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Research Article

Estrogenic activity of *Bryonia dioica* Jacq. through *in silico* and *in vitro* studies on *pS2* gene expression in the breast cancer cell line MCF-7

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Abstract

Bryonia dioica is a member of the Cucurbitaceae that produces bioactive secondary metabolites (PSM), especially steroidal cucurbitacins. Tubers accumulate cucurbitacins, tetrahydrocucurbitacins, bryonioside, and polyphenols such as kaempferol and isovitexin. Through in vitro and simulation studies, this study explores the potential estrogenic and antiestrogenic properties of *Bryonia dioica*. *In silico* study was performed by molecular docking simulation to predict the binding affinity of Bryonia PSM in Estrogen Receptor Alpha using Molegro Virtual Docker ver 6.0. The *in vitro* investigation of a methanolic tuber extract used MCF-7 cells, a breast cancer cell line with estrogen receptor (ER), in a medium with or without estradiol. We also analyzed the effect of the extract on the expression of the estrogen-dependent *pS2* gene using real-time polymerase chain reaction (RT-PCR). Molecular docking showed that Cucurbitacin-E exhibited the best binding affinity to the estrogen receptor with binding sites at His 524, Gly 521, Arg 394, Glu 353, and Leu 391. Methanolic extract of *Bryonia dioica* exhibited cytotoxic activity in MCF-7 cells with IC50 = 185 µg/mL (in medium without E2) and IC50 = 125 µg/mL (in medium with E2). In media with and without estrogen, it can stimulate the expression of the *pS2* gene at a concentration of 50 µg/mL, a marker for estrogenic activity. The results suggest that *Bryonia dioica* contains phytoestrogenic PSM with ER agonistic and cytotoxic activities.

Keywords

Bryonia dioica, estrogenic effect, MCF-7, *pS2* gene, cucurbitacins, cytotoxicity

Introduction

The term phytoestrogen describes a drug or compound from plants that exhibit estrogen-like effects (Mills and Bone 2000). Phytoestrogens exerts estrogenic effects by binding to estrogenic receptors (ERs) or human sex hormone-binding globulins (SHBG) and acting as an agonist, partial agonist, or antagonist competing with estrogen at the ER (Ibieta et al. 2014). At a cellular level, some phytoestrogens can affect protein tyrosine kinase, DNA topoisomerases I and II, matrix metalloproteinase (MMP9), and vascular endothelial growth factor (VEGF), which can inhibit cellular growth,

signal transduction, and cellular proliferation (Hsieh et al. 2018; Mostrom and Evans 2018). Phytoestrogens can mimic the structural and functional properties of 17-estradiol and affect various of molecular targets in breast cancer cells. Phytoestrogens have a lower affinity for estrogen receptors than estrogen, but they can compete with 17-estradiol at the ligand-binding domain of ER; they can exert epigenomic effects that may be helpful in the treatment and prevention of breast cancer (Basu and Maier 2018).

Known phytoestrogens include isoflavones like genistein, biochanin A, and daidzein, coumestans like coumestrol, stilbenes like resveratrol and rhaponticin (Bhat et al. 2001), lignans like enterolactone, matairesinol, enterodiol, and steroids, and sapogenins like dammarane and diosgenin (van Wyk and Wink 2017). Depending on the quantity of circulating estrogen, these substances appear to have an estrogenic or antiestrogenic impact (Chen et al. 2014). Furthermore, phytoestrogens are employed in Hormone Replacement Therapy to reduce menopause and premenstrual syndrome (Moreira et al. 2014). Therapeutically, some phytoestrogens are employed as natural selective estrogen receptor modulators (SERMs), particularly in treating hormone-dependent breast cancer. Some phytoestrogens presently in clinical interest focus are SERM, which do not affect the uterus but typically the hypothalamus/pituitary complex and bones (Wuttke et al. 2003). Only a few plant-produced SERM have been evaluated in clinical trials. However, some traditional herbal medicines, such as extracts of red clover and black cohosh, claim to alleviate premenstrual and postmenopausal symptoms. (Wade et al. 1999; van Wyk and Wink 2017).

Isoflavones, like genistein, have estrogenic and antiestrogenic effects; they also inhibit tyrosine protein kinases (Davis et al. 1999; van Wyk and Wink 2017). In terms of the ability of genistein to bind to ER, genistein is approximately 100 times less potent than 17-estradiol (Ibieta et al. 2005). Genistein and other isoflavones are present in soybeans and other legumes (van Wyk and Wink 2017). Genistein can reach circulation concentrations that modulate estradiol binding at ER or SHBG in humans who consume moderate amounts of soybeans. Remarkably, compared to Western women, Asian women who consume a diet high in soy had a 6-fold decreased risk of developing breast cancer (Key et al. 1990). Several epidemiological studies have suggested that a phytoestrogen-rich diet may protect against prostate, bowel, and cardiovascular disease. (Wiseman 2000). As a result, these phytoestrogens may function as preventive agents (Bhat et al. 2001).

Bryonia dioica Jacq. (Cucurbitaceae) is a perennial herb with a tuberous root, that grows in Europe, North Africa, and Western Asia (Sallam 2010). *B. dioica* roots contain alkaloids, polyphenols, triterpenes, sterols, saponins, and carbohydrates (Akihisa 1999; Bernaba 2012). The main active PSM are cucurbitacins (including cucurbitacin E, B, I, D, J, K, L), dihydro cucurbitacins (E and B), and tetrahydro cucurbitacin I (Pohlmann 1975), bryonolic acid (Saltykova 1968), and flavone C-glycosides (Baranowska 1995). This plant has traditionally been used for internal and external medicinal uses. Orally, *B. dioica* was used to treat inflammatory conditions, asthma, bronchial complaints, arthritis, hypertension, and intestinal ulcers. In folk medicine, this plant is known as a drastic purgative, emetic, bitter tonic, and anti-diabetic agent (Kadhim 2014; van Wyk and Wink 2017). In Africa, *B. dioica* has been employed to treat cancer, hypercholesterolemia, diabetic, fertility disorders, rheumatism and hypertension (Bernaba 2015). Some pharmacological studies showed that this plant can exhibit hepatoprotective (Kadhim 2014), antinociceptive, antimicrobial, antioxidant, hepatoprotective, anticancer (Jasiem 2020), and anti-inflammatory properties (Ukiya 2002). In Burkitt's lymphoma BL41 cell lines, an aqueous extract of *B. dioica* tuber induced apoptosis via the mitochondrial pathway (Bernaba et al. 2012) and caused G2/M cell cycle arrest in MDA-MB 231 breast cancer cells (Benarba et al. 2019).

The present study investigated the binding activity of active compounds in *B. dioica* at the estrogen receptor (ER) using Molecular Docking. The molecular modeling studies confirmed the interactions of these compounds at the active site of ER. We also investigated the cytotoxicity of a methanolic extract of *B. dioica* roots against the estradiol-dependent breast cancer cell line MCF-7 in media with or without estradiol. This cell line enables the discovery of estrogen agonists. The expression of *pS2*, an estrogen-dependent gene, was examined as an agonistic and antagonistic ligand marker (Masiakowski et al. 1982; Agustini et al. 2019).

Materials and methods

Molecular docking

The structures of *B. dioica* PSM were obtained from the simplified molecular-input line-entry system (SMILES) in the Knapsack web version (http://www.knapsackfamily.com/KNApSAcK/) (Shinbo 2006) and converted into 2D and 3D structures minimized in ChemDraw Professional 20.1.1.125 (https://perkinelmerinformatics.com/). The Molegro Virtual Docker 6.0 program was used for protein preparation, detecting cavities, grid construction, and targeted ligand docking. The docking wizard was customized by using a default MolDock optimizer algorithm with 100 numbers of runs. The docking method was validated by extracting estradiol, already present in the protein estrogen receptor alpha (PDB ID: 5WGD), and executing the docking process. Programs that can discard positions that are less than a predetermined Root Mean Square Deviation (RMSD) value from the known conformation (typically 1.5 or 2, depending on ligand size) are regarded as successful (Hevener 2009). Additionally, ligands in the molecule table are substituted with compounds from *B. dioica* using alignment and docking simulations for each of compound.

Samples preparation

Bryonia dioica tuber was obtained from the Department of Biology, Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg University, Germany. It had been obtained from wild plants in a vineyard

near Dossenheim, Germany by MW. It was cut into small pieces and then extracted with methanol. The filtrate was dried in a vacuum rotary evaporator and freeze-dried.

Cell culture

MCF-7, a human breast adenocarcinoma cell line, came from the cell culture collection at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg University, Germany. MCF-7 cells were cultivated and maintained in 75 cm^2 flasks at 37 °C, 5% CO_2, and 95% humidity. RPMI 1640 (Gibco Life Technologies) was supplemented phenol red, 2 mM glutamine, 100 U/ml penicillin, 1 mM sodium pyruvate, 0.1 mg/ml streptomycin, and 10% heat-inactivated Fetal Bovine Serum (FBS, Gibco Life Technologies).

Cell proliferation in media with or without estradiol (E2)

Cells were seeded into 96-well plates (50,000 cells/well) in a medium free of estrogen (phenol-free RPMI 1640 containing 100 U/ml penicillin, 1 mM sodium pyruvate, and 0.1 mg/ml streptomycin) with 10% stripped- FBS. Stripped FBS was prepared as follows; The DCC solution contained 714 mg of dextran T70, 7.14 g of charcoal (Norit A), and 628 mg of NaCl dissolved in 50 ml H_2O. 50 ml FBS was mixed with 1.5 ml DCC solution, then centrifuged at 2100× g for 30 min. The supernatant was separated and incubated with another 1.5 ml DCC solution for 30 min at 37 °C and centrifuged at 210× g for another 30 min. The stripped serum was sterile filtered through a 0.22 µm filter, then frozen at -20 °C until use. The cells were incubated for 48 h, and the medium was changed every 24 h with or without *B. dioica* extracts (twelve different concentrations from 0.25 to 1000 µg/mL; use µg/mL) and E2 (10 nM, as a positive control). After 48 h, the treated cells were washed twice with PBS. Neutral red (5 mg/100 ml in medium) was added and then incubated for three hours at 37 °C, 5% CO_2, and in a 95% humidified atmosphere. After that, cells were washed with PBS twice. The dye was then homogenized for 30 min on a plate shaker with 150 µl of HCl-isopropanol (0.04 N HCl, 100% 2-propanol) to dissolve it. An ELISA reader was then used to measure the absorbance at 540 nm as an indicator of cell metabolism.

pS2 gene expression

Cells were plated into six-well plates containing an estrogen-free culture medium, as previously described (200,000 cells/well). Every 24 h, the medium was replaced with a test compound. Test compounds are 50 µg/mL methanolic extract of *B. dioica*; 50 µg/mL methanolic extract with 10 nM E2 and 10 nM E2 as a positive control. The cells were washed with PBS twice after 48 h of treatment. Trypsinized cells were obtained by using trypsin-EDTA 300 µl/well at room temperature for 3 min. Next, 2 ml of medium (10% FBS) was added to inactivate trypsin. After centrifugation of the cells, RNA was extracted immediately from the cells pellets.

Extraction and purification of RNA transcripts

RNeasy Mini Kit (Qiagen) was used for RNA isolation, while RNase-Free DNase kit was used for DNA degradation (Qiagen). The first step in isolation is to dissolve the pellets. The sample is mixed with standardized buffer RTL (350 µl) (10 µl -mercaptoethanol [14.3 M] is added to 1.990 µl RTL buffer). The lysate was then homogenized by being passed through a gauge needle five times. The homogenized lysate was then added and thoroughly mixed with an equivalent volume of 70% ethanol. A 700 µL sample was then applied onto the RNA isolation column and centrifuged > 8000× g for 15 sec. Washing steps were started by adding 350 µL buffer RW1 onto the column and centrifuged > 8000× g for 15 seconds. Residual DNA was digested by adding 80 µL of DNase I solution [10 µL 2.72 KunitzU/µL DNase) to 70 µL buffer RDD] directly onto the column and then incubated for 15 min at room temperature. After that, 350 µL buffer RW1 was added to the column and centrifuged > 8000× g for 15 sec. Washing was repeated twice by adding 500 µL buffer RPE and centrifuging > 8000× g for 15 seconds. The adsorbed mRNA was eluted from the column and 30 µL of RNase-free water was added and centrifuged > 8000× g for 90 sec. The obtained total RNA is immediately placed at -80 °C. The concentration of total RNA was analyzed using Nanodrop Spectrophotometer (ND-1000 V3.30) at 260 nm. Electrophoresis was performed on agarose gels (0.1% ethidium bromide) for 10 min with 100V to check the quality of total RNA.

Conversion of mRNA to cDNA

Complementary DNA (cDNA), which is more resistant to deterioration and is not damaged by ubiquitous RNAse, was then created from mRNA. Using Avian-Myeloblastosis-Virus reverse transcriptase (AMV-RTase), the mRNA was reverse transcribed and then stored at -20 °C until real-time PCR analysis. The protocol for reverse transcription included two steps. In step 1, the PCR tube contained 0.5 µg oligo (dT), 1 µg total RNA, and 5.6 µl DEPC-H_2O was placed for 5 minutes in RT-PCR 70 °C. Step 2 involved the addition of 1 mM dNTP, 15 units of reverse transcriptase (AMV-RTase), 20 units of RNase inhibitor (RNasin), 5 mM $MgCl_2$, and 4 µL of buffer 5× (10 mM Tris-HCl pH 7.0, 50 mM KCl, and 0.1% Triton X-100). RT-PCR was then conducted. All of the acquired cDNA was kept in storage at -20 °C.

Expression of presenilin 2 (pS2) (Ibieta 2005; Agustini 2019)

PCR primers for *pS2* and -actin (Eurofins mwg operon), a housekeeping gene are represented below:

pS2 (f) 5'- CAT GGA GAA CAA GGT GAT CTG-3'
pS2 (r) 5'- CAG AAG CGT GTC TGA GGT GTC-3'
β-actin (f) 5'-TCA TGA AGT GTG ACG TGG ACA TCC GC-3'
β-actin (r) 5'-CCT AGA AGC ATT TGC GGT GGA CGA TG-3'

For RT PCR, the reaction mixture contained 1 µl cDNA, 200 µM dNTP, 400 nM *pS2* or β-actin primers (f, r), PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.5% Triton X-100, pH 8.5) and 1 U Taq DNA polymerase. The amplicon was separated and visualized by agarose gel electrophoresis.

Results

Molecular docking

Docking studies were carried out to investigate potential binding affinities based on scoring functions. The top-ranked compounds were chosen using docking score and hydrogen interaction. The best RMSD that could be obtained was 0.73. Fig. 1 shows the overlay's reference ligand's crystal and re-docked structure. The bioactive components of *B. dioica* were subjected to molecular docking simulation (PDB ID: 5WGD) on ER alpha, expressed in MCF7 as breast cancer cell model (ECACC 2012). Each substance was positioned as a ligand or an inhibitor that reduced the performance of the receptor. The outcome of each compound's docking score using the Molegro Virtual Docker ver. 6.0 program is shown in Table 1. The docking positions had the following coordinates: x = 61.58; y = -2.95; z = 63.01 with radius 15 A. The visualization method was used for cucurbitacin E (CuE). CuE formed typical hydrogen bonds with several residues, including His 524, Gly 521, Arg 394, Glu 353, and Leu 391, as shown in Fig. 2. The main intermolecular interactions that provide estrogen receptor alpha and ligand complexes a lot of stability are hydrogen bonds.

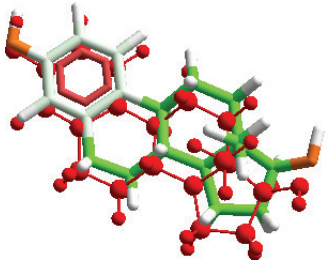

Figure 1. Redocking of estradiol in estrogen receptor alpha.

Table 1. MolDock Score and HBond prediction from bioactive compounds of *B. dioica.* (http://www.knapsackfamily.com/KNApSAcK).

Compound name	MolDock Score	HBond
(24R)-24-Hydroxy-24-vinyllathosterol	-110.12	-3.4872
(24S)-24-Hydroxy-24-vinyllathosterol	-118.48	-4.2734
Bryonioside G	-17.754	-4.4386
Bryonolic acid	-47.391	-1.0481
Cabenoside D	-91.995	-9.6648
Cucurbitacin B	-103.86	-2.723
Cucurbitacin D	-91.817	-4.0925
Cucurbitacin E	**-124.11**	**-2.3166**
Cucurbitacin I	-108.72	-2.3199
Cucurbitacin J	-95.612	0.81081
Cucurbitacin S	-38.353	0.63098
Dihydrocucurbitacin B	-111.79	-2.6133
Bryoamaride	-7.4332	-3.8326
Isovitexin 7-O-(6"'-caffeoyl)-beta-D-glucopyranoside	-3.9544	-3.1047
Saponarin	-47.274	-1.4303
Bryodulcosigenin	-107.27	-3.7409
Bryonioside A	-122.51	-12.296
Bryonioside B	-63.314	-2.9404
Bryonioside C	-14.557	-9.7637
Bryonioside D	-72.044	-5.7088
Bryonioside E	-113.7	-13.078
Bryonioside F	-85.752	-4.939
Kaempferol 3,7 dirhamnoside	-94.012	-9.3677
ESTRADIOL	-102.27	-9.5344

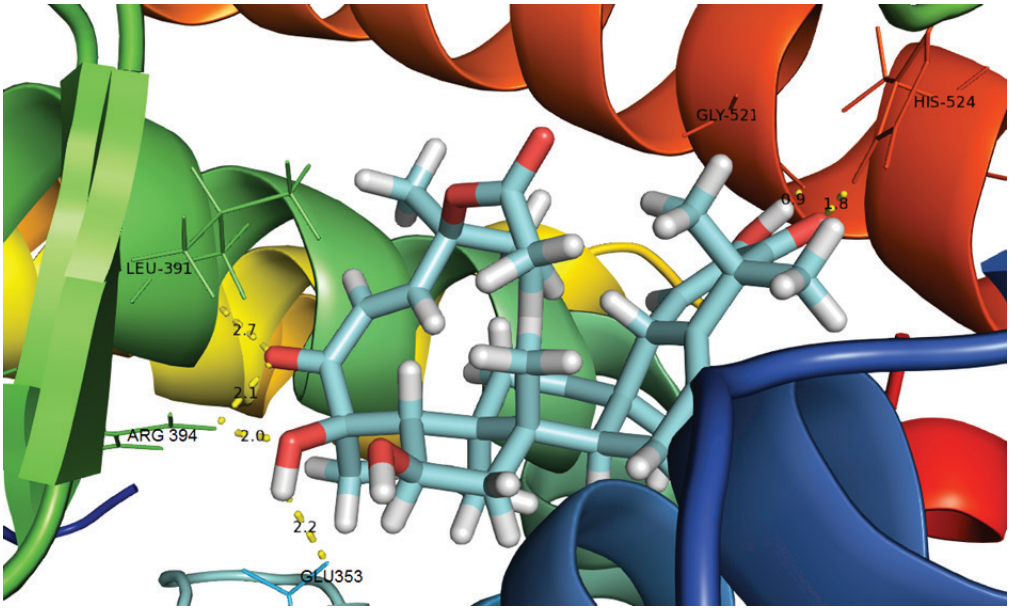

Figure 2. Interaction Cucurbitacin E with amino acid in estrogen receptor alpha.

Influence of a methanol extract from *B. dioica* on proliferation of MCF-7 cells in media with or without estradiol (E2)

This assay found that methanolic extract of *B. dioica* has a cytotoxic effect. High doses of the *B. dioica* extract can prevent MCF-7 cells from proliferating (Table 2). The outcomes of both assays were similar whether the medium contained or lacked E2 10 nM (Fig. 3). Methanolic extract of Bryonia dioica exhibited cytotoxic activity in MCF-7 cells with IC50 = 185 µg/mL (in medium without E2) and IC50 = 125 µg/mL (in medium with E2) (Table 2).

Table 2. IC50 values of *B. dioica* extract against MCF-7 cells in the media with or without 10 nM E2.

Sample	Without E2	With E2
	IC50 (µg/mL)	IC50 (µg/mL)
Methanolic extract of *Bryonia dioica*	185	125

E2: Estradiol.

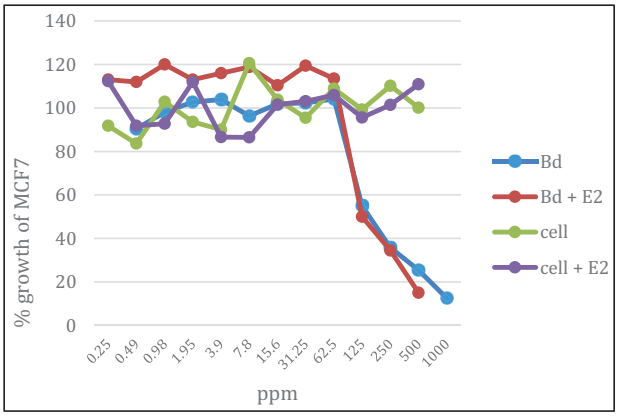

Figure 3. Effects of methanolic extract of *B. dioica* in media with (Bd+E2) and without (Bd) of 10 nM estradiol on the growth of MCF-7 cells. E2 = Estradiol.

Analysis of *pS2* expression

Presenilin-2 (*pS2*) is produced by estrogen-responsive cells after E2 activation. Figs 4, 5 demonstrate the expression of the *pS2* gene. Real-Time PCR was used to determine the relative copy number of the mRNA for *pS2* and ß-actin. In estrogen and estrogen-free media, *pS2* gene expression was induced by a methanolic extract of *B. dioica* (50 µg/mL), with estrogen media exhibiting lower *pS2* expression than estrogen-free media (Fig. 5). In cells with estrogen receptors, E2 can increase *pS2* levels. After three hours, an increase of the expression in presenilin can be observed (Fig. 5). According to relative expression results, all cells treated with E2 or phytoestrogens had higher levels of *pS2* than untreated controls.

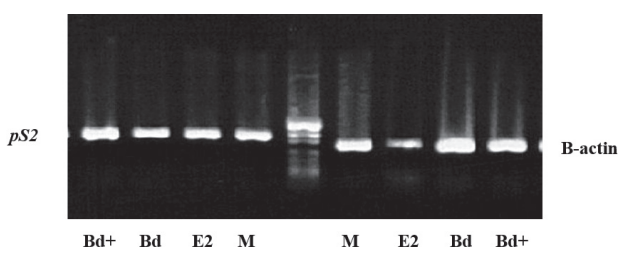

Figure 4. Stimulation of *pS2* expression in MCF-7 cells. *pS2* and β-actin, which are the PCR products, were separated by agarose gel electrophoresis. M = medium, E2 = 10 nM E2, Bd = 50 µg/mL methanol extract of *B. dioica*, Bd+=50 µg/mL methanol extract of *B. dioica* in the presence of E2 10 nM.

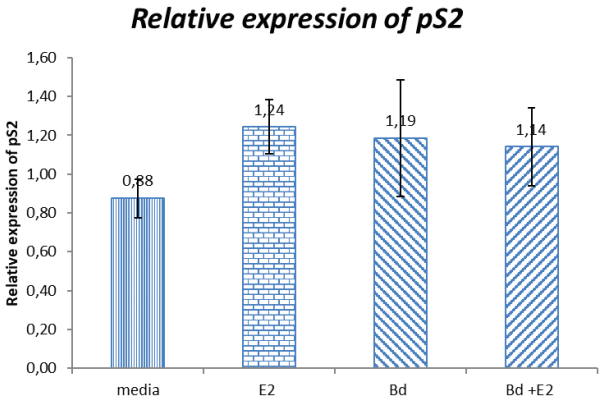

Figure 5. Relative expression of *pS2*. M = medium, E2 = 10 nM E2, Bd = 50 µg/mL methanol extract of *B. dioica*, Bd+E2 = 50 µg/mL methanol extract of *B. dioica* in the presence of E2 10 nM.

Discussion

Molecular docking on estrogen receptor

Due to their powerful ability to suppress a variety of malignancies, cucurbitacins and their derivatives have attracted increased interest in treating cancer. According to the results of an *in-silico* investigation, cucurbitacin E, an oxygenated tetracyclic triterpenoid, had the highest Molecular Docking score among the bioactive components of *B. dioica*. Several mechanisms, such as proapoptotic, the induction of autophagy, cell cycle arrest, the prevention of cancer invasion and migration, and many more, are used by cucurbitacins and their derivatives to inhibit cancer growth. Cucurbitacins also alter several intracellular signaling pathways. Intriguingly, each derivative of cucurbitacins may limit the growth and progression of several cancer cell types by triggering somewhat distinct molecular signaling cascades. The primary avenues through which cucurbitacins induce apoptosis to exhibit their powerful anticancer effect are signal transducers and activators of transcription 3 (STAT3) and Janus kinase (JAK) signaling. The primary targets of cucurbitacins are JAK/STAT pathway, MAPK pathway, and Wnt signaling. Moreover, cucurbitacins can cause G2/M phase cell cycle arrest, which could be another strategy for treating a variety of malignancies (Samuel 2019). According to Wang et al. (2017), cucurbitacin E (CuE) have an anticancer and anti-inflammatory effect by treatment, resulting in STAT3-dependent apoptosis and G1/G0 cell cycle arrest. CuE also acted as a tyrosine kinase inhibitor, interfering with the EGFR/MAPK signalling pathway and antiproliferative effect on A549 cells (Jing 2020). CuE inhibits the phosphorylation of STAT3, but not ERK-1/2. Due to its substantial enhancement of cisplatin's growth-inhibitory effect on

breast cancer, low-dose CuE may have clinical value in detecting and treating human breast cancer (Lan et al. 2013).

According to other publications, proliferation and telomerase activity of some breast cancer cell lines are inhibited by cucurbitacin B, and it also appears to have an inhibitory effect on ER-negative breast cancer cells SKBR-3 (Duangmano 2010). CuE led to cell death and G2/M cell cycle arrest in the Triple Negative Breast Cancer (TNBC) cell lines, MDA-MB-468 and SW527 (Kong 2014). CuE also reduced the percentage of T24 cells viable in human bladder T24 cancer cell lines (Huang 2012).

Effect of *B.dioica* on MCF7 proliferation

Our research has shown that the extract of *B. dioica* can inhibit and kill the aggressive MCF7 breast cancer cell line. Interestingly, the cooperation between *B. dioica* and E2 significantly promotes this cytotoxic activity. On a cellular level, this cell death was frequently accompanied by the induction of apoptosis and a G2/M phase cell cycle arrest (Benarba 2015). Another study that used Burkitt's lymphoma BL51 cell line demonstrated that an aqueous extract of *B. dioica* caused apoptosis via the intrinsic (mitochondrial) mechanism (Benarba et al. 2012). Proliferation assay in media with E2 showed that the methanolic extract of *B.dioica* has cytotoxic activity that inhibit the proliferation of MCF-7 cells. According to Johnston 2005, the ideal SERM should inhibit the proliferation of breast cancer cells dependent on estrogen and have a higher binding affinity for estrogen receptors (ER).

Effect of *B. dioica* on *pS2* gene expression

The *pS2* gene product is a small, secreted polypeptide with properties that resemble several growth factors, but its function is still unknown. According to preliminary research, *pS2* may serve as a marker for hormone-dependent breast cancer. The implicated function of *pS2* in the estrogen-mediated progression of breast malignancies and its potential application as a marker for estrogen-dependent cancer will continue to be the focus of ongoing studies (Stack et al. 1988). Based on the results of our investigation, *B. dioica* (50 µg/mL) reduced the expression of the cancer marker *pS2* in media with estrogen. Interestingly, the administration of *B. dioica* reduced the expression of *pS2* as an anti-cancer signal, following a pattern similar to that of the previous proliferation assay also discovered in this gene expression investigation. Although the exact mechanism by which this extract impacts *pS2* expression is unknown, one idea suggests that it interferes with the *pS2* receptor, which in turn increases transcription of the *pS2* gene by interacting with an ERE in the gene's 5'-flanking region (Stack et al. 1988; Yang et al. 2018).

Tahvilian et al. (2022) reported that a methanol extract from *B. dioica* roots could recover hormonal factors in female rat model polycystic ovary syndrome (PCOS). They demonstrated that as compared to the PCOS group, the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) levels were adjusted in the *B. dioica* groups ($p < 0.05$) adjusted. The potential of *B. dioica* and its PSM, especially CuE, to treat hormone-dependent breast cancer, should be investigated in more detail through in vitro and in vivo assays. Another in vitro assay should be investigated if CuE also can express *pS2* in estrogenic dependent breast cancer cell lines such as MCF-7 in a media with or without E2.

Conclusion

This study showed that Cucurbitacin E in *Bryonia dioica* provides the best binding affinity with ER-α. In contrast, the *in vitro* study showed that the methanolic extract had cytotoxic effects in MCF-7 cells. The cytotoxic activities may be due to a disturbance of the cytoskeleton by curcubitacins (Wang et al. 2017). MCF-7 grown in media with 10 nM estradiol had a lower IC50 value than MCF-7 cultivated in estradiol-free media. This study also demonstrated that *pS2*, an estrogen-regulated gene, could be expressed in MCF-7 cells by a methanol extract of *B. dioica*. We can conclude from these results that *B. dioica* can have both estrogenic and cytotoxic effects in MCF-7 cells.

Authors' contributions

The first author is a participant in an internship program at IPMB Heidelberg, who contributed mainly substantially to the *in vitro* research work presented in the manuscript. The second and third co-author contributed to the molecular docking work. The fourth co-author helped to elaborate the discussion of *in vitro* assay. The last co-author directed all the experiments, facilitated this work in IPMB Heidelberg, and helped to improve the manuscript.

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