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Comparative Evaluation of Phytochemical Composition, Anticancer Potential, and Computational Analysis of various Macroalgae Extracts

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KEYWORDS
Macroalgae extracts, anticancer activity, Molecular docking, Estrogen receptor alpha

ABSTRACT:

Introduction: Breast cancer ranks as the second most prevalent cancer globally, affecting approximately 1 in 8 women worldwide. Seaweeds are recognized for their diverse biological activities, including antioxidant, antimicrobial, antiproliferative, and cytotoxic effects. However, the specific bioactive constituents responsible for these activities have yet to be fully explored.

Objectives: This study aims to investigate the phytochemical composition of various seaweeds and compare their anticancer potential.

Methods: Cytotoxicity assessments were conducted using the MTT assay on the MCF-7 breast cancer cell line. Phytochemical analysis of methanolic seaweed extracts revealed the presence of cardiac glycosides, steroids, terpenoids, and proteins across all samples. Consequently, LC-MS analysis was performed on the T. conoides extract to identify its active constituents.

Results: Among the extracts tested, *Turbinaria conoides* demonstrated notable cytotoxic activity with an IC50 value of 24.68 µg/mL. The chromatogram identified 16 bioactive compounds, of which Mescaline, Quinic acid, Shogaol, and Stachydrine were selected for molecular docking studies with estrogen receptor alpha (PDB ID: 5DXM). Shogaol exhibited the lowest binding energy of -7.15 kCal/mol and displayed stronger binding affinity with key residues at the ligand-binding site of estrogen receptor alpha compared to Mescaline (-6.04 kCal/mol), Quinic acid (-3.51 kCal/mol), and Stachydrine (-4.03 kCal/mol).

Conclusions: These findings suggest that the anticancer properties of *Turbinaria conoides* are attributed to these bioactive compounds, highlighting its potential as a promising candidate for future anticancer therapeutics.

1. Introduction

In 2020, it was found that cancer is the second-leading cause of death and it claims nearly 10 million lives [1]. Breast cancer is the most common cancer and nearly 2–2.5 million women are affected every year. Approximately 627,000 deaths due to breast cancer have been recorded in the year 2018 [2]. Estrogen receptor alpha plays a main role in the development and progression of breast cancer in almost 70% of breast cancer patients [3]. The functional role of estrogenic compounds are modulated to a great extent by the estrogen receptor at the level of regulation of genes. The estrogen receptor regulates the expression of genes by directly interacting with the DNA regulatory elements in the nucleus. There are two different isoforms- estrogen receptor α and β [4]. Activation of estrogen receptor alpha by estrogen will result in the stimulation of ECM remodeling and EMT signaling pathways. Cancer progression in ER+ breast cancer is via activation of the PI3K/AKT pathway. Overall, ERα is found to impart a significant role in the prognosis of breast cancer [3].

Chemotherapy is widely used for the treatment of cancer, however, there are several side effects encountered by cancer survivors. Drug development for the treatment of cancer is currently leaning more heavily towards natural sources in order to counteract these detrimental effects. Compared to the terrestrial ecosystem, the marine environment comprises widely unexplored biodiversity and thus acts as a rich source of bioactive compounds. To date, over 30,000 natural products have been isolated from the marine environment [5].

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Bioactive compounds from marine organisms show novel molecular scaffold, which helps in the development of a wide range of active drugs [6]. At present, 14 drugs of marine origin, are found in the market and among these drugs, 9 drugs were used to treat cancer. In marine environments, the anticancer compounds are derived from sponges, cyanobacterium, sea creatures Seaweeds and fungi [7,8]. Due to its abundance and existence in extreme environmental conditions, algae act as one of the attractive sources for anticancer compounds [9]. The major group of macroalgae that are found in marine ecosystems includes the *Phaeophyta* (brown algae), *Chlorophyta* (green algae), and *Rhodophyta* (red algae). Adaptation to extreme environmental conditions such as pH, temperature, and supply of carbon dioxide confers to the alteration of the chemical composition of the algae, thereby synthesizing bioactive compounds such as terpenoids, flavonoids, alkaloids, phenolics, polysaccharides, etc. These compounds were found to exhibit unique properties and molecular scaffolds, thus showing anti-inflammatory, antioxidant, and anticancer activities [10], [11], [12], [13]

Seaweed has been used as food and for medicinal purposes in Asia since the ancient period. Seaweed supplies the essential micro- and macronutrients required for human health [14]. Recent research suggests that various bioactive components isolated from seaweeds exhibit anticancer activity a different mechanism of action such as inhibition of metastasis, cell cycle arrest, and promotion of cancer cell death by the activation of apoptosis [15,16].

2. Objectives

The current research aims at the identification of the seaweeds found in the coastal regions of Tamil Nadu, their phytochemical analysis, and comparison of the anticancer activity of the identified seaweed extracts in human breast cancer cell MCF-7. Additionally, the chemical composition of the methanolic extract of *Turbinaria conoides* using LC-MS and prediction of anticancer activity via *in silico* docking studies to understand the mechanism of action of bioactive compounds was performed.

3.Materials and Methods

3.1. Collection and identification of seaweeds

The seaweeds were collected along the coast of Tamil Nadu from in Olakuda, Nallupani, Thangachimadam, Mandapam Thonithurai, and Kilakkarai, Tamil Nadu and identified. Red algae- *Gracilaria dichotoma*, *Gracilaria edulis*, *Gracilaria verrucosa*, *Halymenia floresii*, Brown algae- *Dictyota dichotoma*, *Padina boergesenii*, *Sargassum wightii*, *Turbinaria conoides*, and Green algae - *Enteromorpha flexuosa Ulva fasciata*, respectively were used for present study (Table-1).

3.2. Preparation of seaweed extracts

The collected seaweeds were washed thoroughly in several times and allowed to air dry under the shade. Dried seaweeds were finely grounded using blender and sieved 0.8mm sieve ate and stored at refrigerator place until further analysis. The dried powder was soaked in 250 mL of ethanol and allowed to stand for 72hrs. Then, the sample is homogenized in an electric blender with the solvent at room temperature, and filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator and stored at -20° C. The concentrated extract is used for future analysis.

3.3 Phytochemical analysis

A preliminary analysis of the seaweed extracts was done to investigate the presence of different phytochemicals by using standard protocol [17].

3.3.1 Test for alkaloids:

Mayer's Test: 2 mL of Mayer's reagent was added to 1 mL of the seaweed extract. The appearance of reddish-brown precipitate suggests the presence of alkaloids in the extract.

3.3.2 Test for saponins:

Five mL of water and 1 mL of the extract were added to a test tube and the tube is shaken vigorously for a few minutes. The formation of a layer of foam represents the presence of saponins in the sample.

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3.3.3 Test for tannins:

To 1 mL of the extract, a few drops of 5% ferric chloride is added. The appearance of greenish-black or dark blue colour confirms the presence of tannins in the extract.

3.3.4 Test for cardiac glycosides:

Keller-Killani test: Solution containing a drop of ferric chloride and 2 mL glacial acetic acid is added to 1 mL of the extract. 1 mL of concentrated H_2SO_4 is added along the side of the test tube. The formation of a brown ring is evidence of the presence of cardiac glycosides.

3.3.5 Test for flavonoids:

lkaline reagent test: 2 mL of seaweed extract is treated with 1 mL of 10% sodium hydroxide. A deep yellow colour formation confirms the presence of flavonoids.

3.3.6 Test for phenols:

Lead acetate test: 1.5 mL of seaweed extract is taken in a test tube and 3 mL of 10% lead acetate is added to it. A bulky white precipitate is an indication of the presence of phenol.

3.3.7 Test for steroids:

Ten mL of chloroform is added to 1 mL of seaweed extract after which 10 mL of concentrated sulphuric acid is added along the side of the test tube. The upper chloroform layer becomes red and the sulphuric acid layer becomes yellow with green fluorescence which suggests the presence of steroids in the extract.

3.3.8 Test for terpenoids:

Salkowski test: 2 mL of chloroform is added to 5 mL of seaweed extract and along the side of the tube, few drops of sulphuric acid are added. An interface with reddish-brown colour appears confirming the presence of terpenoids in the sample.

3.3.9 Test for Quinones:

Alcoholic potassium hydroxide is added to seaweed extract. The appearance of red to blue colour shows a positive result for quinones.

3.3.10 Test for Proteins:

Ninhydrin test: 3 mL of the seaweed extract is taken in a test tube and 3 drops of ninhydrin reagent were added. The solution is then heated and the appearance of purple or pink colour confirms the presence of amino acids, proteins, or peptides.

3.4 Anticancer activity

3.4.1. Cell culture

The cell line used in the present study is the human breast cancer cell line, MCF-7 obtained from NCCS. The cells were cultured and maintained in DMEM medium, which was supplemented with 10% of Fetal Bovine Serum (FBS), with streptomycin (100 µg/mL), penicillin (100 IU/mL) at 37ºC, in a humidified environment of 5% CO_2. These cells were later used for cell viability tests.

3.4.2 MTT assay

To determine the effect of seaweed extracts on the proliferation of the adherent cells, an MTT assay was performed. The cells were seeded in a flat-bottom 96-well plate with a density of the cells of 1 x 104 cells/well. After 24 h, these cells were treated with 100 µL of varying concentrations of seaweed extracts (6.25 to 100 µg/mL) and incubated for 24 hrs in a 5% CO_2 atmoshere at 37ºC. Cells treated with 5-fluorouracil (6.25 to 100 µg/mL) act as a reference standard and untreated cells act as a control. After the incubation, 20 µL MTT was added to each well and allowed to stand for 4 hrs at 37ºC in a 5% CO_2 atmosphere. The formazan crystals formed were dissolved in 100 µL of DMSO and the absorbance was measured at 570 nm in a microplate reader and the cell viability is calculated.

Cell viability (%) = [Absorbance of the cells treated/Absorbance of the control cell] x 100

IC50 values (µg) are derived from the dose-response curve at which the concentration of the extract exhibits a 50% decrease in the reduction of MTT when compared to that of the untreated control cells.[17]

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3.5 High Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) analysis:

The TC extract was analyzed qualitatively by HR-LCMS in the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Mumbai. Methanol was used for the preparation of the sample and analysis is performed with Agilent's High-Resolution Liquid Chromatography and Mass Spectrometry model- G6550A for the generation of chemical fingerprints with 0.01% mass resolution. The MS acquisition method was set between a minimum range of 50 (m/z) and a maximum of 1000 Da (m/z) with a scanning rate of one spectrum per second. Column 18 (100 × 1.0 mm, particle size 1.8 µm; Waters) is used for the chromatographic separation. The ejection speed is 100 µl/min with an ancillary speed of 100 µl/min, and injection volume of 8 µl and 5 µl of flush out factor were set in order to perform HR-LCMS. Solvent A consists of methanol: acetonitrile (90:10) and Solvent B -10 mM ammonium acetate in water is used in HR-LCMS.

The mass spectra generated are interpreted using the SAIF database which comprises more than 62,000 patterns. The spectrum of the seaweed extract was identified by comparing it with the spectral pattern available in the SAIF library. [18]

3.5 *In silico* analysis

3.6.1 Preparation of Receptor:

The structure of estrogen receptor alpha (PDB ID: **5DXM)** [19] was retrieved from the protein data bank and used to perform molecular docking. The cocrystallized ligand and water molecules are removed and hydrogen atoms were added to the protein using Pymol [20]. Energy minimization of the receptor is done using Avagadro to exclude the bad contacts.

3.6.2 Molecular docking

Molecular docking was carried out with the AUTODOCK tool since it is considered one of the most widely used docking programs [21]. The molecular interaction between estrogen receptor-alpha and four phytochemicals was performed in AUTODOCK 4.2 [22]. Hydrogen atoms were added and active torsions were assigned to the receptor using AUTODOCK 4.2 tools and the ligands were docked into the binding site of the receptor. The binding pocket of the estrogen receptor-alpha was obtained from the literature work [19]. The region of the binding site was generated within a range of 5 Å. Autogrid was used to create the grid maps around the binding site with 50 x 50 x 50 points and 0.375 Å of grid spacing. Docking parameters were assigned as follows: population size was set to 150, maximum number of energy evaluations- 500,000, number of LGA runs- 100, and the maximum number of generations is 27,000. The final structures of the receptor—phytochemical complex were clustered and ranking is done based on the Autodock scoring function. Docking results with high docking score and best conformational pose is considered for further analysis. The binding affinity between the receptor and phytochemical and its interaction was analyzed and estimation of contact analysis, ligand binding energy, and clustering of the docked conformations were noted.

4.Results and Discussion

4.1 Phytochemical analysis

Qualitative analysis of the phytochemicals in different seaweed extracts reveals the presence of alkaloids, cardiac glycosides, steroids, terpenoids, proteins, phenols, flavonoids, and quinones (Table-2). Cardiac glycosides, steroids, terpenoids, and proteins are present heavily in all the 10 seaweed extracts. Phenols are present in all the extracts except DD and HF. Flavonoids are found in the extracts of GE, GV, PB, SW, UF, DD, and TC, and are absent in GD, HA, and EF. Quinones are present in the extracts of GE, GV, GD, PB, SW, DD, and EF. Alkaloids are found in the extracts of GE, GV, SW, UF, TC, and EF. Saponins and Tannins are not found in any of the seaweed extracts (Figure-1).

Table-1: Seaweeds collected from Coastal regions of Tamil Nadu, India

S.No	Voucher specimen No.	Sample code	Name of Seaweeds	Family
1	GGAB 12-A/2019/SPC NO-127	HA	*Halymenia floresii*	Red Algae
2	GGAB 12-A/2019/SPC NO-128	GE	*Gracilaria edulis*	Red Algae
3	GGAB 12-A/2019/SPC NO-129	GV	*Gracilaria verrucosa*	Red Algae
4	GGAB 12-A/2019/SPC NO-130	GD	*Gracilaria dichotoma*	Red Algae
7	GGAB 12-A/2019/SPC NO-133	PB	*Padina boergesenii*	Brown Algae
8	GGAB 12-A/2019/SPC NO-134	DD	*Dictyota dichotoma*	Brown Algae
9	GGAB 12-A/2019/SPC NO-135	TC	*Turbinaria conoides*	Brown Algae
10	GGAB 12-A/2019/SPC NO-136	SW	*Sargassum wightii*	Brown Algae
11	GGAB 12-A/2019/SPC NO-137	UF	*Ulva fasciata*	Green Algae
12	GGAB 12-A/2019/SPC NO-138	EF	*Enteromorpha flexuosa*	Green Algae

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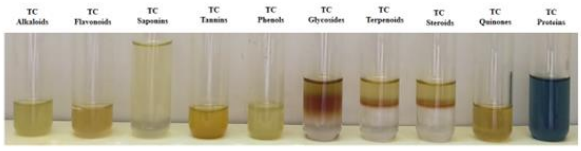
Cardiac glycosides involve in the inhibition of the Na+/K+ pump and rises the calcium ion concentration which causes the contraction of the heart muscle and is hence used in the treatment of heart arrhythmia and heart failure [23]. Terpenoids are found to be a protective factor against oxidative stress and are used to treat many oxidative stress-related diseases [24]. The concentration of protein in the seaweeds varies depending on the seasonal variation and habitat; red algae possess up to 47% of proteins, green algae 9-26%, and in brown algae 3-15% of proteins are seen [25,26].

Table-2 : Phytochemical analysis of different Seaweed extracts

Sample	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
GE	+	+	-	-	+	+	+	+	+	+
GV	+	+	-	-	+	+	+	+	+	+
GD	-	-	-	-	+	+	+	+	+	+
PB	-	+	-	-	+	+	+	+	+	+
SW	+	+	-	-	+	+	+	+	+	+
UF	+	+	-	-	+	+	+	+	-	+
DD	-	+	-	-	-	+	+	+	+	+
HA	-	-	-	-	-	+	+	+	-	+
TC	+	+	-	-	+	+	+	+	-	+
EF	+	-	-	-	+	+	+	+	+	+

These proteins exhibit anti-ageing, anticancer, anti-inflammatory, and antioxidant activities used in the treatment of neurological disease, gastric ulcers, etc [27]. The phenolic compounds in Seaweeds inhibit the growth of tumour cells by inducing alteration in the mitosis at the telophase stage. Some flavonoids change the production of hormones and aromatase inhibition to prevent the progression of cancer cells [28].

Figure -1: Phytochemical analysis of TC



4.2 Anticancer activity

The effects of the seaweed extracts on the viability of the cell were analyzed and compared. MTT assay was performed in estrogen receptor-positive (ER+) MCF-7 cells, in which the cells were treated with 6.25 to 100 µg/mL concentration of different seaweed extracts, and viability of the cells after 24 hrs was determined. [29]. This assay gives information on the metabolic activity of the cell. The cytotoxic activity of different seaweeds against MCF-7 cells with respect to the increase in the concentration of the extract and 5-fluorouracil is shown in Figure 2. From the graph, it is evident that there was a tapering in the intensity which shows a decline in the viability of the cells with an increase in the concentration of the seaweed extracts.

Table-3: IC_{50} values determined by MTT Assay in MCF-7 cell line

Sample	IC_{50} (µg/ml)
PB	>100
DD	31.42
TC	24.68
GE	>100
SW	87.76
GV	>100
UF	>100
GD	90.65
EF	>100
HA	>100
5-FU	4.74

The IC_{50} values of the treated cells were given in Table 3. Following the incubation of 24 hrs with different seaweed extracts, the viability of cells was 71.9% and 45.7% at 12.5 and 25 µg/mL of *T.conoides* extract. This shows that there is a significant decrease in cell growth by 28.1% and 54.3% by *T.conoides* extract when compared to other seaweed extracts. In addition, the IC50 value of *T.conoides* extract was 24.68 µg/mL, which lies between 12.5 and 25 µg/mL, showing high toxicity against the cancer cells. *Tubunaria conoides* is a brown alga that belongs to the family Sargassaceae and order Fucales. Various phytochemicals such as fucosterol, phenolics, and sulfated polysaccharides including fucoidan, alginic acid, neutral glucan, steroids, and flavonoids were present in it [30]. In a study [31], the cytotoxic activity of ethyl acetate extract of *T. conoides* in HepG2 cells lies in the range of 30–83% at the concentration of 80–320 µg/mL, which confirms that TC extract exhibits anticancer properties. The intensity of the cells treated with *T. conoides* extract and 5-Fluorouracil is shown in Figure 3a and 3b respectively. The IC50 values of other seaweed extracts were found to be 31.42 µg for DD extract, 87.76 µg for SW extract, 90.65 µg for GD extract. For PB, GE, GV, UF, EF and HA extracts, the IC50 value is found to be >100 µg.

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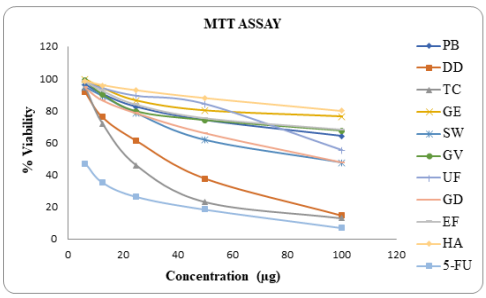

Figure 2: Plot showing Viability of MCF 7 cells after 24 hours treatment with different seaweed extracts and 5 fluorouracil

4.3 Liquid chromatography-mass spectrometry

LC-MS approach is used to identify the semi-polar compounds and active metabolites in the extract of *T. conoides*. Sixteen bioactive compounds were found in the *T. conoides* extract and the LC-MS chromatogram was shown in Figure 4. The retention time, molecular weight, and molecular formula of the identified 16 compounds were given in Table-4.

Table-4: Bioactive compounds determined by LC-MS analysis in the extract *Tubunaria conoides*

S.No	Name of the compound	Molecular formula	Molecular weight	RT
1.	(9-cis)-Retinal	C20 H28 O	284.2143	20.222
2.	DL-**Stachydrine**	C7 H13 N O2	143.0947	1.146
3.	Arecoline	C8 H13 N O2	155.0948	1.163
4.	**Shogaol**	C17 H24 O3	276.1725	17.795
5.	Anacardic acid	C22 H36 O3	348.2666	19.748
6.	9S,13R-12-Oxophytodienoic acid	C18 H28 O3	292.2028	19.082
7.	D-Sphingosine	C18 H37 N O2	299.2825	21.64
8.	Thymine	C5 H6 N2 O2	126.043	1.184
9.	Cholecalciferol	C27 H44 O	384.3392	24.114
10.	6-Gingerol	C17 H26 O4	294.1833	18.059
11.	**Mescaline**	C11 H17 N O3	211.1211	9.639
12.	Betulin	C30 H50 O2	442.3813	23.647
13.	Lignoceric acid	C24 H48 O2	368.3654	25.723
14.	Azelaic acid	C9 H16 O4	188.105	12.894
15.	Suberic acid	C8 H14 O4	174.0893	10.826
16.	D-(-)-**Quinic acid**	C7 H12 O6	192.0636	1.183

Of the 16 compounds, four compounds Mescaline, Quinic acid, Shogaol, and Stachydrine were considered for *in silico* analysis. These compounds were shown to exhibit various biological activities and each compound has a unique sidechain that enables them to exert cytotoxic activity. Mescaline is a phenethylamine alkaloid found in cacti. This compound exhibits psychedelic activity acting mainly on the alpha-2 adrenergic receptor 5-HT1A and 5-HT2A receptors [32]. Quinic acid is a cyclohexane carboxylic acid found to exhibit anticancer, antioxidant, antidiabetic, antimicrobial, antiaging, and analgesic activities. The anticancer effect is exerted by the activation of the apoptosis, reducing the expression of matrix metallopeptidase 9 and inhibition of protein kinase C, activator protein 1 and certain MAPKs [33]. Stachydrine is a pyrrolidine betaine, which has various pharmacological activities such as inhibition of cell proliferation, migration and invasion, reduce oxidative stress, induce apoptosis, decrease inflammation and antifibrotic activity [34]. Shogaol is a polyphenol pungent compound, present in *Zingiber officinale*, derived from gingerols. Various biological activity shown by Shogaol and its isoform 6-Shogaol includes antioxidant, antipyretic, anti-inflammatory, antiobesity, antimicrobial, anticancer, etc [35].

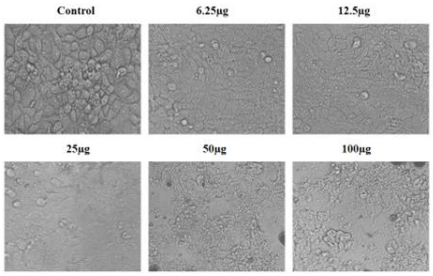

Figure 3a: MTT images showing the intensity of the cells at different concentration of TC extracts (6.25-100µg/mL)

4.4 Molecular Docking

A molecular docking study is essential to find out the interaction between the receptor and ligand. The estrogen receptor alpha structure highlights the key amino acid residues found in the estrogen binding site and its importance in binding. The crystal structure of estrogen receptor alpha (PDB ID: 5DXM) was co-crystallized with a ligand-binding domain complex, cyclofenil derivative, which allowed us to determine the binding affinity of the phytochemicals found in *T. conoides* extract. Based on the co-crystallized ligand, the binding site of the receptor was extended around 5 Å residues. Four phytochemicals namely Mescaline, Quinic acid, Shogaol and Stachydrine were docked into the binding site of the estrogen receptor alpha (Table-5). The phytochemical with the lowest binding energy was found to be Shogaol with -7.15 kCal/mol. The binding energy of other phytochemicals are -6.04 kCal/mol for Mescaline, -3.51 kCal/mol for Quinic acid, -4.03 kCal/mol for Stachydrine (Table-5).

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Table 5: Overall docking results of the ligands with Human estrogen receptor alpha (PDB ID: 5DXM)

Compound name	Binding Energy (Kcal/mol)	Inhibition constant	Hydrogen Bond	Bonding Distance Å	Binding site of Ligand
Shogaol	-7.15	5.78µM	(ARG394)N…H-O (LEU387)O…H-O	2.8 2.8	Hydroxyl group of Shogaol
Mescaline	-6.04	37.65µM	(GLU353)O…H-N (GLU353)O…H-N	3 2.5	Amine group of Mescaline
Stachydrine	-4.03	1.1mM	(LEU387)O…H-O	3.2	Hydroxyl group of Stachydrine
Quinic acid	-3.51	2.69mM	(GLU353)O…H-O (GLU353)O…H-O (LEU346)O…H-O	2.6 2.6 2.8	Hydroxyl group of Quinic acid

The ER-LBD (estrogen receptor-ligand binding domain) contains an estrogen-binding site, ER-LBD dimerization interface and Activation Function-2 (AF-2) site. 12 alpha helices (H1-H12) form the structure of ER-LBD. The H4–6, H8 and H9 are placed in the middle with H7, H10 and H11 on one side and H1 and H3 arranged on the other side. This conformation leads to the generation of a hydrophobic pocket which serves as an estrogen-binding site, into which the estrogen binds. The key amino acids which interacts with the estrogen are MET343, LEU346, ALA350, GLU353, LEU384, LEU387, MET388, LEU391, ARG394, PHE404, MET421, ILE242, LEU428, GLY521, HIS524 and LEU525 [36,37].

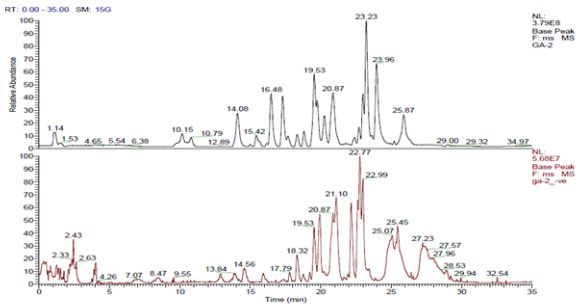

Figure 4: LC-MS Chromatogram of TC extract

In our current study, Shogaol forms two interactions with the residues at the estrogen-binding site of ERα. One hydrogen bond formed between the OH group of Shogaol and N of ARG394 at a bonding distance of 2.8 Å and another hydrogen bond between the OH group of Shogaol and O of LEU387 with a bonding distance of 2.8 Å (figure-5a). These two amino acids were found to be involved in the estrogen-binding site. In addition, hydrophobic interactions with MET343, LEU346, LEU349, LEU387, MET388, LEU391, PHE404, MET421, ILE424, PHE425, LEU428, and LEU525 were found. Shogaol exhibits hydrophilic interactions with THR347, GLU353, ARG394, and HIS524. In vitro studies shows that one of the isoforms of shogaol, 6-shogaol exhibits antiproliferative activity in MCF-7 and T47D cells via the induction of apoptosis and partly contributes to the cell cycle arrest at S- and G2/M-phase[38]. This shows that Shogaol alters the estrogen-mediated signaling pathway and thereby acts as an anticancer agent.

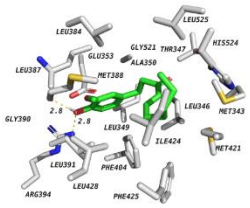

Figure 5a) 3-D Interaction pattern of Human Estrogen receptor alpha and Shogaol

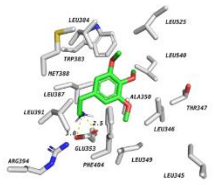

Figure 5b) 3-D Interaction pattern of Human Estrogen receptor alpha and Mescaline

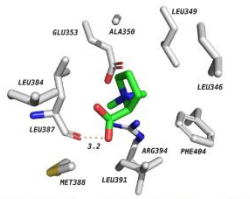

Figure 5c) 3-D Interaction pattern of Human Estrogen receptor alpha and Stachydrine

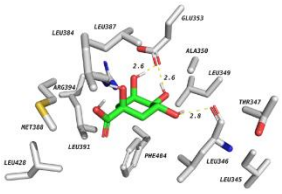

Figure 5d) 3-D Interaction pattern of Human Estrogen receptor alpha and Quinic acid

The 3-D interaction mode of Mescaline was shown in figure 5b. The amine group of Mescaline forms two hydrogen bonds with the OH group of GLU353 at a distance of 3.0 and 2.5 Å and the inhibition constant is found to be 37.65µM. [39]

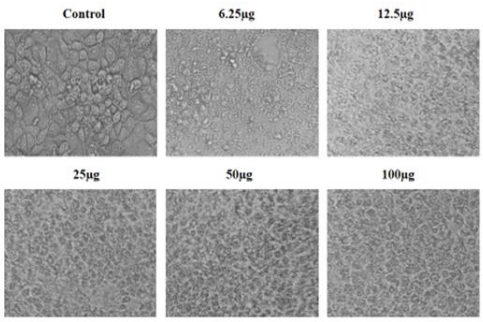

Figure 3b: MTT images showing the intensity of the cells at different concentration of 5-Fluorouracil (6.25-100µg/mL)

Figure 5c shows the 3D interaction of Quinic acid with Human Estrogen receptor alpha. The hydroxyl group of quinic acid is found to form three hydrogen bonds with the key residues at the binding site- one with the OH of GLU353 at a distance of 2.6 Å, with the OH of LEU346 at a distance of 2.8 Å and finally with the OH of GLU353 at a distance of 2.6 Å.

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Stachydrine shows a weak hydrogen bond interaction with LEU387 with a bond distance of 3.2 Å. The bond is formed between the OH of Stachydrine and OH of LEU387 and is shown in figure 5d [40].

5.Conclusion

The results obtained in the current study show the phytochemical richness in different seaweeds, and *Turbinaria conoides* extract has good cytotoxic activity against breast cancer cell line MCF-7 when compared to other extracts. Further, the LC-MS analysis of *Turbinaria conoides* extract identified 16 bioactive compounds, out of which molecular docking was done using Mescaline, Quinic acid, Shogaol and Stachydrine with estrogen receptor alpha. Shogaol has the highest binding affinity with estrogen receptor-alpha, followed by Mescaline, Stachydrine and Quinic acid. From these results, it can be concluded that the cytotoxic activity of *Turbinaria conoides* is due to the synergistic effect of these bioactive compounds and it may act as a source of bioactive compounds that can be used further in in vitro and in vivo analysis for the treatment of cancer.

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Conflict of Interest

Authors have no conflicts to declare

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