Phytochemical and Phytopharmacological Evaluation of *Murraya koenigii* Seeds: A Review

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***Abstract*—Murraya Koenigii is a multipurpose medicinal plant which belongs to the Rutaceae family. It is a small perennial plant or aromatic shrub that grows wild and is found almost in the lowlands and hill forests of Nepal, Bhutan, China, India, and Myanmar except in the higher parts of the Himalayas.[1] *Murraya Koenigii* commonly known as "Curry leaf Plant", used in cooking and traditional medicines have been examined for their remarkable antioxidant potential.[2] The whole plant is a rich source of carbazole alkaloids and these alkaloids have been reported for their various pharmacological activities such as antiemetic, antidiarrhoeal, blood purifier and febrifuge. It is also been reported as antidiabetic, antioxidant, antihypertensive, antibacterial, cytotoxic and also in the treatment of various respiratory tract disorders. The literature survey reveals that limited work has been carried out on the chemical composition of *Murraya koenigii* seeds except for one report which prompted us to carry out the detailed chemical analysis of *M. koenigii* fruit and its seeds. This review encompasses a description of *Murraya Koenigii,* its phytochemical constituents, various pharmacological activities exhibited by isolated compounds, and the bioactivity observed in the extracts. Furthermore, the review highlights the potential of *Murraya Koenigii* to serve as an important nutraceutical for addressing application as a therapeutic agent.**

Index Terms: Murraya Koenigii Seeds, Chemical Constituents, Biological Activity, Toxicity profile.

I. INTRODUCTION

Murraya koenigii (also known as the 'curry tree') is an important medicinal plant, which has been used in diverse forms for centuries and it finds a pride place in Indian Ayurveda, referred as "Krishnanimba". Several parts of this plant constitute the vital ingredients of many Ayurvedic formulations. For example, fresh leaves, fruits, bark, and roots are used to treat several health disorders such as diabetes, bronchial disorders, vomiting and skin ailments.[3] Systematic scientific studies have been conducted regarding the efficacy of different plant parts in the treatment of various diseases.

There is a need to review the information available in literature on *Murraya koenigii*, so that it would aid future research by phytochemists, pharmacologists, clinicians, scientists, researchers and toxicologists. [4] This plant has been reported to have anti-oxidative, cytotoxic, antimicrobial, antibacterial, anti-ulcer, positive inotropic and cholesterol reducing activities. Compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols, simple phenolic compounds have been isolated from different plant parts. The leaves of this plant have been researched for various biological activities. However, research on *Murraya koenigii* seeds is limited, as most studies have concentrated on its fruits. This review provides a comprehensive briefing on the *Murraya Koenigii* seeds, highlights its specification, biological activity, phytochemical constituents and toxicity profile. The review outlines the diverse bioactive compounds reported, correlating them with specific biological properties such as antioxidant, antimicrobial, anticancer and anti-inflammatory, while also addressing safety and toxicity index.

Fig. 1. *Murraya Koenigii* leaves, fruit and seeds

II. PLANT TAXONOMY

Kingdom: Plantae
Sub-kingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliospida
Subclass: Rosidae
Order: Sapindales
Family: Rutaceae
Genus: Murraya
Species*:* *Murraya koenigii*

Murraya Koenigii known in English as 'Curry leaf', has various vernacular names across Indian and Foreign languages. In Marathi it is called Kadipatta, Karipatta, Jhirang; in Hindi it is known as Meetha neem, Kathnim, Bursunga and in Sanskrit it is referred to as Gunimba. In Bengali it is Barsunga, while in Kannada, it is called Karibevu. In Malayalam, the terms Kariveppilei, Karaepela are common, whereas in Gujarati it is known as Mitho limdo. Beyond Indian languages, in Italian it is called Fogli di Cari, in German Curryblatter and in French Feuilles de Cari. [5]

III. PLANT DESCRIPTION

Murraya koenigii is more or less deciduous shrub or small tree reaching up to 6 m in height. [6] The plant has a short trunk with 15-40 cm diameter, smooth, greyish or brown bark and has dense shady crown [7]. The main stem is dark green to brownish in colour. The leaves are bipinnately compound, 15-30 cm long, each bearing 11-25 leaflets alternate on rachis, 2.5-3.5 cm long ovate lanceolate with an oblique base. The leaf margins are irregularly serrate and petiole is 2-3 mm long.[8] Inflorescence is terminal cymes; each bearing 60-90 flowers. Each flower is bisexual, white, funnel shaped sweetly scented, stalked, complete, ebracteate and regular with average diameter of fully opened flower being 1.12 cm. The calyx is deeply lobed with five cleft and pubescent. Petals are five with free, whitish, glabrous dotted glands. Fruits occur in close clusters. They are small ovoid or subglobose, glandular, with thin pericarp enclosing one or two seeds which are spinach green in colour [9]. Fruits are 2.5 cm long and 0.3 cm in diameter wrinkled with glands and turns purplish black after ripening; are edible and yields 0.76% of a yellow volatile oil. The individual seed is 11 mm long, 8 mm in diameter and weights up to 445 mg [9].

IV. PLANT PARTS AND ITS BIOLOGICAL ACITIVITIES

Murraya koenigii is a rich source of diverse phytochemicals distributed across its leaves, roots, stem bark, fruits, and seeds. Extracts from these plant parts have revealed the presence of alkaloids, flavonoids, terpenoids, polyphenols, and essential oils, many of which are associated with significant pharmacological activities. The leaves, in particular, possess notable nutritional value, containing appreciable amounts of carbohydrates, proteins, fats, crude fiber, sugars, and starch, along with essential vitamins such as β-carotene, niacin, and thiamin, as well as minerals including calcium, magnesium, and sodium. In addition, the extractive values of alcohol-soluble, water-soluble, and ash fractions further underscore the chemical richness of the species. Collectively, the abundance of carbazole alkaloids, terpenoids, and flavonoids highlights the therapeutic potential of *M. koenigii* as both a nutritional and medicinal resource.[10]

Leaves

Leaves are the most chemically diverse part of the plant and contain a wide variety of phytochemicals. Terpenoids identified include Icariside B1,(–) Epifoliolide, several monoterpenes such as (–)-α-pinene, (+)-α-pinene, (+)-β-pinene, (+)-sabinene, as well as squalene, β-sitosterol, blumenol A, and loliolide. Flavonoids are abundant in the leaves and comprise quercetin, apigenin, kaempferol, rutin, catechin, myricetin, and more complex glycosides such as 4-O-β-D-rutinosyl-2-R-methoxyphenyl-1-propanone, 1-O-β-D-rutinosyl-2-R-ethyl-1-pentanol, and 8-phenylethyl-O-β-D-rutinoside. In addition, polyphenolic compounds like selir-11-en-4α-ol are reported. Leaves are also particularly rich in alkaloids, including bicyclomahanimbine, phebalosin, isomahanimbine, koenimbidine, euchrestine B, bismurrayafoline E, isomahanine, mahanimbine, girinimbine, glycozoline, isoMurrayazoline, mahanine, O-methylmurrayamine A, koenigine, koenimbine, and murrayone[11- 14]. Thus, leaves serve as the major repository of terpenoids, flavonoids, polyphenols, and alkaloids.

Leaves and Bark

Some phytochemicals are distributed in both leaves and bark. Among terpenoids and steroids, β-sitosterol has been detected in both parts. Polyphenols such as selir-11-en-4α-ol are also present in both tissues. Additionally, certain alkaloids like glycozoline, iso-murrayazoline, and mahanimbine are found in both leaves and bark, highlighting a shared metabolic composition between these two plant parts.[12,13]

Bark

The bark contains both polyphenols and alkaloids. Reported polyphenols include 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid. Alkaloids from bark are chemically diverse and include mukoeic acid, mukolidine, mukoline, and mukonidine, as well as bismurrayaquinone A and several Murrayazoline derivatives (A–D). This suggests that the bark is a key storage site for structurally unique carbazole alkaloids.[13]

Roots

Roots are another important source of alkaloids. Among the compounds reported are koenimbidine, mahanimbine, mukoeic acid, mukolidine, murrayazolinol, and murrayazoline. The presence of multiple carbazole alkaloids in the roots highlights their pharmacological significance and indicates that this plant part contributes strongly to the overall alkaloid diversity.[14]

Stems

The stems are particularly rich in alkaloids, with compounds such as isoigoline, murrayazoline derivatives (A–D), 9-carbethoxy-3-methylcarbazole, and 9-formyl-3-methylcarbazole being reported. These findings emphasize that stems are primarily a source of specialized carbazole alkaloids, rather than terpenoids or flavonoids.[15,16]

Fruits

Fruits are comparatively less diverse but still contain significant alkaloid constituents such as isomahanine and mahanimbine. These alkaloids are commonly associated with bioactivity, suggesting that fruits, though less studied, may also hold pharmacological relevance.[17, 18]

V. SPECIFICATIONS OF SEEDS

Murraya koenigii plant bears fruits from mid- July to the end of August. Seeds are small, dark green, and inedible. [19] Reisch et al. (1994) isolated mahanimbine, girinimbine, koenimbine, isomahanine and mahanine from the seeds of *M. koenigii*. The petroleum ether extract of the seeds showed the presence of 2-methoxy-3-methyl carbazole. Mandal et al. (2010) isolated three bioactive carbazole alkaloids: kurryam, koenimbine, and koenine, with structural confirmation using 2D-NMR spectra. Coumarin-like compounds such as indicolactone, anisoalctone, and 2-epoxy indicolactone (a furocoumarin lactone) were also isolated from the seeds. Adebajo et al. (2000) reported the presence of xanthotoxin, isobyaknagelicol, byakangelicol, and isogosferol as minor furocoumarins in the seeds. Isoheraclenin, isoimperatonin, oxypeucedanin, isopimpinellin, and bergaptan were isolated from the seeds of *M. koenigii*. The seeds contain 4.4% of total lipids, with 85.4% being neutral lipids, 5.1% glycolipids, and 9.5% phospholipids. Neutral lipids consist of 73.9% triacylglycerol, 10.2% free fatty acids, and small amounts of diacylglycerols, monoacylglycerols, and sterols. Sterylglucoside and acylated sterylglucoside are the major glycolipids identified from the seeds. Phospholipids mainly include phosphatidylethan olamine and lysophosphatidyl choline.[19-21]

Chemical Constituents in *Murraya koenigii* Seeds

Lipids: Total seed lipids are ~ 4.4% of the dry seed mass. Breakdown: neutral lipids (85.4%), glycolipids (5.1%), phospholipids (9.5%). Within neutral lipids: 73.9% are triacylglycerols; 10.2% are free fatty acids; smaller amount of diacylglycerols, monoacylglycerol and sterols. Major glycolipids: sterylglucoside, acylated sterylglucoside, Minor glycolipids include digalactosyldiacylglycerol, monogalactosyldiacylglycerol, monogalactosylmonoacylglycerol. Major phospholipids: phosphatidylethanolamine, phosphatidylcholine, lysophosphatidyl ethanolamine, Lysophosphatidylcholine, Minor ones are phosphatidylinositol, phosphatiglycerol, phosphatidic acid. [22]

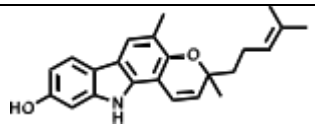
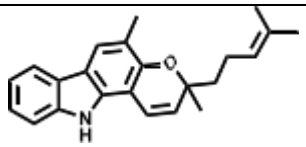
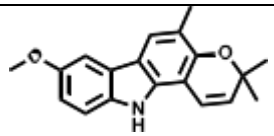
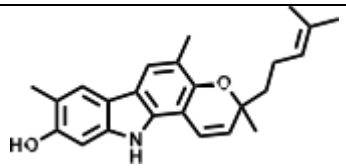
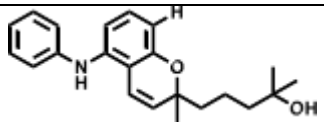
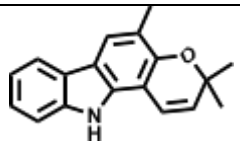
Alkaloids / Carbazole Alkaloids: Several carbazole alkaloids are present in seed extracts. Examples include koenimbine, murrayanine, murrastanine A,

koenine, isomahanine, etc. A newly isolated carbazole alkaloid from *M. koenigii* seeds include *3-geranyl 8-hydroxy-6,7-dimethoxy-3',3'-dimethyl-1,2-pyranocarbazole*. [23,24] [Table 1]

Phenolics / Flavonoids / Other Small Bioactive Molecules The methanolic seed extract profiling (via UPLC-MS/MS) identified ~ 35 phenolics: including flavonoids, phenolic acids, and coumarins. Also identified: organic acids, amino acids, one sugar, one vitamin, a fatty amide, and some fatty acids. [25]

Volatile / Terpenoid / Furocoumarin Compounds Seeds reportedly contain *furocoumarins* (certain lactones) along with terpenoid / volatile compounds such as terpinene, terpinen-4-ol, *ocimene*, etc. Minor furocoumarins: *xanthotoxin*, *byakangelicol*, *isopimpinellin*, *bergaptol*, etc.[26]

Table 1: The major bioactive compounds found in berries and seeds of *M. koenigii* and its pharmacological activities.[27]

Sr. No.	Constituents	Structures	Activity
1.	Mahanine		• Cytotoxicity • anti-microbial • anti-cancer
2.	Mahanimbine		• Cytotoxicity • anti-oxidant • anti-microbial • anti-diabetic • hyperlipidemic
3.	Koenimbine		• Cytotoxicity • anti-diarrhea
4.	Isomahanine		• Cytotoxicity • anti-oxidant • anti-microbial • anti-diabetic • hyperlipidemic
5.	Mahanimbinine		• Anti-oxidant • anti-microbial • anti-diabetic • hyperlipidemic
6.	Girinimbine		• Anti-tumor

VI. BIOLOGICAL ACTIVITY

A. Carbazole alkaloids:

Carbazole alkaloids such as Koenine, Mukoeic acid, Mahanine, Mahanimbine, Koenimbine, Murrayazolidine, Murrayazoline, Murrayacine and Girinimbine have been identified as biologically active compounds with antioxidant, antimicrobial, anti-inflammatory, anthelmintic, antidiarrheal, hepatoprotective, analgesic and cytotoxic properties.

Mahanine (MH)

Samanta et.al (2018) studied the anticancer potential of mahanine against glioma through a series of in vitro and in vivo biological assays. Cell viability was first assessed using the MTT assay, which showed that mahanine inhibited proliferation of glioma cells in a dose-dependent manner, with the lowest IC_{50} of 7.5 µM observed in HS 683 cells, while normal astrocytes were far less sensitive (IC_{50} = 90.5 µM). Apoptosis induction was confirmed by DAPI and AO/EB staining, which revealed nuclear condensation and apoptotic morphology. Annexin V/PI flow cytometry, demonstrated a significant rise in apoptotic cell populations (from 1.8% to 29.9%) (Fig. 2)

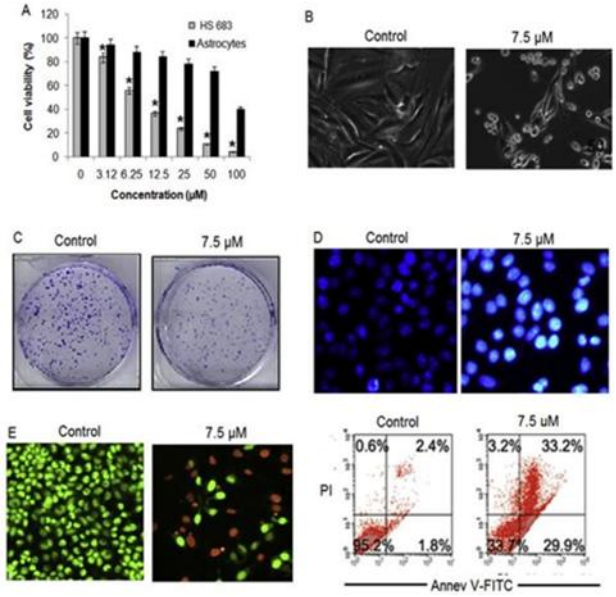


Fig. 2 Antiproliferative effects of Mahanine on Glioma HS 683 cells. (A) Effect on cell viability (B)Cell morphology (C) Colony formation (D) DAPI staining showing apoptotic cells (E) AO/EB staining for apoptosis (E) Estimation of apoptotic cell populations by annexin V/PI staining. The experiments were carried out in triplicates and the values are expressed as mean±SD (*p < 0.05).

Western blot analysis further validated this by showing upregulation of Bax, cytochrome c, cleaved caspase-3, cleaved caspase-9, and cleaved PARP, along with downregulation of Bcl-2, indicating activation of the mitochondrial apoptotic pathway. Cell cycle analysis revealed that MH induced G2/M phase arrest in HS 683 cells, which correlated with suppression of regulatory proteins such as cyclin B1, Cdc25c, p-Cdc25c, Cdc2, and p-Cdc2, while p21 was upregulated, suggesting a p53-independent checkpoint control. (Fig. 3)

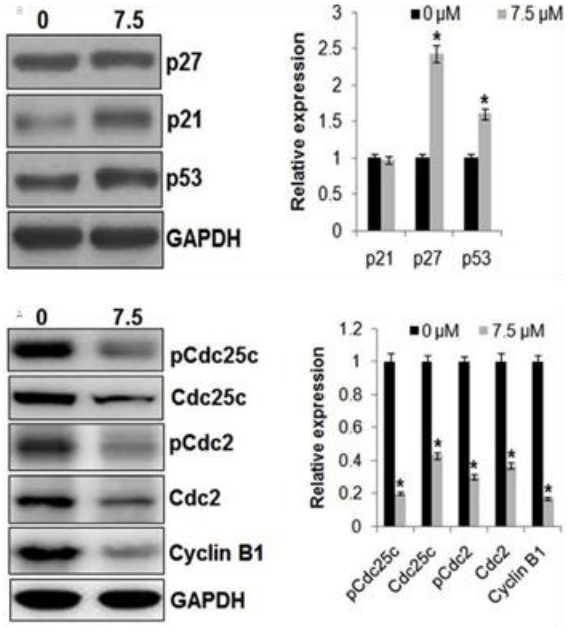


Fig 3. Mahanine triggers G2/M Cell cycle arrest in Glioma HS 683 cancer cells (A) Expression of pCdc25c, Cdc25c, pCdc2, Cdc2, cyclin B1 (B)Expression of p21, p27 and p53 as determined by western blotting. The cells were incubated with 7.5 µM Mahanine The experiments were carried out in triplicates and the values are expressed as mean ± SD (*p < 0.05).

In addition, migration and invasion assays showed that MH significantly inhibited glioma cell motility and invasiveness, pointing to its anti-metastatic potential. Western blotting also revealed that MH exerted its effects through suppression of the PI3K/AKT/mTOR signaling pathway, with reduced phosphorylation of PI3K, AKT, and mTOR but no major changes in their total protein levels. The in vivo xenograft study in BALB/c nude mice confirmed these findings, as MH

treatment at 20–80 mg/kg substantially suppressed tumor volume and weight without noticeable toxicity. (Fig. 4)

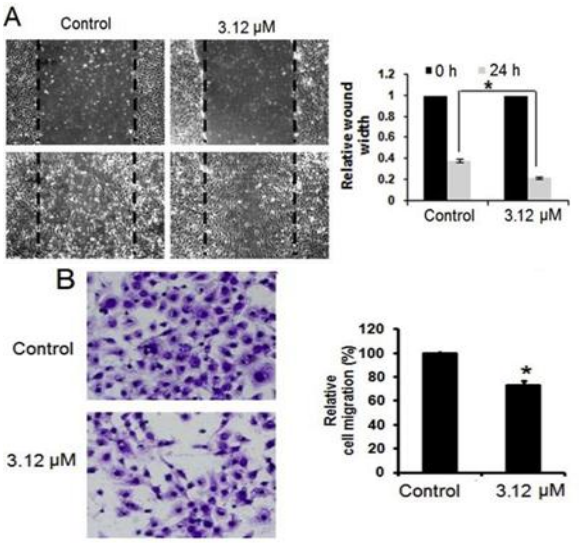


Fig. 4. Mahanine treatment for 24 h inhibits cell migration and invasion of Glioma HS 683 cells. (A)cell migration assay (B) cell invasion assay. The experiments were carried out in three biological replicates and expressed as mean ± SD. (*p < 0.05, **p < 0.01).

Immunohistochemical staining of tumor tissues further demonstrated reduced Ki-67 expression, indicative of decreased proliferation, and elevated cleaved caspase-3, confirming apoptosis induction in vivo (Fig. 5). Collectively, these results establish mahanine as a promising anticancer agent against glioma, acting through apoptosis induction, cell cycle arrest, inhibition of migration and invasion, and suppression of the PI3K/AKT/mTOR pathway, both in vitro and in vivo. These findings establish Mahanine as a potent multi-target anticancer agent with selective cytotoxicity toward cancer cells, effective induction of apoptosis, regulation of cell cycle checkpoints, and inhibition of oncogenic signaling, both in vitro and in vivo.[29]

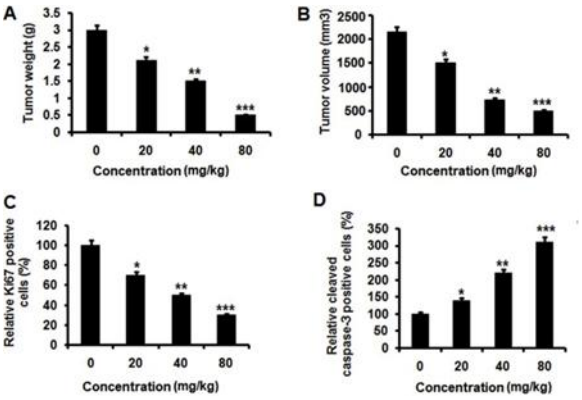


Fig. 5. Mahanine inhibits glioma tumor growth in vivo. Effect of Mahanine on (A) Tumor volume and (B) tumor weight (C) Relative percentages of Ki67 positive cells and (D) cleaved caspase-3. The experiments were performed in triplicate and data are presented as the mean ± standard deviation (*P < 0.05,**P < 0.01 and ***P < 0.001).(Chen et al., 2019)[28]

Mahanimbine

The study of Mahanimbine alkaloid by Xie et.al (2020) on human bladder cancer cells demonstrated that this natural carbazole compound exerts potent anticancer effects through multiple cellular mechanisms. Using WST-1 assays, Mahanimbine significantly reduced the viability of Hs172.T bladder cancer cells in a dose- dependent manner, with an IC_{50} of 32.5 µM, while having minimal cytotoxicity against normal RT24 bladder cells (Fig. 6).

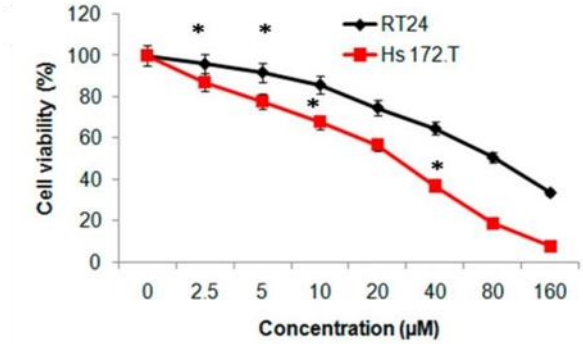


Fig. 6. Antiproliferative effect of Mahanimbine on Hs172.T BC cells as compared to normal RT24 BC cells at various dose concentrations for 24 h. All the experiments were carried in triplicate and data are expressed as mean ± SD values. *p<0.05.

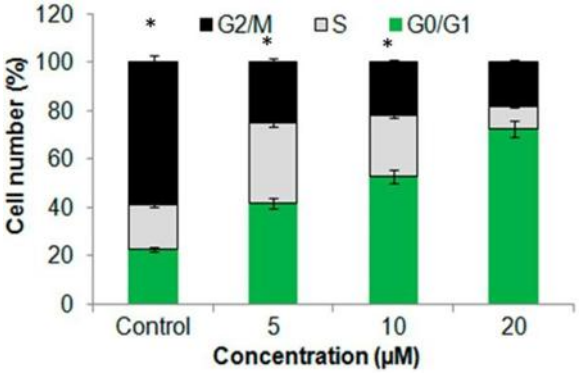


Fig. 7. Cell cycle analysis of Mahanimbine-treated Hs172.T BC cells at varying dose concentrations i.e. control, 5, 10 and 20 μM for one day. All the experiments were carried in triplicate and data are expressed as mean ± SD values. *p<0.05.

Flow cytometric analysis revealed that Mahanimbine induced marked G0/G1 cell cycle arrest, increasing the proportion of cells in this phase up to ~70%, accompanied by downregulation of cyclin D1 and D2 and upregulation of cyclin D3 and E (Fig. 7). Apoptosis induction was confirmed by AO/EB staining and Annexin V/PI flow cytometry, which showed a sharp rise in apoptotic cell populations from 5.2% in control to ~75% at 100 μM, correlating with increased Bax and decreased Bcl-2 protein levels (Fig. 8). Additionally, autophagy induction was observed, with TEM revealing autophagic vacuole formation, mitochondrial degeneration, and nuclear changes, while western blotting confirmed increased LC3-II and decreased p62 expression, indicating autophagy-mediated cell death.

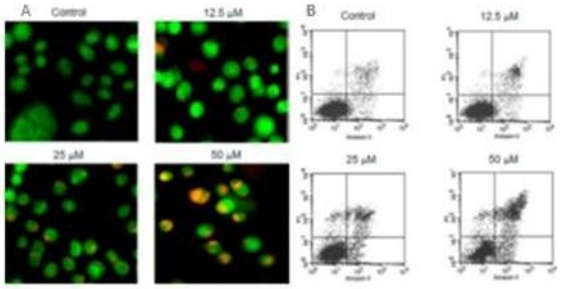


Fig. 8. (A) Morphological changes under fluorescence microscope after exposure to Mahanimbine and AO/EB staining of Hs172.T BC cells. (B) Apoptosis quantification after Mahanimbine treatment using annexin V-FITC/PI staining and flow cytometry. Mahanimbine induced dose-dependent induction of apoptosis.

Together, these findings establish that Mahanimbine inhibits bladder cancer cell proliferation by orchestrating cell cycle arrest, promoting apoptosis, and activating autophagy, highlighting its potential as a promising natural anticancer agent against human bladder carcinoma (Xie et al., *JBUON*, 2020)[30]

Mahanimbine (MN), also exerts significant cytotoxic effects against human breast cancer cells (MCF-7) through mechanisms involving mitochondrial apoptosis and anti- angiogenesis pathways evaluated by Hobani et.al (2022). The compound demonstrated potent antiproliferative activity, with an IC_{50} of 14 μM in MCF-7 cells, while non-cancerous mammary cells were less affected, reflecting selectivity in its cytotoxic action. (Fig. 9)

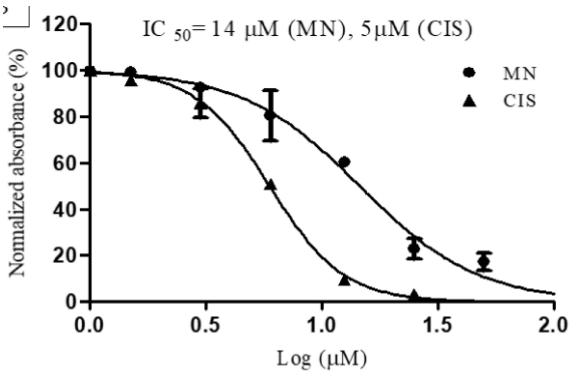


Fig 9. Antiproliferative effect of MN and cisplatin (CIS) on MCF-7 cells at various dose concentrations for 48 h.

Morphological evaluation and acridine orange/propidium iodide staining indicated typical apoptotic features such as cell shrinkage, chromatin condensation, and membrane blebbing, all of which intensified over time. Importantly, MN treatment induced a marked accumulation of reactive oxygen species (ROS), which is a critical mediator of mitochondrial dysfunction and programmed cell death. This was associated with pronounced loss of mitochondrial membrane potential, as evidenced by decreased JC-1 dye aggregation, and with elevated activities of caspase-7 and caspase-9, but not caspase-8, implicating the intrinsic pathway of apoptosis . (Fig. 10)

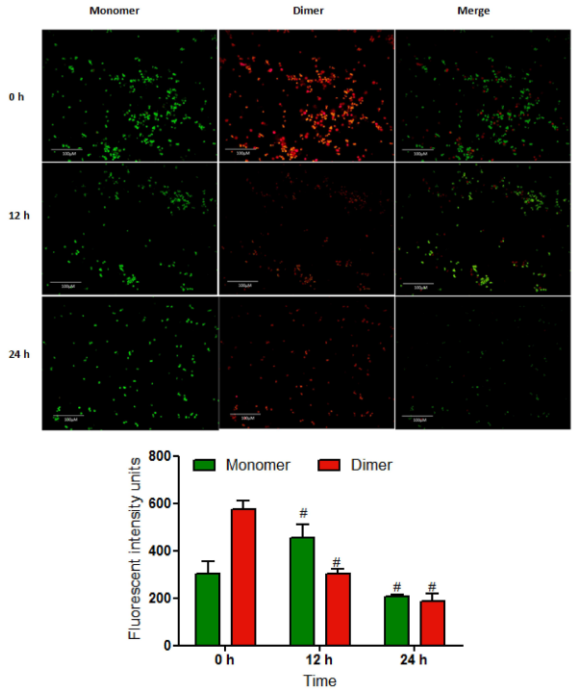


Fig. 10. Effect of MN on the D m in MCF-7 cells stained with JC-1 dye. Pictures shown are JC-1 monomer, J-aggregates dimer (red fluorescence), and merged images. As the pictures show, the accumulation of JC-1 dye in the mitochondria matrix generates red fluorescence. As the D m decreased, JC-1 could not aggregate in the matrix, causing JC-1 to remain as a monomer, resulting in green fluorescence. The quantified data showed that the red fluorescent decreased significantly as time increased. All data are represented as mean _ SD. # $p < 0.05$ vs. control n = 3.

On the molecular level, MN treatment led to upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2, further supporting its role in mitochondrial-mediated cell death. The anti-angiogenic and anti-invasive properties of MN were highlighted by wound healing assays, which showed considerable inhibition of cell migration even at sub-cytotoxic concentrations, and by reduced expression of invasion-related genes and proteins, specifically MMP-2 and MMP-9 (Fig. 11).

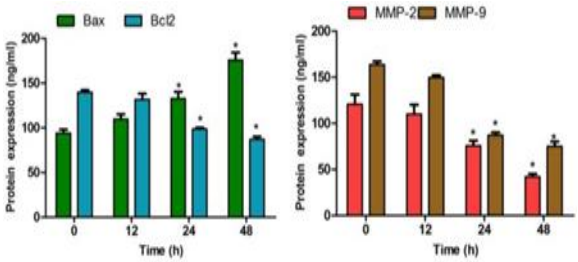


Fig. 11. Bax, Bcl-2, and MMP-2 and -9 protein expression in MCF-7 cells treated with MN for 12, 24, and 48 h evaluated by ELISA.

Overall, these cumulative findings reveal that mahanimbine induces apoptosis in breast cancer cells primarily via the mitochondrial pathway and impairs tumor cell migration by suppressing key metalloproteinases, positioning it as a noteworthy lead compound for future anti-breast cancer drug development based on natural products. [31]

Mahanimbine, also demonstrates significant antidiabetic and hypolipidemic effects in experimental studies involving streptozotocin-induced diabetic rats. Weekly intra-peritoneal administration of mahanimbine at doses of 50 mg/kg and 100 mg/kg for 30 days led to a marked reduction in fasting blood glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels, while high-density lipoprotein (HDL) levels increased notably. These effects were statistically significant when compared to diabetic controls, and comparable to the standard antidiabetic drug glibenclamide.[32]

The mechanism underlying mahanimbine's antidiabetic action is suggested to be similar to glibenclamide, possibly by enhancing insulin secretion from pancreatic beta cells and/or increasing peripheral glucose uptake. Additionally, mahanimbine exhibited appreciable alpha-amylase inhibitory activity (IC_{50} of 83.72 µg/ml) and weaker alpha-glucosidase inhibition (IC_{50} of 99.89 µg/ml), indicating potential utility in managing postprandial hyperglycemia by limiting carbohydrate hydrolysis and absorption in the gut (Fig. 12). Weight loss typically seen in diabetic rats was mitigated by mahanimbine treatment, aligning with its lipid-lowering effects and favorable influence on lipid metabolism. The overall biochemical improvements from mahanimbine administration may reduce the risk

of cardiovascular complications commonly associated with diabetes, such as atherosclerosis and coronary heart disease.

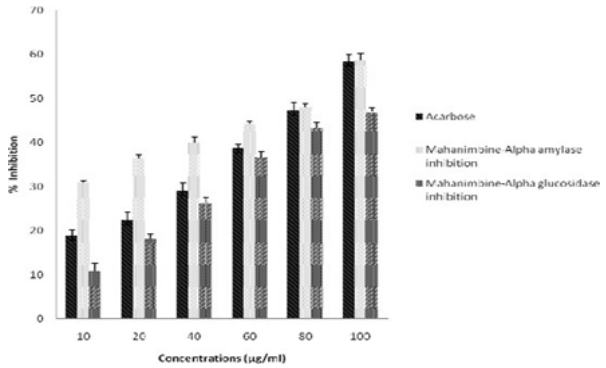


Fig 12. Alpha amylase and alpha glucosidase inhibitory effects of mahanimbine

In conclusion, the study provides strong evidence that mahanimbine possesses both antidiabetic and hypolipidemic properties, making it a promising agent for the management of diabetes and its associated lipid abnormalities and cardiovascular risks. (Dineshkumar et al., International Journal of Phytomedicine 2010)[32]

Girinimbine

Girinimbine, demonstrate notable anticancer and anti-inflammatory activities as shown by both in vitro and in vivo experiments studied by Iman et.al. Anticancer effects were primarily established in human colon cancer cells (HT-29), where girinimbine induced strong apoptosis without being cytotoxic to normal colon cells. Mechanistically, girinimbine caused cell cycle arrest at the G0/G1 phase, upregulating the cyclin-dependent kinase inhibitors p21 and p27, and significantly increasing the tumor suppressor protein p53. Apoptosis induction proceeded via the intrinsic mitochondrial pathway, evidenced by altered mitochondrial membrane potential, increased nuclear condensation, cytochrome c release, higher expression of pro-apoptotic Bax, and decreased anti-apoptotic Bcl-2. Activation and cleavage of caspases 9 and 3 further supported mitochondria-involved apoptosis. These findings were corroborated in vivo using a zebrafish embryo model, where girinimbine exposure led to significantly greater apoptotic cell death compared to controls (Fig. 13).

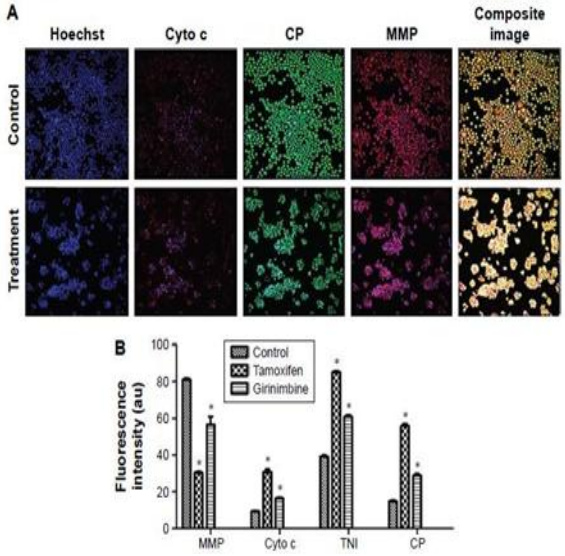


Fig. 13. Effects of girinimbine on nuclear morphology, Cyto c release, CP, and MMP (A) Representative images of HT-29 cells stained with Hoechst 33342, Cyto c, CP, and MMP dyes after 24-hour treatment with girinimbine. Magnification: 4×. (B) Representative bar chart indicating the reduction in MMP and the increased CP, TNI, and Cyto c release in treated HT-29 cells after 24 hours. All data are expressed as mean ± SD of three independent experiments (*P 0.05).

For its anti-inflammatory properties, girinimbine inhibited nitric␣oxide production in LPS/IFN-γ stimulated RAW 264.7 macrophages, without reducing cell viability. It also suppressed the nuclear translocation of NF-κB, indicating blockage of key inflammatory signaling pathways. In mice with carrageenan-induced peritonitis, oral girinimbine pretreatment significantly reduced leukocyte migration (mainly neutrophils) and decreased the levels of pro-inflammatory cytokines IL-1β and TNF-α in peritoneal fluid, confirming its in vivo anti-inflammatory efficacy. Additionally, girinimbine displayed considerable antioxidant activity in ORAC assays, suggesting another beneficial mechanism in inflammation suppression (Fig. 14).

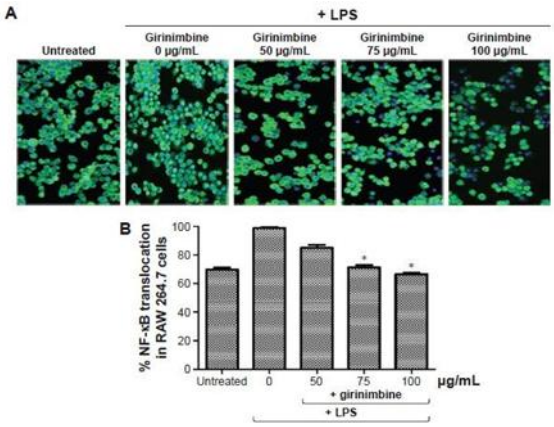


Fig. 14. Effect of girinimbine on NF-κB translocation in RAW 264.7.(A) Images (magnification: 4×) and (B) representative bar chart of RAW 264.7 cells treated with various concentrations of girinimbine for 2 hours, and then exposed to LPS(10 ng/mL) for 30 minutes and analyzed using ArrayScan HCS Reader. Percentage of NF-κB translocation to the nucleus was calculated by using ANOVA. The data are presented as the mean ± SD of three independent experiments (*P∟0.05).

Collectively, these results strongly suggest that girinimbine possesses both chemo-preventive and chemotherapeutic potential, working by simultaneously triggering apoptosis and suppressing inflammation, and should be further investigated for possible applications in early-stage cancer therapy and prevention of inflammation-driven malignancies. [33]

Girinimbine demonstrates significant anti-tumour promoting activity and potent antioxidant properties. In vitro testing revealed that girinimbine effectively inhibited the expression of early antigen of Epstein-Barr virus (EA-EBV) in Raji cells induced by the tumour promoter phorbol 12-myristate 13-acetate. This suppression was particularly strong for the restricted early antigen (EA-R) across all concentrations, while the diffused early antigen (EA-D) was only partially suppressed at higher concentrations (32.0 µg/mL). Notably, girinimbine was not cytotoxic to the Raji cells, as cell viability remained above 90% for all tested concentrations. The compound displayed a 50% inhibition rate at a concentration of 6.0 µg/mL, indicating substantive anti-tumour promoting capability (Fig. 15).

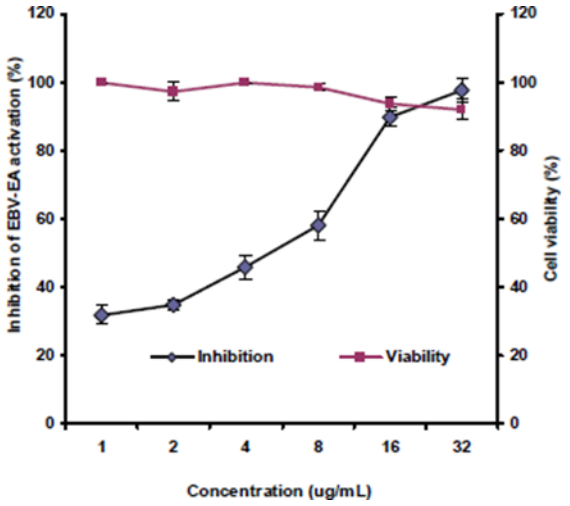


Fig. 15. Effect of girinimbine on the early antigen (EA) of Epstein-Barr virus activation and the viability of Raji cells.

Regarding antioxidant properties, girinimbine exhibited very strong activity in the ferric thiocyanate (FTC) assay, comparable to that of vitamin E (α-tocopherol) (Fig. 16). It also achieved over 95% inhibition of superoxide generation in TPA-induced differentiated premyelocytic HL-60 cells at concentrations of 5.3 and 26.3 µg/mL (Fig. 17). However, girinimbine did not effectively scavenge diphenyl picryl hydrazyl (DPPH) free radicals, highlighting its specific antioxidative mode of action.

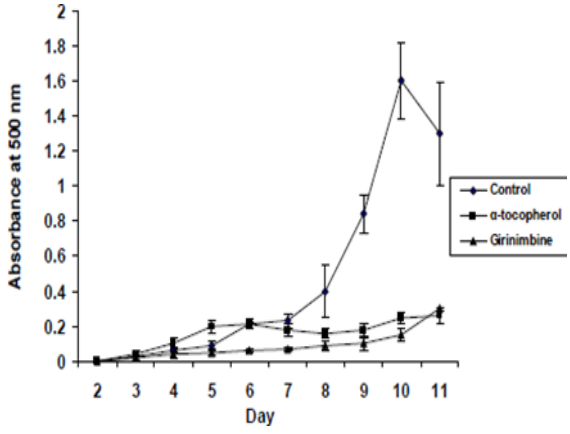


Fig. 16. Antioxidant activity of girinimbine measured by FTC method.

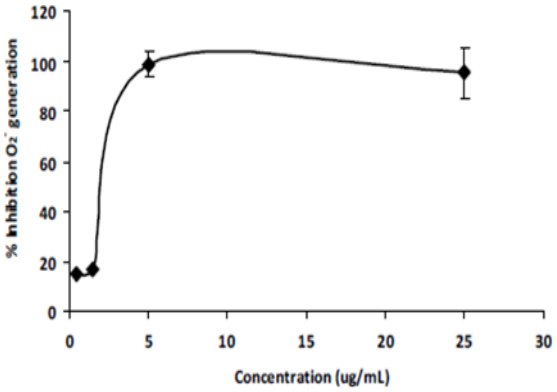


Fig. 17 Inhibitory effect of girinimbine on the superoxide generation in differentiated HL60 cells.

The robust antioxidant and anti-tumour promoting activities of girinimbine are likely related, as the suppression of superoxide and other free radicals can inhibit alterations in gene expression linked to tumour promotion. These findings position girinimbine as a promising compound for further development in cancer chemoprevention and antioxidative therapies. [34]

Antidiarrhoeal activity

The study by Mandal et al. (2010) investigated the antidiarrhoeal potential of carbazole alkaloids isolated from the seeds of *Murraya koenigii* Spreng (Rutaceae). Bioassay-guided fractionation of the n-hexane seed extract yielded three alkaloids—kurryam, koenimbine, and koenine. Among them, kurryam and koenimbine showed significant, dose-dependent inhibitory activity against castor oil-induced diarrhoea, gastrointestinal motility, and PGE_2-induced enteropooling in rats. At a dose of 30 mg/kg, both compounds produced effects comparable to the standard antidiarrhoeal drug diphenoxylate (5 mg/kg), while at 50 mg/kg their efficacy surpassed that of the standard. The findings highlight that these alkaloids not only reduce diarrhoeal frequency but also delay intestinal transit and secretion, thereby justifying the traditional use of *M. koenigii* in diarrhoeal management. This work represents the first report on the biological activity of these seed-derived carbazole alkaloids, supporting their potential as natural antidiarrhoeal agents (Mandal et al., 2010,*Fitoterapia*,81:72.74.doi:10.1016/j.fitote.2009.08.016)[35]

Biological activity of the Methanolic extract of Murraya koenigii seeds (MEMS):

Antioxidant activity: MEMS exhibited strong free radical scavenging and reducing power, demonstrated through DPPH and FRAP assays, confirming its role in oxidative stress reduction.

Antimitotic activity: The extract significantly inhibited sprout growth in *Cicer arietinum* seeds and reduced the mitotic index in *Allium cepa* root tips, showing its ability to suppress abnormal cell division.

Anti-angiogenic activity: MEMS reduced angiogenesis in the chick chorioallantoic membrane (CAM) assay, suggesting its potential in preventing tumor vascularization.

Cytotoxic activity: The extract showed moderate cytotoxicity in the brine shrimp lethality bioassay (LD_{50} = 40.86 mg/mL), confirming its bioactive potency.

Antiproliferative activity: MEMS inhibited the growth of human cancer cell lines (DLD-1 colorectal and MCF-7 breast cancer) in a dose-dependent manner, with higher selectivity toward cancer cells than normal cells.

Apoptosis-inducing activity: MEMS induced apoptosis via phosphatidylserine externalization, mitochondrial membrane depolarization, and significant caspase-3/7 activation, confirming caspase-mediated cell death mechanisms.

Phytochemical contribution: LC–MS analysis identified compounds such as neplanocin A and girinimbine, which were shown (via molecular docking) to interact with caspases and contribute to apoptosis induction. These findings establish that *Murraya koenigii* seeds possess antioxidant, antimitotic, anti-angiogenic, cytotoxic, antiproliferative, and apoptosis-inducing activities, highlighting their therapeutic potential (Nadaf et al., 2020, *South African Journal of Botany*, 132:328–337. doi:10.1016/j.sajb.2020.05.021)[36]

The biological evaluation of *Murraya koenigii* (curry leaf) seeds and pericarps revealed a clear difference in their pharmacological potential. In the in vitro antibacterial assays, the methanolic seed extract showed remarkable inhibition of multidrug-resistant *Acinetobacter baumannii*, producing an inhibition zone of 21 mm, which was slightly higher than the standard antibiotic tigecycline (20 mm), and far superior to the pericarp extract (10 mm). The minimum inhibitory concentration (MIC) confirmed this potency, with seeds active at 32 µg/mL compared to 125 µg/mL for pericarps.

These findings were substantiated by in vivo studies in a murine pneumonia model. Animals treated with seed extracts exhibited significant improvements in lung pathology, including reduced inflammatory cell infiltration, decreased pulmonary necrosis, and lower vascular congestion, closely paralleling the tigecycline-treated group. Pericarp extracts, while offering some protection, showed only moderate improvements. Importantly, bacterial load analysis revealed that seed extracts drastically reduced lung colony counts compared to pericarp extracts.

At the biochemical level, seed treatment modulated immune responses by lowering pro-inflammatory cytokines (TNF-α, IL-6, IFN-γ, MPO) and elevating protective anti-inflammatory cytokines (IL-10, IL-12), highlighting both its antibacterial and anti-inflammatory roles.

Phytochemical profiling linked these activities to abundant carbazole alkaloids (e.g., mukonine, isogirinimbine, murrastinine B), coumarins (e.g., scopolin, murrayanone, 5-methoxymurrayatin), and phenolics (e.g., ferulic acid, quercitrin, sinapine). These metabolites not only demonstrated strong docking affinity to the bacterial enzyme MurF (involved in peptidoglycan biosynthesis) but also possess previously reported antioxidant, hepatoprotective, cytotoxic, antidiarrheal, and anthelmintic effects.

Together, these findings establish that M. koenigii seeds are far more potent than pericarps, offering a dual antibacterial and anti-inflammatory effect against resistant *A. baumannii* infections, and highlighting their potential as a source of novel phytotherapeutics (El-Shiekh et al., 2024).[25]

VI. SEED TOXICITY

Seeds are commonly described as inedible and potentially toxic. Several food/produce sources and botanical references warn that the small dark seeds embedded in the curry berry are bitter and should be discarded as they are *not* used as food. [37]

Most formal toxicology data come from studies of leaves and leaf extracts, not seeds. Leaves (and leaf extracts) have been extensively studied and are generally found to be safe at typical dietary or experimental doses, though very high or chronic doses can produce biochemical or histopathological changes in animals. [38]

Seeds contents are associated with bioactive alkaloids (carbazole-type alkaloids) that show cytotoxic activity in vitro. The genus contains carbazole alkaloids such as mahanine, mahanimbine, koenimbine and related compounds; these have documented cytotoxic/anticancer activity in cell and animal models. Cytotoxic phytochemicals can be beneficial at controlled doses but can also cause toxicity if concentrated, crushed, or consumed inappropriately. [39,40]

Animal studies (high / repeated doses of extracts) report organ changes at high doses. Repeated/chronic administration of methanolic/ethanolic extracts in rats has produced changes in liver enzymes, histopathological signs (e.g., lymphocytic infiltration in liver) and mild kidney changes at higher dose ranges in some studies — indicating potential hepatic and renal stress with excessive dosing. LD_{50} (acute lethal dose) for many extracts has generally been high (i.e., relatively low acute toxicity) but chronic toxicity and organ effects were seen at lower long-term doses in some studies. Human poisoning reports specific to *Murraya koenigii* seeds are scarce in the scientific literature. I did not find peer-reviewed case series clearly documenting severe systemic poisoning from ordinary accidental ingestion of *Murraya* seeds; however, authoritative cautions (horticultural / produce) and the fact that seeds contain concentrated phytochemicals support the conservative advice to avoid ingesting the seeds, especially by children. (Absence of many published human cases is not proof of safety — it may reflect low incidence or underreporting.)[41]

VII. CONCLUSION

To conclude, this review highlights the seeds of *Murraya koenigii* a plant part that remains comparatively unexplored despite the extensive studies on its leaves and other parts. By combining existing reports on their chemical composition, biological activities, and toxicity profiles, it brings attention to both the promising therapeutic potential and the possible risks associated with seed utilization. This comprehensive overview not only addresses current gaps in knowledge but also emphasizes the importance of conducting detailed phytochemical and pharmacological studies to substantiate their bioactivity and ensure their safe application in nutraceutical and therapeutic developments.

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