuously heat extracted for 24 hours using 500 cc of acetone. To get rid of the solvent entirely, the extract was distilled under pressure and vacuum. After drying, it was stored in a decicator until further testing. The yield percentage was computed in terms of air-dried powdered crude material after the extract was weighed.

PHYTOCHEMICALS

LEAF

Ursolic acid (1) and polyphenolic substances such as quercetin (2), kaempferol3Orutinoside

(3), acacetine-7-O-β D glucopyranoside (4), and apigenin 5,7dimethylether 4'galactoside (5) were extracted from the leaves (Rao and Rao, 1983).

Desai et al. (1980) found that leaves are high in Ca, with Fe, Cu, Mn, and Zn levels ranging from 20.20 to 79.28%, 2.67 - 12.67, 0.15 - 7.20, and 6.0 - 45.0 ppm.

BARK AND HEARTWOOD OF MORINDA TINCTORIA:

Alizarin-1 ether, rubiadin (5), and D-mannitol (6) have been discovered in stem bark. In addition to the 6-primeveroside of morindone, anthragallol-2, 3-dimethyl ether, soranjidiol, and ibericin were extracted from the root bark (Rao and Rao, 1983).

According to Murti et al. (1959) and Eswaran et al. (1979), the heartwood produces tinctomorone (8), an anthraquinone ester, morindone, damnacanthal (9), and nordamnacanthal (1, 3-dihydroxy-2-formylan thraquinone). According to research, the stem and bark extracts in n- hexane, dichloromethane, and methanol show antifungal and antibacterial properties (Jayasinghe et al., 2002).

FLOWERS AND FRUITS OF MORINDA TINCTORIA

In Floral nectars, sugars and amino acids were found. Among sugars and amino acids, glutamic acid and fructose were more prevalent.(dore et al., 2001)

Chemical tests have shown that M. citrifolia fruits contain more important elements than M. tinctoria fruits. However, M. tinctoria had a significant concentration of manganese, whereas both fruits had comparable levels of calcium, potassium, phosphorus, and magnesium. The fruits of M. citrifolia had high amounts of reducing sugars and lipids, while M. tinctoria had high levels of total soluble sugars, starch, and crude fibers. (Mathivanan N et al., 2006).

ROOTS OF MORINDA TINCTORIA

Morindone, damnacanthal, nordamnacanthal β-sitosterol, and two unidentified colouring compounds were found in the root of morinda tinctoria. In addition, anthragallol-2, 3-diMethylether, soranjidiol, ibericin, and 6-primeveroside of morindone were identified in Morinda tinctoria (Rao and Reddy, 1977; Rao et al., 1983).

CONCLUSION

Morinda tinctoria, is a medical remedy known as Noni. Various parts of noni plant were traditionally used in Polynesian island as an herbal medicine in various Aliments. In vitro

evaluations revealed promising biological activities, particularly in antimicrobial and antioxidant assays, indicating their potential for medical and pharmaceutical applications. It shows potential antidiabetic activity due to its phytochemicals, including alkaloids, flavonoids, and saponins, which may help regulate blood sugar levels. The first report of fruit extracts from M. tinctoria being utilized to suppress biofilm formation in Amp C-producing *K. pneumoniae*, and **Morinda tinctoria (MEMT) leaves** exhibits potent **anticancer efficacy** in both in vitro and in vivo models. MEMT demonstrated selective cytotoxicity against multiple human cancer cell lines while remaining safe for normal cells. significantly reduced the formation of micronuclei and bi nuclei, improved comet assay parameters, and enhanced antioxidant enzyme activities, while lowering lipid peroxidation levels. These results suggest that the **antiinflammatory and antioxidant properties of M. tinctoria contribute to its antigenotoxic potential**, Morinda tinctoria shows promise in malaria treatment research, its utility in mosquito control is limited, and further phytochemical investigations are needed to identify the active constituents responsible for its anti-plasmodial effects its phytochemical analysis are studied.

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