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Characterization and evaluation ethyl acetate extract of *melochia corchorifolia* leaf- anticancer antibiological and molecular docking studies on breast cancer estrogen receptor

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The present work focused on Phytochemical screening, characterization, anticancer activity and antibiological activity of ethyl acetate extract of *Melochia corchorifolia* leaves followed by molecular docking studies have been carried out. The plant leaves have been collected, weighed, and extracted with the soxhlet apparatus by using ethyl acetate solvent and then extracted are subjected to phytochemical screening. Antibiological activity of plant leaves ethyl acetate extract has been tested against six bacterial and two fungal strains using agar well diffusion methodology. The characterization of phytoconstituents compounds has been carried out using various spectroscopy method such as GC-MS (Gas chromatography Mass spectroscopy), UV-visible and Fourier-transform infrared. Auto dock tool (4.2.0) is used for molecular docking studies. The phytochemical analysis of *Melochia corchorifolia* ethyl acetate leaves, reveals the existence of carbohydrates, glycosides, triterpenes flavonoids and alkaloids. Antimicrobial activity is effective against gram-positive bacterial strains namely *Staphylococcus aureus* (17 mm), *Bacillus subtilis* (16 mm), the gram-negative bacterial strains namely *Salmonella typhi* (15 mm) and *E. coli* (14 mm). Moreover, the extract is also found to be effective against *Aspergillus Niger* (18 mm) fungal species. The GC-MS and FT-IR analysis show bioactive compounds and their functional groups. UV-VIS analysis results reveal that the presence of phytoconstituents derivatives in the range between 206-350 nm. The cytotoxicity activity for the MCF-7 cell line shows that the drug efficacy IC50 value is 148.836 (µg/mL). Further, the predicted bioactive compounds are docked with the cancer estrogen protein receptor (PDB ID: 3s7s) with ligand martidin-15 one shows the highest binding affinity. The study reveals the potential of *Melochia corchorifolia* leaves ethyl acetate extract showed antibiological and anticancer activity.

Keywords: Anticancer activity, Antimicrobial activit, FTIR, GC-MS, Human breast cancer estrogen protein, Molecular Docking, Melochia corchorifolia, UV-VIS

The human system is mainly composed of numerous tiny cells. Normal cells in human system grow and divide in the body for a period of time and then stop dividing and growing. Cellular reproduction plays an extensive role in immune system once the process goes out of govern cancer cell occurs. Therefore, the abnormal division and growth noticed in cancer cells which are caused by damage in the genetic material[1]. Breast cancer is one of the utmost serious complications of oncology. The huge amount of threat factors has been identified which advance chance of cancer, such as exposure to radiation, obesity, smoking chemical mutagens, environmental pollutants, alcohol consumption and lack of physical exercise. A large number of anticancer drugs are currently available in progress; unfortunately, the side effects of the synthetic drugs are an extensive drawback in clinical study. The entire world is facing problem in the enlargement of drugs for various cancer remains extreme clinical challenges[2-4]. On the other hand, Plants are found to be a feasible source in the cancer treatment. India has identified more than 3000 medicinal plants. Natural products are playing major role treatment of various diseases and playing a significant role in drug development[5-7]. The parts of the plants have been used over the last several decades to cure different diseases. The exploration for infectious agents has engaged many research organizations in the territory of ethno pharmacology[8, 9]. Characterization of the phytochemical compounds from the medicinal plants will add information on the various functional groups which are mainly responsible for the therapeutic properties. The drugs from medicinal plants have shown a good antitumor activity against breast cancer, such as Paclitaxel and Docetaxel (Taxus brevifolia), Camptothecin

(Camptotheca acuminata) and Vinblastine (Catharanthus roseus). Drugs are involved in inhibiting the cell cycle mechanism and thereby inhibiting the DNA topoisomerase and microtubule assembly[10-13]. The main purpose of molecular docking is to obtain the preliminary information of any bioactive compound's affinity before the *in-vitro* experiment. In this analysis, three parameters such as H-bonding energy, binding energy, and inhibition constant were evaluated a protein receptor ligand for binding affinity[14,15]. The estrogen receptor used therapeutic importance as a target to prevent osteoporosis and breast cancer[16].

Melochia corchorifolia has been conventionally used for different medicinal purposes. In India, the plants of *Melochia corchorifolia* are used to treat several diseases like to reduce abdominal swelling, headache, ulcer, chest pain, and cancer treatment. *Melochia corchorifolia* will grow tall up to 1.3-2.0 m with stellate hairs. The leaves are ovate in shape and blade of narrow leaves measures up to 7.5 cm × 5.5 cm and veins measured 5 cm to 7 cm long. The petioles are typically 5 cm long and have linear stipules[17-19]. In this study, scrutinized the presence of phyto constituents, analyzed its anti-biological activity and evaluated bioactive phyto constituents from *Melochia corchorifolia* leaves ethyl acetate extract and *In-silico* studies on human breast cancer protein estrogen receptor with bioactive ligand components.

Experimental Section

Ethyl acetate extract

Melochia corchorifolia leaves collected from the various surroundings Vandalur, India. The freshly collected leaves washed and air dried for 15 days in the shade to remove chlorophyll content. Using a mechanical blender, the leaves of *Melochia corchorifolia* are finely grounded into a powder.

The 100 g of air-dried ground leaf material was extracted with ethyl acetate in the ratio of 1:10 (w/v) using soxhlet apparatus for 72 hrs at 50-60°C. The ethyl acetate extract then filtered by using Whatman filter paper No: 1 separate container. The above processes were repeated for three times. The collected ethyl acetate extract concentrated about 40°C (rotary evaporator). Ethyl acetate was then added to increase the extraction volume to 100 mL. The concentrated extract of *Melochia corchorifolia* leaves were weighed lyophilized and kept at 4°C for further studies[20].

Qualitative analysis of phytochemical screening

The ethyl acetate extract of *Melochia corchorifolia* were subjected to preliminary phytochemical screening using standard methods. The sample extract was initially screened for different types of phyto constituents such as phenolic, terpenoids, alkaloids, and steroids etc. using standard reagents[21,22].

Antibacterial activity of *Melochia corchorifolia*

The Ethyl acetate leaf extract have been screened against various microorganisms responsible for the infections[23]. The bacterial strains were obtained by the process of serial dilution and spread over an agar plate. For bacterial growth, the plates were incubated for 3-4 days at 37°C. Species identifications were performed using standard methods. Antibacterial activity of *Melochia corchorifolia* ethyl acetate was evaluated by using both gram positive and negative bacteria including *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermis*, strains *Proteus vulgaris, Salmonella typhi,* and *Escherichia coli* using agar well diffusion assay method. Nutrient agar plates were swabbed with 8 h old culture of each bacterium. Well performed in respective petri plates by sterile cork borer (10mm diameter, 2 cm apart). Approximately 100 µL of ethyl acetate extract added into wells using a sterile syringe kept room temperature for 2 h. The control experiments were carried out without using a plant leaves extract. The positive control ampicillin 100 µg/mL then incubated plates at 37°C about 24 h bacterial growth. A zone of inhibition was observed after 24 h and the activity index was also calculated and above process was carried out thrice[24-26].

Analysis of antifungal activity

The antifungal activity of plant leaves extract screened for agar well diffusion method[27]. Serial dilution method was used to isolate the fungi from soil samples. Pure fungal cultures were swabbed on the agar plates using sterilized cotton buds. Wells are made on the agar plates. Ethyl acetate extract of *Melochia corchorifolia* were tested against *Aspergillus Niger* and *Candida albicans* concentration 100 µg/mL. The Fluconazole as positive control and plates are incubated at 37°C for 48 h and precise and index activity zone of inhibition was calculated[28-30].

FT-IR analysis

FT-IR spectral investigation of the ethyl acetate leaves extract sample accomplished using FT-IR Shimadzu IR Prestige-21 (FT-IR-84005). FT-IR spectrum provides the compositional and functional information of *Melochia corchorifolia* extract. The analysis was performed in range of wave number 500-3500 cm^{-1} at room temperature by using the KBr pellet[31,32].

GC-MS

A Clarus 500 Perkin-Elmer gas chromatography fitted with an Elite-5 capillary column (5 percent phenyl 95 percent dimethyl polysiloxane) (30 nm × 0.25 mm ID × 0.25 µm df) with EI mode is used to test and run the GC-MS[33,34].

UV-VIS spectrum analysis

The *Melochia corchorifolia* ethyl acetate extract was centrifuged at 4000 rpm for 15mins, followed by filtering using Whatmann filter paper No: 1. further dilution process was carried out in the ratio of 1:10 with ethyl acetate. The extract was analyzed using Perkin Elmer Spectrophotometer at a wavelength ranging from 200 to 800 mm, and the characteristic peaks were recorded[35,36].

Protein and ligand preparation

The three-dimensional complex structure of the protein Human Breast cancer estrogen receptor (PDB ID 3s7s) was retrieved from the protein databank[37]. The estrogen protein receptor was converted into native structure by deleting all the water and heteroatom molecules using the Auto dock 4.2.0 software. The 2D structures obtained from GC-MS were changed to 3D clusters by using Open Babel tool[38].

Molecular docking analysis on protein Human Breast cancer estrogen receptor and potent ligand

The computational docking analysis was performed by using software Auto Dock 4.2.0. Based on the literature review the automated process was performed to locate the appropriate active site and binding conformations of estrogen receptor protein by using Autodock 4.2.0 docking tool[39]. The cluster analysis was calculated on the docked results. The binding energy of each cluster is calculated for all different docked pose of conformations with the ligand Sophoridine, Martridin-15-one and Phytol acetate.

Swiss ADME

Swiss ADME web tool is used to computing the physicochemical properties of drug-likeness and pharmacokinetic nature of small ligand. This method used to support drug-designing study[40].

PASS prediction

The (BAS) biological activity spectrum was calculated by using the PASS prediction method. The outcomes of the ligand exhibit pharmacological, physiological, biochemical and specific toxicity activities[41].

Anticancer activity

The MCF-7 cells subculture was subsequently trypsinized in DMEM after that medium of culture eliminated. The flask with disaggregated cells is added with 25 mL of DMEM and 10% FCS[42].

Seeding of cells

About 1 mL of a homogenized suspension of a cell has been applied to about 25 well culture plates along with different concentrations of the test ethyl acetate plant extract sample from 0 to 200µg/mL and 37°C incubated at 5% CO_2 humidified. The cells are observed (inverted tissue culture microscope) after 48 h incubation, with a cytotoxicity assay of 80% confluence of cells[43].

Cytotoxicity assay

Assay test performed (3- (4, 5-dimethyl thiazol-2yl) -2, 5-diphenyl tetrazolium bromide (MTT). The wells added with MTT after 48 hrs incubation left at room temperature for 3 h then wells removed the content using the pipette and added 100 µl SDS in DMSO to dissolve formazan crystals absorbance was reader at 570 nm Read Well Touch microplate reader[44].

Results and Discussion

Phytochemical analysis

Phytochemical scrutiny ethyl acetate extract *Melochia corchorifolia* leaves are show in (Table 1). The analysis report has exposed the occurrence of bioactive secondary metabolites compounds triterpenes, carbohydrates, glycosides, flavonoids and alkaloids.

Antimicrobial activity

The antimicrobial activity *Melochia corchorifolia* ethyl acetate leaves extract evaluated against \ *Staphylococcus epidermis, Staphylococcus aureus,*

proteus vulgaris, Bacillus subtilis, Salmonella typhi, and *Escherichia coli* strains. The *Melochia corchorifolia* was found to be active against with a *Staphylococcus aureus* of 100 µg/mL (Table 2). Antifungal activity was evaluated for ethyl acetate extract against *Aspergillus Niger* and *Candida albicans*. The maximum zone of inhibition showed *Aspergillus Niger* ethyl acetate extract of the *Melochia corchorifolia* leaves. Similarly, *Aerva lanata* solvent organic extracts have shown a significant role in phytochemical and antimicrobial properties studied[46] (Table 3).

FT-IR analysis

The FT-IR showed various peaks -NH stretching (3412 cm-^{1}), sp3 hybridization -CH group (2929), C=C group (1634), C-C combination of stretch & bends group (1391 cm-^{1}) and C-O group (1050 cm-^{1}). Similar research was performed in the FT-IR spectral analysis study of *Ampelocissus latifolia* extract and stated that the occurrence of specific functional groups such as secondary amine, alkanes, and carbonyl compound[45]. The spectral analysis peak showed identification different functional groups and chemical bonds stretches value. (Table 4 and Fig. 1).

Table 1 — Phytochemical screening of ethyl acetate extract of aerial parts of *Melochia corchorifolia*

S.No	Phyto constituents	Test	Result
1	Alkaloids	Hager's reagent & Wagner's test	+
2	Amino acid	Ninhydrin test	-
3	Tannins	Ferric Chloride test	-
4	Triterpenes	Terpenoids test	+
5	Carbohydrates	Molisch's	+
6	Glycosides	Borntrager's	+
7	Flavonoids	Lead acetate	+
8	Proteins	Millon's	-
9	Saponin	Froth forming	-

Table 2 — Zone of inhibition ethyl acetate extract against microorganism

Microorganisms	Zone of inhibition (mm)	Ampicillin (mm) [positive control]	Distilled water [negative control]
Staphylococcus aureus	17	12	–
Bacillus subtilis	16	13	–
Staphylococcus epidermis	13	14	–
Escherichia coli	14	10	–
Salmonella typhi	15	11	–
Proteus vulgaris	12	13	–

Table 3 — Zone of inhibition ethyl acetate extract against fungal organisms

Fungal Organisms	Zone of inhibition (mm)	Fluconazole (mm) [positive control]	Distilled water [negative control]
Aspergillus Niger	18	13	–
Candida albicans	12	11	–

GC-MS spectral analysis

The GC-MS is most specific process to detect numerous secondary metabolites existing in the plant extracts. The ethyl acetate plant extract evaluated by GC-MS to detect various compounds with Mass Spectral library NIST 17 and Wiley 8 and compounds are identified. The chromatograph showed five peaks with five individual compounds shown in (Table 5 and Fig. 2). The plant extract which contain higher level of major constituents is Phytol, acetate (30.43 Per cent), followed by Matridin-15-one (22.73 Per cent), l-(+)-Ascorbic acid 2, 6-dihexadecanoate (22.10 Per cent), 2,6,10-Trimethyl,14-ethylene-14-pentadecane (17.04 Per cent) and low level area is Sophoridine (7.70 Per cent). .

UV-VIS spectrum analysis

The spectrum analyses detect compounds contain σ-bonds, π-bonds (lone pair of electrons) aromatic

Table 4 — FTIR spectrum and functional group obtained from the ethyl acetate extract

S. No	Extract	Peak values cm^{-1}	Functional groups
1	Ethyl acetate extract of *Melochia corchorifolia*	3412	-N-H stretching (secondary amine)
2		2929	-C-H stretching
3		1634	-C=C group
4		1391	-C-C (Combination of stretching and bending)
5		1050	-C-O group

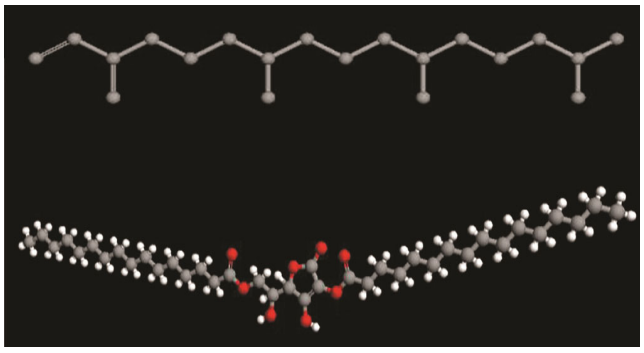

Fig. 1 — FT-IR spectrum of *Melochia corchorifolia* leaves extract in ethyl acetate

Table 5 — Bioactivity phytocomponents detected- *Melochia corchorifolia* leaves

S.no	Retention time	Area	Area Percent	Mol. Formula	Mol. weight g/mol	Compound name	Nature of the compound	Biological activity & Ref
1	35.140	100332	17.04	$C_{20}H_{38}$	254.5	2,6,10-Trimethyl,14-ethylene-14-pentadecane	Olefins	Antiproliferative, Antibacterial[47]
2	39.196	130118	22.10	$C_{38}H_{68}O_8$	652.9	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Fatty acid Ester	Antidiabetic, Antibacterial, Antibronchitic, Anticancer[48]
3	43.735	179154	30.43	$C_{22}H_{42}O_2$	338.6	Phytol, acetate	Diterpene	Antimicrobial, Anti-inflammatory, diuretic, Anticancer [49]
4	48.773	133858	22.73	$C_{15}H_{24}N_2O$	248.36	Matridin-15-one	Alkaloid	Anti-inflammation, Anti-fibrotic Anticancer [50]
5	49.113	45360	7.70	$C_{15}H_{24}N_2O$	248.36	Sophoridine	Tetracyclic quinolizidine alkaloid	Anti-tumor [51]

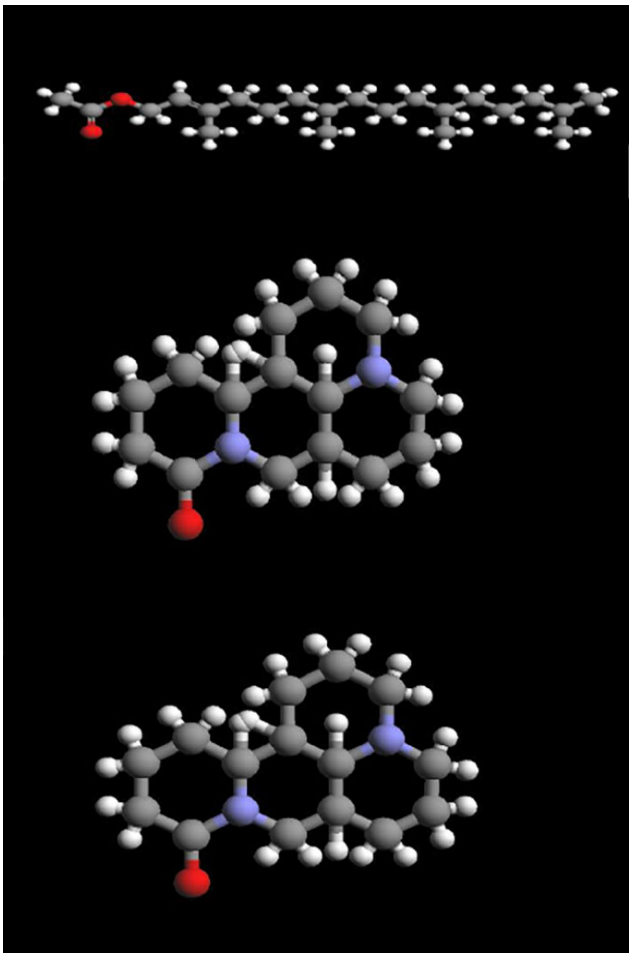

Fig. 2 — Structure of active compounds identified by GC-MS -ethyl acetate extract of *Melochia corchorifolia* leaves

ring and chromophores. UV-VIS spectrum of leaf extract recorded at a wavelength between 200 to 800 nm, because of sharpness peak at 206 nm with a proper baseline. The results showed other peaks 326, 273 and 260 nm with spectral absorption 0.02, 0.027, 0.186 and 0.024. UV-VIS spectroscopic (206-350 nm) result reveals that the existing of flavonoids and phenolic compound derivatives (Table 6).

Table 6 — The Peak values of UV-VIS Spectrum of ethyl acetate extract of *Melochia corchorifolia*

S.No.	Wavelength (nm)	Absorbance
1	326	0.020
2	273	0.027
3	206	0.186
4	260	0.024

Table 7 — The binding affinity of ligand with estrogen breast cancer receptor protein

Protein Pdb ID	Compounds	Binding energy	Inhibition constant
3s7s	Matridin-15-one	-7.75	328.65
	Sophoridine	-6.43	300.75
	Phytol acetate	-1.47	120.08

Molecular Docking analysis

The present analysis reveals that ligand Matridin-15-one showed binding affinity of -7.75 kcal/mol and high inhibition constant value, which indicates better affinity when compared to Phytol acetate. Hence, Matridin-15-one is the potential lead molecule for the inhibition of human cancer estrogen protein amino acid residues Arg86, Tyr52, Gly49, Pro50, Pro48, Gly51, and Arg343. They are the most significant amino acid residues for potential drug targets as carbon-hydrogen bond, conventional hydrogen bond, and Vander Waals

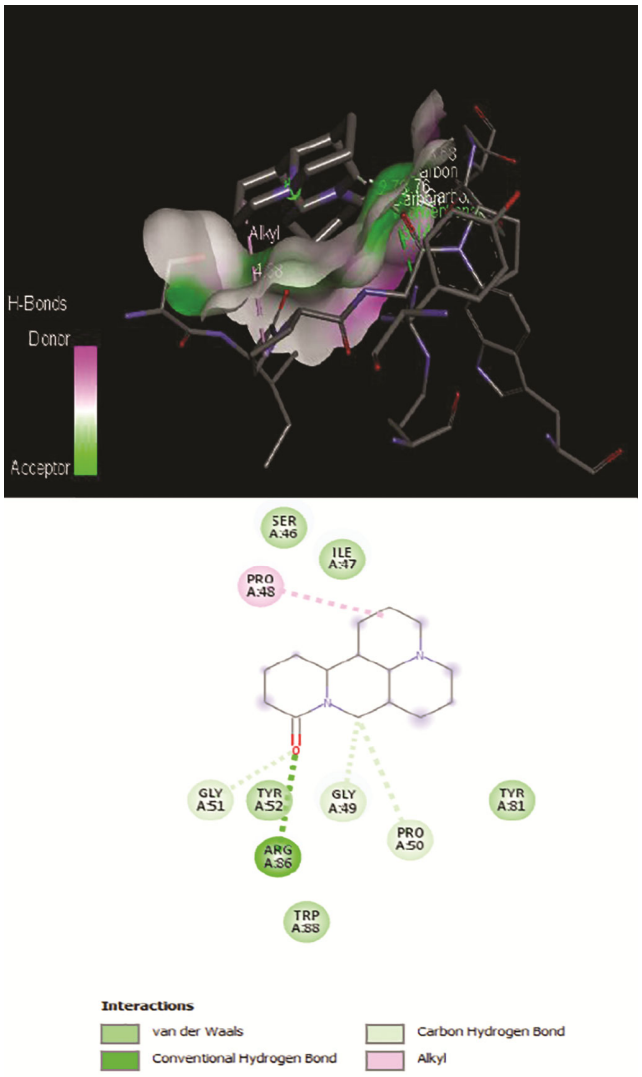

Fig. 3 — Binding interactions of Matridin-15-one in 3s7s Receptor

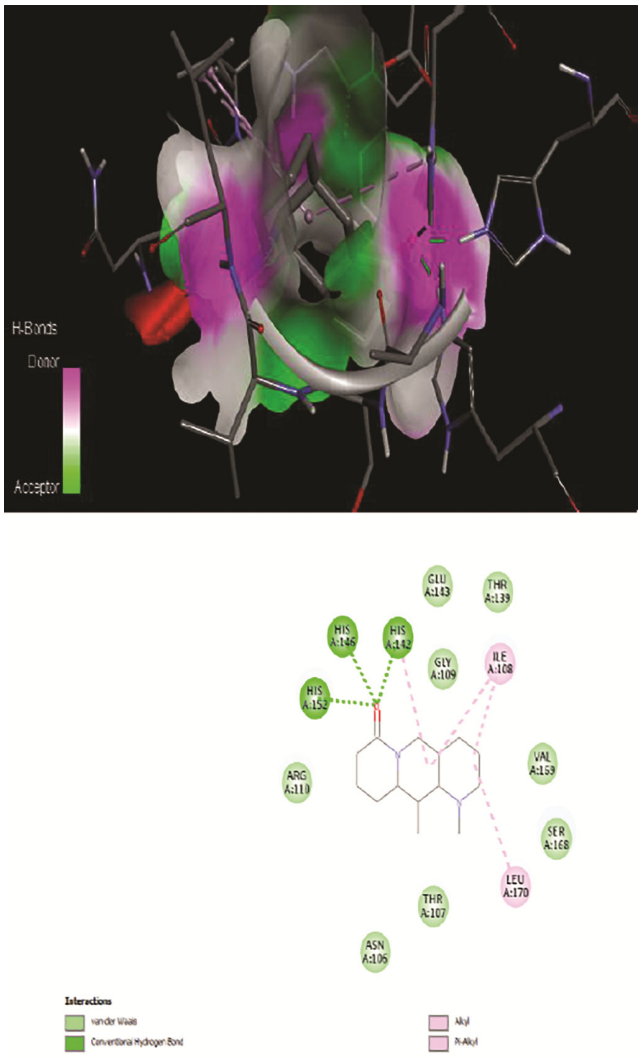

Fig. 4 — Binding interaction of Sophoridine in 3s7s Receptor

interaction. Theoretically, sophoridine also showed moderate binding affinity value of -6.43 (Table 7 and Figure 3, 4 and 5).

ADME toxicity testing

The ADME/TOX analysis of the ligand Martidin-15-one shows drug-likeness, obeys Lipinski rule five, no violation and high score value of bioavailability (Table 8).

PASS prediction activity

The PASS prediction analysis of ligand Martidin-15-one shows higher Pa value (Probability active value) for an anticancer property (Table 9).

Anticancer activity

Cytotoxicity assay

The cytotoxicity effect against suspension adherent cell line MCF-7 is showed (Table 10 & Figure 6 & 7). The compound showed a good anticancer reactivity against MCF-7 cell line. There was dose dependant manner activity was observed. MCF-7 cell lines showed low level of cell viability (55.38%) registered in 400 µg/ml at 48 h, where as 48 h MCF-7 cell line showed low level of cell viability registered (85.46%) in 3.125 µg/mL. The IC50 value ethyl acetate extract of *Melochia corchorifolia* leaves calculated at 148.36 µg/mL cell viability against MCF-7 cell lines. Similarly, ethyl acetate extract of Nepenthes plant reported that a better cell viability against MCF-7 cell line[52].

Table 8 — ADME property of Martidin-15-one drug

Molecule	Canonical SMILES	Formula	MW	#Heavy atoms	#H-bond acceptors	Log Kp (cm/s)	Lipinski #violations	Bioavailability Score
Matridin-15-one	O=C1CCCC2N1CC1CCCN3C1C2CCC3	$C_{15}H_{24}N_2O$	248.36	18	2	-6.69	0	0.55

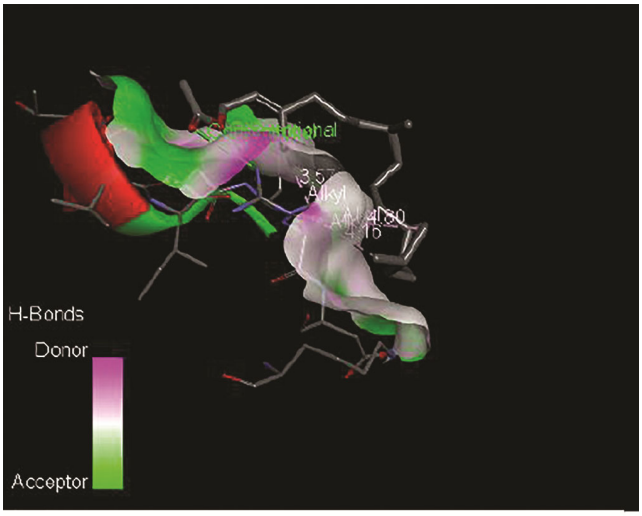

Fig. 5 — Binding interactions of Phytol acetate in 3s7s Receptor

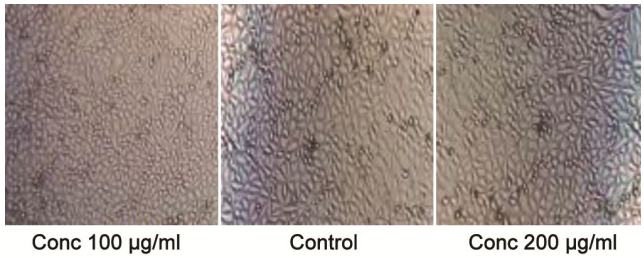

Fig. 6 — Anticancer activities of ethyl acetate *Melochia corchorifolia* leaves extract

Table 9 — Activity prediction for Martidin-15-one- Possible activities at Pa > 30 %

Pa	Pi	Activity
0.898	0.001	Antineoplastic alkaloid, Anticancer
0.841	0.011	Nootropic
0.836	0.006	Nicotinic alpha2beta2 receptor antagonist
0.765	0.015	TP53 expression enhancer
0.743	0.003	Fibrosis treatment
0.740	0.003	CYP2D2 inhibitor
0.726	0.005	Cognition disorders treatment
0.729	0.008	Analgesic
0.723	0.005	Analgesic, non-opioid
0.717	0.004	Polarisation stimulant
0.734	0.025	Nicotinic alpha6beta3beta4alpha5 receptor antagonist
0.709	0.007	Cardiovascular analeptic
0.707	0.006	Antipsychotic, Antivenom
0.702	0.004	Cholinergic

Table 10 — In vitro cytotoxicity effect of ethyl acetate *Melochia corchorifolia* leaves extract against MCF-7 cell lines

Sample Concentrations (µg/mL)	MCF-7 Cell Viability (%)
0	100.00
3.125	85.46
6.25	81.09
12.5	76.32
25	72.25
50	65.75
100	63.10
200	60.77
400	55.38
IC50	148.836

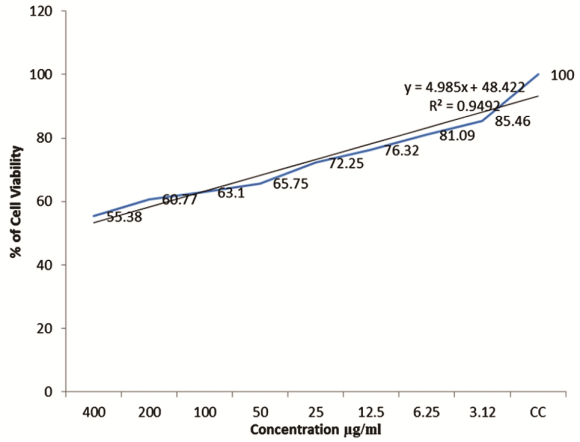

Fig. 7 — Graphical representation- Anticancer activity of ethyl acetate *Melochia corchorifolia* leaves extract

Conclusion

Based on the present study, an antibiological analysis of *Melochia corchorifolia* ethyl acetate leaves extract have been identified which inhibits the growth of selected Gram-negative and Gram-positive bacterial and fungal species. The phytochemical result relies on the occurrence of various phytocomponents. UV-VIS, FT-IR, and GC-MS results reveal that active functional groups and bioactive compounds present in the leaves extract. Furthermore, it showed a significant inhibitory effect on MCF-7 cell line growth and proliferation. The molecular docking result indicated that the ligand compound Matridin-15-one is having better binding affinity and inhibition constant for both aspects. The molecular docking investigation explained that the compound present in the leaves extract highly binding affinity with protein human breast cancer (estrogen receptor) and it might use to reduce lifetime exposure to estrogen. The consequences of various experiments, which were conducted in the present investigation, afford promising principles regarding the potential uses of *Melochia corchorifolia* for the estrogen protein receptor. Further, the ADME/TOX result reveals that the Martidin-15-one ligand shows a potent inhibitor for the targeted protein. PASS prediction results reveal that Martidin-15-one is a potential inhibitor for anticancer activity value. Future research requires a detailed investigation of active chemical constituents like isolation, characterization, and biological activities. This study boosts the efficacy of the bioactive compounds for destiny drug discovery through leading advanced techniques.

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