INTRODUCTION

Breast cancer is the most common cancer diagnosed worldwide, with an estimated 2.3 million new cases in 2020 alone. Due to the long-standing predominance of risk factors related to reproduction, hormones, and lifestyle in North America, Canada, and Western Europe, incidence rates in these countries have been higher. However, Asian nations like China, Japan, and India have also seen an increase in the prevalence of breast cancer. Recent data suggest, 1 in 9 Indian females has a lifelong risk of developing breast cancer. Breast cancer is a genetically and clinically heterogeneous disease. Due to a lack of diagnostic markers, it has been difficult to measure the progression of this disease. Breast cancers were categorized using conventional tumor classification such as fibroepithelial, myoepithelial, and mesenchymal neoplasms. Human epidermal growth factor receptor-2 (HER-2) and hormone receptors (ER and PR) are the most relevant clinical markers that are now frequently employed in stratifying the disease. HER-2 is positive in 1 in 5 individuals with breast cancer, while ER-α is positive in 1 in 3 cases. On that molecular classes, this disease has now recognized as estrogen receptor positive (ER+), HER-2-positive (HER2+), triple-negative (TN), and unclassified. Chemotherapeutics are used for its treatment, which either acts on one or more of these up- or down-regulating receptor signaling pathways to confront the deadly disease, but they are accompanied by serious side effects, including emesis, anorexia, diarrhea, skin rashes, hot flashes, headaches, fever, exhaustion, and hair loss. Numerous herbal medications are used in conjunction with chemotherapy or radiation therapy to overcome these challenges and increase the effectiveness of cancer treatment while minimizing side effects and consequences.

Body Revival® (BR), a polyherbal suspension has been developed for its multi-targeted function. Each 5 ml BR contained dry water extract of Aegle marmelos fruit pulp (150 mg), Acorus calamus root (200 mg), Rubia cordifolia root (200 mg), Symplocos racemosa stem bark (95 mg), Terminalia catappa leaf (75 mg), Ocimum sanctum leaf (10 mg), and Terminalia chebula fruit (15 mg). Aegle marmelos is a medicinal herb known for its antioxidant and anti-inflammatory potential. Acorus calamus is known for its anti-inflammatory and analgesic properties. Rubia cordifolia is known for its anti-inflammatory and anti-tumor potential. Symplocos racemosa is known for its anti-inflammatory and anti-tumor potential. Terminalia catappa is known for its anti-inflammatory and anti-tumor potential. Ocimum sanctum is known for its anti-inflammatory and anti-tumor potential. Terminalia chebula is known for its anti-inflammatory and anti-tumor potential.

The anticancer effect of an Indian herbal preparation was studied under a cancer cell line, as well as the in silico computational methods that explain the probability of protein ligands binding to ER-α and HER-2 receptors. The in vitro anticancer activity of Body Revival® suspension (BR) was determined using cytotoxicity tests, cell invasion and migration assays, and metastatic protein expression assays using MCF-7 breast cancer cells. The computational predictive biological method was applied to find out the pharmacodynamic and pharmacokinetic interactions between the active molecules present in the BR and ER-α/HER-2 of breast cancer.

RESULTS: BR showed significant and dose dependent cytotoxic effects on MCF-7 cells. The 50% effective cytotoxic dose of BR was 34.27 μM. It restricted invasion (26%) and migration (28%) of cancer cells than BSA control. MMP-9 and IL-6 concentration were reduced significantly (p<0.001) after treatment. Cucurbitacin B had maximum in silico binding energy score (-7.8) with ER-α, while symconoside B had with HER-2 (-9.4); but, among the other interactions between the two ligands and receptors, withaferin A had the highest affinity (-15.3). Additionally, withaferin A, symconoside A, and symconoside B curcurbitacin A demonstrated bioavailability and fulfilled safety standards.

Conclusion: Body Revival® showed as a powerful multi-target inhibitor of ER-α and HER-2 that has prospective anticancer action without side effects, and may be useful in the therapy management following a successful trial in breast cancer patients.

Keywords: Breast cancer, Cytotoxicity, MCF-7, ER-α, HER-2, Herbs, In Silico
mg), Withania somnifera root (325 mg), Blumea lacera fruit (115 mg), Rumex vesicatorius whole plant (240 mg), Cucumis melo seed (200 mg), and honey (qqs). These substances, together with their active constituents, have anticancer characteristics, including β-asarone, ucucbitcin B, methylglyoxal, quercetin, symconoside A, symconoside B and withaferin B^{17}. In addition, it has a substantial quantity of polyphenols such gallic acid, p-coumaric acid, quercetin, and apigenin^{18}. BR was reported to prevent myocardial infarction and decrease vascular platelet aggregation in animal models^{19}. Furthermore, it enhanced cellularity in bone marrow and leukocyte, granulocyte, and lymphocyte counts in peripheral blood of immunosuppressive animals^{18}. Recent study suggested BR improved quality of life, especially in the psychological and physical spheres of daily living of breast cancer patients^{20}. However, at this time, its mode of action in cancer environments is a matter of debate. Hence, extensive research was carried out using in vitro and in silico computational methodologies to ascertain the potential effect of BR on breast cancer cells and the underlying protein-ligands binding interaction probabilities with ER- and HER-2 receptors.

**MATERIAL AND METHODS**

**In vitro anticancer activities**

The following verified standard procedures for screening cancer drugs were employed.

**Cytotoxicity Test**

MCF-7 cells were maintained in Dulbecco’s modified eagle medium (DMEM) containing 10% FBS and incubated at 37°C in CO₂ incubator (Thermo Fisher, USA). Streptomycin and penicillin (100µg/ml) was used to avoid any contamination. Approximately, 1×10⁴ cells were grown separately in 96 well plates and treated with varying concentrations of BR (i.e., 0, 6.25, 12.5, 25, 50, 100 µl/ml) at 37°C for 24h. In the following day, post-treated cells were washed with PBS, and incubated with MTT (1 mg/ml stock) at 37°C for 4h. The absorbance was measured at 570 nm using a micro-plate reader (Sinothinker, China). All tests were done in triplicate. The 50% cytotoxic concentration of the test compound was identified for treated cell line^{21}.

**Cell Invasion and Migration Assay**

1×10⁴ MCF-7 cells were cultured in 24 well plates at 37°C in CO₂ incubator for 24h. The post-treated cells were then further incubated for another 24h after constructing scratches on the monolayer cells, and the migration was observed using an inverted microscope (Zeiss, Germany). In invasion assay, 1×10⁴ MCF-7 cells were seeded in Transwell chamber (ECM 555, Sigma, USA) for overnight at 37°C in a CO₂ incubator. Non-invaded cells were stained with Trypan blue dye (Sigma, USA) and washed sequentially to remove death cells. The invasive cells in the matrix were observed under an inverted microscope and the corresponding intensities were measured using a micro-plate reader at 450 nm^{22}.

**Metastatic Protein Expression Assay**

Briefly, 1×10³ MCF-7 cells were incubated with BSA vehicle or BR for 24 h at 37°C. Thereafter, relative protein expression analysis was carried out using matrix metalloproteinase-9 (MMP-9) and inflammatory cytokine, interleukin-6 (IL-6) specific ELISA kits (RayBio, USA) following the supplier's protocol. The micro-plate was read at 450 nm.

**In Silico Molecular Docking**

The computational predictive biological method was applied to find out the pharmacodynamic and pharmacokinetic interactions between the molecules present in BR and breast cancer receptors.

**(i) Receptors preparation:** The crystal structures of ER-α and HER-2 were downloaded from the Protein data bank (https://www.rcsb.org/). The structure of the protein was validated using SAVES 6.0 server. Energy minimization was done using SFDBV software. The structural quality of the target protein was determined using PROCHECK server.

**(ii) Determination of active sites:** The presence of amino acids in the active site was determined by the CASTp web server^{23}.

**(iii) Ligand preparation:** The structure data format of the selected 10 bioactive compounds of BR (apigenin, cucurbitacin B, gallic acid, methylglyoxal, p-coumaric acid, quercetin, symconoside A, symconoside B, withaferin and β-asarone) was retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). Gasteiger charges (polar hydrogen charges) were drafted and non-polar hydrogen molecules were combined with carbons. The protein and ligand were converted to PDBQT format using Autodock 4.2 tools.

**(iv) Molecular docking:** The docking of all 10 compounds was done into a 3D X-ray structure by Autodock 4.2 and AutodockVina. This is a fruitful automated method to investigate the binding of macromolecule and ligands. With the help of Autodock tools, Gasteiger charges and hydrogen atoms were added to the protein and for simulation AutodockVina was used. The algorithm that AutodockVina uses is the Broyden-Fletcher-Goldfarb-Shanno algorithm that improves the accuracy of docking and prediction of the binding mode. Finally, the binding complexes were visualized by Bovia Discovery Studio Visualizer^{24}.

**Pharmacokinetic, toxicity and safety studies:**

**Absorption, Distribution, Metabolism, and Excretion (ADME)**

ADME were measured at the SwissADME website (https://www.swissadme.ch). The following parameters such as aqueous solubility (LogS), skin permeation (Log kp), bioavailability Score, human intestinal absorption, blood-brain barrier and CYP2C9 inhibitors were measured and compared.

**Toxicity and Safety Prediction**

The tolerance capacity of animal models as well as human before application and ingestion are important. To predict the toxicity level, an online server named pkCSM (http://biosig.unimelb.edu.au/pkcsam/) was used where the structure can be drawn otherwise input the SMILES which were downloaded from Drugbank, Pubchem or Zinc15 database. The pkCSM allows the study of toxicological effects by analyzing AMES toxicity, oral rat chronic and acute toxicity and maximum tolerated dose for human.

**Statistical Analysis**

The research results were input in the electronic data-sheet for statistical analysis using SPSS version 20 (IBM, Chicago, USA). Categorical variables were presented as percentages. All data were presented as mean and standard deviation (SD) and statistically analyzed by t-test. P-value ≤0.05 was considered significant.

**RESULTS**

The results of cytotoxic effects of different doses of BR in MCF-7 cells were presented in Fig. 1A. The 50% cytotoxic dose of BR was noted 34.27±µl/ml (y=0.964x+83.04; r²=0.825). In Transwell cell membrane, BR exhibited 34% invasion (34±0.2).
by low dose (10 µl/ml) and 26% by high dose (10 µl/ml) than BSA control (Fig. 1B). Moreover, BR showed 42% (42±9.44) migration at low dose and 28% (28±10.23) at high dose, compared to 70% (70±8.25) migration in the BSA control (Fig. 1C).

MMP-9 or metastatic matrix protein expression of MCF-7 cells showed dose dependent inhibition after BR treatment (Fig. 1D). BR at the dose of 10 µl/ml inhibited 36% (56±5.28 pg/ml) and 52% (42±9.14) of MMP-9 respectively than BSA control (88±3.24). BR demonstrated dose dependent down regulation of IL-6 concentrations in the MCF-7 cells (Fig. 1E). At the dose of 10 µl/ml BR reduced 32.9% (63±8.3) IL-6 and at the dose of 20 µl/ml (48±7.6) diminished 48.9% compared to BSA control (94±5.8).

The result of in silico molecular docking analysis of protein-ligands binding was described in Table 1. The binding energy score of cucurbitacin B (-7.8) was the maximum, followed by withaferin (-7.7), symconoside A (-7.2), symconoside B (-6.8) and quercetin (-6.3) with ER-α indicating their strong therapeutic inhibitory properties with the protein ligands binding. Furthermore, the binding energy score of symconoside B (-8.4) was the highest, followed by symconoside A (-7.9), withaferin A (-7.6), quercetin (-7.5), cucurbitacin B (-6.9), apigenin (-6.9) and gallic acid (-6.4) with HER-2 also. With regard to interactions between ligands and receptors (ER-α and HER-2), withaferin A had the highest affinity (-15.3).

Table 1: In silico protein–ligands binding energy of active components of BR

<table>
<thead>
<tr>
<th>Active Components</th>
<th>ER-α (5J1H)</th>
<th>HER2 (5T92)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>-5.5</td>
<td>-6.9</td>
<td>-12.4</td>
</tr>
<tr>
<td>Cucurbitacin B</td>
<td>-7.8</td>
<td>-6.9</td>
<td>-14.7</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>-5.2</td>
<td>-6.4</td>
<td>-11.6</td>
</tr>
<tr>
<td>methylglyoxal</td>
<td>-2.8</td>
<td>-3.3</td>
<td>-6.1</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>-5.2</td>
<td>-6.1</td>
<td>-11.3</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-6.3</td>
<td>-7.5</td>
<td>-13.8</td>
</tr>
<tr>
<td>Symconoside A</td>
<td>-7.2</td>
<td>-7.9</td>
<td>-15.1</td>
</tr>
<tr>
<td>Symconoside B</td>
<td>-6.8</td>
<td>-8.4</td>
<td>-15.2</td>
</tr>
<tr>
<td>withaferin</td>
<td>-7.7</td>
<td>-7.6</td>
<td>-15.3</td>
</tr>
<tr>
<td>β-asarone</td>
<td>-4.5</td>
<td>-4.9</td>
<td>-9.4</td>
</tr>
</tbody>
</table>
The four most active ingredients present in BR, symconoside A, symconoside B, curcurbitacin, and withaferin A with HER-2, were shown in Fig. 2 as protein-ligand binding sites with amino acid consequences. Figures showed symconoside A binds with 10 amino acids: Ser_381, Glu_385, Met_427, His_513, Arg_515, His_516, Met_517, Ser_518, Lys_520 and Glu_525 of HER-2; symconoside B binds with 11 amino acids: Ser_381, Met_427, Ala_430, Thr_431, Ala_434, His_513, Arg_515, His_516, Met_517, Ser_518 and Arg_519 of HER-2; curcurbitacin B binds with 1 amino acid: Gln_506 of HER-2; and withaferin A binds with 2 amino acids: Arg_434 and Arg_503.

**Table 2. In silico pharmacokinetic, ADME and toxicological properties of active components of BR**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Symconoside A</th>
<th>Symconoside B</th>
<th>Curcurbitacin B</th>
<th>Withaferin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{26}H_{32}O_{14}</td>
<td>C_{26}H_{32}O_{14}</td>
<td>C_{32}H_{46}O_{8}</td>
<td>C_{28}H_{38}O_{6}</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>568.52</td>
<td>568.52</td>
<td>558.7</td>
<td>470.6</td>
</tr>
<tr>
<td>H-bond donor</td>
<td>14</td>
<td>8</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>H-bond acceptor</td>
<td>8</td>
<td>14</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Lipinski violation</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin permeation (LogKp)</td>
<td>-2.73</td>
<td>-2.73</td>
<td>-3.50</td>
<td>-3.02</td>
</tr>
<tr>
<td>Aqueous solubility (LogS)</td>
<td>-2.606</td>
<td>-2.79</td>
<td>-4.28</td>
<td>-4.46</td>
</tr>
<tr>
<td>Bioavailability score</td>
<td>0.17</td>
<td>0.17</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>Human intestinal absorption</td>
<td>32.58 (Low)</td>
<td>30.24 (Low)</td>
<td>79.86 (High)</td>
<td>86.31 (High)</td>
</tr>
<tr>
<td>Caco-2 permeability</td>
<td>-0.651</td>
<td>-0.261</td>
<td>0.582</td>
<td>0.651</td>
</tr>
<tr>
<td>Blood Brain Barrier</td>
<td>-1.45</td>
<td>-1.52</td>
<td>-1.17</td>
<td>-0.03</td>
</tr>
<tr>
<td>CYP2C9 inhibitor</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>AMES Toxicity</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Rat Oral Chronic Toxicity (log mg/kg bw/day)</td>
<td>5.42</td>
<td>5.53</td>
<td>1.66</td>
<td>0.95</td>
</tr>
<tr>
<td>Rat Oral Acute Toxicity (LD50) log mg/kg bw</td>
<td>2.50</td>
<td>2.71</td>
<td>3.82</td>
<td>2.78</td>
</tr>
<tr>
<td>Maximum tolerable dose (human) log mg/kg/day</td>
<td>-0.008</td>
<td>-0.194</td>
<td>-0.77</td>
<td>-0.41</td>
</tr>
</tbody>
</table>
Table 2 shown the in silico anticipated ADME and toxicity evaluations of the four major interacting molecules found in BR, including symconoside A, symconoside B curcubitacin B, and withaferin A. The strongest H-bond donor was symconoside A, although symconoside B also has the ability to act as an H-bond acceptor. The Lipinski rule was only not broken by withaferin A. Withaferin A and curcubitacin B both had a 0.55 biavailability score. Withaferin A showed the highest permeability in Caco-2 cells (0.885 cm/sec). Withaferin A had an intestine absorption rate of 86.32, followed by curcubitacin B at 79 and symconosides at 30 to 32. The blood-brain barrier (BBB) can be more easily crossed by withaferin A than by the other three substances. No active ingredients have been found to be harmful to AMES or to inhibit CYP2C9. Symconoside B, curcubitacin B, and withaferin A all had human tolerated doses (log mg/kg/day) of -0.194, -0.77, and -0.412, respectively, demonstrating their non-toxic nature.

**DISCUSSION**

Alkaloids, flavonoids, terpenoids and polyphenols compounds are a few examples of natural compounds that have been extensively used in preclinical research of breast cancer over the past 20 years due to their abundance in the natural world, low toxicity, and high efficacy. Recently published studies revealed active components of BR possess anticancer effects. These components might improve the immune system’s capacity to defend DNA against damage, reduce oxidative damage, or initiate the apoptotic process. However, its role in cancer has not been explored as a composite. Cancer immunotherapy has gained increasing attention over the past few decades and has grown into an excellent option for cancer treatment. Clinical adjunct therapies for the treatment of cancer have a long history in traditional Indian medicine.

MTT cell proliferation assay is one of the most widely used for evaluating anticancer activity of both synthetic derivatives and natural products. The viable cells contain NAD(P)H-dependent oxidoreductase enzymes, which reduce the MTT to formazan. In the present study, BR dose dependently and significantly enhanced the cytotoxicity and cell viability was seen to drop in MCF-7 breast cancer cells. This single study unequivocally demonstrated BR has anticancer effect. In a previous study, withaferin A, one of the active ingredients in BR, demonstrated cytotoxicity against four human cancer cell lines: DU-145 for the prostate, HCT-15 for the colorectal, A-549 for the lung, and MCF-7 for the breast.26 Similarly, curcubitacin B has been found to have anticancer properties in human leukemia cells. Moreover, symconosides showed cytotoxic action on Hep3B hepatocellular carcinoma cells.27

In the transwell cell migration assay, the ability of cells to chemotactically move in the direction of a chemo-attractant is quantified. Cell migration studies tally the quantity of cells that pass through a porous membrane, whereas cell invasion procedures quantify cell movement across extracellular matrix, a critical step in angiogenesis. The topology of the extracellular environment, adhesion, confinement, and stiffness are the main physical factors affecting cell movement. Additionally, it might evaluate distinct migratory capacities brought on by the over-expression of a receptor.28 Present study demonstrated BR has the ability to prevent cancer cells from spreading over normal cells into the surrounding tissues. Fruit pulp extract of *Aegle marmelos*, one of the important components of BR exhibited anti-proliferative activity through suppressing the breast tumor growth rate.29

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases. MMP-9 plays vital roles in cancer cell invasion and tumor metastasis. It supports angiogenesis by weakening matrix barriers and has the ability to reduce tumor neovascularization. It also plays a part in the breakdown of the basement membrane.23 Breast cancer tissues have high levels of MMP-9, which is directly linked to lymph node metastases and tumor stage.24 Triple-negative and HER-2 positive breast tumors clearly exhibit over expression of MMP-9.35 It can serve as a guide for determining the prognosis and course of treatment for breast cancer. Hence, development of MMP-9 inhibitors is an important area for breast cancer research.35 In the present study, metastatic matrix protein expression, particularly MMP-9 showed dose dependent inhibition after BR treatment in MCF-7. Withaferin A showed a significant correlation with a decrease of MMP-9 mRNA expression levels in metastatic Caski cell line.36 Interestingly, several cancers, including breast cancer, have been shown to over express the cytokine interleukin-6 (IL-6), which is found in the tumor microenvironment. In the tumor microenvironment, fibroblasts associated with the tumor and tumor cells are the main producers of IL-6.37-38 The immunopathogenic role of IL-6 and its signaling in breast cancer tumor development, metastasis, and treatment resistance has been shown in numerous investigations.39 It is also clear that the presence of high levels of IL-6 in breast cancer tissues encouraged the production of Jagged-1, which in turn helped the cancer cells proliferate and maintain their aggressive nature.40 Therefore, it would seem that IL-6 targeting and/or its receptor in combination with other effective anticancer medicines could be a potent therapeutic approach for breast cancer therapy.41 In this study, BR treatment showed significant inhibition of IL-6 concentrations in the MCF-7 metabolites. Other studies confirmed that withaferin A blocked IL-6 and TNF-α-induced cancer cell invasion and thereby eliminated the interactions between STAT3, STAT1, and NF-kB and suppressed STAT3 phosphorylation.42

Breast stem cell proliferation, differentiation, and cell death are regulated by ER-α and HER-2 signaling pathways, and breast cancer is mostly caused by the over expression of these signaling pathways. Hence, ER-α and HER-2 antagonists have received a lot of interest as possible anti-cancer drugs.43 The target of the present grid based in silico docking study was to screen out the potential antagonists of ER-α and HER-2 from the most effective compounds or ligands present in BR. Like apigenin, curcubitacin B, gallic acid, methylglyoxal, p-coumaric acid, quercetin, symconoside A, symconoside B, withaferin A and β-asarone. The present study revealed curcubitacin B has the highest binding score with ER-α, followed by withaferin A, symconoside A, and B, indicating their strong antagonistic properties in breast cancer. In addition, symconoside B has the greatest binding score to HER-2, followed by symconoside A, withaferin A, quercetin, curcubitacin B, and apigenin. Withaferin A also displayed the most encouraging potentiating qualities when taking into account the overall protein-ligands binding affinity for ER-α and HER-2. Withaferin A has previously been shown to treat down-regulation of ER-α protein expression, which correlates with a decline in nuclear level, suppression of mRNA level, and inhibition of E2-dependent activation of ER2e2b-luciferase reporter gene.44 Thus, four of the ten active molecules—symconosides A and B, curcubitacin B, and withaferin A of BR—displayed synergistic therapeutic potentials for breast cancer. In order to predict preclinical toxicological endpoints, clinical side effects, and ADME characteristics of these substances, in silico approaches were further explored. This study offers a powerful system, pharmacology approach for identification of promising and safe molecules from BR for development of breast cancer therapy. Withaferin A interacts with the positively charged residual amino acids of HER-2 at Arg_434 and Arg_503 and possesses 6
hydrogen donor bonds and 2 hydrogen acceptor bonds; whereas symonoside A interacts with 10 residual amino acids, symonoside B with 11, and cucurbitacin B with a single (Gln5,06). Since withaferin A adheres to all five of Lipinski’s principles, it may be termed orally bioavailable than other three components. Withaferin A has the greatest aqueous solubility, whereas symonoside is poorly soluble. The Caco-2 human colon cancer cell line is an example of an experimental screen used in drug discovery to measure membrane permeability and estimate human oral absorption. The most rapid rate of oral absorption and Caco-2 permeability was found in withaferin A. The blood-brain barrier (BBB) has been described as a dynamic interface that regulates the passage of substances between the blood and the brain to maintain the best possible circumstances for neuronal and glial activity,54 BBB prevents the entry of harmful substances into the brain. All four substances were expected to pass the BBB.

The Ames test is typically used in predicted toxicity models to assess potential carcinogenic/mutagenic effects of substances. In the current investigation, the Ames test revealed that none of the bioactive components were carcinogenic. Moreover, Cytochrome P450 2C9 (CYP2C9) enzyme in liver is involved in drug metabolism and excretion. CYP2C9 inhibition may lead to toxic drug accumulation and hazardous drug-drug interactions in the body. Present in silico study highlighted that the components present in BR did not have any role in CYP2C9 inhibition. Additionally, they failed to exhibit oral acute and chronic toxicity in animal models, and they were categorized as class V according to the poisonous class of the Globally Harmonized System of classification of chemical labels.

Therefore, taking into account in vitro and in silico studies, it may be concluded that the bioactive ingredients present in Body Revival® exhibit as potent multi-target inhibitors of ER-α and HER-2 with potential anticancer activity without side effects and may be helpful in the treatment management after successful trial in breast cancer patients.

Acknowledgement
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Conflict of Interest
There is no conflict of interest.

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