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Estrogenicity of essential oils is not required to relieve symptoms of urogenital atrophy in breast cancer survivors

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Abstract

Background: Urogenital atrophy (UA) is a common treatment-limiting side effect of endocrine therapies. Topical estrogen is effective but systemic absorption may counter aromatase inhibitor efficacy. Numerous complementary approaches are marketed for use in UA without rigorous testing of their estrogenicity. We tested multiple essential oils in cancer cell growth and estrogen reporter assays *in vitro* and assessed clinical outcomes with the essential oil pessaries (EOPs) in breast cancer survivors with UA.

Methods: Effects on cell growth were tested in hormone-dependent (MCF-7) and -independent (MDA-MB-231) cell lines using the sulforhodamine-B assay. An estrogen response element (ERE) luciferase reporter assay was used to assess estrogenicity directly. Antifungal activity against two common pathogenic yeasts was assessed using standard microdilution methods. EOPs were offered to breast cancer survivors with symptomatic UA and the service evaluated using serial questionnaires.

Results: Two essential oils, *Cymbopogon martinii* and *Pelargonium graveolens*, demonstrated marked estrogenicity, stimulating ER+ cell growth and ERE-luciferase reporter activity to levels seen with premenopausal estradiol concentrations. Additional oils were screened for estrogenicity and *Lavandula angustifolia* and *Chamaemelum nobile* identified as non/minimally estrogenic. The antifungal activity of this combination of oils was confirmed. A second cohort of breast cancer survivors with UA received the second generation EOP with comparable improvement in symptom scores suggesting that estrogenicity may not be required for optimal therapy of UA.

Conclusion: Certain essential oils demonstrate profound estrogenicity and caution should be exercised before their use in breast cancer survivors. Our minimally estrogenic pessary will be formally tested in clinical trials.

Keywords: breast cancer, essential oil, estrogen, symptomatic, urogenital atrophy

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Introduction

Breast cancer (BC) is the commonest malignancy in women in the developed world. BC mortality rates are falling due, primarily, to significant improvements in systemic treatments, including endocrine therapy (ET).[1] However, ET is best given for prolonged durations (5–10 years or longer) and results in significant toxicity in many women.[2] Cohort studies demonstrate adherence rates to be as low as 50% after 4 years of ET, whilst adherence rates below 80% have been shown to result in a significant increase in BC mortality.[3,4] The predominant reason for women discontinuing ET is side effects, such as urogenital atrophy (UA), and it is essential that acceptable approaches to managing these side effects, to improve both survival and quality of life (QoL), are found.[5,6]

Retrospective studies report the prevalence of UA in BC survivors to be as high as 71% in postmenopausal women.[7,8] These numbers are higher than in the general population, where this condition is reported in 4% of women early in menopause and 47% of those in later menopause.[9] The aetiology of UA is reduction in local estrogen action, resulting in thinning of the vaginal epithelium and reduction in secretions. Common symptoms include pruritus, dyspareunia, dysuria, urinary frequency and incontinence, all of which can have a profound impact on QoL.

The possible management options available for UA in women with BC have been recently reviewed by Sousa and colleagues.[10] In the general population, topical vaginal estrogen is the gold standard treatment for UA and offers significant advantages in terms of symptomatic improvement and vaginal physiology over nonhormonal moisturisers.[11] However, systemic absorption of estrogen has been documented with vaginal estrogen preparations.[12] In women receiving ET for early estrogen receptor (ER)-positive BC, such a rise in systemic estrogen exposure may, in theory, have a negative impact on BC outcomes.[13,14] Unfortunately, in such women, nonhormonal vaginal moisturisers have been shown to be no better than placebo preparations.[15]

Many BC survivors use complementary therapies containing natural products to manage side effects like UA, however most of these therapies have limited evidence of either safety or efficacy.[10,16] Antibacterial, antifungal and anti-inflammatory properties are described for many essential oils, a heterogeneous group of hydrophobic plant compounds, making them potential candidates for the treatment of UA.[17,18] To combat the frequent side effect of UA in allogeneic bone marrow transplant recipients, we developed a cocoa butter (Theobroma) pessary containing three plant extracts/essential oils, each at a concentration of 1% volume/volume (v/v). This first-generation essential oil pessary (EOP1) worked extremely well in alleviating the symptoms of UA in this cohort of women and also in a small cohort of BC survivors receiving ET referred into the team. Many plants are known to contain phytoestrogens, which could potentially have mediated the beneficial effects seen in BC survivors. In this study, we describe our approach to testing the estrogenicity of the plant extracts/essential oils *in vitro* using both cancer cell line growth and estrogen reporter assays. Two of the oils showed considerable estrogenicity and were substituted with oils that did not to create a second-generation EOP (EOP2). Clinical outcomes with EOP1 and EOP2 are described in sequential cohorts of women with BC referred to the service.

Materials and methods

Breast cancer cell line culture and reagents

MCF-7 (ER-positive) and MDA-MB-231 (ER-negative) BC cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM-F12 Glutamax (Invitrogen, Paisley, UK) supplemented with 10% FCS (Invitrogen, Carlsbad, CA, USA). Hormone-deprived experimental medium was phenol-red-free DMEM-F12 Glutamax (Invitrogen) containing 10% dextran-coated charcoal-stripped serum. Stock solutions were prepared in ethanol using 10^{-4} mol/l 17β-estradiol (Sigma, St. Louis, MO, USA) and 10^{-4} mol/l ICI 182780 (fulvestrant; Tocris Bioscience, Bristol, UK).

Essential oils and cocoa butter preparation

Essential oils/extract were obtained from two sources (all oils from Oshadhi Ltd., Cambridge, UK; Calendula CO2 extract from Flavex, Rehlingen, Germany) and diluted by adding 10% v/v to vehicle solvent, dimethyl sulfoxide (DMSO, Sigma). Final concentrations of essential oils above 0.01% v/v in cell culture medium were cytotoxic and this was the highest concentration used. For cocoa butter (Naturallythinking, Croydon, UK), cytotoxicity was not observed up to 0.1% v/v. Cocoa butter and *Calendula officinalis* (CO) CO2 extract are solid at room temperature and were liquefied at 37°C prior to dilution.

Sulforhodamine-B (SRB) assay

The effects of each substance on the cell number in monolayer culture were assessed using the SRB assay, which measures cellular protein content, as described previously.[19] In brief, cells were seeded in 96-well plates and allowed to adhere for 24 h before culture in hormone-deprived media for 72 h. Experimental medium containing the test substances was applied and plates fixed with 50% trichloroacetic acid at the desired time points. Plates were stained with 0.4% SRB in 1% acetic acid and SRB solubilized with 10 mmol/l trisaminomethane base (pH 10.5) prior to absorbance being read at 490 nm with an automated plate

reader (BioTek ELx800 with Software BioTek Gen5 Version 1.04.5, Winooski, VT, USA). Data were standardized to the vehicle solvent group.

Estrogen response element (ERE)-luciferase reporter assay

To measure the activation of ER-dependent transcription, MCF-7 cells, cultured in hormone-depleted medium for 72 h, were transfected with either the pGL4-3xERE-luciferase vector containing three copies of a consensus ERE driving the expression of the firefly luciferase gene, or with the pGL4-luciferase vector as control. A vector expressing renilla luciferase (pGL4-CMV promoter-luciferase) was cotransfected and used as control for the efficiency of transfection. Transfection was performed using Xtremegene® (Roche, Basel, Switzerland) diluted in Opti-MEM medium (Life Technologies, Carlsbad, CA, USA). At 6 hours after transfection, cells were dissociated with phenol red free trypsin (Sigma) and 300,000 cells per well were seeded in hormone-depleted medium in six-well plates together with the test substances. Positive and negative controls were 17β-estradiol (10^{-8} mol/l) and selective estrogen receptor downregulator fulvestrant (10^{-7} mol/l) respectively. After 20 h of treatment, medium was removed and 150 µl of passive lysis buffer (Promega, Madison, WI, USA) was added. Next, 50 µl of this lysate was used for analysis using the Dual-Glo® Reagent (Promega) following the manufacturer's instructions. In short, Dual-Glo® Reagent was added and the firefly luciferase activity was detected using a luminometer (Glomax, Promega). After this, Stop and Glo® (Promega) Reagent was added and the renilla luciferase activity was measured. Firefly luciferase activity was normalized to renilla luciferase; these 3xERE-luciferase relative values were then normalized to the control luciferase vector results and expressed as relative light units. Peanut oil was used as an internal control.

Antifungal testing

The essential oils/extract were tested against one *Candida albicans* laboratory control strain (Mycology Reference Centre Manchester; MRCM 112) susceptible to fluconazole (minimum inhibitory concentration, MIC = 0.25 mg/l) and one *Candida glabrata* control strain (MRCM 4023) resistant to fluconazole (MIC > 64 mg/l) individually, in 1:1 dual combinations and in 1:1:1 triple combination using the European committee on antimicrobial susceptibility testing (EUCAST) standard microdilution method.[20] The stock oils were diluted in DMSO and further diluted into RPMI-1640 (Sigma) to achieve final concentrations of the doubling dilution series of 0.008–4.0%. Growth inhibition was measured spectrophotometrically following the EUCAST standard (50% growth inhibition as MIC). The minimum fungicidal concentration (MFC) was determined by culture after the MIC was established.

Assessment of patient symptom scores

Women with symptomatic UA were referred into The Department of Complementary Health and Wellbeing, The Christie NHS Foundation Trust for EOP therapy and questionnaires used to evaluate this service. Service evaluation in England is exempt from ethics committee review (Health Research Authority guidance, available at: www.hra.nhs.uk). All patients due to start treatment with EOPs provided written informed consent to treatment and recording of symptomatology. A questionnaire was used to assess symptoms at baseline and on treatment. Women were asked to grade discomfort due to UA from clothing, walking, passing urine or other activities, on a continuous scale from 0 (representing no discomfort) to 9 (unbearable). They were also asked to grade their symptom of most concern (if not discomfort) and to record whether or not they were sexually active. The programme is ongoing and complete data for both EOPs are available for early time points (to month 2).

After initial instruction, EOPs were self applied nightly for 6 of 7 days for the first month before reduction in frequency to alternate nights in month 2 and every third night in month 3 if symptomatic improvement was maintained.

Statistical analysis

Two-tailed student's *t* tests were performed to compare groups/treatments with their respective controls. Data are shown as mean ± 95% confidence intervals of at least three independent experiments performed at least in triplicate. A value of probability smaller than 0.05 was considered to be statistically significant.

Results

Essential oils from EOP1 stimulate MCF-7 but not MDA-231 cell growth

Concentration curves for the two individual essential oils [*Cymbopogon martinii* (CM) and

Pelargonium graveolens (PG)] resulted in dose-dependent stimulation of MCF-7 cell growth (Figure 1a, 1b). CO extract and cocoa butter resulted in a minor increase in cell number but without a clear dose–response relationship (Figure 1c, 1d). None of the oils, extract or cocoa butter stimulated MDA-MB-231 cell growth at any dose. However, CM and CO resulted in minor but apparently dose-dependent inhibition of MDA-MB-231 cell growth through unknown mechanisms (Figure 1a–c). At 96 h, CM and PG resulted in greater than 50% of the stimulation in cell growth seen with 10^{-8} mol/l estradiol (Figure 1e). Little or no stimulation in growth was observed with cocoa butter or CO, and fulvestrant, inhibited cell growth as expected in MCF-7 cells. These results suggest estrogenic action of CM and PG oils, although nonestrogenic actions could not be excluded.

Estrogenicity of essential oils was confirmed using an (ERE)-luciferase reporter assay

To further evaluate the mechanism of growth stimulation in MCF-7 cells we measured the activation of ER-dependent transcription using a vector that contains three ERE sequences in the promoter region leading to estrogen-dependent expression of luciferase. As a negative control, the same vector lacking the three ERE sequences was used to account for the basal transcriptional activity. Oils, extract and cocoa butter demonstrated significantly increased estrogenic activity over controls (Figure 2a). Basal luciferase activity itself was ER dependent, evidenced by its inhibition with treatment fulvestrant co-trea. The two oils (CM and PG) which had the most pronounced effect on MCF-7 cell growth also demonstrated the greatest induction of luciferase activity. This induction was inhibited by fulvestrant, confirming it to be mediated through ER activation. The reporter assay may not differentiate between ER alpha and beta isoforms as their DNA binding domains are known to share 96% sequence homology.[21] However, the stimulation is likely to be mediated through ER-alpha as ER-beta stimulation has previously been shown to inhibit the growth of MCF-7 and other ER-positive cell lines.[22]

Assessment of estrogenicity in 11 further essential oils

Eleven additional essential oils were selected based on their anticipated benefits in reducing symptoms of UA. All 11 oils were tested for estrogenicity using the ERE-luciferase reporter assay and, with the exception of *Lavandula angustifolia* (LA), all demonstrated a statistically significant increase in luciferase activity (Figure 2b). Overall, both LA and *Chamaemelum nobile* (CN) oils demonstrated minimal stimulation in ERE-luciferase activity and demonstrated little or no stimulation of MCF-7 cell growth at 0.01% v/v (Figure 2c).

EOP2 has antifungal properties

The three oils/extract with minimal estrogenicity were chosen for the formulation of the second-generation pessaries (EOP2) and were tested *in vitro* for effects of fungal growth (Table 1). When tested individually, CN and LA had MIC for *Candida albicans* of 0.25% and 0.5%, respectively, and for *Candida glabrata* of 0.008% and 0.13%, respectively. In contrast, CO enhanced fungal growth at all concentrations tested. When CN and LA were tested in dual combination, a 3-dilution decrease to 0.03% in the MIC was seen with *C. albicans*, but not with *C. glabrata*. The MIC of the triple combination resembled that of LA alone. Based on MFC comparisons, CN alone or in a blend with LA was fungicidal at 1% for *C. albicans* but not for *C. glabrata*. Mixtures which contained CO were not fungicidal even at the highest concentration tested. Although CO enhanced fungal growth on its own, the triple combination of CO, CN and LA inhibited the growth of both *C. albicans* and *C. glabrata* at concentrations lower than 1% v/v.

EOP1 and EOP2 show comparable efficacy in breast cancer patients

The first generation EOP was used to treat 12 women with BC and symptomatic UA before introduction of the minimally estrogenic EOP2 in a second cohort of 12 women. The demographics and BC treatments are detailed in Table 2. All women have reached at least the 2-month assessment, thus composite change in symptom scores at 2 months are presented (Table 3). With EOP1, 27/32 (84%) baseline symptoms improved compared with 23/32 (72%) for EOP2. There was no significant difference in the proportions of symptoms changing with treatment between the two versions of EOP. New symptoms developing on therapy were generally mild (scored 1 to 3 on the nine-point scale) and transient (where follow-up data are available).

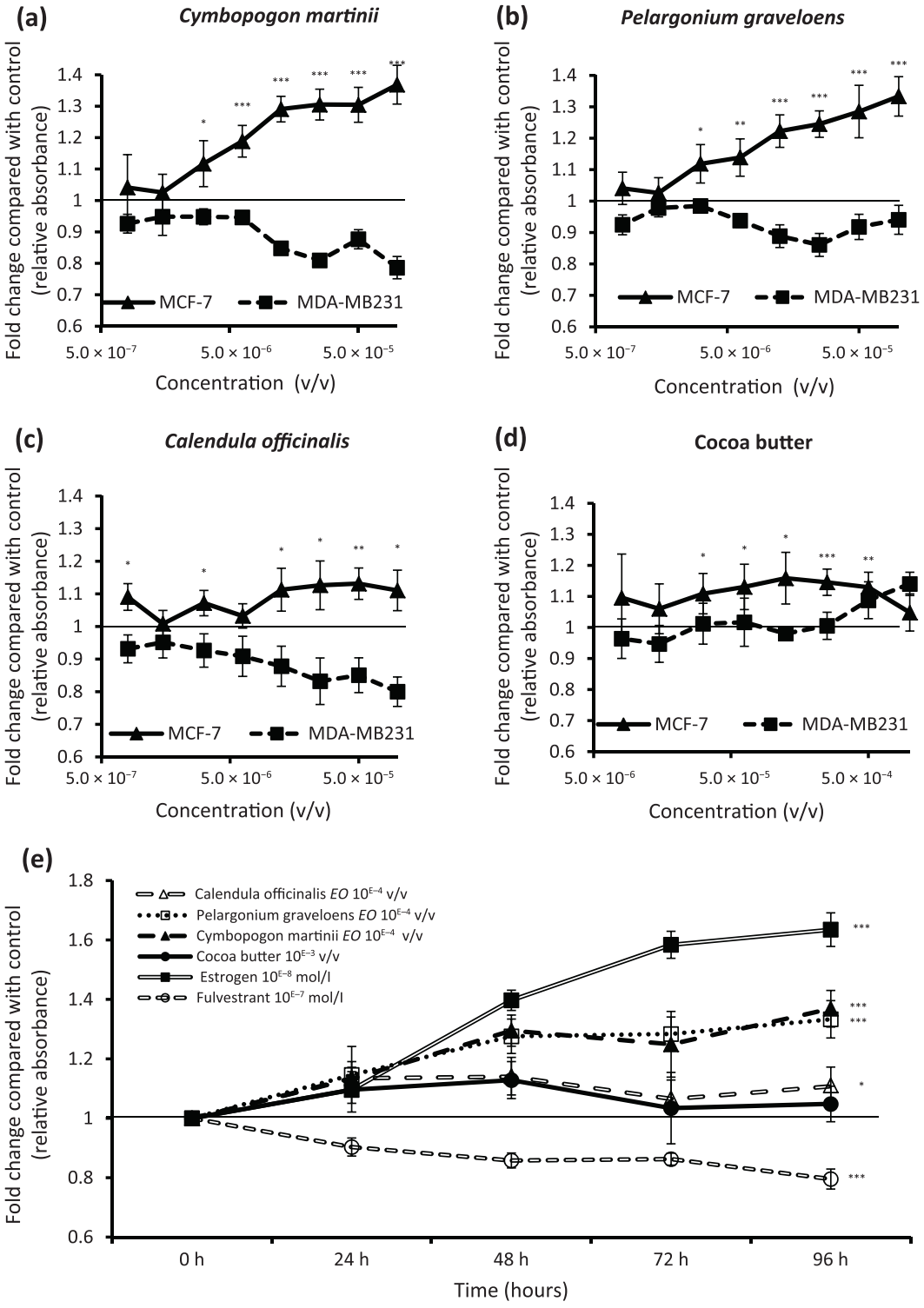

Figure 1. Growth effects of essential oils and cocoa butter on estrogen-dependent (MCF-7) and -independent (MDA-MB-231) cell lines.

(a–d) Cell lines were cultured for 96 h in the conditions described prior to cell density determination using SRB assay. Results are expressed as relative absorbance compared with the respective cell line treated with vehicle control (DMSO); (e) MCF-7 cell growth curves over 96 h. Cells were harvested, and SRB assay performed every 24 h.

Statistical analysis by unpaired *t* test against control values at the same vehicle concentration (a–d) or 96 h time point (e). Data are represented as mean ± 95% confidence intervals.

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

DMSO, dimethyl sulfoxide, a vehicle control; EO, essential oil; SRB, sulforhodamine-B; v/v, volume/volume.

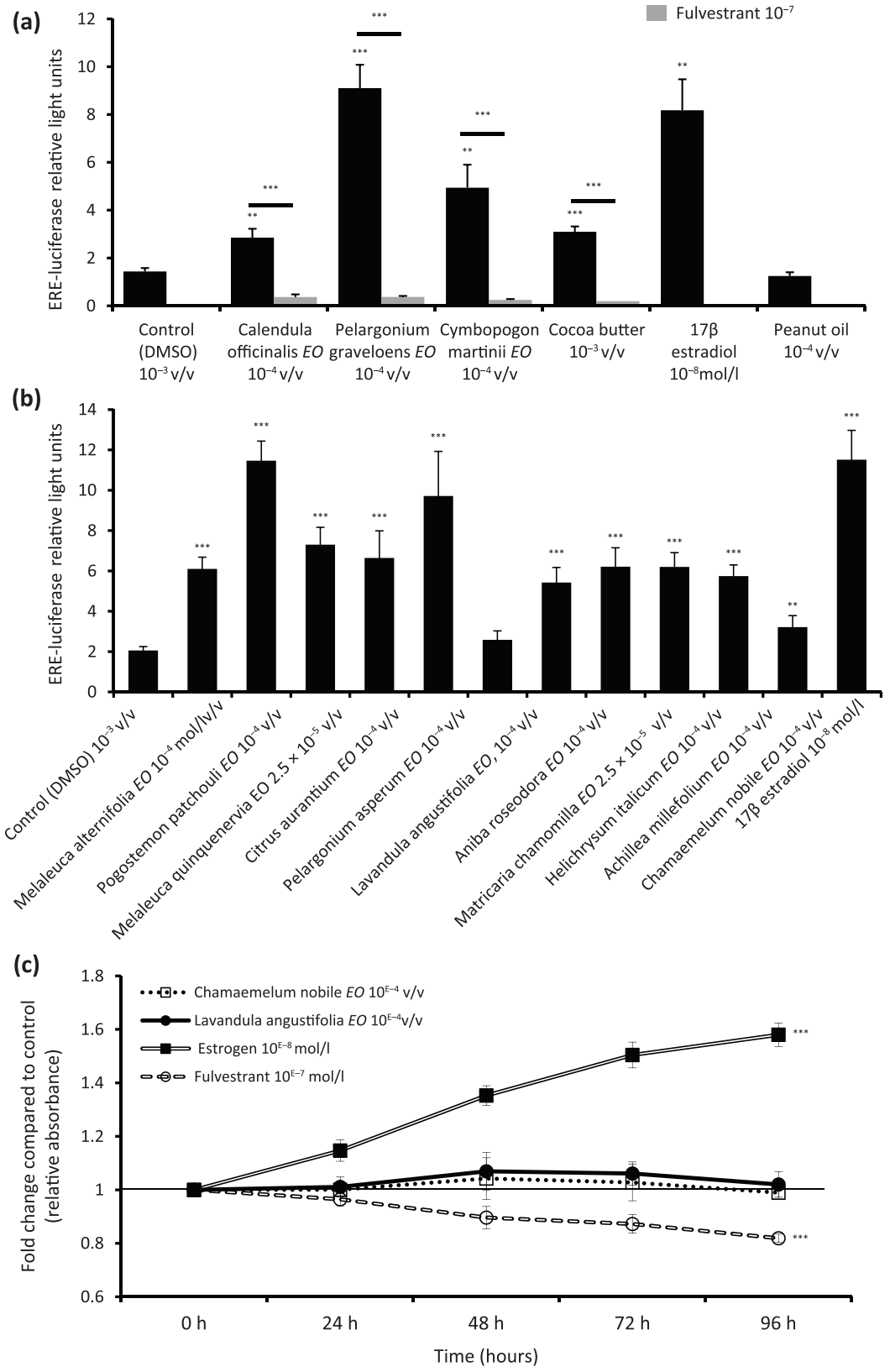

Figure 2. Assessment of estrogen receptor reporter activity of essential oils and cocoa butter, and the effects on cell line growth of nonestrogenic oils identified.

(a) MCF-7 cells were incubated with essential oils with or without fulvestrant for 20 h prior to harvest and measurement of ER transcriptional activity. Results are expressed as relative luciferase activity of 3xERE reporter compared with control (DMSO); (b) MCF-7 cells were incubated with 11 additional essential oils and ER transcriptional activity was measured as described in Figure 2a. Assay was performed at 0.0025% v/v for *Melaleuca quinquenervia* and *Matricaria chamomilla* oils due to cytotoxic effects at higher concentrations; (c) MCF-7 cells growth curves over 96 h in the culture conditions described. Cells were harvested, and SRB assay performed every 24 h. Results are expressed as relative absorbance compared with the values of the vehicle control (DMSO).
Statistical analysis by unpaired *t* test (treated *versus* control). Data are represented as mean ± 95% confidence intervals. $^{**}p < 0.01$, $^{***}p < 0.001$.
DMSO, dimethyl sulfoxide, a vehicle control; EO, essential oil; ER, estrogen receptor; ERE, estrogenic response element; SRB, sulforhodamine-B; v/v, volume/volume.

Table 1. Assessment of antifungal activity of essential oils.

Range MICs	Single oils/extract			Dual combinations			Triple combination
	CN	**LA**	**CO***	**CN + LA**	**CN + CO**	**LA + CO**	**CN + LA + CO**
Candida albicans	0.25%	0.5%	>2%**	0.03%	1%	0.5%	0.5%
Candida glabrata	0.008%	0.13%	>2%**	0.008%	0.25%	0.25%	0.25%
Range MFCs							
Candida albicans	1%	>4%	>2%**	1%	>2%**	>2%**	>4%
Candida glabrata	>4%	>4%	>2%**	>4%	>2%**	>2%**	>4%

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of three essential oils/extract alone and in combination on the growth of one strain of *Candida albicans* and *Candida glabrata*.
*On its own, CO enhanced *Candida* growth.
**The highest soluble concentration that could be obtained for CO was 2%.
CN, *Chamaemelum nobile*; LA, *Lavandula angustifolia*; CO, *Calendula officinalis.*

Discussion

Anti-estrogen therapy represents a significant advance for women with early and advanced BC. However, adverse effects are common and contribute to low adherence rates associated with inferior survival. Vaginal estrogens are effective in treating UA but result in systemic absorption of estrogen which could in theory counter the effect of ET, in particular aromatase inhibitors (AIs), with the potential for detrimental BC outcomes.[13,14] Most oncologists (71%) prefer to prescribe nonhormonal treatments instead of vaginal estrogen therapy.[23] Alternate approaches are required and essential oils with antibacterial, antifungal and anti-inflammatory properties make ideal candidates for testing in the treatment of UA.

To address the issue of UA, the complementary health and wellbeing team at The Christie NHS Trust produced vaginal pessaries made with cocoa butter as a base and containing three essential oils/extract of CM, PG and CO. These first-generation EOPs were effective in reducing symptoms in BC survivors with symptomatic UA secondary to endocrine or chemotherapy-induced menopause. The level of efficacy of the EOPs caused concern that the constituents could be estrogenic. This was confirmed through ER+ cell line growth stimulation and ERE-luciferase assays for CM and PG. The quantitative estrogenic activity of PG in the reporter assay was similar to that seen with premenopausal levels of 17β-estradiol. A major constituent of both CM and PG, but not CO, the terpinoid citral (also known as geraniol) is a major constituent of both CM and PG, but not CO, and has previously been reported to be estrogenic[24,25] and to induce benign prostatic hyperplasia and vaginal mucosal proliferation in rodent models.[26] CM and PG are

Table 2. Baseline characteristics of women treated with first- and second-generation essential oil pessaries.

Characteristics	First-generation EOP ($n = 12$)	Second-generation EOP ($n = 12$)
Age (median, range)	50 (45–63)	50 (32–62)
Premenopausal	4/12 (33%)	3/12 (25%)
Postmenopausal	8/12 (67%)	9/12 (75%)
Previous pregnancies (%)	7/12 (58%)	7/12 (58%)
Stage of disease when referred for EOP		
Adjuvant	9/12 (75%)	10/12 (83%)
Metastatic	3/12 (25%)	2/12 (17%)
Previous adjuvant chemotherapy	11/12 (92%)	11/12 (92%)
Current treatment		
Tamoxifen	4/12 (33%)	1/12 (8%)
AI	7/12 (58%)	8/12 (67%)
Fulvestrant	0/12	1/12 (8%)
Chemotherapy + goserelin*	0/12	1/12 (8%)
Trastuzumab	1/12 (8%)	0/12
None	0/12	1/12 (8%)
Previous HRT	1/12 (8%)	3/12 (25%)
Previous topical treatment for UA	9/12 (75%)	10/12 (83%)

*No other patient was receiving gonadotrophin-releasing hormone analog therapy.
EOP, essential oil pessaries; AI, aromatase inhibitor; HRT, hormone replacement therapy; UA, urogenital atrophy.

currently marketed as preformulated pessaries, single oils and creams for the treatment of vaginal dryness. Furthermore, review of BC forums and chat rooms reveals that they are frequently promoted for use within the BC survivor community and our studies suggest they should be used with caution in this population. Cocoa butter did not promote MCF-7 cell growth but demonstrated some estrogenic activity using the reporter assay. This is compatible with a weak estrogenic action, as already demonstrated for cocoa beans but not for cocoa butter.[27,28] These results suggested that the first-generation EOPs could have been effective due to their estrogenicity. An additional 11 essential oils, with theoretical efficacy in UA, were screened and two with minimal estrogenicity in ERE-luciferase and cell growth assays were identified (*Lavandula angustifolia* and *Chamaemelum nobile*). These oils were combined with CO in the cocoa butter base to form the second generation of EOPs and subsequently tested in a separate cohort of 12 women with early BC and symptomatic UA. Using the same assessment questionnaires, a similar improvement in symptom scores was seen, suggesting that the beneficial effects of the first-generation EOPs may not have been due to their estrogenicity.

There are some weaknesses in our study that we acknowledge. First, the patient population was heterogeneous and included those with early and advanced disease. This is suboptimal, as the clinical, psychological and sexual situations of these patients are likely to be very different, which could have a significant impact on outcomes. A

Table 3. Change in symptoms from baseline in response to treatment with first and second generation essential oil pessaries.

Change in symptoms from baseline	First-generation EOP	Second-generation EOP	Significance by χ^2 analysis
Improvement	27	23	$p = 0.44$
No change	4	6	
Worsening of existing	1	3	
Development of new	4	4	
Total	36	36	

EOP, essential oil pessary.

more homogeneous population would be ideal and will be recruited in subsequent prospective trials. Second, the questionnaires used were developed in house and have not been formally validated. However, these questionnaires were developed through our extensive knowledge of the most prominent symptoms reported by cancer survivors and were administered uniformly throughout. Third, we did not use more extensive global QoL assessments in this study, primarily due to time constraints in a busy service department. In future clinical trials, both validated questionnaires and QoL assessments will be used to formally evaluate the effects of the EOPs on symptoms of UA, sexual activity and QoL. Improving adherence rates to ET and the QoL of BC survivors are of great importance. Three studies have examined the effect of oral administration of hormone replacement therapy (HRT) in women receiving adjuvant ETs.[29–31] The HABITS trial[30] reported an increase in local recurrence with estrogen plus progesterone HRT whilst the LIBERATE study reported an increase in metastatic disease with tibolone (a synthetic androgen with progestogenic activity).[29] The third study was terminated early with no firm conclusions.[31] The predominant ET in these studies was tamoxifen and the detrimental effects may have been greater in a population receiving AIs, which primarily function by reducing serum estrogen levels. Notably the BC-preventive effect of tamoxifen in the IBIS-1 study was lost in women taking HRT.[32]

To date, there is still limited evidence regarding safety of topical vaginal estrogen in BC survivors. A recent clinical trial reported that both a vaginal estrogen ring (7.5 μg/d) and intravaginal testosterone cream can improve symptoms of UA in postmenopausal women receiving AIs to treat BC with almost none showing persistent elevation of systemic estradiol levels.[33] However, without an apparent explanation, baseline levels of estrogen were commonly above the postmenopausal range in the cohort of patients of this study, which complicates the analysis of these data. In another ongoing phase II study, estradiol assays will be used to assess for systemic absorption in women with early BC and UA secondary to AI therapy treated with ultra-low-dose vaginal estrogen tablets (Vagifem 10 μg) [ClinicalTrials.gov identifier: NCT00984399].

To further develop our second generation EOPs, we will conduct a single-arm phase II study to accurately define the effect level on symptoms of UA in a larger cohort of BC survivors on AIs with symptomatic UA resistant to nonhormonal vaginal moisturisers. This study will enable powering of a randomized trial comparing the second-generation EOPs against ultra-low-dose topical vaginal estrogen. The 10 μg vaginal estradiol tablet has already demonstrated comparable efficacy but significantly reduced systemic absorbance compared with the standard 25 μg preparation.[12,34] However, the median peak estradiol level of 80 pmol with the 10 μg preparation is in the high postmenopausal range and at least tenfold higher than the expected serum estradiol level in women receiving AI therapy. We hope that the proposed studies will not only confirm the efficacy and safety of the EOPs but also provide a larger safety assessment of ultra-low-dose vaginal estrogen. Ideally, if both treatments are equally effective and safe, the options for women

with symptomatic UA secondary to ET will have been meaningfully expanded.

Conclusion

EOPs are an effective treatment of UA in BC survivors. However, we have shown that some essential oils have potent estrogenic activity and their safety in BC survivors, on long-term adjuvant ET, needs further evaluation in clinical studies. Caution is advised in recommending untested plant-based topical products for UA in women treated with AIs, as there may be significant, previously unrecognized, estrogenic constituents. Significantly, we report a new minimally estrogenic pessary formulation that is effective in improving symptomatic UA and may be a safer treatment to use in women surviving BC.

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Authors' contributions

BMS and BK: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing;
RBC: Conception and design, data analysis and interpretation, manuscript critique;
JS: Essential oil pessary formulation, collection of clinical data, manuscript critique;
LN-F, KY and RR-R: Collection, assembly and interpretation of data, manuscript critique;
GZ and AA: Essential oil pessary patient recruitment and assessment;
SJH: Conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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