Isolation, characterization and in silico docking studies of synergistic estrogen receptor α anticancer polyphenols from Syzygium alternifolium (Wt.) Walp.

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ABSTRACT

Aim: This study aims to isolate, characterize, and in silico evaluate of anticancer polyphenols from different parts of Syzygium alternifolium. Materials and Methods: The polyphenols were isolated by standard protocol and characterized using Fourier-transform infrared (FT-IR), High performance liquid chromatography - Photodiode array detector coupled with Electrospray ionization - mass spectrometry (MS/MS). The compounds were elucidated based on retention time and molecular ions (m/z) either by [M+H]+/[M-H]− with the comparison of standard phenols as well as ReSpect software tool. Furthermore, absorption, distribution, metabolism, and excretion (ADME)/toxicity properties of selected phenolic scaffolds were screened using OSIRIS and SwissADME programs, which incorporate toxicity risk assessments, pharmacokinetics, and rule of five principles. Molecular docking studies were carried out for selected toxicity filtered compounds against breast cancer estrogen receptor α (ERα) structure (protein data bank-ID: 1A52) through AutoDock scoring functions by PyRx virtual screening program. Results: The obtained results showed two intensive peaks in each polyphenol fraction analyzed with FT-IR, confirms O-H/C-O stretch of the phenolic functional group. A total of 40 compounds were obtained, which categorized as 9 different classes. Among them, flavonol group represents more number of polyphenols. In silico studies suggest seven compounds have the possibility to use as future nontoxic inhibitors. Molecular docking studies with ERα revealed the lead molecules unequivocally interact with Leu393, Arg391, Glu353, Leu391, Arg391, Gly301, Leu325 residues, and Phe404 formed atomic π-stacking with dihydrochromen-4-one ring of ligands as like estradiol, which stabilizes the receptor structure and complicated to generate a single mutation for drug resistance. Conclusion: Overall, these results significantly proposed that isolated phenolics could be served as potential ER mitigators for breast cancer therapy.

KEYWORDS: Estrogen receptor α, Fourier-transform infrared, high performance liquid chromatography-photodiode array detectors - electrospray ionization - mass spectrometry/mass spectrometry, molecular docking, polyphenols, Syzygium alternifolium

INTRODUCTION

The term polyphenol was derived from the Greek word “polus” means many and “phenol” means a chemical structure formed by one or more hydroxyl groups fortified with an aromatic hydrocarbon group [1]. Polyphenols, a group of plant secondary metabolites have increasing considerations in the fields of biological systems to cure different diseases, act as natural antioxidants, have nourishing and play a big part to human beings’ health. These are widespread in apples, berries, coffee, cocoa, onions, tea, and wine [2]. Based on number of phenol units, the plant phenolics were mainly divided into simple phenols and polyphenols. Polyphenols were again divided into two groups, i.e., non-flavonoids and flavonoids. Simple phenols, phenyl alcohols, stilbenes, chalcones, and lignans were categorized under the nonflavonoid compounds. Flavones, flavonols, flavanones, flavanols, dihydroflavonols, anthocyanins, proanthocyanidins, and isoflavones were categorized under the flavonoid group.

They arranged from simple single aromatic ring to complex polymers [3]. Tropical medicinal plants, green leafy vegetables, thick colored fruits, and wines were wealthiest wellspring of polyphenols. The previous studies experienced to the isolation and characterization of polyphenols with the assistance of various chromatographic and mass spectroscopic techniques like high performance liquid chromatography - electrospray ionization - mass spectrometry (HPLC-ESI-MS/MS) in
different medicinal plants such as *Rhus verniciflua* [4], *Citrus limetta* [5], *Juglans regia* [6], and *Annona cherimola* [7]. HPLC is a significant tool for separation of a mixture of polyphenolics in a desired manner and ESI coupled with MS/MS can produce the ions from macromolecules and can fragment them for identification. In this study, polyphenolic compounds were separated by HPLC - PDA detector. Because the equivocal of compounds was effectively separated and is superior in the virtue of flavan-3-ols, flavonoids, nonflavonoids, and their derivatives. HPLC-PDA coupled with ESI-MS/MS was chosen as a sought apparatus for correct identification of polyphenols in this study.

In the recent past, various researchers derived polyphenols from various medicinal plants to prove different biological activities such as anti-inflammatory [8], antibacterial [9], anticancer [10], antihyperglycemic [11], antimutagenic [12], antioxidant [13], hepatoprotective [14], and wound healing activities [15]. Thereafter, the exponential biologically active polyphenols were isolated from processed foods/drinks such as vanillic acid, gallic acid, caffeic acid, ferulic acid, and hydroxycinnamoyltyrosine from *Origanum vulgare*, *Camellia sinensis*, *Prunus virginiana*, *Thymus vulgaris*, and *Olea europaea*, respectively, showed synergistic biological activities [16]. The plant *Syzygium alterifolium* belongs to the family Myrtaceae and is locally known as mogi/adavi neredu. This plant inhabited to high altitude hilly top areas of Tirumala hills, part of the Eastern Ghats, Andhra Pradesh, India, and is recently categorized under the endangered state by IUCN-red data book [17]. The ethnomedical studies state that stem bark powder was utilized for the treatment of external wounds [18] and oral intake regulate blood sugar level [19]. Fruit powder was used for the treatment of diabetes [20] and diarrhea [21]. The previous enormous evidence reveals that leaf part of the plant has anticancer [22], antimicrobial [23], antioxidant [24], hypoglycemic and anti-inflammatory activities [25]. The earlier qualitative and quantitative studies of secondary metabolites from *S. alterifolium* purported that rich in phenols [26].

However, isolation, characterization, and toxicity evaluation of polyphenols from *S. alterifolium* are still disputable. Hence, the present work was conducted to isolate and to characterize the polyphenols through Fourier-transform infrared (FT-IR), HPLC-PDA-ESI-MS/MS from stem bark, leaf and fruit parts of *S. alterifolium*. The pharmacokinetics assets and toxicological etiologies of isolated polyphenols were characterized using *in silico* tools like virtual screening and molecular docking approaches established against estrogen receptor α (ERα) (protein data bank [PDB]-ID: 1A52) ligand binding domain to potentiate the plausible recognized lead scaffolds as for future anticancer therapeutics.

**MATERIALS AND METHODS**

**Chemicals**

The high purity Milli q-MilliPak water (Merck water solutions, France) was used for the preparation of chemicals and ultra-pure Milli q-LCPak water for HPLC analysis. Polyvinylpolypyrroldone was procured from Himedia Laboratories, India. HPLC grade dichloromethane, acetone, methanol, formic acid, and NaOH were purchased from Molychem Laboratories, India. 0.1 mM concentration of stock solution was prepared using 18 standard polyphenols (data not shown) were used as reference compounds for identification of polyphenols. The obtained pseudomolecular ions (m/z values) were cross checked with available previous literature as well as liquid chromatography (LC)/MS database developed by ReSpect-Riken Msn spectral database [27].

**Collection and Extraction of Plant Materials**

Matured plant parts such as stem bark, leaves, and fruits were collected from the Nagatheertham area of Tirumala Hills and authenticated with the help of herbarium (voucher no. 121) deposited in Department of Botany, Sri Venkateswara University, Tirupati. The collected plant materials were washed and shade dried up to 15-20 days at room temperature (37°C). Then, grounded with the help of a blender and sieved it for further studies. Extraction of polyphenols from various parts of *S. alterifolium* was followed by the method of Magalhaes et al. [28].

**HPLC-PDA-ESI-MS/MS Instrumentation**

The chromatographic separation of polyphenols was analyzed using HPLC (Shimadzu lab solutions, Kyoto, Japan) equipped with a LC-20 AD pump, detection with SPD-20A PDA and ultraviolet-visible detectors. The LC solution data acquisition software was retrieved from Shimadzu, Kyoto, Japan, and was installed in the Hewlett Packard system for recording of chromatography and its integrated data. For mass analysis, bench top Triple Quadrupole mass spectrometer (Quattro Micro manufactured by Waters Company, Manchester, UK) was equipped with an ESI source, operated by Masslynx version 4.1 software program.

**Chromatographic Conditions**

Agilent XDB C18 (150 × 4.6 mm, 5 µm) column was used for separation of polyphenols. The mobile phase consists of 0.1% formic acid in 70% methanol for 25-30 min recording time of the column at 210 nm with a speed of 1.0 ml/min at 25°C. Sample injection was performed with the help of Rheodyne 7725 injection valve via 20 µl loop and pH of the mobile phase was adjusted to 3.0 using a Dolphin pH meter. The MS acquisition was performed using ESI in positive and negative modes. For negative mode [M-H]- spectral range was recorded from 100 to 900 m/z range, while in the positive mode [M+H]+ spectral range was recorded from 50 to 750 m/z. The parameters were set as 0.5 s interval period, 10,000 amu/s scan speed of flow rate, heat block and DL temperature was adjusted to 200°C, DL voltage 4.5 kV, qarray voltage 1.0 V, RF voltage 90 V, detection gain at 1.0 kV were maintained, and N2 gas was used as a nebulizer gas at the speed of 1.5 L/min.
Computational Analysis

The pharmacokinetics were exceptionally crucial and advancement strategies for identification of therapeutic potential molecular candidates, especially in ethnopharmacology. The analysis of pharmacokinetics through in vitro and in vivo approaches was captivating a lot of time and more expensive process [29]. Thus, we applied in silico programs for molecular screening and the properties computation of confined polyphenols from S. alternifolium.

Absorption, distribution, metabolism, excretion (ADME)/Tox properties

Initially, toxicity risk assessments such as mutagenic, tumorigenic, irritant, reproductive effects along with fragment-based drug-likeness and the overall drug score of polyphenols were predicted using the OSIRIS property explorer program [30]. The bioactive properties of lead molecules were envisaged using molinspiration server (www.molinspiration.com). Furthermore, the physicochemical properties, n-octanol/water partition coefficient, pharmacokinetics, drug-likeness, and synthetic accessibility of molecules were anticipated within the adaptable range through GB/SA approach by using robust SwissADME server [31]. From the above auspicious consequences, the filtered potential nontoxic compounds were utilized for further screening and docking approaches.

Ligands preparation

The recognized small molecules were retrieved with three dimensional (3D) structure data file format from PubChem database [32]. The protonation state of ligands was charged to neutral position (pH=7) for outline and sustains the hydrogen bond formation. The leads comprises stereochemical clashes were optimized through conjugate gradient energy minimization by using visual molecular dynamics v1.9.1 tool [33] by applying CHARMM27 force fields with an exclusive topology and parameters acquired from SwissParam server [34]. Furthermore, relaxed ligands were converted into AutoDock ligand (PDBQT) format and arranged as a spreadsheet by PyRx virtual screening module [35].

Receptor preparation

Recent enormous investigations reported that polyphenolic compounds were profoundly inhibited the breast cancer cell proliferation and malignant tumor growth through estrogen mediated effects. In the present examination, we screened the isolated compounds against human ERα by using molecular docking approaches. Here, ERα (PDB ID: 1A52) [36] crystal structure was downloaded from PDB (http://www.rcsb.org/pdb). Furthermore, protein structure was neutralized by addition of polar hydrogens to the side chains and main chain. Subsequent to ensuring substance precision, protein 3D structure was subjected to energy minimization to control the crystallography conflicts by applying GROMOS96 force fields. They optimize the bonds, angles, torsions, nonbonded, and electrostatic potentials by using Swiss-PDB Viewer v4.1 software package [37]. The final protein energy was reduced to 0.01 kcal/mol energy consistency. Furthermore, the streamlined protein was used as possibly permitted structure for virtual screening and docking simulations.

Virtual Screening and Molecular Docking Studies

Moreover, to portray the potential dynamic site in prepared protein, we utilized AutoLigand module implicit in AutoDock tools. By applying AutoDock force fields, habilitated pocket was generated for binding of ligands [38]. Pocket grid was generated using AutoGrid module and grid dimensions set as center_x = 101.1269, center_y = 23.015, center_z = 97.0783 (xyz axis Å) and grid points set as 25×25×25 Å with 0.375 Å grid spacing. Initially, we screened segregated polyphenolic compounds onto the ligand binding domain (LBD) of ERα though AutoDock Vina program in PyRx software [35]. In this contest, we used default Lamarckian genetic algorithm parameters and empirical free energy function as scoring algorithms and docked each ligand with 300 maximum exhaustiveness runs against protein grid. The top-ranked ligands were again re-docked by using a flexible docking approach with three replication frameworks as past docking strategies. Finally, the resulted lead phenolics have greater probability for profoundly utilized as templates for ERα as anticancer therapeutics.

RESULTS

FT-IR Analysis

The first and foremost method to know the isolated fractions with functional group analysis was by FT-IR instrument. From the FT-IR consequences, a total number of 6-8 peaks were obtained from each fraction [Figure 1]. Among those, the peaks at 3328.51 cm⁻¹ and 1238.39 cm⁻¹ of stem bark fraction I; 3361.83 cm⁻¹, 1235.55 cm⁻¹, and 1093.48 cm⁻¹ of stem bark fraction II; 3334.42 cm⁻¹ and 1236.38 cm⁻¹ of leaf fraction I; 3357.99 cm⁻¹ and 1235.63 cm⁻¹ of leaf fraction II; 3367.25 cm⁻¹ and 1236.70 cm⁻¹ of fruit fraction I; 3357.99 cm⁻¹, 1235.63 cm⁻¹, and 1093.44 cm⁻¹ of fruit fraction II corresponds to O-H/C-O stretch of phenols. These results paved a clear way for further HPLC-ESI-MS/MS analysis.

Identification of Polyphenols

Identification of polyphenols from stem bark

The identification of polyphenols was done based on their retention time and mass spectra (MS, m/z) determined using HPLC coupled with triple quadrupole mass spectrometer in positive and negative ion modes. These data were tabulated as retention time, peak area (%), m/z values, molecular weight, molecular formula, and name of the compound. Four peaks were obtained from both positive and negative ion modes of stem bark fraction-I. Here in the case of positive [M+H]+ mode showed retention time at 1.40 (m/z 111), 2.45 (m/z 121), 6.05 (m/z 165), and 7.30 (m/z 431) were identified as kaempferol,
flavanone, epicatechin, and homoorientin, respectively [Table 1 and Figure 2 ai]. In negative [M-H]− mode, the retention time at 1.40 (m/z 113), 2.45 (m/z 227), 6.03 (m/z 317), and 7.30 (m/z 329) were identified as palatinose monohydrate, kaempferol-3-glucoside, myricitrin, and syringetin-3-O-galactoside, respectively [Table 1 and Figure 2 aii].

Whereas in the case of fraction-II positive [M+H]+ mode of stem bark gives 6 peaks with the retention time at 1.33 (m/z 111), 1.47 (m/z 291), 1.58 (m/z 159), 7.59 (m/z 197), 8.05 (m/z 225), and 8.71 (m/z 413) were identified as kaempferol, (+)-catechin hydrate,peonidin, formononetin, flavanone, and orientin [Table 1 and Figure 2 aiii]. In negative [M-H]− mode, the retention time at 1.33 (m/z 117), 1.47 (m/z 287), 1.58 (m/z 267), 7.59 (m/z 283), 8.05 (m/z 448), and 8.71 (m/z 577) were identified as apigenin, eriodictyol, formononetin, acacetin, orientin, and rhoifolin, respectively [Table 1 and Figure 2 aiv].

Identification of polyphenols from leaf

The leaf part of the fraction-I gives 4 peaks in both positive and negative modes. Here, the positive [M+H]+ mode demonstrated retention time at 1.38 (m/z 139), 1.63 (m/z 193), 1.82 (m/z 197), and 2.49 (m/z 435) were identified as (-)-epicatechin, palatinose monohydrate, formononetin, and naringenin-7-O-glucoside, respectively [Table 1 and Figure 2 aii].
Table 1: HPLC-ESI-MS/MS characterized polyphenols isolated from different parts of *S. alternifolium*

| RT      | Peak area % | HPLC-ESI-MS/MS (m/z) | Molecular weight | Molecular formula | Name of the compound |
|---------|-------------|----------------------|------------------|------------------|---------------------|
| Stem bark fraction I [M+H]+ | 1.40 | 82.53 | 111 | 286.04 | C_{16}H_{14}O_{6} | Kaempferol |
|         | 2.45 | 14.53 | 121 | 224.25 | C_{16}H_{12}O_{5} | Flavonone |
|         | 6.03 | 0.62  | 165 | 290.26 | C_{16}H_{14}O_{6} | Epicatechin |
|         | 7.30 | 2.05  | 431 | 448.37 | C_{21}H_{23}O_{11} | Homoorientin |
| Stem bark fraction I [M-H]- | 1.4  | 82.53 | 113 | 342 | C_{15}H_{18}O_{8} | Palatinose monohydrate |
|         | 2.4  | 14.53 | 227 | 448.37 | C_{16}H_{14}O_{6} | Kaempferol-3-glucoside |
|         | 6.0  | 0.62  | 317 | 464.37 | C_{16}H_{14}O_{6} | Myricitrin |
|         | 7.3  | 2.05  | 329 | 508 | C_{23}H_{24}O_{13} | Syringetin-3-O-galactoside |
| Stem bark fraction II [M+H]+ | 1.33 | 50.28 | 111 | 286 | C_{15}H_{14}O_{6} | Kaempferol |
|         | 1.47 | 18.46 | 291 | 290.27 | C_{15}H_{14}O_{6} | (+)-Catechin hydrate |
|         | 1.58 | 26.93 | 159 | 301.27 | C_{16}H_{14}O_{6} | Peonidin |
|         | 7.59 | 3.02  | 197 | 268.26 | C_{16}H_{14}O_{6} | Formononetin |
|         | 8.05 | 0.74  | 229 | 224.25 | C_{16}H_{14}O_{6} | Flavonone |
|         | 8.71 | 0.54  | 413 | 448 | C_{23}H_{24}O_{14} | Orientin |
| Stem bark fraction II [M-H]- | 1.33 | 50.28 | 117 | 270.23 | C_{15}H_{12}O_{6} | Apigenin |
|         | 1.47 | 18.46 | 287 | 288.25 | C_{16}H_{14}O_{6} | Eriodictyol |
|         | 1.58 | 26.93 | 267 | 268.26 | C_{16}H_{14}O_{6} | Formononetin |
|         | 7.59 | 3.02  | 283 | 284.26 | C_{16}H_{14}O_{6} | Acacetin |
|         | 8.05 | 0.74  | 448 | 448 | C_{23}H_{24}O_{14} | Orientin |
|         | 8.71 | 0.54  | 577 | 578.51 | C_{23}H_{24}O_{14} | Rhoifolin |
| Leaf fraction I [M+H]+ | 1.38 | 53.46 | 139 | 290.07 | C_{15}H_{14}O_{6} | (-)-Epicatechin |
|         | 1.63 | 10.59 | 193 | 342 | C_{16}H_{14}O_{6} | Palatinose monohydrate |
|         | 1.82 | 35.80 | 197 | 268.26 | C_{16}H_{14}O_{6} | Formononetin |
|         | 2.49 | 0.14  | 435 | 434.39 | C_{21}H_{20}O_{15} | Naringenin-7-O-glucoside |
| Leaf fraction I [M-H]- | 1.38 | 53.46 | 113 | 342 | C_{15}H_{12}O_{6} | Palatinose monohydrate |
|         | 1.63 | 10.59 | 151 | 450.39 | C_{15}H_{14}O_{6} | Marein |
|         | 1.82 | 35.80 | 301 | 302.27 | C_{15}H_{14}O_{6} | Hesperetin |
|         | 2.49 | 0.14  | 434 | 434 | C_{15}H_{14}O_{6} | Quercetin-3-arabinoside |
| Leaf fraction II [M+H]+ | 1.37 | 59.28 | 71  | 610 | C_{20}H_{20}O_{15} | Neohesperidin |
|         | 1.82 | 31.79 | 291 | 290.27 | C_{15}H_{14}O_{6} | (+)-Catechin hydrate |
|         | 2.47 | 5.57  | 127 | 578.52 | C_{21}H_{18}O_{15} | Procyanidin B1 |
|         | 3.39 | 1.66  | 197 | 268.26 | C_{16}H_{14}O_{6} | Formononetin |
|         | 3.67 | 1.32  | 317 | 478 | C_{16}H_{14}O_{6} | Isorhamnetin-3-O-glucoside |
|         | 4.02 | 0.36  | 438 | 436 | C_{16}H_{14}O_{6} | Phloridzin |
| Leaf fraction II [M-H]- | 1.37 | 59.28 | 221 | 342 | C_{15}H_{14}O_{6} | Palatinose monohydrate |
|         | 1.82 | 31.79 | 255 | 464 | C_{15}H_{14}O_{6} | Hyperoside |
|         | 2.47 | 5.57  | 289 | 290.26 | C_{15}H_{14}O_{6} | Epicatechin |
|         | 3.39 | 1.66  | 317 | 480 | C_{16}H_{14}O_{6} | Gossypol |
|         | 3.67 | 1.32  | 329 | 508 | C_{16}H_{14}O_{6} | Syringetin-3-O-galactoside |
|         | 4.02 | 0.36  | 591 | 592.54 | C_{20}H_{20}O_{15} | Fortunellin |
| Fruit fraction I [M+H]+ | 1.34 | 51.14 | 61  | 464 | C_{16}H_{14}O_{6} | Palatinose monohydrate |
|         | 3.41 | 7.54  | 102 | 208 | C_{16}H_{14}O_{6} | Chalcone |
|         | 3.77 | 3.14  | 235 | 446.40 | C_{21}H_{20}O_{15} | Sissotrin |
|         | 4.07 | 1.25  | 277 | 342 | C_{16}H_{14}O_{6} | Palatinose monohydrate |
|         | 4.99 | 2.05  | 291 | 290.27 | C_{15}H_{14}O_{6} | (-)-Catechin hydrate |
|         | 5.49 | 5.62  | 197 | 268.26 | C_{16}H_{14}O_{6} | Formononetin |
|         | 5.60 | 2.04  | 337 | 594 | C_{16}H_{14}O_{6} | Saponarin |
|         | 7.29 | 16.64 | 409 | 578.52 | C_{15}H_{14}O_{6} | Puerarin |
|         | 4.99 | 2.05  | 203 | 290.07 | C_{16}H_{14}O_{6} | Procyanidin B1 |
| Fruit fraction I [M-H]- | 1.34 | 51.14 | 59  | 342 | C_{15}H_{14}O_{6} | Palatinose monohydrate |
|         | 3.41 | 7.54  | 91  | 254 | C_{15}H_{14}O_{6} | Daidzein |
|         | 3.77 | 3.14  | 124 | 304.25 | C_{15}H_{14}O_{6} | (+/-)-Taxifolin |
|         | 4.07 | 1.25  | 151 | 272 | C_{16}H_{14}O_{6} | Naringenin |
|         | 4.99 | 2.05  | 203 | 290.07 | C_{16}H_{14}O_{6} | (-)-Epicatechin |
|         | 5.49 | 5.62  | 227 | 432 | C_{16}H_{14}O_{6} | Kaempferol-3-Rhamnoside |
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In negative [M-H]- mode, the retention time at 1.38 (m/z 113), 1.63 (m/z 151), 1.82 (m/z 301), and 2.49 (m/z 434) were identified as palatinose monohydrate, marein, hesperetin, and quercetin-3-arabinoside [Table 1 and Figure 2 bii]. Whereas in the case of leaf part of the fraction-II gives 6 peaks in both positive [M+H]+ and negative [M-H]- modes. In positive [M+H]+ mode the retention time at 1.37 (m/z 71), 1.82 (m/z 291), 2.47 (m/z 127), 3.39 (m/z 197), 3.67 (m/z 317), and 4.02 (m/z 438) were identified as neohesperidin, (+)-catechin hydrate, procyanidin B1, formononetin, isorhamnetin-3-O-glucoside, and phloridzin (Table 1 and Figure 2 biii). In negative [M-H]- mode, the retention time at 1.37 (m/z 221), 1.82 (m/z 255), 2.47 (m/z 289), 3.39 (m/z 317), 3.67 (m/z 329), and 4.02 (m/z 591) were identified as palatinose monohydrate, hyperoside, epicatechin, gossypin, syringetin-3-O-galactoside, and fortunellin [Table 1 and Figure 2 biv].

Table 1: (Continued)

| RT   | Peak area % | HPLC-ESI-MS/MS (m/z) | Molecular weight | Molecular formula | Name of the compound             |
|------|-------------|----------------------|------------------|------------------|----------------------------------|
| 6.03 | 2.04        | 243                  | 478              | C_{22}H_{22}O_{13} | Isorhamnetin-3-O-glucoside       |
| 6.50 | 7.82        | 327                  | 594              | C_{16}H_{15}O_{6}  | Saponarin                        |
| 7.29 | 16.64       | 593                  | 594.51           | C_{30}H_{26}O_{13} | Saponarin                        |
| Fruit fraction II [M+H]+ | 1.37 | 59.28 | 127 | 290.26 | C_{15}H_{14}O_{6} | (+)-Epicatechin                  |
| 1.82 | 31.79       | 197                  | 268.26           | C_{16}H_{12}O_{4}  | Formononetin                     |
| 2.47 | 5.57        | 235                  | 446.40           | C_{22}H_{10}O_{15} | Sissotrin                        |
| 3.39 | 1.66        | 277                  | 342              | C_{17}H_{11}O_{11} | Palatinose monohydrate           |
| 3.67 | 1.32        | 321                  | 416              | C_{21}H_{10}O_{6}  | Puerarin                         |
| 4.02 | 0.36        | 337                  | 594              | C_{27}H_{30}O_{15} | Saponarin                        |
| Fruit fraction II [M-H]- | 1.37 | 59.28 | 139 | 290.07 | C_{15}H_{14}O_{6} | (+)-Epicatechin                  |
| 1.82 | 31.79       | 165                  | 316              | C_{15}H_{14}O_{6}  | Rhamnetin                        |
| 2.47 | 5.57        | 227                  | 448.37           | C_{16}H_{12}O_{14} | Kaempferol-3-glucoside           |
| 3.39 | 1.66        | 293                  | 578              | C_{16}H_{12}O_{14} | Vitexin-2'-O-rhamnoside          |
| 3.67 | 1.32        | 327                  | 448.37           | C_{16}H_{12}O_{14} | Homoorientin                     |
| 4.02 | 0.36        | 593                  | 740              | C_{21}H_{32}O_{14} | Robinin                          |

HPLC-ESI-MS/MS: High performance liquid chromatography - electrospray ionization - mass spectrometry

Figure 2: (a-c) High performance liquid chromatography chromatograms of a stem bark b leaf c fruit polyphenols. Inset image belongs to respective mass spectra (i) fraction I positive mode [M+H]+, (ii) fraction I negative mode [M-H]-, (iii) fraction II positive mode [M+H]+, (iv) fraction II negative mode [M-H]-.
Identification of polyphenols from fruit

The fruit part of the fraction-I gives 9 peaks in both positive and negative modes. Here, in the positive [M+H]⁺ mode, the retention time at 1.34 (m/z 61), 3.41 (m/z 102), 3.77 (m/z 235), 4.07 (m/z 277), 4.99 (m/z 291), 5.49 (m/z 197), 6.03 (m/z 337), 6.50 (m/z 321), and 7.29 (m/z 409) were identified as hyperoside, chalcone, sissotrin, patalnose monohydrate, (+)-catechin hydrate, formononetin, saponarin, puerarin, and procyanidin B1, respectively [Table 1 and Figure 2 ci]. Whereas in negative [M-H]⁻ mode, the retention time at 1.34 (m/z 59), 3.41 (m/z 91), 3.77 (m/z 124), 4.07 (m/z 151), 4.99 (m/z 203), 5.49 (m/z 227), 6.03 (m/z 245), 6.50 (m/z 327), and 7.29 (m/z 595) were identified as patalnose monohydrate, daidzein, (±)-taxifolin, naringenin, (-)-epicatechin, kaempferol-3-rhamnoside, isorhamnetin-3-O-glucoside, saponarin, and tiliroside [Table 1 and Figure 2 ci]. In fruit part of the fraction-II gives 6 peaks in both positive and negative modes. Here in the case of positive [M+H]⁺ mode, the retention time at 1.35 (m/z 127), 4.90 (m/z 197), 5.73 (m/z 235), 7.66 (m/z 277), 8.01 (m/z 321), and 8.71 (m/z 337) were identified as epicatechin, formononetin, sissotrin, patalnose monohydrate, puerarin, and saponarin, respectively [Table 1 and Figure 2 ci]. Whereas in the case of negative [M-H]⁻ mode, the retention time at 1.35 (m/z 139), 4.90 (m/z 163), 5.73 (m/z 227), 7.66 (m/z 293), 8.01 (m/z 327), and 8.71 (m/z 595) were identified as (-)-epicatechin, rhamnetin, kaempferol-3-glucoside, vitexin-2''-O-rhamnoside, homoorientin, and robinin [Table 1 and Figure 2 ci].

Pharmacokinetics and Structure-based Virtual Screening

ADME/Tox evaluations

Among the 40 isolated polyphenols from S. alternifolium the 7 compounds such as (+)-epicatechin, epicatechin, flavanone, kaempferol-3-rhamnoside, patalnose monohydrate, syringetin-3-O-galactoside, and vitexin-2''-O-rhamnoside have repeated scaffolds and unknown chirality. Due to this, these compounds were discarded for further analysis. Finally, 33 lead phenolics were listed based on principle fragments. Furthermore, they subjected to initial toxicity assessments through in silico pharmacokinetic screening strategies. The chemical scaffolds were retrieved from PubChem database and illustrated in the OSIRIS Property Explorer program, which computes drug-relevant properties of compounds and provides results as color coded features. From the 33 phenolics, apigenin, chalcone, kaempferol, orientin, rhamnetin, and robinin have serious mutagenic properties. The compound sissotrin showed strong tumorigenic effect. The compounds daidzein, gossypin, phloridzin, procyanidin B1, and puerarin consist of reproductive effects [Table 2]. Therefore, these 12 compounds are not profitable for therapeutic usage. The other bioactivity scores against druggable targets such as G protein-coupled receptors ligands, kinase inhibitors, ion channel modulators, nuclear receptor ligands, and enzyme inhibitors were predicted from molinspiration server, revealed that all the compounds act as prominent drug candidates for above targets receptors [Table 2]. The ADME evaluations of drugs were considerably more vital for potential therapeutic ligands prophecy. Here, broadly used Lipinski rule of five (RO5) [39] strategies was implemented for ADME property prediction by SwissADME tool. The physicochemical features of lead scaffolds uncovered that there were only 07 compounds obey the RO5 values. Other 14 compounds such as (+)-catechin hydrate, fortunellin, homoorientin, hyperoside, isorhamnetin-3-O-glucoside, kaempferol-3-glucoside, marein, myricitrin, naringenin-7-O-glucoside, neohesperidin, quercetin-3-arabinoside, rhoifolin, saponarin, and tiliroside are violating the RO5 principles [Tables 3a and b]. Further, the pharmacokinetics resources like drug-likeness and synthetic accessibility of respected lead candidates were likewise analyzed [Table 3b].

Virtual screening and binding mode analysis

Recent in vitro studies reveal that S. alternifolium plant species have great antioxidant and anticancer activities. The previous reports positively revealed that the ERα has identified as a most significant therapeutic target specifically in breast cancer therapy. Hence, the investigations of phenolic scaffolds that can bind to ERα an interesting area for recognition of potential druggable lead compounds. In this study, we used virtual screening and molecular docking methods for 7 screened polyphenols against human ERα (PDB ID: 1A52) LBD with Autodock scoring functions by PyRx virtual screening program. Before going to docking, protein and ligands structure protonation states were adjusted to flexible point (pH=7) via structure optimization algorithms. The docking simulations found that all the ligands were prominently interacting with the core cavity of ERα active site like estradiol with functional residues [Figure 3]. As per docking log files, the compounds naringenin (439246), eriodictyol (440735), (+/-)-taxifolin (459533), (-)-epicatechin (72276), formononetin (5280578), acacetin (5280442), and hesperetin (72281) showed −8.9, −9.9, −8.7, −8.6, −7.4, −7.2, and −7.2 kcal/mol ΔGbinding energies, respectively [Table 4].

Figure 3: The secondary structure view of ligand binding domain of Estrogen receptor with potential isolated phenolics (a) secondary structure (red = helix, green = loops, yellow = sheets) with domain cavity (blue color dotted surface), (b) the structural alignment of lead scaffolds potentially fix in AutoLigand predicted domain cavity (blue color lines = functional residues, blue dotted surface = functional cavity, sticks = phenolic scaffolds)
The compound naringenin framed two H-bonds with Arg^{394} and Phe^{404} residues with 2.81, 3.08 Å bond distances. In H-bond, Arg^{394} and dihydrochromen-4-one both act as H-bond donors and mutually shares the electrons. In another bond, Phe^{404} bound as H-bond acceptor with donor dihydrochromen-4-one ring stacks and one additional atomic π-stacking was observed [Figure 4a]. The compound eriodictyol formed four H-bonds with Glu^{391}, Arg^{394}, and Leu^{521} residues with 3.13, 3.76, 3.11, 3.63 Å bond distances. Here, Glu^{391} acts as H-bond acceptor and it pulls electrons from CO group atoms of dihydrochromen-4-one ring stacks, Arg^{394} and Leu^{521} both are acts as H-bond donors to CO group atoms of dihydrochromen-4-one and dihydroxyphenyl ring stack, respectively, and Phe^{404} bound as hydrophobic residue [Figure 4b]. The compound (+/-)-taxifolin bound like eriodictyol, (+/-)-taxifolin and frame five H-bonds with Leu^{521}, Glu^{391}, Arg^{394} and Gly^{524} residues with 3.80, 3.68, 3.86, 3.19 and 3.29 Å bond distances. Leu^{521} and Arg^{394} bound as donors, their shares electrons to CO group atoms of dihydrochromen-4-one chain, respectively. Leu^{521}, Glu^{391} and Gly^{524} acts as H-bond acceptor, and they pull electrons from CO group atoms of dihydrochromen-4-one and dihydroxyphenyl ring stacks, the Phe^{404} framed one atomic π-stacking with ring stacks of dihydrochromen-4-one ring stacks [Figure 4c]. The compound (+)-epicatechin bound like eriodictyol, (+/-)-taxifolin and frame five H-bonds with Leu^{521}, Glu^{391}, Arg^{394} and Gly^{524} residues with 3.80, 3.68, 3.86, 3.19 and 3.29 Å bond distances. Leu^{521} and Arg^{394} bound as donors, their shares electrons to CO group atoms of dihydrochromen-4-one chain, respectively. Leu^{521}, Glu^{391} and Gly^{524} acts as H-bond acceptor, and they pull electrons from CO group atoms of dihydrochromen-4-one and dihydroxyphenyl ring stacks, the Phe^{404} framed one atomic π-stacking with ring stacks of dihydrochromen-4-one ring stacks [Figure 4d].
| Compound name | MW (g/mol) | Log P | BBB | GI | Bioavailability | Synthetic accessibility | ADME, RO5 violations | Pharmacokinetic properties | Lipophilicity | Water solubility |
|---------------|------------|-------|-----|---|----------------|------------------------|----------------------|--------------------------|-------------|----------------|
| Saponarin     | 285.45     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin     | 288.45     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Marein        | 272.25     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside    | 302.56     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin  | 318.24     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol   | 270.25     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin      | 290.27     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin| 308.45   | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Naringenin    | 298.25     | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin-7-O-glucoside | 320.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside-7-O-glucoside | 342.56 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin-7-O-glucoside | 360.27 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol-7-O-glucoside | 378.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin-7-O-glucoside | 396.27 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin-7-O-glucoside | 414.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Naringenin-3-O-glucoside | 432.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin-3-O-glucoside | 450.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside-3-O-glucoside | 468.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin-3-O-glucoside | 486.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol-3-O-glucoside | 504.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin-3-O-glucoside | 522.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin-3-O-glucoside | 540.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Naringenin-7-O-arabinoside | 558.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin-7-O-arabinoside | 576.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside-7-O-arabinoside | 594.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin-7-O-arabinoside | 612.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol-7-O-arabinoside | 630.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin-7-O-arabinoside | 648.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin-7-O-arabinoside | 666.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Naringenin-3-O-glucuronide | 684.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin-3-O-glucuronide | 702.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside-3-O-glucuronide | 720.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin-3-O-glucuronide | 738.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol-3-O-glucuronide | 756.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin-3-O-glucuronide | 774.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin-3-O-glucuronide | 792.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Naringenin-7-O-glucuronide | 810.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Rhoifolin-7-O-glucuronide | 828.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Hyperoside-7-O-glucuronide | 846.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Homoorientin-7-O-glucuronide | 864.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Eriodictyol-7-O-glucuronide | 882.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| Acacetin-7-O-glucuronide | 900.25 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |
| (+/-)-Taxifolin-7-O-glucuronide | 918.45 | 2.81  | Yes | Yes | Soluble        | Yes                    | No                   | No                       | 0.20        | 3.29           |

Tables 3a-b: ADME, RO5 violations, bioavailability and synthetic accessibility properties of selected lead compounds.

Table 3a: Physicochemical properties of toxicity filtered lead compounds.

- MW: Molecular weight (≤500), Log P: Average hydrophobicity (≤5), N. RB: Number of rotatable bonds (≤5), MR: Molar refractivity, TPSA: Topological polar surface area, N. HBDs: Number of H-bond donors (≤10), N. HBAs: Number of H-bond acceptors (≤10), N. RB: Number of rotatable bonds (≤5), N. HBDs: Number of H-bond donors (≤10), N. HBAs: Number of H-bond acceptors (≤10).
and it pull electrons from CO group atoms of dihydroxyphenyl ring stacks and Phe framed one atomic π-stacking with ring stacks of dihydrochromen-4-one ring stacks [Figure 4f]. The compound hesperetin formed three H-bonds with Glu, Arg and Leu residues of ERLBD with 2.70, 2.86 and 3.91 Å of bond distances. Arg and Leu both are acts as H-bond donors to CO group atoms of dihydrochromen-4-one and dihydroxyphenyl ring stacks. Glu acts as H-bond acceptor and it pulls electrons from CO group atoms of dihydrochromen-4-one framed one atomic π-stacking with ring stacks of dihydrochromen-4-one ring stacks [Figure 4g]. Structural superimposition of all lead scaffolds to LBD of ERα showed that functional Glu, Arg, Phe and other hydrophobic residues are participating to unique H-bond acceptor, H-bond donors and van der Waals contacts (π-stacking) formation, which are crucial for estrogenic 3-hydroxyl group interaction, water-mediated H-bond formation and receptor fixed in specific positions for hormone specificity [Figure 5].

DISCUSSION

A total number of 40 compounds were identified from stem bark, leaf, and fruit parts of the plant. Among them, the compound kaempferol from stem bark fraction I and II of positive [M+H]+ mode shows a noteworthy peak area of percentage. Kaempferol was the principle compound found in Brassica crops. Higher intake of Kaempferol reduces coronary heart disease, has strong antioxidant activity and suppress the growth of human gut cancer cell lines [40]. The compounds palatinose monohydrate from fraction I and II of positive [M+H]+ mode shows a noteworthy peak area of percentage. Kaempferol was the principle compound found in Brassica crops. Higher intake of Kaempferol reduces coronary heart disease, has strong antioxidant activity and suppress the growth of human gut cancer cell lines [40].
Table 4: AutoDock binding energy scoring values and interacting residues of selected polyphenols with ERαLBD

| Compound ID | Name of the compound | ΔGbinding energy (kcal/Mol⁻¹) | 2D structure | Hydrogen Bonds | Distance (Å) | Donor angle (°) | Atomic n-stacking residues |
|-------------|----------------------|-------------------------------|--------------|----------------|--------------|----------------|----------------------------|
| 439246      | Naringenin           | −8.9                          |              | Arg^{384}NH₂-----OC² | 2.81         | 102.96         | Phe¹⁰⁴                     |
|             |                      |                               |              | Phe^{450}O-----OC²  | 3.08         | 124.24         |                            |
| 440735      | Eriodictyol          | −8.9                          |              | Glu^{353}OE₁-----HO² | 3.13         | 134.68         |                            |
|             |                      |                               |              | Glu^{353}OE₁-----HO² | 3.76         | 166.56         |                            |
|             |                      |                               |              | Arg^{384}NH₂-----OC² | 3.11         | 106.20         |                            |
|             |                      |                               |              | Leu^{353}N-----OC²  | 3.63         | 103.13         |                            |
| 439533      | (+/-)-Taxifolin      | −8.7                          |              | Glu^{353}OE₁-----HO² | 3.20         | 130.10         | Phe¹⁰⁴                     |
|             |                      |                               |              | Glu^{353}OE₁-----HO² | 2.81         | 110.89         |                            |
|             |                      |                               |              | Arg^{384}NH₂-----OC² | 3.14         | 109.17         |                            |
|             |                      |                               |              | Gly^{353}CO-----OC² | 2.79         | 136.60         |                            |
| 72276       | (-)-Epicatechin      | −8.6                          |              | Leu^{346}NCO-----OC² | 3.80         | 123.46         | Phe¹⁰⁴                     |
|             |                      |                               |              | Glu^{353}OE-----OC² | 3.68         | 109.43         |                            |
|             |                      |                               |              | Leu^{353}N-----OC²  | 3.86         | 115.71         |                            |
|             |                      |                               |              | Arg^{384}NH₂-----OC² | 3.19         | 128.17         |                            |
|             |                      |                               |              | Gly^{353}CO-----OC² | 3.29         | 131.19         |                            |
| 5280378     | Formononetin         | −7.4                          |              | Arg^{384}NH₂-----OC² | 2.69         | 130.47         | Phe¹⁰⁴                     |
|             |                      |                               |              | Gly^{353}CO-----OC² | 2.70         | 123.37         |                            |
| 5280442     | Acacetin             | −7.2                          |              | Arg^{384}NH₂-----OC² | 2.73         | 130.50         | Phe¹⁰⁴                     |
|             |                      |                               |              | Gly^{353}CO-----OC² | 2.83         | 125.16         |                            |
|             |                      |                               |              | Leu^{353}N-----OC²  | 3.96         | 114.36         |                            |
| 72281       | Hesperetin           | −7.2                          |              | Glu^{353}E-----OC² | 2.70         | 122.06         | Phe¹⁰⁴                     |
|             |                      |                               |              | Arg^{384}NH₂-----OC² | 2.86         | 124.64         |                            |
|             |                      |                               |              | Leu^{353}N-----OC²  | 3.91         | 112.67         |                            |

¹Hydrogen bond donor atoms, ²Hydrogen bond acceptor atoms, ³distance between donor and acceptor atoms, ⁴angle between donor, acceptor and hydrogen atom

Apigenin from fraction II of stem bark extract of negative [M-H]⁻ mode indicated highest peak area of percentage. The compound apigenin was also identified from Acanthophora spicifera as principle compound, possesses potent analgesic, anti-inflammatory, and antiproliferative activities [41]. The compounds (-)-epicatechin and neohesperidin show the highest peak area in leaf part of fraction I and II of positive [M+H]⁺ mode. The compound (-)-epicatechin was found as the main compound in grapes and cocoa [42]. Whereas, neohesperidin as main compound in citrus fruits [43] has anticancer activity. Whereas in the case of leaf fraction I and II of negative [M-H]⁻ mode, the palatinose monohydrate showed astounding peak percentage. The compounds hyperoside and epicatechin from fruit fraction I and II of positive [M+H]⁺ mode show the highest peak area of percentage. The same type of result was obtained from Eucalyptus globulus showed hyperoside as the main compound and act as synergistic antimicrobial and antioxidant activity [44]. The compound epicatechin was isolated from tea leaves as the principle compound has antioxidant activity [45]. The compounds palatinose monohydrate and (-)-epicatechin from fraction I and II of negative mode of fruit part, respectively, showed an elevate peak area of percentage.

Compounds such as kaempferol, flavanone, peonidin, and myricitrin of the positive [M+H]⁺ mode and apigenin, acacetin, eriodictyol, orientin, and rhoifolin of negative [M-H]⁻ mode were solely obtained from stem bark of the plant. The compounds neohesperidin, phloridzin and naringenin-7-O-glucoside of positive [M+H]⁺ mode, marein, hesperetin, gossypin, querectin-3-arabinoside, fortunellin of negative
Flavanones are contrasted with flavonols, by having a chiral C2 center, C2-C3 saturated bond, phenol B ring, and most of them are nonplanar in nature, which have several to multi hydroxylated groups either by glycosylated or methylated [48]. From the isolated compounds, flavanone, naringenin, eriodictyol, hesperetin, naringenin-7-O-glucoside, neohesperidin and palatinose monohydrate comes under flavanone class of flavonoid compounds. Flavanons are one of the major subclasses of flavonoids, which are structurally similar with anthocyanins. Chemically they differ from other flavonoids by lacking of oxygen group at 4 positions and presence of double bond between 2 and 3 positions of C ring is the contrasting characters. The presence of hydroxyl group at 3 position creates two centers of asymmetry for polymerization of flavonol to give brown pigments, which are rich sources in green tea and cocoa [49]. The compounds epicatechin, (-)-epicatechin and (+)-catechin hydrate comes under dihydroflavonol class of flavonoid compound, which is the subclass of flavonols, structurally similar with flavonoids [50]. The compound peonidin comes under anthocyanin group of flavonoid compounds derived from phenylalanine, which are synthesized in cytosol, stored in vacuoles and imparts different colors to flowers, fruits and vegetables. Structurally they contain favylium cation linked either by hydroxyl or methoxyl groups and have one or more sugars [51].

Proanthocyanidins represent the second most abundant class of natural polyphenolics, which are widely distributed in various parts of bark, berries, flowers, fruits, and seeds; they give protection from microorganisms. They have a complex chemical structure being oligomers or polymers. Based on the bonding between monomers of proanthocyanidins, it may forms, either of B-type or A-type of structures [52]. The compound procyanidin B1 comes under proanthocyanidin group of tannin compound. From the isolated compounds, daidzein, formononetin, puercarin, and sissotrin comes under the flavone class of polyphenols, which are colorless polyphenols, predominant in legumes, especially in soybean plants has a significant impact on human health.

They are structurally similar to other flavonoids. However, differ in linking of the B ring to second position of the C ring, it may link via the third position of C ring. This structure is similar to an estrogen that is why they are also known as phytoestrogens [53]. Chalcones are nonflavonoid class of polyphenolic compounds have 1,3-diaryl-2-propen-1-one as backbone structure, found in fruits and vegetables. Chalcones display dimer, oligomer, diels-aldar adducts and other conjugates, which differ from other flavonoids by displaying open chain with three carbon molecules, binds to A and B ring instead of C ring [54]. From the isolated compounds chalcone, chloridzin and mearin are belonging to chalcone class of flavonoid compounds.

The pre-ADME approaches of pharmacological candidates screening are vital potentiated towards moderating different diseases. Here, the in silico ADME/Tox analysis is the profitable approach and safe to examine the promising drugs recognition within low time and cost [29]. The previous outcomes evidenced...
that plant polyphenols were strongly acted as antagonist or agonists for different therapeutic targets to ameliorate the various diseases [55]. However, in vivo and in vitro studies revealed the plant phenols consists some toxicological properties [56]. The plant S. alternifolium has enormous medicinal and bioavailability features [57]. Here, the adaptable polyphenols are isolated and characterized from S. alternifolium through in vitro and in silico approaches are considered as our theme line object. After distinguished polyphenols from S. alternifolium, pharmacokinetics and lead-likeness properties of compounds are implemented to optimize the ADMET toxicological features by using computational programmes. Overall two filtration schemes reveal that 7 potential polyphenols such as naringenin, eriodictyol, (-/-)-taxifolin, (-/-)-epicatechin, formononetin, acacetin, and hesperetin were obeying the RO5 principles and have a reliable ADMET features without toxicity. Here, we put our efforts to screen nontoxic next generation druggables for reducing the breast carcinoma. Some RO5 fluctuations of polyphenols are acceptable; however, the resulted compounds are potentiate for further in vitro analysis; only versatile compounds are considered in our study.

Molecular docking simulations of virtually screened compounds against the predicted druggable pocket of the ERα structure showed that binding affinities occupied in between −8.9 and −7.2 kcal/mol−1 energies. The three replication methods indicated that naringenin, eriodictyol, (+/-)-taxifolin, (+/-)-epicatechin, formononetin, acacetin, and hesperetin were bound with −8.9, −8.9, −8.7, −8.6, −7.4, −7.2, and −7.2 kcal/mol−1 ΔGbinding energies within the core cavity of receptor, respectively. From the docking consequences, the specificity of screened phenolic compounds resulted that they possibly repositioning the estradiol and has strong binding affinities with ERα like naringenin. Molecular interaction profiles reveal that compounds are from probable hydrogen bonds, atomic π-contacts, salt bridges like reference drug estradiol. Henceforth, they may serve as inhibitors to ERα for mitigating the breast invasive carcinoma. From the previous studies, naringenin inhibits or antagonize the respective biochemical or biosynthesis action of estrogen substrates by estrogen-mediated mechanism. It potentially reduces the breast carcinoma by preventing and suppresses the transforming growth factor beta 1 secretion [58] and protein kinase-C activation inhibition [59]. Moreover, naringenin, eriodictyol, (+/-)-taxifolin, (+/-)-epicatechin prompts the apoptosis in cancer cells through associated inhibition of fatty acid biosynthesis [60]. Isoflavone and formononetin are arresting the cell cycle through interferes with several cell signaling pathways to reduce breast cancer cell viability [61]. Acacetin prompts the apoptosis in breast cancer cells through interactive with various cell signaling pathways [62]. The flavonone, hesperetin, and naringenin have highest inhibitory potency in aromatase-expressing MCF-7 tumor cell viability [63]. Overall, our docking outcomes strongly agree with the previous enormous evidences. Therefore, these hopeful leads are reliable for further clinical and preclinical approaches. Thus, these compounds could use as anticancer agents against potential therapeutic targets toward various malignant disorders.

CONCLUSION

In this study, an investigation has been made to isolate polyphenols from the medicinal plant S. alternifolium and to characterize those with FT-IR and extended with HPLC-PDA-ESI-MS/MS. Our selected protocol and characterization tools were best suitable for isolation of polyphenols from S. alternifolium. Overall, a total number of 40 compounds were obtained, and most of them are belongs to flavonol class of polyphenolics. The result of this study screened out a selective number of compounds has anticancer activity. There is a good phenomenon about the polyphenols, acts as natural anticancerous agents. However, the isolation and standardization of polyphenols are meager in the present scenario. This study extends the use of natural polyphenols available in S. alternifolium in cancer-related diseases, especially in the case of breast cancer. The present computational methodologies revealed that the 7 phenolics consist of best drug-like properties, virtual screening, and molecular docking studies against ER indicate ligands potentially interact with functional cleft residues by forming prominent H-bonds and atomic π-contacts with flexible residues. The obtained results were agreed with previous evidence. Thus, overall in silico strategies indicate that the lead scaffolds could be served as future anticancer agents to ameliorate the breast carcinoma.

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