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In-Vitro Evaluation of Anticancer Potential of Cinnamon Bark Oil and Cinnamonaldehyde in Adjuvant Endocrine Therapy for Hormone Receptor Positive Breast Cancer by Using Human Cell Line

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Authors' Contribution
SS developed the idea and study design, undertook the experimental work, and prepared the manuscript. MA and AA oversaw the experimental work and offered valuable input on the research design, analyzed data, and helped with the writing of the manuscript. MOO engaged in statistical modeling and helped to discuss the outcomes, sourced the materials necessary for the research and took part in the editing of the paper. AAA helped with the writing of the manuscript.

Key words
Breast cancer, Hormone receptor-positive, Combination therapy, *Cinnamomum zeylanicum* essential oil, Cinnamaldehyde, Tamoxifen, Anastrozole, Synergistic interaction, Cytotoxicity, Estrogen receptors

ABSTRACT

The purpose of this study is to evaluate the combinatorial effects of *Cinnamomum zeylanicum* essential oil (CEO) and its principal compound, derived from cinnamon, 'cinnamaldehyde' (CA) alongside standard hormonal anti-cancer therapies such as tamoxifen (TAM) and anastrozole (ANA), for the management of estrogen receptor-positive (ER+) breast cancer. The CEO contains about 69.57% of cinnamonaldehyde, which showed high antioxidant, anticancer and medicinal activities. The most pronounced synergy was found when combined tamoxifen and CEO (CI = 0.205), causing potent cell death, G0/G1 phase arrest and modulation of estrogen receptor mRNA levels. Real-time PCR analysis demonstrated that the levels of the mRNAs for the estrogen receptors alpha (ERα) and beta (ERβ) were significantly lower in the treated cells. Moreover, the treated cells did not show any noteworthy damage as per the DNA damage assessment conducted, thus showing a positive safety window. These results indicate that the effectiveness of hormone therapies in treating ER+ breast cancer can be improved with the addition of drugs such as CEO and CA hence the combination regimen is likely to be very effective and less toxic.

INTRODUCTION

Breast cancer is one of the most gendered cancers which is leading to death in the world with breast cancer which has estrogen receptors being present in nearly 70% of the women affected. Commonly used drugs include tamoxifen (TAM) which target estrogen receptors and anastrozole (ANA) which functions by inhibiting aromatase, an enzyme responsible for the synthesis of estrogen, without directly interacting with the estrogen receptor both work accordingly to suppress the growth of the tumors (Cancer Research UK, 2023). Nonetheless, factors like drug resistance, recurrence, and drug toxicity calls for the investigation of different treatment approaches (Bardia *et al*., 2023). Employing the use of natural products in addition to standard treatment methods can be helpful in targeting better treatment response without increasing toxicity levels.

Cinnamon, which is scientifically *Cinnamomum zeylanicum*, has been applied in traditional medicine for centuries especially attributed of its active component; Cinnamaldehyde which has anti-inflammatory, antimicrobial and anticancer properties (Siriwardhana *et al*., 2021). Cinnamon essential oil (CEO) which is a product made from cinnamon bark is also known to be high in cinnamaldehyde and has been reported to suppress the growth of certain types of cancer cells by manipulating pathways, inducing cell death, and alleviating oxidative stress levels in human cells (Tiwari *et al*., 2022).

Despite the fact that previous publications illustrated

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anticancer activities of both infused cinnamon oil (CEO) and cinnamaldehyde, the research on their impact when administered alongside breast cancer drugs such as tamoxifen and anastrozole is limited. The recent publications indicate that the usage of natural ingredients such as CEO or CA, will increase the efficacy of the conventional treatments by enhancing oxidative stress and apoptosis induction as well as the modulation of estrogen receptors (Gucbilmez *et al*., 2023). Still, the molecular mechanisms and the effective dose of such combinations remain a mystery.

MATERIALS AND METHODS

Study design and setting

This study was conducted in collaboration between the Department of Pharmacology and Toxicology, Institute of Microbiology, Quality Operation Laboratory (QOL), and Department of Parasitology, University of Veterinary and Animal Sciences (UVAS), Lahore. The investigation aimed to assess the synergistic effects of *Cinnamomum zeylanicum* essential oil (CEO) in combination with hormone therapies (tamoxifen and anastrozole) for estrogen receptor-positive (ER+) breast cancer treatment.

Plant material and extraction

Dried pieces of *Cinnamomum zeylanicum* bark were procured from the Sri Lankan market, and their identification was done by the Department of Botany, Govt. College University, Lahore (voucher No. GC. Herb. Bot. 3877). The essential oil was extracted using a modified Clevenger apparatus and hydro-distillation (Wong et al., 2014). Briefly, 300g of dried and powdered cinnamon bark was immersed in distilled water and boiled for 6 hrs. at 100°C. The distillate was then obtained by using dichloromethane, then dehydrated with anhydrous sodium sulfate and filtered. Moreover, phytochemical screening, GC-MS, HPLC and FTIR was performed on CEO and its principal bioactive component, Cinnamonaldehyde, to assess the chemical characteristics.

In vitro *antioxidant activity*

The antioxidant capacity of the extract was measured using DPPH radical scavenging methods based on Mensor *et al*. (2023) with a few changes. A variety of test sample concentrations were studied and percentage inhibition was determined from the absorbance at 517 nm of the test sample versus absorbance of the methanol blank.

Cell culture

MCF-7 cells (breast cancer cells that are positive for human estrogen receptor) and Vero cells (African green monkey kidney cells that are not cancerous) were acquired from the American type culture collection (ATCC). MCF-7 cells were grown in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin and Vero cells were grown in M-199 medium with 10% FBS. The cells were kept in an incubator that maintains a temperature of 37 °C and humidified atmosphere containing 5% CO_2.

Cytotoxicity assay

The cytotoxicity of CEO and essential compounds such as CA, drugs TAM, ANA and there monotherapy and polytherapy regimens combinations were determined using MTT assay. Cells were plated in 96-well plates at a density of 5 × 10^3 cells per well and exposed to different amounts of the compounds from (1.95 to 500 µg/mL). After a treatment period of 24, 48 and 72 h, 20 µL of MTT solution (5 mg/mL in PBS) was appropriately spaced into each well and the absorbance read at 540nm using a microplate reader (Mosmann, 1983). The IC50 values were calculated with GraphPad Prism using non-linear regression (log(inhibitor) vs. response). The dose-response graphs for MCF-7 and Vero cells with R^2 and 95% confidence intervals were indicated. This method is widely used for evaluating cytotoxicity (Zhang *et al*., 2024; Li *et al*., 2023).

Flow cytometric assay

Stock solutions of CEO, CA, and drugs TAM and ANA was prepared from IC90 data from MTT assay: 0.01 µg/mL CEO + TAM, 0.13 µg/mL CA + TAM, 1.09 µg/mL CEO + ANA, and 3.06 µg/mL CA + ANA. The cellular treatment was done with the test mixtures for 24hrs, after which the cells were collected, washed with PBS, and stained with potassium iodide (PI), rhodamine 123, and acridine orange. The analysis of stained cells was performed using FACS Attune X™ NxT cytometer (Invitrogen, USA) (Inala and Pamidimukkala, 2021).

Gene expression analysis by RT-PCR

To make androgen receptor antagonists more effective for use against breast cancer, the hormone regulator estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and GAPDH gene expression levels were investigated in the test of CEO or CA treatment with TAM and ANA (Osborne *et al*., 2021, 2023). The total RNA was extracted with TRIzol™ Reagent and purified. The concentration and purity of the extracted RNA were determined using a spectrophotometer. The first stand cDNA was obtained by from 1 ug RNA using superscript IV reverse transcriptase. Agarose gel electrophoresis was performed to analyze the PCR products and the fragments were stained with ethidium bromide before being visualized under UV light (Lim *et al*., 2023).

Genotoxicity and mutagenicity assessment

Comet assay was performed to determine the DNA damage in peripheral blood lymphocytes caused by treatment with the CEO, TAM and ANA. Cells were treated with the test compounds and embedded in agarose gel. DNA fragmentation was examined using a fluorescent microscope after ethidium bromide staining (Arnold *et al*., 1983; Johnson *et al*., 2020).

Mutagenicity testing was done using the Ames test and *Salmonella typhimurium* TA100 strain. The test compounds (CEO, TAM and ANA) were placed on minimal agar plates containing histidine, with or without S9 metabolic activation (Mortelmans and Zeiger, 2020).

Data analysis

Data were analyzed through SPSS software, version 20. IC50 values, selectivity index (SI), and combination index (CI) were computed with the use of GraphPad Prism and Compusyn software (Chen *et al*., 2024; Chou, 2022). A statistical analysis was carried out using a one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

RESULTS

Extraction and characterization of CEO

Hydro-distillation of *Cinnamomum zeylanicum* bark yielded 3.1 g of essential oil (1.03%), consistent with previous studies (Adams, 2023). Preliminary screening detected flavonoids, phenols, aldehydes, and other compounds (Table I), supporting the diverse phytochemical profile of cinnamon bark (Nogueira et al., 2023). GC-MS revealed cinnamaldehyde (69.57%) as the predominant component, with 6-octadecenoic acid (29.48%) and anthraquinone derivatives (12.8%) (Table II). HPLC confirmed cinnamaldehyde's presence, matching the standard (Fig. 1) (Huang *et al*., 2018). FTIR spectra exhibited a characteristic absorption peak at 1.5882 cm^{-1} for cinnamaldehyde's carbonyl group (Fig. 2). TFC was 2.82 mg QE/g, and TPC was 10.74 mg GAE/g, reflecting the oil's significant antioxidant capacity (Chen *et al*., 2024).

Antioxidant activity

To assess the antioxidant property of cinnamon oil, DPPH assay was performed. Table III demonstrate that among the test samples, ascorbic acid exhibited the strongest activity (IC50: 17.13 µg/mL) while CEO, followed with a higher concentration of 58.06 µg/mL and the lowest concentration being given to were compounded with TAM and ANA and increased antioxidant effects were observed (Shreelalitha et al., 2018).

Table I. Preliminary phytochemical screening of *C. zeylanicum* bark oil.

Phytochemical test	*C. zeylanicum* bark oil
Lead acetate test (flavonoids)	++
Schiff's test (aldehyde)	++
Benedicts reagents (ketone)	++
Litmus test (carboxylic acid)	++
Igner's test (alkaloids)	++
Ferric chloride test (tannins)	++
Foam test (saponins)	++
Anthocyanin	--
Salkowiski test (terpenoids)	++
Borntrager's test (glycosides)	++
Lead acetate test (phenolic compounds)	++
Alkaline reagent test (coumarins)	+

(+) high concentration (+) moderate concentration (-) Not detected.

Table II. Chemical constituents with their relative retention times and respective concentrations of the constituents present in the *Cinnamomum zeylanicum* bark essential oil.

Constituent	Con-centra-tion %	RT (min)
2 (5) Furanone	1.21	8.761
1, 5- Hexadien-3-yne	0.54	13.337
1, 4-Anthracenedione, -8, 8-dimethyl-1-Tri-methylsilyloxy-3, 4-diemthylcy clohexane (Anthraquinone glycosides)	12.8	15.645
1, 4-Anthracenedione (Anthraquinone glyco-sides) derivates	4.34	15.682
1, 4-Anthracenedione derivates	7.92	15.844
Phenylethanol amines (aromatic ketone)	0.187	17.157
3-Phenyl-2-propyn-1-1,2-Propenal, 3-Phenyl (Trans-cinnamonaldehyde)	0.185	20.679
Cinnamic aldehyde (cinnamonaldehyde)	69.57	20.950
Oleic acid	2.25	22.339
1, 3-Benzodiozole derivatives	4.55	23.094
6-Octadecenoic acid methyl ester (saturated fatty acid stearic acid)	29.48	23.300
Butanedioic acid, phenyl- 1, 3, 7-Octa-trien-5-yne (Succinic acid)	6.35	23.509
Phenol 2-methoxy-4-(2-propenyl)-methyl (Phenol)	0.36	24.619
Trans 6-Octadecenoic acid methyl ester (long chain saturated fatty acid)	6.45	26.902
3- methoxy-d-homoestra-1,3,5(10)-trien-17a-one (8.alpha., 9.alpha.) (estratrien derivative)	0.52	27.197
1,3-diethenyl-1,4-dihydronahthalene	0.45	27.703
3-Eicosene, (E) Chloroacetic acid	1.53	28.094

RT: Retention time

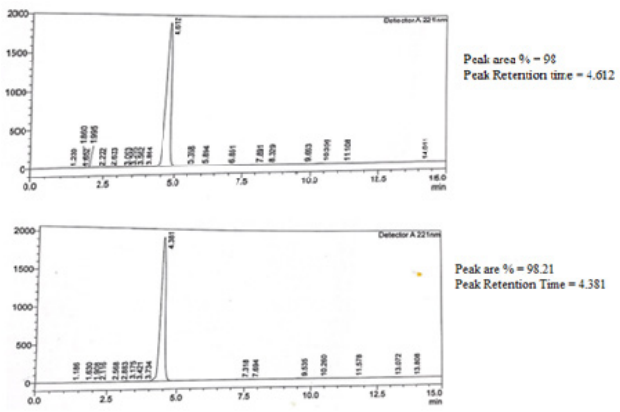

Fig. 1. Chromatogram of standard Cinnamonaldehyde and in *C. zeylanicum* essential oil.

IC50, selectivity index (SI), and combination index (CI) with anticancer drugs

Table IV shows that the CEO had a marked anticancer effect with an IC50 of 47.28 µg/mL and a selectivity index (SI) of 2.56. In comparison, CA showed an IC50 value of 168.1 µg/mL (SI: 2.34). The IC50 values for TAM and ANA were 5.76 µg/mL (SI: 2.44) and 52.7 µg/mL (SI: 2.11) respectively. In terms of the effects of combined treatments, the greatest synergy was achieved with CEO + TAM (CI = 0.205), and with CEO + ANA (CI = 0.770), suggesting that this combination has a higher anticancer effect (Adel and Karakaya, 2021) The dose-response graphs for MCF-7 and Vero cells are shown in Supplementary Figures S1-S4, with R^2 and 95% confidence intervals indicated.

Table III. Inhibitory concentration (IC_{50}) of ascorbic acid, *C. zeylanicum* essential oil (CEO), cinnaonaldehyde (CA), tamoxifen, anastrozole, CEO + tamoxifen, CA + tamoxifen, CEO + anastrozole, CA + anastrozole, CEO + tamoxifen+ anastrozole, CA + tamoxifen + anastrozole.

Control and samples	IC_{50} (ug/mL)
Ascorbic acid	17.13
C. zeylanicum essential oil (CEO)	58.06
Cinnaonaldehyde (CA)	81.76
Tamoxifen	38.18
Anastrozole	58.86
CEO+*tamoxifen* (1:1)	30.14
CA+*tamoxifen* (1:1)	36.74
CEO+*anastrozole* (1:1)	41.74
CA+*anastrozole* (1:1)	48.86
CEO+ *tamoxifen*+*anastrozole* (1:1:1)	47.72
CA+ *tamoxifen*+*anastrozole* (1:1:1)	38.16

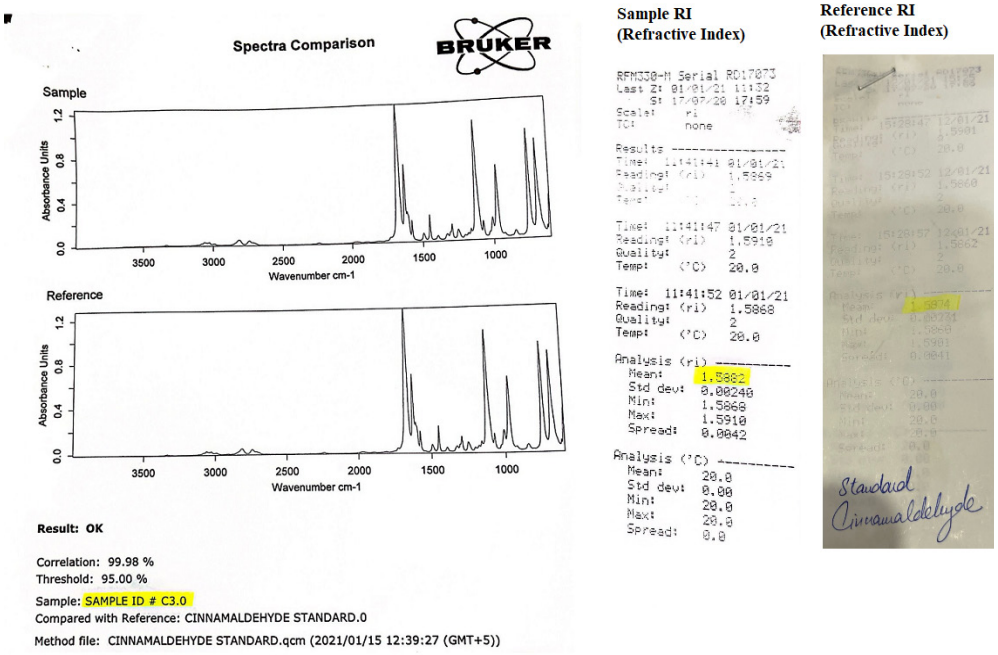

Fig. 2. Comparative FTIR spectrum and refractive index of Cinnamonaldehyde in *C. zeylanicum* bark essential oil and standard cinnamonaldehyde.

Table IV. Inhibitory concentration (IC50), selectivity index (SI), and combination index (CI) of *C. zeylanicum* essential oil (CEO), cinnamonaldehyde (CA), *tamoxifen*, *anastrozole*, CEO + *tamoxifen*, CA + *tamoxifen*, CEO + *anastrozole*, CA + *anastrozole*, *tamoxifen* + *anastrozole* + CEO, and *tamoxifen* + *anastrozole* + CA.

Treatment	IC_{50} (µg/mL)[a, b]		SI	Combination index (CI)		Remarks
	Vero cell line	MCF-7 cell line		At IC50 of constant ratio (Fa=0.50)	1:1 (Fa= 0.50~0.95)	
C. zeylanicum essential oil (CEO)	122.7±0.010	47.28±0.124	2.56	-		Significant anticancer potential
Cinnamonaldehyde (CA)	393.2±0.019	168.1±0.012	2.34	-		Significant anticancer potential
Tamoxifen (TAM)	14.1±0.043	5.76±0.001	2.44	-		Significant anticancer potential
Anastrozole (ANA)	111.6±0.011	52.7±0.018	2.11	-		Significant anticancer potential
TAM+CEO (1:10)	20.02±0.031	8.89±0.002	2.22	0.205	0.780 – 0.007	Synergistic effect
TAM+CA (1:34)	37.4±0.020	17.93±0.010	2.08	0.236	1.608 - 1.589	Synergistic effect
ANA+CEO (1:1)	81.9±0.025	38.69±0.003	2.11	0.770	0.770 – 0.014	Middle synergistic
ANA+CA (1:3.4)	133.8±0.009	64.5±0.008	2.07	0.574	0.803 – 0.461	Middle synergistic
TAM+ANA+CEO	72.83±0.025	36.19±0.010	2.01	0.985	2.573 – 2.321	Synergistic effect
TAM+ANA+CA	152.3±0.007	76.11±0.029	2.00	1.018	5.031 – 3.667	Synergistic effect

[a] IC_{50} values were presented as the mean ± SD of three independent experiments. [b] Statistically significant differences were noted using a one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Cell death and mitochondrial integrity

CEO + TAM induced the highest cell death (58.9%), followed by CEO + ANA (49.7%) (Fig. 3A). Mitochondrial membrane integrity was significantly disrupted by CEO + TAM and CA + ANA treatments, with intact membranes dropping to 0.98% and 0.87%, respectively (Fig. 3B) (Adel and Karakaya, 2021).

Table V. Percentages of cell cycle arrest in MCF cell line after treatment.

Treatment	Phases of cell cycle	% Cell arrest
Untreated cells	G0/G1	52.61
	S	5.71
	G2/M	6.69
CEO+TAM	G0/G1	71.21
	S	10.21
	G2/M	2.36
CA+TAM	G0/G1	42.15
	S	27
	G2/M	6.85
CEO+ANA	G0/G1	71.25
	S	16.5
	G2/M	3.9
CA+ANA	G0/G1	40.1
	S	31.13
	G2/M	3.5

Cell cycle analysis

Table V shows treatment with CEO + TAM resulted in significant G0/G1 arrest (71.21%) and minimal effects on the S (10.21%) and G2/M (2.36%) phases. CEO + ANA induced similar G0/G1 arrest (71.25%), with moderate effects on the S phase (16.5%). CA combinations showed moderate G0/G1 arrest (40.1%) and S phase arrest (31.13%) (Fig. 3C) (Nimavat *et al*., 2020a; Zhang *et al*., 2023).

Mechanistic insight – RT-PCR

RT-PCR analysis indicated that CEO and CA treatments reduced ERα and ERβ expression when combined with TAM enhancing its efficacy ($p < 0.05$) (Fig. 4). ANA showed minimal effects on ERα and no significant impact on ERβ.

Safety profile

Genotoxicity tests revealed no significant DNA damage for TAM + CEO and ANA + CEO at concentrations up to 1000 µg/mL ($P > 0.05$). Over 80% of cells were classified as Class 0, indicating no genetic damage, supporting the safety of these combinations (Fig. 5) (Adel and Karakaya, 2021; Sushma *et al*., 2020). The Ames test revealed low mutagenic activity for TAM + CEO and ANA + CEO in *S. typhimurium* TA100. TAM + CEO exhibited a dose-dependent increase in mutagenic index (MI), ranging from 0.3 to 1.5, while ANA + CEO showed MI values between 1.4 and 1.6. Both treatments had lower MI values than the positive control (Sodium Azide, MI = 3.47), indicating minimal mutagenic potential (Fig. 6).

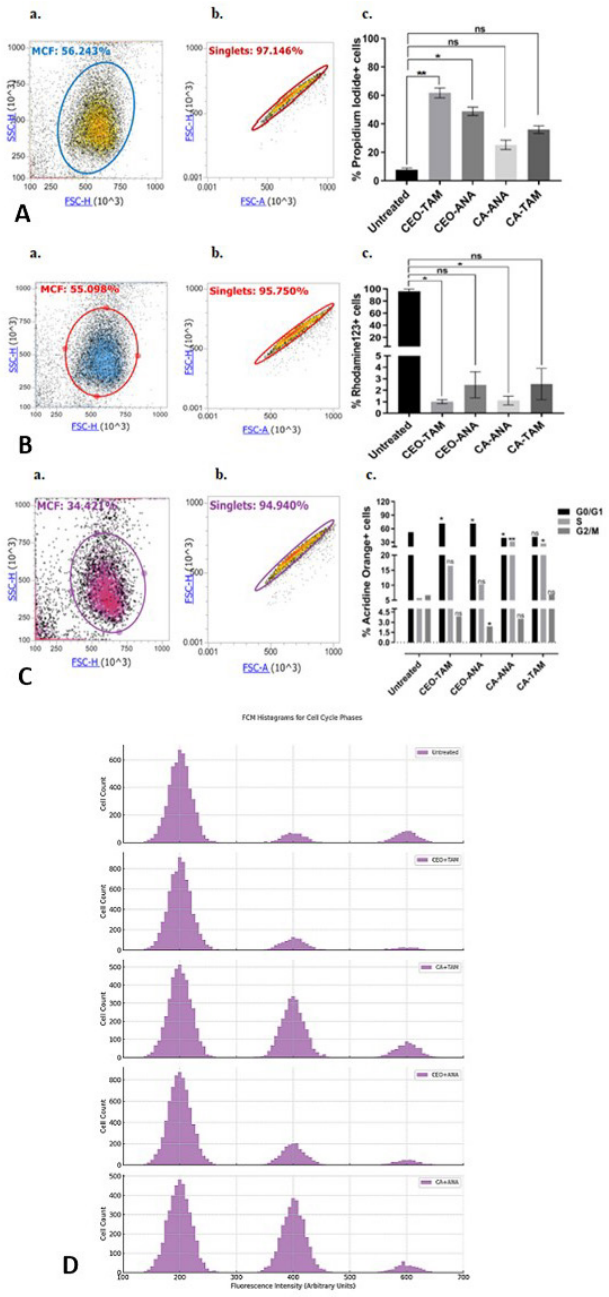

Fig. 3. Effect of CEO and CA combinations with TAM and ANA on cell death, mitochondrial integrity, and cell cycle progression in MCF-7 cells. (A) Flow cytometry-based detection and quantification of cell death (%) induced by treatments. (B) Mitochondrial integrity analysis, including flow cytometry detection and quantification of intact membranes (%) after treatments. (C) Cell cycle analysis showing flow cytometry detection, quantification of cell cycle stages (%), and distribution after treatments. (D) Representative FCM histogram for cell death, mitochondrial membrane integrity and cell cycle phases.

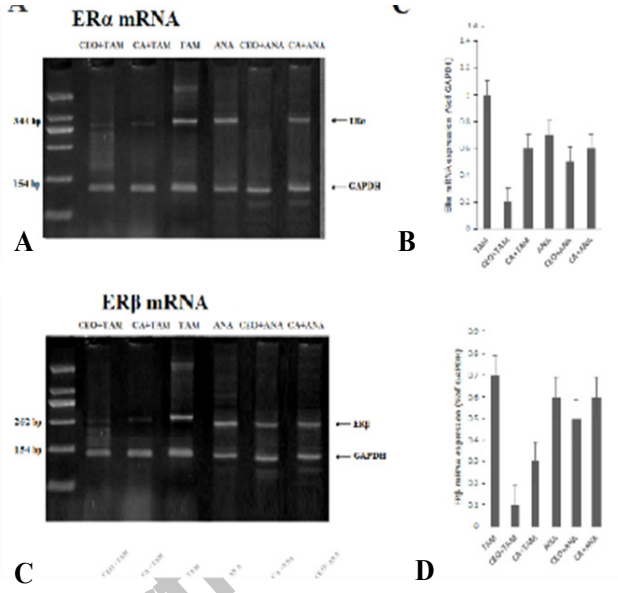

Fig. 4. Gel electrophoresis results of RT-PCR products.

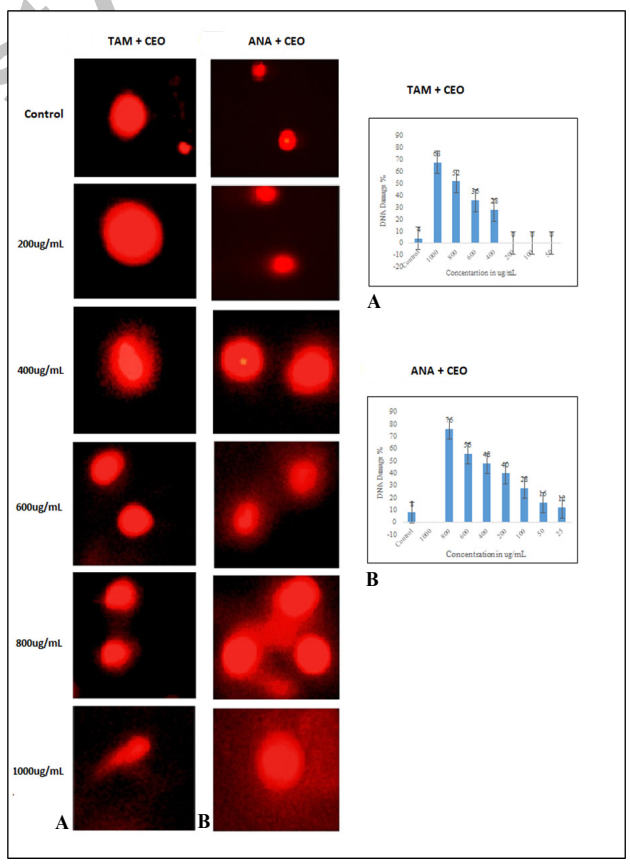

Fig. 5. Percentage DNA damage in two tested combinations.

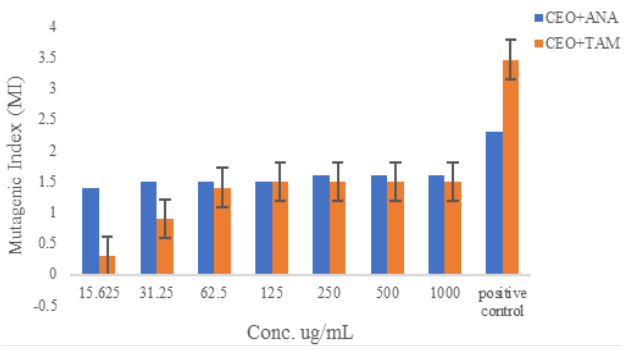

Fig. 6. Percentage DNA damage in two tested combinations.

DISCUSSION

Research explored the scope of anticancer activity of *Cinnamomum zeylanicum* essential oil (CEO) as well as the concomitant effects of *Tamoxifen* (TAM) and *Anastrozole* (ANA) in breast cancer of estrogen receptor positive type (ER+). CEO had remarkable anticancer properties especially with TAM combination which resulted to increased cell death, G0/G1 phase arrest and reduced expression of ERα/ERβ in MCF-7 cells. This supports the work of Tiwari *et al*. (2022) who indicated that essential oils, in particular from Cinnamomum species, have the ability to enhance the anticancer activity of traditional cancer treatments. The best synergy was exhibited by CEO + TAM (CI = 0.205) demonstrating the possibility of drug resistance reversal with little dose increment of TAM minimizing its side effects.

When paired with ANA, the CEO demonstrated moderate synergy as well, although less so than with TAM. This lower measure of synergy (CI = 0.770 to 0.014) could be due to the fact that the two drugs use different ways to achieve their effects. TAM, which is a selective estrogen receptor modulator (SERM), modifies pathways of the estrogen receptor (Gucbilmez *et al*., 2023). On the other hand, ANA only inhibits the aromatase enzyme which causes a fall in estrogen levels, which is probably the reason for the stronger observed results of the combination of CEO + TAM. Past investigations have also indicated the separate mechanisms of actions of these drugs which enhance the regard of their interaction with TAM in combination with the administration of the CEO.

On the other hand, CA, which is one of the primary components of oils of cinnamon, showed less synergism in tandem with both TAM and ANA. The synergism exhibited with CA + TAM (CI = 0.236) and CA + ANA (CI = 0.574) combinations was lower than that of the combination of CA and oils from cinnamon bark where CA evident stronger activity than oils result showing more diverse activity of oils compared to CA (Zhang *et al*., 2023). This implies that all the compounds present in the CEO have a stronger anti-cancer effect than simply treating with CA.

The results of three-agent combinations including TAM + ANA + CEO and TAM + ANA + CA showed synergism; nonetheless the effects were less pronounced as compared to two-agent combinations. This implies that although the combination of either CEO or CA with both TAM and ANA may have enhanced anticancer efficacy, the extent of additional benefit may be lesser due to the tautological mechanisms of action of TAM and ANA. The results are consistent with earlier studies wherein adding more than one treatment option actually diminishes the added effect (Jordan, 2021; Smith and Dowsett, 2022).

In this regard, the combination of CEO with either TAM or ANA did not promote any appreciable genotoxic effect. The GDI analysis revealed that at higher concentrations as well, most cells stayed in Class 0 (no damage) which further supports the non-toxic nature of CEO as an adjunct treatment for cancer (Adel and Karakaya, 2021). This is especially beneficial for the sustained therapies for cancer since it indicates that the use of CEO may be able to alleviate the adverse effects of the conventional treatment regimens.

The combination of TAM and CEO is based on the hypothesis that bioactive compounds in CEO may influence estrogen receptor signaling, including modulation of ERα expression and related genes. This combination could enhance TAM's therapeutic effect, potentially overcoming resistance and improving treatment outcomes in ER+ breast cancer. This hypothesis is supported by previous research indicating that natural compounds, including those found in *Cinnamomum* species, can interact with estrogen receptor pathways, thereby enhancing the effectiveness of TAM and potentially reducing resistance (Tiwari *et al*., 2022).

Overall, this study underscores the potential of CEO, especially in combination with TAM, as an effective and safe adjunct to conventional ER+ breast cancer therapies. CEO enhances the efficacy of TAM and ANA, potentially reducing side effects and improving therapeutic outcomes. Further in vivo and clinical trials are necessary to validate these findings and explore the full potential of CEO in cancer treatment.

CONCLUSION

This research suggests a considerable promise of *Cinnamomum zeylanicum* (CEO) essential oil and its principal active component Cinnamaldehyde, as a supplement to normal hormonal therapies, Tamoxifen (TAM) and Anastrozole (ANA) when treating breast

cancer that is positive for estrogen receptors (ER+). The results show that CEO in particular when used with TAM has very potent synergistic effects including causing cell death, G0/G1 phase arrest and downregulation of estrogen receptor mRNA levels. Noteworthy, such combinations did not show any significant genotoxicity, proving their safety. This research creates a model for the application of natural bioactive compounds to the standard hormone treatments for the patients, which is otherwise not possible due to drug resistance and other side effects. It is worth noting that further studies in the form of in vivo tests and clinical trials would be necessary to affirm the effectiveness and safety of CEO in the management of breast cancer.

DECLARATIONS

Acknowledgment

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IRB approval

Approved by the Advanced Studies and Research Board of the University of Veterinary and Animal Sciences, Lahore (Approval Ref Notification No. DAS/935 dated 10/06/2021.

Ethical statement

All experimental procedures were conducted using established cell lines and adhered to institutional ethical guidelines. No human or animal subjects were directly involved in this study.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20241121132000

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

In-Vitro Evaluation of Anticancer Potential of Cinnamon Bark Oil and Cinnamonaldehyde in Adjuvant Endocrine Therapy for Hormone Receptor Positive Breast Cancer by Using Human Cell Line

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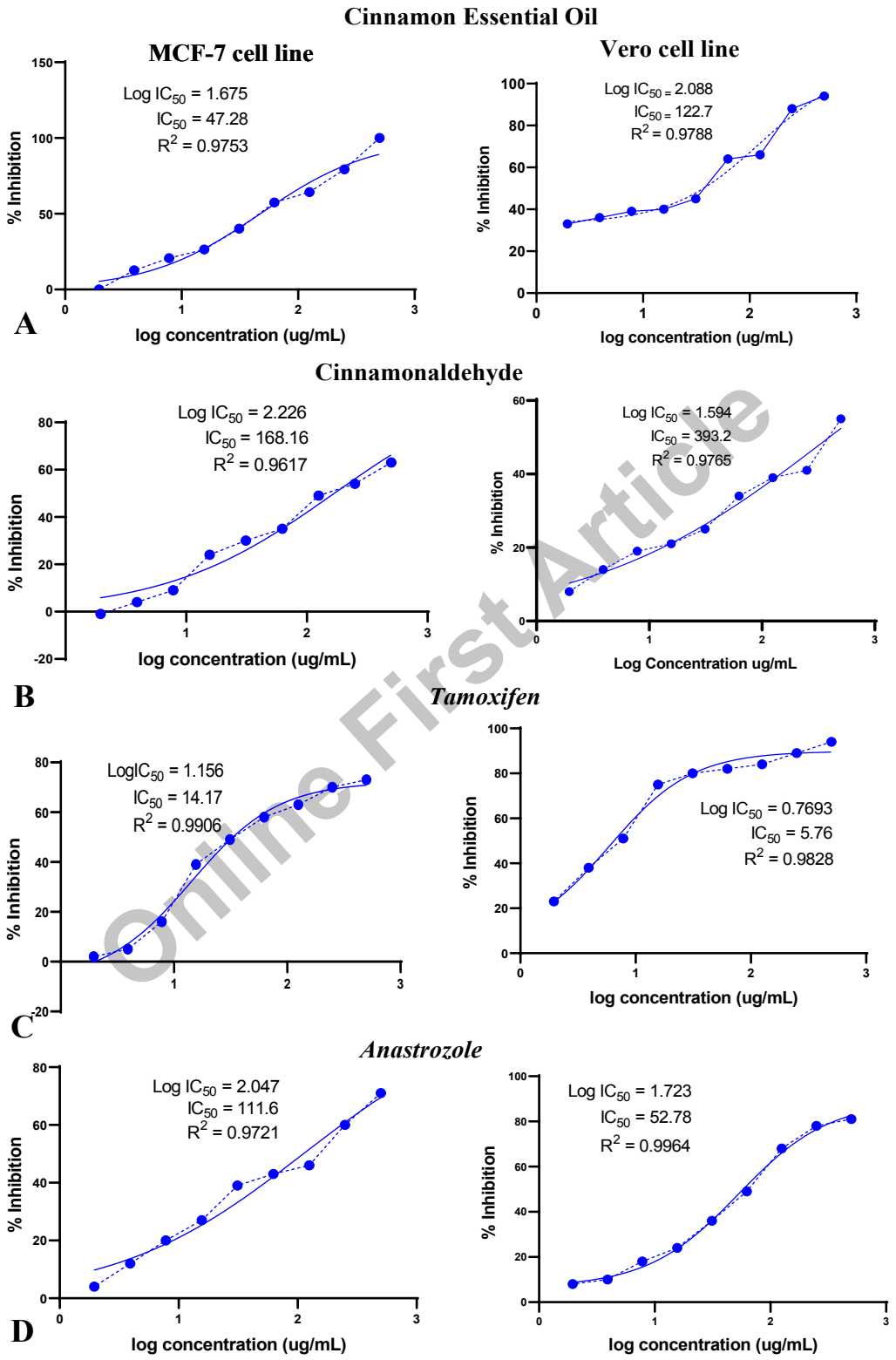
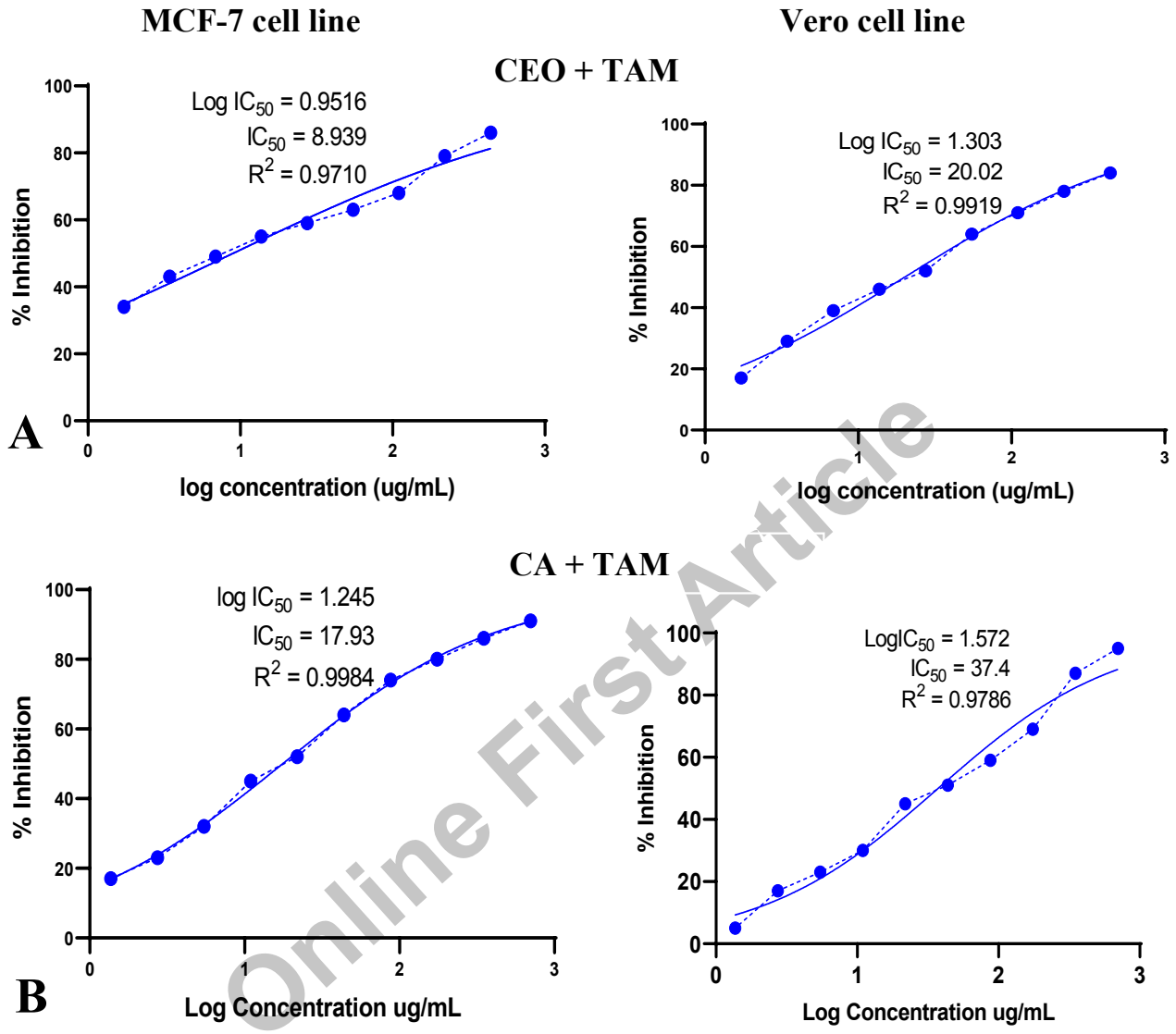
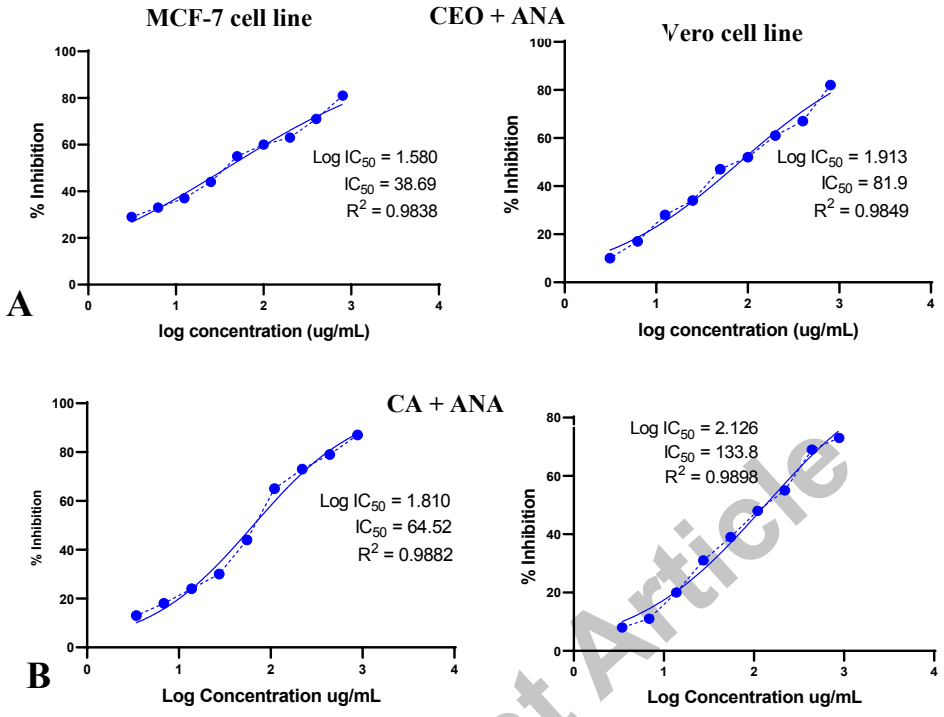


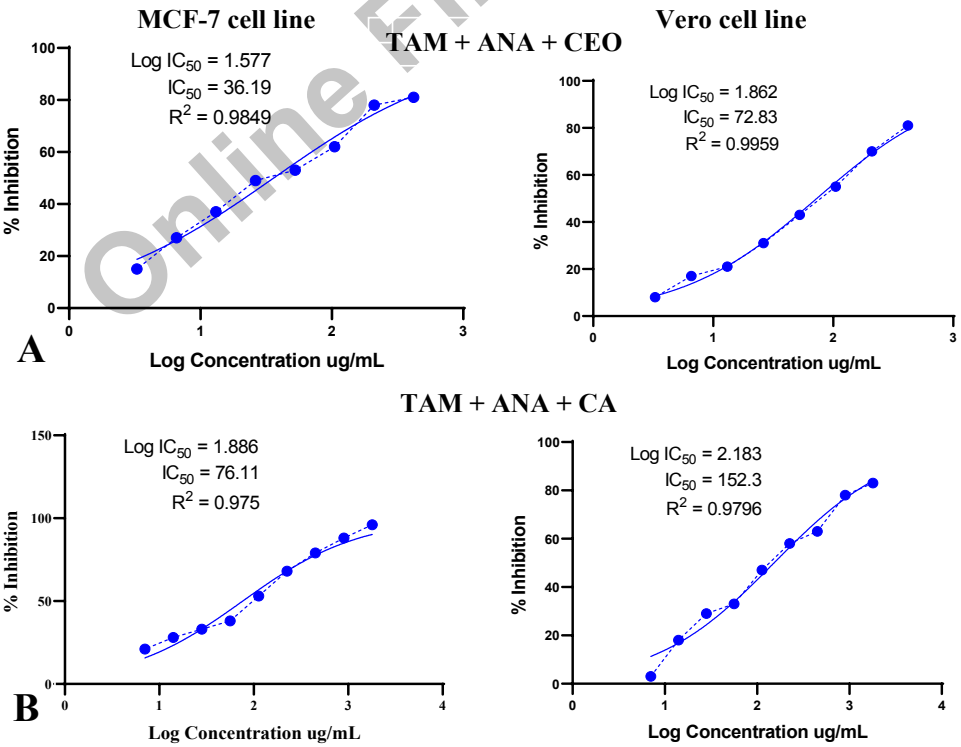
Supplementary Fig. S1. Cytotoxicity activity of cinnamon essential oil (A), cinnamonaldehyde (B), tamoxifen (C), and *Anastrozole* (D) on MCF-7 and Vero cell lines.



Supplementary Fig. S2. Cytotoxicity activity of CEO + TAM (10:1) (A) and CA + TAM (34:1) (B) on MCF-7 and Vero cell lines.



Supplementary Fig. S3. Cytotoxicity activity of CEO + ANA (34:1) (A) and CA + ANA (34:1) (B) on MCF-7 and Vero cell lines.



Supplementary Fig. S4. Cytotoxicity activity of TAM + ANA + CEO (1:10:34) (A) and TAM + ANA + CA (1:10:34) (B) MCF-7 and Vero cell lines.