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Invitro And Insilico Anticancer Activity Of *Murraya Koenigii* (L) Against Breast Cancer

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Abstract: *Phytochemical screening study well showed the presence of alkaloids, steroids, protein, saponins and flavonoids. Murraya koenigii leaves have potent antioxidant. Murraya koenigii leaves possess anticancer activity against MCF- 7 cell lines, like wise Murraya koenigii leaves possess antibacterial agent against Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli. Docking studies were performed for the four proteins with four marker compounds. The interaction of protein and ligands for the docked ligands with least binding energy was calculated. Mahanimbicine has least binding energy with HER2 protein. Further research has to be conducted for components present in the methanolic extract of Murraya koenigii which may act as active principles. In future the components present on Murraya koenigii may act as a drug, for the above mentioned cell line.*

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I. Introduction

Medicinal plants are considered to be source of healthy human life. Herbs is always principal form of medicine in India and presently they are becoming popular throughout the developed world as strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with bodies on defence.

In more than 80% of developed countries depends the medicinal plants as traditional medicine for various diseases. Different parts of the plants like roots, leaves, stem, bark and fruits have been used for many infections.

Many plants have been used for their antimicrobial traits, which are chiefly due to the synthesis of secondary metabolites and their inhibitory effect against the growth of human pathogens. Keeping this in view, efforts are under way to search for economic and safe photochemical for disease control. Despite the existence of potent antibiotic and antifungal agents, resistant microbial strains are continuously appearing, suggesting the need for permanent search and the development of new drugs.

Breast cancer is the most commonly diagnosed cancer and the primary cause of cancer death in females worldwide. In India, breast cancer is the second with a rate of 17.2 per 100,000. The latest statistics indicated that about 1.3 million women are diagnosed with breast cancer annually worldwide and about 465,000 die from the disease (Elangovan *et al*., 2008). Despite the fact that many tumors initially respond to chemotherapy, breast cancer cells can subsequently survive and gain resistance to the treatment. The prevalence of breast cancer in Indian women is more at the age of forty. Some breast tumors stay resistant to conventional treatment and may have many side effects which affect the quality of the treatment.

II. Materials and Methods

Preparation of Plant Extract

Murraya koenigii (L) was collected in an around Trichy district, Tamilnadu. The leaves of the plant *Murraya koenigii* (L) leaves were carefully removed and washed thoroughly 2-3 times with running water and with distilled water to remove dust particles. The leaves were air-dried in a shade under room temperature for seven days and then crushed into coarse powdery substance by using mortar and pestle.100 gram of the powdered leaves were subjected to maceration in methanol (100 g/250 mL). The extracts were stored at 5°C for further experimental study.

The phytochemical screening of the plant extract was carried by the methods of Trease and Evens (1978) and Harborne (1973).

Phytochemical Screening of the Leaves Extract

Qualitative screenings for the presence of various phytochemical compounds were performed using the methanol extract.

Antioxidant activity

In vitro method to determine the free radical scavenging activity frequently involve the in vitro radical scavenging ability of the test antioxidant can be determined by use of chemicals which generate free radicals (Aroma *et al*., 2002).

DPPH radical scavenging activity

The ability of *M. koenigii* extracts to scavenge the DPPH radical was assessed using (Blois et al., 1958) method with modification. Percentage of inhibition (%) = $[(A_1–A_2)/A_1]$ X 100
Where A_1-absorbance of DPPH and A_2 -absorbance of reaction mixture (sample with DPPH).

$ABTS^+$ radical cation decolourisation assay

The methanolic extract of *M. koenigii* leaf was evaluated their $ABTS^+$ radical scavenging by following the method described by (Re *et al*., 1999) with modification. Percentage of inhibition (%) = $[(A_1–A_2)/A_1]$ X 100
Where A_1-absorbance of reaction mixture without sample and A_2 –absorbance of reaction mixture with sample.

Fe^{2+} chelating activity assay

The chelating activity of *M. koenigii* leaf extracts were evaluated by measuring the Fe^{2+} chelating activity according to the method of (Dinis *et al*., 1994) with some modification. The percentage of chelating activity calculated using the formula: % of chelating activity = $[(A_1–A_2)/A_1]$ X 100
Where A_1 -absorbance of the reaction mixture without extract and A_2 -absorbance of the reaction mixture with extract.

Ferrous reducing power

The reducing ability of methanolic extracts of *M. koenigii* leaf was assed according to the method of Oyaizu (1986).

Antimicrobial Activity
Antibacterial Screening
Test organism: *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* were used for antibacterial activity test.

In-Vitro Anti Cancer Activity

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10 % fetal bovine serum (FBS). Maintenance cultures were passage weekly, and the culture medium was changed twice a week. 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt.
The % cell inhibition was determined using the following formula. % Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

III. Docking Studies

Software used
PDB Database, PUBCHEM Database, Autodock tools (ADT), Autodock (ver 4.2.2), Autogrid (ver 4.2.2).

Method

- The proteins were searched in PDB and 3D structure was downloaded as pdb text file.
- The ligand was searched in PUBCHEM.
- The protein is prepared for docking by adding Kollman charge and polar hydrogen bonds and saved as .pdbqt file.
- The ligand is modified for docking and is saved as .pdbqt file.
- Grid parameter file (.gpf) was generated by selecting the ligand and protein and setting the grid box.
- Docking paramerter file was generated by selecting the ligand and protein and Search parameter was chosen as Genetic algorithm and output was saved as Lamarckian GA (.dpf).
- AutoGrid was run and output was generated as Grid Log File (.glg)
- Similarly AutoDock was run and the output was generated as Docking Log File (.dlg)
- The output was analysed and minimum binding energy is noted for the best docked pose.

- This process is repeated for four proteins with four ligands each.

IV. Results & Discussion

Phytochemical screening

Qualitative phytochemical analysis of methanolic extracts of *Murraya koenigii* leaves

Table.1: Qualitative phytochemical analysis of *Murraya koenigii*

S.No:	Phytochemical compound	Presence/Absence
1	Steroids	+
2	Tannins	-
3	Alkaloids	+
4	Saponins	+
5	Quinones	-
6	Amino acids	+
7	Flavonoids	+

ANTIOXIDANT ACTIVITY

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity of the methanolic extract of *M. koenigii* leaves possess ability to scavenge DPPH free radicals as equal to the standard antioxidant L-ascorbic acid. It produced hydrazine by converting the unpaired electrons to paired electron due to the hydrogen donating ability of the extract. The photometric evaluation of free radical scavenging ability of the methanolic extract of *M. koenigii* leaves showed good antioxidant capacity (Figure.1). More reduction of absorbance was observed in the DPPH radical scavenging activity due to the scavenging ability of the extracts. The IC50 value calculated for the leaf extracts and compared to the standard antioxidant L-ascorbic acid (Std) such as 10.15 ± 0.85, > 160, and 07.55 ± 0.45 µg/ml respectively (Table-3). A lower IC 50 value indicates a higher free radical scavenging activity. DPPH is a stable free radical that accepts an electron of hydrogen radical to become stable diamagnetic molecule. It produced hydrazine by converting the unpaired electrons to paired electron due to the hydrogen donating ability of the extract. The reduction capacity of DPPH radicals was determined by the decrease in the absorbance at 517 nm. Hence DPPH is usually used as a substrate to evaluate antioxidative capacity of antioxidants. All analyses are the mean of triplicate measurements ± standard deviation.

DPPH radical scavenging activity involves H atom transfer and ABTS+ radical involve an electron transfer process and reduction were recorded at 517 nm. In ABTS+ blue chromophore produced by the reaction between ABTS+ and potassium persulfate in the presence of the plant extract or L-ascorbic acid, where cation radicals reduced and the remaining cation concentration in the reaction were recorded at 734nm.

Table.2: IC50 values of *M. koenigii* leaf for In vitro antioxidant assays

S.No	Methods	Compounds (m/ml)	
		M. koenigii leaf	Standard (L-ascorbic acid)
1	DPPH radical scavenging activity	10.15 ± 0.85	07.55 ± 0.45
2	ABTS radical scavenging activity	164.65 ± 3.05	102.25 ± 2.35
3	Fe2+ chelating activity	172.45 ± 2.15	97.50 ± 2.15

ABTS+ radical cation decolourisation assay

The ABTS+ radical scavenging ability of *M. koenigii* methanolic extracts of leaves were screened and found to have significant radical scavenging activity and the IC50 value found to be 164.65 ± 3.05, >250 and 102.25 ± 2.35 respectively (Table.2).

Fe2+ chelating activity assay

The IC50 value of methanol extract of *M. koenigii* leaves and L-ascorbic acid found to be 172.45 ± 2.15, >250 and 97.50 ± 2.10 respectively (Table.2). It either was reported that chelating activity of a particular extract is mainly due to the presence of chelated metal ions or suppressed reactivity, by occupying all coordination sites of metal ion, by the antioxidants present in the extract.

Ferrous reducing power assay

The presence of reductant antioxidants in the tested samples would result in the reduction of Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The ferrous ion can therefore be monitored by measuring

the formation of Perl's Prussian blue at 700 nm. The reducing power of methanol extracts of *M. koenigii* leaves has been revealed.

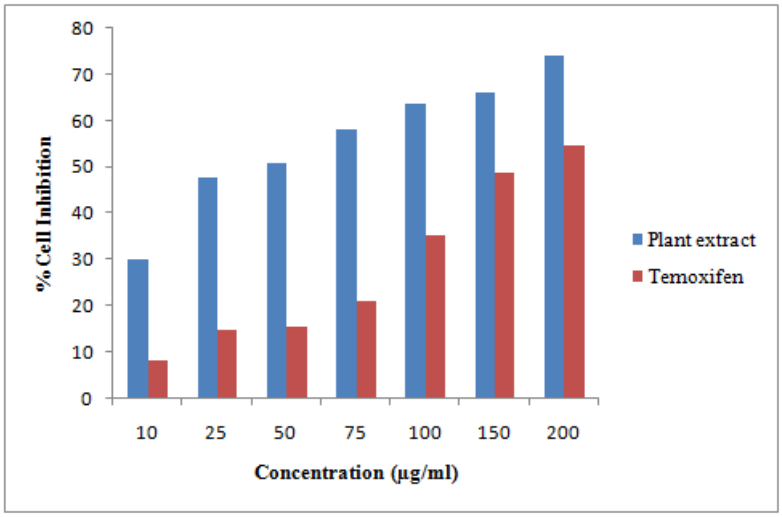
Antibacterial Activity

The *M. koenigii* leaves at the dose level of 50 µg /ml was active against *Escherichia coli* (31.05 ± 2.15), *Pseudomonas aeruginosa* (33.45 ± 2.05) and *Bacillus subtilis* (34.25 ± 3.12). All the test samples were compared with standard antibiotic Streptomycin (25µg/ml). The result shows the *M. koenigii* possess antibacterial agent.

In vitro Anti-Cancer Activity

Viability and Characterization of Cell lines The cytotoxicity activity of the methanolic extract of *M. koenigii* leaves was carried out by using MTT assay with different concentration on MCF-7 cell lines. Results of anticancer activity on MCF-7 are shown in (Figure 1).

Figure 1: Comparison of cell inhibition percentage with Temoifen



Docking with active marker compounds

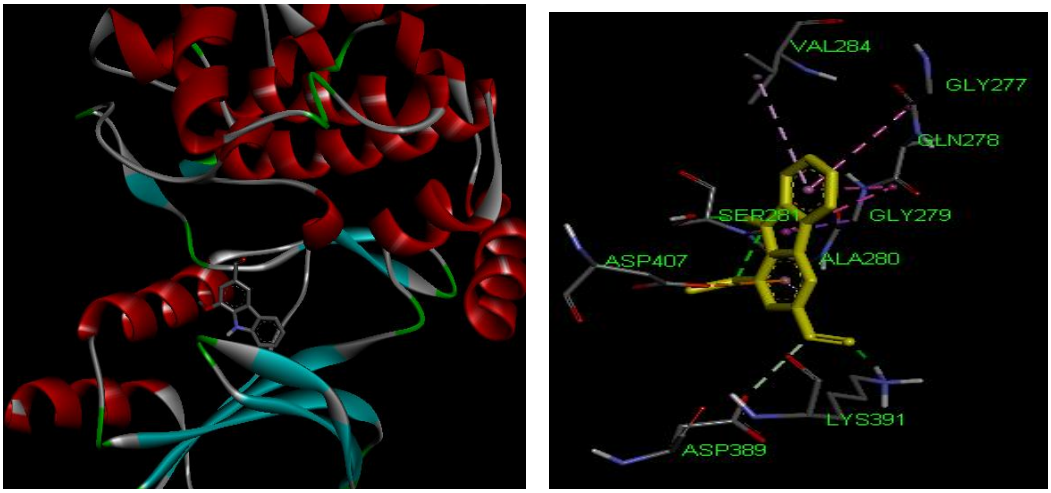
Docking studies were performed for the four proteins with four marker compounds using AutoDock 4 and binding energy was calculated. Mahanimbicine has least binding energy with HER2 protein.

Table 3: Docking of breast cancer proteins with *Murraya koenigii* active compounds

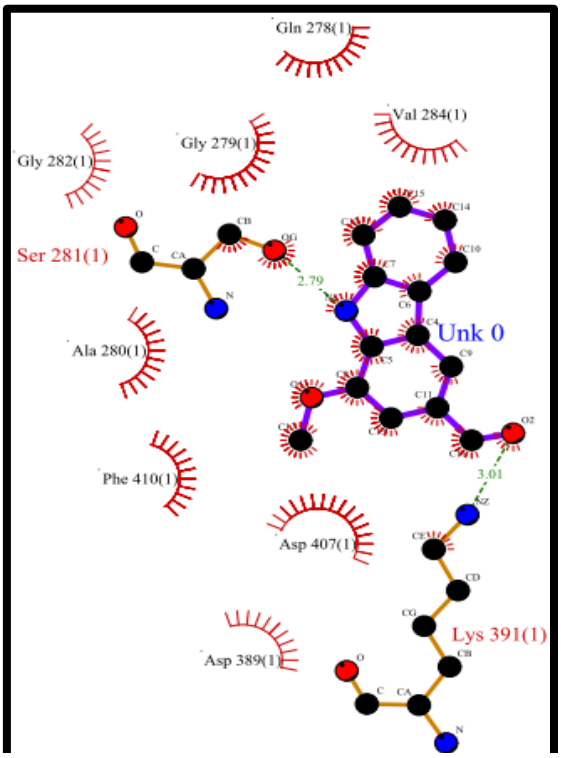
S.No	Target protein	Active Components	Binding energy (kcal/mol)	Interaction with amino acids residues
1	PAK1 (PDB ID:2HY8)	Murrayanine	-4.9	ASP389, LYS391, ASP407, GLY277, GLY279, ALA284, GLN278, SER281, ALA280
		Mahanine	-7.15	THR406, VAL284, ALA297, LEU347, GLU345, LEU396
		Koenimbine	-5.17	ASP407, ALA280, GLU315
		Mahanimbicine	-6.34	VAL284, ILE276, LEU396
2	HER2 (PDB ID:3WSQ)	Murrayanine	-5.65	SER26, LEU13, PHE29
		Mahanine	-6.56	
		Koenimbine	-5.11	ARG332, ARG12, TYR54, THR30
		Mahanimbicine	-7.39	GLN424, GLY425, ALA392, PRO356, ARG100
3	BRCA1 (PDB ID: 3PXB)	Murrayanine	-3.92	LYS1702, LEU1701, ALA1700, ASN1774
		Mahanine	-5.5	ASN 1774, ALA1700, LEU1839, GLU1836, ARG1835
		Koenimbine	-4.96	VAL1654, GLY1656, LYS1702, LEU1701, ALA1700
		Mahanimbicine	-6.46	LEU1839, ALA1700, ASN1774, MET1775
4	ESR-α (PDB ID: 1ERE)	Murrayanine	-4.34	LYS520, MET427, HIS516, CYS 381, HIS547
		Mahanine	-6.18	LYS520, MET427, HIS547, GLY420, GLU423
		Koenimbine	-5.5	ALA546, LYS520, CYS381, HIS516, MET427
		Mahanimbicine	-6.58	MET427, LYS520

Table 4: Docking results of breast cancer proteins with standard drug

S.No	Active Component	Target proteins	Binding energy (kcal/mol)	Interaction with amino acids residues
1	Temoxifen	PAK1 (PDBID:2HY8)	-6.23	MET344, ARG299, VAL284, ASP407
		HER2 (PDBID:3WSQ)	-6.56	PRO356, TYR32, ARG98, ASP106, VAL2, HIS448, PRO356, TYR32, ARG98, ASP106, VAL2, HIS448
		BRCA1 (PDB ID: 3PXB)	-4.31	LYS1702, LEU1701, ALA1700, ARG1699
		ESR-α (PDB ID: 1ERE)	-5.53	ASP426, HIS547, CYS381



PAK1 (PDBID: 2HY8) docked with Murrayanine



LIGPLOT-PAK1 Interaction with Murrayanine

V. Conclusion

In the present study leaf samples were collected and air dried and their crude extract was isolated using methanol. *Murraya koenigii* leaves have potent antioxidant and anticancer activity against MCF- 7 cell lines. Docking studies were performed for the four proteins with four marker compounds. Mahanimbicine has least binding energy with HER2 protein.

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