Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Detection of Estrogenic, Antiestrogenic, and Drug Synergistic Activities of Seven Commercially Available Fruits by *In Vitro* Reporter Assays

Paramita Basu, Dinu Dixon, Sherin Varghese, Camelia Maier

Department of Biology, Texas Woman's University, Denton, TX 76204-5799, USA

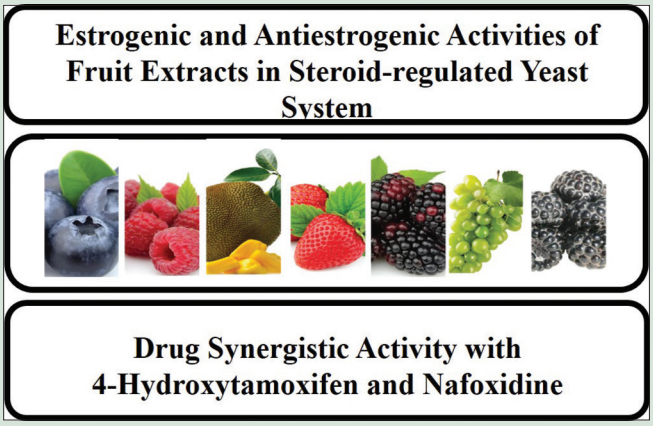
ABSTRACT

Background: Fruits are known to possess antiosteoporotic and anticancer properties in part due to their estrogenic and antiestrogenic activities. **Objective:** In this study, estrogenic, antiestrogenic, and drug synergistic activities of seven commercially available fruits were evaluated. **Materials and Methods:** A steroid-regulated transcription system in *Saccharomyces cerevisiae* containing a human estrogen receptor alpha expression plasmid, and a β-galactosidase gene reporter plasmid was employed for the estrogenic, antiestrogenic, and drug agonistic studies. **Results:** California table grape extract showed the highest estrogenic activity. The estrogenic activities of other extracts ranked as follows: blackberry, red raspberry, strawberry > blueberry > jackfruit, black raspberry. The transcriptional activities of the combination estradiol-fruit extracts (FEs) (400E equivalents) ranked as follows: blueberry (95.9%), blackberry (86.2%), black raspberry (88.9%), and California table grape (81.5%) > jackfruit (72.2%), and red raspberry (73.2%) > strawberry (60.7%). Black and red raspberry extracts showed the highest synergistic activities with 4-hydroxytamoxifen (4-OHT). Black and red raspberry extracts in combination with 4-OHT lowered the estradiol activity by 74.9% and 73.9%, respectively. The highest synergistic activity with nafoxidine (NAF) was displayed by red raspberry extract. Together, NAF and red raspberry extract lowered estradiol activity by 77.9%. Fold changes were calculated for drug synergistic activities of FEs, and they ranged from 1.3 to 15.3 for 4-OHT and 1.5–17.4 for NAF, respectively. **Conclusions:** The active compounds in the FEs studied may be useful in enhancing the antiestrogen activities of chemotherapy drugs and be used as chemopreventive agents for patients at high risk of estrogen-induced cancers.

Key words: 4-hydroxytamoxifen, antiestrogenic, drug synergism, estrogenic, nafoxidine, steroid-regulated yeast system

SUMMARY

- California table grape extract showed the highest estrogenic activity in a steroid-regulated transcription system in *Saccharomyces cerevisiae* containing a human estrogen receptor alpha expression plasmid and a β-galactosidase gene reporter plasmid. Strawberry extract showed the highest antiestrogenic activity
- Black and red raspberry extracts showed the highest synergistic activities with 4-hydroxytamoxifen. The highest synergistic activity with nafoxidine was displayed by red raspberry extract
- The active compounds in the fruit extracts studied may be useful in enhancing the antiestrogen activities of chemotherapy drugs and be used as chemopreventive agents for patients at high risk of estrogen-induced cancers.



Abbreviations Used: 4 OHT: 4 hydroxytamoxifen, Abs: Absorbance, CAA-glucose medium: Casamino acid glucose medium, E: Estrogen/Estradiol, ER: Estrogen receptor, ERα: Estrogen receptor alpha, FE: Fruit extract, LBD: Ligand binding domain, MU: Miller Unit, NAF: Nafoxidine, RQC: Resveratrol, quercetin, and catechin.

Correspondence:
Dr. Camelia Maier,
Department of Biology, Texas Woman's University,
Denton, TX 76204-5799, USA.
E-mail: cmaier@mail.twu.edu
DOI: 10.4103/pr.pr_111_17

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Estrogens play important roles not only in the development and function of gonadal organs but also in the functions of extragonadal tissues such as the bone, brain, heart, liver, and muscles.[1] Long-term exposure to estrogen is associated with an increased risk of breast cancer,[2] whereas estrogen deficiency leads to the development of cardiovascular disease, osteoporosis, and obesity during menopausal.[3-5]

Estrogen controls expression of target genes through the classical estrogen nuclear receptors (ER), as well as by nongenomic signaling mechanisms through plasma membrane estrogen receptors and intracellular transmembrane G-protein-coupled receptors, thus contributing to normal estrogen physiology as well as pathophysiology.[6,7] Nearly 70% of breast cancers are known to express ER, progesterone receptor, and/or estrogen-responsive genes.[8] Therefore, targeting ERs using antiestrogens has become important therapeutic treatment for cancer patients.[9] Antiestrogenic compounds are those capable of inhibiting estrogenic responses by competing with estradiol for the

Cite this article as: Basu P, Dixon D, Varghese S, Maier C. Detection of estrogenic, antiestrogenic, and drug synergistic activities of seven commercially available fruits by *In Vitro* reporter assays. Phcog Res 2018;10:137-42.

ligand-binding domain (LBD) of the ER and/or favoring interactions with corepressor complexes.[10] Some of known antiestrogens are tamoxifen, nafoxidine (NAF), chlorinated estrogens, and the pesticides chlorfluazuron, imazalil, and chlorfenapyr.[11-13] Tamoxifen is a triphenylethylene derivative classified as a selective estrogen receptor modulator as it acts as an agonist in the uterus and as an antagonist in the breast tissues.[9] Tamoxifen is commonly used as a chemotherapeutic agent in treating ER-positive breast cancer since it competes with estradiol for binding to the LBD of ER.[9]

NAF, a derivative of tamoxifen, is a nonsteroidal antiestrogenic drug, which had been developed for the treatment of advanced breast cancer. In their review, Legha *et al*. indicated that the cumulative data for all published clinical trials of NAF treatments of breast cancer showed a positive response of 31%, concluding that it was as satisfactory as any of the hormonal therapies.[14] However, NAF toxicity (mostly dermatologic but others as well) limited its practical usefulness.[14] It has been reported that demethylation of NAF resulted in the formation of lasofoxifene for treatment of osteoporosis showing high affinity for the ER and rapid Phase II metabolism followed by increased rate of excretion.[15] In the future, it could be possible that chemical modifications of NAF will reduce its side effects to be used as a potent antiestrogenic drug.

Berry phytochemicals are known to possess health enhancing properties as antioxidant, anti-cancer, anti-inflammatory, and antimicrobial agents.[16-18] It has been shown that fruit intake reduced the rate of bone fracture and increased bone mineral density.[19,20] In a previous study, we evaluated the polyphenolic contents and *in vitro* antioxidant activities of the seven commercially available fruits: blueberry (*Vaccinium corymbosum*, *Ericaceae*); jackfruit (*Artocarpus heterophyllus*, *Moraceae*); blackberry (*Rubus fruticosus*, *Rosaceae*); black raspberry (*Rubus occidentalis*, *Rosaceae*); red raspberry (*Rubus idaeus*, *Rosaceae*); strawberry (*Fragaria ananassa, Rosaceae*); and California grape (*Vitis californica*, *Vitaceae*).[21] In the current study, the estrogenic, antiestrogenic, and drug synergistic activities of the seven fruit extracts were assessed. Secondary metabolites in FEs may be useful for the treatment or prevention of osteoporosis, menopausal symptoms, and breast cancer. To the best of our knowledge, this is the first report of estrogenic, antiestrogenic, and drug synergistic activities of the seven fruits extracts by employing an estrogen-regulated transcription system.

MATERIALS AND METHODS

Chemicals

Ortho-nitrophenyl-β-galactoside, 17β-estradiol, sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \times 7H_2O$), sodium phosphate monobasic, monohydrate ($NaH_2PO_4 \times H_2O$), potassium chloride, magnesium sulfate ($MgSO_4 \times 7H_2O$), D-(+)-glucose, 2-mercaptoethanol (C_2H_6OS), and 200-proof ethyl alcohol (CH_3CH_2OH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Yeast nitrogen base was from the United States Biological (Salem, MA, USA). Casamino acids were from BD Difco (San Jose, CA, USA). Adenine sulfate ($C_5H_5N_5O_5H_2SO_4$) was from Acros Organics (NJ, USA).

Preparation of fruit extracts

FEs were prepared according to the method of Basu and Maier.[21] FEs are listed in the results tables [Tables 1-4], according to the alphabetical order of their respective plant families.

Estrogenic and antiestrogenic assays

The estrogenic and antiestrogenic assays were performed according to the method of Maier *et al*., 1995.[22] A steroid-regulated *Saccharomyces cerevisiae* system (BJ3505 [MAT a, pep4:His 3, prbl-D1.6R, his3-D200, lys2-801, trpl-D101 [gal3], ura3-52 [gal2], can1]) containing a human estrogen receptor alpha (ERα) expression plasmid and a β-galactosidase gene reporter plasmid was used to determine the estrogenic, antiestrogenic, and drug synergistic activities of FEs. Yeast cultures were grown in a casamino acid-glucose medium (CAA-glucose medium – 20% glucose, 10% YNB, and 5% adenine sulfate) at 230 rpm, 30°C, overnight in an incubator-shaker. The estrogen (E) equivalents of each FE were estimated based on a 17β-estradiol (E) standard curve. The cultures were inoculated with 100 μg, 200 μg, 300 μg, or 400 μg E equivalents of FEs. Cultures inoculated with 2 μg estradiol or with 2 μg genistein were used as positive controls and yeast cultures without any treatment were used as negative controls for all experiments. For antiestrogenic assays, cultures were inoculated with 2 μg estradiol and 100 μg, 200 μg, 300 μg, or 400 μg E equivalents of FEs. The antiestrogenic activities of the FEs translate in the reduction of transcriptional activity induced by estradiol in the steroid-regulated yeast system. All cultures were allowed to grow in the incubator-shaker for 6 h. After 6 h, the cells were disrupted with glass beads (0.5 mm dia; BioSpec Products, Inc.,) and the protein concentrations in supernatants were estimated spectrophotometrically.

Estrogenic activity was calculated according to the following equations and expressed in Miller Units (MU):

Equation 1: MU = (Abs. at 420 nm)/(Protein concentration [g] × Time [min]) × 1000 and expressed in MU.

Abs = Absorbance

The transcriptional activity induced by the combination E-FEs was expressed as percentage of estradiol activity using the equation:

Equation 2: Activity of E + FE (MU)/E activity (MU) × 100

Drug synergistic assays

For drug synergistic assays, cultures were inoculated with different combinations of estradiol, drugs, and FEs as follows: E (2 μg) + FE (100–400E Equiv.) + 4-hydroxytamoxifen or NAF (4-OHT) (2 μg). Positive control cultures were inoculated with E (2 μg), 4-OHT (2 μg), or NAF (2 μg) by themselves and E (2 μg) + drug (2 μg) combinations. The drug synergistic activities of FEs were determined based on the following calculations:

- Equation 3a: % Decrease of E activity by FE or drug (4-OHT or NAF) = 100 – (FE/drug + E activity [MU]/E activity [MU] × 100)
- Equation 3b: % Decrease of E activity by drug + FE = 100 – (Activity of E + FE + 4-OHT/NAF [MU]/Activity of estradiol [MU] × 100)
- Equation 3c: Fold change by FE = % Decrease of E activity by 4-OHT or NAF + FE/% Decrease of E activity by FE
- Equation 3d: Fold change by drug (4-OHT or NAF) = % Decrease of E activity by 4-OHT or NAF + FE/% Decrease of E activity by drug.

Statistical analysis

Each experiment with two replicates each was repeated three times and means and standard deviations were calculated. One-way ANOVA was performed, and significance of differences among means was determined with Tukey's test ($^*P \leq 0.05$) using GraphPad Prism 6. Pearson correlation was performed between the antiestrogenic and drug synergistic activities of FEs at 400E equivalents.

RESULTS

Estrogenic activities of fruit extracts and drugs

Compared to estradiol and genistein activities, all FEs showed low-to-medium estrogenic activities in the estrogen-regulated transcription system in yeast. California table grape extract showed the highest estrogenic activity (296 ± 1.6 MU) among FEs

at 400E equivalents. The estrogenic activity of the other FEs at 400E equivalents ranked as follows: blackberry, red raspberry, and strawberry > blueberry > jackfruit, and black raspberry [Table 1]. All FEs displayed increasing estrogenic activity with increasing concentrations, and the highest activities were induced by 400E equivalents of each FE [Table 1]. The estrogenic activities of 4-OHT and NAF were 209.68 ± 4.4 and 200.3 ± 8.9 MU, respectively, indicating low estrogenic activities of both drugs. The estrogenic activities of the drugs were in the same range with those of most FEs at 400E equivalents [Table 1]. For that reason, the 400E equivalents of FEs were used in determining the drug synergistic activities of the FEs.

Antiestrogenic activities of fruit extracts and drugs

FEs showed antiestrogenic properties by reducing the transcriptional activity induced by estradiol in the transgenic yeast expressing human ERα. The transcriptional activities of the combinations estradiol-FEs (400E equivalents) ranked as follows: blueberry (95.9%), blackberry (86.2%), black raspberry (88.9%), and California table grape (81.5%) > jackfruit (72.2%), and red raspberry (73.2%) > strawberry (60.7%) [Table 2]. The transcriptional activities of the combinations estradiol with 4-OHT or NAF were 73.4% and 73.5% of estradiol activity, respectively (calculated according to Eq. 2). The drugs decreased the estradiol activity by 26.5 ± 2.9% (4-OHT) and 26.6 ± 1.6% (NAF), respectively (calculated according to Eq. 3a).

Drug synergistic activities of fruit extracts

In this study, we investigated whether the FEs can enhance the antiestrogenic activities of 4-OHT and NAF for better adjuvant therapy of breast cancer [Table 3]. The highest synergistic activities with 4-OHT were induced by black and red raspberry extracts at 400E equivalents [Table 3]. Black raspberry extract with 4-OHT lowered estradiol activity by 74.9%. Red raspberry extract in combination with 4-OHT decreased estradiol activity by 73.9%. The drug synergistic activities of the other FEs in combination with 4-OHT ranked as follows: blueberry (62.7%) and California table grape (60.7%) > jackfruit (54.5%), blackberry (51.6%), and strawberry (50.4%) [Table 3]. Similarly, the highest drug synergistic activity with NAF was induced by the red raspberry extract, which in combination with the drug lowered the estradiol activity by 77.9%. The drug synergistic activities of the other FEs with NAF ranked as follows: blueberry (71.2%) >

Table 1: Estrogenic activities (Miller units) of fruit extracts

Fruit extracts	100 E equivalents	200 E equivalents	300 E equivalents	400 E equivalents
Blueberry	106.9±12.4[a]	125.4±2.5	166.7±5.1	181.4±4.2
Jackfruit	29.3±0.7[b]	69.3±1.3[b]	78±0.7[b]	83±2.5[b]
Blackberry	88.7±3.7[a,c]	115.6±2.4[c]	129.8±3.9	218.5±9.1[c]
Black raspberry	40.4±3.6[b,d]	72±3.1[b]	85.4±0.5[b]	100.6±4.7[b]
Red raspberry	149.7±4.2	162.6±2.9	183.4±3.5[e]	223.7±12.2[c,e]
Strawberry	73.9±8[c]	111.3±2[c]	183±4[e]	212.9±28.9[c,e]
California table grape	47.2±1.6[b,d]	182.3±2.3	235.2±0.3	296±1.6

Results represent means±SD of three experiments (each experiment had two replicates). In each column, mean values with no superscript letters are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different from each other at $P \leq 0.05$ (Tukey's test). The average estrogenic activities for the positive controls estradiol and genistein were 3358.2±142.5 and 1406.1±63.9 MU, respectively. MU: Miller units; SD: Standard deviation

Table 2: Transcriptional activities (percentage of estradiol activity) of fruit extracts-estradiol combinations

Fruit extracts	100 E equivalents	200 E equivalents	300 E equivalents	400 E equivalents
Blueberry	62±2.5[a]	76.3±10.1	86.2±5.7	95.9±5.6[a,c]
Jackfruit	47.3±1.9[b]	50.9±2.3[b]	54.5±2.8[b,f]	72.2±4.6
Blackberry	44.5±2.3[c]	54.5±1.9[b,c]	70±3.8[c]	86.2±9.1[a,c]
Black raspberry	43.2±2.4[b,c]	60.2±2.8	63.9±3	88.9±4.7[a,c]
Red raspberry	45.2±1.8[b,c,d]	54.5±1.7[b,c,e]	70.3±2.2[c]	73.2±2.6[b]
Strawberry	39.7±2.4[b,d,e]	50.5±0.3[b,c,e,f]	55±0.7[b,f]	60.7±1.6
California table grape	33.5±2.1[b,e]	49.1±4.7[b,f]	55.1±4.1[b,f]	81.5±12.8[c]

Results represent means±SD of three experiments (each experiment had two replicates). In each column, mean values with no superscript letters are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different from each other at $P \leq 0.05$ (Tukey's test). The average activities for the positive controls estradiol and genistein were 3358.2±142.5 and 1406.1±63.9 MU, respectively. MU: Miller units; SD: Standard deviation

Table 3: Synergistic activities of fruit extracts with 4-hydroxytamoxifen and nafoxidine

Fruit extracts	Percentage decrease of estradiol activity by FE (400 estrogen equivalents)[a]	Percentage decrease of Estradiol activity by 4-OHT+FE[b]	Fold change by FEc	Fold change by 4-OHT[d]	Percentage decrease of estradiol activity by NAF+FE	Fold change by FE[c]	Fold change by NAF[d]
Blueberry	4.1±5.6[a,c]	62.7±1.6[a]	15.3	2.4	71.2±0.8	17.4	2.6
Jackfruit	27.8±4.6[b]	54.5±1.8[b]	2	2.1	50.9±2.1[b]	1.9	1.9
Blackberry	13.8±9.1[a,c]	51.6±1.1[b,c]	3.7	2	50.3±1.4[b,c]	3.6	1.8
Black raspberry	11.1±4.7[a,c]	74.9±0.8[d]	6.8	2.8	63.8±1.2	5.8	2.3
Red raspberry	26.8±2.6[b]	73.9±1[d]	3	2.8	77.9±1	2.9	2.9
Strawberry	39.3±1.6	50.4±2[b,c]	1.3	1.9	56.4±1.9[f]	1.5	2.2
California table grape	18.5±12.8[b,c]	60.7±1.2[a]	3.3	2.3	53.8±1.5[b,c,f]	2.9	2.1

Results represent means±SD of three experiments. In each column, mean values with no superscript letters are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different from each other at $P \leq 0.05$ (Tukey's test). The average activities for estradiol, estradiol with 4-hydroxytamoxifen, and estradiol with nafoxidine were as follows: 1835.03±41 MU (100%), 1346.14±23 MU (73.4%), and 1347.2±24.1 MU (73.5%), respectively. a, b, c, d: Equations for calculations in Materials and Methods. 4-OHT: 4-hydroxytamoxifen; NAF: Nafoxidine; FE: Fruit extract; MU: Miller units; SD: Standard deviation

black raspberry (63.8%) > jackfruit (50.9%), blackberry (50.3%), strawberry (56.4%), and California table grape (53.8%) [Table 3]. Fold changes between percentage decrease of E activity by 4-OHT or NAF + FE and percentage decrease of E activity by FE were calculated and ranged from 1.3 to 15.3 for 4-OHT and 1.5–17.4 for NAF. Blueberry extract in combination with 4-OHT and NAF induced the highest fold changes of 15.3 and 17.4, respectively [Table 3]. Fold changes between percentage decrease of E activity by 4-OHT or NAF + FE and percentage decrease of E activity by drug (4-OHT/NAF) were calculated and ranged from 1.9 to 2.8 for 4-OHT and 1.8–2.9 for NAF. Results clearly show drug synergistic activities of FEs with both drugs.

Correlation between antiestrogenic activity and drug synergistic activities of fruit extracts

The antiestrogenic activity of FEs at 400E equivalents was correlated with their drug synergistic activities and the results are presented in Table 4. The antiestrogenic activities of blueberry, black raspberry, and California Table grape extracts displayed high correlation with drug synergistic activities for both 4-OHT and NAF. Blackberry, red raspberry, and strawberry antiestrogenic activities showed high correlation only with NAF synergistic activity. Jackfruit antiestrogenic activity showed low correlation with drug synergistic activities of both 4-OHT and NAF, whereas the antiestrogenic activities of blackberry, red raspberry, and strawberry extracts showed low correlation only with 4-OHT synergistic activity [Table 4].

DISCUSSION

Our results demonstrate the *in vitro* synergistic effects of seven FEs with 4-OHT and NAF. The combined drug and FEs significantly lowered the estradiol activity in steroid-regulated transcription *S. cerevisiae* cells expressing the human ERα. No reports of the estrogenic, antiestrogenic, and drug synergistic activities of the seven fruits by employing an estrogen-responsive yeast system were found in the literature. This highlights the novelty of the present study. Few studies speculated on the estrogenic activities of some FEs by reporting their bone-sparing and cardioprotective effects. Grape and blueberry extracts have been shown to increase bone calcium retention and BMD in ovariectomized rats.[23,24] Iowa Women's Health Study on postmenopausal women (n = 34.489) found a correlation between strawberry intake and reduced cardiovascular disease mortality during a 16-year period of follow-up.[25] Häkkinen *et al*. reviewed the chemical composition of 25 edible berries and found that blueberry, red raspberry, and strawberry contained the flavonoids quercetin. Besides quercetin, blueberry, and strawberry also contained other flavonoids such as myricetin and kaempferol, respectively.[26] Resende *et al*. reported the estrogenic activity of kaempferol by employing a recombinant yeast assay expressing human ERα.[27] The presence of flavonoids in the FEs used in the present study is most likely responsible for the estrogenic and antiestrogenic activities in the estrogen-regulated transcription system.

Table 4: Correlation between antiestrogenic and drug synergistic activities of fruit extracts

Fruit extracts (400 estrogen equivalents)	E+4-OH-T+FE	E+NAF+FE
Blueberry	0.960	0.934
Jackfruit	0.204	0.365
Blackberry	0.982	0.923
Black raspberry	0.633	0.550
Red raspberry	0.988	0.994
Strawberry	0.190	0.376
California table grape	0.999	0.975

4-OHT: 4-hydroxytamoxifen; E: Estradiol; NAF: Nafoxidine; FE: Fruit extract

The antiproliferative activities of some of the FEs employed in this study were reported in cancer cell lines containing ERα. Studies on the effect of blackberry, black and red raspberry, blueberry, and jackfruit FEs on the proliferation of MCF-7 cells found that all FEs inhibited cell proliferation and induced apoptosis.[28-31] Lu and Serrero reported the antiestrogenic activity of resveratrol, a stilbene found in grapes, which acted as an estradiol antagonist leading to the inhibition of MCF-7 cell growth.[32] It is possible that the antiestrogenic activities of the FEs contributed to the antiproliferative effects on MCF-7 cancer cells in the above study[28-32]. In our study, different FEs showed different degrees of transcriptional activities in the presence of estradiol *in vitro*, ranging from 33.5% estradiol activity by California table grape extract at 100E equivalents to 95.9% estradiol activity by blueberry extract at 400E equivalents.

Globally, breast cancer is reported as being the most frequently diagnosed cancer in women and is responsible for the highest number of cancer-associated deaths.[33] Various treatments such as surgery, radiotherapy, adjuvant chemotherapies, and hormonal therapies help reduce mortality rates of breast cancer patients.[34] Although pharmaceutical agents show preventive and chemotherapeutic actions toward breast cancer, the use of phytochemicals extracted from different fruits and vegetables has been explored to target molecular subtypes of breast cancer and breast cancer stem cells.[35] There are not many studies reporting the drug synergistic activities of FEs with breast cancer drugs; however, Castillo-Pichardo and Dharmawardhane explored the effects of grape polyphenols, such as resveratrol, quercetin, and catechin (RQC) on potentiating the drug gefitinib, an epidermal growth factor receptor-specific tyrosine kinase inhibitor, which is used for treatment of certain breast cancers. Gefitinib together with RQC decreased the cell viability in gefitinib-resistant cells to a greater extent as compared to gefitinib or RQC alone. Furthermore, this combination reduced growth and metastasis of a mammary tumor in nude mice.[36] Another study by Woode *et al*. found that both red raspberry extract and its active compounds delphinidin and ellagic acid enhanced the effect of sublethal dose (1 nM) of fulvestrant (ICI 182,780; ICI), an antiestrogen introduced clinically, and inhibited cell proliferation in ICI-sensitive and resistant cell lines LCC1, ZR75-1, and BT474.[37] Shi *et al*. reported the synergistic activity of resveratrol with tamoxifen in that together they enhanced apoptosis in tamoxifen-resistant MCF-7/TR cells.[38] In our study, red raspberry extract showed the highest drug synergistic activities with both 4-OHT and NAF. Red raspberry extract in combination with 4-OHT lowered the estradiol activity by 73.9% and with NAF by 77.9%. Black raspberry extract also showed the highest synergistic activity with 4-OHT, lowering the estradiol activity by 74.9%. The results show that some FEs were more effective in their drug synergistic activities than others. In the present study, the decrease of estradiol activity by FEs ranked from 39.3% (strawberry) to 4.1% (blueberry) [Table 3]. Although blueberry extract induced a very low decrease of estradiol activity, in combination with each drug, it showed the highest synergistic activity expressed in the fold change. On the other hand, red and black raspberry extracts induced a significant higher antiestrogenic activity by themselves, 11.2% and 26.8%, respectively, compared to the antiestrogenic activity of blueberry extracts (4.1%) but showed a much lower synergistic activity in combination with the drugs.

The antiestrogenic activities of blueberry, blackberry, red raspberry, and California table grape extracts have been found to highly correlate with their synergistic activities with both 4-OHT (0.960, 0.982, 0.988, and 0.999, respectively) and NAF (0.934, 0.923, 0.994, and 0.975, respectively) [Table 4]. The results indicate that the antiestrogenic activities of blueberry, blackberry, red raspberry, and California table grape extracts at 400E equivalents contributed to the drug synergistic activities. In the cases of jackfruit and strawberry extracts, low correlations

have been observed between their antiestrogenic activities and synergistic activities with 4-OHT (0.204 and 0.190, respectively) and NAF (0.365 and 0.376, respectively) [Table 4]. Black raspberry extract shows medium correlation between its antiestrogenic activity and synergistic activity with 4-OHT (0.633) and NAF (0.550). The low correlations between antiestrogenic and drug synergistic activities indicate the presence of other mechanisms for decreasing the estradiol activity in the estrogen-regulated steroid yeast system. It can be speculated that the phytochemicals present in jackfruit and strawberry extracts interact differently than the other FEs with 4-OHT and NAF in the estrogen-regulated steroid system. Yang and Liu reported the synergistic activity of apple extract with quercetin 3-β-D-glucoside on the antiproliferative activity in MCF-7 cells. The EC50 values of apple extract were 2-fold lower, and of quercetin, 3-β-D-glucoside were 4-fold lower were combined compared to when each of them were used individually in MCF-7 antiproliferative assays.[39] In another study, Huang *et al*. showed that chrysin present in the water-ethyl acetate FEs of cheese fruit, *Morinda citrifolia*, in combination with apigenin synergistically reduced cell viability, and induced apoptosis in HepG2 and MDA-MB-231 cells. Furthermore, cotreatment of apigenin and chrysin reduced tumor growth in human MDA-MB-231 breast cancer cells xenografts.[40] The present study shows that the FEs under study, with the exception of jackfruit and strawberry extracts, induced high-fold changes (3–17.4) in the antiestrogenic activities of both 4-OHT and NAF [Table 3]. The drugs by themselves induced fold changes in the range of 1.9–2.9 [Table 3]. The results showed that phytochemicals present in the FEs enhanced the antiestrogenic activities of both drugs in estrogen-regulated transcription system in yeast. More research is needed on the mechanism of synergistic activities of FEs and identification of active compounds to establish their efficacy in cancer treatment.

CONCLUSIONS

The current study presents evidence of the *in vitro* estrogenic, antiestrogenic, and drug synergistic activities of seven commercially available fruits. To the best of our knowledge, this is the first report on the estrogenic, antiestrogenic, and drug synergistic activities of the seven FEs by employing an estrogen-regulated transcription system in yeast. This is also the first report of *in vitro* synergistic activities of blueberry, jackfruit, blackberry, black raspberry, red raspberry, strawberry, and California table grape extracts with 4-OHT and NAF. The data on antiestrogenic and drug synergistic properties of the FEs offer opportunities for exploring new drug targets and could be useful for scientists and nutritionists in search of new effective ways for preventing or treating cancers, osteoporosis, and menopausal symptoms.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: From periphery to brain. Trends Mol Med 2013;19:197-209.
2. Yue W, Yager JD, Wang JP, Jupe ER, Santen RJ. Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. Steroids 2013;78:161-70.
3. Murphy E. Estrogen signaling and cardiovascular disease. Circ Res 2011;109:687-96.
4. Klein-Nulend J, van Oers RF, Bakker AD, Bacabac RG. Bone cell mechanosensitivity, estrogen deficiency, and osteoporosis. J Biomech 2015;48:855-65.
5. Lizcano F, Guzmán G. Estrogen deficiency and the origin of obesity during menopause. Biomed Res Int 2014;2014:757461.
6. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 2005;307:1625-30.
7. Renoir JM, Marsaud V, Lazennec G. Estrogen receptor signaling as a target for novel breast cancer therapeutics. Biochem Pharmacol 2013;85:449-65.
8. Ariazi EA, Ariazi JL, Cordera F, Jordan VC. Estrogen receptors as therapeutic targets in breast cancer. Curr Top Med Chem 2006;6:181-202.
9. Ali S, Buluwela L, Coombes RC. Antiestrogens and their therapeutic applications in breast cancer and other diseases. Annu Rev Med 2011;62:217-32.
10. Pike AC. Lessons learnt from structural studies of the oestrogen receptor. Best Pract Res Clin Endocrinol Metab 2006;20:1-14.
11. Núñez NP, Jelovac D, Macedo L, Berrigan D, Perkins SN, Hursting SD, *et al*. Effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole on serum hormones and bone characteristics in a preclinical tumor model for breast cancer. Clin Cancer Res 2004;10:5375-80.
12. Yaz G, Kabadere S, Oztopçu P, Durmaz R, Uyar R. Comparison of the antiproliferative properties of antiestrogenic drugs (nafoxidine and clomiphene) on glioma cells *in vitro*. Am J Clin Oncol 2004;27:384-8.
13. Kojima M, Fukunaga K, Sasaki M, Nakamura M, Tsuji M, Nishiyama T, *et al*. Evaluation of estrogenic activities of pesticides using an *in vitro* reporter gene assay. Int J Environ Health Res 2005;15:271-80.
14. Legha SS, Slavik M, Carter SK. Nafoxidine – An antiestrogen for the treatment of breast cancer. Cancer 1976;38:1535-41.
15. Craig Jordan V, McDaniel R, Agboke F, Maximov PY. The evolution of nonsteroidal antiestrogens to become selective estrogen receptor modulators. Steroids 2014;90:3-12.
16. Juranić Z, Zizak Z. Biological activities of berries: From antioxidant capacity to anti-cancer effects. Biofactors 2005;23:207-11.
17. Heinonen M. Antioxidant activity and antimicrobial effect of berry phenolics – A Finnish perspective. Mol Nutr Food Res 2007;51:684-91.
18. Folmer F, Basavaraju U, Jaspars M, Hold G, El-Omar E, Dicato M, *et al*. Anticancer effects of bioactive berry compounds. Phytochem Rev 2004;13:295-322.
19. Shen CL, von Bergen V, Chyu MC, Jenkins MR, Mo H, Chen CH, *et al*. Fruits and dietary phytochemicals in bone protection. Nutr Res 2012;32:897-910.
20. Hardcastle AC, Aucott L, Reid DM, Macdonald HM. Associations between dietary flavonoid intakes and bone health in a Scottish population. J Bone Miner Res 2011;26:941-7.
21. Basu P, Maier C. *In vitro* antioxidant activities and polyphenol contents of seven commercially available fruits. Pharmacognosy Res 2016;8:258-64.
22. Maier CG, Chapman KD, Smith DW. Differential estrogenic activities of male and female plant extracts from two dioecious species. Plant Sci 1995;109:31-43.
23. Hohman EE, Weaver CM. A grape-enriched diet increases bone calcium retention and cortical bone properties in ovariectomized rats. J Nutr 2015;145:253-9.
24. Devareddy L, Hooshmand S, Collins JK, Lucas EA, Chai SC, Arjmandi BH, *et al*. Blueberry prevents bone loss in ovariectomized rat model of postmenopausal osteoporosis. J Nutr Biochem 2008;19:694-9.
25. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, *et al*. Flavonoid intake and cardiovascular disease mortality: A prospective study in postmenopausal women. Am J Clin Nutr 2007;85:895-909.
26. Häkkinen SH, Kärenlampi SO, Heinonen IM, Mykkänen HM, Törrönen AR. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J Agric Food Chem 1999;47:2274-9.
27. Resende FA, de Oliveira AP, de Camargo MS, Vilegas W, Varanda EA. Evaluation of estrogenic potential of flavonoids using a recombinant yeast strain and MCF7/BUS cell proliferation assay. PLoS One 2013;8:e74881.
28. Seeram NP, Adams LS, Zhang Y, Lee R, Sand D, Scheuller HS, *et al*. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. J Agric Food Chem 2006;54:9329-39.
29. Madhusoodhanan R, Natarajan M, Singh JV, Jamgade A, Awasthi V, Anant S, *et al*. Effect of black raspberry extract in inhibiting NFkappa B dependent radioprotection in human breast cancer cells. Nutr Cancer 2010;62:93-104.
30. Olsson ME, Gustavsson KE, Andersson S, Nilsson A, Duan RD. Inhibition of cancer cell proliferation *in vitro* by fruit and berry extracts and correlations with antioxidant levels. J Agric Food Chem 2004;52:7264-71.
31. Li F, Li S, Li HB, Deng GF, Ling WH, Wu S, *et al*. Antiproliferative activity of peels, pulps and seeds of 61 fruits. J Funct Foods 2013;5:1298-309.
32. Lu R, Serrero G. Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells. J Cell Physiol 1999;179:297-304.
33. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D, *et al*. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
34. Scharl A, Kühn T, Papathemelis T, Salterberg A. The right treatment for the right patient – Personalised treatment of breast cancer. Geburtshilfe Frauenheilkd

2015;75:683-91.

35. Siddiqui JA, Singh A, Chagtoo M, Singh N, Godbole MM, Chakravarti B, *et al.* Phytochemicals for breast cancer therapy: Current status and future implications. Curr Cancer Drug Targets 2015;15:116-35.
36. Castillo-Pichardo L, Dharmawardhane SF. Grape polyphenols inhibit Akt/mammalian target of rapamycin signaling and potentiate the effects of gefitinib in breast cancer. Nutr Cancer 2012;64:1058-69.
37. Woode DR, Aiyer HS, Sie N, Zwart AL, Li L, Seeram NP, *et al.* Effect of berry extracts and bioactive compounds on fulvestrant (ICI 182,780) sensitive and resistant cell lines. Int J Breast Cancer 2012;2012:147828.
38. Shi XP, Miao S, Wu Y, Zhang W, Zhang XF, Ma HZ, *et al.* Resveratrol sensitizes tamoxifen in antiestrogen-resistant breast cancer cells with epithelial-mesenchymal transition features. Int J Mol Sci 2013;14:15655-68.
39. Yang J, Liu RH. Synergistic effect of apple extracts and quercetin 3-beta-d-glucoside combination on antiproliferative activity in MCF-7 human breast cancer cells *in vitro*. J Agric Food Chem 2009;57:8581-6.
40. Huang C, Wei YX, Shen MC, Tu YH, Wang CC, Huang HC, *et al.* Chrysin, abundant in *Morinda citrifolia* fruit water-etOAc extracts, combined with apigenin synergistically induced apoptosis and inhibited migration in human breast and liver cancer cells. J Agric Food Chem 2016;64:4235-45.