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Aromatase Inhibition by Polyphenolic Extracts and Isolated Compounds from Seeds and Shoots of Georgian Grapevine (*Vitis vinifera* L.) Varieties

Mariam Tatanashvili[1]*, Malkhaz Jokhadze[2], Koba Sivsivadze[1], Natia Bokuchava[1], Ia Pantsulaia[3], Tamaz Murtazashvili[1], Tamar Masiukovichi[1]

[1]*Department of Pharmaceutical, Toxicological and Medical Chemistry, Faculty of Pharmacy, Tbilisi State Medical University, Tbilisi, Georgia*

[2]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Tbilisi State Medical University, Tbilisi, Georgia*

[3]*Vladimer Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia*

Abstract

Breast cancer remains a significant global health challenge, with estrogen-dependent subtypes accounting for the majority of postmenopausal cases. Aromatase inhibitors (AIs) are central to current therapeutic strategies, yet synthetic AIs are associated with adverse effects and resistance, necessitating the search for safer alternatives. This study investigates the in vitro aromatase inhibitory activity of crude extracts and isolated polyphenolic compounds from grapevine (Vitis vinifera L.) by-products, specifically seeds and shoots from various Georgian cultivars. Polyphenol-enriched extracts were obtained using ultrasound-assisted extraction, and individual compounds were isolated via chromatographic techniques and identified by nuclear magnetic resonance spectroscopy. Aromatase inhibition was assessed using a cell-free fluorometric assay. Results demonstrated that seed extracts, particularly from the Kisi and Saperavi cultivars, exhibited the highest inhibitory activity (up to 41.9% inhibition; $IC_{50} \approx 452$ µg/mL), while shoot extracts showed comparatively lower activity. Among isolated compounds, resveratrol displayed the most potent inhibition (43.0%), followed by kaempferol (26.1%), whereas quercetin showed lower but measurable activity under the applied assay conditions. These findings highlight grape seeds as a promising source of natural aromatase inhibitors, with resveratrol contributing significantly to the observed activity. The study supports valorization of viticultural by-products as potential agents for prevention and adjunctive treatment of estrogen-dependent breast cancer, warranting further *in vivo* and mechanistic investigations.

Keywords: Breast cancer, Aromatase inhibition, cell-free assay, Grape by-products, Polyphenols

Full length article **Corresponding Author, e-mail: m.tatanashvili@tsmu.edu, Doi # https://doi.org/10.62877/1-IJCBS-25-28-22-1,*
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1. Introduction

Breast cancer remains a major global health problem due to its persistently high incidence and mortality rates across diverse populations. According to the International Agency for Research on Cancer (IARC), approximately 1 in 20 women worldwide has been diagnosed with breast cancer, and 1 in 70 dies from the disease. These statistics underscore the urgent need for improved prevention, early detection, and therapeutic strategies. If current epidemiological trends persist, projections indicate that by 2050, there will be an estimated 3.2 million new cases of breast cancer annually, resulting in approximately 1.1 million deaths among women each year [1]. Such alarming figures highlight global burden of breast cancer and the necessity for ongoing research into its molecular mechanisms and treatment modalities. Breast cancer is a heterogeneous disease, classified into three major molecular subtypes based on the presence or absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (ERBB2; formerly HER2): hormone receptor-positive (ER+ and/or PR+), ERBB2-positive, and triple-negative breast cancer [2]. This molecular classification is clinically significant, as it guides therapeutic decision-making and prognostic assessment. Notably, approximately 80% of postmenopausal breast cancer cases are estrogen receptor positive (ER+), indicating

that tumor progression in this subtype is strongly influenced by estrogen concentration in body [2-5].

The predominance of ER+ tumors in postmenopausal women emphasizes the importance of hormonal regulation in breast cancer pathogenesis. Estrogens play essential physiological role in regulating normal cell growth, differentiation, and homeostasis in mammary glands, ovaries, and uterine tissues. These steroid hormones are involved in a variety of cellular processes, including proliferation, apoptosis, and tissue remodeling. However, abnormal estrogen signaling, whether due to excessive production, altered receptor activity, or dysregulated downstream pathways, can promote tumor initiation, progression, and metastasis [6-7]. The dual nature of estrogen as both a vital physiological regulator and a potential oncogenic factor necessitates careful modulation in therapeutic contexts. There are two main strategies to control the pathological activity of estrogens in breast cancer: (1) direct targeting of the estrogen receptor, which has led to the development of antagonists such as tamoxifen, and (2) inhibition of aromatase (CYP19), key cytochrome P450 enzyme that catalyzes conversion of androgens to estrogens through aromatization of steroid A-ring [2-8-10]. Aromatase inhibition is particularly effective in postmenopausal women, where peripheral tissues become primary source of estrogen biosynthesis. By reducing local estrogen production in breast tissue, aromatase inhibitors lower risk of estrogen-dependent breast cancer development and recurrence [7-11]. Third-generation nonsteroidal aromatase inhibitors (AIs), such as anastrozole and letrozole, have demonstrated higher efficacy and fewer side effects compared to tamoxifen in the treatment of both early- and advanced-stage breast cancer.

These agents have become standard-of-care for hormone receptor-positive breast cancer, especially in postmenopausal patients. Nonetheless, clinical use of synthetic AIs is associated with several challenges, including possible inhibition of other P450 enzymes, development of resistance during prolonged treatment, and notable side effects such as musculoskeletal disorders, osteoporosis, hot flashes, and insomnia. These adverse effects can significantly impact patient quality of life and adherence to therapy. Furthermore, emergence of resistance mechanisms, such as upregulation of alternative estrogen biosynthetic pathways or mutations in the aromatase enzyme, underscores the need for ongoing research into novel inhibitors with improved pharmacological and toxicological profiles [7-12-16]. Natural products have gained considerable attention as potential sources of novel anticancer agents due to their structural diversity, pleiotropic biological activities, and generally lower toxicity compared to synthetic drugs [17-18]. The exploration of plant-derived compounds has led to the successful introduction of several agents into breast cancer therapy, including vinca alkaloids (vinblastine, vincristine), taxanes (docetaxel, paclitaxel), colchicine alkaloids, and podophyllotoxin derivatives, as well as topotecan, irinotecan, and etoposide [19-21]. Among bioactive plant metabolites, phenolic compounds—including flavonoids, stilbenes, and phenolic acids—are of particular interest due to their antioxidant, anti-inflammatory, cardioprotective, and anticancer properties [19-27].

Importantly, some polyphenols and phytoestrogens have been shown to inhibit aromatase activity, thereby offering a promising mechanism for prevention or the treatment of hormone-dependent breast cancer [28-35]. The multitarget effects of these compounds, including modulation of cell signaling pathways, induction of apoptosis, and inhibition of angiogenesis, further enhance their therapeutic potential. Grapevine (Vitis vinifera L.) is a rich source of polyphenolic compounds, not only in grapes and wine but also in by-products generated during cultivation and processing, such as seeds, skins, stems, leaves, and shoots. The valorization of such viticultural by-products aligns with both environmental sustainability and the search for novel bioactive agents [36-42]. By utilizing these residues, researchers can contribute to waste reduction and resource optimization while simultaneously identifying new compounds with therapeutic potential. In this context, the present study aimed to investigate the in vitro aromatase inhibitory activity of crude extracts obtained from grapevine shoots and seeds, as well as individual compounds isolated from these extracts. By exploring these viticultural residues and their bioactive constituents as potential sources of natural aromatase inhibitors, this work contributes to the ongoing search for safer and more effective agents for the prevention and treatment of estrogen-dependent breast cancer. The findings of this research may inform future studies on the development of nutraceuticals, adjuvant therapies, and environmentally sustainable approaches to cancer prevention and management.

2. Materials and Methods

2.1. General Material

Analytical-grade solvents, including methanol, ethanol, ethyl acetate, dimethyl sulfoxide (DMSO) and chloroform, were obtained from Sigma-Aldrich (Germany). Ultrapure water was prepared using a Milli-Q purification system (Millipore, Bedford, USA). Chromatographic materials included silica gel 60 F_{254} (particle size 0.04–0.063 mm; Merck, Germany) and Diaion HP-20 resin (Mitsubishi Chemical, Japan). Extraction procedures were performed using an ultrasonic water bath (Runyes Clean-02, Spain). The structure elucidation of isolated compounds was conducted using nuclear magnetic resonance (NMR) spectroscopy on a Bruker Avance NEO 500 MHz spectrometer (Bruker, Germany). The fluorometric Aromatase (CYP19A) Inhibitor Screening Kit (ab284522) was purchased from Abcam (Cambridge, UK). Measurements of fluorescence intensity were performed using BioTek Synergy HTX multifunctional microplate reader (BioTek Instruments, USA) and 96-well flat-bottom microplates (Sigma-Aldrich, Germany). Sample incubation during the enzymatic assay was carried out using a PST-100HL Biosan plate shaker-thermostat (Riga, Latvia).

2.2. Plant material

Grapevine by-products were collected in 2025 in the Sagarejo district, Kakheti region (N 41°44′00″, E 45°20′00″). Shoots were harvested in July at the end of the flowering phase, while seeds were obtained in October, three weeks after fermentation of the pressed grapes**.**

- Seeds from three grapevine varieties: *Saperavi, Rkatsiteli,* and *Kisi.*
- Shoots from five grapevine varieties: *Saperavi, Rkatsiteli, Kisi, Khikhvi,* and *Mtsvane*.

Drying was performed under mild conditions: seeds were air-dried at temperatures not exceeding 40-45 °C to preserve thermolabile compounds, whereas shoots were dried

naturally in a well-ventilated environment at ambient temperature (20-25 °C).

2.3. Extraction and Isolation

To obtain polyphenol-enriched extracts, an ultrasound-assisted extraction (UAE) methodology was employed, following a comprehensive preliminary study aimed at optimizing the extraction parameters for maximum yield and preservation of bioactive compounds [43]. UAE is recognized for its efficiency in disrupting plant cell walls, thereby facilitating the release of intracellular polyphenols into the solvent medium. This technique utilizes ultrasonic waves to generate cavitation bubbles, which, upon collapse, produce localized high temperatures and pressures, enhancing mass transfer and extraction efficiency. The final extraction protocol was meticulously developed to ensure reproducibility and optimal recovery of polyphenolic constituents. The protocol included the following parameters:

- Solvent: 70% ethanol (v/v) was selected as the extraction solvent due to its proven efficacy in solubilizing a broad spectrum of polyphenols while maintaining food-grade safety and minimizing toxicity.
- Particle Size: Plant material was milled to a particle size of 0.3–0.5 mm for seeds and 2.5–3.0 mm for shoots. This size reduction increases the surface area available for solvent penetration, thereby improving extraction kinetics and yield.
- Solvent-to-Material Ratio: A ratio of 20:1 (v/w) was employed, ensuring sufficient solvent volume to fully immerse the plant matrix and facilitate efficient extraction of target compounds.
- Extraction Conditions: The extraction was conducted at 60°C for 30 minutes, a temperature and duration chosen to balance the enhanced solubility and diffusion rates of polyphenols with the need to avoid thermal degradation of sensitive compounds.
- Extraction Cycle: A double extraction cycle was implemented, wherein the plant material was subjected to two consecutive extraction steps. This approach was adopted to maximize the recovery of polyphenols, as residual compounds may remain after the initial cycle.

This optimized method was selected based on its demonstrated efficiency in extracting polyphenolic compounds from plant matrix, as confirmed by preliminary analytical assessments. The use of UAE, combined with carefully controlled parameters, resulted in extracts with high concentrations of bioactive polyphenols, suitable for subsequent fractionation and analysis. Following extraction, fractionation of research material and subsequent isolation of individual constituents were performed using advanced column chromatography techniques. Diaion HP-20 resin (Mitsubishi Chemical, Japan) was employed as the stationary phase due to its high affinity for phenolic compounds and its capacity for efficient separation based on polarity. Gradient elution was carried out using water–methanol mixtures in varying proportions (100:0, 50:50, and 0:100 v/v), as well as pure ethyl acetate as mobile phases. This gradient system enabled the sequential elution of compounds with differing polarities, facilitating the isolation of distinct polyphenolic fractions. For further purification of obtained fractions, silica gel 60 $F_{(254)}$ (particle size 0.04–0.063 mm; Merck, Germany) utilized as stationary phase in a secondary chromatographic step. Elution was achieved using a series of chloroform–methanol–water solvent systems with varying ratios: 60:10:1, 50:10:1, 45:12:1, 40:12:1, 30:12:2, 40:10:1, 26:12:1, and 30:12:1 (v/v/v).

This stepwise gradient was specifically designed to facilitate the separation of target compounds into highly purified forms, suitable for further structural characterization and bioactivity assessment. Comprehensive extracts were obtained from all categories and varieties of raw materials, ensuring a broad representation of the polyphenolic profile present in grapevine by-products. In contrast, specific compounds were selectively isolated from the seeds and shoots of the Vitis vinifera cultivar Saperavi, chosen for its known richness in bioactive phenolics. The isolation of these compounds enabled detailed investigation of their individual contributions to overall bioactivity of the extracts. Structural elucidation of isolated compounds was performed using nuclear magnetic resonance (NMR) spectroscopy, a powerful analytical technique that provides detailed information on molecular structure, functional groups, and stereochemistry. NMR analysis was conducted under optimized conditions to ensure high-resolution spectra and accurate identification of isolated substances. The combination of advanced extraction, fractionation, and analytical techniques employed in this study underscores the rigorous approach taken to characterize grapevine-derived polyphenols and assess their potential as natural aromatase inhibitors.

2.4. Identification of Isolated Substances

The structures of isolated individual substances were determined using a 298 K, 500 MHz nuclear magnetic resonance spectroscope equipped with a cryogenic probe. Solutions of the analysis samples were prepared with deuterated methanol. The scanning protocol was meticulously optimized to ensure thorough acquisition of both proton (^{1}H) and carbon (^{13}C) spectra, as well as multidimensional correlation spectra required for comprehensive structural characterization. Details of the scanning parameters for each nucleus and technique are provided in Table №1. Typically, the ^{1}H spectra were recorded with 128 scans to achieve a robust signal-to-noise ratio, while the ^{13}C spectra required 5120 scans due to the lower sensitivity and natural abundance of carbon nuclei. Additionally, two-dimensional NMR experiments—including COSY (Correlation Spectroscopy), HMBC (Heteronuclear Multiple Bond Correlation), and HSQC (Heteronuclear Single Quantum Coherence)—were conducted to reveal proton-proton and proton-carbon connectivities, which are critical for constructing the molecular framework and identifying functional groups. This comprehensive NMR approach enabled accurate determination of the chemical structures of the isolated substances, including identification of key structural motifs, stereochemistry, and substitution patterns. The integration of high-field instrumentation, cryogenic technology, and advanced multidimensional techniques ensured reliable characterization, even for minor components and complex mixtures. Such detailed structural insights are vital for linking the chemical identity of these compounds to their biological activities and for advancing our understanding of their functional roles.

2.5. Aromatase Inhibition Assay

Aromatase (CYP19A) inhibitory activity of crude extracts and selected individual compounds was determined *in vitro* (direct, cell-free) using a fluorometric Aromatase (CYP19A) Inhibitor Screening Kit (ab284522; Abcam, Cambridge, UK). The assay is based on the conversion of a fluorogenic substrate by recombinant human aromatase, producing a fluorescent metabolite (Ex/Em = 488/527 nm). Prior to the experiments, all reagents supplied with the kit were prepared according to the manufacturer's protocol. Letrozole served as the standard positive inhibitor, while vehicle controls were matched to the solvent used for each sample: acetonitrile (ACN) for letrozole and dimethyl sulfoxide (DMSO) for extracts and isolated compounds. Dry extracts and isolated compounds did not fully dissolve in ACN and were therefore prepared in DMSO. The final DMSO concentration in assay wells did not exceed 0.1%, as concentrations above 0.25% are known to interfere with aromatase activity based on the manufacturer's instructions. Fluorescence calibration curves were constructed using Fluorescence Standard IV. Background controls lacking substrate were included to correct for autofluorescence.

Stock solutions of crude grape seed and shoot extracts (prepared from Saperavi, Rkatsiteli, Kisi, Mtsvane, and Khikhvi cultivars) and individual compounds (kaempferol, resveratrol, quercetin) prepared in and dimethyl sulfoxide (DMSO) and diluted in assay buffer to obtain multiple concentrations. For each sample, concentration–response curves were generated to calculate IC_{50} values. Test plates were assembled in 96-well format with following groups: (i) assay wells containing extracts/compounds, (ii) vehicle controls, (iii) background controls (without substrate), and (iv) positive inhibition controls (letrozole, 1 μM). Following 15 min pre-incubation at 37 °C on a plate shaker (PST 100HL Biosan), substrate/$NADP^+$ mixture was added to initiate the reaction. Fluorescence was measured kinetically for 60 min using a BioTek Synergy HTX microplate reader under controlled conditions (Ex/Em = 488/527 nm, 37 °C, 1-min intervals). Reaction slopes were derived from linear portion of progress curves (25–60 min). Background-corrected values were normalized to vehicle controls. Percent inhibition for each extract or compound was calculated relative to letrozole and expressed as mean ± SD from independent triplicate experiments.

3. Results and discussion

3.1. NMR

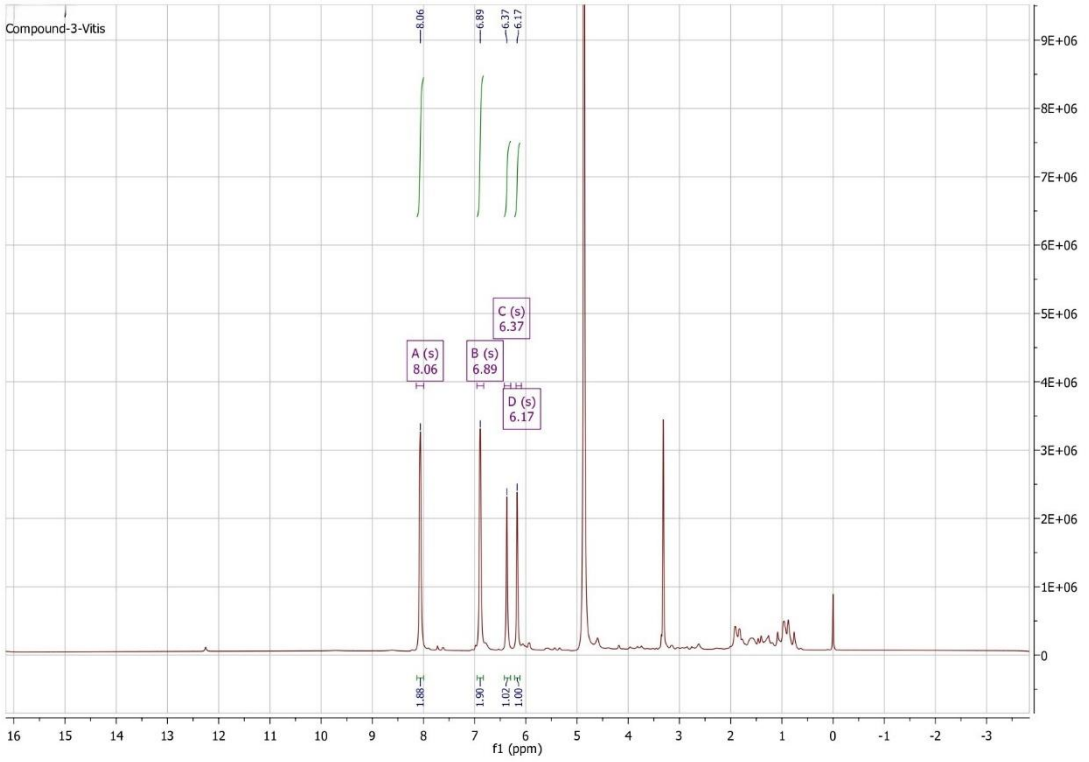
Three individual substances were isolated from the obtained extracts, which were given conditional names – "Substance 1", "Substance 2" and "Substance 3" and analyzed using nuclear magnetic resonance spectroscope. The spectra of individual substance, with the conditional name - substance 1, ^1H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) are given in Figure №1. Based on the comparison of the results obtained by nuclear magnetic resonance and the data available in the literature, individual "Substance 1" was identified as a phenolic compound - Kaempferol ($C_{15}H_{10}O_6$), with a molecular mass of 286.24 g/mol (M/z: 285 $[M-H]^-$).
The spectra of individual substance, with the conditional name - substance 2, ^1H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) are given in Figure №2. Based on the analysis of the obtained results and comparison with literature data, individual substance 2, a light-yellow powder, was found to be trans-3,5,4′-trihydroxystilbene -Resveratrol ($C_{14}H_{12}O_3$), with a molecular weight of 228.24 g/mol (M/z: 227.1 $[M-H]^-$). The spectra of individual substance, with the conditional name - substance 3, ^1H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) are given in Figure №3. Based on the comparison of the results obtained by nuclear magnetic resonance and the data available in the literature, the yellow amorphous powder, designated as individual substance 3, is 3,3′,4′,5,7-pentahydroxyflavone, Quercetin ($C_{15}H_{10}O_7$), with a molecular weight of 302.23 g/mol (M/z: 301.1 $[M-H]^-$).

3.2. Aromatase Inhibition

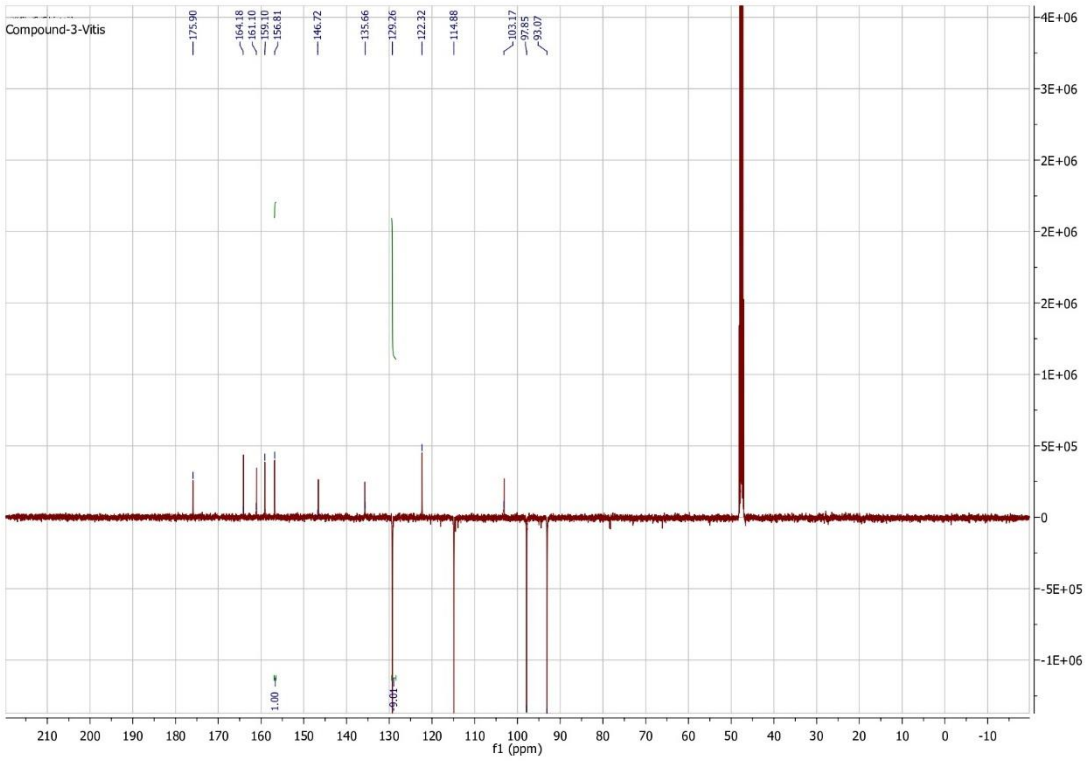
The standard calibration curve of the fluorescence standard demonstrated a linear relationship across the tested range ($R^2 \geq 0.9971$), validating the quantification procedure. Progress curves confirmed that the reactions followed a linear kinetic phase under the applied assay conditions. Letrozole, used as a reference inhibitor, demonstrated potent inhibition of aromatase activity (97.47 ± 7%). Among grape-derived samples, seed extracts generally showed higher inhibitory activity compared to shoot extracts. The Kisi seed extract exhibited the strongest effect within this group (41.90 ± 14% inhibition; IC_{50} = 452 μg/mL), closely followed by Saperavi seed extract (39.51 ± 14%; IC_{50} = 456 μg/mL). Rkatsiteli seed extract displayed comparatively weaker inhibition (23.36 ± 5%; IC_{50} = 527 μg/mL). Shoot extracts showed lower overall activity. Saperavi (17.93 ± 9%; IC_{50} = 646 μg/mL) and Kisi (14.76 ± 6%; IC_{50} = 300 μg/mL) exhibited modest inhibition, whereas Khikhvi shoots displayed the weakest inhibition (7.96 ± 3%; IC_{50} = 1152 μg/mL). Among the tested individual compounds, resveratrol demonstrated the highest inhibitory effect (43.02 ± 3%), followed by kaempferol (26.14 ± 10%). whereas quercetin showed low but measurable inhibition (12.28 ± 5%) under the applied assay conditions. Overall, the findings indicate that grape seed extracts - particularly those from Kisi and Saperavi cultivars - as well as resveratrol contribute significantly to aromatase inhibition, whereas shoot extracts generally display weaker activity.

These results highlight seeds as a more promising source of aromatase-inhibiting phytochemicals compared to other plant parts. Overall, the findings indicate that grape seed extracts - particularly those from Kisi and Saperavi cultivars - as well as resveratrol contribute significantly to aromatase inhibition, whereas shoot extracts generally display weaker activity. These results highlight seeds as a more promising source of aromatase-inhibiting phytochemicals compared to other plant parts. The results of this study offer new proof that grapevine byproducts, especially seeds, are a potential source of naturally occurring aromatase inhibitors. Our findings demonstrate that extracts derived from grape seeds (especially Kisi and Saperavi varieties) exhibited stronger aromatase inhibitory activity than those obtained from shoots, with relative inhibition values approaching 40–42%. This is consistent with previous reports highlighting the high polyphenolic load of grape seeds, notably flavonoids and stilbenes, which are implicated in diverse bioactivities including estrogen-modulating effects [34-40-44-45]. The lower activity of shoot extracts, despite their phenolic richness, may be explained by compositional differences in phenolic subclasses. Seeds are enriched in proanthocyanidins and stilbenes, while shoots contain high levels of phenolic

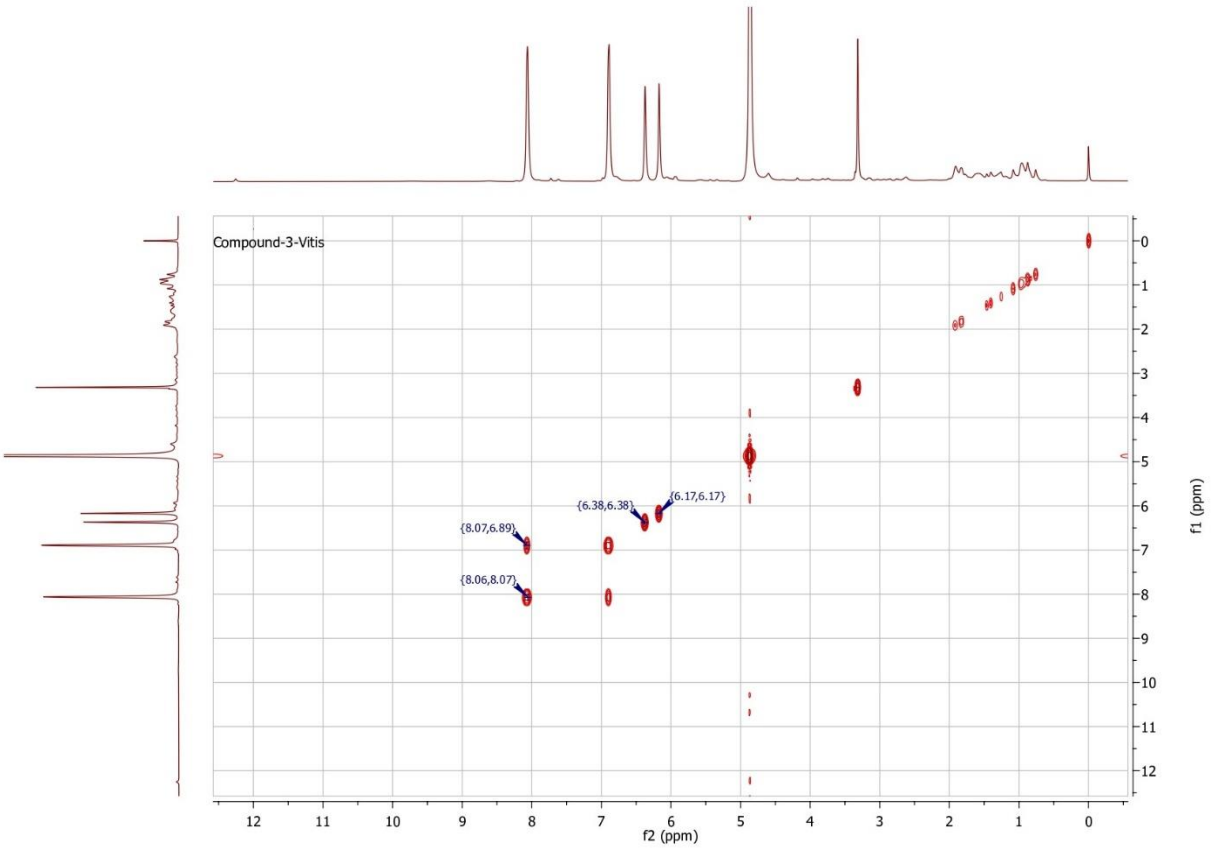
acids and flavonol glycosides, which may have weaker direct interaction with the aromatase active site [41-46].



A



B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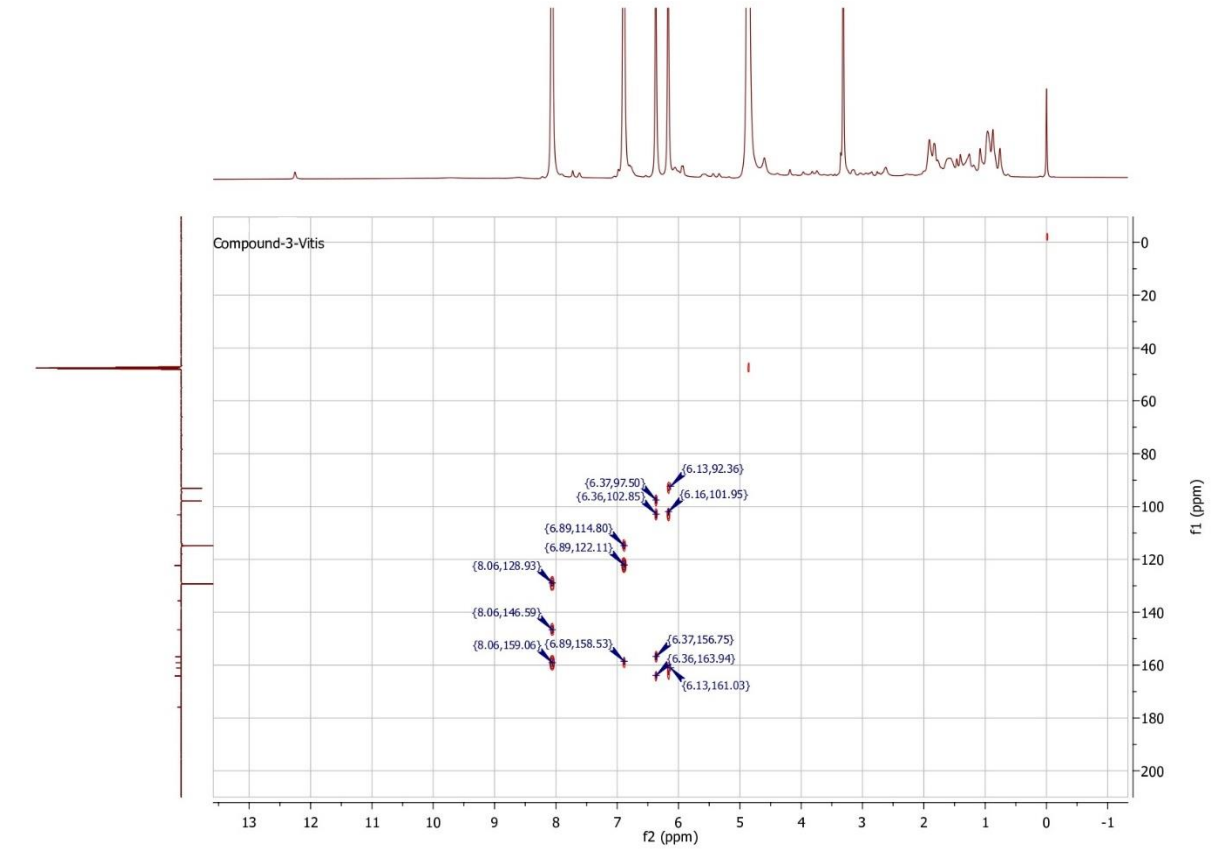
{6.17,6.17}
{8.07,6.89}
{8.06,8.07}
f2 (ppm)
f1 (ppm)

C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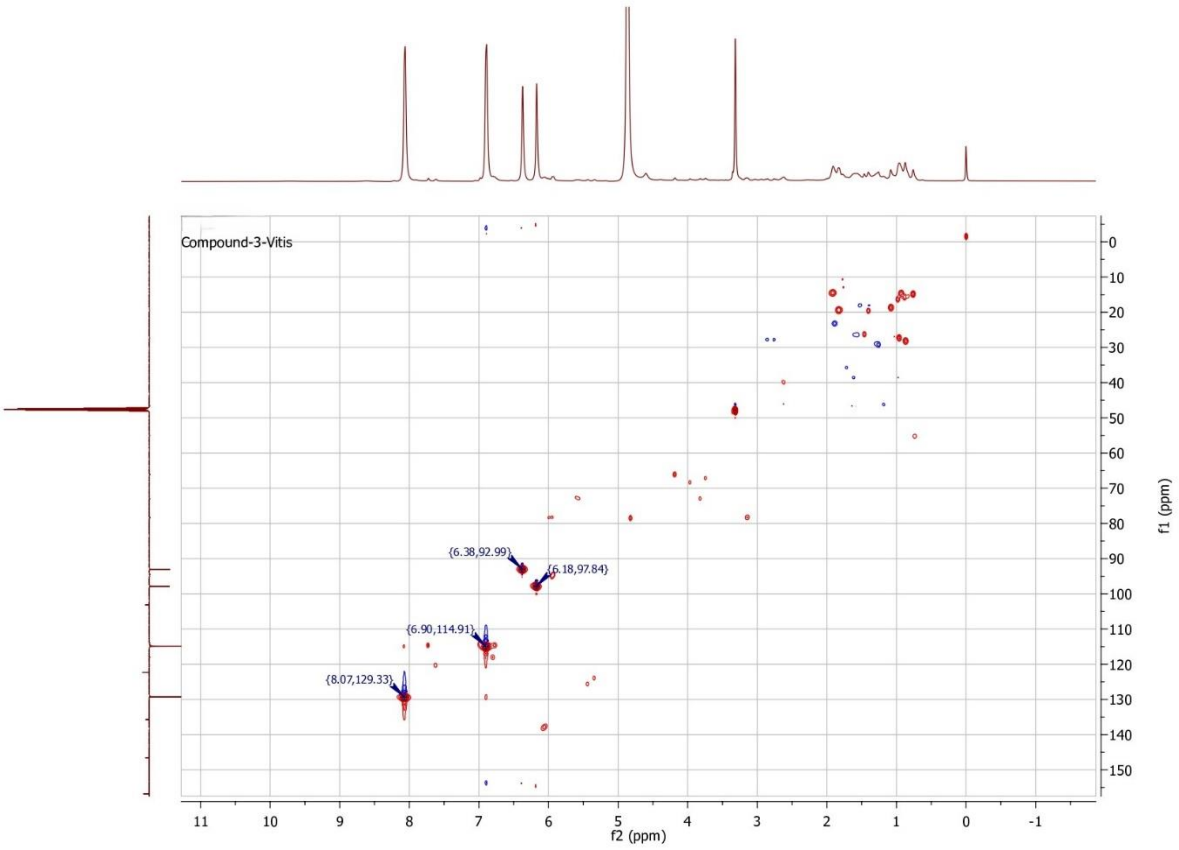
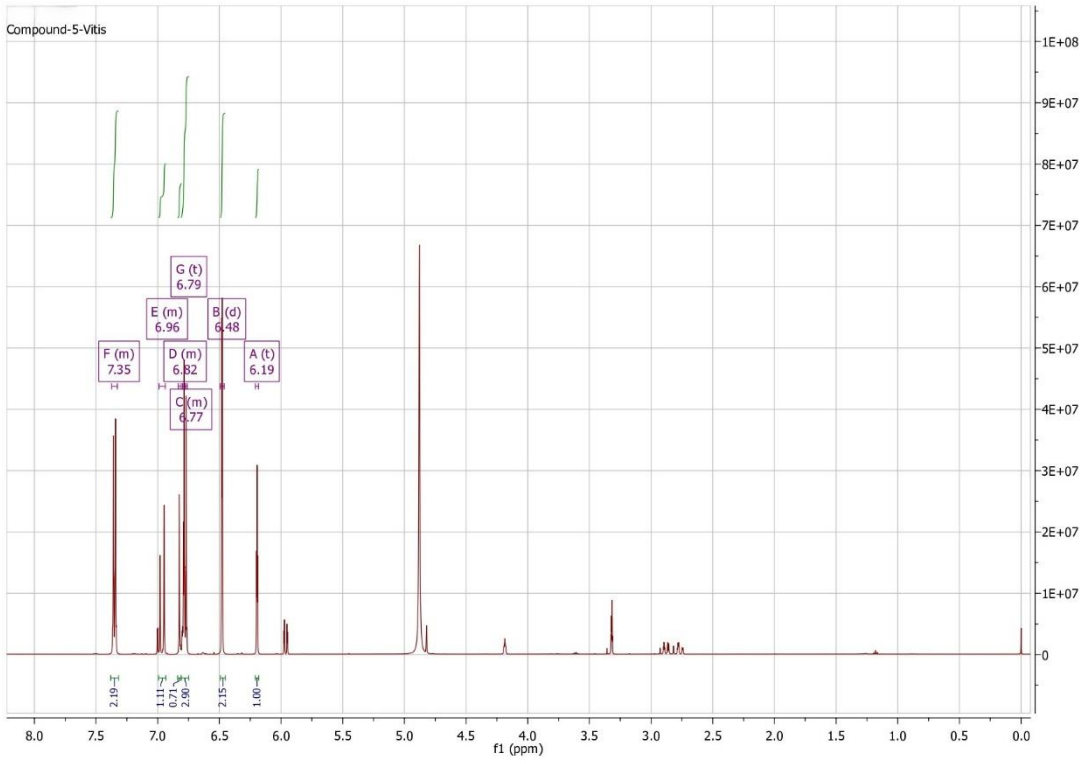
f2 (ppm)
f1 (ppm)

D

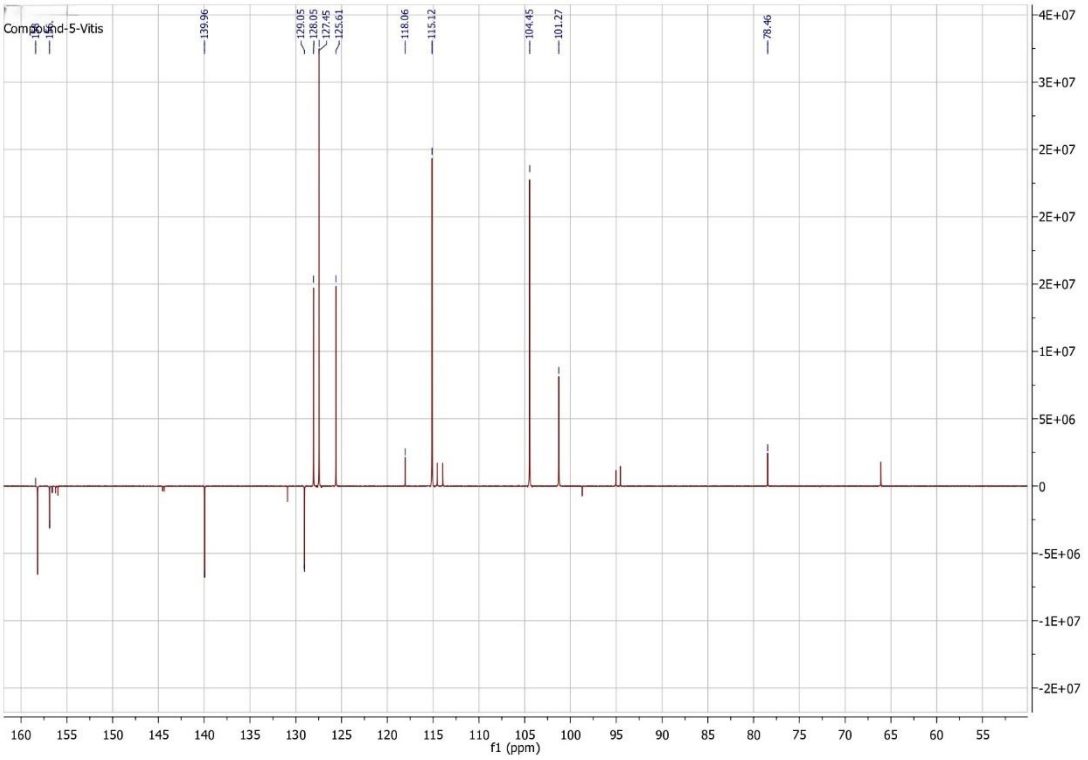


E

Figure 1 – Substance 1 ^{1}H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) spectra



A


Compound-5-Vitis
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128.05
127.45
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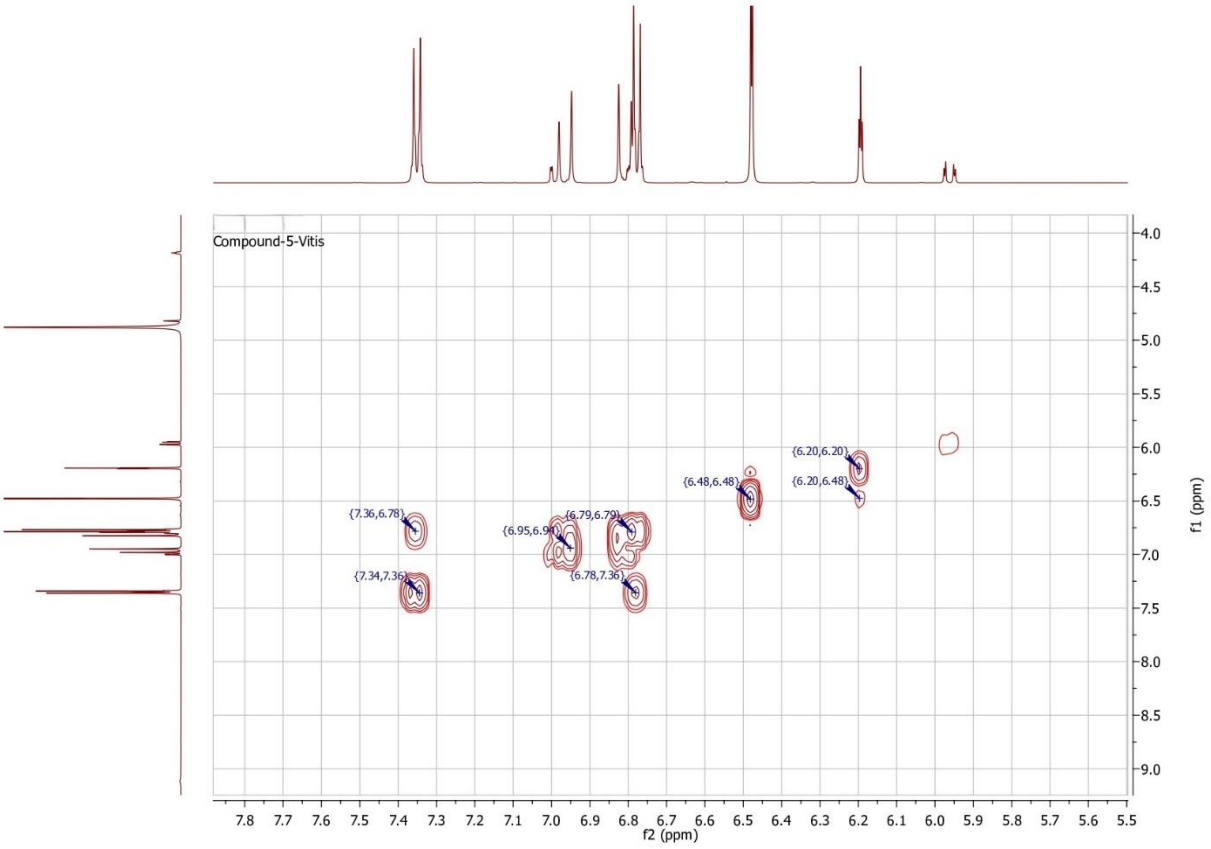
118.06
115.12
104.45
101.27
78.46
f1 (ppm)

B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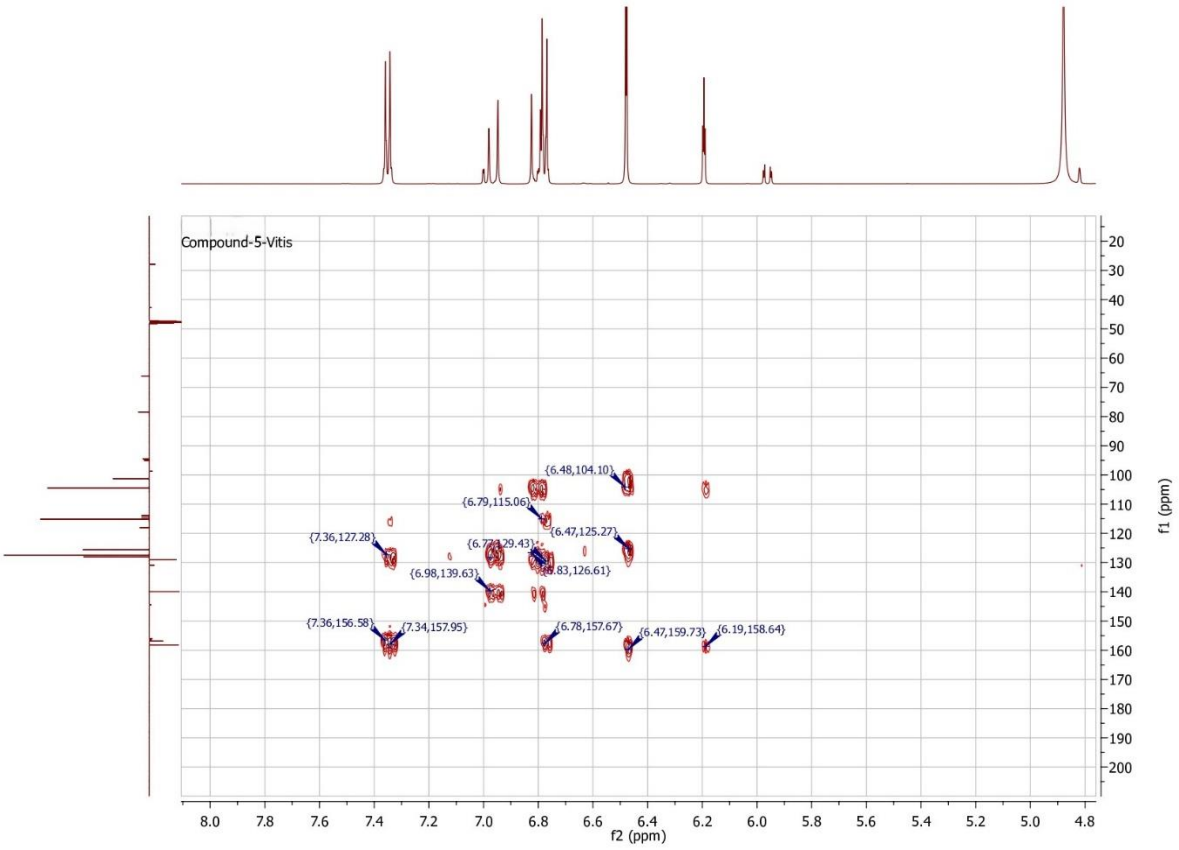
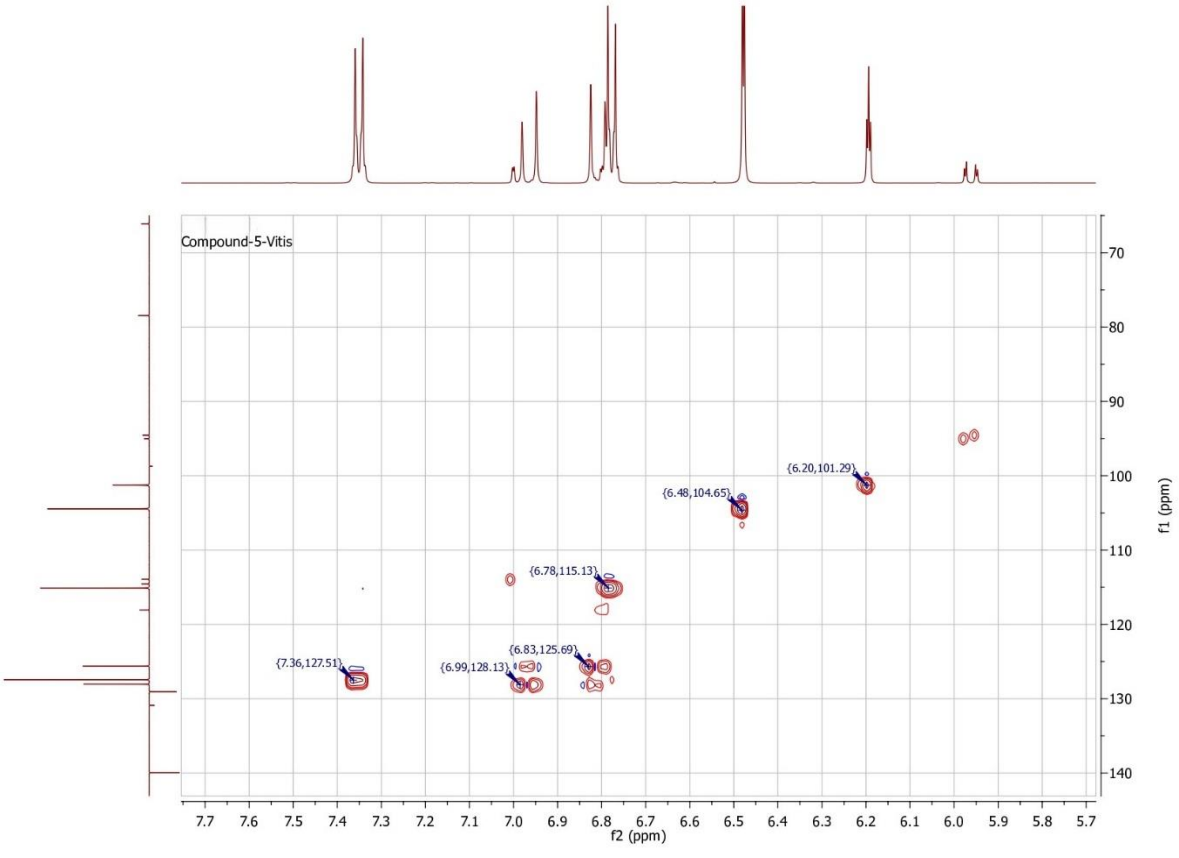
{6.20,6.20}
{6.20,6.48}
f2 (ppm)
f1 (ppm)

C

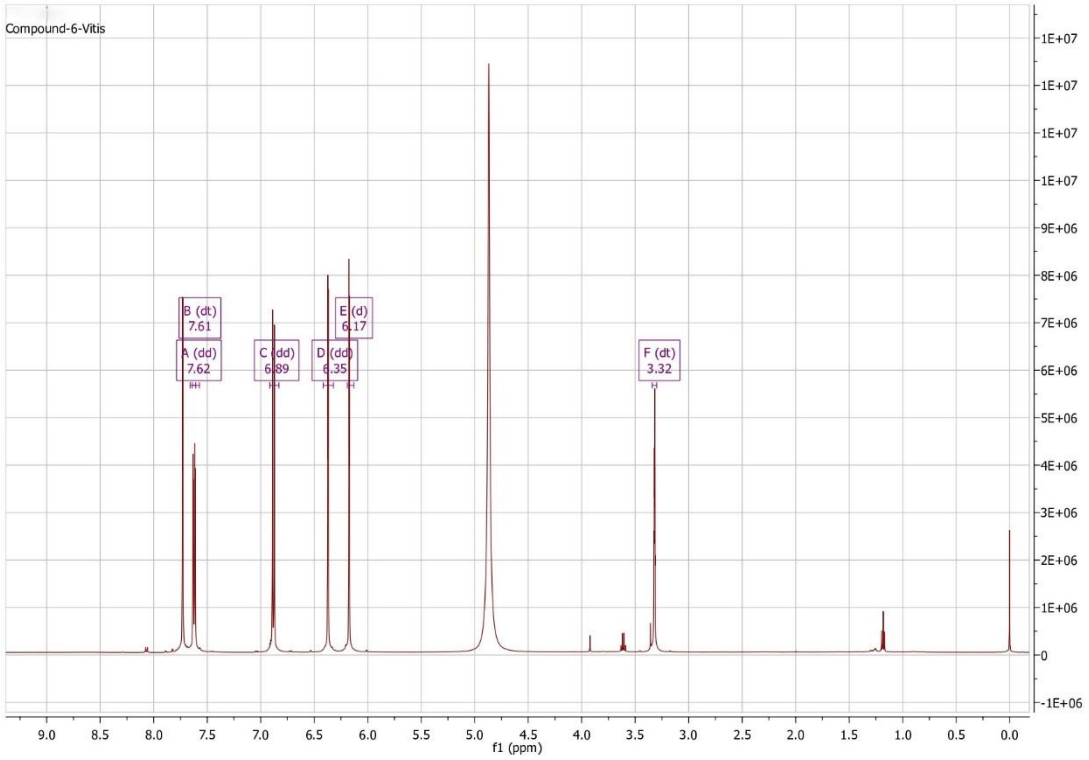


D

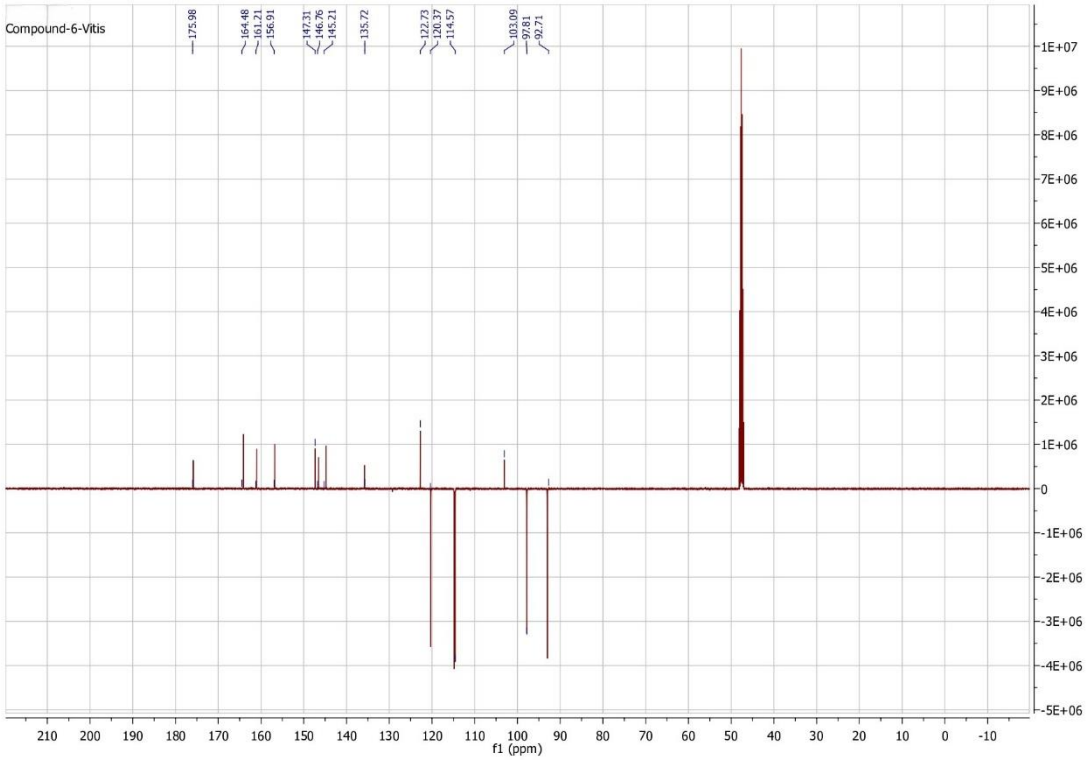


E

Figure 2 – Substance 2 ^{1}H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) spectra


Compound-6-Vitis
B (dt)
7.61
A (dd)
7.62
C (dd)
6.89
D (dd)
6.35
E (d)
6.17
F (dt)
3.32
f1 (ppm)

A


Compound-6-Vitis
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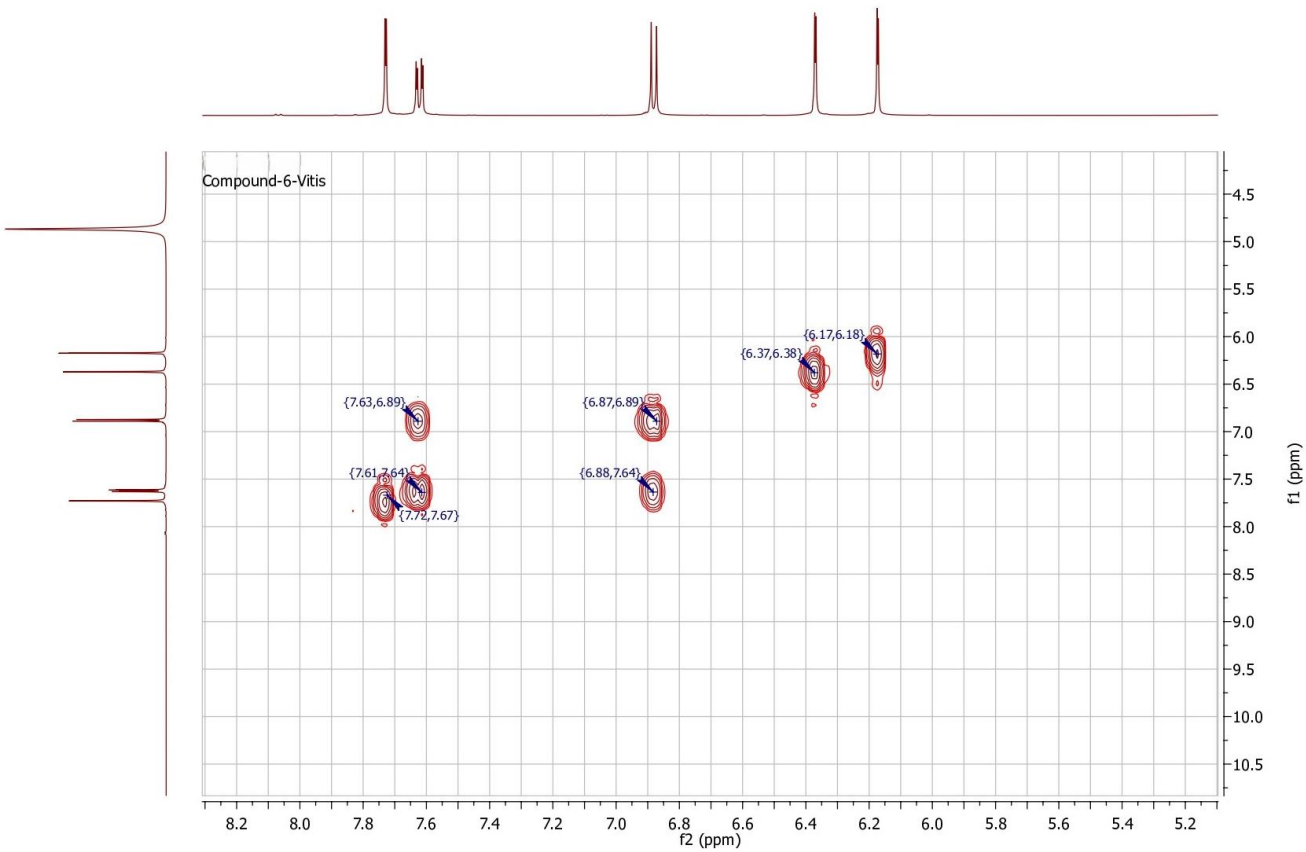
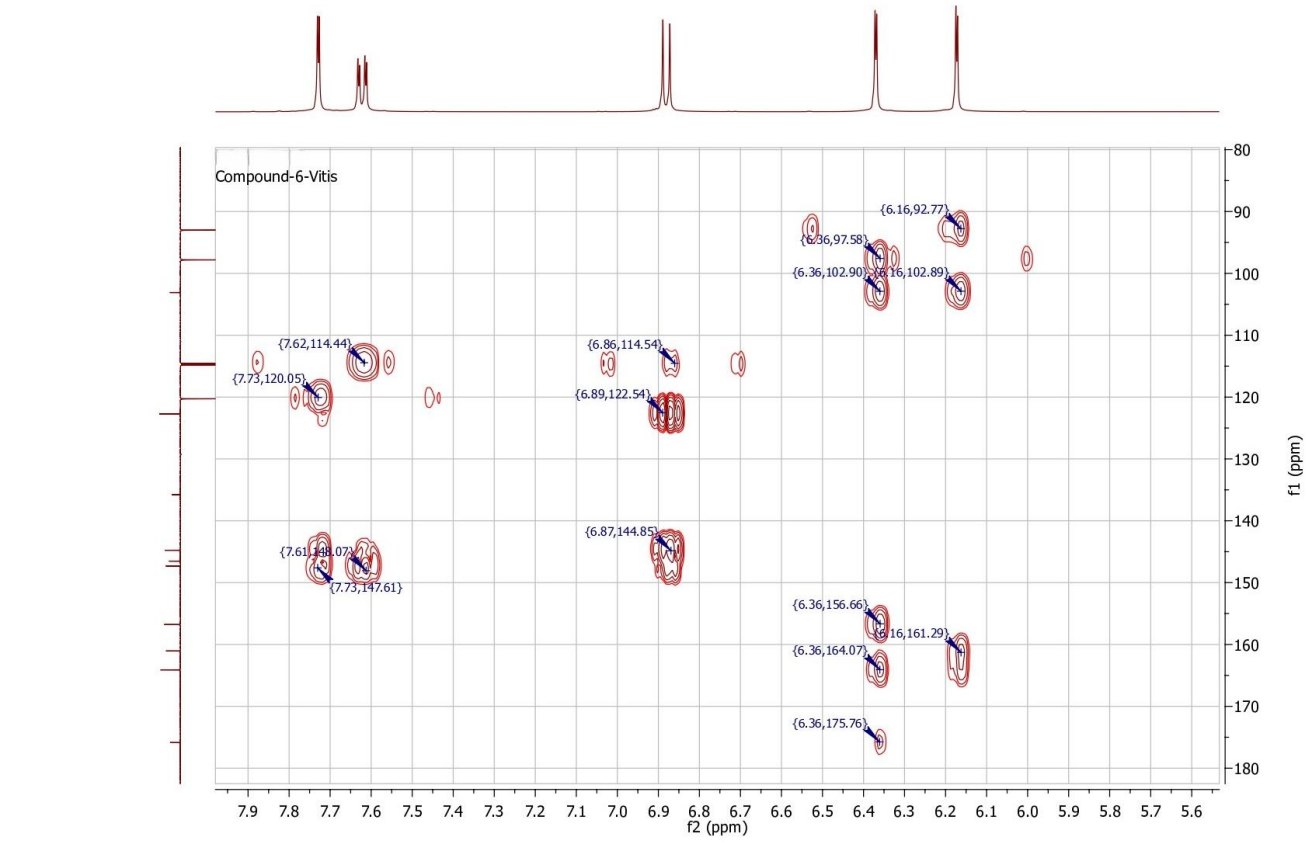
164.48
161.21
156.91
147.31
146.76
145.21
135.72
122.73
120.37
114.57
103.09
97.81
92.71
f1 (ppm)

B


Compound-6-Vitis
{6.17,6.18}
{6.37,6.38}
{7.63,6.89}
{6.87,6.89}
{7.61,7.64}
{6.88,7.64}
{7.72,7.67}
f2 (ppm)
f1 (ppm)

C


Compound-6-Vitis
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{6.16,102.89}
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{6.86,114.54}
{7.73,120.05}
{6.89,122.54}
{6.87,144.85}
{7.61,148.07}
{7.73,147.61}
{6.36,156.66}
{6.16,161.29}
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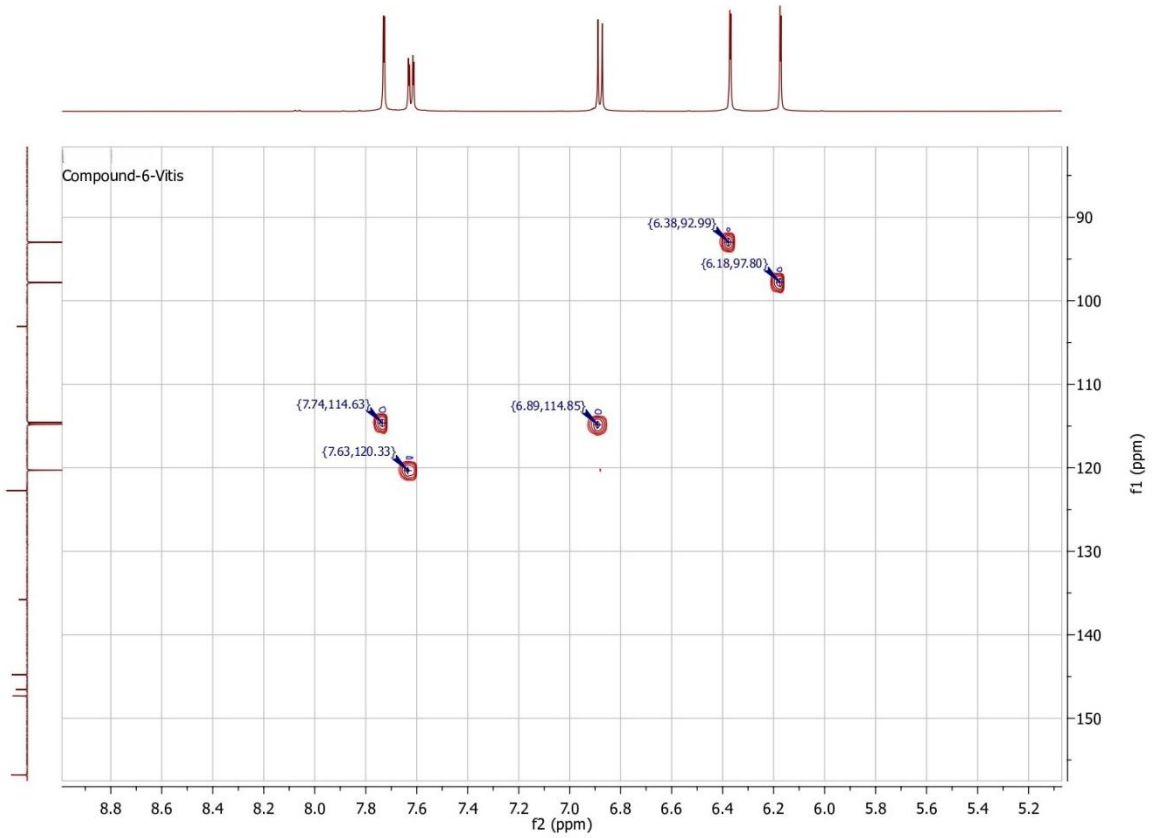
{6.36,175.76}
f2 (ppm)
f1 (ppm)

D



E

Figure 3 – Substance 3 ^{1}H (A), ^{13}C (B), COSY (C), HMBC (D), HSQC (E) spectra

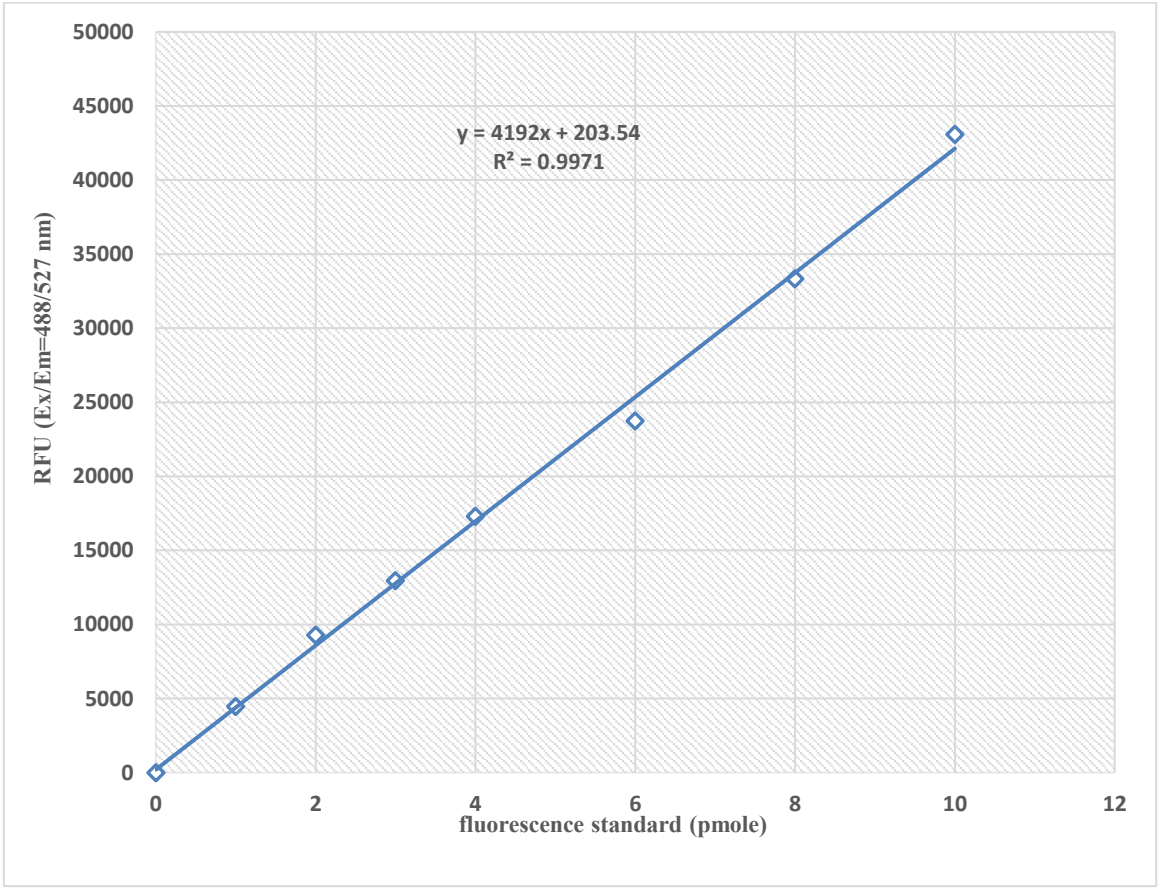


Figure 4 - The standard calibration curve of the fluorescence standard

Table 1: Nuclear magnetic resonance spectroscopy scanning parameters

^{1}H Proton spectrum	128 scans
^{13}C Carbon spectrum	5120 scans
Correlation spectroscopy (COSY)	4 scans
Heteronuclear multiple bonds correlation (HMBC)	16 scans
Heteronuclear single-quantum correlation (HSQC)	8 scans

Table 2: Aromatase inhibitory activity of total extracts and individual compounds derived from grape raw materials

Analysis sample	%Relative inhibition	IC_{50} (µg/mL)
Positive control (letrozole)	97.47±7	-
Extract of Saperavi seeds	39.51±14	456 ± 8
Extract of Rkatsiteli seeds	23.36±5	527 ± 9
Extract of Kisi seeds	41.90±14	452 ± 8
Extract of Saperavi shoots	17.93±9	646 ± 8
Extract of Rkatsiteli shoots	15.21±2	712±5
Extract of Kisi shoots	14.76±6	300 ± 5
Extract of Mtsvane shoots	12.78±11	686 ± 12
Extract of Khikhvi shoots	7.96±3	1152 ± 10
Kaempferol	26.14±10	>60
Resveratrol	43.02±3	>30
Quercetin	12.28±5	>41

These structural considerations align with earlier studies showing that planar polyphenols with multiple hydroxyl groups, such as resveratrol and certain flavonols, display stronger enzyme binding through hydrogen bonding and hydrophobic interactions [28-34-47]. Among the individual compounds isolated, resveratrol exhibited the most potent inhibitory effect (43.02%), consistent with its well-documented role as a modulator of estrogen metabolism and aromatase inhibition [32-35]. Kaempferol showed moderate inhibition (26.14%), which supports previous evidence that flavonols may act as competitive inhibitors of CYP19 [29-34-47]. Quercetin exhibited low but measurable inhibitory activity under the applied assay conditions, which is consistent with previous reports describing its moderate aromatase inhibition potential [33-48]. The relatively weak effect observed in our study may reflect concentration-dependent behavior, partial instability in the assay medium, or limited interaction with the enzyme in a cell-free system. Further optimization of assay parameters - such as solvent composition and incubation time - could provide additional insight into quercetin's inhibitory dynamics.

Comparison with synthetic inhibitors such as letrozole highlights the relatively modest potency of the tested natural extracts and compounds (IC_{50} in the hundreds of µg/mL range versus nanomolar activity for letrozole). Nevertheless, the advantage of natural inhibitors lies in their multitarget biological effects, lower systemic toxicity, and potential for combination therapy [29-31]. The observed moderate inhibition suggests that grape-derived polyphenols may not serve as individual therapeutic agents but could complement existing endocrine therapies as adjuvants or preventive nutraceuticals. Taken together, our results support the hypothesis that grapevine by-products represent a valuable, underutilized source of bioactive molecules with aromatase-inhibiting potential. The higher activity of seed-derived extracts and the strong effect of resveratrol underline the importance of variety selection and compound-specific contributions. Further *in vivo* studies and mechanistic analyses are required to confirm clinical relevance of these findings, particularly concerning bioavailability, metabolism, and synergistic interactions among polyphenolic constituents.

4. Conclusion

This study provides compelling evidence that grapevine by-products, particularly seeds from the Kisi and Saperavi cultivars, are a promising natural source of aromatase inhibitors. The extracts derived from grape seeds demonstrated significantly stronger aromatase inhibitory activity compared to those obtained from shoots, with relative inhibition values approaching 40–42%. Among the isolated compounds, resveratrol exhibited the most potent inhibitory effect, followed by kaempferol, while quercetin displayed low but measurable activity under the experimental conditions. The lower activity observed in shoot extracts may be attributed to differences in phenolic composition, with seeds being richer in proanthocyanidins and stilbenes. Although the potency of these natural extracts and compounds is modest compared to synthetic inhibitors such as letrozole, their multitarget biological effects, lower systemic toxicity, and potential for use as adjuvants or nutraceuticals in endocrine therapy are notable advantages. Overall, the findings support the valorization of grapevine by-products as a valuable and underutilized source of bioactive molecules with aromatase-inhibiting potential. Further in vivo studies and mechanistic analyses are warranted to confirm the clinical relevance of these results, particularly regarding bioavailability, metabolism, and synergistic interactions among polyphenolic constituents.

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Conflicts of interest

The authors report no financial or any other conflicts of interest in this work.

Ethical approvals

This study does not involve experiments on animals or human subjects.

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