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Research Article

In-Vitro and *In-Silico* Approach Distinguish ER-α and HER-2 Antagonistic Properties of Indian Herbal Formulation on Breast Cancer

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Abstract

Objectives: 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.

Method: 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/and 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 34.27µl/ml. 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 (-8.4); 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*

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[1-2]. However, Asian nations like China, Japan, and India have also seen an increase in the prevalence of breast cancer[3]. Recent data suggest, 1 in 9 Indian female has a lifelong risk of developing breast cancer[4].

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[5]. Breast cancers were categorized using conventional tumor classification such as fibroepithelial, myoepithelial, and mesenchymal neoplasms[6-7]. 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[8]. HER-2 is positive in 1 in 5 individuals with breast cancer, while ER-α is positive in 1 in 3 cases[9]. On that molecular classes, this disease has now recognized as estrogen receptor positive (ER^+), HER-2-positive ($HER2^+$), triple-negative (TN), and unclassified[10]. 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[11-13]. 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[14-16].

Body Revival® (BR), a polyherbal suspension has been developed (M/s Health Reactive, Mumbai) to treat cancers. Each 5 ml BR contained dry water extract of *Aegle marmelos* fruit pulp (150 mg), *Acorus calamus* rhizome (175 mg), *Rubia cordifolia* root (200 mg), *Symplocos racemosa* stem bark (95

mg), *Withania somnifera* root (325 mg), *Blumea lacera* fruit (115 mg), *Rumex vesicarius* whole plant (240 mg), *Cucumis melo* seed (200 mg), and honey (qs). These substances, together with their active constituents, have anticancer characteristics, including β-asarone, cucurbitacin B, methylglyoxal, quercetin, symconoside A, symconoside B and withaferin[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 CO_2 incubator (Thermo Fisher, USA). Streptomycin and penicillin (100µg/ml) was used to avoid any contamination. Approximately, 1×10^4 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

1×10^4 MCF-7 cells were cultured in 24 well plates at 37°C in CO_2 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, 1×10^4 MCF-7 cells were seeded in Transwell chamber (ECM 555, Sigma, USA) for overnight at 37°C in a CO_2 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, 1×10^3 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 SPDBV 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/pkcsm/) 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 34.27µl/ml ($y=-0.964x+83.04$; $r^2=0.825$). In transwell cell membrane, BR exhibited 34% invasion (34±8.2)

by low dose (10µl/ml) and 26% by high dose (10µl/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 of10 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).

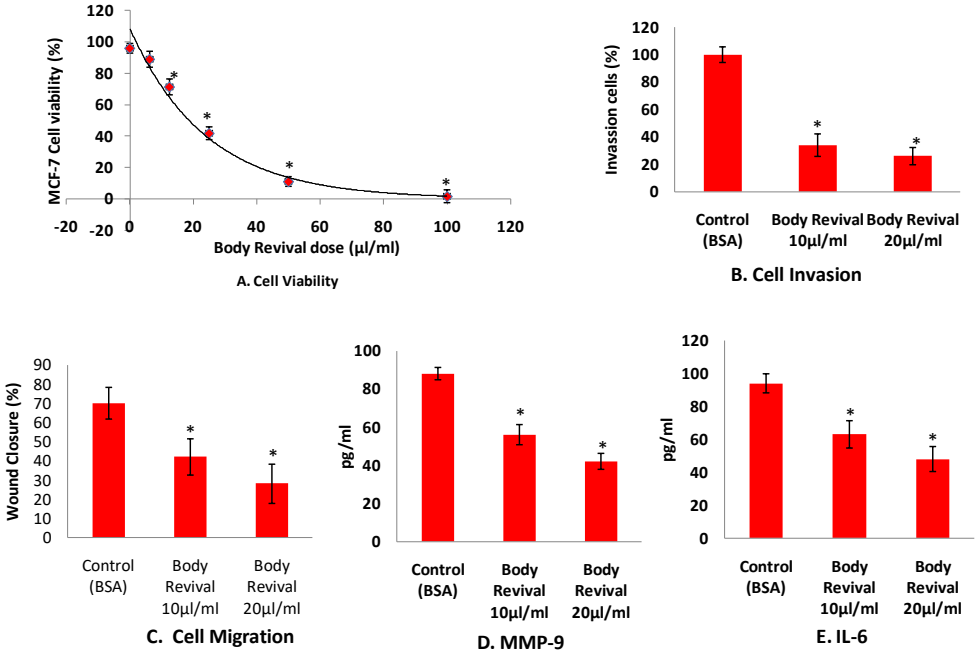

Figure 1: *In vitro* anti-cancer effect of BR on MCF-7 cells

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

Active Components	Protein – Ligands binding energy (kcal/mol)		
	ER-α (5JIH)	HER2 (5T92)	Total
Apigenin	-5.5	-6.9	-12.4
Cucurbitacin B	**-7.8**	-6.9	-14.7
Gallic acid	-5.2	-6.4	-11.6
methylglyoxal	-2.8	-3.3	-6.1
p-Coumaric acid	-5.2	-6.1	-11.3
Quercetin	-6.3	-7.5	-13.8
Symconoside A	-7.2	-7.9	-15.1
Symconoside B	-6.8	**-8.4**	-15.2
withaferin	-7.7	-7.6	**-15.3**
β-asarone	-4.5	-4.9	-9.4

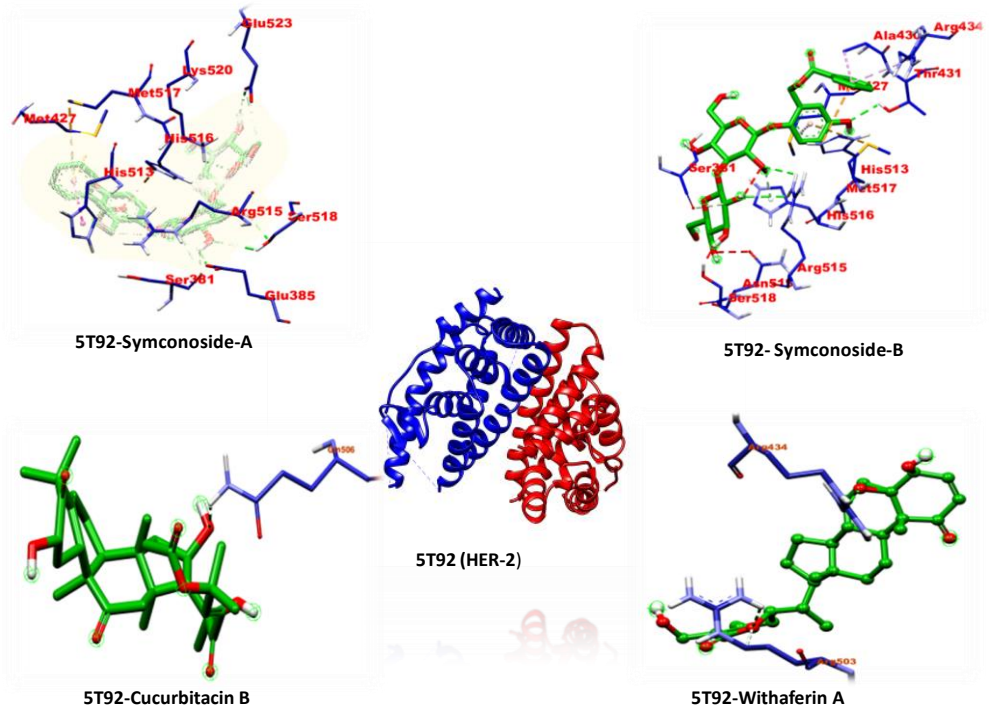

Figure 2: *In silico* HER-2 receptor binding sites of 4 most active components of BR

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; cucurbitacin 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

Properties	Symconoside A	Symconoside B	Curcurbitacin B	Withaferin A
Molecular formula	$C_{26}H_{32}O_{14}$	$C_{26}H_{32}O_{14}$	$C_{32}H_{46}O_8$	$C_{28}H_{38}O_6$
Molecular weight (g/mol)	568.52	568.52	558.7	470.6
H-bond donor	14	8	3	6
H-bond acceptor	8	14	8	2
Lipinski violation	3	3	1	0
Skin permeation (LogKp)	-2.73	-2.73	-3.50	-3.02
Aqueous solubility (LogS)	-2.606	-2.79	-4.28	-4.46
Bioavailability score	0.17	0.17	0.55	0.55
Human intestinal absorption	32.58 (Low)	30.24 (Low)	79.86 (High)	86.31 (High)
Caco-2 permeability	-0.651	-0.261	0.582	-0.651
Blood Brain Barrier	-1.45	-1.52	-1.17	-0.03
CYP2C9 inhibitor	No	No	No	No
AMES Toxicity	No	No	No	No
Rat Oral Chronic Toxicity (log mg/kg bw/day)	5.42	5.53	1.66	0.95
Rat Oral Acute Toxicity (LD_{50}) log mg/kg bw	2.50	2.71	3.82	2.78
Maximum tolerable dose (human) log mg/kg/day	-0.008	-0.194	-0.77	-0.41

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 curcurbitacin 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 cucurbitacin B both had a 0.55 bioavailability 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 cucurbitacin 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, cucurbitacin 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[17-18]. 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[25]. 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 oxido-reductase 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 colon, A-549 for the lung, and IMR-32 for the neuroblastoma[26]. Similarly, cucurbitacin B has been found to have anticancer properties in human leukemia cells[27].Moreover, symconosides showed cytotoxic action on Hep3B hepatocellular carcinoma cells[28].

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[29-30]. Additionally, it might evaluate distinct migratory capacities brought on by the over-expression of a receptor[21]. 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[31].

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[32]. Breast cancer tissues have high levels of MMP-9, which is directly linked to lymph node metastases and tumor stage[33]. Triple-negative and HER-2-positive breast tumors clearly exhibit over expression of MMP-9[34]. 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].

Furthermore, many 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 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, cucurbitacin B, gallic acid, methylglyoxal, p-coumaric acid, quercetin, symconoside A, symconoside B, withaferin A and β-asarone[17]. The present study revealed cucurbitacin 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, cucurbitacin 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 ERE2e1b-luciferase reporter gene[44]. Thus, four of the ten active molecules—symconosides A and B, cucurbitacin B, and withaferin A of BR—exhibited 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 systems 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 symconoside A interacts with 10 residual amino acids, symconoside B with 11, and cucurbitacin B with a single (Gln_506). 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 symconoside 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[45]. 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.

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Conflict of Interest

There is no conflict of interest.

REFERENCES

1. American Cancer Society, 2021. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71:209-249. https://doi.org/10.3322/caac.21660 PMid:33538338

3. Cardoso F, Spence D, Mertz S, Corneliussen-James D, Sabelko K, Gralow J et al. Global analysis of advanced/metastatic breast cancer: Decade report (2005-2015). The Breast 2018; 39:131-138. https://doi.org/10.1016/j.breast.2018.03.002 PMid:29679849

4. Kumar SK, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 and projection for 2025: Result from National Cancer Registry Programme, India. Indian J Med Res 2022; 156(4&5):598-607. https://doi.org/10.4103/ijmr.ijmr_1821_22 PMid:36510887 PMCid:PMC10231735

5. Riaz M, van Jaarsveld MT, Hollestelle A, Prager-van der Smissen WJ, Heine AA, Boersma AW, et al. miRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs. Breast Cancer Res BCR. 2013; 15: R33. https://doi.org/10.1186/bcr3415 PMid:23601657 PMCid:PMC3672661

6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74. https://doi.org/10.1016/j.cell.2011.02.013 PMid:21376230

7. IARC. WHO classification of tumours of the breast. Eds. Lakhani S, Ellis I, Schnitt S, Tan PH, van de Vijver MJ. 4th ed. Lyon, IARC Press, 2012.

8. Sengal AT, Mukhtar NS, Elhaj AM, Bedri S, Kantelhardt EJ, Mohamedani AA, et al. Immunohistochemistry defined subtypes of breast cancer in 678 Sudanese and Eritrean women; Hospitals based case series. BMC Cancer 2017;17:804. https://doi.org/10.1186/s12885-017-3805-4 PMid:29191181 PMCid:PMC5710067

9. Chand P, Garg A, Singla V, Rani N. Evaluation of immune histochemical profile of breast cancer for prognostics and therapeutic use. Niger J Surg 2018; 24:100-106. https://doi.org/10.4103/njs.NJS_2_18 PMid:30283220 PMCid:PMC6158994

10. Shawarby MA, Al-Tamimi DM, Ahmed A. Molecular classification of breast cancer: An overview with emphasis on ethnic variations and future perspectives. Saudi J Med Med Sci 2013; 1:14-9. https://doi.org/10.4103/1658-631X.112908

11. NIH, National Cancer Institute. Side Effects of Cancer Treatment. 2023. https://www.cancer.gov/about-cancer/treatment/side-effects

12. American Cancer Society, Chemotherapy Side Effects. 2023. https://www.cancer.org/content/dam/CRC/PDF/Public/8419.00.pdf

13. CDC: Cancer Survivors, Side Effects of Cancer Treatment. 2023. https://www.cdc.gov/cancer/survivors/patients/side-effects-of-treatment.htm

14. Yin SY, Wei WC, Jian FY, Yang NS. Therapeutic applications of herbal medicines for cancer patients. eCAM 2012; 302426. https://doi.org/10.1155/2013/302426 PMid:23956768 PMCid:PMC3727181

15. Subramaniam S, Selvaduray KR, Radhakrishnan AK. Bioactive compounds: natural defence against cancer. Biomol 2019; 9:758-768. https://doi.org/10.3390/biom9120758 PMid:31766399 PMCid:PMC6995630

16. Sinha K, Som Chaudhury S, Ruidas B, Majumder R, Pal T, Sur TK et al. Role of modern biological techniques in evidence-based validation of Ayurvedic herbometallic preparations. In: Evidence Based Validation of Traditional Medicines: A Comprehensive Approach. Eds. Mondal SC, Chakraborty R, Sen S. Springer Nature, Singapore, 2021. pp. 313. https://doi.org/10.1007/978-981-15-8127-4_16

17. Khan M, Pandit S, Saha A, Joshi V, Biswas TK, Sur TK. Perspective of using Indian polyherbal medicine in the treatment of cancer. Curr Res Compl Alter Med 2022; 6:165 https://doi.org/10.29011/2577-2201.100065

18. Khan M, Somani R, Sur TK. Assessment of immunopotentiation action of standardized Indian herbal formulation (Body Revival). Eur J Med Health Sci 2020; 2:1-6. https://doi.org/10.24018/ejmed.2020.2.2.229

19. Sur TK, Auddy B, Bhattacharyya D. Effects of Body Revival (herbal formulation) on human platelet aggregation and myocardial ischemia in rats. Zhong Xi Yi Jie He Xue Bao 2011; 9:746-51. https://doi.org/10.3736/jcim20110708 PMid:21749825

20. Joshi V, Khan M, Somani R, Pandit S, Sur TK. Indian medicine can improve quality of life in breast cancer patients: case studies. Nat Ayurvedic Med 2023; 7: 000395. DOI: https://doi.org/10.23880/jonam-16000395

21. Ruidas B, Sur TK, Das Mukhopadhyay C, Sinha K, Som Chaudhury S, Sharma P, et al. Quercetin: Silent retarder of fatty acid oxidation in breast cancer metastasis through steering of mitochondrial CPT1. Breast Cancer. 2022; 29:748-760. https://doi.org/10.1007/s12282-022-01356-y PMid:35511410

22. Ruidas B, Sur TK, Pal K, Som Chaudhury S, Prasad P, Das Mukhopadhyay C. Herbometallic nano-drug inducing metastatic growth inhibition in breast cancer through intracellular energy depletion. Mol Biol Rep 2020; 47:3745-3763. https://doi.org/10.1007/s11033-020-05467-7 PMid:32361897

23. CASTp web server. http://sts.bioe.uic.edu/castp/index.html?2011

24. Hazra AK, Ghosh C, Chatterjee S, Biswas TK, Pandit S, Sur TK. In silico studies of essential oil components from Indian herbal formulation against RdRp enzyme in Covid-19 virus. J Analytical Pharmaceutic Res 2022; 11:95-100.

25. Cai C, Wu Q, Hong H, He L, Liu Z, Gu Y, et al., In silico identification of natural products from Traditional Chinese Medicine for cancer immunotherapy. Scientific Reports 2021; 11:3332. https://doi.org/10.1038/s41598-021-82857-2 PMid:33558586 PMCid:PMC7870934

26. Yadav B, Bajaj A, Saxena M, Saxena AK. In vitro anticancer activity of the root, stem and leaves of Withania somnifera against various human cancer cell lines. Indian J Pharm Sci 2010; 72:659-663. https://doi.org/10.4103/0250-474X.78543 PMid:21695006 PMCid:PMC3116319

27. Chan KT, Li K, Liu SL, Chu KH, Toh M, Xie WD. Cucurbitacin B inhibits STAT3 and the Raf/MEK/ERK pathway in leukemia cell line K562. Cancer Lett 2010; 289:46-52. https://doi.org/10.1016/j.canlet.2009.07.015 PMid:19700240

28. Acharya N, Shah U, Hingorani L, Acharya S. Antioxidant and anticancer potential of Symplocos racemosa bark against Hep3b cell line. Int J Pharm Sci Res 2015; 6:4529-4533.

29. Albini A. Tumor and endothelial cell invasion of basement membranes: The matrigel chemoinvasion assay as a tool for dissecting molecular mechanisms. Pathol Oncol Res 1998; 4:230-241. https://doi.org/10.1007/BF02905254 PMid:9761943

30. Radu CG, Yang LV, Riedinger M, Au M, Witte ON. T cell chemotaxis to lysophosphatidylcholine through the G2A receptor. Proc. Natl Acad Sci 2004;101:245-250. https://doi.org/10.1073/pnas.2536801100 PMid:14681556 PMCid:PMC314170

31. Akhouri V, Kumari M, Kumar A. Therapeutic effect of Aegle marmelos fruit extract against DMBA induced breast cancer in rats. Scientific Rep 2020; 10:18016. https://doi.org/10.1038/s41598-020-72935-2 PMid:33093498 PMCid:PMC7581526

32. Farina AR, Mackay AR. Gelatinase b/MMP-9 in tumour pathogenesis and progression. Cancers 2014; 6:240-296. https://doi.org/10.3390/cancers6010240 PMid:24473089 PMCid:PMC3980597

33. Leifler KS, Svensson S, Abrahamsson A, Bendrik C, Robertson J, Gauldie J, et al. Inflammation induced by MMP-9 enhances tumor regression of experimental breast cancer. J Immunol 2013; 190: 4420-4430. doi: 10.4049/jimmunol.1202610. Epub 2013 Mar 15. https://doi.org/10.4049/jimmunol.1202610 PMid:23509357 PMCid:PMC3619527

34. Yousef EM, Tahir MR, St-Pierre Y, Gaboury LA. MMP-9 expression varies according to molecular subtypes of breast cancer. BMC Cancer 2014; 14: 609. https://doi.org/10.1186/1471-2407-14-609 PMid:25151367 PMCid:PMC4150970

35. Huang H. Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: Recent advances. Sensors 2018: 18, 3249. https://doi.org/10.3390/s18103249 PMid:30262739 PMCid:PMC6211011

36. Lee DH, Lim IH, Sung EG, Kim JY, Song IH, Park YK, Lee TJ. Withaferin A inhibits matrix metalloproteinase-9 activity by suppressing the Akt signaling pathway. Oncol Rep. 2013; 30:933-938. https://doi.org/10.3892/or.2013.2487 PMid:23708780

37. Ahmed OI, Adel AM, Diab DR, Gobran NS. Prognostic value of serum level of Interleukin-6 and Interleukin-8 in metastatic breast cancer Patients. Egypt J Immunol 2006; 13:61-68. PMID: 18689272.

38. Wolfe AR, Trenton NJ, Debeb BG, Larson R, Ruffell B, Chu K, et al. Mesenchymal stem cells and macrophages interact through IL-6 to promote inflammatory breast cancer in pre-clinical models. Oncotarget 2016; 7:82482-82492 https://doi.org/10.18632/oncotarget.12694 PMid:27756885 PMCid:PMC5347707

39. Tawara K, Scott H, Emathinger J, Wolf C, LaJoie D, Hedeen D, et al. High expression of OSM and IL-6 are associated with decreased breast cancer survival: synergistic induction of IL-6 secretion by OSM and IL-1b. Oncotarget 2019; 10:2068-2085. https://doi.org/10.18632/oncotarget.26699 PMid:31007849 PMCid:PMC6459341

40. Chen J, Wei Y, Yang W, Huang Q, Chen Y, Zeng K, Chen J. IL-6: The link between inflammation, immunity and breast cancer. Front Oncol 2022; 12:903800. https://doi.org/10.3389/fonc.2022.903800 PMid:35924148 PMCid:PMC9341216

41. Manore SG, Doheny DL, Wong GL, Lo HW. IL-6/JAK/STAT3 signaling in breast cancer metastasis: biology and treatment. Front Oncol 2022; 12:866014. https://doi.org/10.3389/fonc.2022.866014 PMid:35371975 PMCid:PMC8964978

42. Chung SS, Wu Y, Okobi Q, Adekoya D, Atefi M, Clarke O, et al. Proinflammatory cytokines IL-6 and TNF-α increased telomerase activity through NF-κB/STAT1/STAT3 activation, and Withaferin A inhibited the signaling in colorectal cancer cells. Mediators Inflamm 2017; 2017:5958429. https://doi.org/10.1155/2017/5958429 PMid:28676732 PMCid:PMC5476880

43. Rajagopal K, Kalusalingam A, Bharathidasa AR, Sivaprakash A, Shanmugam K, Sundaramoorth M. In silico drug design of anti-breast cancer agents. Molecules 2023; 28:4175. https://doi.org/10.3390/molecules28104175 PMid:37241915 PMCid:PMC10223712

44. Hahm ER, Lee J, Huang Y, Singh SV. Withaferin A suppresses estrogen receptor-α expression in human breast cancer cells. Mol Carcinog 2011; 50:614-24. https://doi.org/10.1002/mc.20760 PMid:21432907 PMCid:PMC3129407

45. Carpenter TS, Kirshner DA, Lau EY, Wong SE, Nilmeier JP, Lightstone FC. A method to predict blood-brain barrier permeability of drug-like compounds using molecular dynamics simulations. Biophys J 2014: 5;107:630-641. https://doi.org/10.1016/j.bpj.2014.06.024 PMid:25099802 PMCid:PMC4129472