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Original Research

Potential effect of *Olea europea* leaves, *Sonchus oleraceus* leaves and *Mangifera indica* peel extracts on aromatase activity in human placental microsomes and *CYP19A1* expression in MCF-7 cell line: Comparative study

N. Z. Shaban[1], W. A. Hegazy[1], S. M. Abdel-Rahman[2*], O. M. Awed[1], S. A. Khalil[1]

[1] Department of Biochemistry, Faculty of Science, Alexandria University, Alexandria, Egypt
[2] Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt

Abstract: Aromatase inhibitors (AIs) provide novel approaches to the adjuvant therapy for postmenopausal women with estrogen-receptor-positive (ER^+) breast cancers. In this study, different plant extracts from *Olea europaea* leaves (OLE), *Sonchus oleraceus* L. (SOE) and *Mangifera indica* peels (MPE) were prepared to identify phytoconstituents and measure antioxidant capacities. The effects of these three extracts on aromatase activity in human placental microsomes were evaluated. Additionally, the effects of these extracts on tissue-specific promoter expression of *CYP19A1* gene in cell culture model (MCF-7) were assessed using qRT-PCR. Results showed a concentration-dependent decrease in aromatase activity after treatment with OLE and MPE, whereas, SOE showed a biphasic effect. The differential effects of OLE, SOE and MPE on aromatase expression showed that OLE seems to be the most potent suppressor followed by SOE and then MPE. These findings indicate that OLE has effective inhibitory action on aromatase at both the enzymatic and expression levels, in addition to its cytotoxic effect against MCF-7 cells. Also, MPE may be has the potential to be used as a tissue-specific aromatase inhibitor (selective aromatase inhibitor) and it may be promising to develop a new therapeutic agent against ER+ breast cancer.

Key words: Breast cancer, aromatase inhibitors, MCF-7 cell line, *CYP19A1*, *Olea europea*, *Sonchus oleraceus*, *Mangifera indica*.

Introduction

Estrogens and the estrogen receptors (ERs) play a significant role in the development and progression of breast cancer and 70 percent of breast cancers are ER^+, approximately (1). Estrogens have various effects throughout the body, including helpful effects on the bone, brain, heart, liver and vagina. Prolonged estrogen exposure is associated with harmful effects such as increased risk of breast, uterine and endometrial cancers (2). Estrogens are thought to influence breast cancer risk by increasing cell proliferation, thereby increasing the probability of replication errors and DNA damage, as well as, promotion of cancer growth (3, 4). Aromatase (oxidoreductase, EC 1.14.14.14) is a cytochrome P450 enzyme and responsible for catalyzing the biosynthesis of estrogens (estrone and estradiol) from androgens (androstenedione and testosterone). Mammalian aromatase cytochrome P450 is mostly expressed in the ovaries of pre-menopausal women and in the placenta of pregnant women, as well as, in some peripheral tissues (5). After menopause, the ovaries lose the ability to express aromatase and the adipose tissue becomes the main aromatase-expressing body site, in addition to, the other peripheral tissues.

Aromatase gene expression is regulated in a tissue-specific manner by the use of ten alternative non-coding first exons (I.1, I.2, I.2a, I.3, I.4, I.5, I.6, I.7, I.f and PII) with separate promoters. Where; exon I.1 is responsible for aromatase gene expression in the placenta major; exons I.3 and I.4 in adipose tissue; exon I.5 in fetal tissue; exon I.6 in bone; exon I.7 in endothelial cells; exons I.2 and I.2a in placenta minor; exon I.f in brain; and exon PII is responsible for aromatase expression in gonad-specific (6-8). Aromatase expression and activity levels, and as a result estrogen level, are markedly higher in breast cancer tissue than in normal breast tissue (9). Malignant epithelial cells secrete some factors (e.g. IL-6, IL-11 and TNFα) that inhibit the differentiation of pre-adipocyte fibroblasts to mature adipocytes resulted in over-expression of aromatase (10). At the same time, other factors secreted by malignant epithelial cells (mainly PGE2) activates aromatase expression driven by promoters I.3 and PII, via cyclic adenosine monophosphate (cAMP), in surrounding adipose fibroblasts (10-12). In normal breast adipose stromal cells and fibroblasts, aromatase expression is driven by promoter I.4 (glucocorticoid dependent), and that the action of promoters I.3 and II is suppressed by the silencer negative regulatory element S1 (located near PI.3 and PII). However, in cancer cells, the cAMP level increases, and aromatase promoters are switched to cAMP-dependent promoters I.3 and II. In the presence of cAMP, a positive regulatory element (cAMP responsive element (CREaro)) acts as an enhancer element when cAMP responsive element binding protein 1 (CREB1) binds and overcome the action of the silencer S1 on the function of promoter I.3 (13). On the other hand, promoter 1.7 is upregulated in vascular endothelial cells of breast cancer (14). It contains endothelial-type *cis*-acting ele-

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*** Corresponding author:** Salah M. Abdel-Rahman, Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt. Email: salahmaa@gmail.com

ments that interactwith endothelial-type transcription factors (e.g. GATA-2). Excessive aromatase expression via promoters I.3, II and I.7, and consequent increase in estrogen biosynthesis in malignant epithelial cells, undifferentiated adipose fibroblasts and adjacent endothelial cells contribute to the development and progression of breast cancer (14).

Two approaches are used in endocrine therapy to ameliorate the growth effects of estrogens on ER+ breast cancers; through interfering with the estrogen binding to its receptor using selective estrogen receptor modulators (SERMs), such as tamoxifen or via decreasing circulating levels of estrogen by using aromatase inhibitors (AIs) (15). AIs are superior to tumor-associated macrophages (TAMs) as adjuvant hormonal therapy for postmenopausal ER+ breast cancer (16). The occurrence probability of side effects due to long-term administration of AIs has motivated new efforts for development of a new generation of AIs based on natural products (17). Natural products that have been used traditionally for nutritional or medicinal purposes may also provide inhibitory effect on aromatase without deleterious traces. The noticeable good impact of these natural products may be the result of compounds within the natural product that inhibit aromatase, while other compounds (e.g. phytoestrogens) may mitigate some of the side effects of estrogen deprivation (18). As such, natural product AIs may be important for the translation of AIs from their current clinical uses as chemotherapeutic agents to future clinical uses in breast cancer chemoprevention.

In the current study, three different plants extracts: *Olea europea* (Olive) leaves, *Sonchus oleraceus* leaves and *Mangifera indica* (Mango) peels, were tested as AIs in human placental microsomes and as selective modulators of aromatase expression in MCF-7 cells. This study was focused on the characterization of the different extracts, impact of these extracts on aromatase activity and their anti-proliferative effect against MCF-7 cells. The effects of these extracts on total aromatase expression level and expression driven from different promoters were evaluated.

Materials and Methods

Chemicals and cell lines

Testosterone, Gentamycin, NADPH, Arimidex® (Anastrozole), Dimethylsulfoxide (DMSO), Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and all other chemicals with high purity were obtained. Also Estradiol ELISA kit (BioCheck, USA) and MCF-7 cell line were obtained from VACSERA Tissue Culture Unit, Cairo, Egypt.

Plant materials and extracts preparation

Olea europaea leaves extract (OLE). Olive leaves were selected randomly from a tree at Borg El-Arab, Alexandria, Egypt. Dried leaves were powdered and sieved, then, OLE was prepared according to the method described by (19). 200 g of olive leaves powder was soaked in 600 ml of methanol and water (4:1 v/v) overnight under agitation in the dark. The extract was filtered and concentrated to dryness at 40ºC. The extract was re-dissolved in 10 volumes of methanol/water (4:1 v/v) and hydrolyzed at 100ºC for 1 h using a 2M HCl solution (2:1 v/v).

Sonchus oleraceus L. extract (SOE). Fresh *Sonchus oleraceus* L. (SO) was purchased from local market, Alexandria, Egypt. Aerial parts of plant (leaves, stem and flowers) were washed and dried at room temperature. The plants parts milled mechanically to a fine powder of mesh size l mm for preparation of methanolic extract. 100 g of SO powder was extracted twice with two volumes of absolute methanol with occasional shaking, and the extract was filtered after one week. The obtained filtrate was mixed and dried in rotary evaporator at 40°C and then the SOE was stored at 4°C for further investigations (20).

Mangifera indica L. peel extract (MPE). Mango was brought from a local market at Alexandria, Egypt. The peel was manually separated from the edible flesh. According to (21), MPE samples were lyophilized, crushed and extracted with 80% ethanol (1:5 w/v) by sonication for 3 days at room temperature (25°C). The extract was filtered and concentrated using rotary evaporator at 40°C till dryness. Finally, powdered MPE was obtained using lyophilization and then stored in a dark bottle at -20°C until used.

Characterization of plants extracts

Quantitative phytochemical analysis of the previous extracts (OLE, SOE and MPE) were analyzed for total phenolic contents (22), HPLC for phenolic compounds (23, 24), total flavonoid contents (25), total triterpenoids (26), total alkaloids (27) and total antioxidant capacities (28). Additionally, anti-lipid peroxidation assay (29), Nitric oxide (NO) scavenging activity (30), diphenyle–α-picrylhydrazyl (DPPH) radical scavenging assay (31) and reducing power (32) were evaluated.

Enzyme inhibition assay performed on aromatase in placental microsomes

Human placenta

A full-term human placenta was obtained from El-Shateby hospital, Alexandria, Egypt, from two healthy women in the thirties of age with known history of disease according to the rules of scientific research ethics. Placentas were obtained immediately after delivery and placed on ice during transportation to the laboratory.

Preparation of placental microsomes

Microsomal fractions were prepared according to the method of (33). Where, the microsomes were re-suspended in 50 mM potassium phosphate buffer, pH 7.4 containing 0.25 M sucrose, 20% glycerol and 0.05 mM DTT. Microsomal fractions were stored at -80ºC until used.

Determination of protein concentration

The total protein content in microsomal fraction was estimated using Bradford Coomassie brilliant blue assay (34).

Aromatase assay

Aromatase activity can be measured by incubating the substrate (testosterone) for a certain time in a mix-

ture including NADPH. According to (35), the amount of the formed estradiol was measured by the competitive enzyme-linked immunosorbent assay (ELISA). Concentrated microsomal protein (1500 µg/ml) was diluted in 0.1% BSA, 50 mM PB, pH 7.4 to obtain 0.45 µg protein/10 µl, just prior to starting the P450 aromatase reaction. Where, the aromatase activity was expressed as pmol µg-1 min-1.

Effects of different plants extracts on aromatase activity

Effects of the OLE, SOE and MPE extracts were evaluated on human placental aromatase using the optimized ELISA assay. Different concentrations of each plant extract (100, 50, 25, 12.5, 3.125 µg/ml final concentrations) and the positive control (Anastrozole) were dissolved in DMSO. The activity was expressed in percent of control and the IC_{50} values were calculated by non-line fit analysis using GraphPad prism 6.

Evaluation of cytotoxicity of the different plants extracts against MCF-7 cell line

Cell proliferation studies using the MCF-7 cells were performed to determine the consequences of aromatase inhibition by the tested extracts in breast cancer cells. MCF-7 cells are ER+ breast cancer cell line and were characterized by high expression levels of aromatase gene. Hence, MCF-7 cells are often used as a model to demonstrate the estrogen dependence of the breast cancer growth. MCF-7 cells were propagated in DMEM which supplemented with 10% heat inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/ml gentamycin. All cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

The potential cytotoxic (antitumor) effect of each extract was evaluated against MCF-7 cells by the crystal violet staining (CVS) method described by (36). Briefly, the cells were seeded at a cell concentration of 1×10^4 cells/well in 100 µl of growth medium in the conditions described above. After 24 h, the cells were simultaneously treated with 100 µl of each tested extract, the positive control or DMSO in fresh maintenance medium in triplicate wells individual dose and incubated for a period of 48 hours. At the end of the incubation period, media were aspirated and the quantitative analysis was performed. Then, the IC_{50} was determined by non-linear regression analysis.

Quantitative real time polymerase chain reaction (qRT-PCR)

MCF-7 cells were cultured in a 100 mm polystyrene tissue culture plates with the growth conditions and growth media as described before. Cells were allowed to attach and grow for 2 days until they reached 80% confluence. Then, cells were treated separately in duplicate with two different concentrations of each extract or DMSO (as a control). After 24 h treatment, cells were trypsinized, washed and resuspended in a very small volume of PBS (50 µl PBS for 1 × 106 cells). Finally, cells were stored in RNA*later* reagent at -80°C until used for RNA isolation.

Total cellular RNA was purified from MCF-7 cells with the GeneJET RNA Purification Kit (Thermo Scientific, USA), according to the manufacturer's instructions. The extracted RNAs from different cultures were quantified and qualified using NanoDrop Spectrophotometer. Finally, all RNAs samples were normalized to avoid any false increase in gene expression levels.

Aromatase mRNA was reverse transcribed and quantified using Verso™ SYBR® Green 1-Step QRT-PCR Kit (Thermo Scientific, USA) in qRT-PCR apparatus (Thermo Scientific PikoReal). The effect of the tested extracts on the level of aromatase mRNA expression and the exon I were detected using specific primers (Table 1), and GAPDH gene was used as internal control. qRT-PCR was performed in a reaction mixture of 10 µl using 0.1 µl verso enzyme mix, 5 µl 1-step QPCR SYBER mix (1X), 0.5 µl RT-enhancer, 0.5 µl forward and reverse primers (10 pm), 0-2.9 µl water (PCR grade) and 0.5-3.4 µl RNA template (50 ng). qRT-PCR program was applied as one cycle of cDNA synthesis at 50°C for 15 min, one cycle of Thermo-start enzyme activation at 95°C for 15 min and followed by 40 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 1 min and extension at 72°C for 30 sec.

Results

Characterization of plant extracts

The ethanolic extract of mango peel (MPE) yielded 14% of the starting material. Whereas, the methanolic extracts of dried leaves of both olive (OLE) and *S. Oleraceus* (SOE) yielded 4.7% and 2.3% starting material, respectively. Quantitative estimation of the phytochemical constituents of OLE, SOE and MPE showed that one gram of each dry extract contains different amounts of phenolics, flavonoids, alkaloids and triterpenoids as listed in Table 2. Figure 1 and Table 3 showed HPLC analysis of polyphenolic compounds of these extracts and their concentrations. The total antioxidant capacity of OLE, SOE and MPE were expressed in terms of ascorbic acid (AA) equivalent and were found to be 371.55, 56.22 and 366.87 mg AA/g of extract, respecti-

Table 1. Oligonucleotide primer sequences for qRT-PCR.

Coding region	Primer sequence 5' - 3' (Forward/Reverse)
Exon II	CCTCTGAGGTCAAGGAACAC/GTGCCCTCATAATTCCACAC
Exon I.3	GGGCTTCCTTGTTTTGACTTG/GTGCCCTCATAATTCCACAC
Exon I.6	CACAGCAGAACCAGCACATC/GTGCCCTCATAATTCCACAC
Exon I.7	GGCTCCATCTACAAGGATGA/GTGCCCTCATAATTCCACAC
Exon I.f	GGCACACGAAGAGTGATTG/GTGCCCTCATAATTCCACAC
Exon PII	GCAACAGGAGCTATAGATGAAC/GTGCCCTCATAATTCCACAC
GAPDH	GAAGGTGAAGGTCGGAGTC/GAAGATGGTGATGGGATTTC

Primer sequences for aromatase gene expression were designed using UCSC Genome Bioinformatics Site and were tested for their Tm's and primer dimers using Multiple Primer Analyzer program (Thermo Scientific); For GAPDH mRNA (Chen et al., 2007).

Table 2. Phytochemical composition of OLE, SOE and MPE extracts.

Plant extract	Total phenolics (mg/g extract)[a]	Total flavonoids (mg/g extract)[b]	Total alkaloids (mg/g extract)[c]	Total triterpenoids (mg/g extract)[d]
OLE	314.81	19.81	5.94	1.24
SOE	81.09	28.71	0.23	1.34
MPE	111.84	31.54	11.22	1.55

Values were represented as means of triplicates. (a) Values expressed as gallic acid equivalents/g of dry extract, (b) Values expressed as quercetin equivalents/g of dry extract, (c) Values expressed as berberine equivalents/g of dry extract and (d) Values expressed as ursolic acid equivalents/g of dry extract.

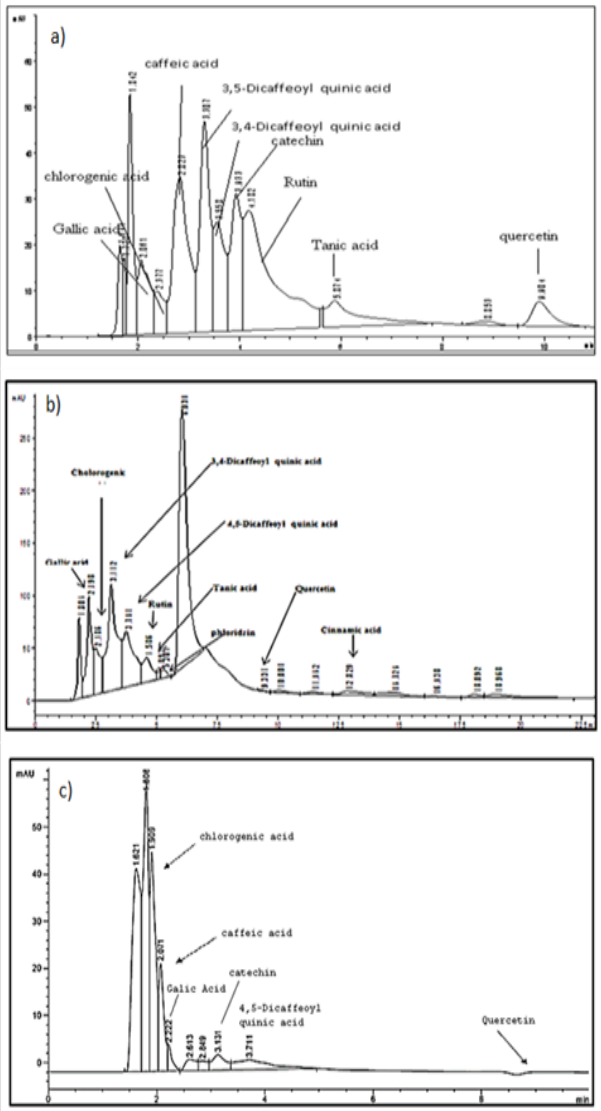

Figure 1. HPLC analysis of polyphenolic compounds. The chromatogram of some polyphenolic compounds identified by HPLC in SOE (a), OLE (b) and MPE (c).

vely. All extracts showed anti-lipid peroxidation, reducing power and DPPH radical and Nitric oxide (NO) scavenging activities as presented in Figure 2.

Enzyme inhibition assay performed on aromatase in placental microsomes

The activity of human placental aromatase was decreased in the presence of OLE, SOE and MPE. IC_{50} values for OLE, SOE and MPE were shown to be 45.31, 38.55 and 86.09 µg/ml, respectively (Figure 3). In OLE and MPE treated microsomes, aromatase inhibition was in a concentration-dependent manner. On the other hand, 3.75 µg/ml and 12.5 µg/ml concentrations of SOE caused significant elevation in aromatase activity by ~110% and 120%, respectively, but the aromatase activity was significantly decreased at higher concentration of SOE (≥ 50 µg/ml).

Evaluation of cytotoxicity against MCF-7 cell line

The results showed that the exposure of MCF-7 cells to all three extracts decreased cell viability in a dose-dependent manner (Figure 4). Where, treated cells with 50 µg/ml of OLE, SOE, MPE and vinblastine sulfate drug (as standard), separately, showed cell death by about 83.56%, 84.46%, 81.87% and 92.44 %, respectively.

Effect of MPE, OLE and SOE on aromatase gene expression in MCF-7 cells

Aromatase gene expression (*CYP19A1*) was significantly decreased by 87.5% and 98.1%, as a result of treatment with 33 and 66 µg/ml of MPE, respectively, compared to control. Also, *CYP19A1* expression level was significantly decreased by 99.7% and 99.8%, as a result of treatment with 16 and 32 µg/ml of OLE, respectively, compared to control. On the other hand, the results showed that low dosages of SOE (20 µg/ml) appeared to up-regulated *CYP19A1* expression by 570%

Table 3. HPLC analysis of polyphenolic compounds of OLE, SOE and MPE.

Compound	Concentration (mg/g OLE)	Concentration (mg/g SOE)	Concentration (mg/g MPE)
Chlorogenic acid	4.987	0.110	0.223
Caffeic acid	nd	1.026	0.488
3,4-Dicaffeoyl quinic acid	20.597	nd	0.952
3,5-Dicaffeoyl quinic acid	nd	2.690	nd
4,5-Dicaffeoyl quinic acid	12.218	1.892	1.170
Catechin	nd	16.910	6.630
Cinnamic acid	0.004	0.002	nd
Gallic acid	11.63	0.440	0.173
Phloridzin	0.001	nd	nd
Quercetin	0.006	0.007	0.006
Tanic acid	0.678	nd	nd
Rutin	3.19	1.021	nd

All data expressed as mg/g of dry extract. nd: non-detected.

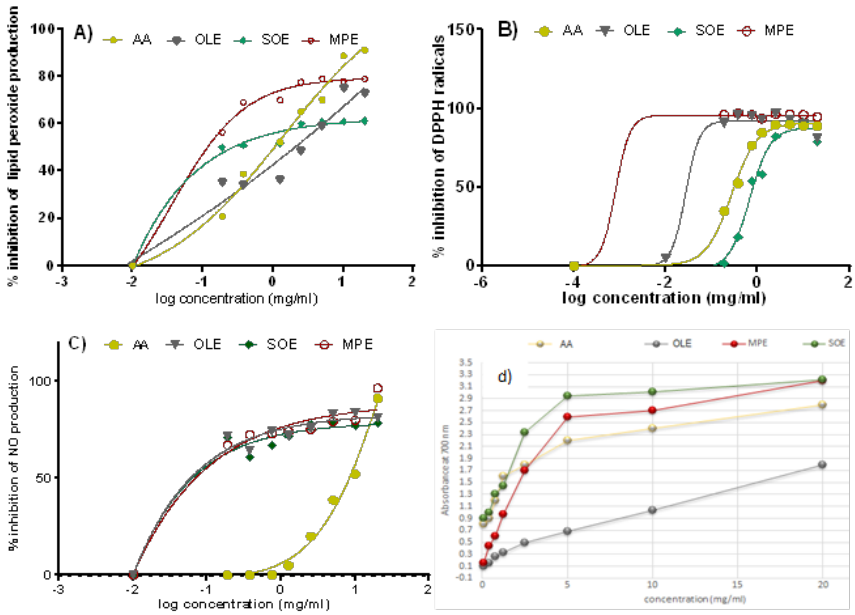

Figure 2. Plants extracts characterization. Where, a: Lipid peroxidation activity of SOE, OLE and MPE, b: DPPH radical scavenging effect of SOE, OLE and MPE, c: Nitric oxide scavenging activities of SOE, OLE and MPE and d: Ferric reductive abilities of SOE, OLE and MPE. Ascorbic acid was taken as a standard. All points of data were plotted as mean values (n=3).

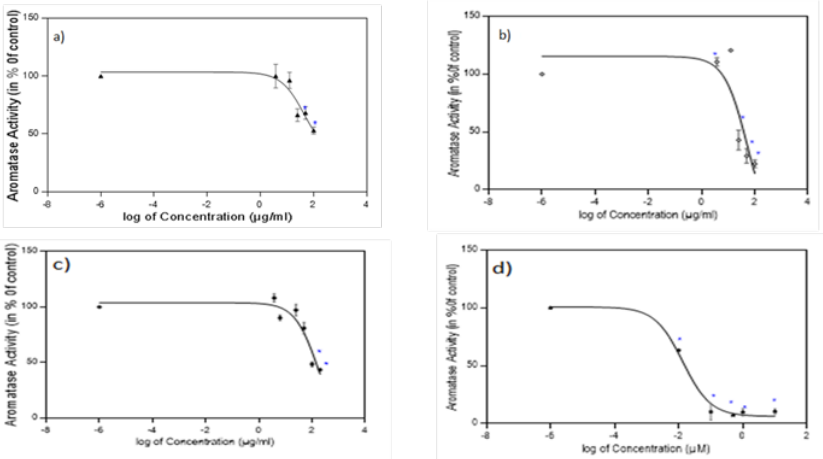

Figure 3. Effect of the evaluated plant extracts on aromatase activity. SOE (a), OLE (b), MPE (c) and (d): anastrozole (+ve control). The % aromatase activity in human placental microsomes plotted against logarithmically transferred concentrations; Aromatase activity without chemicals (0.1% DMSO) was 12.05±0.5 pmol/min/µg protein and was taken as 100%. All points of data were plotted as average values± SE (n=2). *Means were significantly ($p < 0.05$) different from those of the control.

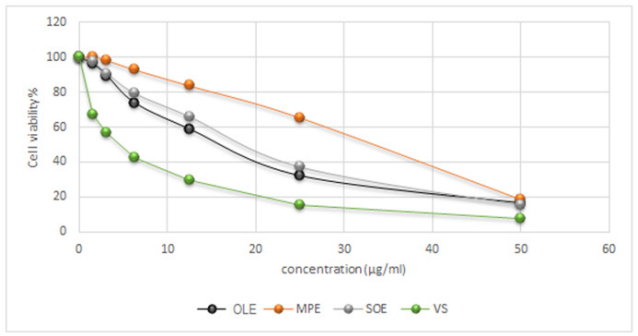

Figure 4. Effect of different concentrations of SOE, OLE, MPE and VS on MCF-7 cell viability.

compared to control. While, the high dosages of SOE (40 µg/ml) significantly decreased the expression in treated cultures by 99.5% compared to control (Figure 5).

Effect of OLE, SOE and MPE on aromatase exon I's/promoter's gene expression

The results showed that OLE concentration (16 and 32 µg/ml) exerted a significant and potent inhibition in aromatase expression that is specific to the aromatase promoters I.3, I.6, I.7, I.f and PII. Where, higher concentration (32 µg/ml) suppressed the levels of all transcripts: I.3, I.6, I.7, I.f and PII containing mRNAs in treated MCF-7 cells by 99.9%, 80%, 99.6%, 95% and 98.7%, respectively, compared to control. Also, the lower concentration of OLE (16 µg/ml) significantly decreased the levels of transcripts containing promoters: I.3, I.6, I.7, I.f and PII by 99.7%, 88%, 99.4%, 41% and 86.8% respectively, compared to control. Higher concentration of SOE (40 µg/ml) suppressed the levels of all transcripts: I.3, I.6, I.7, I.f and PII containing mRNAs in treated MCF-7 cells by 100%, 94%, 100%, 97% and 100%, respectively, compared to control. While, the lower concentration of SOE (20 µg/ml) significantly increased the levels of transcripts containing promoters: I.6, I.7 and I.f by 399%, 602% and 363%, respectively, and non-significantly increased I.3 and PII activities by 50% and 158%, respectively, compared to control. Treatment of MCF-7 cells with 66 µg/ml of MPE signi-

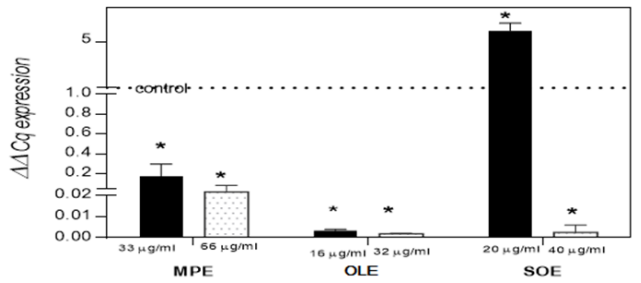

Figure 5. Effect of SOE, OLE and MPE on *CYP19* mRNA expression levels. Total aromatase mRNA expression in MCF-7 cells treated with MPE, OLE and SOE. The level of aromatase mRNA in cells treated with DMSO was normalized to 1. The data represents the means ± SE of two samples isolated from independent cultures. *Mean values were significantly ($p < 0.05$) different from those of the control cultures with no treatment.

ficantly decreased aromatase expression driven from 1.3, 1.7 and PII promoters by 94%, 89.7% and 82%, respectively, compared to control. While, MPE treatment (66 µg/ml) significantly enhanced the expression driven from P1.6 by 500%, but non-significantly driven from P1.f by 100%. Cells treated with 33 µg/ml of MPE, showed a significant reduction in I.3 promoter by 78% compared to control and a significant increase in 1.6 promoter by 1478%, but a non-significant increase in I.7 and I.f promoters by 294% and 212%, respectively, compared to control. Treatment of MCF-7 cells with 33 µg/ml of MPE has no significant effect on PII promoter level (Figure 6).

Discussion

In the current study, experimental evaluation of phytochemicals indicated that OLE, SOE and MPE contain many antioxidant compounds. OLE contains high content of polyphenolic compound (314.81 mg/g extract), flavonoids, alkaloids and triterpenoids compounds. HPLC analysis of phenolic compounds revealed the presence high concentration of 3,4-dicaffeoyl quinic acid, 4,5-dicaffeoyl quinic acid, gallic acid, chlorogenic and rutin. SOE contains high content of polyphenolic compound (81.09 mg/g extract), flavonoids, alkaloids and triterpenoids compounds. The phenolic profile of SOE revealed the presence of catechin, rutin, gallic acid, qurcetin, caffeic acid and 3,5- and 4,5-dicaffeoylquinic acid. The results of SOE analysis agree with (37). Additionally, (38) found that the major flavonoids in SOE are apigenin and luteolin derivatives. MPE contains high content of polyphenolic compound (111.84 mg/g extract), flavonoids, alkaloids and triterpenoids compounds. HPLC analysis of these phenolics showed the presence of catechin, gallic acid, quercetin, caffeic acid, chlorogenic acid and its isomers, in addition to other unidentified compounds. These extracts also showed radical scavenging activities, since they decreased the levels of lipid peroxidation as well as NO and DPPH radicals. These findings agree with the results of previous studies (19, 21, 37). All phenolic compounds revealed by HPLC analysis have the structural requirements for free radical scavengers since they can act as hydrogen or electron donors, capable to stabilize unpaired electrons and scavenge ROS, finish Fenton reaction, reduce the N-nitrosation reaction and prevent oxidative damage. Moreover, triterpenoids have a radical scavenging power and inhibitory effect on lipid peroxidation (23).

The results of the current study showed that OLE and MPE inhibited human placental aromatase activity and this inhibition was concentration dependent manner. On the other hand SOE at low concentrations (3.75 and 12.5 µg/ml extract) activated aromatase activity, while, at high concentrations (≥ 50 µg/ml extract) inhibited the enzyme activity. Considering the dual effect of SOE on aromatase activity, the same pattern was reported for the natural flavone (quercetin) of SOE (39).

IC_{50} values for OLE, SOE and MPE were shown to be 45.31, 38.55 and 86.09 µg/ml, respectively. The results showed that 100 µg of SOE had the maximum aromatase inhibitory effect (80%) which may be due to the effect of the high contents of polyphenolics and flavonoids compounds (see Table 2). Previous studies showed that flavone (apigenin and luteolin derivatives), phytoestrogens and polyphenolics compounds inhibit aromatase activity in a competitive manner. Where, these compounds can bind to aromatase active site in an orientation in which their rings-A and -C mimic rings-D and -C of the androgen substrate, respectively (38, 40). Inhibition of aromatase by OLE may be related to the effect of flavonoids, alkaloids and polyphenolic compounds (3,4-dicaffeoyl quinic acid, 4,5-dicaffeoyl quinic acid, gallic acid, chlorogenic and rutin). These results were in agreement with the findings of (41) who reported that gallic acid is an aromatase inhibitor with IC_{50} in the range of 10^{-5}M. Concerning, inhibition of aromatase by MPE may be due to the effect of phenolic

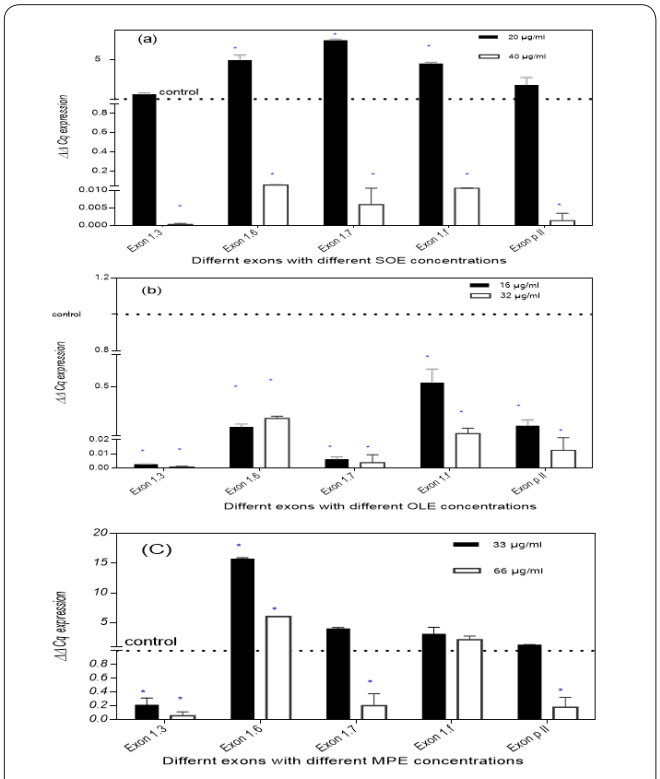

Figure 6. Exon I specific QRT-PCR analysis of SOE (a), OLE (b) and MPE (c) treated MCF-7 cultures. The level of aromatase mRNA in cells treated with DMSO was normalized to 1 and The GAPDH was amplified as an internal control. The data represents the means±SE of two samples isolated from independent cultures. *Mean values were significantly ($p < 0.05$) different from those of the control cultures.

compounds, especially catechin, 3,4-dicaffeoyl quinic acid, flavonoids and alkaloids. Xanthones (major bioactive constituent of phenolic compounds) were reported to have moderately to strongly aromatase inhibitory activities (42). As can be seen, the differences in the inhibitory activities between all tested extracts arise from the variation in their phytoconstituents.

The results of the present study showed that of OLE, SOE and MPE decreased cell viability with different degrees. As a consequence, these extracts have cytotoxic activity and anti-cancer properties, and this may be due to the effect of phenolics, flavonoids, alkaloids and triterpenoids compounds. Where, the phenolic compounds (powerful antioxidants) were considered as interesting molecules with anti-inflammatory, antimutagenic and anticancer activities (43). In a previous study, (44) reported that polyphenols exhibit anticancer effect through both inhibition of cell growth and induction of death in the cancer cells. In another study to (45), anticancer effect of polyphenols is concentration dependent, where, at low concentrations of polyphenols cell growth arrest has been attributed to both the inhibition of cell proliferation and the induction of apoptotic cell death. While, at high concentrations cell growth arrest has been attributed to a direct toxic effect, which leading to necrotic cell death. In particular, OLE was the most efficient in decreasing cell viability (IC_{50} 16.60 µg/ml). This result agree with previous studies which reported that hydroxytyrosol present in OLE has a cytotoxic effect, inhibited cell proliferation and induced cell apoptosis (46-48). The results showed that SOE has anti-tumor activity (IC_{50}19.40 µg/ml). These results are in agreement with (49) who found that SOE hydroalchoholic extract decreases the cell viability. Moreover, apigenin, one of the major constituents of SOE, could effectively induce apoptosis and overproduction of ROS in MCF-7 cells. Also, catechin has antiproliferative action, and this is related to the increased expression of pro-apoptotic genes (50). Cytotoxic effect of MPE in the current study (81.59% cell death at 50 µg/ml) may be related to the effect of phenolics, flavonoids, alkaloids and triterpenoids compounds. This result agrees with (51) who reported that MPE (100 µg/ml) significantly reduced the number of viable MCF-7 cells, and this is related to the presence of gallic acid and its galloylated derivatives. Additionally, mangiferin, the major constitute of MPE, showed a potent antimutagenic activity on MCF-7 (52).

In the present study, the differential effects of OLE, SOE and MPE on aromatase expression showed that OLE seems to be the most potent suppressor followed by SOE and then MPE. The suppressor effects of OLE, SOE and MPE may be related to the beneficial effects of their contents which include polyphenolic, flavonoid, alkaloids and triterpenoids compounds. These results agree with previous studies which demonstrated that most polyphenolic compounds and flavonoids inhibit aromatase at the enzymatic level and/or at the transcriptional level with comparable (53). Also, previous studies showed that OLE, SOE and MPE have good anti-inflammatory activities, since they inhibit COX-2 and prostaglandin E2 (PGE2) production (21, 46, 54). Also it has been reported that there is a strong association between *CYP19A1* gene expression and COX gene expression (55). This indicates that these extracts effect on *CYP19A1* gene expression. Likewise, it was reported that COX inhibitors suppress the mRNA expression of *CYP19* (56). PGE2 is a powerful stimulator of adenylate cyclase (AC) in adipose stromal cells. Thus increases intracellular cAMP levels stimulate aromatase expression from PI.3/PII in adjacent breast adipose fibroblasts, leading to increased local concentrations of estrogen [5, 6]. The observed down regulation of aromatase gene mediated by these extracts may attributed to their anti-inflammatory activities. From the overall results it is possible to point out that, hydroxytyrosol rich olive leaves extract (OLE) demonstrates effective inhibitory action on aromatase at both the enzymatic and expression levels, in addition to its cytotoxic effect against MCF-7 cells. The results also indicates that mango peel extract (MPE) may has the potential to be used as a tissue-specific aromatase inhibitor (i.e. selective aromatase inhibitor) and it may be promising to develop a new therapeutic agent against ER+ breast cancer.

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