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Phytochemical Screening, *In Silico* Molecular Docking, ADME Properties, and *In Vitro* Antioxidant, Anticancer, and Antidiabetic Activity of Marine Halophyte *Suaeda maritima* (L.) Dumort

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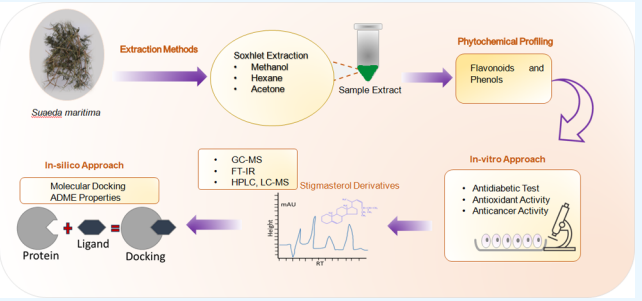
ABSTRACT: Medicinally valuable components derived from natural resources are highly desirable as prospective alternatives to synthetic drugs to treat fatal diseases, such as cancer and diabetes mellitus. *Suaeda maritima* (L.) Dumort (*Amaranthaceae*) (*S. maritima*) is a halophyte plant that can thrive in saline environments and possesses excellent medicinal properties. Hence, for the present investigation, *S. maritima* has been chosen, and its phytochemical constituents have been extracted utilizing various solvents, including hexane, acetone, and methanol, and identified by GC-MS, LC-MS, and HPLC analyses. The antioxidant activity of the compounds using DPPH, ABTS, and reducing power assays demonstrated that all three extracts of *S. maritima* possessed significant radical scavenging activity comparable to standard ascorbic acid with lower IC_{50} values (69.20−95.58 μg/mL). In addition, the evaluation of antidiabetic activity by α-amylase inhibition and α-glucosidase inhibition methods revealed that the acetone extract of *S. maritima* (SMAE) displayed equipotent activity of standard acarbose with an IC_{50} of 32.6 μg/mL. Advantageously, SMAE also exhibited better inhibition activity against the growth of lung cancer cells with an IC_{50} of 78.19. μg/mL and less toxicity on the noncancerous HUVEC cells with a high IC_{50} of 300 μg/mL. In addition, the cancer cell death mechanism via the apoptotic pathway induced by SMAE was confirmed by DAPI staining and ROS analysis. The analysis of ADME properties, including absorption, distribution, metabolism, and excretion, witnessed that the physicochemical and druglikeness factors were best catered by stigmasterol, γ-sitosterol, and vitamin E. Further, the key phytochemicals identified from SMAE were docked with CtBP1 and SOX2 bound to importin-α target proteins associated with carcinogenic pathways using Schrodinger software. The results showed that the phytochemicals, scilicet, stigmasterol, γ-sitosterol, octadecadienoic acid, and vitamin E, showed a good binding affinity with Glide scores in the range −2.845−4.018 kcal/mol. Overall, the findings support that the least investigated traditional edible medicinal mangrove-related *S. maritima* is high in pharmacologically active constituents and might be one of the finest sources of naturally derived molecules for drug development and delivery systems.



1. INTRODUCTION

Cancer and diabetes mellitus, recognized as formidable and life-threatening health challenges in the modern era, significantly influence the global public health domain, resulting in 9.6 million and 1.6 million annual deaths worldwide, respectively.[1] Lung cancer stands as the most commonly diagnosed cancer in both men and women, and is also the leading cause of cancer-related deaths. Nevertheless, there exists significant disparity in the occurrence and fatality rates of lung cancer globally, which can be attributed to diverse trends in tobacco consumption, exposures to environmental risks, and hereditary factors.[2] Besides, diabetes mellitus, a chronic metabolic disorder characterized by elevated blood sugar levels, is associated with various disease complications, including cardiovascular disease, atherosclerosis, arterial disease, kidney failure, retinopathy, and neurological disorders.[3] Clinicians have been reporting the prevalence of patients with diabetes and cancer for over 50 years. Epidemiologic data indicate that type 2 diabetes and cancer share several risk factors and that people with diabetes have a significantly increased risk of several types of cancer. Additionally, observational studies witness that several drugs used to treat

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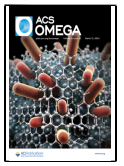

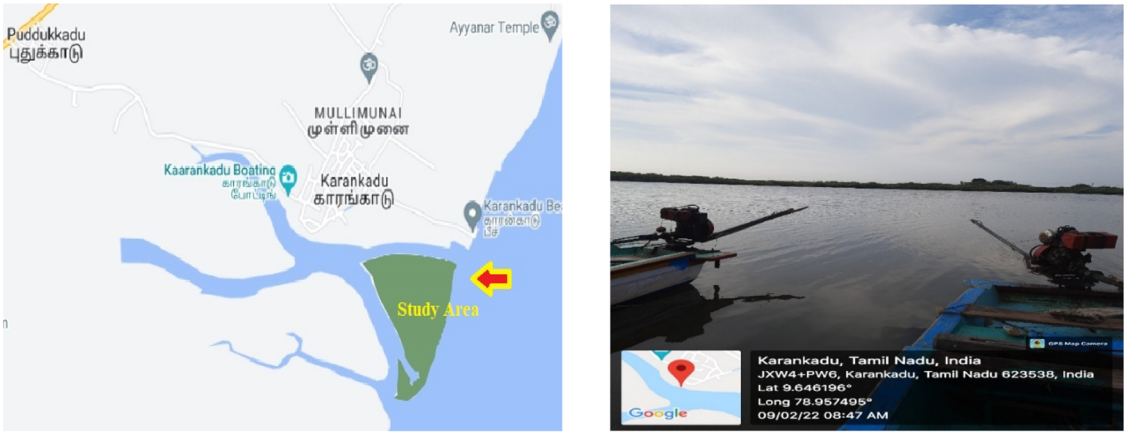

Figure 1. Study area of *S. maritima* plants from Karankaadu, Chitruvadi, and Ramnad district (Lat 9.646196°, Long 78.957495°) using QGIS Software (version 3.26).

hyperglycemia may be associated with a risk of liver and pancreatic cancer.[4]

Although numerous modern treatment methods have been developed, cancer is frequently futile due to medication resistance and distant metastases.[5] Further, chemically produced medications are used to mitigate the effects of diabetes, causing unwanted side effects such as obesity, gastrointestinal difficulties, and heart diseases. Consequently, contemporary synthetic and chemical medicines are cautiously approached due to their propensity to manifest adverse effects.[6]

Active pharmaceutical compounds derived from natural products and their synthetic derivatives significantly involved in treating human ailments are well-known. Nature bestows enormous bioactive compounds, and plants are a prodigious source of these bioactive compounds utilized for food and herbal medicine. For instance, nearly 25% of commercial drugs originate directly or indirectly from plants.[7] Dietary phytochemicals are secondary metabolites predominantly found in plant-derived raw foods, such as green leaves and vegetables. These compounds demonstrate a variety of biological and medicinal properties, such as antioxidant, antidiabetic, and anticancer properties.[8] Also, minor nutritional constituents such as phytosterols, tocopherols, polyphenols, and small squalane ingredients conferred remarkable physiological actions on plants. α-Tocopherol is a natural antioxidant found in plants that prevents the formation of free radicals and inhibits the formation of singlet oxygen.[9] Phytosterols, specifically stigmasterol, β-sitosterol, and campesterol, are plant membrane ingredients that successfully lower serum low-density lipoproteins (LDLs) and atherosclerosis risk.[10]

Alternative therapeutic compounds generated from plants are currently of great interest due to their eco-friendly nature, accessibility, high bioavailability, affordability, and lack of side effects. *In vivo* and *in vitro* investigations have revealed that anticancer actions of tannins are primarily directed through negative regulation of transcription factors, growth factors, receptor kinases, and several tumorigenic entities.[11] Natural products and conventional herbal medicines possess significant antidiabetic efficiency. Some of the phytochemicals can inhibit α-amylase and are utilized to regulate blood glucose levels in type 2 diabetes mellitus with lesser side effects compared to synthetic drugs.[12] Every plant includes unique phytoconstituents from its different parts that have the potential to assist in mitigating difficulties linked to different diseases. A vast reservoir of biologically active secondary metabolites is present in many plant species; however, only a limited fraction of these compounds have been thoroughly investigated and confirmed as valuable sources of therapeutic agents.[13]

Mangroves are an example of a diverse ecosystem with climatically sensitive range limitations. They are halophytic intertidal vegetation found globally on tropical and subtropical coasts and are represented mainly by shrubs and trees along the sea−land interface.[14] *S. maritima* (L.) Dumort (*S. maritima*) (Ramanathapuram district, Tamil Nadu), a grassy mangrove-associated plant is widely dispersed on the landward borders. Apart from being a feasible source of food, fodder, and conventional medicine in certain regions, the plant also has potential uses in phytoremediation. This plant can withstand high levels of NaCl in its native environment and typically accumulates significant concentrations of ions in shoots.[15] It is reported to exhibit antioxidant, antiviral, antimicrobial, and hepatoprotective activities.[16] Recently, gold and silver nanoparticles designed using galangin (a flavonoid) derived from *S. maritima* have been analyzed for antimicrobial and anticancer activities.[17] Despite the increasing interest in natural compounds for drug development, there remains a critical gap toward the exploration of the pharmacologically active constituents present in traditional edible Indian mangrove-related plants like *S. maritima*. In addition, only minimal reports are available on the identification, isolation of phytocompounds, characterization, *in silico* molecular docking, and evaluation of *in vitro* biological activities, including antioxidant, antidiabetic, and anticancer properties. Therefore, the primary aim of our investigation is to identify, characterize, and evaluate the pharmacological properties of phytochemical constituents in *S. maritima*. In order to analyze the biological potential of any plant species, the preparation of different extracts from the plant is essential. According to previous research, organic extracts prepared employing methanol, ethanol, and ethyl acetate have more significant concentrations of phytochemical components with strong antibacterial and antidiarrheal activities *in vitro* and *in vivo*.[18] Following that, spectrometric and chromatographic screening of the extracts from medicinal plants offers fundamental details of their biochemical and pharmacological activity. However, several drugs did not enter the market due to inadequate pharmacological attributes, resulting in substantial losses for

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the pharmaceutical industries. Computational prediction tools play a crucial role in selecting protocols directing pharmaceutical research and are employed in the *in silico* assessment of a drug's pharmacokinetics and pharmacodynamic characteristics. Molecular docking is currently a successful and low-cost method for engineering and evaluating pharmaceuticals, which also offers insights into drug−receptor interactions, aiding in the prediction of the binding mode and mechanism of the bioactive compound with the target protein receptors. In light of this, the current study emphasizes the identification of bioactive compounds from various extracts of *S. maritima* and analysis by GC-MS and HPLC techniques. We have also evaluated the efficacy of the extracts for their antiproliferative, antioxidant (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and reducing power assays), and antidiabetic (α-glucosidase and α-amylase assays) activities. Besides, *in silico* molecular docking was performed for the identified phytocompounds with C-terminal binding protein (CtBP) and SOX2 bound to importin-α3 target proteins. This report aims to forecast the structure and biological activities of phytochemical components present in the halophyte plant *S. maritima* and underscores its potential as a source of naturally derived molecules for drug development and delivery systems.

2. RESULTS AND DISCUSSION

Samples of *S. maritima* were procured from Karankaadu, Chitruvadi, Ramanathapuram district, Tamil Nadu (Figure 1). Phytochemical profiling and *in vitro* antioxidant, antidiabetic, and anticancer activities have been evaluated for three different solvent extracts of *S. maritima*: hexane, acetone, and methanol. The quantitative existence of phenolics and flavonoids was investigated using the GC-MS technique. Further, *in silico* and ADME analyses have been performed for the phytochemicals identified from SMAE by the GC-MS technique.

2.1. Yield of Extract. The amount of desirable components that can be extracted from a specific amount of plant material is termed the plant extract yield, typically indicated as a percentage. Soxhlet extractor, a standard leaching method for over a century, was used to execute successive solid−liquid extractions to get high yields of chemicals from insoluble fractions.[19] We obtained the extracts with different constituents based on polarity. The methanol extract (7.3%) had the highest yield, whereas the acetone and hexane extracts had comparatively lower yields (Table 1). In *S. maritima*, the ability of polar solvents to draw out the most phytochemicals may be influenced by solvent efficiency and environmental factors like temperature.

Table 1. Extract Yield of *S. maritima* Extracts

s. no	extraction technique	solvent	total extract	total extract yield %
1	Soxhlet	hexane	1.75 g	2.3
2	Soxhlet	acetone	4.53 g	5.6
3	Soxhlet	methanol	5.72 g	7.3

2.2. Evaluation of Phytochemical Profiling. *2.2.1. Qualitative Assessment of S. maritima Extracts.* The three solvent extracts were analyzed for the existence of saponins, anthraquinones, flavonoids, alkaloids, phenols, steroids, and glycosides. The qualitative evaluation focused on precipitation processes, foamy appearance, and color change, all of which are features of the chemical components of the *S. maritima* plant, which are the presence and absence of secondary metabolites shown in Table 2. The acetone extract recorded the presence of most of the phytochemicals, except glycosides. The presence of saponins was noticed in all of the extracts, and phenols and steroids were present in hexane and acetone extracts.

Table 2. Presence and Absence of Phytochemical Test of *S. maritima* Extracts[a]

s. no	test	*S. maritima* hexane extract	*S. maritima* acetone extract	*S. maritima* methanol extract
1	saponins	+	+	+
2	flavonoids	−	+	−
3	glycosides	−	−	−
4	quinones	−	+	+
5	phenols	+	+	−
6	anthraquinones	−	+	+
7	steroids	+	+	−

[a](+ represents a presence and − represents an absence).

2.3. Quantitative Assessment of *S. maritima* Extracts. All *S. maritima* extracts underwent quantitative determination of nonenzymatic antioxidant components (phenolics and flavonoids),[20] and the findings are displayed in Figure 2. The amount of nonenzymatic antioxidants found in the SMAE was the highest, which revealed the increased efficacy of midpolar solvents for extracting polyphenolic substances from the plant sample.

2.4. Thin-Layer Chromatography (TLC) and Column Chromatography. TLC was done for the *S. maritima* sample, and the new spot was observed by an UV transilluminator at 365 nm in acetone with an Rf value of 0.54 (SI, Figure S1).[21] Compounds were collected from the whole *S. maritima* plant sample by column chromatography using hexane, chloroform, ethyl acetate, and methanol eluents (SI, Figure S2).[22]

2.5. HPLC Analysis. Twenty-five μL of the *S. maritima* acetone extract in 20 ppm concentration was injected into reversed-phase high-performance liquid chromatography (RP-HPLC) for analytical purpose, with β-sitosterol as the reference standard, and peak was observed under 256 nm (mobile phase ratio methanol (75%) and HPLC gradient water (25%)) (Figure 3). The spectrum confirmed the presence of trace amounts of stigmasterol derivatives in the SMAE at the retention times of 0.684, 1.052, 1.405, 3.830, and 9.736 min.[23]

2.6. Spectroscopic Study. The FT-IR spectral technique is typically employed to detect the different functional groups in the plant extract and is confirmed by comparing it with the standard IR.[24] The FT-IR spectrum of SMAE (Figure 4) displayed a broad band around 3400 cm^{-1}, demonstrating the presence of several hydroxyl (−OH) and amino (−NH) groups. The peaks at 1258 and 1060 cm^{-1} revealed the skeletal C−C stretching, and the former peak includes the mixed $\nu_{\text{aryl C−O}}$ vibrations, and the latter peak designated the existence of both aliphatic $\nu_{\text{C−F}}$ and $\nu_{\text{C−N}}$ stretching. The spectrum also confirmed the presence of active methylene and olefinyl aromatic C=C functional motifs, attributed to the stretching peaks around 2920 and 1640 cm^{-1}, respectively. The symmetric stretching of the COOH emerged around 1420 cm^{-1}. The less intense peaks around 622 cm^{-1} revealed the alkynyl C−H and aliphatic C−Br moieties in the SMAE. The prevalence of these chemical stretches and linkages for various functional groups raises the possibility that the extract

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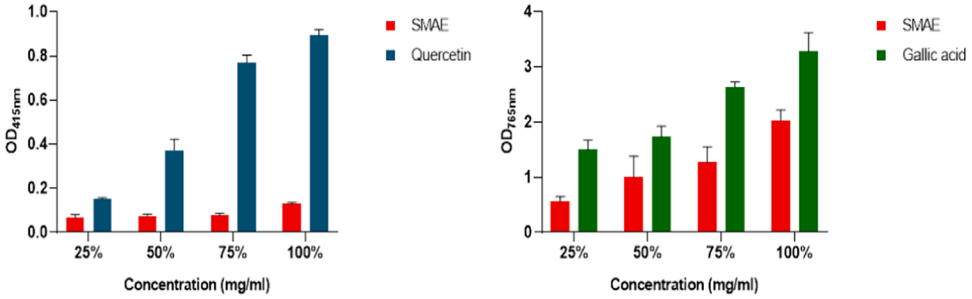

Figure 2. Quantitative assessment of phenolics and flavonoids (positive control: GAE (gallic acid for phenols and quercetin for flavonoids)).

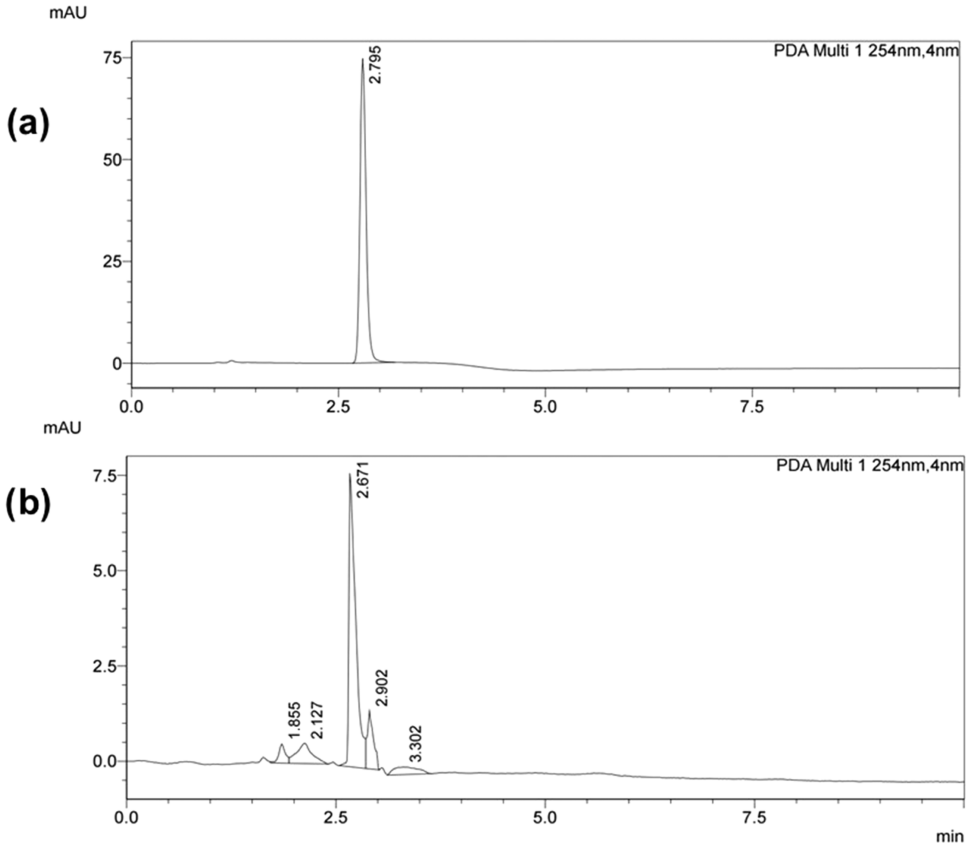

Figure 3. HPLC of the *S. maritima* acetone extract. (a) Reference standard β-sitosterol and (b) SMAE-identified compound stigmasterol.

components may bind to the target proteins that trigger the apoptotic signaling cascade in antitumor activity. A comparable FT-IR investigation on the acetone extract of *S. maritima* was related to the molecular docking of volatile chemicals based on GC-MS.[25,26]

2.7. GC-MS Analysis. The GC-MS chromatogram (Figure 5) of SMAE displayed the potential phytochemicals present in that extract (Table 3), and its mass spectrum and relative retention times (Rt) were compared with those of the standard NIST library. The phytocompounds, stigmasterol and γ-sitosterol, were observed at retention times of 16.21 and 17.09, with retention index values of 2970 and 2950, respectively, possessing the high peak area %. The existence of octadecanoic acid, octacosane, and vitamin E has been confirmed by the retention times of 17.985, 22.262, and 14.123, along with retention index values of 1823, 2987, and 2987, respectively.

The occurrence of tritetracontane and 17-pentatriacontene has been determined by analyzing their respective retention times, measured as 21.029 and 21.751. Additionally, the maximum retention index values of these compounds were 4381 and 3598, respectively. The phytochemicals shown in the GC-MS chromatogram have significant biological and therapeutic effects. For instance, stigmasterol is shown to have antidiabetic properties by enhancing GLUT4 translocation, insulin resistance, and anticancer activities by cell cycle arrest and cell growth inhibition.[27] γ-Sitosterol derived from *Acacia nilotica* has been reported to induce cell cycle arrest at the G2/M phase and apoptosis in A549 and MCF-7 cells.[28] Octacosane exhibited antibacterial and wound-healing properties.[29] 17-Pentatriacontene has shown anti-inflammatory, anticancer, antiatherogenic, and antiarthritic activities.[30] However, mechanisms by which many of these phytochemicals exhibit biological activities have not yet been fully understood. Hence, we focused on evaluating the *in silico* molecular docking; ADME properties; and *in vitro* antioxidant, antidiabetic, and anticancer activities for phytochemicals identified from SMAE.

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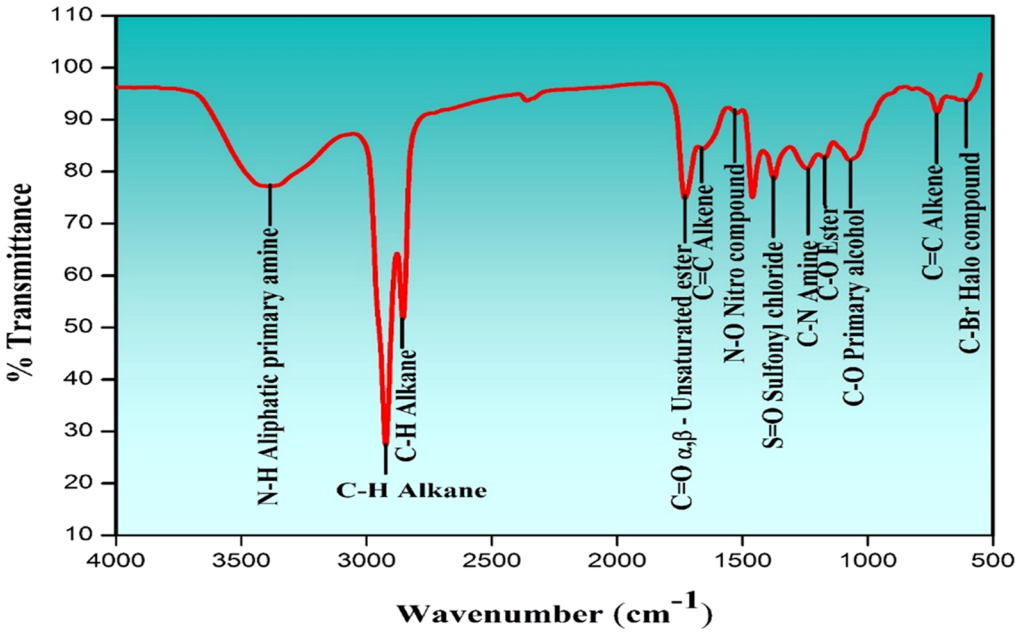

Figure 4. FT-IR spectrum of *S. maritima* acetone extract.

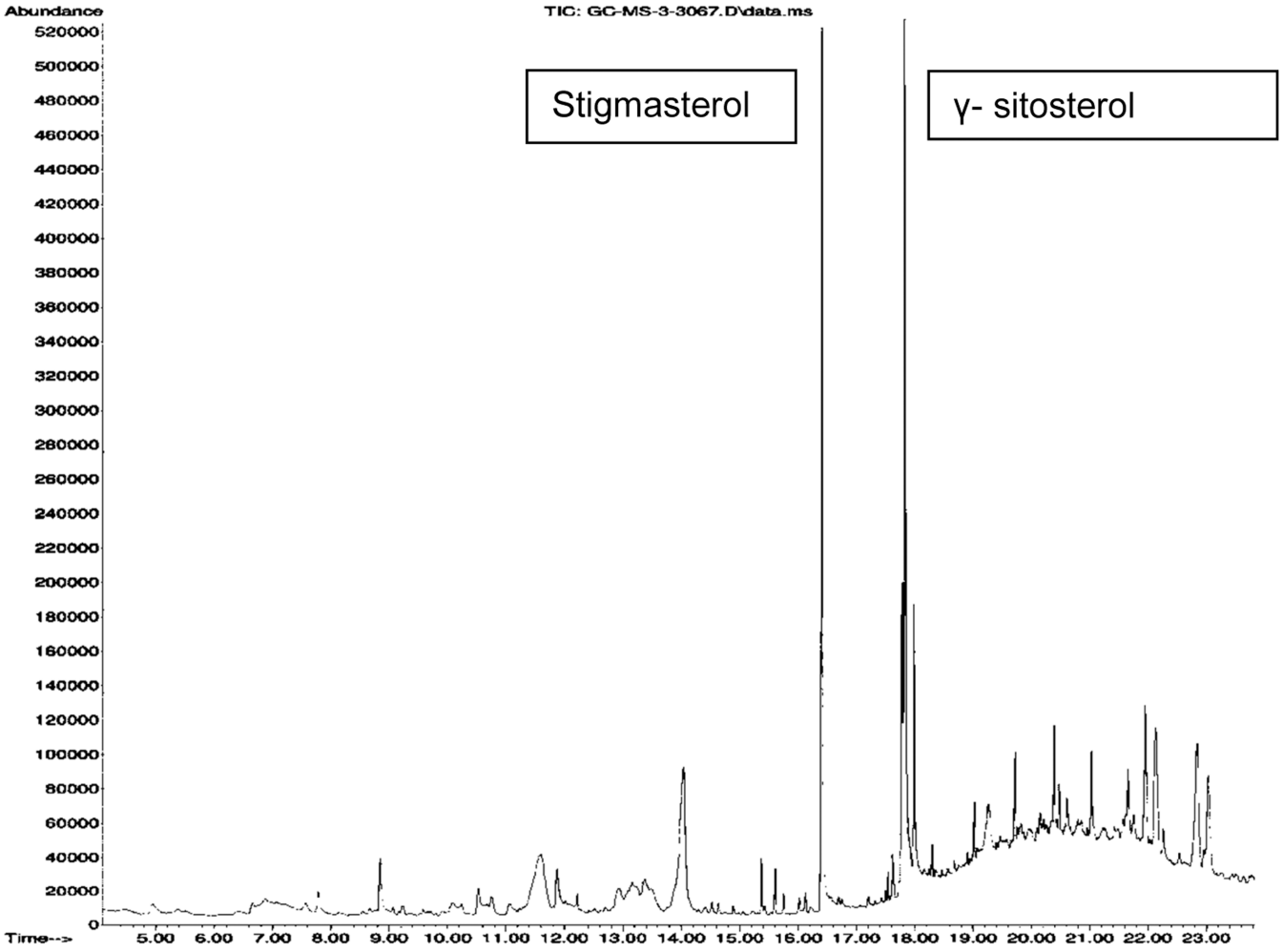

Figure 5. GC-MS chromatogram of the *S. maritima* acetone extract.

2.8. LC-MS Analysis. LC-MS analysis was carried out for SMAE to determine the phytochemical composition. The SMAE displayed a base peak at m/z 412.3757 and a less abundant peak at m/z 605.2368 (SI, Figure S3). The mass

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Table 3. GC-MS Analysis of *S. maritima* Acetone Extract

s.no	retention time	peak name	height	molecular formula/weight	retention index	biological activity
1	16.210	stigmasterol	200422019466-47-8 99	$C_{29}H_{52}O$/416.7 g/mol	2970	anticancer, antidiabetic, anti-inflammatory[27]
2	17.090	γ-sitosterol	199879000083-47-6 99	$C_{29}H_{52}O_2$/432.7 g/mol	2950	anticancer, anti-inflammatory[28]
3	22.262	octacosane	193569000630-02-4 91	$C_{28}H_{58}$/394.8 g/mol	2798	antibacterial, wound healing[29]
4	21.751	17-pentatriacontene	212522006971-40-0 91	$C_{35}H_{70}$ /490.9 g/mol	3598	antiatherogenic[30]
5	21.029	tritetracontane	217983007098-21-7 91	$C_{43}H_{88}$ /605.2 g/mol	4381	antioxidant[31c]
6	14.123	vitamin E	203745000059-02-9 89	$C_{29}H_{50}O_2$/430.7 g/mol	2987	antioxidant, α-tocopherol, important to vision, reproduction, and health of your brain, blood, and skin[31d]
7	17.985	octadecanoic acid	124556000057-11-4 99	$C_{18}H_{36}O_2$/284.5 g/mol	1823	anti-inflammatory, antiandrogenic, anticancer, antileukotriene-D4[31e]

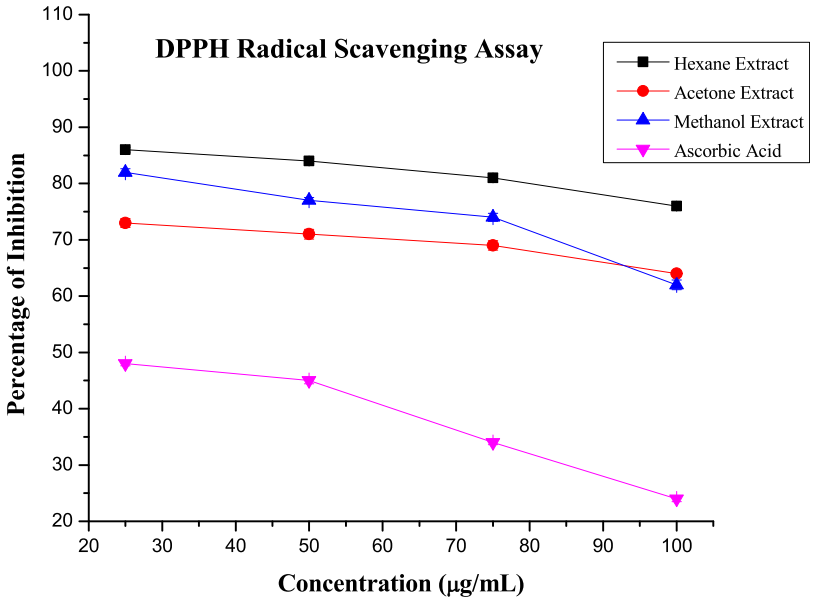

Figure 6. DPPH radical scavenging ability of *S. maritima* extracts.

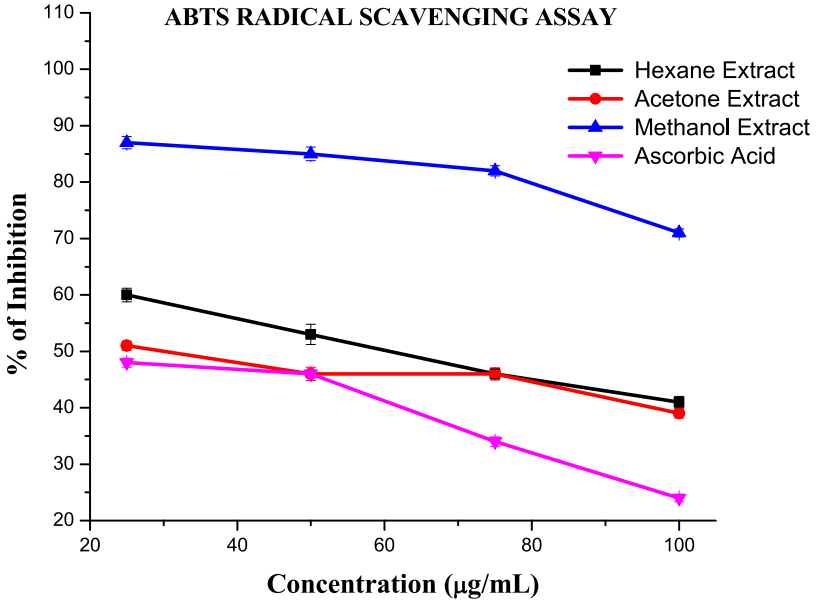

Figure 7. ABTS$^{\bullet+}$ radical scavenging ability of *S. maritima* extracts

values of the two peaks coincide well with the formula weights of stigmasterol (412.69 g/mol) and tritetracontane (605.20 g/mol), respectively.[32]

2.9. *In Vitro* Antioxidant Activity. *2.9.1. DPPH Assay.* Free radicals are reactive and unstable intermediates that can cause DNA damage in humans. DNA damage is believed to

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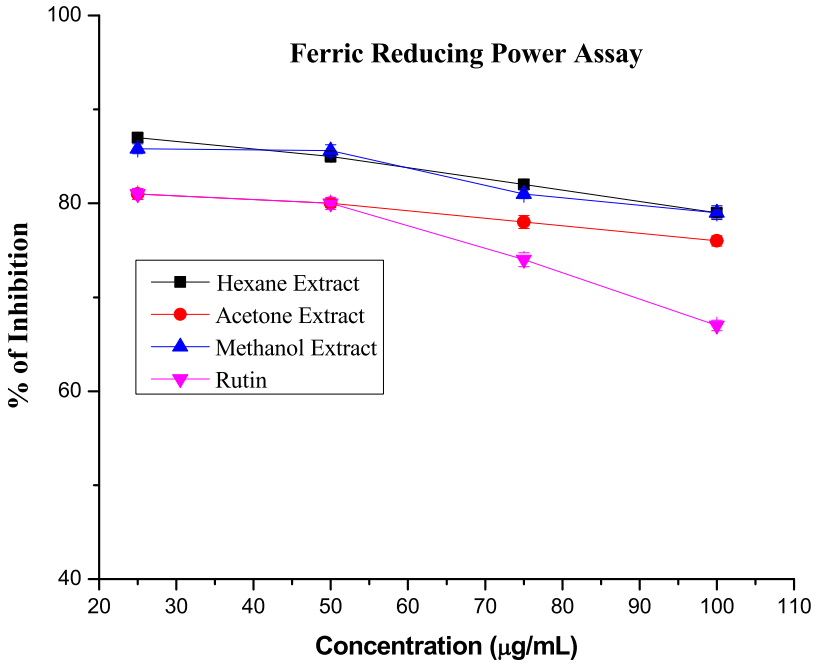

Figure 8. Reducing power ability of *S. maritima* extracts.

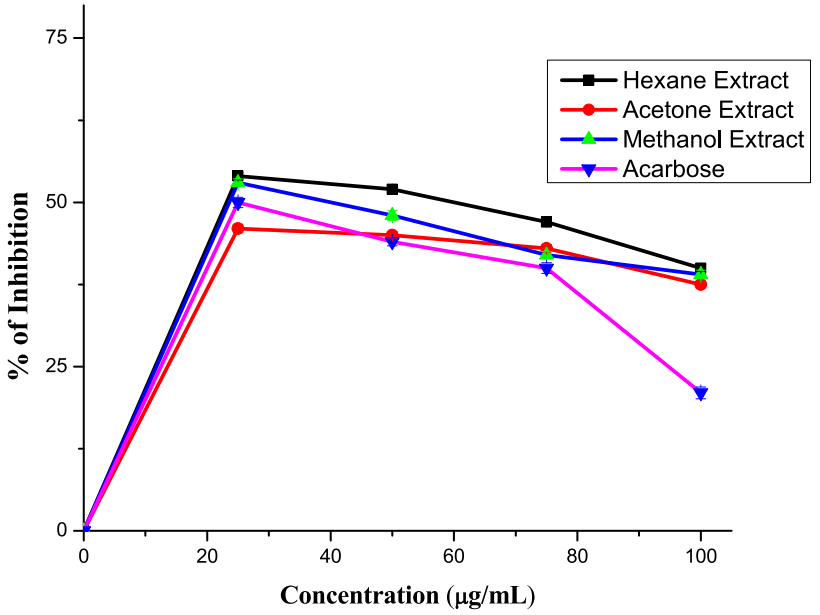

Figure 9. Anti-α-amylase activity of the *S. maritima* extracts compared with acarbose.

contribute to aging and numerous diseases, such as carcinoma and chronic inflammation. Scavenging free radicals may help to prevent such diseases. DPPH radical is extensively utilized to evaluate the antioxidant activity of substances as scavengers of harmful free radicals or proton donors.[33] The odd electron of DPPH free radical produces a bright purple color in methanol with a maximum absorption at 517 nm. The color changes to yellow with a decrease in molar absorptivity of the DPPH radical when it pairs with hydrogen to generate the reduced DPPH-H species. The number of collected electrons and the consequent decolorization are stoichiometric. Hence, the free-radical scavenging property of *S. maritima* extracts has been tested employing the DPPH radical in methanol solution in a dose-dependent manner, with ascorbic acid as a standard to compare the antioxidant activity of the extracts. The different concentrations of the extracts used for the antioxidant activity are 25, 50, 75, and 100 μg/mL. The IC_{50} value of the positive control ascorbic acid is 31.3 μg/mL, whereas that of the acetone extract was 95.58 μg/mL. Among the extracts, the hexane extract has lower activity (Figure 6). The greater antioxidant property of the SMAE depicts its capacity to supply hydrogen atoms and scavenge the free radicals, which can be endorsed to the existence of polyphenolic and flavonoid contents as indicated by the qualitative phytochemical analysis and FT-IR spectroscopy.[26,31,34]

2.9.2. ABTS Assay. When ABTS interacts with potassium persulfate, a blue chromophore ($ABTS^{\bullet+}$ radical) is created. With the inclusion of a component possessing antioxidative

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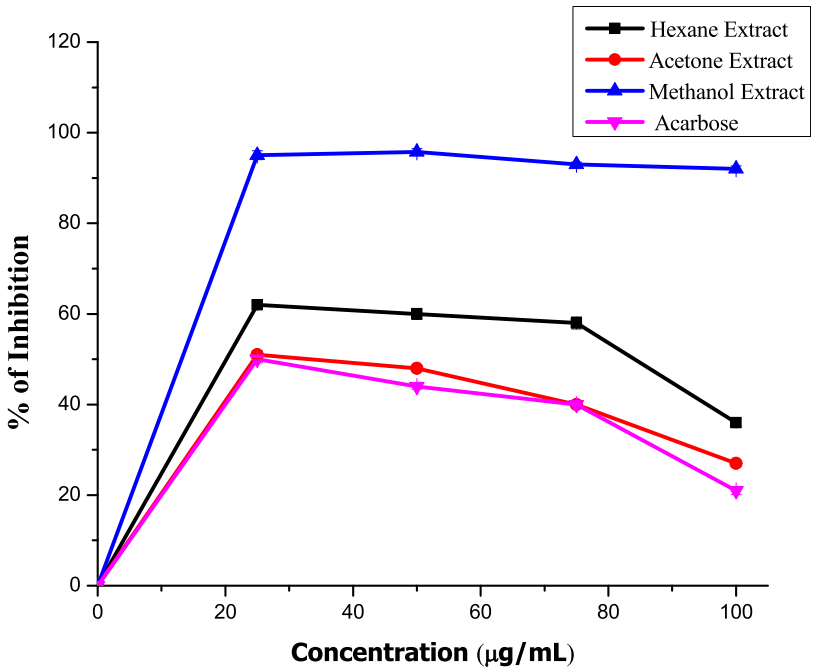

Figure 10. Anti-α-glucosidase activity of the *S. maritima* extracts compared with acarbose.

potential, the concentration of $ABTS^{\bullet+}$ radical falls and gets decolourized.[35] For the various solvent extracts examined in this investigation, a dose-dependent suppression of the $ABTS^{\bullet+}$ radical was observed. The IC_{50} value of the SMAE was 69.2 μg/mL, and the standard ascorbic acid has an IC_{50} value of 33.24 μg/mL. Contrary to the DPPH assay, the methanol extract displayed the least scavenging ability. The maximum concentrations (100 μg/mL) of all of the extracts and ascorbic acid (the positive control) showed a modest (statistically nonsignificant) variation in percent inhibition (Figure 7).

2.9.3. Reducing Power Ability. A key mechanism for putative free-radical scavenging activity is the reducing power of the plant's extract, which can serve as a valuable indicator of its antioxidant potential. Reducing power ability in antioxidant activity refers to the capacity of a substance to donate electrons and reduce oxidative species, particularly free radicals (electron transfer from the antioxidant to the free radicals), within a biological or chemical system. The antioxidant effect of SMAE is manifested through the reduction of ferric ions (Fe(III)) within the ferricyanide complex, converting them to their ferrous (Fe(II)) form. The reducing power of the extracts exhibited varying degrees of activity, ranging from high to moderate, when compared to rutin, as shown in Figure 8. SMAE established a high reducing power ability, with an IC_{50} of 163.78 μg/mL at an absorbance of 700 nm. The results from DPPH, $ABTS^{\bullet+}$, and reducing power radical scavenging assays of the SMAE indicated a positive correlation between the observed antioxidant activity and the presence of phenols and flavonoids in the acetone extract, which were not present in others. A similar observation was noticed in the antioxidant studies of the related genus *Suaeda japonica*.[36]

2.10. *In Vitro* Antidiabetic Activity. *2.10.1. α-Amylase Inhibition Assay.* α-Amylase is an enzyme that digests carbohydrates (mainly polysaccharides) by hydrolyzing their α-linkages and converting them into oligosaccharides. It is responsible for the increase in the level of postprandial glucose in diabetic patients, and hence, inhibiting its activity may control hyperglycemia and reduce diabetic development.[37] Evaluation of the α-amylase inhibitory activity of the extracts in a dose-dependent trend indicated better inhibition by the acetone extract, among others, and the activity of acarbose was also studied as a comparative standard (Figure 9). The concentrations of the tested samples were expressed in terms of μg/mL, and the acetone extract exhibited the lowest IC_{50} value of 32.6 μg/mL. Overall, the α-amylase inhibition activity of the extracts followed the order acarbose > acetone > hexane > methanol. In addition, the acetone extract matched the inhibition activity of acarbose under 25 and 75 μg/mL concentrations. The antidiabetic activity via the α-amylase inhibition mechanism of the various extracts of *S. maritima* is consistent with previous reports.[26]

2.10.2. α-Glucosidase Inhibition Assay. α-Glucosidase is a crucial catabolic enzyme that controls plasma glucose levels, enabling essential energy sources for the body's healthy functioning. Its primary role lies in carbohydrate digestion and the disintegration of complex sugars into simpler forms such as glucose. Inhibiting α-glucosidase can result in delayed or diminished carbohydrate absorption, offering significant benefits in controlling postmeal blood glucose levels, and α-glucosidase-inhibiting drugs lower the blood glucose levels to treat type 2 diabetes.[38] The acetone, hexane, and methanol extracts of *S. maritima* revealed a potent inhibitory capability of α-glucosidase with IC_{50}values of 86.53 $\pm$ 0.06, 55.50 $\pm$ 0.07, and 54.40 $\pm$ 0.08 μg/mL, respectively, compared to the standard acarbose with IC_{50} = 27.09 $\pm$ 0.09 μg/mL (Figure 10). In contrast to the α-amylase inhibition activity, SMAE demonstrated IC_{50} values higher than those of the other extracts and the acarbose. It is well-known that some phytochemicals, including polyphenols, alkaloids, terpenoids, saponins, lignans, phenolic acids etc., have been identified as responsible for α-amylase inhibition, contributing to their antidiabetic activity. Since α-amylase and α-glucosidase require metal ions like calcium for their proper functioning, certain phytochemicals can bind to these metal ions, preventing them

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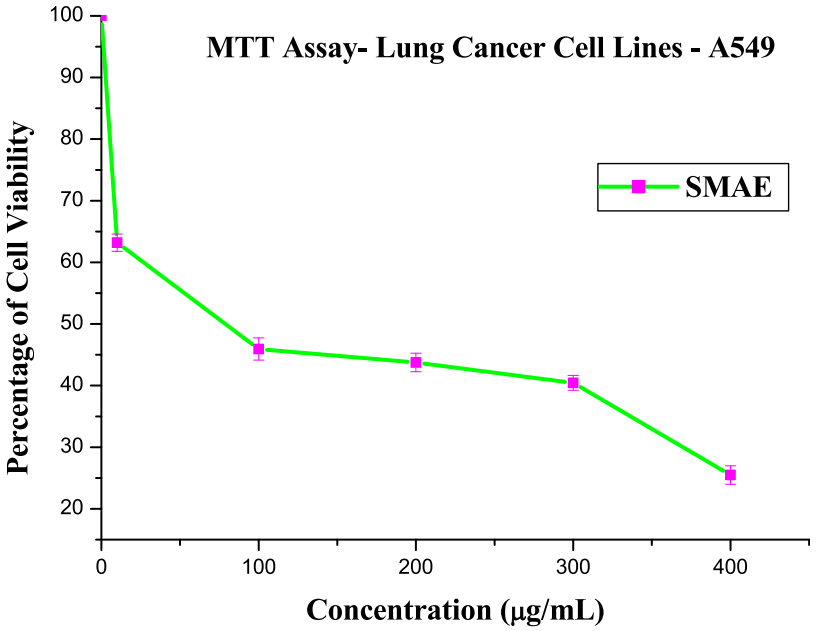

Figure 11. Effect of different concentrations of acetone extract on the death of A549 cells calculated using MTT assay.

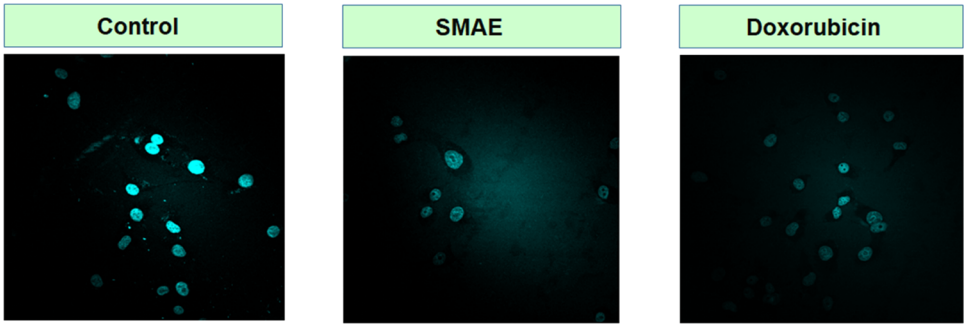

Figure 12. Identification of the A549 cells treated with SMAE and the standard drug doxorubicin using DAPI staining.

from interacting with the enzyme and inhibiting its activity. Further, they exert antidiabetic activity also via competitive inhibition, allosteric inhibition, and binding and modifying the enzyme conformation via hydrogen bonding.[39] In our investigation, the SMAE was also found to have maximum phytochemicals, specifically saponins, anthraquinones, flavonoids, alkaloids, phenols, and steroids and showed almost equipotent antidiabetic activity when compared to the standard acarbose.[26]

2.11. *In Vitro* Anticancer Activity. *2.11.1. MTT Assay.* Many plant extracts have been reported to exhibit cancer cell growth inhibition,[40−42] and hence, we have analyzed the anticancer potential of SMAE in lung cancer cell proliferation. In order to monitor the development and morphological modifications in lung cancer (A549) cells, it is more common to utilize 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as a formazan test. Since the acetone extract dominated the other extracts in phytochemical analyses and antioxidant studies, its anticancer potential was analyzed against lung cancer cells using the MTT assay and compared with doxorubicin (positive control). A plot of concentration vs percentage of cell death shown in Figure 11 indicated the increase in cell death upon increasing the concentration of the sample from 100 to 400 μg.

The IC_{50} values of doxorubicin (SI, Figure S4) and SMAE against the proliferation of lung cancer cells are 12.64 ± 0.86 and 78.19 ± 1.46 μg/mL, respectively. Also, the degree to which various doses of SMAE inhibit cancer growth is displayed in the SI, Figure S5. With an increase in the concentration of SMAE, the cell debris increased due to increased cell death (SI, Figure S5). Selectivity toward cancerous cells is the major challenge in advancing new antitumor drugs. Ideally, an effective anticancer agent should exclusively target cancer cells without harming healthy cells. In light of this, cytotoxic selectivity has been analyzed using noncancerous HUVEC cells (SI, Figure S6). Advantageously, the SMAE showed specific antiproliferative activity only toward the proliferation of cancer cells, which has been witnessed by its higher IC_{50} value, 300 ± 1.12 μg/mL, on noncancerous HUVEC cells. Contrary to the previous report, the extracts of *S. maritima* unveiled significant *in vitro* cancer cell growth inhibition activity against lung cancer cells.[43]

2.11.2. DAPI Staining. Apoptosis is the preferred pathway for therapeutic drugs to trigger cancer cell death. Hence, the apoptosis induction capacity of SMAE in lung cancer cells has been examined through nuclear morphological alterations by DAPI staining analysis. For this experiment, lung cancer cells were treated with different concentrations of SMAE (1, 2, 4, and 6 μM) for 48 h and compared with the standard anticancer

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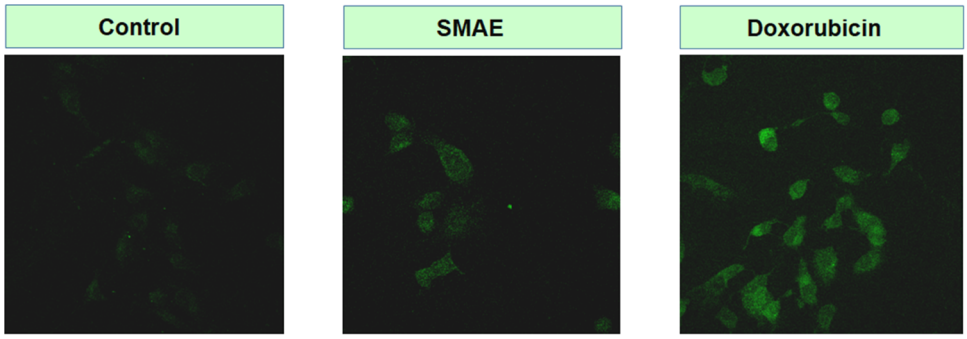

Figure 13. Fluorescence images of ROS levels in A549 cells treated with SMAE and the standard drug doxorubicin using DCFH-DA staining.

Table 4. ADME Characteristics of Phytochemicals Identified by GC-MS from the Acetone Extract of *S. maritima*

s. no.	molecule	MW	log Po/w	log *S*	PMDCK	HOA%
1	stigmasterol	416.729	7.455	−5.640	1881.395	100
2	γ-sitosterol	414.713	4.473	−8.353	1880.763	100
3	octacosane	394.8	5.540	−8.305	1881.395	83.26
4	17-pentatriacontene	490.9	4.473	−8.353	1654.210	78.56
5	tritetracontane	605.2	6.320	−7.023	123.650	81.56
6	vitamin E	430.713	6.983	−8.334	2630.822	100
7	octadecanoic acid	284.5	5.370	−4.621	144.366	88.54

drug doxorubicin. In the course of apoptosis, cells undergo a myriad of transformations. One notable change involves the shift from genetically active chromatin to a more condensed state. Subsequently, during the ensuing stages, chromatin within the internucleosomal DNA linker regions experiences fragmentation orchestrated by DNA endonucleases.

As a result, compacted nodes show heightened luminosity and reveal fragmented nuclei, a phenomenon readily discernible through fluorescence microscopy, especially in DNA regions marked with chromatin-specific dyes, such as DAPI. The nuclear morphological changes mentioned above were discerned in lung cancer cells when exposed to SMAE and doxorubicin, as visually depicted in Figure 12. These observations signify that apoptosis induced by SMAE predominantly constitutes the mechanism underlying cancer cell death.

2.11.3. ROS Analysis. Intracellular reactive oxygen species (ROS) are oxygen-encompassing reactive species that play pivotal roles in regulating fundamental cellular processes. These molecules are biologically indispensable with a profound impact on cell functions. The oxidation reactions stemming from aerobic metabolism lead to the generation of ROS. External stimuli trigger the oxidation of DNA and lipids, organelle degradation, and expedited apoptosis. Dysregulated ROS production profoundly influences the cellular architecture, potentially resulting in various disorders. Notably, a prevalent mechanism of action for anticancer therapeutics involves the augmentation of the ROS levels. Thus, we have undertaken an assessment of the capacity of SMAE to induce ROS production in lung cancer cells, employing the dichlorodihydrofluorescein diacetate (DCFH-DA) methodology, and compared with doxorubicin. The cellular enzyme esterase can convert the compound DCFH-DA into DCFH (2′,7′-dichlorodihydrofluorescein), a nonfluorescent molecule.

In the presence of intracellular reactive oxygen species (ROS), the nonfluorescent DCFH initially undergoes rapid conversion into a highly luminous 2′,7′-dichlorofluorescein (DCF). The intensity of the fluorescence of DCF is likely to be correlated with the quantity of ROS found within cells. Consequently, an evaluation was conducted on the intracellular levels of reactive oxygen species (ROS) in lung cancer cells following treatment with SMAE (1, 2, 4, 6 μM) for 48 h. The treatment of SMAE with lung cancer cells resulted in a noticeable rise in intracellular levels of reactive oxygen species (ROS) compared to those of the negative control group. Upon cellular uptake, the nonfluorescent compound DCFH-DA is rapidly hydrolyzed by intracellular esterases, resulting in the formation of the fluorescent compound DCFH. In addition, it is worth noting that nonfluorescent DCFH has the ability to undergo oxidation in the presence of intracellular reactive oxygen species (ROS), resulting in the formation of fluorescent DCF. According to the findings depicted in Figure 13, the application of SMAE and doxorubicin resulted in a visible augmentation of green fluorescence. The outcomes suggest that SMAE, similar to doxorubicin, can potentially facilitate the production of reactive oxygen species (ROS) within cells.

2.12. Pharmacological Evaluation. Finally, we delve into a comprehensive analysis of the pharmacological properties of the compounds identified from the GC-MS analysis of SMAE, employing ADME (absorption, distribution, metabolism, and excretion) analysis. Understanding the ADME characteristics is pivotal in predicting the potential efficacy and safety of phytochemicals found in *S. maritima* as drug candidates. Additionally, we explore the molecular interactions of these compounds with C-terminal binding protein, CtBP1 (PDB 4U6Q), and SOX2 bound to importin-α3 (PDB 6WX8) target proteins using molecular docking techniques. The *in silico* approach allows us to elucidate the binding affinities and binding orientations of the identified compounds, shedding light on their potential as therapeutic agents. Together, these analyses provide valuable insights into the pharmacokinetic and pharmacodynamic profiles of the investigated compounds, paving the way for drug development.

2.12.1. ADME Analysis. The ADME characteristics were examined to assess the possible clinical potential of the seven phytocomponents detected by GC-MS in the acetone extract

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Table 5. Molecular Docking Results for 4U6Q Target

s. no	ID	compound name	Glide score (kcal/mol)	Glide energy (kcal/mol)	no. of H-bonds	interacting residues
1	241572	stigmasterol	−3.852	−32.706	1	THR264, ARG266
2	457801	γ-sitosterol	−3.487	−27.894	1	THR264
3	12408	octacosane	−3.022	−34.876		no interactions
4	5365022	17-pentatriacontene	−2.842	−27.907		GLY101, ARG97(SB)
5	522398	tritetracontane	−2.788	−33.019		ARG184, ASN119
6	14985	vitamin E	−0.449	−41.335		THR128
7	5281	octadecadienoic acid	−3.404	−38.635	2	VAL185

Table 6. Molecular Docking Results for 6WX8 Target

s. no	ID	compound name	Glide score (kcal/mol)	Glide energy (kcal/mol)	no. of H-bonds	interacting residues
1	5280794	stigmasterol	−2.845	−26.594-	1	SER144R
2	457801	γ-sitosterol	−2.987	−30.437		ARG103
3	12408	octacosane	−1.665	−37.035		no interactions
4	5365022	17-pentatriacontene	−2.185	−37.618		no interactions
5	522398	tritetracontane	−2.451	−28.23		no interactions
6	14985	vitamin E	−4.018	−38.051	1	ASP261, TRP22 (pi−pi)
7	5281	octadecanoic acid	−0.591	−26.113		ARG103

of *S. maritima*, and the results are given in Table 4. A quantitative multiple linear regression model was used to predict the proportion of human oral absorption (over 80% indicates high absorption and below 25% indicates poor absorption). The ADME assessment employs a set of knowledge-based guidelines that include determining appropriate values for % human oral absorption, number of metabolites, number of rotatable bonds, partition coefficient, solubility, molecular weight, and cell penetration.[44] Solubility (log *S*) of a drug determines its ability to dissolve in biological fluids and profoundly influences its pharmacological behavior. Optimal solubility ensures effective absorption and distribution of the compounds within the body. Generally, a log *S* value close to 0 or slightly negative is desirable for favorable drug formulation. In our case, the solubility of the phytocompounds ranges from −4.621 to −9.168, indicating their suboptimal dissolution characteristics in water.

However, efficient intracellular drug delivery relies on the transport of therapeutic molecules across the lipophilic cell membrane. In addition, permeation is a key factor impacting crucial pharmacological aspects of drugs including absorption, distribution, metabolism, and elimination. The outstanding logPo/w (lipophilicity in terms of partition coefficient between octanol and water system) and PMDCK (predicted apparent MDCK cell permeability in nm/s) of the identified compounds have been evidenced from the derived log Po/w (4.473−7.455) and PMDCK values (129.663−2630.822), respectively. Ideally, drug candidates should have high human oral absorption (HOA) %, indicating good oral bioavailability.[45] A value of 80% or higher is often considered favorable for oral drugs, and all the examined phytocompounds showed >80% HOA, which dictated their remarkable HOA and, consequently, their bioavailability. Overall, the physicochemical and druglikeness factors were best met (100% human oral absorption; MW 250−500, often known as the Rule of 5) by almost all of the phytocompounds identified from the SMAE.

2.12.2. Molecular Docking. Molecular docking is crucial in modern drug discovery and the rational design of bioactive compounds. It allows for investigating binding interactions between possible drug candidates and specific target proteins, providing crucial insights into their binding affinities and therapeutic applications.[46] Cancer is marked by intricate and multifaceted molecular processes, frequently entailing a cascade of genetic changes and the heightened expression of particular proteins. Targeted chemotherapeutic strategies aim to address this complexity by precisely identifying and targeting the overexpressed proteins, thereby enhancing the selectivity and efficacy of therapeutic drugs. In this study, we used molecular docking to investigate the interactions of the important compounds identified through GC-MS analysis of the SMAE and two significant target proteins related to cancer, CtBP1 (PDB Code: 4U6Q)[47] and SOX2 bound to importin-α3 (PDB Code: 6WX8).[48] CtBP1 (C-terminal binding protein 1) stands as an evolutionarily conserved transcriptional corepressor with a multifaceted role in gene regulation. Its impact extends to the transcription of genes that are critical in cancer progression. Notably, CtBP1 exerts a negative regulatory influence on several tumor suppressor genes, including BRCA1, p16INK4a, and E-cadherin, thus promoting cell migration, invasion, and imparting antiapoptotic traits to tumor cells. Moreover, CtBP1 significantly contributes to processes such as epithelial−mesenchymal transition, tumor metastasis, glucose metabolism, and the self-renewal of cancer stem cells. Its overexpression in various tumor tissues underscores its close association with tumorigenesis, progression, and prognosis.[49]

On the other hand, SOX2, an oncogenic transcription factor, exhibits overexpression in nearly half of basal-like triple-negative breast cancers and is also associated with copy number amplification and promoter overactivity in various malignancies, including lung cancer subtypes like squamous cell carcinoma and adenocarcinoma. The clinical relevance of targeting and inhibiting SOX2 is underscored by the positive correlation between high SOX2 mRNA levels and reduced overall survival and progression-free survival in cancer patients. Hence, CtBP1 and SOX2 have been chosen as the focal points of our docking investigations, seeking to unravel their potential as therapeutic targets for targeted cancer therapy. The docking was performed to bind the protein and ligand in the standard precision (SP).[50] A total of 15 GC-MS compounds were docked into the 4U6Q and 6WX8 proteins; however, only 7 compounds were bound to the target proteins. Based on the

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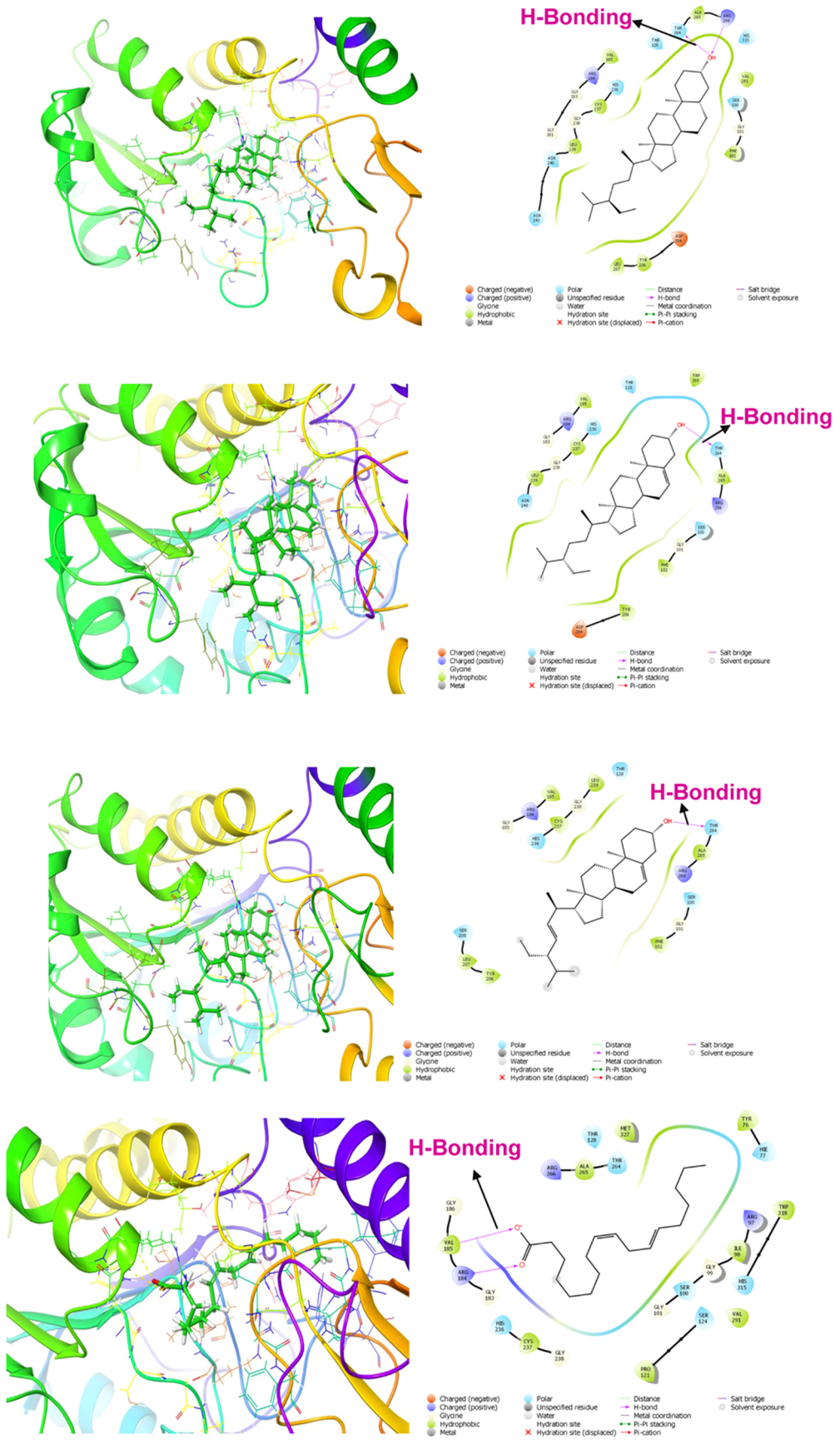

Figure 14. Three- and two-dimensional 4U6Q docking interaction images with stigmastanol, stigmasterol, γ-sitosterol, and octadecadienoic acid.

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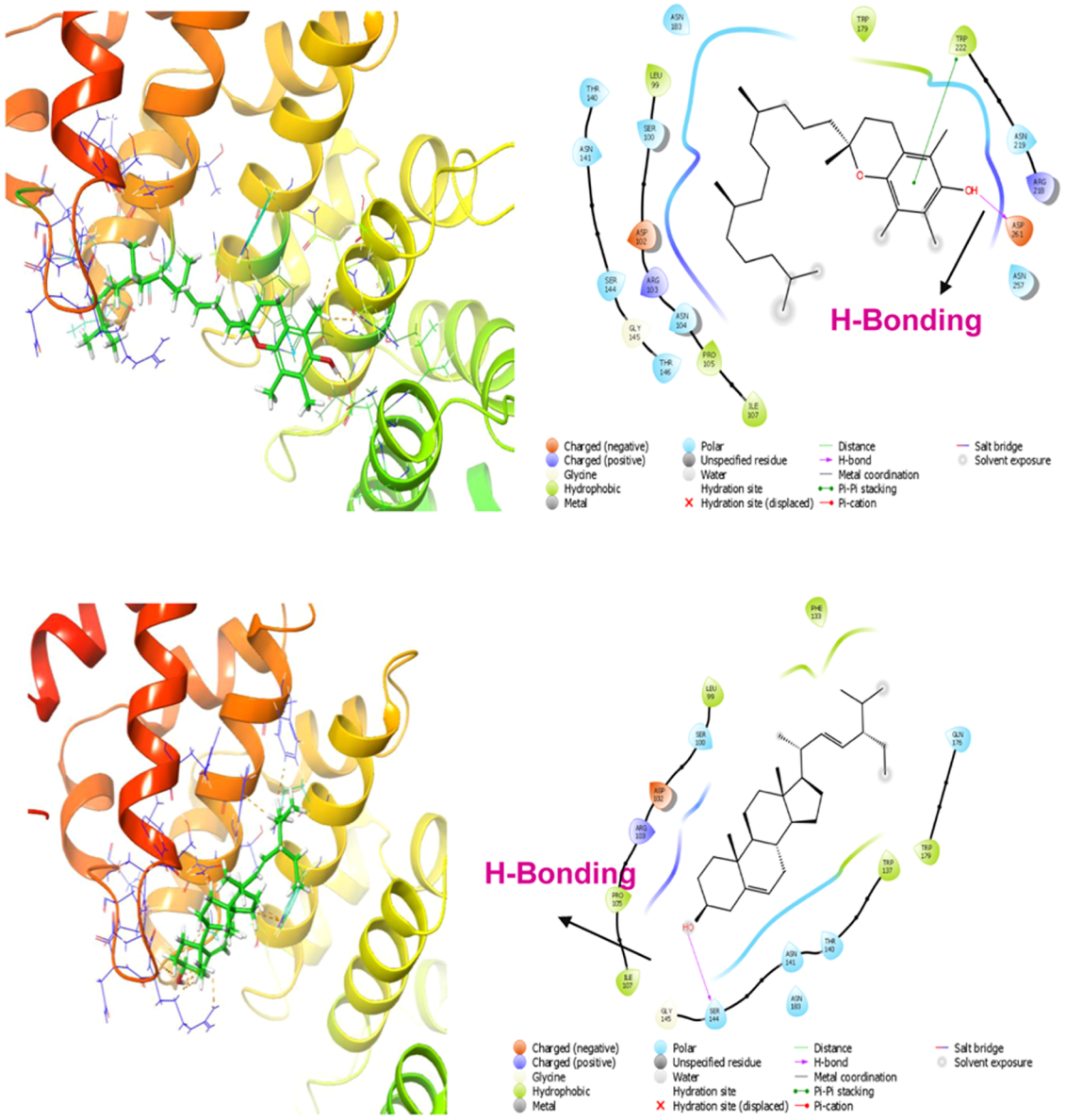

Figure 15. Three- and two-dimensional 6WX8 docking interaction images with vitamin E and stigmasterol.

Glide score (kcal/mol) and Glide energy (kcal/mol), the best binding poses were selected, and analysis was carried out using Schrodinger software. Tables 5 and 6 represent the docking score and energy as well as interacting residues of the protein and ligand. Based on the results, hydrogen-bonding interactions were analyzed, and the best compounds were selected. The interactions of the stigmasterol, γ-sitosterol, octadecadienoic acid, stigmastanol, and vitamin E with the target proteins are provided in Figures 14 and 15. Stigmasterol, γ-sitosterol, and octadecadienoic acid demonstrated the best Glide scores of 3.852, −3.487, and −3.404 kcal/mol, respectively, with their interaction with the CtBP1 protein target.

Stigmasterol, γ-sitosterol, and vitamin E unfolded excellent interactions with SOX2 bound to importin-α3 protein with the Glide scores of −2.845, −2.987, and −4.018 kcal/mol, respectively. Threonine (THR), arginine (ARG), and valine (VAL) are the prevalent amino acid residues that demonstrated good affinity with the phytocompounds via hydrogen-bonding interactions. In addition, the compounds revealed multiple hydrophobic, polar electrostatic, and pi−pi stacking interactions with various amino acid residues in the target proteins. Hydrogen-bond interactions are essential for ensuring the stability and specificity of ligand−receptor interactions, significantly influencing their overall binding affinity. Additionally, hydrophobic and pi−pi stacking interactions contribute to the stability of the ligand−receptor complexes. In our study, γ-sitosterol and stigmasterol displayed hydrogen bonding between the sterol hydroxyl group and the THR264 amino acid residue in the CtBP1 protein. In addition, with the same protein, octadecadienoic acid formed a couple of hydrogen bonds with VAL185 and ARG184 via the carbonyl oxygen and hydroxyl moieties of the carboxylic acid functional group. For SOX2 bound to importin-α3, stigmasterol interacted with SER144 via hydrogen bonding. Vitamin E displayed hydrogen bonding between the hydroxyl group of the phenolic−chromanol ring with ASP261 and pi−pi stacking interaction among the arene ring and TRP222. Overall, the outcomes of the docking simulations emphasize the crucial involvement of these specific complexes in facilitating molecular recognition and binding with the chosen protein

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receptors, attesting to their potential to disrupt critical tumor-related pathways. To validate their therapeutic effectiveness and safety, further *in vivo* studies are imperative.

3. MATERIALS AND METHODS

3.1. Glasswares and Chemicals. All analytical grade chemicals used in this investigation were purchased from HiMedia, Mumbai. The borosil grade glass was used for all objects.

3.2. Sample Collection. *S. maritima* was gathered in its entirety from the Karankaadu, Chitruvadi, Ramnad district (11° 2′ 46″ N, 76° 51′ 7″ E) (Figure 1). The taxonomic identity of the plant was confirmed by the Southern Regional Center of the Botanical Survey of India (BSI), Coimbatore. The whole plant was shade-dried, chopped, and utilized for further analysis.

3.3. Extraction of *S. maritima*. To prepare the extract, Soxhlet equipment was used. With increasing order of polarity, hexane, acetone, and methanol solvents were used to derive extracts from the dried *S. maritima* powder (30 g). The solvent extraction was performed several times with hexane, acetone, and methanol individually. Then, the solvent extracts were condensed using a rotary vacuum evaporator and air-dried. After air drying, the sample was macerated in boiling water for 2 h. Finally, an estimate of the extract's yield % was made.

$$\text{extract yield}(\%) = (\text{weight of extract obtained}/\text{weight of p}$$
$$\text{lant material used}) \times 100$$

3.4. Assessment of Phytochemical Profiling. Belazougui et al. described the protocol for phytochemical screening and prospection. It was investigated whether saponins, anthraquinones, flavonoids, alkaloids, phenols, steroids, and glycosides were present. The qualitative examination was based on precipitation processes, foamy appearance, and color change, which are characteristics of the chemical components that make up the *S. maritima* plant.[51]

3.5. Quantitative Assessment of *S. maritima* Extracts. *3.5.1. Total Phenol Content (TPC).* TPC of SMAE was determined employing the Folin−Ciocalteu reagent and gallic acid (GA) standard from Sigma-Aldrich, with minor modifications to the methods reported. One mL of the sample extract, 0.3 mL of a saturated solution of Na_2CO_3, and 0.1 mL of Folin−Ciocalteu reagent were added to a volumetric flask. Finally, double-distilled water was utilized to complete the volume. The solution was incubated at room temperature in the dark for 1 h. A UV−visible spectrophotometer set to 765 nm was used to determine TPC. Using GA as a reference, a calibration curve was fashioned, and the outcomes were given in milligrams of GA equivalents (GAE) per gram.[52]

3.5.2. Total Flavonoid Content. The total flavonoid content (TFC) in the extract was determined using the colorimetric technique with minor modifications. Aliquots (50 L) of each extract's accessions were deposited in a 96-well plate containing 10% aluminum chloride, 96% ethanol, and 10% sodium acetate. The mixes were incubated at room temperature in the dark for 40 min. A UV−visible spectrophotometer (JASCO UV) was used to detect the absorbance at 415 nm. TFC was reported as mg of quercetin equivalents per gram of dry weight (mg QE/g DW) using a quercetin calibration curve.[53]

3.6. Biological Studies. *3.6.1. Antioxidant Assays.* Antioxidant activities of the *S. maritima* extracts in hexane, methanol, and acetone have been evaluated using different assays such as DPPH, $ABTS^{\bullet+}$ radical, and reducing power assay (FRAP)[55] using the reported methods with different concentrations of stock solutions of extracts (20, 40, 60, 80, and 100 μg/mL).[54,55] In the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical assays, ascorbic acid acts as the positive control, whereas in the FRAP assay, rutin was used as the positive control. In all of the above assays, methanol was used as a negative control.

3.6.2. In Vitro Antidiabetic Activity. Antidiabetic activity of the *S. maritima* extracts in hexane, methanol, and acetone was examined by α-amylase inhibition assay and α-glucosidase inhibition assay according to the literature methods[56] with different concentrations of *S. maritima* extracts (20, 40, 60, 80, and 100 μg/mL) and positive control acarbose (20, 40, 60, 80, and 100 μg/mL). The sample-free reaction system was employed as a negative control, and the enzyme-free system as a blank to rectify background absorbance.

3.6.3. Anticancer Activity (MTT Assay). Evaluation of anticancer potency of the acetone extract of *S. maritima* (SMAE) has been examined by MTT assay with the most common cancer cells, A549 (lung carcinoma), and one normal HUVEC cells according to the reported method[57] with various concentrations (10, 100, 200, 300, and 400 μg/mL) of the SMAE and positive control doxorubicin (12.5, 25, 50, 100, and 200 μg/mL) for 48 h. In addition, the apoptosis induction capacity of the SMAE was examined with A549 cells by DAPI staining and ROS analysis with doxorubicin as the positive control. In both assays, the medium without the extract acted as the negative control.

4. CONCLUSIONS

In the present investigation, the medicinal potential of *S. maritima* (L.) Dumort (Amaranthaceae) and its pharmacologically active compounds have been explored. GC-MS, LC-MS, and HPLC analyses revealed the presence of various phytochemicals, including saponins, anthraquinones, flavonoids, alkaloids, phenols, and steroids from the extracts of *S. maritima*. These phytochemicals underlie diverse therapeutic and pharmacological properties, as evidenced by their *in vitro* antioxidant, antidiabetic, and anticancer activities. The electron- and proton-transfer abilities of the plant extract facilitate the excellent radical scavenging activity to neutralize the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals and reduction of oxidative species. Further, the equipotent antidiabetic activity of the extracts with the standard acarbose was alluded to by the various interactions of phytochemicals and the α-amylase and α-glucosidase enzymes. The *in vitro* anticancer activity of the *S. maritima* against lung cancer cells was demonstrated by MTT, DAPI, and ROS assays. Finally, the molecular docking exemplified the binding propensity of the key phytochemicals with CtBP1 and SOX2 bound to importin-α target proteins via hydrogen bonding, various hydrophobic interactions, and good Glide scores of −2.845−4.018 kcal/mol. Our research underscores the potential of plant-based drug development for future medical treatments beyond cancer and diabetes, emphasizing the importance of phytochemical identification and comprehensive pharmacological assessments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05591.

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The experimental procedures for thin-layer chromatography, column chromatography, HPLC analysis, FT-IR analysis, GC-MS, radical scavenging assays (DPPH, ABTS, reducing power), α-amylase assay, α-glucosidase assay, MTT assay, DAPI staining, ROS analysis, *in silico* evaluation (ADME properties, target protein preparations, binding site detection, and grid generations), preparation of ligand, molecular docking, and statistical analysis are provided in ESI. Images of TLC and column chromatographic analysis of *S. maritima*, LC-MS analysis of SMAE, concentration−cytotoxicity curve for doxorubicin and SMAE on A549 cells and noncancerous HUVEC cells, and photomicrograph of control and SMAE-treated A549 cells (PDF)

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Notes

The authors declare no competing financial interest.

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