Journal of Experimental Biology and Agricultural Sciences, February - 2025; Volume – 13(1) page 97 – 107

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Carbazole alkaloids Koenigicine, Koenigine, Mahanine and Mukonicineas Multi-Target Inhibitors in Triple-Negative Breast Cancer: Insights into MMP9, MMP13, EGFR, and NUDT5 Interactions through Molecular Docking

Anupam Sharma[1], Narinder Kumar[1], Seema Ramniwas[2], Ritu Chauhan[3], Moyad Shahwan[4,5], Ammar Abdulrahman Jairoun[6,7], Hardeep Singh Tuli[8], Anil Kumar Sharma[9*]

[1]Department of Physics, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, 151302, India
[2]Marwadi University Research Centre, Faculty of Sciences, Marwadi University, Rajkot 360003, Gujarat, India
[3]Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun 248002, Uttarakhand, India
[4]Department of Clinical Sciences, College of Pharmacy and Health Sciences, Ajman University, Ajman 346, United Arab Emirates
[5]Centre of Medical and Bio-Allied Health Sciences Research, Ajman University, Ajman 346, United Arab Emirates
[6]Health and Safety Department, Dubai Municipality, Dubai 67, Dubai, United Arab Emirates
[7]Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM), Pulau Pinang, 11500, Malaysia
[8]Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to Be University), Mullana, Ambala, 133207, India
[9]Department of Biotechnology, Amity School of Biological Sciences, Amity University Punjab, Mohali, Punjab, 140306, India

Received – November 24, 2024; Revision – February 18, 2025; Accepted – February 20, 2025
Available Online – March 05, 2025

DOI: http://dx.doi.org/10.18006/2025.13(1).97.107

KEYWORDS

Carbazolealkaloids

Triple-negative breast cancer (TNBC)

Molecular docking

Multi-target inhibitors

MMP9

MMP13

ABSTRACT

Plant-based natural products have been widely used for treating and preventing diseases due to their nutritional and pharmacological benefits, significantly improving the health and well-being of individuals. These medicinal plants are also easily accessible and offer a low-cost, less harmful source for developing new medications. Breast cancer is the second most common form of cancer reported in women worldwide. The treatment of triple-negative breast cancer (TNBC) remains challenging, as this subtype lacks targeted therapeutics. TNBC accounts for approximately 15-20% of newly diagnosed breast cancer cases. Because TNBC tumors do not express estrogen receptors (ER), progesterone receptors (PR), or human epidermal growth factor receptor 2 (HER2), patients with TNBC do not benefit significantly from treatments aimed at ER, PR and HER2-positive breast tumors. While TNBC initially responds well to chemotherapy, it often develops resistance over time, complicating disease management and presenting a significant clinical challenge. To address therapy resistance and improve patient outcomes, exploring new therapeutic options for TNBC is essential. This molecular docking study

* Corresponding author
E-mail: anibiotech18@gmail.com (Anil Kumar Sharma)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

EGFR

NUDT5

shows strong interactions between the carbazole alkaloids Koenigicine, Koenigine, Mahanine, and Mukonicine with key oncogenic protein targets such as MMP9, MMP13, NUDT5, and EGFR, which are associated with TNBC progression. The binding energy of these molecules ranges from -7.4 to -9.9 kcal/mol, indicating a very high potential for inhibition. Mahanine exhibits the highest binding affinity for all tested targets, demonstrating strong interactions with NUDT5 (-9.8 kcal/mol) and EGFR (-9.9 kcal/mol). This suggests its potential role as a multi-target inhibitor. The primary non-covalent interactions that contribute to the binding of carbazole alkaloids with target proteins include Van der Waals forces, hydrogen bonds, alkyl interactions, π-alkyl interactions, and π-π stacking. These interactions are crucial for stabilizing the ligand-protein complexes, enhancing binding affinity, and likely influencing the inhibitory effects of the compounds on TNBC-associated oncogenic proteins. The results of this study highlight the potential role of carbazole alkaloids in TNBC treatment, warranting further experimental validation.

1 Introduction

In the modern era, researchers and the general public are increasingly interested in plant-derived natural products for their roles in disease prevention, treatment, and overall health improvement. This surge in interest is mainly due to their nutritional value and significant pharmacological properties. Medicinal plants are widely accessible and provide a low-cost, less harmful alternative for new medication development (Atanasov et al. 2015; Yuan et al. 2016). With a long history dating back to ancient civilizations, plant-based traditional medicine has been a cornerstone of healthcare in many Asian societies. Nearly all traditional medicines rely on plant materials to formulate and synthesize various medications. Current medical practices are profoundly influenced by the rapid development of pharmacologically active drugs derived from medicinal herbs (Thomford et al. 2018). For example, natural ingredients or their analogs account for approximately 60% of all medications currently used to treat cancer (Newman and Cragg 2020). Due to their remarkable pharmacological properties, the use of plants in medicine has grown exponentially in recent years.

The Rutaceae family, which includes 1,600 species and 150 genera, features *Murraya koenigii* (commonly known as curry leaf) (Gaikwad et al. 2025). Research indicates that this plant is native to South Asia, particularly in Bangladesh, India, and Sri Lanka (Gaikwad et al. 2025). *M. koenigii* has been utilized between the first and fourth centuries AD and is traditionally considered stomachic and tonic (Ajay et al. 2011). The leaves, roots, bark, fruits, and seeds of *M. koenigii* contain various natural compounds identified through chromatography and spectroscopy techniques. Phytochemical analyses have isolated bioactive compounds such as terpenoids, alkaloids, flavonoids, coumarins, polyphenols, and essential oils (Balakrishnan et al. 2020).

Experimental studies on animals have shown that *M. koenigii* has hypolipidemic effects, resulting in significant reductions in triglyceride levels when included in their diet. The hypolipidemic properties of *M. koenigii* can be attributed to its phytochemicals, including carbazole alkaloids, terpenoids, flavonoids, saponins, and phenols (Pinto and Cojandaraj 2024). Additionally, *M. koenigii* exhibits hepatoprotective, antioxidant, anti-inflammatory, antimicrobial, nephroprotective, gastroprotective, cardioprotective, neuroprotective, wound-healing, Anticancer, and immunomodulatory properties, which can aid in recovery from liver damage (Gangawat et al. 2024).

Sadwal et al. (2023) demonstrated a significant protective effect against breast cancer induced by DMBA (dimethylbenz[α]anthracene) in rats. The hydroethanolic extract of *M. koenigii* (curry leaves) significantly reduced tumor incidence and volume compared to the group treated with DMBA alone. This extract could be a valuable ingredient in animal feed and medicinal applications. The presence of various bioactive compounds suggests that curry leaves may enhance health and nutrition. Notably, they contain 23.73% alkaloids, 1.24% flavonoids, 8.74% saponins, 4.4% phenolics, and 5.2% tannins (Arif et al. 2024). Alkaloids in plants play a crucial role in defending against biotic and abiotic stressors (Arif et al. 2024). Various parts of the *M. koenigii* plant, including the bark, roots, and leaves, are rich in beneficial compounds, particularly carbazole alkaloids, contributing to its medicinal properties (Patil et al. 2023). Additionally, *M. koenigii* extract is a promising new ingredient for cosmetic formulations to enhance skin elasticity and reduce sagging. Its ability to boost the production of key proteins involved in skin structure positions it as a valuable addition to anti-aging products (Lorion et al. 2023). Research has also found that this extract supports the recovery of biochemical markers and enzymes involved in metabolism, indicating its potential to improve liver function and overall health (Gangawat et al. 2024).

Breast cancer (BC) is the most commonly diagnosed cancer in women worldwide. Molecular subtypes of breast cancer are classified based on varying gene expressions, which influence their prognosis and available treatments. Triple-negative breast cancer (TNBC) is the only BC subtype that currently lacks targeted

therapies, accounting for 15-20% of new breast cancer cases (Zagami and Carey 2022). The standard treatment for TNBC is chemotherapy because the tumors do not express the estrogen receptor (ER), progesterone receptor (PR), or HER2. Although TNBC often shows a good initial response to conventional chemotherapy treatments, it frequently develops resistance to these medications, leading to a clinically challenging situation. Unfortunately, targeted therapies aimed at ER and HER2 are ineffective for these patients (Cheang et al. 2008; Leidy et al. 2014). Furthermore, TNBC has the lowest 5-year survival rate among breast cancer subtypes, underscoring the need for novel treatment options.

Chemotherapy resistance is a significant contributor to breast cancer mortality; despite initial positive responses, 50-80% of women with TNBC may relapse or develop resistance to chemotherapeutic drugs. The lack of targeted treatments and the emergence of chemotherapy resistance are two key factors contributing to higher mortality rates among TNBC patients. Chemoresistance can develop through various mechanisms, including complex interactions between the tumor microenvironment, drug efflux, cancer stem cells, and bulk tumor cells. Specific proteins such as ABC transporters can hinder the effectiveness of chemotherapy drugs by actively transporting the drugs out of cancer cells (Nedeljković and Damjanović 2019). Furthermore, chemotherapy can cause damage that DNA repair pathways can rectify, allowing cancer cells to survive (Bai et al. 2021). The epithelial-mesenchymal transition (EMT) may further enhance cancer cell resistance by enabling them to adopt a more invasive and migratory phenotype (Bai et al. 2021).

There is an urgent need to identify and characterize additional molecular mechanisms and downstream pathways crucial for the development of triple-negative breast cancer (TNBC), chemotherapy resistance, and recurrence. Chemotherapy not only destroys cancer cells but also harms healthy normal cells. Therefore, anticancer medications derived from plants with therapeutic qualities could be better alternatives to current treatment options, as they typically cause fewer side effects (Suvarna et al. 2024). Recently, there has been a growing awareness of the adverse effects of synthetic pharmaceuticals, leading more people to favor natural treatments or those made from plants or alkaloids. Pradhan et al. (2024) demonstrated through *in-silico* docking analysis that flavonoid molecules, particularly rutin, show significant binding energy with butyrylcholinesterase (BChE). This finding supports the potential therapeutic applications of the phytochemical constituents of *M. koenigii* for neurodegenerative diseases (Pradhan et al. 2024). An *in-silico* docking study by Samanta et al. (2024) established a high binding potential between important phytochemical constituents of *M. koenigii*, such as flavonoids and carbazole alkaloids, and caspase-3. Mahanine, a carbazole alkaloid from *M. koenigii*, has shown anticancer activity against most subtypes of breast cancer, likely due to its ability to alter cell cycle genes, particularly CDK4 and CDK6 (Sadwal et al. 2023). These results emphasize the significant potential of carbazole alkaloids to bind with proteins, further validating their biological functions and pharmaceutical applications. The current study aims to provide a viable solution based on the structural, biological, and pharmacological significance of the carbazole derivatives of *M. koenigii*. In this research, we selected four protein targets associated with TNBC: Matrix Metalloproteinase-9 (MMP9), Matrix Metalloproteinase-13 (MMP13), Nudix Hydrolase 5 (NUDT5), and Epidermal Growth Factor Receptor (EGFR). The carbazole alkaloids Koenigicine, Koenigine, Mahanine, and Mukonicine were chosen for docking studies to assess their binding affinity.

2 Computational Methods

In the current study, carbazole alkaloids Koenigicine, Koenigine, Mahanine, and Mukonicinewere taken from PubChem's biological database (NCBI 2024). IR spectra of the titled compounds were computed by using the online computational chemistry server WebMO (Version 24) and Mopac engine on webmo.net (https://www.webmo.net/demoserver/cgi-bin/webmo/jobmgr.cgi)(Polik and Schmidt 2022). Molecular docking was conducted using Mcule.com (Mcule 2024), an online drug discovery platform for molecular design and drug discovery (https://mcule.com/apps/1-click-docking/). The ligand-protein interactions were extracted and visualized using the Discovery Studio 4.5 software (Dassault Systèmes BIOVIA 2016).

3 Results and Discussion

3.1 IR Spectra Analysis

IR spectroscopy is a powerful bioanalytical method that can provide qualitative and quantitative information from various biological samples (Beć et al. 2020). The two most common types of vibrations observed in IR spectra are stretching vibrations, which occur between approximately 4000 and 1000 cm^{-1}, and bending vibrations, which range from around 1500 to 400 cm^{-1}. These vibrations are primarily examined in mid-infrared spectroscopy and are crucial in understanding molecular interactions (Ozaki 2021). For instance, in the case of aromatic rings, C=C stretching vibrations are detected in the range of 1400 to 1600 cm^{-1}. C−H stretching absorption occurs between 3100 and 3000 cm^{-1} for alkenes and aromatic compounds. In alkanes, the C−H stretching is observed around 3000 to 2850 cm^{-1}. Additionally, O−H and N−H stretching vibrations typically appear in the range of 3200 to 3600 cm^{-1} (Wang et al. 2023).

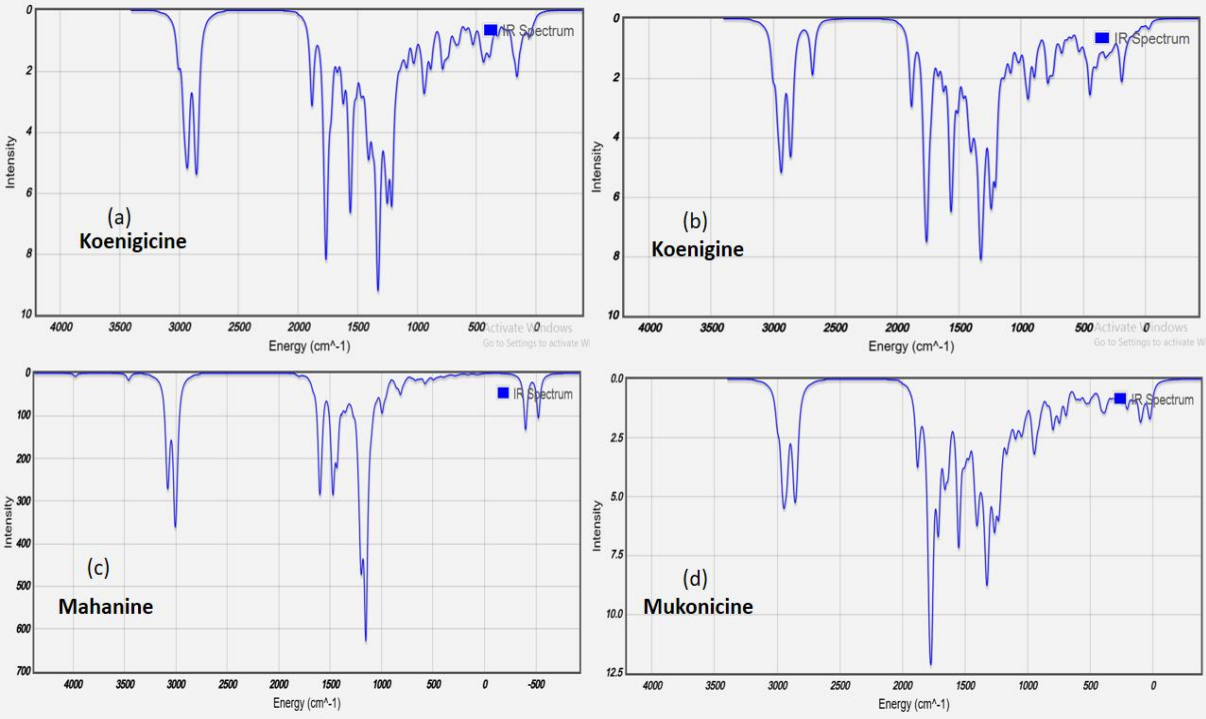


Figure 1 IR (infrared) spectra of the compounds Koenigicine, Koenigine, Mahanine, and Mukonicine. Each plot depicts the IR absorption intensities (y-axis) versus the energy (or wavenumber) in cm^{-1} (x-axis).

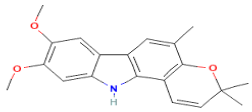
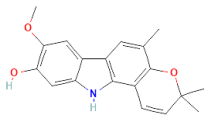
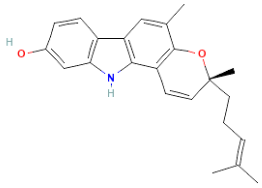
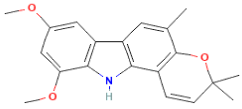
The computed IR spectra provide valuable information about the structural features of each compound, which in turn informs us about their interactions with the MMP9, MMP13, NUDT5, and EGFR proteins related to triple-negative breast cancer (TNBC). The IR spectra illustrated in Figure 1 confirm the presence of functional groups in each compound, suggesting potential binding interactions. The stretching frequencies of C-H, N-H, and C=C bonds observed in the IR spectra are crucial for determining the possibilities of hydrogen bonding, π-π interactions, and van der Waals forces within the molecules (Ghosh et al. 2020; Hansen et al. 2021). All the compounds studied exhibit C-H stretching in the 3000–2800 cm^{-1} region, indicating the presence of aliphatic or aromatic hydrogens. These hydrogens are likely to bind noncovalently within the hydrophobic binding pocket of the proteins. The IR spectra provide clear evidence of C-H, N-H, and C=C stretching, indicating that these molecules can form hydrogen bonds with carbonyl groups or other hydrogen bond acceptors present in the protein framework, as well as engage in π-π stacking interactions with aromatic residues.

3.2 Molecular Docking - Binding Energy

Molecular docking was performed to elucidate the type of interaction and the strength of the binding energy between the identified compounds and the target proteins MMP9, MMP13, NUDT5, and EGFR. The binding energy values for the carbazole alkaloids Koenigicine, Koenigine, Mahanine, and Mukonicine with the proteins MMP9, MMP13, NUDT5, and EGFR are presented in Table 1. These values reflect the strength of the interactions, with more negative values indicating stronger binding affinities. Substantial evidence shows that matrix metalloproteinases (MMPs), a family of secreted, zinc-dependent endopeptidases, play a crucial role at various stages of malignant tumor growth. MMPs can degrade extracellular matrix components, contributing to cancer cell survival (Gonzalez-Avila et al. 2019). Specifically, MMP9 and MMP13 break these matrix components essential for cancer cell invasion and metastasis. Inhibiting MMP9 and MMP13 may reduce triple-negative breast cancer (TNBC) cell dissemination (Mandel et al. 2018). The NUDT5 protein is linked to regulating cellular energy metabolism and stress responses; its inhibition could disrupt the pathways required for rapid cell division, thereby affecting cancer cell viability. Additionally, EGFR is significantly involved in cell signaling and is often overexpressed in TNBC, contributing to aggressive tumor growth. Targeting EGFR could interfere with the proliferation and survival of TNBC cells (Lejeune et al. 2023).

Koenigicine ($C_{20}H_{21}NO_3$) exhibits the highest binding affinity with NUDT5 at -9.2 kcal/mol, followed closely by MMP9 at -9.0 kcal/mol and EGFR at -9.1 kcal/mol. Its interaction with MMP13 is weaker at -7.9 kcal/mol. These results suggest that Koenigicine can form stable complexes with several targets, particularly MMP9, EGFR, and NUDT5. The binding affinity of Koenigicine to EGFR indicates its potential as an anticancer agent. Its affinity

Table 1 Carbazole alkaloids Koenigicine, Koenigine, Mahanine and Mukonicine and their Binding Energy with Protein targets MMP9, MMP13, NUDT5 and EGFR

S. No.	Name of ligand	Molecular formula	2-D Structures	Target Proteins and Binding Affinity (kcal/mol)		Hydrogen bonds Interacting residues
1	Koenigicine	$C_{20}H_{21}NO_3$		MMP9 MMP13 NUDT5 EGFR	-9.0 -7.9 -9.2 -9.1	TYR B132 HIS A148 ARG A38 ASP A146
2	Koenigine	$C_{19}H_{19}NO_3$		MMP9 MMP13 NUDT5 EGFR	-8.5 -9.2 -8.8 -9.2	TYR B132 LEU B130 GLY A134 THR A 142 ARG A38 ASP A146
3	Mahanine	$C_{23}H_{25}NO_2$		MMP9 MMP13 NUDT5 EGFR	-9.2 -9.4 -9.8 -9.9	TYR B132 HIS A148 GLN B2 PHE A154
4	Mukonicine	$C_{20}H_{21}NO_3$		MMP9 MMP13 NUDT5 EGFR	-7.4 -8.3 -8.8 -8.6	GLN B114 GLU B34 LYS A40

for MMP13 and EGFR suggests a multifaceted therapeutic effect, impacting both matrix remodeling and cell proliferation, offering potential implications for cancer treatment (Szczygielski et al. 2024). This indicates that Koenigicine may serve as a multi-target agent, affecting proteins involved in signaling, metabolism, and cancer pathways. Follow-up experiments conducted in vitro and in vivo are needed to support these findings further and clarify the specific mechanisms by which Koenigicine affects these targets (Endo et al. 2018; Almutairi et al. 2023).

In the case of Koenigine ($C_{19}H_{19}NO_3$), a binding affinity of -8.5 kcal/mol was observed for MMP9 and -9.2 kcal/mol for MMP13, indicating a strong interaction with these matrix metalloproteinases, which are key players in extracellular matrix degradation and are associated with increased invasion and metastasis. Notably, the strong binding affinity to MMP13 suggests that Koenigine could inhibit tumor cell migration and invasive pathways, which represent critical mechanisms in the pathobiology of TNBC.

Given the role of NUDT5 in energy homeostasis within the cancer microenvironment, it is noteworthy that Koenigine binds to NUDT5 with an affinity of -8.8 kcal/mol. In triple-negative breast cancer (TNBC), NUDT5 plays a crucial role in preventing oxidative DNA damage, and its inhibition can suppress cancer cell growth (Qian et al. 2024). The interaction between Koenigine and NUDT5 may disrupt these adaptive processes, subsequently inhibiting TNBC progression. Additionally, EGFR has been linked to poor prognosis and resistance to therapy (Zang et al. 2024). The binding affinity of Koenigine to EGFR is -9.2 kcal/mol, indicating that it may act as a potent EGFR inhibitor. Inhibiting EGFR could help shut down proliferative and survival pathways in TNBC cells, making this a rational strategy for limiting their growth and proliferation.

The binding affinity results demonstrate Koenigine's significant interaction with four key protein targets: MMP9, MMP13, NUDT5, and EGFR, all contributing to tumor growth, metastasis, and survival mechanisms in TNBC. By inhibiting MMP9 and MMP13 while downregulating NUDT5 and EGFR, Koenigine may disrupt critical cellular pathways involved in TNBC progression. Its multi-target potential makes it an attractive candidate for drug development, whether alone or combined with other agents targeting various pathways in tumorigenesis.

The present study also examined the molecular interactions of Mahanine ($C_{23}H_{25}NO_2$) with the major pharmaceutical targets MMP9, MMP13, NUDT5, and EGFR. Mahanine's binding

affinities with these targets were measured at -9.2 kcal/mol (MMP9), -9.4 kcal/mol (MMP13), -9.8 kcal/mol (NUDT5), and -9.9 kcal/mol (EGFR). These results indicate a strong interaction between Mahanine and the four targets, essential for its therapeutic effect. In the context of TNBC, MMPs like MMP9 and MMP13 are significantly involved in tumor invasion and metastasis. Mahanine's strong affinity for MMP13 and MMP9 suggests a potential reduction in extracellular matrix degradation, supporting tumor cell migration and positioning Mahanine as an effective anti-metastatic agent. The binding affinity with EGFR indicates that Mahanine may influence pathways contributing to tumor proliferation and survival, particularly in remodeling EGFR-positive TNBC cases (Zhang et al. 2024). Furthermore, NUDT5 correlates with NAD+-related signaling in cancer cell proliferation and survival (Dubey et al. 2024). The strong affinity of Mahanine for NUDT5 could help explain its ability to inhibit metabolic processes necessary for the survival of TNBC cells. This study suggests that Mahanine could be a potential multi-target agent suppressing tumor cell growth and survival in TNBC. Further investigations, including in vitro and in vivo studies, must validate these computational findings and determine Mahanine's effectiveness and the mechanisms involved in TNBC treatment (Dubey et al. 2024).

Additionally, Mukonicine ($C_{20}H_{21}NO_3$) has shown binding affinities of -7.4 kcal/mol and -8.3 kcal/mol for MMP9 and MMP13, respectively. Mukonicine is expected to significantly prevent invasion and metastasis in TNBC cells, as demonstrated by its strong interactions with MMP9 and MMP13. Its affinity for NUDT5 at -8.8 kcal/mol suggests a potent interaction with this target, which could impede regulatory pathways and impact cellular proliferation. Mukonicine also exhibits a binding affinity of -8.6 kcal/mol towards EGFR, indicating a significant inhibitory effect on cell proliferation (Kar et al. 2024).

3.3 Molecular Docking-Interactions Maps

We have examined the 2-D interaction maps of the targeted compounds with specific proteins: MMP9, MMP13, NUDT5, and EGFR. Figure 2 illustrates the molecular interactions of Koenigicine with these protein targets: MMP9 (PDB ID: 2ow2), MMP13 (PDB ID: 1xuc), EGFR (PDB ID: 1xkk), and NUDT5 (PDB ID: 2dsc). The interaction diagrams highlight critical binding characteristics and the relationships between Koenigicine and the active sites of these targeted proteins. They highlight meaningful non-covalent interactions, including van der Waals forces, hydrogen bonds, and π-π stacking interactions. These findings

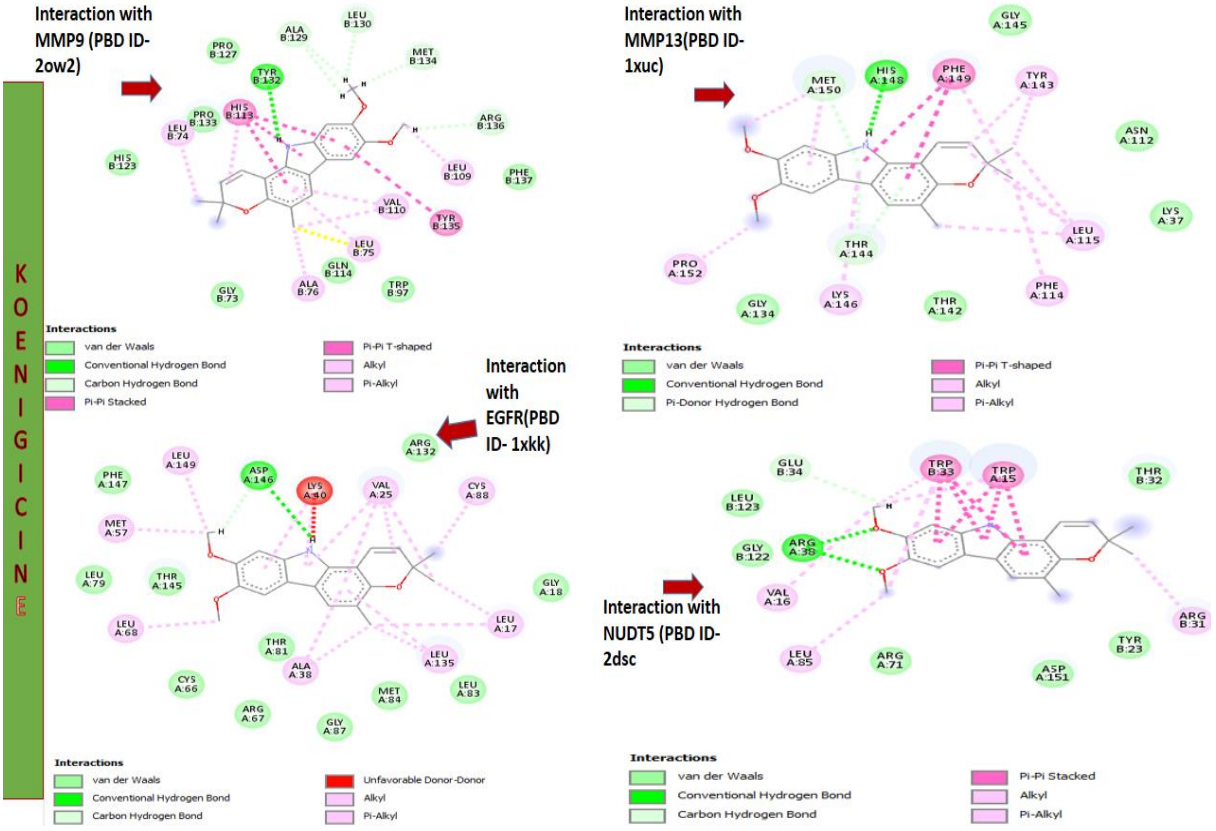


Figure 2 Koenigicine's interaction with MMP9(PBD ID- 2ow2), MMP13(PBD ID- 1xuc),EGFR(PBD ID- 1xkk), NUDT5 (PBD ID- 2dsc ADP-sugar pyrophosphatase).

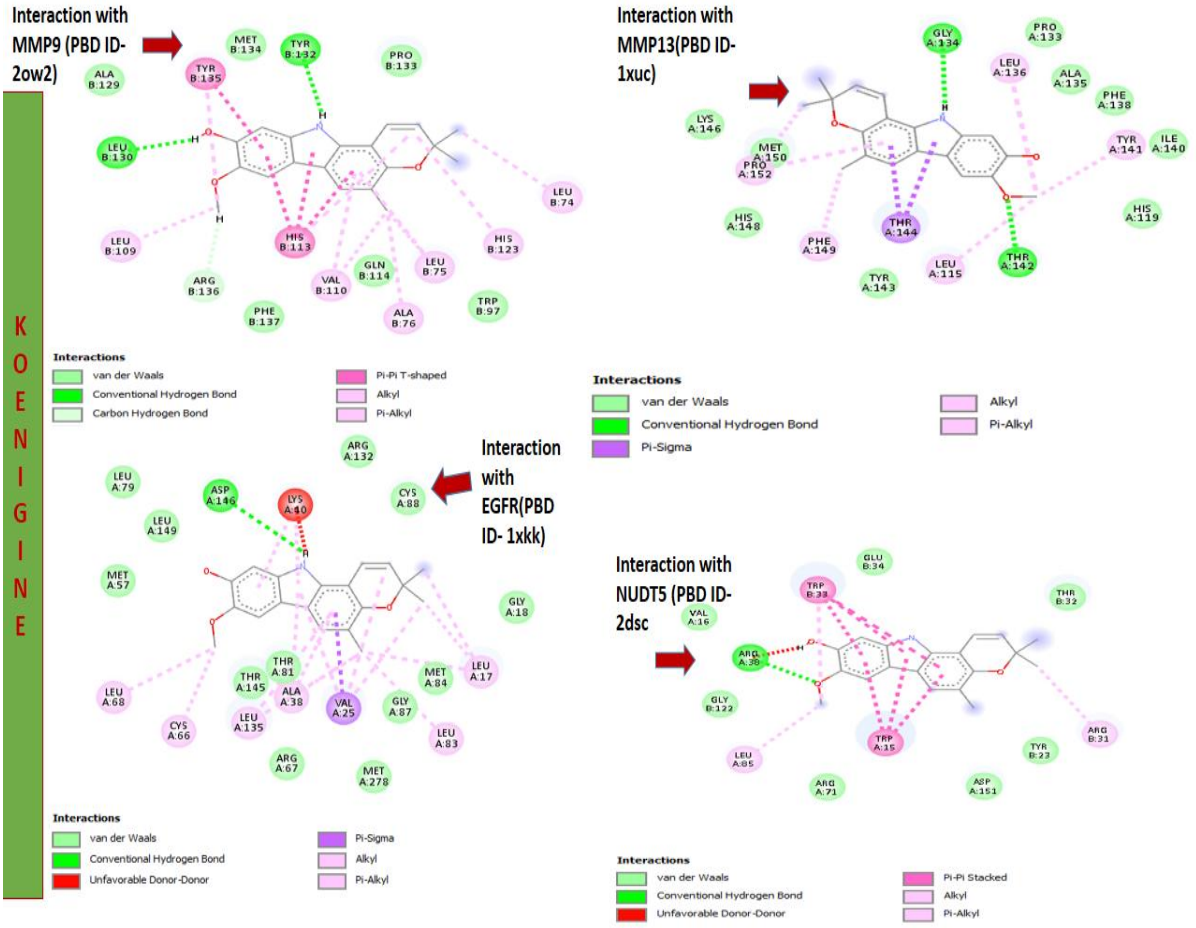


Figure 3 Koenigine interaction with MMP9(PBD ID- 2ow2),MMP13(PBD ID- 1xuc),EGFR(PBD ID- 1xkk),NUDT5 (PBD ID- 2dsc ADP-sugar pyrophosphatase).

demonstrate the binding modes of Koenigicine with the protein targets and suggest that it could serve as a potential inhibitor in therapeutic applications related to triple-negative breast cancer (TNBC).

Additionally, Figure 3 illustrates the molecular docking interactions of Koenigine with four target proteins: MMP9 (PDB ID: 2ow2), MMP13 (PDB ID: 1xuc), EGFR (PDB ID: 1xkk), and NUDT5 (PDB ID: 2dsc). The primary non-covalent binding interactions between Koenigine and the active sites of these proteins include van der Waals forces, hydrogen bonds, and π-π stacking, all depicted in the interaction maps. Together, these docking interactions highlight the binding profile of Koenigine across various targets associated with cancer pathogenesis, supporting its potential as a multi-target inhibitor in treatment strategies for triple-negative breast cancer (TNBC).

Figure 4 illustrates Mahanine's interactions with targeted proteins: MMP9 (PDB ID - 2ow2), MMP13 (PDB ID - 1xuc), EGFR (PDB ID - 1xkk), and NUDT5 (PDB ID - 2dsc, ADP-sugar pyrophosphatase). These interactions occur through van der Waals forces, hydrogen bonding, and π-based interactions. This diversity suggests that Mahanine has broad-spectrum inhibition or modulation potential, particularly against proteins linked to cancer progression and degenerative diseases. Understanding these interactions with specific residues may reveal key insights into the therapeutic potential of Mahanine as a promising agent.

Mukonicine interacts with MMP9, MMP13, EGFR, and NUDT5, as illustrated in Figure 5. This study highlights Mukonicine's effectiveness as a multi-target agent against triple-negative breast cancer (TNBC). The interactions occur through van der Waals forces, hydrogen bonds, and various π-based interactions, creating a stable binding configuration with each protein. The involvement of specific amino acid residues suggests that Mukonicine could modulate the activities of these proteins, potentially affecting cancer cell invasion, migration, proliferation, and survival. This analysis provides insight into the treatment efficacy of Mukonicine and supports further investigation into its role in targeted therapy for TNBC.

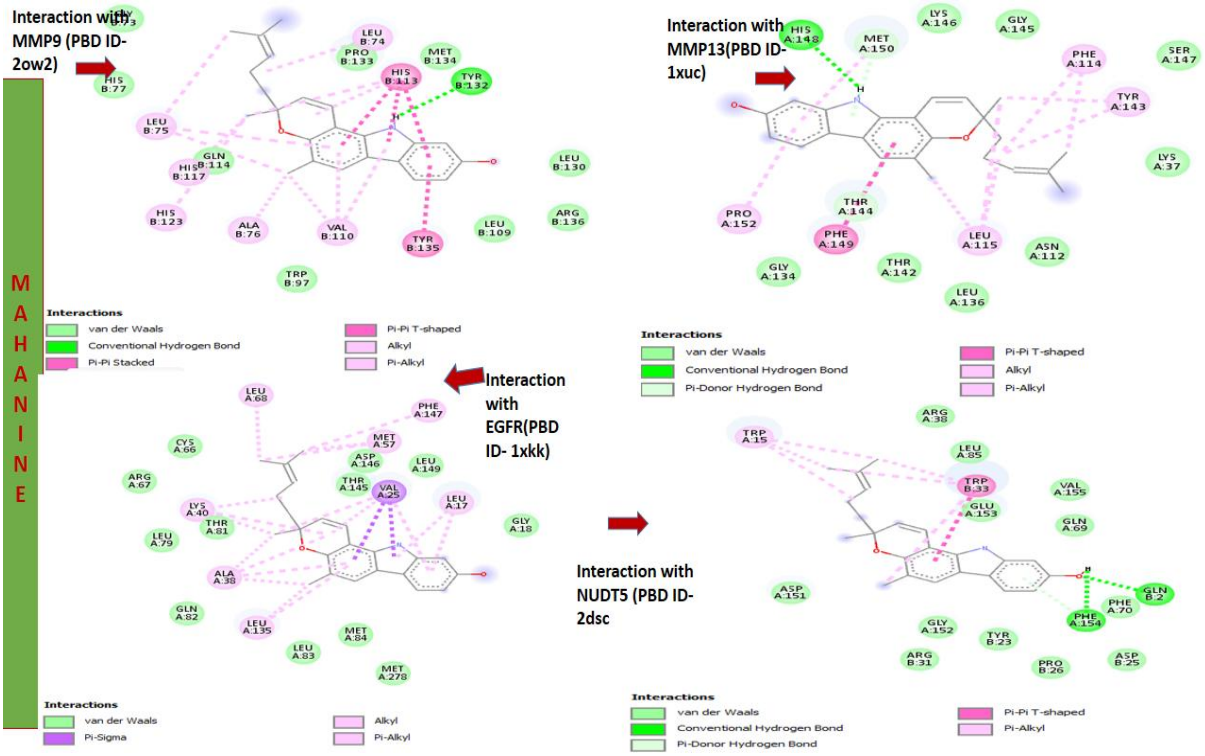


Figure 4 Mahanine interaction with MMP9 (PBD ID- 2ow2),MMP13(PBD ID- 1xuc), EGFR(PBD ID- 1xkk) and NUDT5 (PBD ID- 2dsc ADP-sugar pyrophosphatase).

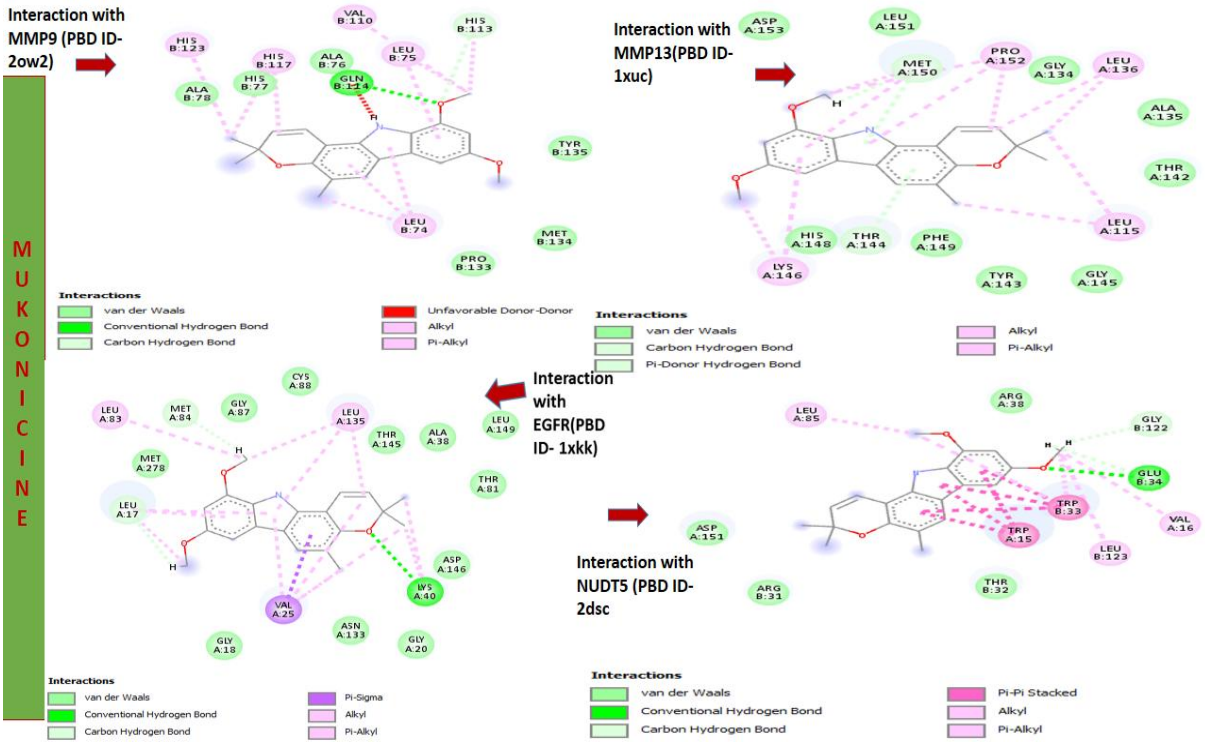


Figure 5 Mukonicine's interaction with MMP9(PBD ID- 2ow2),MMP13(PBD ID- 1xuc),EGFR(PBD ID- 1xkk),NUDT5 (PBD ID- 2dsc ADP-sugar pyrophosphatase).

Conclusion and Future Prospects

The combination of infrared spectral data and molecular docking studies suggests that the carbazole alkaloids Koenigicine, Koenigine, Mahanine, and Mukonicine may serve as effective inhibitors for triple-negative breast cancer (TNBC) due to their interactions with proteins MMP9, MMP13, NUDT5, and EGFR. The structural features of these alkaloids facilitate binding to the respective proteins, which may inhibit cancer progression by slowing down cell proliferation and metastasis. The higher binding affinities of Koenigicine, Koenigine, Mahanine, and Mukonicine indicate that they could act as promising multi-target inhibitors for TNBC treatment. Mahanine is particularly potent against NUDT5 and EGFR, suggesting it may provide the most significant inhibition of TNBC cells and could be considered a lead compound for future investigation. Koenigicine and Koenigine also demonstrate promising multi-target binding, particularly against EGFR and MMPs, which are relevant to TNBC proliferation and metastasis.

Mukonicine, while exhibiting relatively lower binding affinities, is less effective across the targets than the other ligands and may have limited therapeutic potential for TNBC. Therefore, further experimentation is necessary to confirm these inhibitory effects and their potential applications in treatment.

Additionally, these carbazole alkaloids show strong van der Waals forces, hydrogen bonds, and π-π stacking interactions with the mentioned proteins. Optimizing these interactions may enhance selectivity and potency. The interactions identified in this docking study could be crucial for improving binding specificity and affinity in drug development. Consequently, these compounds should be included in experimental studies to validate their obstructive roles and clarify their antitumor efficacy in treating TNBC by targeting cancer proliferation (EGFR and NUDT5) and metastasis (MMP9 and MMP13).

Acknowledgments

Authors acknowledge the Chancellor, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, for providing the requisite platform to complete this article.

Author contributions

AS: Conceptualization, Investigation, data collection, Writing original draft, Figure Preparation, writing review and editing; NK, SR & RC: Analysis, visualization, arranging references; MS, AAJ and HST Figure and Table preparation, reviewing and editing; AKS: Conceptualization, Supervision, Writing-Reviewing, and Editing. All authors read and approved the submitted version.

Funding

No funding was received for the said study.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

Not applicable.

Consent to publish

All the authors consented to publish the manuscript.

Data Availability Statement

All the findings have been disclosed in the manuscript, which is supported by the data. Additional data can be made available upon subsequent request.

References

Ajay, S., Rahul, S., Sumit, G., Paras, M., Mishra, A., & Gaurav, A. (2011). Comprehensive review: *Murraya koenigii* Linn. *Asian Journal of Pharmacy and Life Science*, *1*(4), 417-425.

Almutairi, S., Kalloush, H. M., Manoon, N. A., & Bardaweel, S. K. (2023). Matrix Metalloproteinases Inhibitors in Cancer Treatment: An Updated Review (2013–2023). *Molecules*, *28*(14), 5567. https://doi.org/10.3390/molecules28145567.

Arif, M. A. A., Warsito, S. H., Lamid, M., Lokapirnasari, W. P., Khairullah, A. R., et al. (2024). Phytochemical Analysis of Curry Leaf Extract (*Murraya koenigii* L.) as a Potential Animal Feed and Medicinal Ingredient. *Pharmacognosy Journal*, *16*(2), 471–477. https://doi.org/10.5530/pj.2024.16.75

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E., Linder, T., Wawrosch, C., et al. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, *33*(8), 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Bai, X., Ni, J., Beretov, J., Graham, P., & Li, Y. (2021). Triple-negative breast cancer therapeutic resistance: Where is the Achilles' heel?. *Cancer Letters*, *497*, 100–111. https://doi.org/10.1016/j.canlet.2020.10.016.

Balakrishnan, R., Vijayraja, D., Jo, S. H., Ganesan, P., Su-Kim, I., & Choi, D. K. (2020). Medicinal profile, phytochemistry, and pharmacological activities of *Murraya koenigii* and its primary bioactive compounds. *Antioxidants*, *9*(2), 101. https://doi.org/10.3390/antiox9020568

Beć, K., Grabska, J., & Huck, C. (2020). Biomolecular and bioanalytical applications of infrared spectroscopy - A review. *Analytica Chimica Acta*, *1133*, 150–177. https://doi.org/10.1016/j.aca.2020.04.015.

Cheang, M. C., Voduc, D., Bajdik, C., Leung, S., McKinney, S., Chia, S. K., Perou, C. M., & Nielsen, T. O. (2008). Basal-Like Breast Cancer Defined by Five Biomarkers Has Superior Prognostic Value than Triple-Negative Phenotype. *Clinical Cancer Research*, *14*(5), 1368–1376. https://doi.org/10.1158/1078-0432.ccr-07-1658.

Dassault Systèmes BIOVIA (2016). Discovery Studio Modelling Environment, Release 2017; Dassault Systèmes: San Diego, CA, USA.

Dubey, A., Alanazi, A.M., Bhardwaj, R., & Ragusa, A. (2024). Identification of potential NUDT5 inhibitors from marine bacterial natural compounds via molecular dynamics and free energy landscape analysis. *Molecular Diversity*, *29* (1), 1-15, https://doi.org/10.1007/s11030-024-10950-5.

Endo, H., Owada, S., Inagaki, Y., Shida, Y., & Tatemichi, M. (2018). Glucose starvation induces LKB1-AMPK-mediated MMP-9 expression in cancer cells. *Scientific Reports 8*,10122. https://doi.org/10.1038/s41598-018-28074-w.

Gaikwad, S., Gawande, V., Mhaske, S., & Sharma, T. (2025). A review on the medicinal significance of *Murraya koenigii*. *International Journal of Pharmaceutical Sciences*, *3*(1), 166–174. https://doi.org/10.5281/zenodo.14590932.

Gangawat, R., Parashar, R., & Yadav, R. K. (2024). Hepatoprotective Potential of *Murraya koenigii* (Curry Leaves) against Xenobiotics, Heavy Metals, and Hepatotoxic Agents: A Comprehensive Review. *Current Drug Discovery Technologies*, (in press). https://doi.org/10.2174/0115701638310869240628060001.

Ghosh, S., Ghosh, S., & Wategaonkar, S. (2020). C–H•••Y (Y=N, O, π) Hydrogen Bond: A Unique Unconventional Hydrogen Bond. *Journal of the Indian Institute of Science*, *100*(1), 101–125. https://doi.org/10.1007/S41745-019-00145-5.

Gonzalez-Avila, G., Sommer, B., Mendoza-Posada, D. A., Ramos, C., Garcia-Hernandez, A. A., & Falfan-Valencia, R. (2019). Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Critical reviews in oncology/hematology*, *137*, 57-83.

Hansen, P.E., Vakili, M., Kamounah, F.S., & Spanget-Larsen, J. (2021). NH Stretching Frequencies of Intramolecularly Hydrogen-Bonded Systems: An Experimental and Theoretical Study. *Molecules*, *26*(24), 7651. https://doi.org/10.3390/molecules26247651.

Kar, T., Dugam, P., Shivhare, S. C., Shetty, S. R., Choudhury, S., et al. (2024). Epidermal growth factor receptor inhibition potentiates chemotherapeutics-mediated sensitization of metastatic breast cancer stem cells. *Cancer Reports*, *7*. https://doi.org/10.1002/cnr2.2049.

Leidy, J., Khan, A., & Kandil, D. (2014). Basal-Like Breast Cancer: Update on Clinicopathologic, Immunohistochemical, and Molecular Features. *Archives of Pathology & Laboratory Medicine*, *138*(1), 37–43. https://doi.org/10.5858/arpa.2012-0439-ra.

Lejeune, M., Reverté, L., Gallardo, N., Sauras, E., Bosch, R., et al. (2023). Matrix Metalloproteinase-9 Expression is Associated with the Absence of Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer Patients. *International Journal of Molecular Sciences*, *24*(14), 11297. https://doi.org/10.3390/ijms241411297

Lorion, C., Bardin, V., Bonnet, S., Lopez-Gaydon, A., Vogelgesang, B., & Bechetoille, N. (2023). Elastogenic potential and antisagging properties of a novel *Murraya koenigii* extract. *Journal of Cosmetic Dermatology*, *23*(3), 1036–1044. https://doi.org/10.1111/jocd.16059.

Mandel, A., Larsson, P., Sarwar, M., Semenas, J., Khaja, A. S. S., & Persson, J. L. (2018). The interplay between AR, EGF receptor and MMP-9 signaling pathways in invasive prostate cancer. *Molecular Medicine*, *24*(1) 34. https://doi.org/10.1186/s10020-018-0035-4.

Mcule (2024). Mcule: A Platform for Molecular Design and Drug Discovery. Available at mcule.com.

National Center for Biotechnology Information. (2024). Compound summary for CID -278055 (Koenigicine), 5318825 (Koenigine), 36689305 (Mahanine), 86242003 (Mukonicine). PubChem. Retrieved October 10, 2024, from https://pubchem.ncbi.nlm.nih.gov/compound/.

Nedeljković, M., & Damjanović, A. (2019). Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge. *Cells*, *8*(9), 957. doi: 10.3390/cells8090957.

Newman, D. J., & Cragg, G. M. (2020). Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285.

Ozaki, Y. (2021). Infrared spectroscopy—Mid-infrared, near-infrared, and far-infrared/terahertz spectroscopy. *Analytical Sciences*, *37*(9), 1193–1212. https://doi.org/10.2116/ANALSCI.20R008.

Patil, R., Mandlik, S., & Mandlik, D. (2023). *Murraya koenigii* (Curry Tree): A review of its Phytochemistry, Ethnomedicinal uses, and Pharmacology with Respect to Molecular Mechanisms. *Current Traditional Medicine*, *10*(5), 73-79. https://doi.org/10.2174/2215083810666230609163404.

Pinto, P., & Cojandaraj, L. (2024). Protective Effects of *Murraya koenigii*: Focus on Antihyperlipidemic Property. *Current Drug Therapy*, *20*(1), 24-32. https://doi.org/10.2174/0115748855278592240131105512.

Polik, W.F., & Schmidt, J.R. (2022). WebMO: Web-based computational chemistry calculations in education and research. *WIREs Computational Molecular Science*, *12*, e1554. https://doi.org/10.1002/wcms.1554.

Pradhan, S. P., Subedi, I., Adhikari, K., Gc, A., Pradhan, S. P., Aryal, M. R., Ghimire, G. P., & Pandey, B. P. (2024). In vitro and in silico approach for the evaluation of enzyme inhibitory potential of Kadipatta (*Murraya koenigii*) collected from western Nepal. *Deleted Journal*, *5*(3), 200161. https://doi.org/10.1016/j.ctmp.2024.200161.

Qian, J., Ma, Y., Tahaney, W. M., Moyer, C. L., Lanier, A., et al. (2024). The novel phosphatase NUDT5 is a critical regulator of triple-negative breast cancer growth. *Breast Cancer Research* : *BCR*, *26*(1), 23. https://doi.org/10.1186/s13058-024-01778-w.

Sadwal, S., Bharati, S., Dar, Z. A., & Kaur, S. (2023). Chemopreventive potential of hydroethanolic *Murraya koenigii* leaves extract against DMBA induced breast carcinogenesis: In-silico and in-vivo study. *Journal of Ethnopharmacology*, *319*, 117124. https://doi.org/10.1016/j.jep.2023.117124.

Samanta, S.K., Choudhury, P., Kandimalla, R., Aqil, F., Moholkar, D.N., et al. (2024). Mahanine mediated therapeutic inhibition of estrogen receptor-α and CDK4/6 expression, decipher the chemoprevention-signaling cascade in preclinical model of breast cancer. *Journal of Ethnopharmacology*, *319* (2), 117235. https://doi.org/10.1016/j.jep.2023.117235.

Suvarna, E., Setlur, A. S., Chandrashekar, S., & Niranjan, V. (2024). Computational molecular perspectives on novel carbazole derivative as an anticancer molecule against CDK1 of breast and colorectal cancers via gene expression studies, novel two-way docking strategies, molecular mechanics and dynamics. *Computational Biology and Chemistry*, *108*, 107979. https://doi.org/10.1016/j.compbiolchem.2023.107979.

Szczygielski, O., Dąbrowska, E., Niemyjska, S., Przylipiak, A., & Zajkowska, M. (2024). Targeting Matrix Metalloproteinases and Their Inhibitors in Melanoma. *International Journal of Molecular Sciences*, *25*(24), 13558. https://doi.org/10.3390/ijms252413558.

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. (2018). Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *International Journal of Molecular Sciences*, *19*(6), 1578. https://doi.org/10.3390/ijms19061578.

Wang, T., Tan, Y., Chen, Y., & Tan, C. (2023). Infrared spectral analysis for prediction of functional groups based on feature-aggregated deep learning. *Journal of Chemical Information and Modeling*, *63*(15): 4615-4622. https://doi.org/10.1021/acs.jcim.3c00749.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*, *21*(5), 559. https://doi.org/10.3390/molecules21050559.

Zagami, P., & Carey, L.A. (2022). Triple negative breast cancer: Pitfalls and progress. *NPJ Breast Cancer*, *8*(1), 95. https://doi.org/10.1038/s41523-022-00468-0.

Zhang, H., Qi, Y., Mao, Y., & Zheng, J. (2024). PDZK1 suppresses TNBC development and sensitizes TNBC cells to erlotinib via the EGFR pathway. *Cell Death and Disease*, *15*(3), 199. https://doi.org/10.1038/s41419-024-06502-w.