Supercritical Carbon Dioxide Extracts of *Schinus terebinthifolia* Fruits and their Utilization against Microbial Illness, Lipase, and Butyrylcholinesterase Activities *in Vitro*

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The extraction methods used to obtain natural products face some problems, such as solvent toxicity, high extraction time, and low yields. Supercritical carbon dioxide fluid extraction (SFE-CO_2) is an encouraging extraction system for obtaining high-yield of natural extracts. In this work, *Schinus terebinthifolia* fruits were extracted *via* SFE-CO_2 using two conditions: A (static extraction) (SE) for 15 min, followed by dynamic extraction (DE) for 45 min, and B (without SE but with DE for 60 min). The extract yield was 0.205 g and 0.236 g *via* condition A and B, respectively. High-performance liquid chromatography assessment revealed the occurrence of several constituents with high quantities in the extract at condition B. The well diffusion test showed inhibition of 26 ± 0.1, 25 ± 0.2, 29 ± 0.1, 33 ± 0.2, 27 ± 0.1, and 8.0 ± 0.1 mm zones using the extract at condition B, while at condition A there were low inhibition zones towards *Staphylococcus aureus*, *Pseudomonas areginosa*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*, correspondingly. Lipase (obesity stimulant) and butyrylcholinesterase (Alzheimer stimulant) were inhibited by the extract at condition B with IC_{50} quantities of 27.03 and 4.83 µg/mL, while it was 37.45 and 17.57 µg/mL, respectively at condition A.

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INTRODUCTION

Natural plant products display large roles in medicinal, pharmaceutical, agricultural, and food industry applications for several purposes (Abdelghany 2014; Asaduzzaman *et al*. 2014; Lopa *et al*. 2021; Al-Rajhi *et al*. 2023a; Mashraqi *et al*. 2023; Sahiner *et al*. 2023). A species of the genus *Schinus*, *Schinus terebinthifolia* is intrinsic to Africa, and Central America, besides South America. It is a member of the Anacardiaceae family. It is a decorative tree found in Egypt, where it is prized for its tasty fruits and attractive foliage. This plant is widely used as a common spice as well as for coloring, tanning, and decorating purposes (da Silva Dannenberg *et al.* 2016). Diverse parts of *S. terebinthifolia*, such as leaves and roots, are employed in traditional drugs to manage a variety of sicknesses, including cancer, arthritis, skin issues, diarrhea ulcers,

gastroduodenal illnesses, hypertension, urinary and respiratory issues, and mucous membrane injuries. Furthermore, it has been found to inhibit the progress of microorganisms, and inflammation, besides oxidative stress. It is also traditionally employed in the management of endometriosis in addition to sexually transmitted diseases (Dos Santos *et al.* 2015).

Sarjit *et al*. (2015) documented that fruit extract of *S. terebinthifolia* displayed inhibitory potentials on *C. albicans, S. aureus*, and *E. coli* growth, in addition to the fact that its activity to prevent microbial growth may be related to the occurrence of phenolic constituents, particularly separated phenols such as ellagic and gallic acids, which have earlier been described to have antimicrobial action. Toxicity of *S. terebinthifolia* was reported against *Escherichia coli*, *Acinetobacter baumannii*, *Micrococcus flavus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Sarcina lutea* (Salem *et al.* 2018). Growth and biofilm of various species of *Candida* containing *C. krusei*, *C. albicans*, *C. glabrata*, and *C. dubliniensis* were inhibited significantly by *S. terebinthifolia* extract (de Jesus Viegas *et al.* 2020).

According to Alqathama *et al*. (2023), a molecule, 4′-methoxy-6-vinyl-7-O-rhamnosyl-ampelopsin, was isolated for the first time from *S. terebinthifolia* fruit, in addition to other compounds including afzelin, gallic acid, quercetin 3-O-β-D-glucopyranoside, ellagic acid, quercetin, kaempferol, and genistein 7-O-α-L-rhamnopyranoside. The extract containing these compounds showed notable antimicrobial, cytotoxic, antioxidant, and anti-inflammatory qualities. It also repressed the propagation of MCF-7 cancer cells. Its anti-inflammatory and immunomodulatory characteristics may be the reason for its ability to raise glutathione levels in diabetic rats. Essential oils of *S. terebinthifolia* fruit were investigated to suppress numerous human tumor cell lines. All lines of cancer cells were inhibited particularly leukemia, ovarian, kidney, and prostate (Guzzo da Silva *et al*. 2019).

Obesity represents an increase in total body fat mass and is considered a serious disease and is classified as the 5th reason of death according to Rahman *et al*. (2017). According to Okoro *et al*. (2023), several constituents of plant origin, such as quercetin, thiacremonone, Z-ajoene, and allyl mercaptan, possess pharmacological actions including anti-obesity, and anti-Alzheimer functions. From previous investigation, the inhibition of lipase activity contributed to the treatment of obesity (Shi *et al*. 2014). D'Costa *et al* (2024) found that phenolic materials modulate the bio-function of lipase, which is vital in the degradation of dietary lipids, and so has an indirect influence on their absorption. Understanding the effect of phenolic compounds on activity of lipase is thus crucial to understanding their biological activity, including their potential health benefits, and has been widely investigated.

Alzheimer's disease (AD) represents a worldwide major illness problem among older adults with cerebrovascular and neurodegenerative conditions. The etiology of this disease has been related to the exhaustion of acetylcholine in the central nervous system. Inhibition of acetylcholinesterase and butyrylcholinesterase has been shown to be effective in the management of AD (Unzeta *et al*. 2016). Natural plant extracts and its contents of phenolic acids, flavonoids, alkaloids, has increased attention in recent decades for its vital role in management of AD *via* reducing the activity of acetylcholinesterase and butyrylcholinesterase (Al-Rajhi *et al*. 2023b). Sahiner *et al*. (2023) reviewed the role of several phenols and flavonoids in AD management *in vitro* and *in vivo*. For instance, apigenin, rutin, and quercetin improved the β-amyloid peptides (Aβ) load, repressed the amyloidogenic progression, suppressed oxidative stress, and enhanced AD-associated

memory and understanding impairment besides restoration of acetylcholine levels with inhibition of AChE. Moreover, caffeic acid repressed Aβ aggregation and disturbed fibrils in aqueous besides cellular lipid membrane-like surroundings. Further, from the review report of Sahiner *et al*. (2023), hesperidin plays a vital role in improving cerebral blood flow, cognitive job and memory functions.

Many traditional methods, such as Soxhlet extraction, hydrodistillation, steam distillation, and dynamic maceration, are used to extract natural ingredients from plants, and despite their widespread use, they may have some drawbacks. For instance, some problems were associated with the utilization of Soxhlet extraction such as low temperature of extraction process; moreover it is not appropriate to employ materials that are easily degraded when heated. So scientists are searching for alternative methods or developing the methods used (Abdelghany *et al*. 2019; Alghonaim *et al*. 2023; Al-Rajhi and Abdelghany 2023). One of the alternative methods that scientists are focusing on is the supercritical carbon dioxide fluid extraction (SFE-CO_2) method. The utilization of this method is quite valuable, as it permits the utilization of a less ecologically aggressive, non-flammable, low toxicity, inert solvent, namely supercritical CO_2. Moreover, it provides a simple method of solvent separation from the end product. As might be expected with organic solvent extraction, there is no solvent residual in the extract during SFE-CO_2. Supercritical CO_2 has a solvating power that is easily changed by small changes in pressure and temperature, unlike liquid solvents, which makes it feasible to extract specific chemicals of interest. Because supercritical CO_2 has a relatively low critical temperature, it is also ideal for the extraction of chemicals that are sensitive to severe circumstances (Qanash *et al.* 2024). Additionally, the choosiness of SFE-CO_2 can be adjusted by several parameters such as temperature, pressure, and content of co-solvent management (da Silva *et al*. 2017). *S. terebinthifolia* has potential for commercial use due to its medicinal properties. However, only a few numbers of studies have looked into the extraction of constituents from *S. terebinthifolia* employing SFE-CO_2. This study aims to estimate the yield extract of the *S. terebinthifolia* fruits *via* SFE-CO_2, with estimate its contents of phytochemicals and their activity for combating microbial pathogens, preventing the activators of Alzheimer's, and obesity diseases *in vitro*.

EXPERIMENTAL

Plant Collection

The fruits of *Schinus terebinthifolia* were collected in December 2023 from the Monufia Governorate in Egypt. The collected fruits were washed by water, dried in the air, and followed by drying at low heat 40 °C in a desiccating cabinet. Subsequently, the dried fruits were ground to a fine powder by a Wiley Mill and sieved using 2-mm mesh. The fruits powder was stored at 25 °C until additional processing of extraction.

Supercritical Carbon Dioxide Extraction (SFE-CO_2) of *S. terebinthifolia*

CO_2 is the supercritical fluid that is used the most frequently. Supercritical CO_2 (SFE-CO_2) is a desired medium for isolating active compounds from natural sources due to its special solvent characteristics. It is subsequently easy to separate the CO_2 from the extract.

The prepared powder of *S. terebinthifolia* was subjected to SFE-CO_2 (ISCO-Sitec modified SFX 220 SFE system) at two operating conditions to optimize the extraction yield and its constituents of flavonoids and phenols, in addition to biological activities of the resulted extract at the two operating conditions. The 1st operating condition consisted of static extraction for 15 min, followed by 45 min as dynamic extraction for 4 g of *S. terebinthifolia.* The 2nd operating condition was as follows: without static extraction but only with dynamic extraction for 60 min for 4 g of *S. terebinthifolia* powder. The powder of *S. terebinthifolia* was statically soaked in a fixed quantity of supercritical fluid for 15 min in the vessel of SFE-CO_2. The CO_2 solvent was pumped into the vessel accompanying with the closing of needle valve. After this duration, the dynamic valve was opened for 45 min at a flow rate 50 mL/min (for 1st operating condition) or opened directly from the beginning for 60 min (2nd operating condition), where the supercritical fluid was continuously passes through the powder of *S. terebinthifolia* into the vessel of extraction. The yield of the extract which dissolved in the supercritical fluid CO_2 was harvested in separators. The recovered CO_2 was then reused in a closed ring and utilized all over again. Other conditions during the operation were constant including temperature (50 °C) and pressure (20.68 MPa); the releasing of pressure will cause the dissolved materials to come out of solution so that they can be collected (da Silva *et al.* 2017).

Schinus terebinthifolia Extract Analysis *via* High-performance Liquid Chromatography (HPLC)

The HPLC assessment was achieved *via* an Agilent 1260 series. The separation extract constituents were performed utilizing a 4.6 mm × 250 mm i.d., 5 μm of Zorbax Eclipse Plus C8 column at 40 °C. The employed mobile phase was mixture of water (A) and trifluoroacetic acid (0.05%) in acetonitrile (B). The flow level of the mobile phase was 0.9 mL/min in a line gradient in this way: [82% A for 0 min; 82% A for 0 to 1 min; 75% A for 1 to 11 min; 60% A for 11 to 18 min; 82% A for 18 to 22 min; 82% A for 22 to 24 min. The injection volume was 5 μL of *S. terebinthifolia* extract solution. At 280 nm, the detector of multi-wavelength was adjusted (Alsalamah *et al*. 2023). Quantitative finding of the separated substances was measured depending on the identified data of standard substances. Standard stock solution of constituents including flavonoid and phenolic was made at different doses (10 to 80 μg/mL) in the solvent of methanol, next by inoculated in the device of HPLC.

Inhibition of Microbial Growth by *S. terebinthifolia* Extract

A well diffusion technique was functioned to evaluate the plant extracts' antimicrobial potential (Al-Rajhi and Abdelghany 2023). The crude extract of *S. terebinthifolia* was dissolved in 10% solution of sterile dimethyl sulfoxide (DMSO). The autoclaved growth media potato dextrose agar/nutrient agar for fungi/bacteria were cooled and then inoculated with fungi/bacteria namely *Candida albicans* (ATCC 10221), *Aspergillus niger* (AUMC 14260), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 90274). Width wells (6 mm) were cut from the inoculated agar, and then filled with the extracts (100 μL/well from 10 mg/mL) under aseptic conditions. As positive standards for bacteria and fungi, correspondingly, 100 μL/well of fluconazole and 100 μL/well of rifampcin (Oxoid) were utilized, while wells injected with the corresponding solvents (100 μL) functioned as negative controls. For bacteria and fungi, the Petri plates were incubated for 20 h at 37 °C and 28 °C for 48 h and 72 h, respectively. The inhibition zones that

appeared in the inoculated plates at the end of the incubation periods were measured. For MIC detection, the extract of *S. terebinthifolia* was prepared in a different dilution. Based on the NCCLS standards approach, 10 µL of each dilution was added to Mueller Hinton broth (170 µL) in 96 wells of a microplate fortified with bacterial inoculum (20 µL having 5×10^5 CFU/mL). After that, the microtiter plates were incubated for 1 day at 37 °C. The tested microbe's growth is designated by the developed turbidity, and the minimum inhibitory concentration (MIC) of the tested extract is the concentration at which no discernible growth occurs. The possibility of the minimum bactericidal concentration (MBC) is detected by the dilution representing the MIC. At least two concentrations of the dilution containing MBC are tested to completely inhibit the microbial growth and assayed to determine the viable CFU/mL of the examined microorganisms, the MBC is found. The MBC is the lowest amount that decides a pre-noticed discount (99.9%) of CFU/mL after compared to the dilution of MIC.

Assay of Hemolysis Inhibition by *S. terebinthifolia* Extract

The technique of Rossignol *et al*. (2008) was used to measure the hemolysin activities of *S. terebinthifolia* extracts in sub-MIC (25% and 50% of MIC) treated with examined bacteria. *E. coli*, *S. aureus*, *P. areginosa*, and *B. subtilis* were among the tested bacteria that were treated with 25%, 50%, and 75% of MIC (sub-MIC). The treated and untreated cultures with the extract were adjusted to an OD 600 of 0.4 and centrifuged for 20 min at 21,000× g. Fresh erythrocyte suspension (2%) in saline (0.8 mL) (obtained from the corresponding author of the present study as volunteer) was mixed with supernatants (500 µL), preserved for 60 min at 37 °C, and centrifuged at certain conditions (11,000× g, 10 min, and 4 °C). The positive control (complete hemolysis) was created *via* mixing of 0.1% sodium dodecyl sulphate with the suspension of erythrocyte. The negative control (unhemolyzed erythrocytes) was created by keeping the erythrocytes in the broth of LB at the same conditions of the positive control. The absorbance at 540 nm was used to measure the hemoglobin release. The hemolysis that transpired in the extract of cultures treated with sub-MIC was expressed as the mean error of ± standard of the % change from that of control cultures that were not treated. After comparing the released hemoglobin with the positive and negative controls, the subsequent formula (Eq. 1) was employed to detect the % of hemolysis:

$$\text{Inhibition of Hemolysis (\%)} = \frac{\text{Sample with bacterial culture} - \text{Negative control (NC)}}{\text{Positive control} - \text{NC}} \times 100 \quad (1)$$

Assessment of Lipase Inhibition by *S. terebinthifolia* Extract

Different concentrations of *S. terebinthifolia* extract (7.8 to 1000 µg/mL) were tested to inhibit the lipase activity based on the amended method of Kim *et al*. (2010). At the same time, Orlistat at the same doses was applied as a positive control. Each extract and Orlistat were mixed with the lipase [10 mg of the enzyme was dissolved in 10 mL of ppb solution (1 mg/mL)] in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) and kept for 60 min at 30 °C. Then, for starting the reaction mixture, 0.1 µL of *p*-nitrophenyl butyrate (pNPB) as a substrate was added. The last volume of all contents was completed to 100 µL, and incubated for 5 min at 30 °C. Then, the released *p*-nitrophenol was measured *via* a Biosystem 310-plus UV-visible spectrophotometer at 405 nm. The negative control activity was recorded in the presence or lack of inhibitor. The inhibitory potential of lipase was measured using Eq. 2,

$$\text{Lipase Inhibition (\%)} = 100 - \frac{A - B}{C - D} \times 100 \quad (2)$$

where *A* denotes the absorbance of lipase activity with inhibitor; *B* represents the absorbance of negative control (extract in DMSO) with inhibitor and lacking lipase; *C* denotes the absorbance of lipase activity lacking inhibitor; *D* is the absorbance of DMSO (negative control) lacking inhibitor lipase.

Butyrylcholinesterase (BChE) Activity Inhibition by *S. terebinthifolia*

The 3.47 unit/mL of enzyme BChE were prepared as stock solution *via* its dissolution in 20 mM of sodium phosphate buffer (SPB) with pH 7.6. The solutions were then kept at -80 °C until needed. A 10 mg/10 mL of the extract was dissolved in phosphate buffer (PB) solution with pH 7.6, resulting in a final dose of 100 µg/mL. Before every experiment, all the stock solutions were diluted using a 20 mM SPB solution (pH 7.6) to a range of doses. The DTNB-phosphate-ethanol reagent was made by liquefying 12.4 mg of DTNB in 120 mL of C_2H_5OH (96%), adding 50 mL of 0.1 mM PB (pH 7.6), and 80 mL of distilled water. Based on a colorimetric technique, an assay of BChE activity was performed with the substrate of butyrylthiocholine iodide. The flowing reaction mixture consisted of 10 µL of the *S. terebinthifolia* extract solution mixed with 0.2% DMSO, 79 µL of 20 µM SPB with pH 7.6, and 1 µL of the BChE (with final doses of 0.2 units/mL of BChE, and 0.2 to 100 µg/mL for *S. terebinthifolia* extract) was pre-incubated for 15 min. An amount og 10 µL of 4 mM butyrylthiocholine iodide solution was mixed with the reaction mixture, and then incubated 30 min. By adding 900 µL of DTNB-phosphate-ethanol reagent, the reaction was stopped. Immediately, the absorption at 405 nm was measured on a microplate reader. Employing an enzyme inhibition quantity response curve, the dose of the extract needed to prevent BChE activity by 50% (IC_{50}) was determined (Gorun *et al*. 1978).

$$\text{BChE Inhibition(\%)} = \frac{\text{Control absorbance–Sample absorbance}}{\text{Control absorbance}} \times 100 \quad (3)$$

Statistical Tests

The experimental outcomes were validated as mean ± standard deviation (SD) of three results. The software Graph Pad Prism V5 (San Diego, CA, USA) was employed to estimate the finding for one-way of variance (ANOVA).

RESULTS AND DISCUSSION

SFE-CO_2 and Phytochemical Description

Figure 1 summarizes the performed experiments in the existing examination, which included the extraction of *S. terebinthifolia* fruit *via* SFE-CO_2 at 2 conditions (static extraction and dynamic extraction), where the obtained extract was analyzed by HPLC, and tested for evaluating their biological activities. Approximately 4 g of dried powdered *S. terebinthifolia* fruits were subjected to SFE-CO_2 in the current investigation under two conditions: static extraction for 15 min and dynamic extraction for 45 min at a constant pressure of 20.68 MPa (condition code A). Additional plant sample of dried *S. terebinthifolia* fruit powder was extracted using a dynamic extraction for 60 min at a constant pressure of 20.68 MPa without static extraction (condition code B). When the

sample of dried *S. terebinthifolia* fruit was exposed to static extraction for 15 min and dynamic extraction for 45 min, the extraction yield was 0.205 g, but when exposed to dynamic extraction for 60 min without static extraction the extraction yield was 0.236 g (Table 1). *S. terebinthifolia* has potential for commercial use because of its medicinal properties. However, few studies were reported about the extraction of *S. terebinthifolia* using SFE-CO_2, moreover it focused on the influence of temperature and pressure on the extraction yield and composition. However, the current study concentrated on the effect of extraction period comprising static and dynamic extraction. SFE-CO_2 was used for *S. terebinthifolia* extraction, there is a noticeable pressure effect, which raises the solvent density and, as a result, the extraction yield. Both solute vapour pressure and solvent density are complexly impacted by temperature (da Silva *et al*. 2023). According to da Silva *et al*. (2023), supercritical extracts achieved at 50 to 60 °C exhibited strong anti-kidney cancer activity with total growth inhibition less than 3.9 µg/mL, regardless of pressure. Moreover, extracts at 50 °C and 200 bar demonstrated strong efficacy against glioma, prostate, and ovarian tumor cell lines that were resistant to drugs. The final structure and the extract quality are directly influenced by the extraction method used to obtain natural product extracts. The group of compounds to be extracted and the process objective—whether qualitative or quantitative —determine the extraction technique. Another way the extraction technique and solvent are used is to determine the process yield and extract composition. The effect of dynamic extraction on the yields of extract from spearmint leaves was investigated by Bimakr *et al*. (2009). The yield of the extraction was improved as the time increased until 90 min of dynamic extraction but reached its maximum yield at 60 min. They estimated the dynamic extraction employing different periods after the static extraction for 30 min, all at a constant 10 MPa.

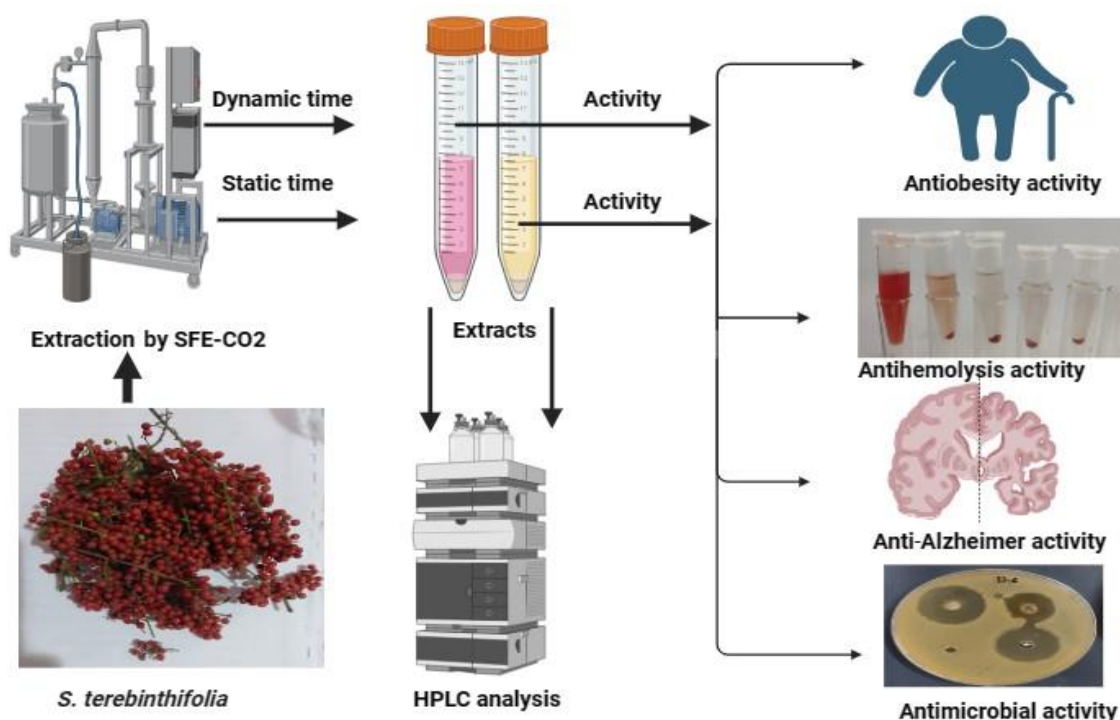

Fig. 1. Scheme of the performed processes including extraction of *S. terebinthifolia* fruit using SFE-CO_2, analysis by HPLC, and biological activities

Table 1. Yield of *S. terebinthifolia* Fruit Extraction by SFE-CO_2 Under Static and Dynamic Extraction for Different Times at Constant Pressure and Temperature

Condition Code	Pressure MPa	Temp. (°C)	Static Extraction	Dynamic Extraction	Quantity (g)	Extract Quantity (g)
A	20.68	50	15 min	45	4.0	0.205
B	20.68	50	0.0 min*	60	4.0	0.236

*, without static extraction

The extraction method utilized in obtaining extracts of natural products clearly affected the final constituents and properties of the extracts. The SFE-CO_2 at condition B was favourable for releasing several compounds of phenols and flavonoids compared to SFE-CO_2 at condition A, as indicated by HPLC chromatograms (Figs. 2 and 3). From the data in Table 2, high concentrations were associated to numerous compounds, such as gallic acid, catechin, ellagic acid, methyl gallate, syringic acid, caffeic acid, pyro catechol, coumaric acid, and naringenin, which were detected in the extract at condition B of SFE-CO_2 compared to their low concentrations at condition A of SFE-CO_2.

Table 2. HPLC Analysis of *S. terebinthifolia* Extract at Static Extraction (Condition A) and Dynamic Extraction (Condition B) for Detection of Phenols and Flavonoids

Detected Constituent	Condition A			Condition B		
	RT (min)	Area (mAU*s)	Conc. (µg/mL)	RT	Area (mAU*s)	Conc. (µg/mL)
Gallic acid	3.57	441.46	1952.41	3.580	1431.52	6331.08
Chlorogenic acid	4.13	40.07	259.94	4.232	0.00	0.00
Catechin	4.46	50.80	548.01	4.456	174.13	1878.31
Methyl gallate	5.49	24.33	61.31	5.478	1434.72	3614.55
Caffeic acid	5.86	38.55	149.16	6.185	101.77	393.75
Syringic acid	6.25	20.64	75.48	6.469	32.29	118.07
Pyro catechol	6.66	10.25	73.88	6.646	69.23	499.03
Rutin	6.85	17.41	128.03	6.848	17.46	129.11
Ellagic acid	7.15	6.27	31.30	7.255	18.90	94.42
Coumaric acid	8.65	85.62	152.35	8.536	782.20	1391.88
Vanillin	9.08	19.50	36.24	9.051	25.96	48.24
Ferulic acid	9.59	86.51	251.28	9.672	276.45	802.96
Naringenin	10.44	39.29	179.55	10.290	469.73	2146.72
Rosmarinic acid	11.87	68.99	369.84	11.818	62.63	335.74
Daidzein	15.70	4.85	13.61	15.736	3.04	8.52
Quercetin	17.30	32.96	222.45	17.270	0.00	0.00
Cinnamic acid	19.16	5.98	5.35	19.567	12.10	10.84
Kaempferol	20.61	11.82	37.27	20.618	19.03	60.03
Hesperetin	21.22	8.29	20.38	21.141	15.32	37.65

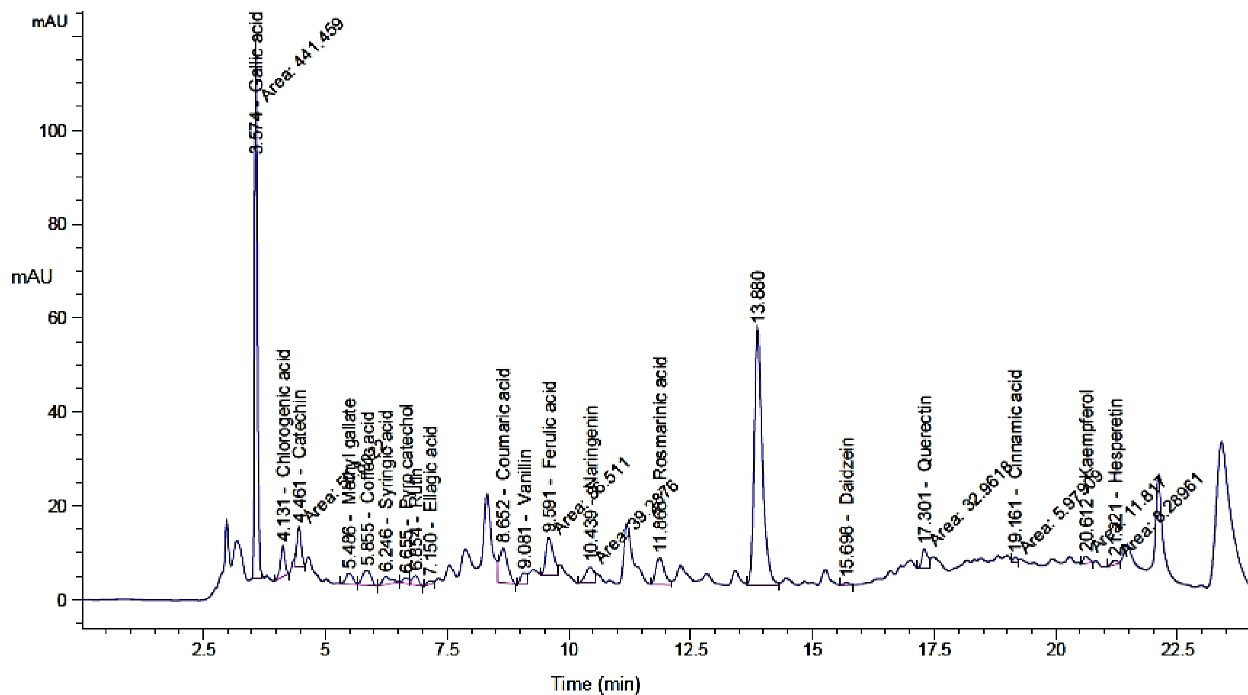

Fig. 2. Recorded chromatograms via HPLC of phenols and flavonoids included in the extract of *S. terebinthifolia* at condition A of SFE-CO_2 by HPLC

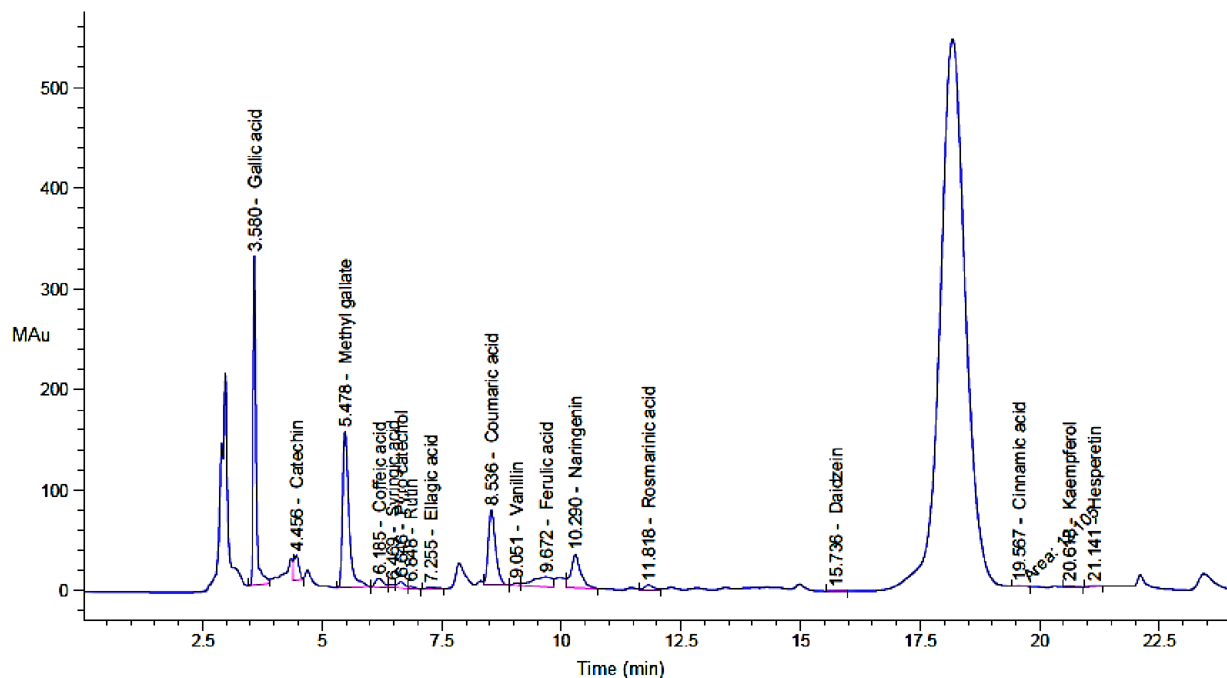

Fig. 3. Recorded chromatograms via HPLC of phenols and flavonoids included in the extract of *S. terebinthifolia* at condition B of SFE-CO_2

Unlike certain compounds including daidzein and rosmarinic acid, which were noticed in high concentrations of 13.6 and 369.8 μg/mL in the extract at condition A of SFE-CO_2 compared to its concentrations of 8.5 and 335.7 μg/mL in the extract at condition

A of SFE-CO_2. Surprisingly, chlorogenic acid (259.9 µg/mL) and quercetin (222.4 µg/mL) were detected only in the extract at condition A of SFE-CO_2. According to de Araujo Gomes *et al*. (2020), phytochemical investigations defined the existence of quercitrin, gallic acid, ethyl gallate, and methyl gallate in *S. terebinthifolia* fruit. *Via* HPLC analysis, afzelin, 3-*O*-*β*-D-glucopyranoside, ellagic acid, gallic acid, quercetin, genistein 7-*O*-*α*-L-rhamnopyranoside, kaempferol, and quercetin, in addition to the new compound namely 4′,methoxy-6-vinyl-7-*O*-rhamnosyl-dihydromyricetin, were detected in *S. terebinthifolia* fruit (Alqathama *et al*. 2023).

Antimicrobial Activities

Extract of *S. terebinthifolia* inhibited the growth of tested microorganisms with various levels of inhibition zones, dependent on the extraction condition of SFE-CO_2 and tested microorganisms (Table 3, Fig. 4). At condition B of SFE-CO_2 for *S. terebinthifolia*, high zones of inhibition were the following, 26 ± 0.1, 25 ± 0.2, 29 ± 0.1, 33 ± 0.2, 27 ± 0.1, and 8.0 ± 0.1 mm visualized versus *S. aureus*, *P. areginosa*, *B. subtilis*, *E. coli*, *C. albicans*, and *A. niger,* respectively. Extraction at condition A showed low inhibition zones toward the same microorganisms except for *A. niger*, which showed complete resistance to this extract. The large inhibition zone (33 ± 0.2 mm) associated with *E. coli* indicated that it was the most sensitive bacteria to the extract at condition B, unlike *P. areginos*a. According to Sarjit *et al*. (2015), growth of *C. albicans, E. coli*, and *S. aureus* was suppressed at levels of 63.2, 58.6, and 43.8% using fruit extract of *S. terebinthifolia* compared to the standard drug. Gallic and ellagic acids were detected in fruit extract of *S. terebinthifolia* in the present study. The current investigation suggested that these acids may play a vital role in antimicrobial activity. The current findings agree with Sarjit *et al*. (2015), who mentioned that these compounds have formerly possessed antimicrobial activities.

Such results underscore the important potential of *S. terebinthifolia* extract as a natural agent for bacterial suppression. Previously, hydroalcoholic extracts of *S. terebinthifolia* (Costa *et al*. 2012) exhibited *in vitro* antimicrobial potential, even at low doses, toward *Enterococcus faecalis*, a nosocomial pathogen. According to these results, Schinus extracts may be a viable supplier of active constituent(s) for microbial pathogens control. Additionally, *Agrobacterium tumefaciens*, a plant pathogen, was inhibited *in vitro* and *in vivo* by *S. terebinthifolia* extract (Ghanney and Rhouma 2015). The determined MIC of the extract was reduced to 25% of extract at condition B toward *S. aureus*, *B. subtilis*, and *E. coli*; 50% toward *P. areginosa* and *C. albicans* compared to levels of MIC of the extract at condition A (Table 4). Moreover, the values of MBC and MFC of the extract at condition B versus all investigated bacteria and *C. albicans* extended from 7.8 to 62.5 µg/mL were less than the MIC and MFC values ranged from 62.5 to 250 µg/mL of the extract at condition A.

It is significant to observe that the MIC of this extract was consistently detected to be less than 500 µg/mL. As mentioned in other studies, the antimicrobial potential of natural extracts depends on numerous factors including the method of extraction, solvent employed, plant part used, tested microorganisms, and geographical and cultivated area of plants. For instance, ethanol extract of *S. terebinthifolia* reflected more activity with MIC of 16 and 32 µg/mL than acetone extract that showed MIC values of 4 to 128 µg/mL against *S. aureus* and *P. aeruginosa*, respectively, while hexane extract reflected weak inhibitory activity (Salem *et al*. 2018).

Table 3. Antimicrobial Activity of *S. terebinthifolia* Extract Under Static Extraction (Sample code A) and Dynamic Extraction (Sample Code B)

Investigated Microorganisms	Radius of Inhibition Zones (mm)			
	Sample Code		Control	
	A	B	*Positive	**Negative
S. aureus	22 ± 0.2	26 ± 0.1	25 ± 0.3	0.0
P. areginosa	20 ± 0.1	25 ± 0.2	19 ± 0.1	0.0
B. subtilis	20 ± 0.2	29 ± 0.1	26 ± 0.1	0.0
E. coli	25 ± 0.1	33 ± 0.2	28 ± 0.2	0.0
C. albicans	18 ± 0.1	27 ± 0.1	25 ± 0.3	0.0
A. niger	0± 0.0	8.0 ± 0.1	33 ± 0.1	0.0

*Positive control namely Gentamycin/Nystatin as antibacterial/antifungal; **negative control (DMSO)

Table 4. Effect of Static Extraction (Sample Code A) and Dynamic Extraction (Sample Code B) of SFE-CO_2 on the MIC, MBC, and MFC as well as MIC or MFC/MBC Index of *S. terebinthifolia* Extract

Investigated Microorganisms	MIC of Sample Code		MBC or MFC of Sample Code		MBC or MFC/MIC Index of Sample Code	
	A	B	A	B	A	B
S. aureus	31.25	7.8	125	15.62	4	2
P. areginosa	31.25	15.62	125	31.25	4	2
B. subtilis	31.25	7.80	62.5	7.80	2	1
E. coli	62.5	31.25	250	62.5	4	2
C. albicans	125	31.25	250	62.5	2	2

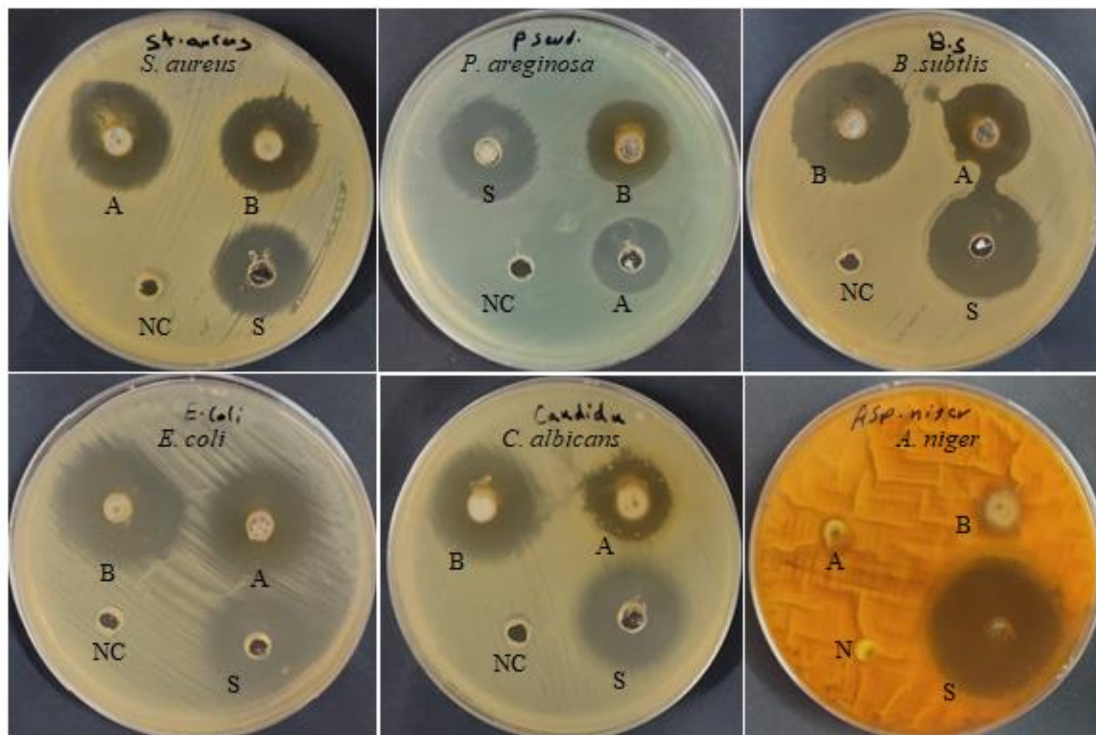

Fig. 4. Antimicrobial activity of *S. terebinthifolia* extract under static extraction (A); dynamic extraction (B); S: namely Gentamycin/Nystatin as antibacterial/antifungal; and NC: negative control (DMSO)

da Silva *et al*. (2018) investigated the inhibitory potential of fruits and leaves extracts of *S. terebinthifolia* versus bacteria and yeasts. They mentioned that the MIC of ethanol extract of *S. terebinthifolia* fruits against *E. coli*, *C. albicans*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Cryptococcus neoformans* was 78, 626, 625, and > 1000 µg/mL, respectively, while ethyl acetate extract reflected MIC values of 312 µg/mL for both *E. coli* and *Cryptococcus neoformans*; 625 µg/mL for both MRSA and *C. albicans*. Ethanol extract of *S. terebinthifolia* leaves provided MIC values of 78, 156, > 1000 µg/mL against *E. coli*, *C. neoformans* and MRSA, respectively, while ethyl acetate extract reflected MIC values of 39 µg/mL for *E. coli*, 625 µg/mL against MRSA, 156 µg/mL against both *C. albicans* and *C. neoformans*. According to da Silva *et al*. (2018), different values of MBC were detected against *E. coli* depending on the used solvents ranging from 39 to 156 µg/mL. Compared to the results of da Silva *et al*. (2018), the current results reflected the efficacy of SFE-CO_2 for enhancing the antimicrobial properties of *S. terebinthifolia* fruit extract.

Bacterial Hemolysis Inhibition

Certain bacteria secrete various toxins, such as α-toxin, which causes hemolysis activity when it attaches to the membranes of erythrocytes, according to Song *et al*. (1996). Therefore, it was investigated how the *V. agnus-castus* extract affected the hemolysis of red blood cells in the existence of tested bacteria. The effect of the extract on the hemolysis percentage in the presence of tested bacteria was visualized (Figs. 5 and 6).

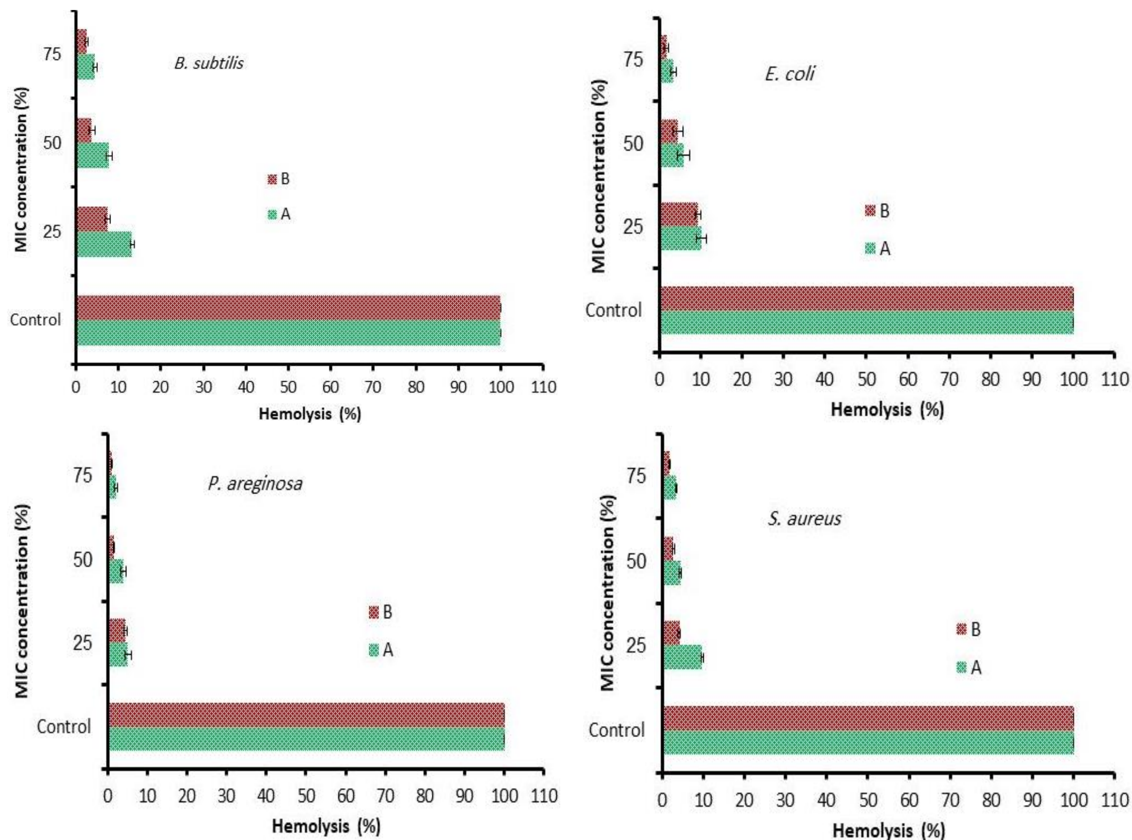

Fig. 5. Effect of different MIC values of *S. terebinthifolia* extract on hemolytic activity of tested bacteria (*B. subtilis*, *E. coli, P. areginosa*, and *S. aureus*). Static extraction (condition A) and dynamic extraction (condition B) of SFE-CO_2

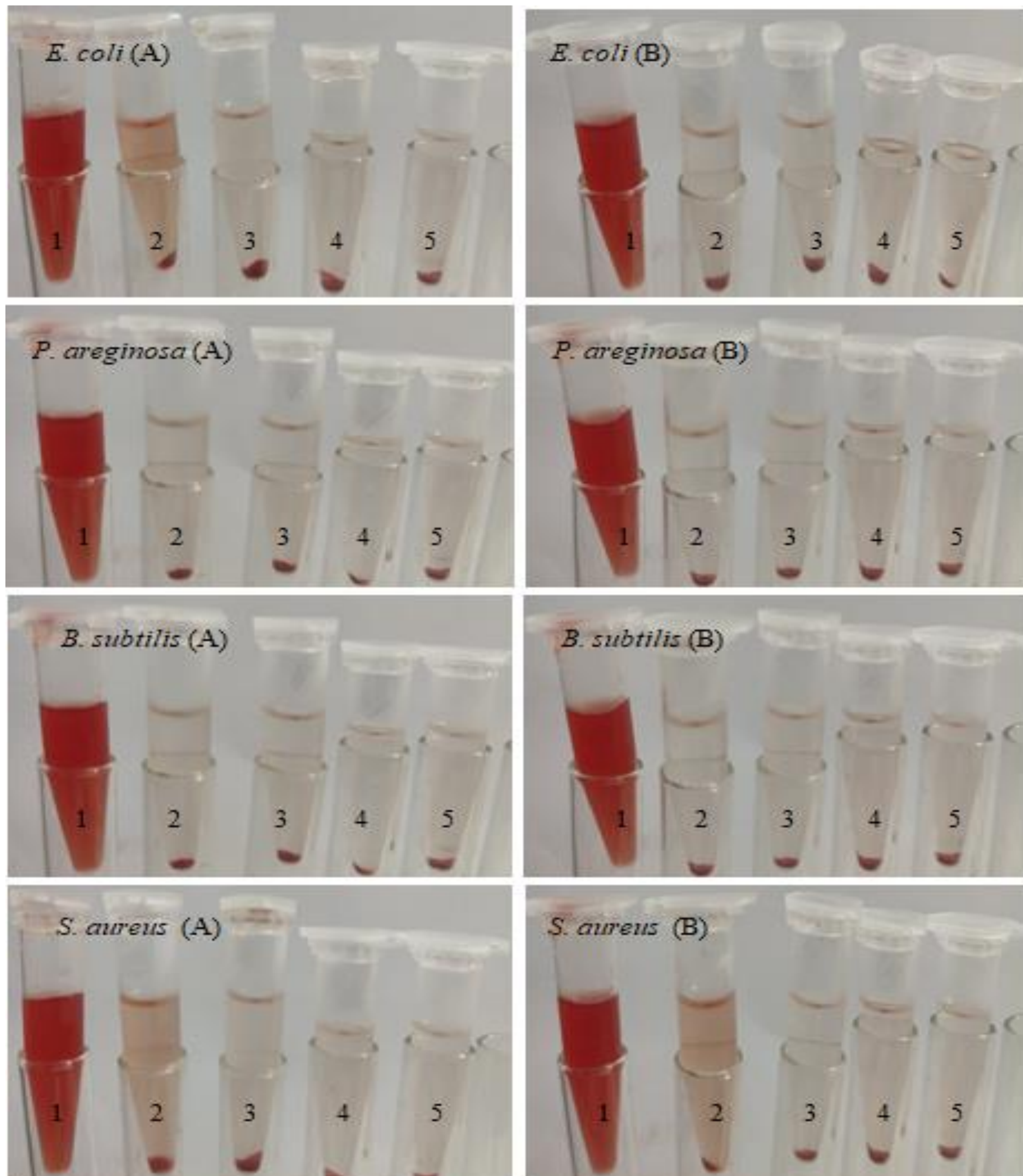

Fig. 6. Effect of *S. terebinthifolia* extract at different concentrations of their MIC on hemolytic activity of tested bacteria. Static extraction (Sample code A), dynamic extraction (Sample code B) of SFE-CO_2, Negative control (1), 25% MIC (2), 50% MIC (3), 75% MIC (4), and positive control (5)

The obtained results showed that hemolysis percentage decreased with increasing the concentration of extract MIC in the existence of all tested bacteria. From the obtained results, the extract at condition B (The extracted *S. terebinthifolia* fruit at a dynamic extraction for 60 min without static extraction) exhibited more preventive effects on the hemolysis than the extract at condition A (The extracted *S. terebinthifolia* fruit using static extraction for 15 min and dynamic extraction for 45 min). At 75% of MIC, the low

hemolysis percentage was observed for *B. subtilis* (1.0% and 2.1%), followed by *S. aureus* (1.8% and 3.5%), *E. coli* (1.9% and 3.5%), and *P. areginosa* (2.5% and 4.5%) using the extract at conditions (B and A), respectively. According to Muhs *et al*. (2017), the extract of *S. terebinthifolia* reduces the hemolytic potential caused by *S. aureus*. This is because *S. aureus* carries the α-hemolysin gene, which is responsible for producing δ-toxins. Kim *et al*. (2015) informed that hemolytic potential of *Pseudomonas aeruginosa* was reduced and that the ability of these bacteria to form biofilms was inhibited by the oil of cinnamon bark.

In a different study, whole blood was more easily eliminated by *S. aureus* but the herring oil reduced the hemolytic effect of the bacteria (Lee *et al*. 2022). The current results show that the tested extract suppressed hemolysis in the occurrence of the tested bacteria, which offers significant implications for the management of bacterial virulence.

Lipase Inhibition

Lipase inhibition was recorded under the effect of *S. terebinthifolia* extract at conditions A and B of SFE-CO_2 (Fig. 7). The enzyme inhibition increased as the dose of extract increased with dose-dependent manner. As noticed from the results, the extract at condition B reflected higher inhibition of lipase than that the extract at condition A at all tested doses. Less IC_{50} value (27.03 µg/mL) of extract at condition B than that IC_{50} value (37.45 µg/mL) of extract at condition A was obtained.

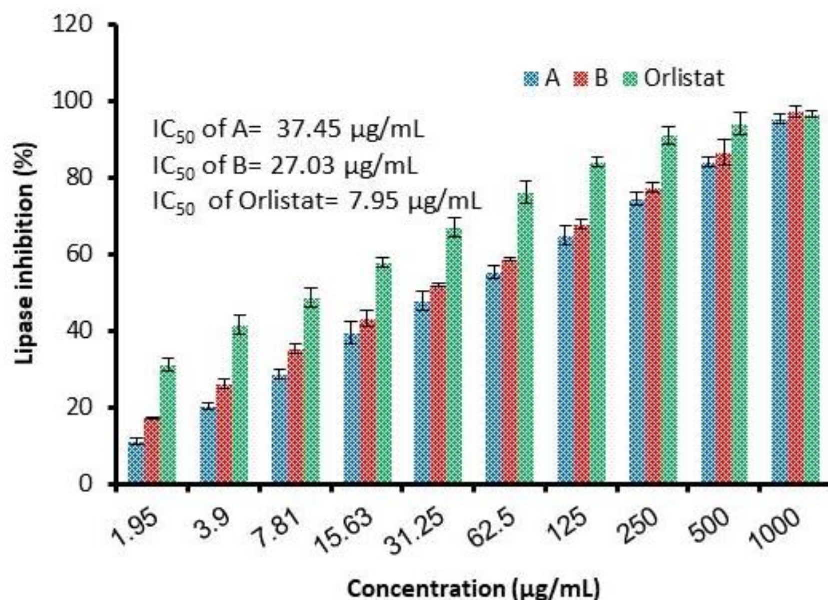

Fig. 7. Effect of different concentrations of *S. terebinthifolia* extract on lipase inhibition. Static extraction (Sample code A) and dynamic extraction (Sample code B) of SFE-CO_2

The activity of the extract was compared to Orlistat, a standard drug that provides IC_{50} of 7.95 µg/mL for lipase inhibition. However, 1000 µg/mL of the extract at condition B caused more inhibition of lipase (97.4%) than that caused by Orlistat (96.5%) at the same concentration. Inhibition of lipase is one of the most explored mechanisms employed to detect the probable influence of natural ingredients as anti-obesity tablets (Shi *et al*. 2015). While several therapeutic approaches for the management of obesity have been studied, they are rarely found to be harmless, and the greatest of these possesses unfavorable effects.

Therefore, an alternative is to find anti-obesity compounds in plants. The presence of chlorogenic acid, quercetin, and rutin in *S. terebinthifolia* with quantities of 259.94, 222.45, and 128.03 µg/mL, respectively is well documented via HPLC investigation. These compounds, according to previous studies, perform a significant function in obesity regulation (Meng *et al*. 2013; Seo *et al*. 2015).

Butyrylcholinesterase Inhibition

Cholinesterase inhibitors that increase the endogenous rate of acetylcholine have been acknowledged as the first line in the management of AD because the quantity of acetylcholine gradually decreases with the disease and appears to relate to the injury of cognition and memory (Anand and Singh 2013). It has been discovered that inhibition of butyrylcholinesterase (BChE) increases brain acetylcholine, which enhances memory and cognition. The activity of BChE was inhibited by *S. terebinthifolia* extract either extracted at condition A or B as illustrated in Fig. 8. Rivastigmine was applied as the standard BChE inhibitor in this investigation that presented an IC_{50} of 3.74 µg/mL. *Schinus terebinthifolia* extract either extracted at condition A or B exerted dose-dependent suppression of BChE enzyme. The extract IC_{50} at condition B was found to be 4.83 µg/mL, while the extract IC_{50} at condition A was 17.57 µg/mL. The lower IC_{50} values of the extract at condition A against BChE compared with the extract at condition B suggest that this extract have more phytoconstituents specificity for BChE inhibition. Generally, the findings revealed the BChE inhibitory properties of *S. terebinthifolia* extract. The potential of the *S. terebinthifolia* extract for BChE inhibition was notable when compared with *Aegle marmelos*, *Enhydra fluctuans*, and Laurel leaf, which are utilized traditionally to improve memory and management of AD (Asaduzzaman *et al*. 2014; Al-Rajhi *et al*. 2023b). The current findings suggest that *S. terebinthifolia* extract particularly the one extracted at condition B possesses a considerable BChE inhibitory activity. Lopa *et al*. (2021) recorded a major link among the content of phenols and BChE inhibition.

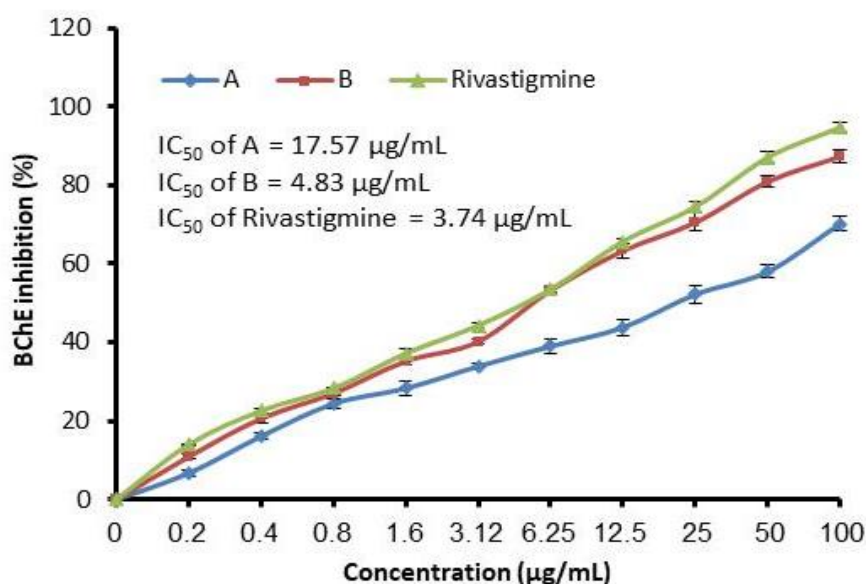

Fig. 8. Effect of different concentrations of *S. terebinthifolia* extract on butyrylcholinesterase inhibition. Static extraction (Sample code A) and dynamic extraction (Sample code B) of SFE

CONCLUSIONS

1. The above outcomes indicate that supercritical fluid extraction with carbon dioxide (SFE-CO_2) by dynamic extraction for 60 min and without static extraction was able to achieve effective extract yield, as well as flavonoid and phenolic contents, and biological activity.
2. *Schinus terebinthifolia* demonstrated varying degrees of microbial inhibition depending on the extraction time and tested microbial species.
3. The extracts of *S. terebinthifolia* demonstrated notable anti-hemolysis effects caused by the tested bacteria. In terms of anti-obesity and anti-Alzheimer effects, *S. terebinthifolia* extract caused a decrease in lipase and butyrylcholinesterase activity with increasing extract concentration.
4. From the results of this investigation, it is possible to conclude that *S. terebinthifolia* can inhibit microbial infection in obesity and Alzheimer's development.
5. The outcomes in the present investigation can be considered as beginning point for further exploration to identify and separate specifically the constituents responsible for the illness treatment *in vitro* and *in vivo*.

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REFERENCES CITED

Abdelghany, T. M. (2014). "Eco-friendly and safe role of *Juniperus procera* in controlling fungal growth and secondary metabolites," *J. Plant Pathol. Microbiol.* 5(3), article ID 1000231. DOI: 10.4172/2157-7471.1000231

Abdelghany, T. M., Ganash, M., Alawlaqi, M. M., and Al-Rajhi, A. M. (2019). "Antioxidant, antitumor, antimicrobial activities evaluation of *Musa paradisiaca* L. pseudostem exudate cultivated in Saudi Arabia," *BioNanoScience* 9, 172-178. DOI: 10.1007/s12668-018-0580-x

Alghonaim, M. I., Alsalamah, S. A., Alsolami, A., and Abdelghany, T. M. (2023). "Characterization and efficiency of *Ganoderma lucidum* biomass as an antimicrobial and anticancer agent," *BioResources* 18(4), 8037-8061. DOI: 10.15376/biores.18.4.8037-8061

Alqathama, A., Abdelhady, M. I., Al-Omar, M. S., Barghash, M. F., and Shallan, A. I. (2023). "Antioxidant, anti-inflammatory and cytotoxic activity of *Schinus terebinthifolia* fruit and isolation of a new immunomodulatory polyphenolic compound," *Pharmacognosy Magazine* 19(1), 13-22. DOI: 10.1177/09731296221138632

Al-Rajhi, A. M., and Abdelghany, T. M. (2023). "Nanoemulsions of some edible oils and their antimicrobial, antioxidant, and anti-hemolytic activities," *BioResources* 18(1),

1465-1481. DOI: 10.15376/biores.18.1.1465-1481
Al-Rajhi, A. M. H., Bakri, M. M., Qanash, H., Alzahrani, H. Y., Halawani, H., Algaydi, M. A., and Abdelghany, T. M. (2023a). "Antimicrobial, antidiabetic, antioxidant, and anticoagulant activities of *Cupressus sempervirens in vitro* and *in silico*," *Molecules* 28(21), article ID 7402. DOI: 10.3390/m olecules28217402
Al-Rajhi, A. M. H., Qanash, H., Almashjary, M. N., Hazzazi, M. S., Felemban, H. R., and Abdelghany, T. M. (2023b). "Anti-*Helicobacter pylori*, antioxidant, antidiabetic, and anti-Alzheimer's activities of laurel leaf extract treated by moist heat and molecular docking of its flavonoid constituent, naringenin, against acetylcholinesterase and butyrylcholinesterase," *Life* 13(7), article 1512. DOI: 10.3390/life13071512
Alsalamah, S. A., Alghonaim, M. I., Jusstaniah, M., and Abdelghany, T. M. (2023). "Anti-yeasts, antioxidant and healing properties of Henna pre-treated by moist heat and molecular docking of its major constituents, chlorogenic and ellagic acids, with *Candida albicans* and *Geotrichum candidum* proteins," *Life* 13(9), article 1839. DOI: 10.3390/life13091839
Anand, P., and Singh, B. (2013). "A review on cholinesterase inhibitors for Alzheimer's disease," *Archives of Pharmacal Research* 36(4), 375-399. DOI: 10.1007/s12272-013-0036-3
Asaduzzaman, M., Uddin, M. J., Kader, M. A., Alam, A. H., Rahman, A. A., Rashid, M., and Sadik, G. (2014). "*In vitro* acetylcholinesterase inhibitory activity and the antioxidant properties of *Aegle marmelos* leaf extract: Implications for the treatment of Alzheimer's disease," *Psychogeriatrics* 14(1), article 12031. DOI: 10.1111/psyg.12031
Bimakr, M., Rahman, R. A., Taip, F. S., Chuan, L. T., Ganjloo, A., Selamat, J., and Hamid, A. (2009). "Supercritical carbon dioxide (SC-CO_2) extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves," *Eur. J. Sci. Res.* 33(4), 679-690.
Costa, E. De Brito, M. M., De Araújo Evangelista, A. P., De Medeiros, A. C. D., Dametto, F. R., and De Carvalho, R. A. (2012). "*In vitro* evaluation of the root canal cleaning ability of plant extracts and their antimicrobialaction," *Brazilian Oral Research* 26, 215-221. DOI: 10.1590/S1806-83242012000300006
da Silva Dannenberg, G., Funck, G. D., Mattei, F. J., da Silva, W. P., and Fiorentini, Â. M. (2016). "Antimicrobial and antioxidant activity of essential oil from pink pepper tree (*Schinus terebinthifolia* Raddi) *in vitro* and in cheese experimentally contaminated with *Listeria monocytogenes*," *Innovative Food Science & Emerging Technologies* 36, 120-127. DOI: 10.1016/j.ifset.2016.06.009
da Silva, B. G., do Prado, J. M., Fileti, A. M., Foglio, M. A., and Rosa, P. D. (2023). "Kinetic models for extraction with supercritical carbon dioxide from pink pepper: Theoretical, empirical, and semi-empirical models and artificial neural network approach," *Chemical Engineering Journal Advances* 15, article ID 100514. DOI: 10.1016/j.ceja.2023.100514
da Silva, B. G., Fileti, A. M., Foglio, M. A., Ruiz, A. L., and Rosa, P. D. (2017). "Supercritical carbon dioxide extraction of compounds from *Schinus terebinthifolia* Raddi fruits: Effects of operating conditions on global yield, volatile compounds, and antiproliferative activity against human tumor cell lines," *The Journal of Supercritical Fluids* 130, 10-16. DOI: 10.1016/j.supflu.2017.07.006
da Silva, J. H., Simas, N. K., Alviano, C. S., Alviano, D. S., Ventura, J. A., de Lima, E.

J., and Kuster, R. M. (2018). "Anti-*Escherichia coli* activity of extracts from *Schinus terebinthifolia* fruits and leaves," *Nat. Prod. Res.* 32(11), 1365-1368. DOI: 10.1080/14786419.2017.1344657

D'Costa, A. S., Chen, A. A., Hamann, E., El Iraki, R., Venugopal, K., Bordenave, N. (2024). "Impact of potato starch on the inhibition of pancreatic lipase by potato phenolic acids," *Food Bioscience* 57, article ID 103414. DOI: 10.1016/j.fbio.2023.103414

de Araujo, Gomes, R. B., de Souza, E. S., Barraqui, N. S. G., Tosta, C. L., Nunes, A. P. F., Schuenck, R. P., and Kuster, R. M. (2020). "Residues from the brazilian pepper tree (*Schinus terebinthifolia* Raddi) processing industry: Chemical profile and antimicrobial activity of extracts against hospital bacteria," *Industrial Crops and Products* 143, article ID 111430. DOI: 10.1016/j.indcrop.2019.05.079

de Jesus Viegas, D., Amêndola, I., Botrel, T. M., de Oliveira, F. E., Figueira, L. W., Sper, F. L., and de Oliveira, L. D. (2020). "*Schinus terebinthifolia* (Brazilian Peppertree) extract used as antifungal to control *Candida* spp. in planktonic cultures and biofilms," *Brazilian Dental Science* 23(4), article 2077. DOI: 10.14295/bds.2020.v23i4.2077

Dos Santos, M. R., Da Silva, J. H., and Caxito, M. L. (2015). "Brief review on the medical uses and antimicrobial activity of different parts of *Schinus terebinthifolia* Raddi," *Int. J. Pharm. Pharm. Sci.* 7(12), 1-7.

Ghanney, N., and Rhouma, A. (2015). "*Schinus terebinthifolia* Raddi (Anacardiaceae) leaf extracts: Antibacterial activity against two *Agrobacterium tumefaciens* strains," *Journal of Crop Protection* 4(1), 85-96.

Gorun, V., Proinov, I., Baltescu, V., Balaban, G., and Barzu, O. (1978). "Modified Ellman procedure for assay of cholinesterases in crude enzymatic preparations," *Anal. Biochem.* 86(1), 324-326. DOI: 10.1016/0003-2697(78)90350-0

Guzzo da Silva, B., Foglio, M. A., Vieira e Rosa, P. T., Taranto, O. P., and Frattini Fileti, A. M. (2019). "Optimization of hydrodistillation and *in vitro* anticancer activity of essential oil from *Schinus terebinthifolia* Raddi fruits," *Chem. Eng. Commun.* 206(5), 619-629. DOI: 10.1080/00986445.2018.1515074

Kim, Y. G., Lee, J. H., Kim, S. I., Baek, K. H., and Lee, J. (2015). "Cinnamon bark oil and its components inhibit biofilm formation and toxin production," *Int. J. Food Microbiol.* 195, 30-39. DOI: 10.1016/j.ijfoodmicro.2014.11.028

Kim, Y. S., Lee, Y. M., Kim, H., and Kim, J. (2010). "Anti-obesity effect of *Morus bombycis* root extract: Anti-lipase activity and lipolytic effect," *J. Ethnopharmacol.* 130(3), 621-624. DOI: 10.1016/j.jep.2010.05.053

Lee, J. H., Kim, Y. G., and Lee, J. (2022). "Inhibition of *Staphylococcus aureus* biofilm formation and virulence factor production by petroselinic acid and other unsaturated C18 fatty acids," *Microbiology Spectrum* 10(3), article e01330-22. DOI: 10.1128/spectrum.01330-22

Lopa, S. S., Al-Amin, M. Y., Hasan, M. K., Ahammed, M. S., Islam, K. M., Alam, A. H. M. K., Tanaka, T., and Sadik, M. G. (2021). "Phytochemical analysis and cholinesterase inhibitory and antioxidant activities of *Enhydra fluctuans* relevant in the management of Alzheimer's disease," *Int. J. Food Sci.* 2021(1), article ID 8862025. DOI: 10.1155/2021/8862025

Mashraqi, A., Modafer, Y., Al Abboud, M. A., Salama, H. M., and Abada, E. (2023). "HPLC analysis and molecular docking study of *Myoporum serratum* seeds extract with its bioactivity against pathogenic microorganisms and cancer cell

lines," *Molecules* 28(10), article ID 4041. DOI: 10.3390/molecules28104041

Meng, S., Cao, J., Feng, Q., Peng, J., and Hu, Y. (2013). "Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review," *Evid.-Based Compl. Alt.* 2013, article ID 801457. DOI: 10.1155/2013/801457

Muhs, A., Lyles, J. T., Parlet, C. P., Nelson, K., Kavanaugh, J. S., Horswill, A. R., and Quave, C. L. (2017). "Virulence inhibitors from Brazilian peppertree block quorum sensing and abate dermonecrosis in skin infection models," *Sci. Rep.* 7, article ID 42275. DOI: 10.1038/srep42275

Okoro, B. C., Dokunmu, T. M., Okafor, E., Sokoya, I. A., Israel, E. N., Olusegun, D. O., Bella-Omunagbe, M., Ebubechi, U. M., Ugbogu, E. A., and Iweala, E. E. (2023). "The ethnobotanical, bioactive compounds, pharmacological activities and toxicological evaluation of garlic (*Allium sativum*): A review," *Pharmacological Research - Modern Chinese Medicine* 8, article ID 100273. DOI: 10.1016/j.prmcm.2023.100273

Qanash, H., El-Fadl, S.R.A., Binsaleh, N.K., Aljahdali, I. A., Altayar, M. A., Khalel, A. F., and Abdelghany, T. M. (2024). "Ecofriendly extraction approach of *Moringa peregrina* biomass and their biological activities *in vitro*," *Biomass Conv. Bioref*. DOI: 10.1007/s13399-024-05916-4

Rahman, H. A., Sahib, N. G., Saari, N., Abas, F., Ismail, A., Mumtaz, M. W., and Hamid, A. A. (2017). "Anti-obesity effect of ethanolic extract from *Cosmos caudatus* Kunth leaf in lean rats fed a high fat diet," *BMC Complement Altern. Med.* 17, 1-17. DOI: 10.1186/s12906-017-1640-4

Rossignol, G., Merieau, A., Guerillon, J., Veron, W., Lesouhaitier, O., Feuilloley, M. G., and Orange, N. (2008). "Involvement of a phospholipase C in the hemolytic activity of a clinical strain of *Pseudomonas fluorescens*," *BMC Microbiology* 8, article 189. DOI: 10.1186/1471-2180-8-189

Sahiner, M., Yilmaz, A. S., Gungor, B., and Sahiner, N. A. (2023). "Review on phyto-therapeutic approaches in Alzheimer's disease," *Journal of Functional Biomaterials* 14(1), article 50. DOI: 10.3390/jfb14010050

Salem, M., El-Hefny, M., Ali, H., Elansary, H. O., Nasser, R. A., El-Settawy, A. A., El Shanhorey, N., Ashmawy, N. A., and Salem, A. Z. (2018). "Antibacterial activity of extracted bioactive molecules of *Schinus terebinthifolia* ripened fruits against some pathogenic bacteria," *Microb. Pathog*. 120, 119-127. DOI: 10.1016/j.micpath.2018.04.040

Sarjit, A., Wang, Y., and Dykes, G. A. (2015). "Antimicrobial activity of gallic acid against thermophilic Campylobacter is strain specific and associated with a loss of calcium ions," *Food Microbiology* 46, 227-233. DOI: 10.1016/j.fm.2014.08.002

Seo, M. J., Lee, Y. J., Hwang, J. H., Kim, K. J., and Lee, B. Y. (2015). "The inhibitory effects of quercetin on obesity and obesity-induced inflammation by regulation of MAPK signaling," *The Journal of Nutritional Biochemistry* 26(11), 1308-1316. DOI: 10.1016/j.jnutbio.2015.06.005

Shi, D., Chen, C., Zhao, S., Ge, F., Liu, D., and Song, H. (2014). "Walnut polyphenols inhibit pancreatic lipase activity *in vitro* and have hypolipidemic effect on high-fat diet-induced obese mice," *J. Food Nutr. Res*. 2(10), 757-763. DOI: 10.12691/jfnr-2-10-16

Song, L., Hobaugh, M. R., Shustak, C., Cheley, S., Bayley, H., and Gouaux, J. E. (1996). "Structure of staphylococcal α-hemolysin, a heptameric transmembrane pore," *Science* 274(5294), 1859-1865. DOI: 10.1126/science.274.5294.1859

Unzeta, M., Esteban, G., Bolea, I., Fogel, W. A., Ramsay, R. R., Youdim, M. B., and Marco-Contelles, J. (2016). "Multi-target directed donepezil-like ligands for Alzheimer's disease," *Front. Neurosci.-Switz.* 10, article ID 197722. DOI: 10.3389/fnins.2016.00205

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