Dynamic Extraction Time's Effect on Phytochemical Characterization of *Vitex agnus-castus* Dry Biomass with Healing Properties and their Activity Against Microorganisms and Ovarian Cancer

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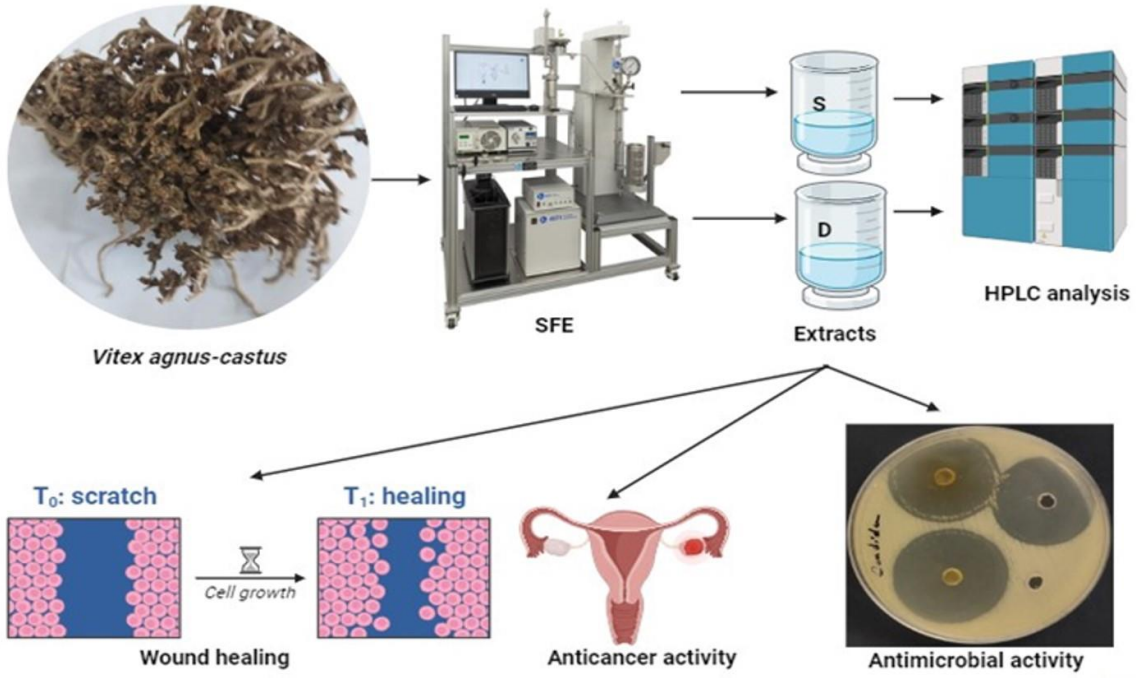
DOI: 10.15376/biores.19.3.5793-5810

GRAPHICAL ABSTRACT



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Efficacies of plant metabolites are known to be dependent on their extraction methods. Yields and compositions of phytoconstituents in the extract were evaluated following supercritical fluid extraction (SFE) of *Vitex agnus-castus* leaves, static extraction times (SET) for 30 min, subsequently dynamic extraction time (DET) for 30 min (condition A) and SET for 0 min followed by DET for 60 min (condition B). The extract exposed to condition B gave an extraction yield of 0.169 g compared to 0.115 g for condition A. High-performance liquid chromatography analysis revealed compounds including cinnamic acid, kaempferol, ferulic acid, rutin, and caffeic acid, in high concentrations in the extract exposed to condition B. *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Candida albicans* were more affected by the condition B with 32 ± 0.1, 20 ± 0.2, 32 ± 0.2, 35 ± 0.2, and 40 ± 0.1 mm inhibition zones, respectively. Less MIC and MBC were noticed of the exposed extract to condition B than to condition A against *C. albicans* and bacteria. The IC_{50} of the extract exposed to condition B was high against ovarian tumor cells. Presently the efficacy of the exposed extract to condition B for wound healing process was documented.

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Keywords: Vitex agnus-castus; Antimicrobial; Supercritical fluid extraction; SKOV3; Wound healing

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INTRODUCTION

Natural products, particularly from plants, have gained great scientific consideration in current decades because of their various therapeutic possessions, including numerous biological activities (Abdelghany *et al*. 2014, 2016; Qanash *et al*. 2022; Alghonaim *et al*. 2023). *Vitex agnus-castus*, which belongs to the Lamiaceae family, has been broadly employed in traditional medicine. Within the Mediterranean regions such as Southern Europe, Western Asia, and North Africa, it grows along seacoasts and riverbanks as a wild plant. It is a tall shrub (3 to 6 m) or low tree. It appears in the shape of an erect shrub or a prostrate habit. The shoots of the young plant are tetrahedral with gray color. Its leaves consist of 3 to 7 leaflets, with white developed tomentum on its lower surface (Adamov *et al*. 2020). According to several investigations, *V. agnus-castus* has several utilizations in biological systems. Its antiseptic, digestive, diuretic, anti-anxiety, aphrodisiac, anti-estrus, emmenagogus, aperitif, analgesic, and antispasmodic effects have been used in traditional treatment. Moreover, *V. agnus-castus* is an effective, traditional plant used to minimize uterine cramps during menstruation regulation (Mari *et al*. 2012; Zahid *et al*. 2016). The *Vitex agnus-castus* fruit is frequently used for a range of reproductive illnesses in females, comprising of female hormonal imbalances and premenstrual syndrome (PMS), such as depression, mood swings, cramps, weight gain, and water retention linked with the disorder of premenstrual dysphoric, PMS, lactation problems, and menopause-related complaints besides low fertility (Shaw *et al*. 2018).

Antioxidant and antihyperglycemic activities, besides antibacterial properties of *V. agnus-castus* were reported by Berrani *et al*. (2021). Moreover, its seeds extract exhibited suppressive effect against bacteria, besides antioxidant, and anti-alzahimer activities (Kavaz *et al*. 2022). Al-Otibi *et al*. (2022) reported the inhibitory potential of *V. agnus-castus* toward numerous species of yeasts comprising *Candida krusei*, *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. dublinesis*, *C. famatai*, and *C. rhodotorula*.

Many secondary constituents, such as iridoids, terpenoids, flavonoids, oils, as well as ketosteroids, are found in various organs of *V. agnus-castus*, including the flowering stems, fruits, and leaves based on phytochemical investigations (Chen *et al*. 2011). The majority of plant materials naturally contain these bioactive constituents, which are recognized to have intriguing biological properties including anti-inflammatory, cancer suppressive, antibacterial, antiviral, and antioxidant agents (Teugwa *et al*. 2013; Al-Rajhi *et al*. 2022a,b; Al-Rajhi *et al*. 2023a; Al-Rajhi and Abdelghany 2023a,b; Alsalamah *et al*. 2023). However, there have been few investigations into *V. agnus-castus* for its pharmaceutical uses. The presence of various bioactive chemical groups and compounds, such as terpenes, polyphenols, terpenoids, fatty acids, steroids, alcohols, aldehydes, and esters, were documented in *V. agnus-castus* extract *via* Fourier transform infrared and gas chromatography-mass spectrometry analyses (Al-Otibi *et al*. 2022).

Supercritical fluid extraction (SFE) is an excellent technique for the extraction of natural compounds because it allows the extraction of heat-susceptible compounds without causing any degradation and is also recognized as an environmentally friendly technology. Through adjusting the extraction temperature and pressure, SFE allows for the manipulation of the yield of extracted compound and selectivity (Jokić *et al*. 2017). Because carbon dioxide (CO_2) (solvent used in the supercritical fluid extraction) is nontoxic, and is easily accessible, affordable, and has low critical point requirements for both pressure and temperature, it has been the most widely used solvent (Bimakr *et al*. 2009). It is unlike the conventional approaches of extraction, which are commonly

performed at high temperatures that can be responsible for the damage of appreciated ingredients. This method also has the advantage that the extraction can be performed under different conditions of temperature, pressure, and extraction time (Bimakr *et al*. 2011; Chamali *et al*. 2023). Currently, there are no data about the influence of static extraction time and dynamic extraction time *via* SFE on *V. agnus-castus*. Therefore, the present study focuses on the influence of extraction time on constituent's analysis of *V. agnus-castus* L. leaves by HPLC. Additionally, this study investigates the antimicrobial, anticancer, and healing activity of maximum yield of *V. agnus-castus* extract.

EXPERIMENTAL

Supercritical Fluid Extraction

According to the description of Žitek *et al*. (2020), the SFE leaves extraction of *V. agnus-castus* (collected from market in Egypt and identified by Prof. Tarek Mohamed, Botany and Microbiology department) was carried out in an ISCO-Sitec modified SFX 220 supercritical fluid extraction system. In this study, 6.0 g of *V. agnus-castus* dried powder were subjected to SEF at two conditions including static extraction time (SET) for 30 min, followed by dynamic extraction time (DET) for 30 min at constant pressure (206.84 bar) and temperature (50 °C) (sample code A). Another sample of *V. agnus-castus* dried powder was extracted at SET for 0 min, followed by DET for 60 min at constant pressure (206.84 bar) and temperature (50 °C) (sample code B). In every run, the supercritical CO_2 consumption and the solvent flow rate remained constant (Hassim *et al*. 2020).

HPLC Analysis

The extract was subjected to HPLC analysis (Waters 2695 Alliance, Waters Inc., Milford, CT, USA) for determining the phenolic and flavonoids contents, which was furnished utilizing an ultraviolet-visible (UV-Vis) DAD. A Waters SunfireTM C18 reverse-phase chromatography column (dimensions: 250 mm length, 4.6 mm width, and 5 µm particle size) was employed to perform the separation. The extract solution and mixture of standard compounds were introduced into the apparatus using an autoinjector. A variety of gradient and isocratic mobile phases were tested at various column temperatures and flow rates to determine an effective separation technique for the standards. The gradient approach was ultimately selected after a sequence of initial investigations. A combination of acetonitrile as mobile phase A and phosphoric acid as mobile phase B was used. The phosphoric acid was prepared by adding 85% orthophosphoric acid dropwise to HPLC grade water until pH = 2. The concentration gradient was changed in the following ways during the method's 60-minute total runtime: A) 5% A + 95% B at first; b) 35% A + 65% B for 15 min; c) 35% A + 65% B for 20 min; d) 40% A + 60% B for 30 min; e) 40% A + 60% B for 35 min; f) 50% A + 50% B for 40 min; g) 70% A + 30% B for 52 min; and h) 5% A + 95% B for 60 min. There was a static flow rate (0.5 mL/min) and temperature (5 °C). Following the examination of the UV-Vis spectra of separate standards, three wavelengths—minimum 210, median 280, and maximum 360 nm—were selected for HPLC examination in this study.

Antimicrobial Screening

The antibacterial and antifungal potential of *V. agnus-castus* extract under different conditions of SFE (static and dynamic extraction times) were examined by the agar well

diffusion technique as designated by Qanash *et al*. (2023b) against the microorganisms: *Mucor circinelloid* (AUMMC 11656), *Candida albicans* (ATCC 10221), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 90274), and *Enterococcus faecalis* (ATCC 29212). Dimethyl sulfoxide (DMSO) was utilized as a solvent for the extract, and then tested as antimicrobial agent. One mL of freshly cultured bacteria/fungi was placed into the midpoint of a sterile petri plates. After cooling, liquefied Mueller-Hinton/potato dextrose for bacteria/fungi was added to the Petri plate comprising the inoculum and mixed thoroughly. In the solidified agar, sterile cork borers of 6 mm diameter were applied to some wells in the agar plates containing the microbial inoculum. Subsequently, 100 µL (extract) was added to the corresponding well. The Petri plates were cooled for 30 min to thoroughly diffuse the extracts in the layer of agar, and then incubated for 1/4 days at 37/30 °C for bacteria/fungi. Measuring the inhibition zone was recorded at the end of incubation period. A 10% of DMSO was used as a negative control while nystatin (500 µg/mL) and ampicillin (1000 µg/mL) were employed against fungi and bacteria, respectively, as positive controls (Abdelghany *et al*. 2019). Minimum inhibitory concentration (MIC) was assayed as follows: the bacterial and fungal inoculum activation and preparation were performed using Mueller Hinton broth for 24 h at 37 °C and potato dextrose broth for 48 h at 30 °C, respectively. The microbial culture was diluted using the appropriate broth to modify the inoculum dose to an optical density of 0.5 McFarland standards. Subsequently, 100 µL of the inoculum was added to each well of a 96-well microtiter plate. Various doses of the extract were then introduced to the wells through serial dilution. The wells containing only media + extract (negative controls) were used, while the wells with microbial inoculum without extract were employed as positive control to estimate the maximum growth. Absorbance of plates was documented at 0 h of inoculum time and again after 24 h at a wavelength of 570 nm. Finally, the MIC value was estimated employing log it analysis. Fungal growth was assessed using the MIC method, where 100 µL of fungal inoculum (adjusted to 0.5 McF of 1.5×10^8 CFU/mL) was spread on a petri dish containing sabouraud dextrose agar medium. The extract was diluted in a DMSO solution (0.1%) to obtain different concentrations ranging from 7.8 to 1000 µg/mL. Subsequently, 10 µL of each dose was applied to a 6 mm agar well, and the fungal culture was then incubated for 4 days at 30 °C. To determine the minimum bactericidal concentration (MBC), certain dilution of examined microbes in MH broth at a concentration of 1×10^6 CFU/mL. The *V. agnus-castus* extract was then diluted at 100% of the MIC and added to 96 microtiter plates in equal volumes (1:1 dilutions). Each concentration of the extract was injected with equal volumes of the examined microbes. Controls (positive and negative) were included in some wells to ensure proper growth during the incubation period. The MBC was determined by observing the dilution that showed a defined decrease in CFU/mL, along with at least two more determined test product dilutions (French 2006). The MBC/MIC index was used to determine whether the *V. agnus-castus* extract had a static or cidal effect. If the ratio of MBC/MIC value was less than or equal to 4, the extract was considered to have a cidal value (Al-Rajhi *et al*. 2023b).

Anticancer Activity

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was utilized to measure the *V. agnus-castus* extract cytotoxicity on human ovarian tumor cell line (SKOV3). In summary, a 96-well plate containing 200 µL of DMEM and 2×10^4 cells/well was seeded with the SKOV3, and the cells were cultured for 12 h. Subsequent treatment, the cells were preserved at 37 °C for 48 h and 5% of CO_2 while being exposed

to varying concentrations of *V. agnus-castus* extract (31.25 up to 1000 µg/mL). Following incubation, 5 mg/mL MTT reagent (20 µL) was added to the cells, and subsequently incubated for 2 h in a CO_2 incubator after the removal of spent medium. After solubilizing the crystals of formazan in 100 µL of DMSO, the wavelength at 570 nm was measured *via* a microplate reader. The cells that were only treated by DMEM were regarded as 100% viable negative controls (Qanash *et al*. 2023a). Using the next formula No:1, the cell viability (%) was determined:

$$\text{Viability (\%)} = 1 - \frac{\text{Optical density of treated cell by extract}}{\text{Optical density of control (untreated cells)}} \times 100 \quad (1)$$

Wound Healing *via* Cell Scratch Test

Using an earlier described method for *in vitro* cell migration studies on L929 cells, the wound healing capabilities of *V. agnus-castus* extract was evaluated. In a nutshell, 6-well plates were seeded by 2×10^4 cells/mL, and then cultured for an entire night. After that, the cells were cleaned using DPBS (Delbucco's Phosphate Buffered Saline) and a sterile 200 µL tip was utilized to make a scratch. The tested cells were washed with DPBS to get rid of the detached cells and cellular debris. After applying 100 µg/mL of *V. agnus-castus* extract, the cells were incubated for 24 h. Negative control cells were untreated. Images captured with an inverted microscope to show cell migration and changes in the morphological profile (Alsalamah *et al*. 2023). Three duplicates of each experiment were run. Analysis was done on the width of the scratch and the wound closure at various time periods (0, 24, and 48 h). The next analysis was computed using Eqs. 2, 3, and 4:

$$\text{Migration rate (MR)} = \frac{\text{Initial width of wound (um)} - \text{Final width of wound (um)}}{\text{Period span of the test (h)}} \times 100 \quad (2)$$

$$\text{Closure of wound (\%)} = \frac{\text{Initial area of wound} - \text{Area of wound after n hours}}{\text{Initial area of wound}} \times 100 \quad (3)$$

$$\text{Difference of area (\%)} = \text{Intial area} - \text{Final area} \quad (4)$$

RESULTS AND DISCUSSION

According to Khaw *et al*. (2017), the SFE was selected as the extraction technique because it guarantees rapid and effective extraction that does not need purification steps and does not include the use of unsafe organic solvents. The extracts of *V. agnus-castus* were subjected to HPLC analysis and biological activities, as presented (Fig. 1). The yield of extraction was higher (0.169 g) at 60 min of DET than the yield of extraction (0.115 g) of the sample exposed to DET (30 min) (Table 1). *Via* SFE, *Vitex negundo* L. leaves were extracted at different conditions of operation including temperature (40 to 65 °C), and pressure (20 to 30 MPa) at constant time (60 min), where the yield of the extract was increased with increasing of temperature up to 50 °C (Mohd *et al.* 2014). Regarding the pressure, the yield of the extract increased at the pressure range from 20 to 30 MPa and at temperature up to 55 °C. Previously, olive leaves were exposed to static condition (for 1 min) of SFE followed by dynamic extraction (5 min up to 140 min), and the quantity extracted yield was 6.7 mg/g and 8.0 mg/g at 20 and 140 min, respectively. Le Floch *et al.*

(1998) and Bimakr *et al*. (2009) studied the impact of DET on the yields of spearmint leaves extract. At constant pressure of 100 bar, they estimated dynamic extraction at different times after the static extraction up to 30 min. The yield of extraction was improved as the dynamic time increased until 90 min, but it reached a maximum yield at 60 min and at 300 bar of pressure.

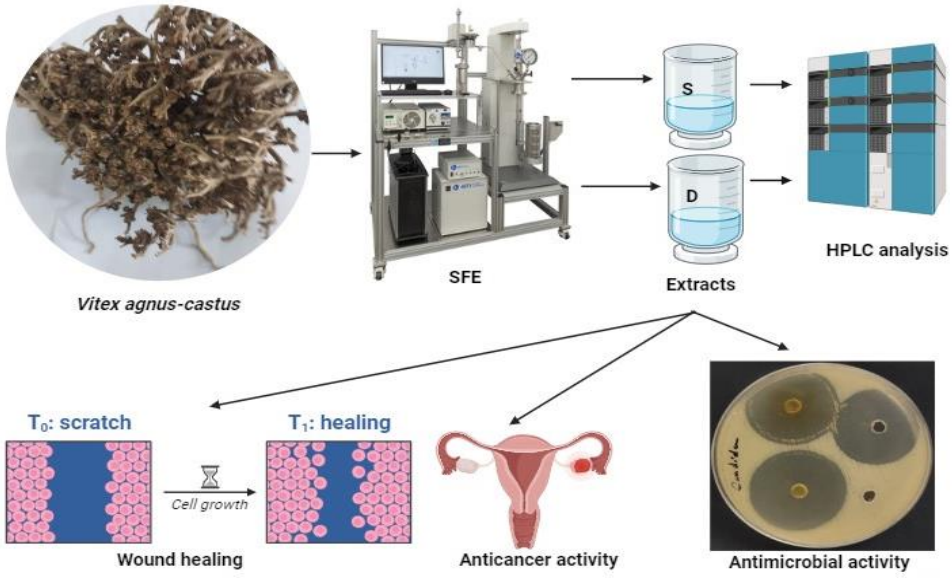

Fig. 1. Extraction of *V. agnus-castus via* SFE at two conditions including static extraction time (S) and dynamic extraction time (D), followed by HPLC analysis of flavonoid and phenolic contents with wound healing, antimicrobial, and anticancer activities of the extracts. Created with BioRender.com

After SFE extraction, the collected extract of *V. agnus-castus* was subjected to HPLC analysis (Figs. 2 and 3) to recognize the contents of phenols and flavonoids in the extract. In sample code B, all constituents of phenols and flavonoids were detected with high concentrations compared to its concentrations in sample code A except one compound namely gallic acid (Table 2). For instance, in the sample code B, the concentrations of cinnamic acid, kaempferol, ferulic acid, rutin, and caffeic acid were respectively 88.7, 1950, 198, 172, and 1210 µg/g, while in the sample code A it was 3.32, 286, 39.0, 35.2, and 302 µg/g with decreasing levels of 96.3%, 85.4%, 80.3%, 79.6%, and 75.1%, respectively. Moreover, hesperetin was detected only in the sample code A. Generally, gallic acid, querectin, chlorogenic acid represent the main detected compounds with high concentration in the extract. The authors' result indicated that the DET was effective in releasing the active constituents in the extract. From recent investigation, vanillic acid represented the major content (22800 µg/L) in the ethanol extract of *V. agnus-castus* seeds besides other phenols, including luteolin, quercetin, fumaric acid, 4-hydroxybenzoic acid, caffeic acid, kaempferol, salicylic acid, butein, resveratrol, ellagic acid, catechin hydrate, phloridzin dehydrate, and naringenin (Kavaz *et al*. 2022). Berrani *et al*. (2021) reported the presence of 25 flavonoids and phenols *via* HPLC-DAD-QTOF-MS analysis with a notable variability among plant parts. Hesperidin, chlorogenic, luteolin, vanillic, 3-hydroxy-benzoic, and 3,4-dihydroxybenzoic were registered with high levels in *V. agnus-castus*. Regarding the effect of static and extraction dynamic time on the phenolic and flavonoid compounds in *V. agnus-castus* extract, previous reports indicated that the extraction yield

of phenolic constituents is affected by pressure, time, temperature, and addition of co-solvents (Junior *et al*. 2010; Bimakr *et al*. 2011). Moreover, in the SFE mode (static or dynamic), the solvent flow rate (Pourmortazavi and Hajimirsadeghi 2007; Leal *et al*. 2008) affected the extraction yield of natural extracts.

Table 1. Extraction Yield of *V. agnus-castus via* SFE at Two Different Conditions (SET and DET) at Constant Temperature and Pressure

Condition Code	Temp. (ºC)	Pressure (bar)	SET (min)	DET (min)	Plant Quantity (g)	Yield of Extract (g)
A	50	206.84	30 min	30	6.0	0.115
B	50	206.84	0 min	60	6.0	0.169

Table 2. Phenols and Flavonoid Compounds of *V. agnus-castus* Extracted *via* SFE in SET (Sample Code A) and DET (Sample Code B) Conditions

Detected Constituent	Sample Code A			Sample Code B		
	*RT	Area (mAU*s)	**Conc. (µg/g)	RT	Area (mAU*s)	Conc. (µg/g)
Gallic acid	3.582	896.90	3966.66	3.585	692.44	3062.41
Chlorogenic acid	4.293	229.55	1489.30	4.297	327.03	2121.70
Catechin	4.652	139.40	1503.75	4.709	180.24	1944.31
Methyl gallate	5.608	100.72	253.75	5.603	401.06	1010.40
Caffeic acid	5.794	78.05	301.98	6.179	313.94	1214.69
Syringic acid	6.380	177.10	647.60	6.375	248.71	909.47
Pyro catechol	6.631	0.00	0.00	6.631	0.00	0.00
Rutin	7.083	4.77	35.21	7.081	23.36	172.28
Ellagic acid	7.318	40.04	199.96	7.347	91.43	456.67
Coumaric acid	8.688	6.73	11.98	8.693	14.91	26.53
Vanillin	8.989	352.12	654.30	8.989	970.21	1802.82
Ferulic acid	9.982	13.42	38.98	9.982	68.00	197.52
Naringenin	10.332	1.23	5.63	10.331	3.22	14.74
Rosmarinic acid	11.902	49.54	265.57	11.895	131.40	704.44
Daidzein	15.756	119.51	335.12	15.755	431.70	1210.53
Querectin	17.341	83.84	565.80	17.338	322.55	2176.85
Cinnamic acid	19.268	3.71	3.32	19.284	99.08	88.72
Kaempferol	20.633	90.80	286.38	20.635	619.83	1954.91
Hesperetin	21.223	0.00	0.00	21.462	9.55	23.48

*RT: retention time, **Conc., Concentration

In the present investigation, the extraction *via* SFE focused on the effect of extraction time on the yield of the extract. Several studies were reported on other plants, for instance, the best conditions were 60 °C, 60 min, and 200 bar for spearmint flavonoids extraction comparable to other conditions, primarily temperature (40 °C and 50 °C), extraction time (30 min and 90 min), and pressure (100 bar and 300 bar) *via* SFE (Bimakr *et al*. 2011). According to the result of Hassim *et al*. (2020), 60 min of SET was the best condition for total yield of the extract of *Phyllanthus niruri*. Influence of temperature and time of extraction was studied on phytochemical characterization, extraction yield, anti-xanthine oxidase, and antioxidant activities. Dynamic time (36 min) and a temperature (179 °C) were the optimum conditions for extraction and biological activities of *Eucalyptus intertexta* (Chamali *et al*. 2023).

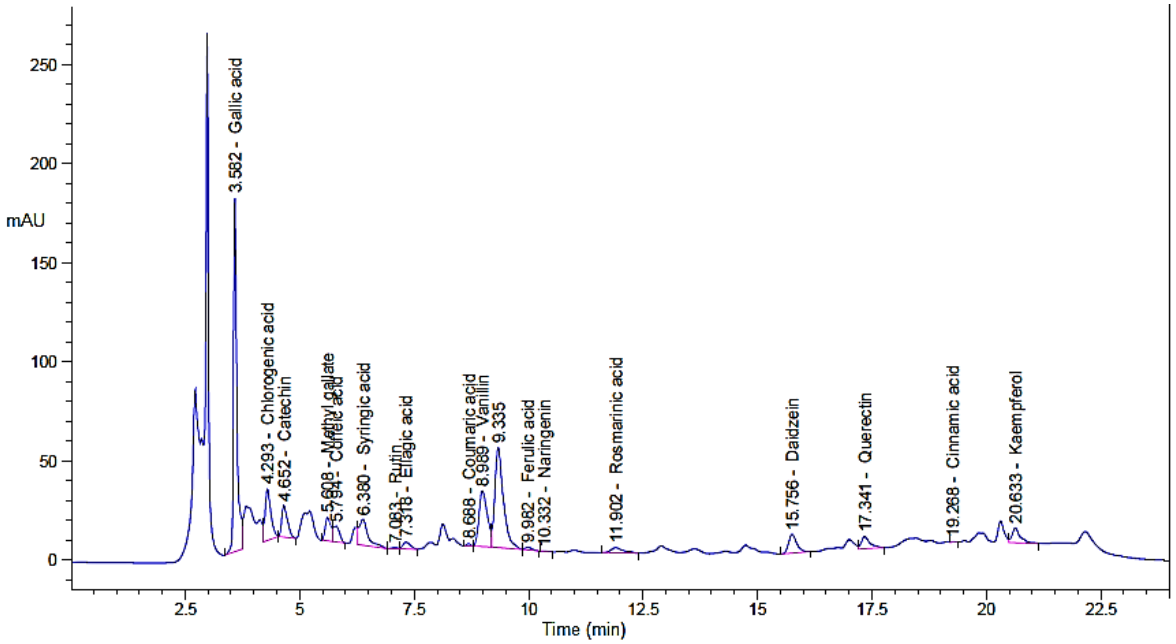

Fig. 2. HPLC analysis of metabollites namely flavonids and phenols in *V. agnus-castus* extractred *via* SFE at static extraction time

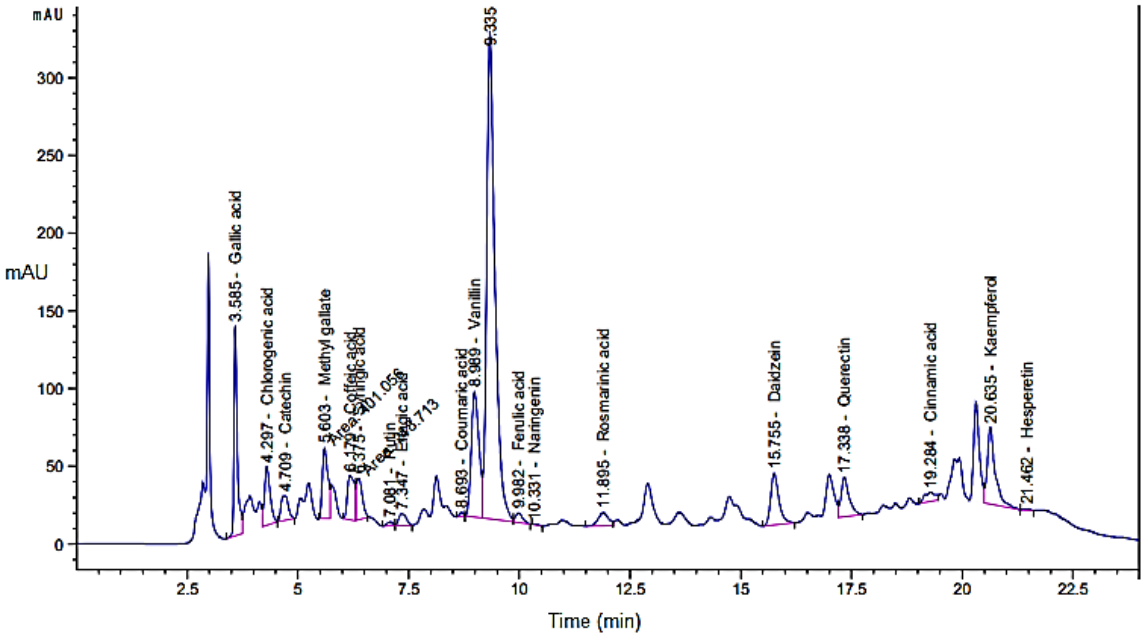

Fig. 3. HPLC analysis of metabollites namely flavonids and phenols in *V. agnus-castus* extractred *via* SFE at dynamic extraction time

The antimicrobial properties of *V. agnus-castus* extracts under SET and DET against *S. aureus*, *P. areginosa*, *K. pneumoniae*, *E. faecalis*, *C. albicans*, and *M. circinelloid* were evaluated in this study (Table 3 and Fig. 4). The obtained findings revealed that the *V. agnus-castus* extracts are effectively suppressing the microbial growth with variable potency based on the conditions of the extraction process. As stated in the results, sample code B of the *V. agnus-castus* extract had high zones of inhibition 32 ± 0.1, 20 ± 0.2, 32 ± 0.2, 35 ± 0.2, and 40 ± 0.1 mm, whereas sample code A of the extract showed

less zones of inhibition 30 ± 0.1, 16 ± 0.1, 25 ± 0.1, 33 ± 0.1, and 35 ± 0.1 mm against *S. aureus*, *P. areginosa*, *K. pneumoniae*, *E. faecalis*, and *C. albicans*, respectively. The extract failed to suppress the growth of *M. circinelloid*. This result may be due to the structure of cell wall that differed from the other tested microorganisms, or because of the incapacity of the extract to pass through the cell membrane. The antimicrobial activity of *V. agnus-castus* extract is attributed to their content of phenols and flavonoids, particularly in the sample of code B that could join to cell membrane proteins *via* hydrophobic and hydrogen bonding. Currently from HPLC analysis, the extract of *V. agnus-castus* contains high concentration of caffeic acid. In another report, Alfarrayeh *et al*. (2021) noted that leaves of *V. agnus-castus* contained a great concentration of caffeic acid. It was discovered that caffeic acid inhibited the growth of various strains of *Candida* by influencing their capacity to form biofilms and mature, ultimately leading to their mortality. Based on findings of Kavaz *et al*. (2022), *Escherichia coli*, *Salmonella typhimurium*, and *S. aureus* were inhibited by seed extract of *V. agnus-castus*. Morphological and ultrastructure alterations were observed *Candida famata* as a result of exposure to *V. agnus-castus* extract (Al-Otibi *et al*. 2022). The results in Table 3 showed that *P. areginosa* was less affected by the extract than other bacteria. This may be explained by the ability of this bacterium to form biofilm that represent one of the mechanisms of drug resistance. Moreover, the extracellular matrix of bacterial biofilm is commonly impermeable and may control the diffusion of antibacterial compounds *via* attaching to the antibacterial compound and obstructing target locations (Alsolami *et al.* 2023).

Table 3. Activity of *V. agnus-castus* extractred *via* SFE at SET (Sample Code A) DET (Sample Code B) against Different Microorganisms

Tested Microorganisms	Inhibition Zones (mm)			
	Sample Code A	Sample Code B	*Positive Control	Negative Control
S. aureus	30 ± 0.1	32 ± 0.1	28 ± 0.1	0.0
P. areginosa	16 ± 0.1	20 ± 0.2	16 ± 0.1	0.0
K. pneumoniae	25 ± 0.1	32 ± 0.2	27 ± 0.1	0.0
E. faecalis	33 ± 0.1	35 ± 0.2	30 ± 0.1	0.0
C. albicans	35 ± 0.1	40 ± 0.1	36 ± 0.3	0.0
M. circinelloid	NA	NA	23 ± 0.1	0.0

* Ampicillin /Nystatin was applied as positive control

Sample code A of the *V. agnus-castus* extract possesses higher MIC and MBC values than the sample of code B against tested bacteria and *C. albicans* (Table 4). The highest MIC and MBC extract were associated with *P. areginosa* with MIC quantities of 250 and 62.5 µg/mL, MBC quantities of 1000 and 125 µg/mL, correspondingly. Gonçalves *et al*. (2017) mentioned that the extract of *V. agnus-castus* from ethanol had promising growth inhibition against *Lactobacillus casei* and *Streptococcus mutans* with an MIC value of 15.6 µg/mL and *Streptococcus mitis* with an MIC value of 31.2 µg/mL. Bouyahya *et al.* (2017) mentioned that the antibacterial potential of *Vitex agnus-castus* extracts is perhaps due to the main phenolic constituents such as chlorogenic acid and luteolin that display antibacterial activity. Moreover, analysis of the different parts of *V. agnus castus* showed the presence of 25 compounds associated to phenols, where the registered compounds with high levels were vanillic acid, chlorogenic acid, hesperidin, luteolin, 3-hydroxybenzoic acids and 3,4-dihydroxybenzoic. The growth of five species of bacteria was inhibited by the extracts with MIC values ranging from 7.81 and 31.2 µg/mL (Berrani *et al.* 2021).

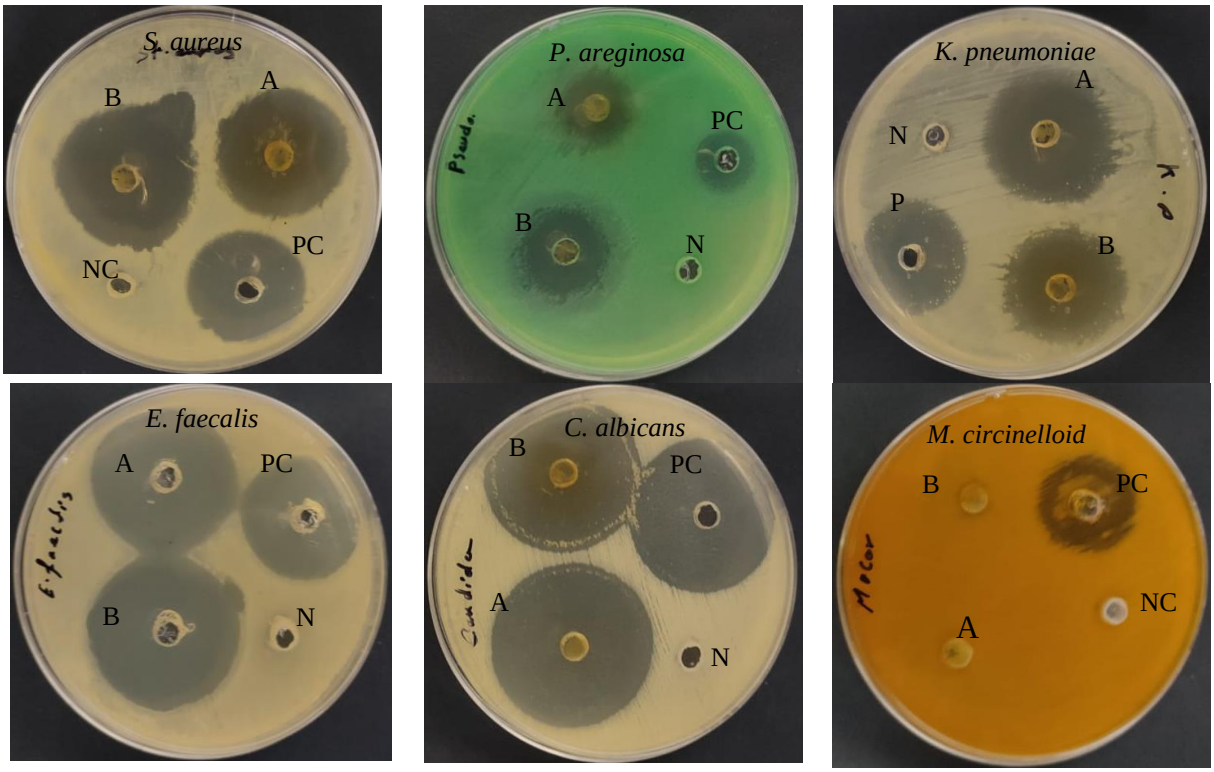

Fig. 4. Activity of *V. agnus-castus* extracted *via* SFE at SET (A) DET (B) against different microorganisms. Negative control (NC) (10% of DMSO), positive control (PC) nystatin (500 µg/mL) and ampicillin (1000 µg/mL)

Table 4. Values of MIC and MBC Besides the MIC/MBC Index of *V. agnus-castus* Extracted *via* SFE at SET (Sample code A) and DET (Sample Code B) against Different Microorganisms

Tested Microorganisms	MIC of Sample Code		MBC of Sample Code		MBC/MIC Index of Sample Code	
	A	B	A	B	A	B
S. aureus	15.62	7.8	62.5	7.8	4	1
P. areginosa	250	62.5	1000	125	4	2
K. pneumoniae	31.25	15.62	62.5	31.25	2	2
E. faecalis	7.8	15.62	15.62	7.8	2	1
C. albicans	7.8	3.9	31.25	7.8	4	2

The extract showed anticancer activity against SK-OV3 cells line, but extraction condition of the sample code B reflected better toxicity than the sample code A (Table 5 and Fig. 5). No toxicity was observed at 31.2 and 62.5 µg/mL of the extract of sample code A. Moreover, less IC_{50} value was recorded, 164.51 ± 1.2 µg/mL for the extract of the sample code B than the IC_{50} value of 209.02 ± 4.11 µg/mL for the extract of the sample code A. As presented in Table 5, the cytotoxic effect was linearly associated with the dose of the extract. Previously, Ohyama *et al*. (2003) reported the DNA fragmentation and apoptosis of SKOV-3 cells treated by *V. agnus-castus* extract, which may be attributed to amplified intracellular oxidation. Another type of cancer cells, namely MCF-7 breast cells was suppressed by seeds extract of *V. agnus-castus* (Sultan and Aşkın 2013). From the detected flavonoids in the extract of *V. agnus-castus*, daidzein was detected in high

concentration. Hua *et al*. (2018) found that daidzein stimulated the morphological alteration in SKOV3 cells and mitochondrial apoptosis with IC_{50} value of 20 µM, while it reflected high IC_{50} value (100 µM) against normal ovarian cells. Data from Hamza *et al*. (2019) recorded ameliorative effects of *Vitex agnus-castus* extract on the syndrome of polycystic ovary *via* the modulation of lipid and hormonal profile in addition to oxidative stress. Furthermore, the promising effects of these constituents are comparable to metformin. As the dose of the extract increased, particularly the extract of the sample code B, the cancer cells of SKOV3 became shrunken, rounder, and detached from the substratum, which are vital morphological alterations linked with apoptosis (Fig. 5). The several changes in the treated MCF-7 breast cells by *V. agnus-castus* extract were observed by Sultan and Aşkın (2013) including condensation of chromatin, cell shrinkage, nuclear fragmentation, and visualization of membrane-linked apoptotic bodies.

Table 5. Cytotoxicity of *V. agnus-castus* Extract *via* SFE at SET (Sample Code A) and DET (Sample Code B) against SK-OV3 Cells

Concentration (µg/mL)	Sample Code A				Sample Code B			
	Mean O.D	SE (±)	% of Viability	% of Toxicity	Mean O.D	SE (±)	% of Viability	% of Toxicity
0.0	0.74	0.010	100.0	0.00	0.74	0.01	100.0	0.00
31.25	0.68	0.001	100.0	0.00	0.68	0.002	99.85	0.146
62.5	0.69	0.002	100.0	0.00	0.61	0.006	88.56	11.44
125	0.53	0.008	78.00	22.00	0.41	0.008	59.90	40.10
250	0.25	0.007	36.25	63.75	0.15	0.006	21.46	78.54
500	0.09	0.006	13.72	86.28	0.05	0.004	7.93	92.07
1000	0.01	0.002	4.28	95.72	0.02	0.001	3.26	96.74
IC_{50} ± SD	209.02 ± 4.11 µg/mL				164.51 ± 1.2 µg/mL			

Management of inflammation is a critical agent of wound-healing stages because extreme inflammation minimizes healing of wounds. The anti-inflammatory influence of *V. doniana* may be assistance for wound repair. The present investigation showed the presence of chlorogenic acid in the *V. agnus-castus* fruit extracts, and according to Rohrl *et al*. (2017), this acid reflected good antioxidant and minimize the inflammations of tissues. In the present investigation, sample code B of the *V. agnus-castus* extract was tested for healing of wounds *in vitro* (Fig. 6 and Table 6) because it contains high concentration of active compounds. From the obtained results, treatment by *V. agnus-castus* extracts reflected wound healing. The indicated signs of wound healing involving migration rate (13.4 um), wound closure % (75.5 μm^2), and area difference (489,000 %) were tabulated (Table 6) as a result of exposure to *V. agnus-castus* extract.

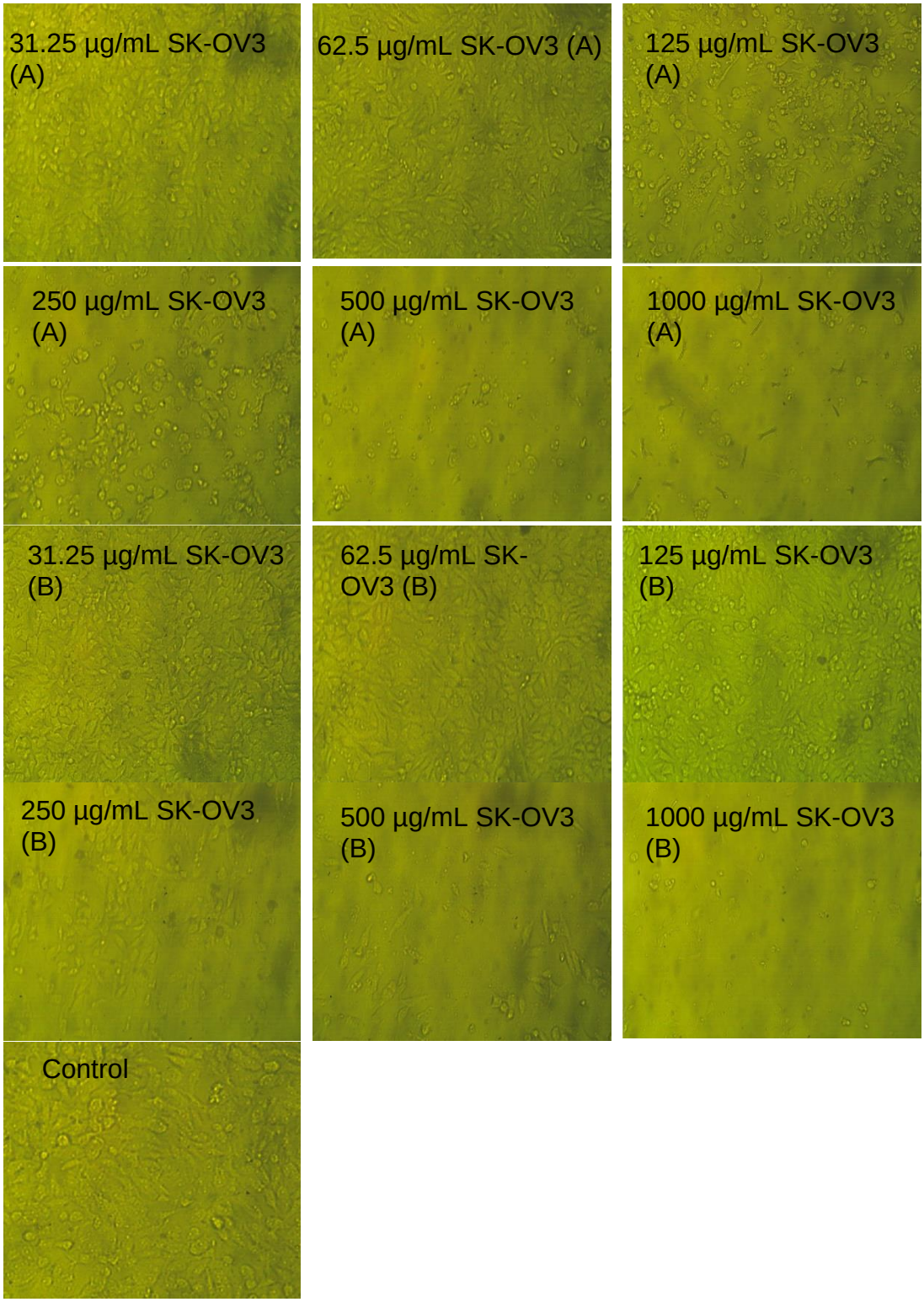

Fig. 5. Morphological changes of SK-OV3 cells exposed to various doses (31.25 to 1000 µg/mL) of extracted *V. agnus-castus via* SFE at SET (A) DET (B). Photos were imagined prior to 24 h cultivation at magnification 40X

Table 6. Healing Activity of *V. agnus-castus* extracted *via* SFE at Dynamic Extraction Time (Sample Code B)

Treatment	Time (h)				RM (µm)	Wound Closure (% µm²)	Area Difference (%)
	0.0 h		48 h				
	Area	Width	Area	Width			
Control (without treatment)	856.0	648884.5	326.0	247120.4			
	826.0	629476.4	218.1	166188.4			
	866.0	654705.4	294.0	222285.3			
	872.1	661059.8	280.0	212268.7			
	836.0	630427.8	180.0	135765.1			
	856.0	648884.5	326.0	247120.4			
	Mean						
	853.4	647450.5	237.0	179727.5	12.8	72.2	468,000
Sample Code B	856.0	648884.5	88.0	66706.11			
	826.0	629476.4	176.1	134153.7			
	866.1	654705.4	260.0	196584.2			
	872.1	661059.8	240.7	182461.7			
	836.0	630427.8	116.1	87523.69			
	864.1	660149.0	374.0	285755.8			
	Mean						
	853.4	647450.5	209.1	158864.2	13.4	75.5	488586.3

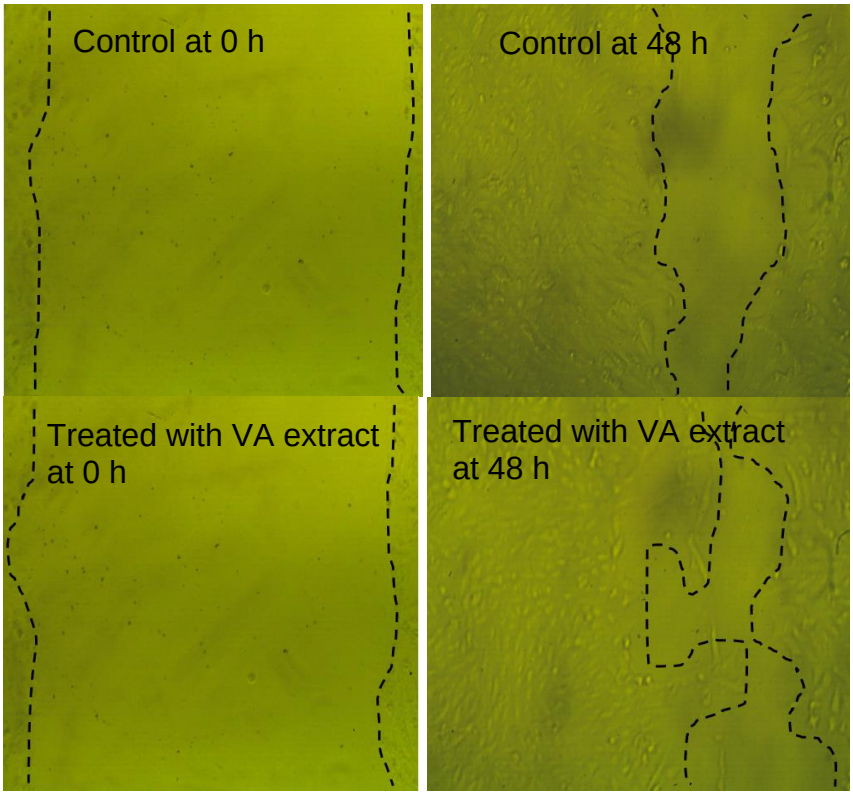

Fig. 6. Descriptions of scratch exam showed the influence of *V. agnus-castus* (VA) extracted *via* SFE at dynamic extraction time (Sample code B) compared to control on the area of wounds at 0 and 48 h

Untreated cells exhibited less values of migration rate (12.8 µm), wound closure % (72.2 µm^2), and area difference (468,000%). The mechanism of *V. agnus-castus* extracts may improve the healing stages through reducing the inflammation and oxidative stress.

Previously, Nyiligira *et al*. (2008) mentioned that several species of *Vitex*, including *V. agnus-castus*, *V. piramidata*, *V. pubescens*, and *V. cienkowskii*, have been registered to be applied in traditional treatments of an extensive variety of ailments, such as venereal diseases, depression, allergy, malaria, skin diseases, wounds, and inflammation.

CONCLUSIONS

1. In the present study the dynamic extraction time was positively efficient relative to extraction yield except for flavonoid and phenolic compounds.
2. Moreover, anticancer activity against SK-OV3 cells, healing process, and antimicrobial activity against different microorganisms represented with inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values of the *V. agnus-castus* extract was highest at the extraction dynamic time of 60 min.
3. This study helps to authenticate the traditional application of *V. agnus-castus* particularly when extracted by SFE under the effect of extraction time as well as encourages investigators to further research the separated compounds from *V. agnus-castus*.

ACKNOWLEDGEMENT

This work was funded by the Deanship of Graduate Studies and Scientific Research at Jouf University under grant No. DGSSR-2023-01-02002.

REFERENCES CITED

Abdel Ghany, T. M., Mahmoud, M. S., Alawlaqi, M. M., Reyad, A. M., Al-Rajhi, A. M. H., and Abdkareem, E. M. (2021). "Physicochemical characterization of agricultural run-off and groundwater inoculated by *Trichoderma asperellum* and its effect on anti-oxidative enzymes production by irrigated *Trifolium alexandrinum* L.," *BioResources* 16(2), 3272-3284. DOI: 10.15376/biores.16.2.3272-3284

Abdelghany, T. M. (2014). "Eco-friendly and safe role of *Juniperus procera* in controlling of fungal growth and secondary metabolites," *J. Plant Pathol. Microbiol*. 5, article 231. DOI: 10.4172/2157-7471.1000231

Abdelghany, T. M., Ganash, M. A., Bakri, M. M., Al-Rajhi, A. M. H., and Al Abboud, M. A. (2016). "Evaluation of natural sources for repress cytotoxic Trichothecenes and Zearalenone production with using Enzyme-linked immunosorbent assay," *Life Sci. J*. 13(8), 74-86. DOI: 10.7537/marslsj130816.13

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.

Adamov, G. V., Rendyuk, T. D., Saybel, O. L., Dargaeva, T. D., Tsitsilin, A. N., and Bokov, D. O. (2022). "*Vitex agnus-castus*: Botanical features and area, chemical

composition of fruit, pharmacological properties, and medicinal uses," *Journal of Applied Pharmaceutical Science* 12(3), 034-044. DOI: 10.7324/JAPS.2022.120304

Alfarrayeh, I., Pollák, E., Czéh, Á., Vida, A., Das, S., and Papp, G. (2021). "Antifungal and anti-biofilm effects of caffeic acid phenethyl ester on different candida species," *Antibiotics* 10(11), article 1359.

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

Al-Otibi, F. O., Alrumaizan, G. I., and Alharbi, R. I. (2022). "Evaluation of anticandidal activities and phytochemical examination of extracts prepared from *Vitex agnus-castus*: A possible alternative in treating candidiasis infections," *BMC Complement Med. Ther.* 22, article 69. DOI: 10.1186/s12906-022-03552-x

Al-Rajhi A. M., Qanash, H., Bazaid. A. S., Binsaleh, N. K., and Abdelghany ,T. M. (2023b). "Pharmacological evaluation of *Acacia nilotica* flower extract against *Helicobacter pylori* and human hepatocellular carcinoma in vitro and in silico," *Journal of Functional Biomaterials* 14(4), article 237. DOI:10.3390/jfb14040237

Al-Rajhi, A. M., and Abdelghany, T. M. (2023a). "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., and Abdelghany, T. M. (2023b). "*In vitro* repress of breast cancer by bio-product of edible *Pleurotus ostreatus* loaded with chitosan nanoparticles," *Appl. Biol. Chem.* 66, article 33. DOI: 10.1186/s13765-023-00788-0

Al-Rajhi, A. M., 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 7402. DOI: 10.3390/molecules28217402

Al-Rajhi, A. M., Yahya, R., Abdelghany, T. M., Fareid, M. A., Mohamed, A. M., Amin, B. H., and Masrahi, A. S. (2022b). "Anticancer, anticoagulant, antioxidant and antimicrobial activities of *Thevetia peruviana* latex with molecular docking of antimicrobial and anticancer activities," *Molecules* 27(10), article 3165. DOI: 10.3390/molecules27103165

Al-Rajhi, A. M., Yahya, R., Alawlaqi, M. M., Fareid, M. A., Amin, B. H., and Abdelghany, T. M. (2022a). "Copper oxide nanoparticles as fungistat to inhibit mycotoxins and hydrolytic enzyme production by *Fusarium incarnatum* isolated from garlic biomass," *BioResources* 17(2), 3042-3056. DOI: 10.15376/biores.17.2.3042-3056

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

Alsolami, A., Bazaid, A. S., Alshammari, M. A., Qanash, H., Amin, B. H., Bakri, M. M., and Abdelghany, T. M. (2023). "Ecofriendly fabrication of natural jojoba nanoemulsion and chitosan/jojoba nanoemulsion with studying the antimicrobial, anti-biofilm, and anti-diabetic activities *in vitro*," *Biomass Conv. Bioref*. DOI: 10.1007/s13399-023-05162-0

Berrani, A., Marmouzi, I., Bouyahya, A., Kharbach, M., El Hamdani, M., El Jemli, M., Lrhorfi, A., Zouarhi, M., Faouzi, M. E. A., and Bengueddour, R. (2021). "Phenolic compound analysis and pharmacological screening of *vitex agnus-castus* functional parts," *Biomed. Res. Int.* 21, article ID 6695311. DOI: 10.1155/2021/6695311

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.

Bimakr, M., Rahman, R. A., Taip, F. S., Ganjloo, A., Salleh, L. M., Selamat, J., and Zaidul, I. S. M. (2011). "Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves," *Food and Bioproducts Processing* 89(1), 67-72. DOI: 10.1016/j.fbp.2010.03.002

Bouyahya, A., Dakka, N., Et-Touys, A., Abrini, J., and Bakri, Y. (2017). "Medicinal plant products targeting quorum sensing for combating bacterial infections," *Asian Pacific Journal of Tropical Medicine* 10(8), 729-743. DOI: 10.1016/j.apjtm.2017.07.021.

Chamali, S., Bendaoud, H., Bouajila, J., Camy, S., Saadaoui, E., Condoret, J. S., and Romdhane, M. (2023). "Optimization of accelerated solvent extraction of bioactive compounds from *Eucalyptus intertexta* using response surface methodology and evaluation of its phenolic composition and biological activities," *Journal of Applied Research on Medicinal and Aromatic Plants* 35, article ID 100464. DOI: 10.1016/j.jarmap.2023.100464

Chen, S. N., Friesen, J. B., Webster, D., Nikolic, D., van Breemen, R. B., Wang, Z. J., Fong, H. H., Farnsworth, N. R., and Pauli, G. F. (2011). "Phytoconstituents from *Vitex agnus-castus* fruits," *Fitoterapia* 82(4), 528-533.

French, G. L. (2006). "Bactericidal agents in the treatment of MRSA infections—The potential role of daptomycin," *J. Antimicrob. Chemother.* 58, article 1107.

Gonçalves, R., Ayres, V. F. S., Carvalho, C. E., Souza, M. G. M., Guimarães, A. C., Corrêa, G. M., Martins, C. H. G., Takeara, R., Silva, E. O., and Crotti, A. E. M. (2017). "Chemical composition and antibacterial activity of the essential oil of *Vitex agnus-castus* L. (Lamiaceae)," *An Acad Bras Cienc*. 89(4), 2825-2832. DOI: 10.1590/0001-3765201720170428

Hamza, A. H., AlBishri, W. M., and Alfaris, M. H. (2019). "Effect of *Vitex agnus-castus* plant extract on polycystic ovary syndrome complications in experimental rat model," *Asian Pacific Journal of Reproduction* 8(2), 63-69. DOI: 10.4103/2305-0500.254647

Hassim, N., Markom, M., Rosli, M. I., and Harun, S. (2020). "Effect of static extraction time on supercritical fluid extraction of bioactive compounds from *Phyllanthus niruri*," *Journal of Computational and Theoretical Nanoscience* 17(2-3), 918-924. DOI: 10.1166/jctn.2020.8742

Hua, F., Li, C. H., Chen, X. G., and Liu, X. P. (2018). "Daidzein exerts anticancer activity towards SKOV3 human ovarian cancer cells by inducing apoptosis and cell cycle arrest, and inhibiting the Raf/MEK/ERK cascade," *Int. J. Mol. Med.* 41(6), 3485-3492. DOI: 10.3892/ijmm.2018.3531

Jokić, S., Jerković, I., Rajić, M., Aladić, K., Bilić, M., and Vidović, S. (2017). "SC-CO_2 extraction of *Vitex agnus-castus* L. fruits: The influence of pressure, temperature and water presoaking on the yield and GC–MS profiles of the extracts in comparison to

the essential oil composition," *The Journal of Supercritical Fluids* 123, 50-57. DOI: 10.1016/j.supflu.2016.12.007

Junior, M. R. M., Leite, A. V., and Dragano, N. R. V. (2010). "Supercritical fluid extraction and stabilization of phenolic compounds from natural sources–review (supercritical extraction and stabilization of phenolic compounds)," *The Open Chemical Engineering Journal* 4(1), 51-60

Kavaz, A., Işık, M., Dikici, E., and Yüksel, M. (2022). "Anticholinergic, antioxidant, and antibacterial properties of *Vitex Agnus-Castus* L. seed extract: Assessment of its phenolic content by LC/MS/MS," *Chem. Biodivers*. 19(10), article ID e202200143. DOI: 10.1002/cbdv.202200143

Khaw, K.-Y., Parat, M.-O., Shaw, P. N., and Falconer, J. R. (2017). "Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review," *Molecules* 22(7), article 1186. DOI: 10.3390/molecules22071186

Le Floch, F., Tena, M. T., Ríos, A., and Valcárcel, M. (1998). "Supercritical fluid extraction of phenol compounds from olive leaves," *Talanta* 46, 1123-1130.

Leal, P. F., Maia, N. B., Carmello, Q. A. C., Catharino, R. R., Eberlin, M. N., and Angela M. A. (2008). "Sweet basil (*Ocimum basilicum*) extracts obtained by supercritical fluid extraction (SFE): Global yields, chemical composition, antioxidant activity, and estimation of the cost of manufacturing," *Food Bioprocess. Technol*. 1, 326-333.

Mari, A., Montoro, P., Pizza, C., and Piacente S. (2012). "Liquid chromatography tandem mass spectrometry determination of chemical markers and principal component analysis of *Vitex agnus-castus* L. fruits (Verbenaceae) and derived food supplements," *J. Pharm. Biomed. Anal.* 70, 224-230.

Mohd, T. A. T., Alias, N. H., Ghazali, N. A., Azizi, A., Adeib, I. S., and Sauki, A. (2014). "Potential of five-leaved chaste tree (*Vitex negundo* L.) leaves as source of natural dye from supercritical carbon dioxide (sc-CO_2) extraction," *Key Engineering Materials* 594, 207-213. DOI: 10.4028/www.scientific.net/KEM.594-595.207

Nyiligira, E., Viljoen, A. M., Van Heerden, F. R., Van Zyl, R. L., Van Vuuren, S. F., Steenkamp, P. A. (2008). "Phytochemistry and in vitro pharmacological activities of South African *Vitex* (Verbenaceae) species," *J. Ethnopharmacol.* 119, 680-685.

Ohyama, K., Akaike, T., Hirobe, C., and Yamakawa, T. (2003). "Cytotoxicity and apoptotic inducibility of *Vitex agnus-castus* fruit extract in cultured human normal and cancer cells and effect on growth," *Biological and Pharmaceutical Bulletin* 26(1), 10-18.

Pourmortazavi, S. M., and Hajimirsadeghi, S. S. (2007). "Supercritical fluid extraction in plant essential and volatile oil analysis," *J. Chromatogr. A* 1163, 2-24.

Qanash, H., Al-Rajhi, A. M. H., Almashjary, M. N., Ammar, A. B., Mohannad, S. H., and Abdelghany, T. M. (2023a). "Inhibitory potential of rutin and rutin nano-crystals against *Helicobacter pylori*, colon cancer, hemolysis and Butyrylcholinesterase *in vitro* and *in silico*," *Appl. Biol. Chem.* 66, article 79. DOI: 10.1186/s13765-023-00832-z

Qanash, H., Bazaid, A. S., Aldarhami, A., Alharbi, B., Almashjary, M. N., Hazzazi, M. S., Felemban, H. R., and Abdelghany, T. M. (2023b). "Phytochemical characterization and efficacy of *Artemisia judaica* extract loaded chitosan nanoparticles as inhibitors of cancer proliferation and microbial growth," *Polymers* 15(2), article 391. DOI: 10.3390/polym15020391

Qanash, H., Yahya, R., Bakri, M. M., Bazaid, A. S., Qanash, S., Shater, A. F., and Abdelghany, T. M. (2022). "Anticancer, antioxidant, antiviral and antimicrobial activities of Kei Apple (*Dovyalis caffra*) fruit," *Sci Rep* 12, article 5914. DOI: 10.1038/s41598-022-09993-1

Rohrl, J., Werz, O., Ammendola, A., and Kunstle, G. (2017). "*Vitex agnus-castus* dry extract BNO 1095 (Agnucaston®) inhibits uterine hyper-contractions and inflammation in experimental models for primary dysmenorrhea," *Clin. Phytoscience* 2, article 20. DOI: 10.1186/s40816-016-0034-3

Shaw, S., Wyatt, K., Campbell, J., Ernst, E., and Thompson-Coon, J. (2018). "*Vitex agnus castus* for premenstrual syndrome," *Cochrane Database Syst Rev*. 3, article ID CD004632.

Sultan, A., Ö., and Aşkın, Ç., T. (2013). "Antioxidant activity and anticancer effect of *Vitex agnus-castus* L. (Verbenaceae) seed extracts on MCF–7 breast cancer cells," *Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics* 66(3), 257-267. DOI: 10.1080/00087114.2013.850797

Teugwa, C. M., Mejiato, P. C., Zofou, D., Tchinda, B. T., and Boyom, F. F. (2013). "Antioxidant and antidiabetic profiles of two African medicinal plants: *Picralima nitida* (*Apocynaceae*) and *Sonchus oleraceus* (*Asteraceae*)," *BMC Complement Altern Med*. 13, article 175.

Zahid, H., Rizwani, G. H.m and Ishaqe, S. (2016). "Phytopharmacological review on *Vitex agnus-castus*: A potential medicinal plant," *Chinese Herb. Med.* 8, 24-29.

Žitek, T., Leitgeb, M., Golle, A., Dariš, B., Knez, Ž., and Knez Hrnčič, M. (2020). "The influence of hemp extract in combination with ginger on the metabolic activity of metastatic cells and microorganisms," *Molecules* 25(21), article 4992. DOI: 10.3390/molecules25214992

Article submitted: April 28, 2024; Peer review completed: June 8, 2024; Revised version received and accepted: June 10, 2024; Published: July 12, 2024.
DOI: 10.15376/biores.19.3.5793-5810