Unveiling Mahanine from *Murraya koenigii* as a Potential Chemopreventive Agent in Breast Cancer: A Comprehensive Exploration from Traditional Wisdom to Modern Perspectives

Suman Kumar Samanta*[1], Dhritismita Deka[1], Bikas Chandra Pal[2], Narayan C Talukdar[1]

[1]Faculty of Science, Assam down town University, Sankar Madhab Path, Panikhaiti, Guwahati, 781026, Assam, India.
[2]CSIR-Indian Institute of Chemical Biology, 4 Raja S C Mullic Road, Jadavpur, Kolkata, 700032, India.

*Correspondence to:

Suman Kumar Samanta; Email: sumansamanta699@gmail.com, Tel: 0361227361.Fax: 0361-2273062. ORCHID: https://orcid.org/0000-0002-2892-653X.

Abstract:

Murraya koenigii, colloquially known as "Krishnanimba" in Indian Ayurveda, stands as a symbol of diverse applications and profound healing attributes. Embedded in the culinary fabric of Indian cuisine, this aromatic and flavorful plant has garnered attention for its multifaceted therapeutic potential. Over the last two decades, extensive research has delved into unraveling the phytochemical constituents of *Murraya koenigii*, shedding light on its promising therapeutic benefits across various health disorders. This chapter amalgamates traditional Ayurvedic wisdom with contemporary scientific insights, providing a holistic view of the health-promoting properties inherent in *Murraya koenigii*.

With a specific focus on cancer, particularly the pervasive threat of breast cancer, prevalent in resource-limited settings, this chapter emphasizes the significance of exploring cost-effective and safe avenues for prevention. Lifestyle factors, mental stress, and environmental pollutants contribute to the escalating incidence of cancer, necessitating innovative approaches. In this context, researchers have turned their attention to dietary phytochemicals derived from natural sources.

The spotlight of this chapter is on Mahanine, a carbazole alkaloid extracted from the leaves of *Murraya koenigii*, commonly referred to as "curry patta." A thorough examination of Mahanine's chemopreventive potential unravels its promising role in mitigating breast cancer risks. The integration of traditional knowledge and modern scientific findings serves as a foundation for

understanding Mahanine's mechanisms of action and potential applications in natural medicine and functional foods.

This exploration of Mahanine's chemopreventive attributes not only contributes to the evolving comprehension of *Murraya koenigii's* therapeutic potential but also paves the way for further research and development. The findings presented herein advocate for the integration of Mahanine into preventive strategies, offering a beacon of hope in the endeavor to address the global health burden imposed by breast cancer.

Key words: Mahanine; Breast Cancer; Natural phytochemical; Chemoprevention; Translational Research;

Introduction:

The people of developing countries are less prepared to carry the huge burden and treatment cost of cancer like diseases. Due to the irregular life style, mental stress and environmental pollution the cancer incidence is now become quite common to all family. From the ancient time, many phytochemicals have been developed from the natural or dietary source for management of several health disorder and found outmost promising. Hence, cancer prevention with dietary phytochemicals is the safest and cost effective among all the existing option. The cancer prevention mechanism may work in three different way; primarily it may act against the initiation of any type of cancer; secondarily, it can stop the recurrence and finally it can work against the spread or metastasis of cancer. When the solution comes from the dietary sources, then it would be more easy to use because of its safety sketch. Many such type of phytochemicals like, lycopene, diallylsulphide, capsaicin, betulinic acid, chlorogenic acid, phenethylisothiocyanate, benzyl isothiocyanate, etc. isolated from different food beverages have been investigated for their chemoprevention activity and showed very promising in the preliminary research. Besides that, many phytonutrients, like vitamin A, B6, B9, linolenic acid, etc. have also been investigated as chemopreventative agents in different research study (Samanta, S.K. et al., 2022).

Murraya koenigii leaves, commonly known as “curry patta” have being extensively used in many Indian cuisine for its signature aroma. In “Indian Ayurveda” (mentioned as “Krishnanimba”), it has been reported for its profound bioactivity against many health disorders

including vomiting, skin ailment, diabetics, etc. Natural product researchers have found many phytochemicals including phenolics, polyphenolics, alkaloids, etc. from the different parts of curry tree (Samanta, S.K. et al., 2018).

Murraya koenigii and Its Bioactive Compounds:

Murraya koenigii, commonly known as curry leaves, is a tropical to subtropical plant native to India and a member of the Rutaceae family. Renowned for its aromatic leaves that add a unique flavor to dishes, curry leaves hold a dual role in both culinary and medicinal domains. With small, shiny, and highly aromatic leaves containing bioactive compounds, the plant finds significance in traditional medicine systems like Ayurveda. This synergy between kitchen and pharmacy highlights the holistic health approach embedded in traditional practices, where savoring the flavors of curry leaves also entails partaking in their medicinal benefits. The therapeutic potential of *Murraya koenigii* is ascribed to its rich phytochemical composition, with alkaloids, flavonoids, tannins, and essential oils being identified as key constituents in the leaves. A diverse array of health benefits is provided through the synergistic action of these compounds, rendering curry leaves a valuable resource in herbal medicine. Details regarding the reported phytoconstituents have been outlined in our published review article (Samanta, S.K. et al., 2018). Among them, the major bioactive phytochemicals, such as Mahanine, mahanimbine, iso-mahanimbine, koenimbine, girinimbine, koenigine, koenoline, isolongifolene, and O-methylmurrayamine, have been documented (Samanta, S.K. et al., 2018). Additionally, several biological activities are attributed to the synergistic activity of the whole extract of *Murraya koenigii* leaves.

Isolation and Characterization of Mahanine:

Mahanine was isolated by washing the fresh leaves of *Murraya koengii* with distilled water and blending them with methanol using a Mixer grinder. Maceration was achieved by soaking in methanol for 96 hours, and the resulting mixture was later filtered to collect the crude methanolic extract. A second maceration was carried out for 96 hours using of methanol, and a second

filtration was performed to collect the crude extract. The methanol extract was concentrated to dryness using a Rotary evaporator at 50°C and freeze-dried at 100 mbar to yield a residue, which was then suspended in water and successively extracted with ethyl acetate and n-butanol. Each fraction obtained was evaporated to dryness in a rotary evaporator. The crude ethyl acetate fraction was chromatographed over a packed column using Silica gel (70-230) and Petroleum ether. The column was initially run with Petroleum ether, followed by gradient elution using petroleum ether and chloroform (100:0 to 5:95), respectively. The resulting bioassay-guided fraction was subjected to repeat chromatography on silica gel [solvent: petrol–chloroform (9:1)] three times. Almost purified fractions were then taken for High-Performance Thin-Layer Chromatographic (HPTLC) separation; spots similar to reference spots were scraped along with silica gel and run in a semi-preparative HPLC column using MeOH:$CHCl_3$ (90:10) to obtain the most active pure compounds. The isolated compound was characterized through spectral data analysis (1D and 2D NMR and Mass spectrometry) and confirmed as 100% pure Mahanine (Samanta et al., 2013; Das et al., 2019).

Mechanistic Action of Mahanine against Cancer:

In accordance with an earlier study, the key structural reasons for the induction of cytotoxicity against various subtypes of cancer locations by Mahanine are attributed to the presence of C7–OH and –NH functional groups and its binding to DNA-minor grooves (Samanta et al., 2013). The growth inhibition of different cancer cells by Mahanine primarily occurs through the blocking of the STAT-3, RASSF1A, and AKT/mTOR pathways, inactivating the Hsp90-cdc37 complex and mitochondrial complex-III, and generating reactive oxygen species (ROS) (Sarkar et al., 2013; Das et al., 2013; Das et al., 2014; Bhattacharya et al., 2014; Samanta et al., 2018). Additionally, MAHANINE activates the caspase cascade, causes PARP cleavage, positively regulates the tumor suppressor gene p53, and induces the formation of the Fas-FasL-FADD-caspase-8 heterotetramer (Bhattacharya et al., 2010).

A MAHANINE-enriched fraction (~40%) was created for cancer treatment by Satyavarapu et al. (2020), who also conducted tests for acute and chronic toxicity, temperature/pH stability, and pharmacokinetics. The fraction was demonstrated to be utilizable as medicine in all respects (Satyavarapu et al., 2020).

Mechanistic Action of Mahanine against Breast Cancer:

Anti-proliferative efficacy of Mahanine against two distinct subtypes of breast cancer (BC) was previously demonstrated. Treatment with Mahanine in MNU-induced mammary-bearing rats resulted in a significant reduction in mammary tumor burden in a curative manner. The effect of Mahanine on tumor reduction and inhibition of BC cells was associated with G0/G1 cell cycle arrest, upregulation of cyclin-dependent kinase inhibitor-1 (p21Cip1) and 1 B (p27Kip1) proteins, and reduction of the self-renewal of BC stem-like cells (Das et al., 2019). However, the exact molecular mechanism of Mahanine-mediated cytotoxicity in BC cell proliferation and its efficacy remains unknown.

Furthermore, dose-dependent cell viability against drug-sensitive (MCF-7 and MDA-MB-231) and paclitaxel-resistant (MCF-7TR and MDA-MB-231TR) BC cells was demonstrated by Mahanine. Mahanine also exhibited synergistic activity with tamoxifen (TAM) against estrogen receptor-positive (ER+) BC cells by inhibiting ERα expression in MCF-7 cells and N-Methyl-N-nitrosourea (MNU)-induced mammary tumors in a dose-dependent manner, while having no effect on vinculin expression (Samanta et al., 2024).

Future Research and Implications:

This preliminary research underscores the potential of Mahanine from curry patta as a chemopreventative option for breast cancer. The government's role in providing proper nutrients and awareness is crucial. Further validation through human trials can solidify the acceptance of curry patta or its isolated compound Mahanine as a practical and effective chemopreventative option, offering hope for breast cancer prevention in developing regions. Ongoing research aims to identify Mahanine's common targets for different breast cancer subsets, aiding clinicians in developing treatment strategies. The study suggests the use of authenticated curry patta, especially from southern India, as a chemopreventative option against breast cancer.

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