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BINDING POTENTIAL OF METHYL 3B- HYDROXYL-BISNORELLOCHOLANOATE WITH HER 2 FOR AN EFFECTIVE TREATMENT OF BREAST CANCER

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ABSTRACT

Breast cancer is prevailing among the women at the rate of 18% of total population worldwide. In the study Coconut shell oil was used to treat breast cancer MCF cell lines. The Coconut plant is also knows as Kalpa Viriksha in India which means the tree gives all the necessities of human life. It is rich in alkaloids, carbohydrates, phenols, tannins, flavonoids, aminoacids, quinines, terpenoids, oxalate and carboxylic acids. The antiproliferative activity of Coconut shell oil was performed by 0.5% 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) method. The highest non-toxic concentration was recorded at 125µg/ml concentration. Molecular docking was predicted using Autodock and Autodock Vina in the PyRx platform on Human Epidermal Growth Factor Receptor 2. The molecule, Methyl 3β-hydroxyl-bisnorellocholanoate obtained from Coconut shell oil has successful and potential binding with the target molecule. Hence, this may be a potent lead molecule for the treatment of breast cancer and further clinical confirmation is needed to prove it as a valid drug.

KEYWORDS: Coconut shell oil, breast cancer, antiproliferative, molecular docking, Methyl 3β- hydroxyl- bisnorellocholanoate.

INTRODUCTION

In tropical countries like India, Coconut shell is available in large quantities and considered as waste from agricultural sector. But various studies reported that the coconut shells contain certain organic materials such as polyphenols and organic acids.[1,2] The main bioactive

components in coconut are tocopherol, fatty alcohol, triterpenealcohol and sterol. The ethanolic extract of CSO has antibacterial and antifungal activity.[3] The oil extracted from the kernel of Coconut inhibited bacterial activity against *Escherichia coli* and *Bacillus subtilis.*[4]

Cancer is one of the severe common disease with high mortality worldwide. The damage of DNA develops cancer cells. US Natural Cancer Institute recognized natural products usage, identification and preparation of anticancer drugs.[5] Approximately 100 different types of cancer were reported, 12.7million new cancer cases reported every year and deaths over 7million every year in the developing countries.[6] The increased risk of breast cancer linked with the high level of estrogen, which mediates its biological effects such as cell apoptosis, genesis, malignant progression.[7] Estrogen Receptor α is present in the liver, pituitary gland, mammary gland, vagina and uterus.[8] Abnormal expression of Estrogen Receptor – α is responsible for 70% of the primary breast cancer patients.[9]

Molecular docking is an essential tool for the drug discovery. Various methods are available for the efficient modeling of peptide flexibility during the docking of the protein-peptide bond.[10] The Human Epidermal Growth Factor Receptor 2 (HER 2) plays an important role in the cell growth and differentiation process associated with the development of human cancers such as breast, gastrointestinal tract and ovarian cancers.[11] Approximately 20 to 50 HER2 gene copies have been found in breast cancers.[12] They have increased sensitivity to cytotoxic agents such as doxorubicin, relative resistance to hormonal agents to metastasize the brain and viscera.[13]

MATERIALS AND METHODS

Invitro assay for cytotoxicity activity (MTT assay)

Pyrolysis method was used to extract Coconut shell oil. The powdered shell (250g) was heated in an earthern pot at 200^{o}C to 250^{o}C temperature for a span of 3h that yields 25cc of oil. The obtained Coconut shell oil (CSO) was extracted with ethanol in 1:3v/v ratio. The extract was concentrated by vacuum evaporator and the crude extract was used.

The cytotoxicity effect of the CSO was evaluated by MTT assay.[14] The cells (1 x 10^{5}/well) were cultured into the plates in 24 well plates and incubated overnight at 37^{o}C for 24 h with 5% CO_2 condition. After the cell reaches the confluence, different concentrations of the extract were added and incubated for 24 h. At the end of the treatment, the extract was

washed with Phosphate Buffered Saline (PBS) at pH 7.4. After this wash 100µl/well (5mg/mL) of 0.5% 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation period, 1mL of DMSO was added to all the wells. The absorbance was measured at 570 nm with UV spectrophotometer using DMSO as the blank. Measurement was performed to evaluate the effect of the extract on the MCF -7 proliferated cells and the % cell viability was calculated.

Molecular docking against Human Epidermal growth factor Receptor 2

Human epidermal growth factor receptor 2 (HER2) is one of the major targets against breast cancer and other carcinomas. In the cell growth and differentiation process HER 2 show an over expression to develop carcinomas. It is over expressed in 15–30% of invasive breast cancer development.[15] Acetamide 2, 2, 2-trifluoro (Ligand-1), Hexadecenoic acid (Ligand 2), Methyl 3β-hydroxy-bisnorallocholanoate (Ligand-3), 9-octadecenoic acid (Ligand-4) were selected for docking against Human Epidermal Growth Factor (HER2). These four ligands was selected based on their peak values obtained from the Gas Chromatography and Mass Spectrometry analysis of CSO.[16]

The crystal strucure of HER2 was available in Protein Data Bank (https://www.rcsb.org/structure/3pp0) (Resolution: 2.25A^{o}). Crystal strucutre of HER 2 (3PP0) was obtained from Protein Data Bank for molecular docking.[17] It was performed using Autodock and Autodock Vina in the PyRx (0.8) version. Binding affinity in terms of energy value (kcal/mol) of research molecules with HER2 protein was compared with 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy] pyridin-3-yl}amino)-5H–pyrrolo[3,2-d] pyrimidin -5-yl]ethoxy}ethanol as the inhibitor which is crystallized with the complex structure 3PP0.

RESULTS AND DISCUSSION

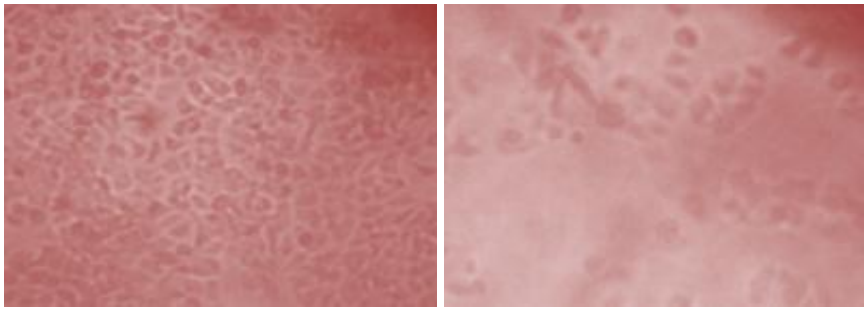
Anticancer Activity

Anticancer activity of CSO depends on the concentration and it works even at low doses. The cancer cell viability decreases as the concentration of CSO increases. The least concentration that checks the growth of cancer cells was 27.43% (Table-1). The IC_{50} value of CSO was 51.58 at 125µg/mL. The survival of the cell was 100% in control. The death of cancer cells was observed from the morphological changes and shrinkage of cells induced by the CSO in the breast cancer cell lines (Fig. 1. A-D).

Table 1: Anticancer effect of CSO *on MCF 7* cell line.

S. No	Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.217	27.43
2	500	1:1	0.282	35.65
3	250	1:2	0.343	43.36
4	125	1:4	0.408	51.58
5	62.5	1:8	0.472	59.67
6	31.2	1:16	0.533	67.38
7	15.6	1:32	0.595	75.22
8	7.8	1:64	0.662	83.69
9	Cell control	-	0.791	100

A) Normal MCF 7 cell line B) Toxicity-1000µg/ml.



C) Toxicity-125µg/mL. D) Toxicity-7.8µg/ml.

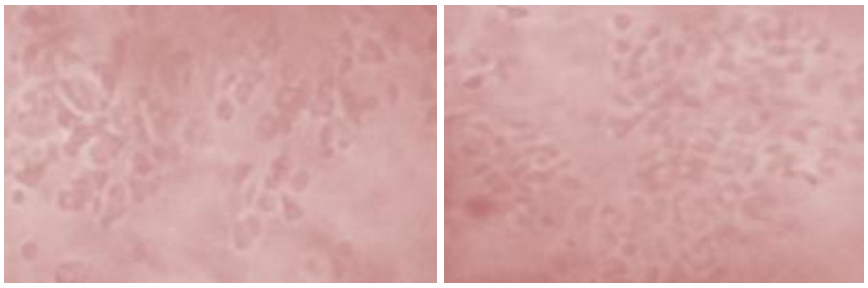


Fig. 1: (A-D) Anticancer effect of CSO on *MCF 7 cell* line.

Docking Studies

The selected four ligands namely Acetamide 2, 2, 2-trifluoro (Ligand-1), Hexadecenoic acid (Ligand-2), Methyl 3β-hydroxy-bisnorallocholanoate (Ligand-3), 9-octadecenoic acid (Ligand-4) were docked against Human Epidermal Growth Factor (HER2). These molecules showed better binding affinity than the inhibitor in Autodock docking tool and three molecules out of four show better binding affinity than the inhibitor in Autodock Vina tool.

The molecule, Methyl 3β- hydroxyl- bisnorallocholanoate (Ligand-3) was found to bind with stronger affinity than all other molecules with target protein HER2. The binding affinity of the molecule in Autodock is -7.06kcal/mol and for Autodock Vina is -9.8kcal/mol. (Table -

2). The inhibitor which resolved to this crystal structure was also docked to the drug target. The binding affinity of the inhibitor HER2 (2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy] pyridin-3-yl} amino)- 5H-pyrrolo [3,2-d]pyrimidin-5-yl] ethoxy} ethanol) for Autodock is -3.08 kcal/mol and Autodock Vina is -8.2 kcal/mol(Fig. 2). The $C_{16}H_{36}O_2$ molecule shows better binding affinity than the inhibitor molecule.

Table 2: Binding affinity of the biomolecules obtained from the CSO and the crystallized inhibitor molecule against HER2 receptor.

Ligand No.	GCMS peak for CSO	Molecular formula	Molecular Weight	Autodock Binding energy (kcal/mol)	Autodock Rank	Autodock Vina Binding energy (kcal/mol)	Autodok Vina Rank
1	Acetamide,2,2,2-trifluoro	$C_5H_3F_6NO_2$	223.073	-3.57	3	-8.5	2
2	Hexadecenoic acid,	$C_{17}H_{34}O_2$	270.457	-3.96	2	-7.3	3
3	Methyl 3β-hydroxy-bisnorallocholanoate	$C_{16}H_{14}O_3$	254.285	-7.06	1	-9.8	1
4	9-octadecenoic acid(Z)	$C_{19}H_{36}O_2$	296.487	-3.31	4	-7	4
	Inhibitor	$C_{22}H_{19}ClF_3N_5O_3$	493.87	-3.08	-	-8.2	-

This study was undertaken to efficiently use the agrowaste to determinate the anticancer activity of Coconut shell oil against MCF- 7 cell lines. The cytotoxic studies of ethanolic extract of CSO showed maximum inhibition due to the presence of many phytochemicals.[18,19] Breast cancer is known as a death sentence and the ratio of breast cancer is one in nine in women. Using Accelrys Discovery Studio software package docking was performed to find out the appropriate binding orientations that allows receptor (protein) flexibility and confirmations of ligands.

In the present docking study on HER2 the molecule with better binding affinity for CSO is Methyl 3β-hydroxyl – bisnorellocholanoate (Ligand - 3) that formed the hydrogen bonding interactions between the drug target and the VAL734 amino acid. Totally eight amino acids were found to fall in the interaction site with that of the inhibitor namely VAL734, PHE864, ALA751, LEU852, MET801 and ASP863.[20] Among them, two amino acids were found to make hydrogen bond interactions (MET801 and ASP863).

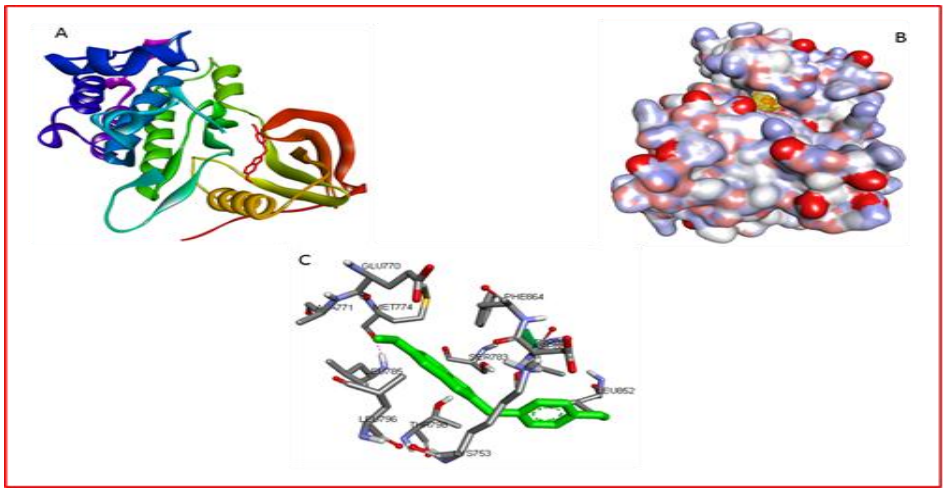


Fig.2: Docking interaction of HER2 protein with ligand -3 from CSO.

A. Interaction between the HER2 protein and the molecule.
B. The surface view of the interaction of the protein and the molecule.
C. Hydrogen bonding interaction between the protein HER2 and the Molecule.

CONCLUSION

The Coconut shell discarded as waste indeed comprises a primary place as agricultural waste. Therefore this research was undertaken to efficiently use the agrowaste and find out their phytochemicals to treat cancer. The results of the present work can be useful for the design and development of the novel compounds that has better inhibitory activity against breast cancer. The molecular docking was done to identify the binding mechanism. The Protein-Ligand interaction plays a necessary role in structural based designing. In the present study we have taken the receptor Methyl 3β-hydroxyl – bisnorellocholanoate and a bioactive anticancer phytoconstituent identified. Further research is required to take forward on the molecule namely Methyl 3β- hydroxyl- bisnorellocholanoate (Ligand-3) towards designing of drugs for breast cancer treatment.

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