Molecular docking analysis of estrogen receptor binding phytocomponents identified from the ethyl acetate extract of *Salicornia herbacea* (L)

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Abstract:
It is of interest to evaluate the secondary metabolites using high performance thin layer chromatography (HPTLC) fingerprinting and Gas chromatography-Mass spectroscopy (GC-MS) in *S. herbacea* extract. The powdered plant material extracted using different solvents were...
used for the qualitative analysis of alkaloids, flavonoids, terpenoids and saponins followed by HPTLC finger printing and GC-MS analysis. The components identified in the GC-MS were docked with estrogen receptor (ER) to identify the binding specificity of isolated compounds. The ethyl acetate extract of \textit{S. herbacea} showed the presence of high number of secondary metabolites when compared to other solvent system. The qualitative analysis of the plant material also showed the presence of carbohydrates, protein, amino acid, phenol, flavonoids, terpenoids, glycosides, saponins and steroids. The HPTLC finger printing analysis revealed the existence of alkaloid, flavonoid, terpenoid and saponin compounds and GC-MS. GC-MS was performed to identify the phytoconstituents constituents in the extract. 8 phyto compounds were identified to analyse binding with ER. The binding affinity score (-6.8 kcal/mol) and interacting ER residues (28) the phyto compound di-n-octyl phthalate showed best docking score with ER α than the standard drugs lasofoxifene, and 4-hydroxytamoxifen. The binding affinity and number of interacting ER residues was -6.9 kcal/mol; 10 and -6.2; 11, respectively. The results identified the presence of ER antagonist in \textit{S. herbacea} and warrants further investigation to explore for treating ER regulated diseases.

**Keywords:** \textit{S. herbacea}, HPTLC, GC-MS, Phytoconstituents, ER, and di-n-octyl phthalate.

**Background:**

Plants are genuinely utilized as a tool for the extraction and isolation of active compounds that resulted in the discovery of new drugs with high therapeutic values [1]. Medicinal plants can act in a symbiotic manner within the human body and present exclusive therapeutic properties with minimal or no desired side effects [2]. Different phytoconstituents of herbal products are documented to be beneficial for the treatment of diseases caused by free radicals. It also protects the body from tissue injury [3]. Phytochemicals comprises the secondary compounds of plant where as the chlorophyll, proteins and common sugars are included in the primary constituents. Phytochemicals include terpenoids, alkaloids, glycosides and phenolic compounds [4]. The latter exhibits the various important pharmacological activities in oxidative stress, inflammation, diabetes, asthma, hepatitis, cancer and gastro [5, 6]. HPTLC and High performance liquid chromatography (HPLC), emerge as the efficient tools for phytochemical evaluation, and enable the analysis of several samples simultaneously [7]. It reduces both time and cost analysis and ensures the reusability of identified spots for quantification by densitometry in a specific track, called as a fingerprint [8-10]. \textit{S. herbacea}, commonly known as pickle wees glasswort, belongs to \textit{Amaranthaceae} family [11]. The name \textit{S. herbacea} has been originated from the Latin word meaning ‘salt’. Studies have been reported that some species, of \textit{S. herbacea} shows tolerance towards salinity as high as 3 % NaCl [12]. This plant is found at the edges of wetlands, marshes, sea shores, and mudflats [13]. ER has provided us with a powerful prognostic and therapeutic marker as well as a promising target for anti-estrogen treatment for hormone-dependent breast cancer [14,15] for drug discovery [16-17]. Therefore, it is of interest to evaluate the secondary metabolites using high performance thin layer chromatography (HPTLC) finger printing and Gas chromatography-Mass spectroscopy (GC-MS) in \textit{S. herbacea} extract.

**Materials and Methods:**

**Plant collection and authentication:**

\textit{S. herbacea} was collected from Ennore area, Chennai. The species was authenticated by Professor Jayaraman, Plant Anatomy Research Center, West Thambaram, and Chennai and assigned a (Voucher number: PARC/2014/2028).
become as follows: 60°C (2 min); observed by using 300°C on the charge of 10°C min–1; and 300°C, where it became held for six minutes. The mass detector situations including switch line temperature 240°C; ion source temperature 24°C; and ionization mode electron effect at 70 eV, an experiment time 0.2 sec and test c language of 0.1 sec. The fragments are from 40 to 600 Da. The spectrums of the components have been as compared with the database of the spectrum of the recognized additives saved in the GC-MS NIST (2008) library [27] [21].

**Target preparation and Ligand Library:**
The crystallographic structure of ER (PDB ID: 6VJD) alpha ligand-binding domain was retrieved from the protein data bank (PDB) with the ligand lasfoxofene. The downloaded ER structure was edited to remove the ligand lasfoxofene and water molecules using the Discovery Studio Visualizer v19.1.0.18287 (www.accelerys.com) and again saved in PDB format. The major phytoconstituents present in *S. herbacea* were retrieved from the protein data bank (PDB) = 76.405641152 for molecular docking. The significant interaction were set as size x = 60.6778268703, size y = 72.6589010655 and size z = 32.9641152 for molecular docking. Also, the native ligand lasfoxofene and 4-hydroxymxifen were selected as standard drugs and docked with ER to compare the effect of *S. herbacea* phytoconstituents.

**Molecular Docking:**
After preparing the phytoconstituents as ligands and ER as a target, PyRx was implied with the Autodock Vina option using the new scoring function [23]. It analyzes the docking orientations and interactions between the ligands and ER. The gird box properties were set as size x = 60.6778268703; size y = 72.6589010655 and size z = 76.405641152 for molecular docking. The significant interaction between the ligands and the receptors-binding site were acquired in 2D and 3D format by importing the docked results into the LigPlot+, PyMol and Discovery studio visualizer v19.1.0.18287 (www.accelerys.com).

**Evaluation of Ligands Drug likeness and Toxicity:**
The screened ligands were evaluated for the drugability, physicochemical properties, toxicity, toxicity classes, and lethal dose using Molinspiration server (www.molinspiration.com/cgi-bin/properties). The drugability properties were analyzed based on the Molar Weights (MW), Total polar surface area (TPSA), lipophilicity (log P), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD) to identify Lipinski’s rule of the drug-like compounds. In addition, the Simplified Molecular Input Line Entry System (SMILES) were downloaded from the PubChem Database to calculate ADMET properties with toxicity class. The ADMET properties were calculated by implementing ADME Tlab 2.0 [24].

### Table 1: Phytochemical screening of *S. herbacea*

| Solvent extraction | AL | FL | TP | AP | CH | CS | SA | OF | TN | ST |
|--------------------|----|----|----|----|----|----|----|----|----|----|
| Petroleum ether    | -  | +  | -  | -  | +  | +  | +  | +  | +  | +  |
| Chloroform         | -  | +  | -  | -  | +  | -  | +  | +  | +  | +  |
| Ethyl acetate      | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| Ethanol            | -  | -  | -  | +  | -  | -  | +  | +  | +  | +  |
| Aqueous            | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  |

AL = Alkaloids; GS-Glycosides; SA = Saponins; OF = Oils and Fats; TP = Tannin and phenolic compounds; TN = Terpenoids; FL = Flavonoids; AP = Amino acids and Proteins; ST = Steroids; CH = Carbohydrates; “+” Present; “-” Absent

### Table 2: Percentage yields of *S. herbacea*

| S.No | Solvents     | % yield of *S. herbacea* |
|------|--------------|-------------------------|
| 1    | Petroleum ether | 0.628                  |
| 2    | Chloroform    | 2.418                   |
| 3    | Ethyl acetate | 5.885                   |
| 4    | Ethanol       | 4.548                   |
| 5    | Water         | 3.121                   |

### Table 3: Peak Table of alkaloids and unknown compounds in ethyl acetate extract of *S. herbacea*

| Track | Peak | Rf  | Height | Area | Assigned substance |
|-------|------|-----|--------|------|-------------------|
| Sample A | 1    | 0.06 | 444.6  | 3263.1 | Unknown           |
| Sample A | 2    | 0.84 | 274.1  | 15988.4 | Unknown           |
| Sample A | 3    | 0.92 | 352.7  | 16028.5 | Alkaloid 1       |
| Sample A | 4    | 0.94 | 352.8  | 13731.2 | Unknown           |
| STD    | 1    | 0.34 | 516.4  | 49307.7 | Colchicine       |

### Table 4: Peak Table of flavonoids and unknown compounds in ethyl acetate extract of *S. herbacea*

| Track | Peak | Rf  | Height | Area | Assigned substance |
|-------|------|-----|--------|------|-------------------|
| Sample A | 1    | 0.07 | 59.7   | 482  | Unknown           |
| Sample A | 2    | 0.23 | 18.8   | 677.6 | Unknown           |
| Sample A | 3    | 0.38 | 16.4   | 147.7 | Unknown           |
| Sample A | 4    | 0.92 | 294.3  | 13630.8 | Saponin         |
| STD    | 1    | 0.41 | 98.2   | 4235.7 | Saponin 1       |

### Table 5: Peak Table of saponin and unknown compounds in ethyl acetate extract of *S. herbacea*

| Track | Peak | Rf  | Height | Area | Assigned substance |
|-------|------|-----|--------|------|-------------------|
| Sample A | 1    | 0.07 | 159   | 7192.2 | Unknown           |
| Sample A | 2    | 0.16 | 15    | 177.1  | Unknown           |
| Sample A | 3    | 0.22 | 18.8  | 677.6  | Unknown           |
| Sample A | 4    | 0.38 | 16.4  | 147.7  | Unknown           |
| Sample A | 5    | 0.92 | 294.3 | 13630.8 | Saponin         |
| STD    | 1    | 0.41 | 98.2  | 4235.7 | Saponin 1       |

### Table 6: Peak Table of terpenoid and unknown compounds in ethyl acetate extract of *S. herbacea*

| Track | Peak | Rf  | Height | Area | Assigned substance |
|-------|------|-----|--------|------|-------------------|
| Sample A | 1    | 0.01 | 41.7   | 307.5 | Unknown           |
| Sample A | 2    | 0.07 | 716.2  | 26263.9 | Unknown          |
| Sample A | 3    | 0.2  | 64.2   | 2550.3 | Unknown           |
| Sample A | 4    | 0.25 | 54.6   | 1651   | Unknown           |
| Sample A | 5    | 0.44 | 16.5   | 399.4  | Terpenoid 1      |
| Sample A | 6    | 0.54 | 21.9   | 622.6  | Terpenoid 2      |
| Sample A | 7    | 0.7  | 38.2   | 968.8  | Unknown           |
| Sample A | 8    | 0.76 | 104.5  | 4353.1 | Terpenoid 3      |
| Sample A | 9    | 0.86 | 239.7  | 101611.4 | Terpenoid 4     |
| Sample A | 10   | 0.97 | 160.2  | 3035.3 | Terpenoid 5      |
| STD    | 1    | 0.89 | 325.2  | 91486.1 | Luteolin        |

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Table 7: Important bioactive compounds in *S. herbaea* ethyl acetate extract identified through GC-MS

| S. No | Compound Name | % of Peak Area | Retention time (RT) | Molecular formula (MF) | Molecular weight (MW) |
|-------|---------------|----------------|---------------------|------------------------|-----------------------|
| 1.    | 2-PENTADECANONE, 6,10,14-TRIMETHYL | 23.68 | 17.85 | C₉H₁₈O | 168 |
| 2.    | N-HEXADECANIC ACID | 23.68 | 19.15 | C₉H₁₈O₂ | 166 |
| 3.    | 9,9-DIMETHYLBICYCLO[3.3.1]NONA-2,4-DIONE | 7.05 | 20.02 | C₁₀H₁₄O₂ | 174 |
| 4.    | OLEIC ACID | 39.65 | 20.75 | C₁₈H₃₁O₂ | 282 |
| 5.    | 1-HEXYL-2-NITROCYCLOHEXANE | 5.26 | 21.91 | C₁₈H₂₉O₄N | 213 |
| 6.    | 16-HEPTADECEN | 4.67 | 22.30 | C₁₈H₃₆O₂ | 252 |
| 7.    | DI-N-OCTYL PHENAL | 3.50 | 22.96 | C₂₀H₃₃O₄ | 390 |
| 8.    | BICYCLO[3.2.1]OCT-3-EN-2-ONE, 3,8-DIHYDROXY-1-METHOXY-7-(7-METHOXY-3,5- | 2.94 | 23.04 | C₇H₁₉O₄ | 388 |

Table 8: Binding affinity, RMSD and interacting residues of the screened phytocompounds against ER

| Plant | Physicochemical properties | Sigma /Cation-Excluded Interaction | Hydrophilic interaction |
|-------|---------------------------|----------------------------------|-------------------------|
|   | | | |

Table 9: The identification of drug-likeness and ADMET properties for the selected standard drug and phytocompound inhibits ER against breast cancer

| ADMET Properties | Standard Drug | S. herbaea |
|------------------|---------------|------------|
| **Physicochemical properties** | Lasofoxifene | 4-hydroxytamoxifen | Di-n-octyl phthalate |
| MW | 413.5 | 387.52 | 390.56 |
| mLogp | 6.07 | 5.58 | 8.39 |
| TPSA | 32.70 | 32.70 | 52.61 |
| Natoms | 31 | 29 | 28 |
| Non | 3 | 3 | 4 |
| Nohm | 1 | 1 | 0 |
| Nolb | 6 | 8 | 18 |
| Nivcad | 1 | 1 | 1 |

| **Absorption** | **Papp** (Caco-2 Permeability) | **Pgp** (Pgp-inhibitor) | **Pgp** (Pgp-substrate) |
|----------------|-------------------------------|-------------------------|------------------------|
| | -5.141 cm/s | -5.022 cm/s | 4.733 cm/s |
| | 0.935 | 0.706 | 0.647 |
| | 6.57 | 0.09 | 0.019 |
| | 0.516 | 0.089 | 0.019 |

| **Distribution** | **PB** (Plasma Protein Binding) | **VD** (Volume Distribution) | **BBB** (Blood-Brain Barrier) |
|------------------|--------------------------------|-----------------------------|-----------------------------|
| | 89.32 % | 93.815 % | 89.022 % |
| | 0.962 L/kg | 0.869 L/kg | -0.574 L/kg |
| | 0.915 | 0.7 | 0.995 |

| **Metabolism** | **P450 CYP1A2 inhibitor** | **P450 CYP1A2 Substrate** | **P450 CYP2C19 inhibitor** |
|----------------|---------------------------|---------------------------|---------------------------|
| | 0.218 | 0.337 | 0.976 |
| | 0.618 | 0.722 | 0.561 |
| | 0.19 | 0.088 | 0.051 |
| | 0.654 | 0.356 | 0.4 |
| | 0.287 | 0.697 | 0.369 |
| | 0.466 | 0.839 | 0.443 |

| **Elimination** | **T1/2 (Half-Life Time)** | **CL (Clearance Rate)** |
|-----------------|---------------------------|------------------------|
| | 2.073 h | 2.243 h | 1.649 h |
| | 1.826 mL/min/kg | 1.704 mL/min/kg | 1.394 mL/min/kg |

| **Toxicity** | **hERG (hERG Blockers)** | **H-HT (Human Hepatotoxicity)** | **Skin sensitization** |
|--------------|---------------------------|-----------------------------|---------------------|
| | 0.091 | 0.095 | 0.064 |
| | 0.868 | 0.96 | 0.2 |
| | 0.375 | 0.509 | 0.002 |
| | 2.576 log mol/kg (1097.841 mg/kg) | 2.581 log mol/kg (1016.945 mg/kg) | 0.484 |
| | 0.19 | 0.62 | 0.352 |
Results and Discussion:
The phytochemical screening of the medicinal plants are important since it have commercial interest in both research institutes and pharmaceutical companies in manufacturing the novel drugs used for treatment of various diseases. The preliminary phytochemical screening of *S. herbacea* shows the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, glycosides, saponins, steroids, phenol, tannins and terpenoids. The results of phytochemical analysis are tabulated in the Table 1. Among the various extracts most of the phytochemicals are found in ethyl acetate extract of *S. herbacea*. The Table 2 shows how the percentage yield of the different extracts of *S. herbacea* is. HPTLC is an effective analytical method. This method is visual, speedy and reasonable as it utilizes smaller quantities of solvents with minimum sample smooth up. Particularly, in a brief duration a huge number of samples are analyzed simultaneously [25]. HPTLC profile of ethyl acetate extract of *S. herbacea* was recorded in Tables 3, 4, 5, 6 and Figure 1-8 for alkaloids, flavonoids, saponins and terpenoids respectively. The extracts had been run at the side of the standards consisting of colchicine, quercetin, lupeol and saponin respectively.

Figure 1: HPTLC chromatogram showing the presence of fractionated alkaloids from ethyl acetate extract of *S. herbacea*; Before derivatization under visible light, 366 nm and 254 nm and after derivatization under day light 1, 2 and 366 nm.

GC-MS evaluation led to the identity of wide variety of compounds from the GC fractions of the ethyl acetate extract of *S. herbacea*. These compounds were diagnosed through MS attached with GC. The compounds gift within the ethyl acetate extract of *S. herbacea* diagnosed via GC-MS evaluation as shown in Figure 9. The active standards with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) within the ethyl acetate one extract of *S. herbacea* are presented in Table 7. The prevailing compounds in the ethyl acetate extract were identified using library prediction as 2-PENTADECANONE, 6, 10, 14-TRIMETHYL23.68 % N-HEXADECANOIC ACID 23.68% OLEIC ACID 39.65%, 1-HEXYL-2-5.26%, 16-HEPTADECENAL 4.67%, DI-N-OCTYL PHthalate 3.50%, BICYCLO [3.2.1] OCT-3-EN-2-ONE, 3,8-DIHYDROXY-1-METHOXY-7-(7-METHOXY-1,3-2.94%. The phyto compounds and their biological activities obtained through GC-MS study of *S. herbacea*

Figure 2: Densitogram display for the alkaloid profile of *S. herbacea* (B) and standard (A)

Figure 3: Chromatograms of ethyl acetate extract of *S. herbacea* in HPTLC analysis. Before derivatization under visible light, 366 nm and 254 nm; after derivatization under visible light and 366 nm.

Figure 4: Densitogram display for the flavonoid profile of *S. herbacea* (B) and standard (A)
**Figure 5:** Chromatograms of ethyl acetate extract of *S. herbacea* in HPTLC analysis—Before derivatization under visible light, 366 nm and 254 nm and after derivatization under visible light and 366 nm.

**Figure 6:** Densitogram display for the saponin profile of *S. herbacea* (B) and standard (A)

**Figure 7:** Chromatograms of ethyl acetate extract of *S. herbacea* in HPTLC analysis—Before derivatization under visible light, 366 nm and 254 nm and after derivatization under visible light and 366 nm.

**Figure 8:** Densitogram display for the terpenoid profile of *S. herbacea* (B) and standard (A)

**Figure 9:** GC-MS Spectrum of *S. herbacea* ethyl acetate extract

**Figure 10:** The structure of the study protein PDB: 6VJD consisted Chain A, B, C and D. The phytocomponent of DI-N-OCTYL PHTHALATE has highly binding affinity to ER as evidenced through molecular docking analysis. The crystallographic structure of ER (PDB ID: 6VJD) a ligand-binding domain consisting of chain A, chain B, chain C and Chain D (Fanning, 2020) were depicted.
Figure 11: The Ligplot for the interaction of ER and the screened phyto-compounds extracted from S. herbacea

According to the phyto-component of DI-N-OCTYL PHTHALATE has highly binding affinity to ER as evidenced through molecular docking analysis. The crystallographic structure of ER (PDB ID: 6VJD) α ligand-binding domain consisting of chain A, chain B, chain C and Chain D [26] were depicted in Figure 10. The docked phyto compounds and target interacting residues with their binding affinity were tabulated in Table 8. The assessment of crude extract is an imperative a part of accurate identification. HPTLC is useful as a phytochemical marker and more effective in the field of plant taxonomy and also for the identification of plant secondary metabolites [27]. HPTLC finger printing is proved to be a linear, unique, and correct technique for herbal identification. Such finger printing is useful in the quality control of herbal products and checking for the adulterants. Therefore, it may be beneficial for the assessment of various advertised pharmaceutical preparations. HPTLC profiles also show the occurrence of secondary metabolites of medicinal importance which support the traditional therapeutic uses of the plant species [28]. The qualitative analysis of ethyl acetate extracts of S. herbacea through HPTLC confirmed the presence of many secondary metabolites like alkaloids, flavonoids, saponins, and terpenoids (Figure 1-8).

Four compounds with Rf values of 0.06, 0.84, 0.92, 0.94, are detected along with 3 unknown compounds (Table 3). In S. herbacea ethyl acetate extract in chromatogram (Figure 1 and 2). Orange, brown colored zone at visible mode is observed in the tracks which after a derivatization of brownish violet at 366 nm confirms the presence of alkaloid compound in the samples. The Table demonstrates that alkaloid numbered as 3 found to be maximum in its concentration. Alkaloids constitute one of the major groups of plant constituents. The mobile phase of used was ethyl acetate: methanol: water (10: 1.35: 1) for the alkaloid profiling. They represent one of the largest and most diverse families of the natural compound [29].

Figure 12: The docking pose of the ER with the most effective phytocompounds based on the binding affinity and interacting residues. (A, E, I) The docking pose with lasofoxifene, 4-hydroxytamoxifen and di-n-octyl phthalate respectively. (B, F, J) The hydrogen donar and acceptor of the intercting residues (green : acceptor; purple : donor). (C, G, K) The type of bonds involved in interacting phytocompounds ER residues. (D, H, L) The Ligplot interaction for the phytocompounds docked with ER residues.

HPTLC of the ethyl acetate extract of S. herbacea plant Table 4. Shows four peak areas, with four different Rf values. Among them, one peak shows the presence of flavonoid. With a Rf value of 0.85. The Figure 3 and 4 exhibit the chromatogram and yellow and yellowish blue colored fluorescent zone at the 366 nm mode after derivatization confirms the presence of flavonoids in the sample Figure 3. The mobile phase used is Ethyl acetate-Butanone-Formic acid-Water (5:3:1:1). Flavonoids are the most important natural phenolic and they possess a broad spectrum of chemical and biological activities including free radical scavenging properties. The flavonoids in plants have been reported to exert multiple
biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic effect, etc. [30].

The Table 5 represents the saponin HPTLC profile of ethyl acetate extract of S. herbacea plant. In this profile, one standard is used, and one saponin is identified in the chromatogram of the extract. The Rf values in the reference standard and extract are found to be 0.07, 0.16, 0.22, 0.38, 0.92 and 041. Figure 5 and 6 and peak 5 indicates the presence of saponin in S. herbacea plant. The band reveals the presence of saponin by its green, yellow, and blue colored zones at daylight mode after derivatization. Mobile phase consisting of chloroform: glacial acetic acid : methanol: water (6.4: 3.2: 1.2: 0.8) was used for profiling. Presence of saponins is important, since it exhibits a wide range of biological activities in controlling diabetes, cancer, bone health and stimulation of the immune system [31].

The chromatographic fingerprinting for terpenoids is well resolved at 366 nm after derivatization Figure 7 and 8. The plates are sprayed with anisaldehyde sulphuric acid reagent followed by heating and visualized in day light which shows 10 prominent peaks in ethyl acetate extract. The 5, 6, 8, 9, 10 peptides detect in the ethyl acetate extracts are identified as terpenoid and the best solvent system to scrutinize the above partition is n-hexane: ethyl acetate (7.2: 2.9). Most of the terpenoids are of plant origin; however, they are also synthesized by other organisms, such as bacteria and yeast as part of the primary or secondary metabolism. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including oxidative stress, inflammation, diabetes, asthma, hepatitis, and cancer and gastroenteritis. A number of terpenoids exhibit cytotoxicity against an expansion of tumor cells and most cancers preventive in addition to anticancer efficacy in preclinical animal model [32].

GC-MS analysis caused the identification of wide variety of compounds from the GC fractions of the ethyl acetate extract of S. herbacea. These compounds have been diagnosed via MS connected with GC. The compounds present within the ethyl acetate extract of S. herbacea identified through GC-MS evaluation as shown in Figure 9. The active concepts with their RT, molecular formula, MW and awareness (%) inside the ethyl acetate one extract of S. herbacea are supplied in Table 7. The prevailing compounds in the ethyl acetate extract were 2-PENTADECANONE, 6,10,14-TRIMETHYLET2.68 % N-HEXADECANOIC ACID 23.68 % OLEIC ACID 39.65 %, 2-PENTADECANONE, 6, 10, 14-TRIMETHYL2.68 % N-HEXADECANOIC ACID 23.68 % OLEIC ACID 39.65 %, 1-HEXYL-2-5.26%,16-HEPTADECANOL 4.67 %, DI-N-OCTYL PHTHALATE 3.50 %, BICYCLO[3.2.1]OCT-3-EN-2-ONE, 3,8-DIHYDROXY-1-METHOXY-7-(7-METHOXY-1,3)-significantly formed the hydrogen bond with SER512 and pi-sigma bond with ARG515. It also extends the alkyl interactions with HIS516, LEU511 and is surrounded by the hydrophobic residues ASN455 and ASN519 with binding affinity -5.5 and RMSD 2.366 Å. The interacting residues with the selected standard drugs and other phytocompounds extracted from the S. herbacea were analyzed by LigPlot (Figure 11). The results explain that the compound di-n-octyl phthalate significantly inhibits ER than other phytocompounds from S. herbacea. The standard drug lasofoxifene-ER (Figure 11a), 4-hydroxytamoxifen-ER (Figure 11e), and di-n-octyl phthalate-ER (Figure 11f) docked complex structure depicted in Figure 11. Also, the nature of hydrogen bondonar and acceptor (Figure 11b, d, i), the 2D structure of the drug complex (Figure 11c, g, k), and interacting residues by LigPlot (Figure 11d, h, l) were delineated to identify the selected drug efficacy. The results evidently demonstrates that di-n-octyl phthalate shows potential inhibition effect against ER based on the binding affinity (-6.8 kcal/mol), RMSD (1.68 Å), and number of residues (No. 24) than the lasofoxifene (binding affinity = -6.9; RMSD = 4.741; No. = 10) and 4-hydroxytamoxifen (binding affinity = -6.2; RMSD = 1.941 ; No. = 11).

The native ligand lasofoxifene formed the hydrogen bond interaction with ARG434, alkyl interaction with ALA505, LEU509, ILE510 and hydrophobic interaction with ALA430, THR431, GLN506 and SER512 residues with the binding affinity of -6.9 kcal/mol and RMSD 4.741. The standard drug 4-hydroxytamoxifen formed a carbon-hydrogen bond with SER512* and alkyl interaction with LEU508 and LEU509. It is also surrounded by the hydrophobic residues ILE451, ASN455, TYR459, LEU479, THR483, ALA505, LEU511, and ARG515 with the predicted binding affinity of -6.2 kcal/mol and RMSD 1.941 Å. The phytocompound 2-Pentadecanone, 6,10,14-trimethyl interacted with HIS513* and HIS513 via carbon-hydrogen and pi-sigma bond. Also, it extends alkyl interaction with ARG434, LEU509, ILE510, HIS513 and is surrounded by the hydrophobic residues ALA430, THR431, SER512, and HIS516. The predicted binding affinity was noted as -4.2 kcal/mol with the RMSD 2.431 Å. The N-Hexadecanoic acid exhibits alkyl interaction with ARG434, ILE510, HIS513 and is surrounded by hydrophobic residues such as ALA430 and THR431 LEU509, SER512, ARG515, HIS516, and ASN519. Also, the predicted binding affinity was observed as -3.9 kcal/mol with the RMSD 1.780 Å. The 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione interacted with LEU346*and GLY521* via carbon-hydrogen bond and extended alkyl interactions with ALA350 and LEU525. It is also surrounded by the hydrophobic residues MET343, THR347, TRP383, LEU384, LEU387, MET388, PHE404, MET421, and ILE424 with the predicted binding affinity of -5.8 kcal/mol and RMSD of 1.946. The oleic acid interacted with ALA430, ARG434, ILE510 and HIS513 through the
alkyl interactions and was surrounded by the hydrophobic residues THR431, SER433, LEU509, SER512, and HIS516. Also, the predicted binding affinity was identified as -4.8 kcal/mol and the RMSD 2.02 Å. The 1-Hexyl-2-nitrocyclohexane formed the carbon-hydrogen bond with GLY521 and establishes alkyl interaction with LEU346, ALA350, MET388, LEU391, PHE404, ILE424, and LEU428. It is also surrounded by hydrophobic residues such as MET343, LEU384, LEU387, MET421, PHE425, and LEU525 with the predicted binding affinity of -5.8 kcal/mol and RMSD 1.581 Å.

In recent years, the complexity and risks of drug discovery and development procedures have grown significantly, resulting in lower productivity. The pharmaceutical industry's productivity is declining due to poor development procedures have grown significantly, resulting in lower productivity. The pharmaceutical industry's productivity is declining due to poor development procedures [34, 35]. The ADME (absorption, distribution, metabolism, excretion, and biopharmaceutical industry's productivity is declining due to poor development procedures. The ADME properties for di-n-octyl phthalate and also for the selected standard drug lasofoxifene, 4-hydroxytamoxifen, and di-n-octyl phthalate is -5.141 cm/s, -4.8 kcal/mol, and the RMSD 2.073 h, 2.243 h and 1.649 h, respectively, which explains the necessity of frequent doses in treatment. However, the phyto compound di-n-octyl phthalate extracted from the S. herbacea might be consumed as a decoction. Clearance rate (CL) is a proportionality factor that relates the concentration of drug measured in the body to the elimination rate. The identified clearance rate for lasofoxifene, 4-hydroxytamoxifen, and di-n-octyl phthalate was 1.826, 1.704, and 1.394 ml/min/kg. It described that the clearance rate was low might sustain in plasma for a long time.

The hepatotoxicity (from hepatic toxicity) implies chemical-driven liver damage. The predicted hepatotoxic probability for the selected standard drugs lasofoxifene, 4-hydroxytamoxifen, and di-n-octyl phthalate was 0.868, 0.96, and 0.2. It distinctly describes that the standard drug will lead to high liver injury, and the phyto compound di-n-octyl phthalate is a hepatic-friendly drug.

Conclusion:
Phyto constituents are identified by qualitative methods and the identified phyto constituents are ascertained using HPTLC. Ethyl acetate extract of S. herbacea plant is rich in terpenoids compounds with biological activities. The data based on the HPTLC finger print approach which can also be proposed as a quick and reliable analytic model for the pharmacognostic study of plant raw materials used in commercial products. Hence, the extracted phyto compounds from the plant S. herbacea using ethyl acetate was analyzed using molecular docking with ER. The phyto compound di-n-octyl phthalate extracted from the S. herbacea had the highest docking score towards ER. Furthermore, the ADME/T characteristics of the di-n-octyl phthalate revealed that it might be deemed a potential drug-like chemical. The di-n-octyl phthalate is widely accessible, allowing for the earlier development of appropriate medications against estrogen driven breast cancer.

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Conflict of interests:
We declare that we have no conflict of interest
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