Influence of *Duranta erecta* Fruits Extract Prepared *via* Supercritical Fluid Extraction on Microbial Growth, Ultrastructure, *in-vitro* Wound Healing and Oxidant Stress

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Diverse metabolites of plants exhibit various biological activities. Supercritical fluid extraction (SFE) was applied at various temperatures (40, 60, and 80 ºC) to extract *Duranta erecta* fruits. Maximum yield of extract (0.456 g) was obtained at 60 ºC; besides, at this temperature the release of gallic acid, chlorogenic acid, methyl gallate, rutin, naringenin, rosmarinic acid, daidzein, quercetin, and kaempferol were promoted in high concentrations of 3510, 277, 326, 571, 7460, 1060, 31000, 7770, and 103 µg/mL, respectively. Moreover, *S. aureus*, *S. typhi*, *B. subtilis*, *E. coli*, and *C. albicans*, were inhibited with highest inhibition zones such as 28±0.1, 27±0.2, 30±0.1, 25±0.1, and 30±0.2 mm, respectively at 60 ºC than that at other temperatures of the SFE. Low quantities of minimum inhibitory and minimum bactericidal concentrations of the extract were recorded at 60 ºC. Ultrastructural changes were observed in the exposed *B. subtilis* to *D. erecta* fruits extract at 60 and 80 ºC including irregular, and rupture of cell wall. Antioxidant potential of *D. erecta* fruits extract *via* DPPH was recorded with promising IC_{50} value of 9.66 µg/mL. Moreover, FRAP antioxidant activity was confirmed with 355 equivalent (AAE) µg/mg at 60 ºC. The fruits extract from *D. erecta* at 60 ºC of SFE conditions reflected excellent wound healing property.

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INTRODUCTION

The utilization of pharmaceutical sources from natural origin has gained traction amongst communities and individuals in developing nations. The adoption of herbal remedies has witnessed a surge due to their affordability, cultural acceptance, accessibility, effectiveness, and purportedly reduced side effects when compared to synthetic drugs (Qanash *et al*. 2022). Numerous plants such as *Cupressus sempervirens, Thevetia peruviana*, and *Musa paradisiaca* commonly contain bioactive constituents such as phenols and flavonoids that exhibit various bio-beneficial utilizations such as cancer-suppressive, antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral effects (Al-Rajhi *et al*. 2022a,b; Al-Rajhi *et al*. 2023; Al-Rajhi and Abdelghany 2023a,b; Alsalamah *et al*. 2023). *Duranta* is classified under the Verbenaceae family and consists of

35 various species, and its origin can be traced back to Africa, Asia, and Central and South America (Aymard and Grande 2012). *Duranta erecta*, also known as *D. repens*, is widely recognized as the "golden dew drop." This vertical, sprawling shrub generally reaches a height of 1 to 3 m. It is frequently cultivated as a hedge or decorative plant in numerous residences across Ghana. According to Agawane *et al*. (2019), *D. erecta* contains various metabolites including both primary and secondary, including glycosides, sterols, saponins, flavonoids, tannins, phenols, proteins, alkaloids, and carbohydrates. Moreover, it displays antimicrobial properties against certain bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogens* (Al-qaysi and Ali 2006). Prior research has indicated the utilization of *D. erecta* for treating headache, toothache, and promoting wound healing, protecting the liver, and acting as diuretics (Ogbuagu *et al*. 2015). Donkor *et al*. (2019) documented the antioxidant and antimicrobial properties of *D. erecta* leaves extract. Several bioactive constituents, including naringenin, β-sitosterol, were extracted from *D. repens* (Abou-Setta *et al*. 2007). Various organs of the plant are utilized in the management of a range of illnesses (Alawlaqi *et al*. 2023; Selim *et al*. 2024). In the traditional herbal medicine, leaves and fruits are employed to treat intestinal worms, malaria, and abscesses, and are sometimes used as a diuretic or vermifuge (Rahmatullah *et al*. 2011). *Duranta erecta* is believed to exhibit antitumor properties and notable antibacterial effects (Bhar *et al*. 2016). The hepato-protective role of *D. erecta* fruit extract was documented by Shadrack *et al*. (2020) *via* minimization of oxidative stress that can be attributed to its contents phyto-constituents.

Developed extraction technologies are often touted as effective alternatives to methods of traditional extraction due to their reliance on commonly recognized as harmless solvents, reduced period of extraction, selective extraction capabilities, and cost-effectiveness. These technologies are increasingly being utilized in the development of functional food ingredients, resulting in a lower environmental impact and are therefore considered environmentally friendly (Pimentel-Moral *et al*. 2018; Bazaid *et al*. 2025). Supercritical fluid extraction (SFE) in particular has gained attention as a sustainable technology that eliminates the need for organic solvents. It can be employed to extract specific phytochemicals or revalorize food by-products (Roselló-Soto *et al*. 2015). SFE involves dissolving analytes in a supercritical fluid, which exhibits properties of both a liquid and a gas. This unique characteristic allows for the recovery of effective constituents and selective extraction *via* adjusting the density and viscosity of the solvent used (Koubaa *et al*. 2015). CO_2 is the most commonly utilized supercritical fluid due to its advantageous properties. It serves as an ideal solvent, as it is non-toxic, nonflammable, and cost-effective. With a decisive level of pressure (73.8 bar) and temperature (31.1 °C), investigations can be conveniently carried out. This sets it apart from other solvents such as H_2O (pressure of 218.3 bar and temperature of 347 °C) or methanol (pressure of 79.8 bar and temperature of 239 °C). Furthermore, CO_2 can be easily separated under common atmospheric conditions from the extract. Its widespread application lies in the extraction of lipophilic compounds, as its nonpolar nature limits its ability to dissolve polar compounds (Bogolitsyn *et al*. 2019). Using traditional extraction methods, the phytoconstituents and pharmacological investigations of *D. erecta* were previously described; but using SFE, only few investigations have been reported.

Up to now, the utilization of SFE has not covered all plants. So, this study was designed to evaluate the impact of the SFE temperature on the extraction yield and

phytochemical characterization of *D. erecta* by High-performance liquid chromatography (HPLC) to investigate their biological utilizations.

EXPERIMENTAL

Preparation of Plant Material and Supercritical Fluid Extraction

The used plant organ *viz.*, ripe fruits, was gathered from pharm campus at Jazan (17°00'54"N, 42°51'03"E) in Saudi Arabia, which is characterized with temperature ranges from 22.2 °C to 36.1 °C and is rarely lower than 20.6 ℃ or higher than 38.3 °C. The period of rain often starts at 6 July to 25 August. The collected *D. erecta* ripe fruits were cleaned with drinking water to eliminate any foreign particles from dust or sand. The collected ripe fruits of *D. erecta* were then dried for 14 days without exposure to direct sun light. The dried fruits were indelicately converted to powder by means of a grinder (Electric blender from Bosch Limited, Stuttgart in Germany). The ISCO-Sitec modified SFX 220 supercritical fluid extraction (SFE) system was utilized for the extraction of *D. erecta* fruits, as described by Žitek *et al.* (2020). About 3 g of dehydrated *D. erecta* fruits were subjected to SFE under various temperatures, including 40, 60, and 80 °C at a constant pressure of 20.7 MPa, and extraction time (15 min of extraction static period and 45 min of extraction dynamic period). Both the rates of supercritical CO_2 and the solvent flow were constant throughout each run.

HPLC Investigation

The phenolic and flavonoids compounds of *D. erecta* ripe fruits extract were identified by HPLC analysis utilizing a Waters 2695 Alliance HPLC, which was equipped with a UV-Vis DAD (Waters Inc., Milford, CT, USA). A Waters SunfireTM C18 reverse-phase chromatographic column with measurements of 250 mm in length, 4.6 mm in width, and 5 µm in size of particle was used to carry out the separation. Using an autoinjector, the phenolic standard solutions and mixtures were introduced into the apparatus. 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 mode included a mixture of two mobile phases A and B, namely acetonitrile and phosphoric acid, respectively, which was formulated *via* dropwise addition of orthophosphoric acid (85%) to HPLC grade water until the pH reached 2. The concentration gradient was changed in the following ways during the method's 60-min total run time: a) 5% A and 95% B at first; b) 35% A and 65% B for 15 min; c) 35% A and 65% B for 20 min; d) 40% A and 60% B for 30 min; e) 40% A and 60% B for 35 min; f) 50% A and 50% B for 40 min; g) 70% A and 30% B for 52 min; finally 5% A and 95% B for 60 min. There was a constant flow rate (0.5 mL/min) and temperature (5 °C). Subsequent the examination of the UV-Vis spectra was applied for the standards of individual phenolic at wavelengths of 210, 280, and 360 nm).

Agar Diffusion Assay for the Antimicrobial Activity

The method of agar diffusion was applied to measure the antimicrobial activity of *D. erecta* ripe fruits extract. The tested microorganisms involving *Candida albicans* (ATCC 10221), *Penicillium glabrum* (Op6941), *Salmonella typhi* (ATCC 6539), *Escherichia coli*

(ATCC 8739), *Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (ATCC 6538) were activated via re-cultured on nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. Saline suspensions of microbial colonies (2×10^8/2×10^4 colony-forming units/mL of bacteria/ fungi) were prepared from a 20/72 h old cultivated nutrient agar/PDA plate of bacteria/fungi. Using sterile Mueller-Hinton agar (MHA) for bacteria and PDA for fungi, the inoculums of tested microorganisms were swabbed onto the agar. Five holes were cut from the growth medium, employing a crock borer with an internal diameter of roughly 5 mm. A 100 µL volume of *D. erecta* ripe fruits extract was poured into each hole. A disc containing 30 µg of chloramphenicol was utilized as the positive control for bacteria while 500 µg mL^{-1} of nystatin was utilized as the positive control for fungi, and three duplicates of DMSO (20% v/v from Sigma, Germany) were utilized as negative control. After allowing the extract to diffuse completely, the plates containing bacteria and yeast were incubated at 37°C for 24 h, while the fungus *P. glabrum* was incubated at 30 °C for 72 h. Following this, the radius of the developed zone of inhibition (clear zone) was measured using a sterile ruler (Qanash *et al*. 2023).

Measurement of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Extract

Minimum inhibitory concentration (MIC) of the handled *D. erecta* ripe fruits extract was detected against the examined bacteria. In this work, *C. albicans* was determined employing the broth micro-dilution technique with slight adjustments. A range-fold dilution series of the extract (3.9 to 1000 µL/mL) was prepared in Muller Hinton broth. Following the guidelines, the suspension of microbes was added to every tube containing the dilution. The tubes containing the inoculants were then placed at 37 °C/24 h and at 28 °C/48 h, respectively for bacteria and *C. albicans* in the incubator. The positive control was medium without the *D. erecta* ripe fruits extract. The MIC was determined as the dose with no visible growth (turbidity). To detect the MBC of the *D. erecta* ripe fruits extract, the 96-well microtiter plate was consecutively diluted and then used for micro dilution assay. This assay involved the inoculated plate with the examined microorganisms (1×10^6 CFU/mL) and then incubated (37 °C/24 h and at 28 °C/48 h, respectively for bacteria and *C. albicans*). Microbial growth was determined utilizing a microtiter plate reader (Manufactured in Jinan, China by SciTech Global Co. Ltd.) to assess the absorbance at 660 nm. The contents were transferred onto plates containing MHA, and after the incubation at appropriate conditions, wells with >90% inhibition were considered. In this study, the lowest dose of the extract needed to eradicate the studied microbes was defied as minimum bactericidal concentration (MBC). The calculated MBC/MIC index was performed to assess the bactericidal effectiveness of the tested extract (Abdelghany *et al*. 2019).

Effect of Fruits Extract on Ultrastructure of *B. subtilis* by Transmission Electron Microscopy (TEM)

Using a transmission electron microscope (TEM), the ultrastructure of *B. subtilis* treated with the *D. erecta* ripe fruits extract at two different SFE temperatures—60 °C and 80 °C—was investigated. Both the treated and untreated *B. subtilis* were fixed for 5 min using a potassium permanganate solution at lab temperature 22 °C using 3% glutaraldehyde, followed by washing in phosphate buffer. The fixed *B. subtilis* cells were dehydrated for 15 min in each of the prepared ethanol dilutions (10% to 90%). The loaded

samples were exposed to a mixture composed of acetone and epoxy resin within a graded series until they were completely covered in pure resin. The prepared ultrathin sections were placed on copper grids, and then stained twice with uranyl acetate, then with lead citrate. Utilizing a TEM (model JEOL-JEM 1010, USA) operating at 70 kV, the ultrastructure-stained sections were examined (Abdelghany *et al*. 2021).

Antioxidant Properties of Fruits Extract *via* 1-Diphenyl-2-Picrylhydrazyl Radical (DPPH)

The procedure for evaluating free radical scavenging *via* radical DPPH of *D. erecta* fruits extract was conducted. About 12.5 mg of *D. erecta* fruits extract was dissolved in methanol to prepare a 25 mL of diluted solution. This solution served as the stock for preparing various test solutions with different doses ranging from 100 up to 1000 µg/mL. In every test tube, 5 mL of *D. erecta* fruits extract dose was mixed with DPPH (1 mL of 0.001 M) solution. A controlled solution was made by mixing 5 mL of methanol to 1.0 mL of DPPH without *D. erecta* fruits extract. Then the reaction mixture was kept in dark for 30 min, followed by measuring their antioxidant potential absorbance at 517 nm employing an Optima UV Visible spectrophotometer (Abdelghany *et al*. 2019). The IC_{50} quantity (the amount of *D. erecta* fruits extract requisite to prevent 50% of the DPPH free radical) was estimated *via* a log dose of prevention curve. Ascorbic acid was used as the guideline drug of the antioxidant activity. The inhibition % of DPPH scavenging was calculated applying the following Eq. 1:

$$\text{DPPH scavenging inhibition (\%)} = \frac{\textbf{Absorbance control} - \textbf{Absorbance extract}}{\textbf{Absorbance control}} \times \mathbf{100} \quad (1)$$

Assay of *D. erecta* Fruits Extract *via* Ferric Reducing Antioxidant Power (FRAP)

The reaction mixture used for FRAP assay of *D. erecta* fruits extract consisted of 1.0 mL of the extract, 2.5 mL phosphate buffer (0.2 M with pH 7) besides 2.5 mL of potassium ferricyanide (1%). The mixture was kept for 30 min at 45 ℃. Then, 2.5 mL of trichloroacetic acid at level of 10% dilution was mixed with reaction mixture. This was followed by centrifugation (at 6000 rpm up to 12 min), and collection of supernatants. Distilled H_2O (2.5 mL) with 0.1% of $FeCl_3$ (0.5 mL) was mixed with 2.5 mL of the collected supernatant (Benzie and Strain1996). The solution absorbance at 700 nm was recorded. Ascorbic acid equivalent (AAE) mg/mg of *D. erecta* fruits extract was employed to express the results.

Cell Scratch Test to Assess Wound Healing Potential of *D. erecta* Fruit Extract

The ability of *D. erecta* fruit extract (at 60 ℃ of SFE extraction) to heal wounds was assessed using an already established methodology for *in vitro* cell migration studies on human skin melanocytes (HFB4) cells. Briefly, 2×10^4 cells/mL were cultivated for the full night in a multi-well plate. 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 well plates, and cellular debris. After adding 125 and 250 µg/mL of *D. erecta* fruits extract, the cells were incubated for 24 h. Images were captured with an inverted microscope to show cell

migration and changes in the morphological profile (Alsalamah *et al*. 2023). The width of the scratch and the wound closure at different times were analyzed (0 and 48 h). The following equations were used to calculate the next analysis:

$$\text{Migration rate} = \frac{\text{Wound width at initial (μm)} - \text{Wound width at final (μm)}}{\text{Time span of the test (h)}} 100 \quad (2)$$

$$\text{Closure of wound \%} = \frac{\text{Wound area at initial} - \text{Wound area after 48 h}}{\text{Wound area at initial}} 100 \quad (3)$$

$$Difference\ of\ area\ \% = Intial\ area - Final\ area$$

Statistical Investigation

SPSS version 15.0 was used to analyses the obtained results (SPSS Inc. Chicago, Illinois, USA). The data was displayed as the average of three independent analyses to calculate the standard deviation (±SD).

RESULTS and DISCUSSION

Phytochemical Characterization of *D. erecta* Fruits Extract

The harvested *D. erecta* fruits were subjected to different operating temperatures (40, 60, and 80 °C) of SFE for obtaining high yield of extract. The assessment of their constituents *via* HPLC, antimicrobial, anti-biofilm, and anti-oxidant activities are presented in Fig. 1.

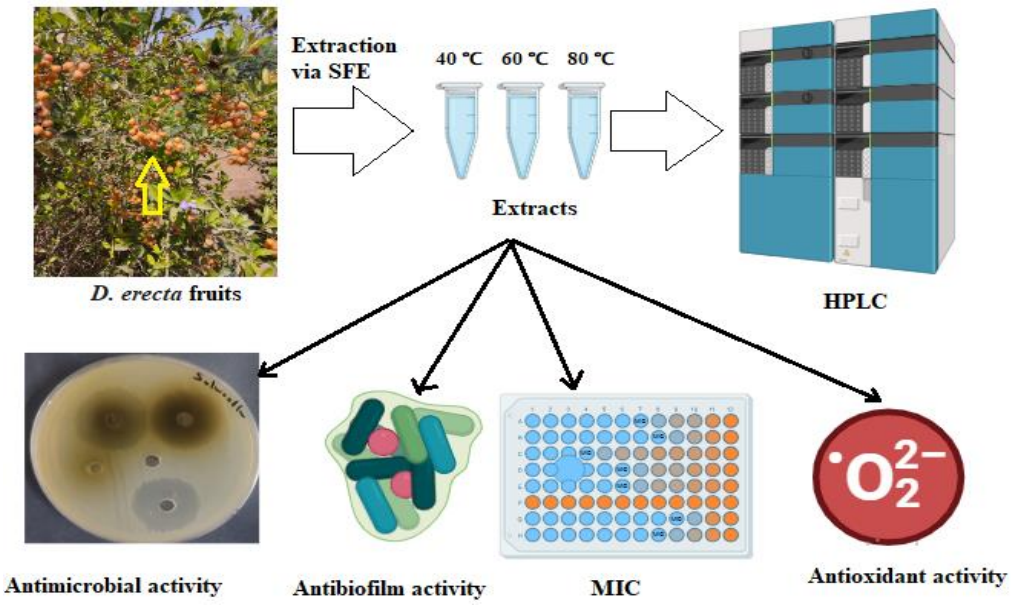

Fig. 1. Histogram presenting some of the performed investigations on *D. erecta* fruits extracts including SFE at different operating temperatures (40, 60, and 80°C), HPLC analysis of extracts and their biological activities namely antimicrobial, antibiofilm, MIC, antioxidant

Regarding extraction yield, it was found that temperature significantly affected the results, as shown by Fig. 2. The results indicated that high temperatures caused plant cells to decompose and release all of the compounds stored inside, including both desired and undesired elements. The highest yield quantity of the extract was 0.456 g at 60 ℃, followed by 0.412 g at 80 ℃, while the lowest quantity of the yield was obtained at 40 ℃. Over the last decade, SFE has been applied widely as a sustainable and eco-friendly technology. For every kind of fruit extract, a different set of factors is vital. The utmost vital factors in the extraction and releasing of bioactive ingredients are extraction time, temperature, and pressure (Čižmek *et al*. 2021). Some advantageous were associated to SFE approach compared to conventional processes for the extraction of natural products which performed at high temperatures, where high temperatures caused destruction of valued constituents, while this destruction did not occur with SFE at operating conditions of high temperature (Bimakr *et al*. 2011; Chamali *et al*. 2023).

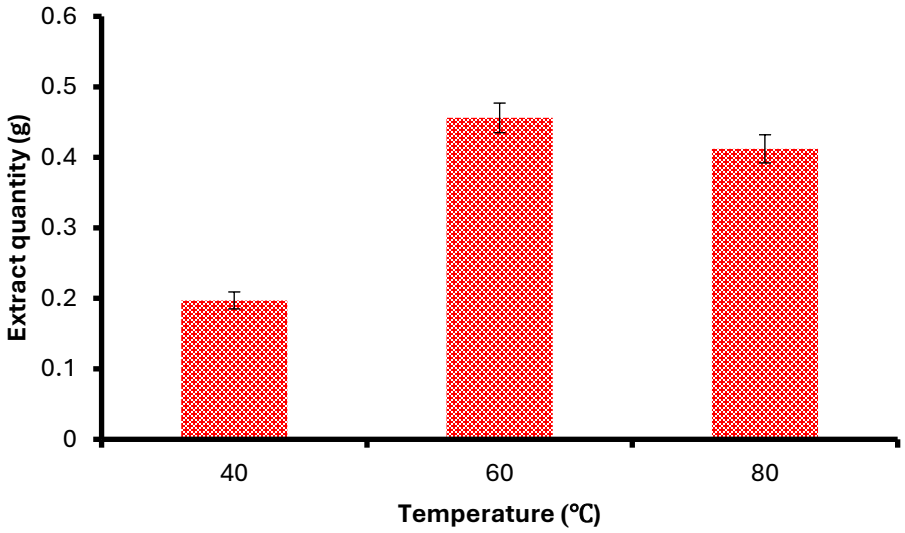

Fig. 2. Yield quantity of the extracted *D. erecta* fruits at different operating temperatures (40, 60, and 80 ℃)

The analysis of *D. erecta* fruit extract and the contents of phenols and flavonoid compounds present at different conditions of SFE temperatures *via* HPLC is illustrated in Table 1 and Figs. 3A-C. Within the extraction at 60 ℃, 50% of the detected compounds, specifically daidzein, gallic acid, chlorogenic acid, methyl gallate, rutin, naringenin, rosmarinic acid, quercetin, and kaempferol, were obtained in high quantity at levels of 30900, 3510, 2770, 326, 571, 7460, 1060, 7770, and 103 µg/mL, respectively, if compared with its concentrations at 40 ℃ and 80 ℃. In the course of extraction at 80 ℃, high concentrations of some compounds namely ellagic acid, catechin, and coumaric acid were recorded if compared with their concentrations at 40 ℃ and 60 ℃, while others sharply decreased in the extract at this temperature, for instance, rosmarinic acid and methyl gallate. In contrast, the quantity of ingredients for instance, caffeic acid, syringic acid, vanillin, and ferulic acid were remarkably increased at 40 ℃. These outcomes indicated that temperature represented a critical factor for control in the releasing of compounds from the extract. The SFE protocol was judged to be suitable for extraction of selective

compounds. Generally, *D. erecta* fruits extract was rich with ellagic acid (37900 µg/mL) and daidzein (30900 µg/mL) at all studied extraction temperatures, particularly at 60 °C and 40 °C, respectively. The temperature plays a crucial role because elevated temperatures lead to changes in the SFE density, which in turn affects how well they can penetrate materials. Additionally, when the temperature goes up, the ability of certain substances to dissolve in the fluid also goes up (Leyva-Jiménez *et al*. 2020). These conditions allow for the extraction of substances from their original sources. Thus, when the pressure remains the same, elevation in temperature results in a decline in the density of the SFE, which in turn enhances the ability of the substances to dissolve in the fluid (Cádiz-Gurrea *et al*. 2019).

Table 1. Contents of Phenols and Flavonoids in *D. erecta* Fruits Extract at Different Temperatures of SFE

Detected Constituent	40 °C			60 °C			80 °C		
	RT (min)	Area (mAU)	Conc. (µg/mL)	RT (min)	Area (mAU)	Conc. (µg/mL)	RT (min)	Area (mAU)	Conc. (µg/mL)
Gallic acid	3.58	743	2990	3.58	870	3500	3.58	792	3189
Chloro-genic acid	4.26	103	658	4.12	434	2770	4.15	251	1603
Catechin	4.62	320	3645	4.56	279	3180	4.60	321	3657
Methyl gallate	5.24	15.9	40.9	5.58	127	326	5.23	14.8	37.9
Caffeic acid	5.87	207	846	5.83	160	653	5.88	152	621
Syringic acid	6.34	70.8	210	6.29	16.3	48.4	6.32	18.2	53.9
Rutin	6.74	43.8	266	6.67	93.8	571	6.73	88.6	539
Ellagic acid	7.45	5130	31600	7.36	4570	28100	7.44	6150	37900
Coumaric acid	8.78	141	234	8.66	145	241	8.75	151	252
Vanillin	9.03	2190	4030	8.98	888	1640	9.03	1190	2190
Ferulic acid	9.64	220	615	9.57	49.6	139	9.62	75.6	212
Naringenin	10.09	1380	6210	9.97	1660	7469	10.0	1600	7160
Rosmarinic acid	11.64	8.9	44.5	11.97	212	1060	11.6	10.1	50.2
Daidzein	15.94	7970	24900	15.83	9890	30900	15.9	8870	27800
Quercetin	16.63	1690	5610	16.53	2330	7770	16.6	1830	6090
Cinnamic acid	19.19	552	472	19.12	411	352	19.2	412	353
Kaempferol	20.68	19.9	71.4	20.66	28.8	103	20.7	25.0	89.4
Hesperetin	21.30	35.3	84.4	21.27	19.6	46.8	21.3	12.5	29.9

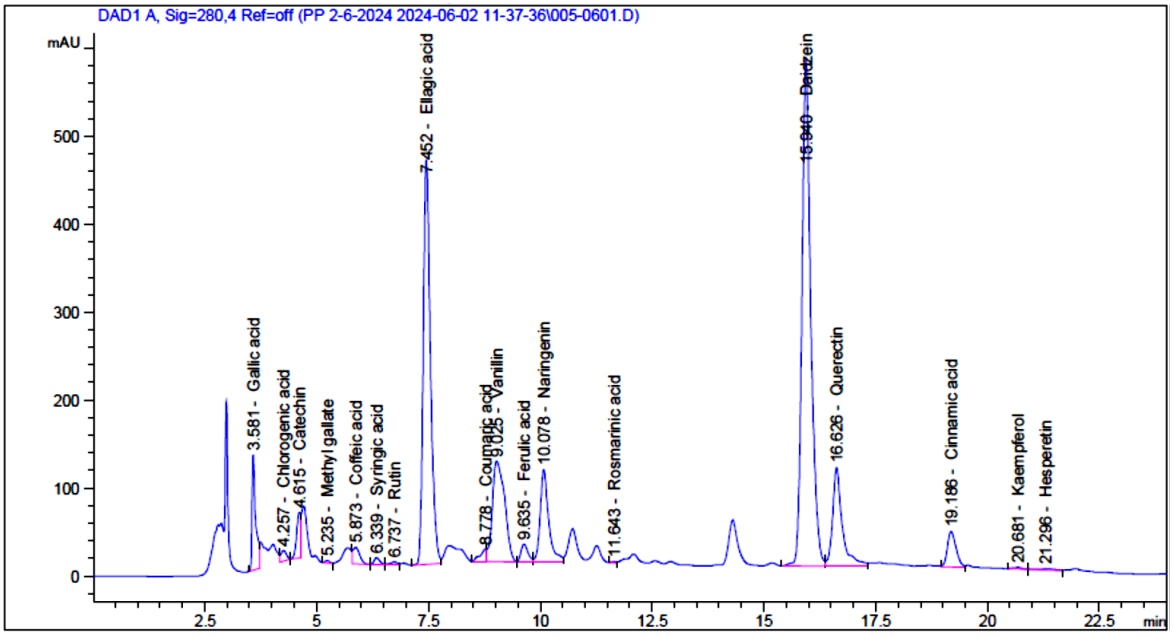

Fig. 3A. HPLC Chromatogram of analysed phenols and flavonoids in *D. erecta* fruits extract obtained from SFE at 40 ºC

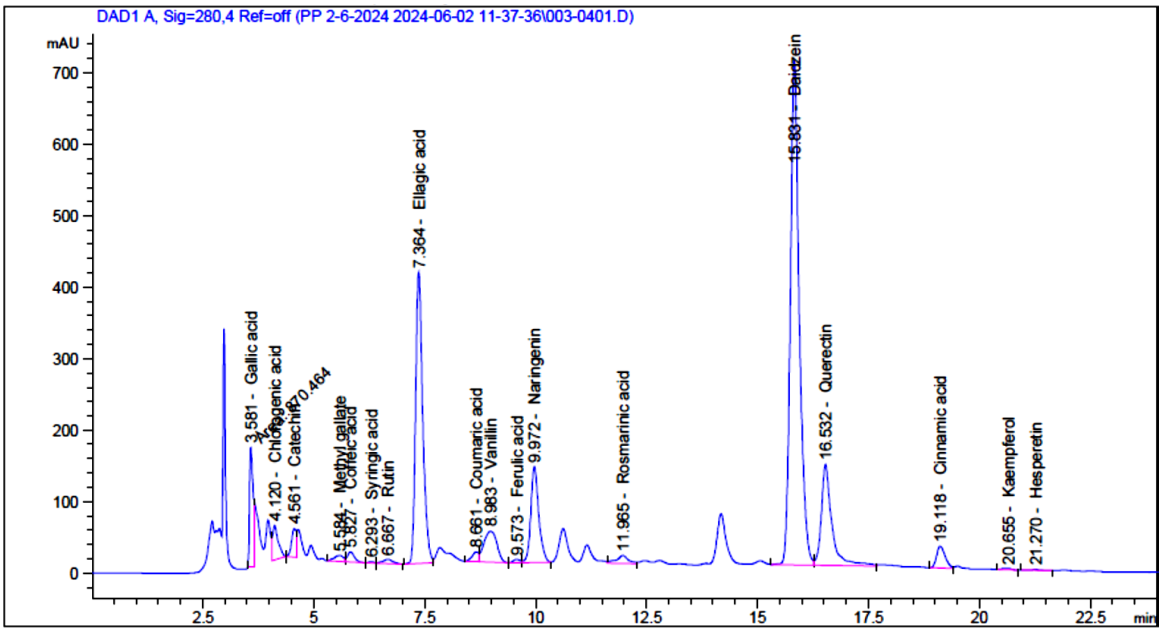

Fig. 3B. HPLC Chromatogram of analysed phenols and flavonoids in *D. erecta* fruits extract obtained from SFE at 60 ºC

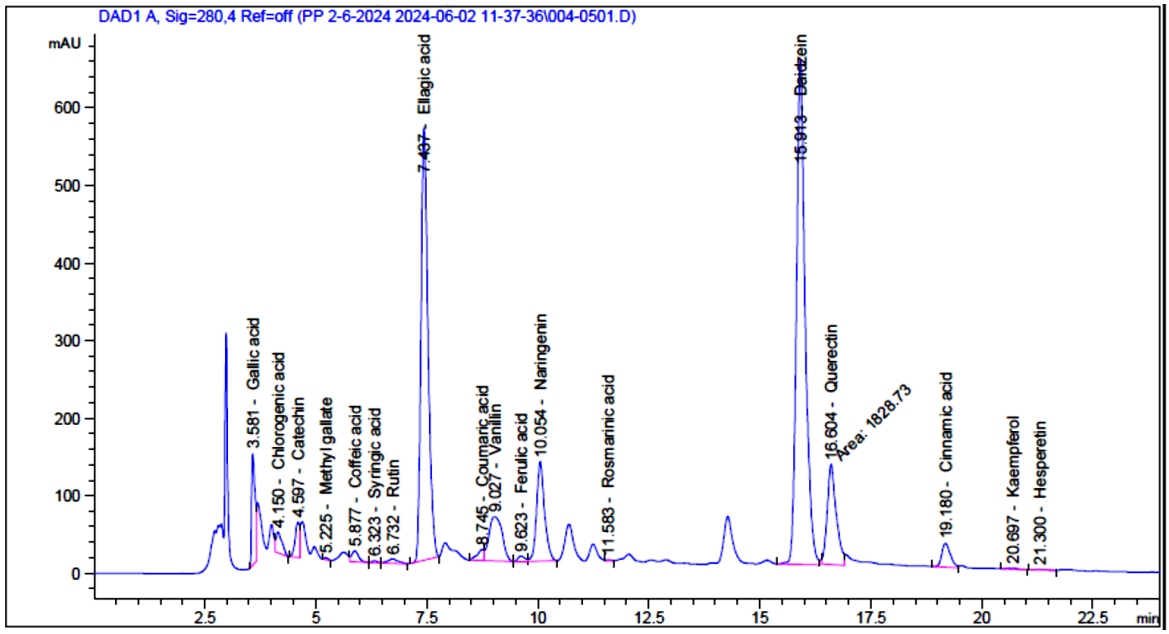

Fig. 3C. HPLC Chromatogram of analyzed phenols and flavonoids in *D. erecta* fruits extract obtained from SFE at 80 ºC

Antimicrobial Properties of *D. erecta* Fruits Extract

At 60 ℃ of the SFE condition, the inhibition of microbial growth was recorded with maximum inhibition zones of 28±0.1, 27±0.2, 30±0.1, 25±0.1, and 30±0.2 mm *versus S. aureus*, *S. typhi*, *B. subtilis*, *E. coli*, and *C. albicans*, correspondingly, it gave higher inhibition zones than that of positive control (Table 2 and Fig. 4). Though unicellular fungus *C. albicans* was inhibited by the *D. erecta* extract, the filamentous fungus *P. glabrum* was not inhibited. At 40 ℃ of the SFE condition, the extract didn't exhibit a notable effect on *S. typhi* growth. Other *D. erecta* plant parts including roots, stem, and leaves extracts by methanol reflected antifungal activity towards *Rhizopus* sp., *Aspergillus flavus*, *Penicillium* sp., *Alternaria* sp., *Trichoderma* sp., and *Fusarium oxysporum* (Sharma *et al*. 2012). The main variables that could account for the differences in their activities that have been observed are the differences in the plants' locations, chemical compositions, handling, and maturity as well as extraction method and solvent used.

Table 2. Activity of *D. erecta* Fruit Extract against Various Microorganisms' Growth at Different Temperatures of SFE

Tested Bacteria/Fungi	Zones of Inhibition (mm)				
	40 ºC	60 ºC	80 ºC	*Positive Control	**Negative Control
S. aureus	24 ± 0.2	28 ± 0.1	20 ± 0.2	25 ± 0.2	0.0
S. typhi	0.0	27 ± 0.2	22 ± 0.1	25 ± 0.2	0.0
B. subtilis	25 ± 0.1	30 ± 0.1	28 ± 0.1	26 ± 0.2	0.0
E. coli	16 ± 0.2	25 ± 0.1	19 ± 0.2	24 ± 0.1	0.0
C. albicans	18 ± 0.2	30 ± 0.2	15 ± 0.2	27 ± 0.2	0.0
P. glabrum	0.0	0.0	0.0	33 ± 0.4	0.0

*Positive control for bacteria/fungi inhibition includes chloramphenicol/Nystatin; and ** DMSO was employed as a negative control

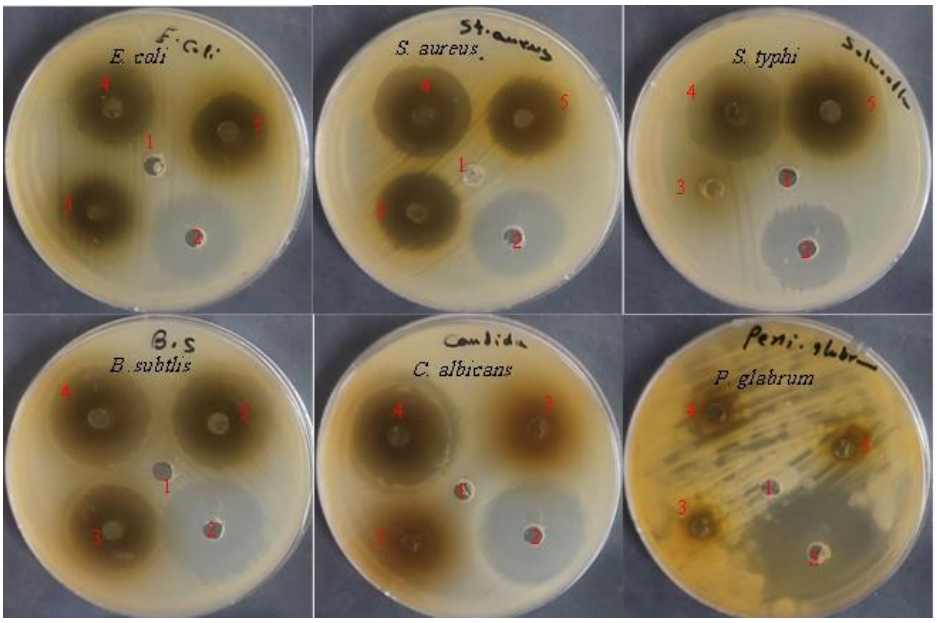

Fig. 4. Action of *D. erecta* extract against various microorganism' growth at different temperatures of SFE (1: negative control; 2: positive control; 3: 40 ºC; 4: 60 ºC; 5: 80 ºC

Noteworthy values of MIC were recorded for *D. erecta* extract depending on the extraction temperature of SFE and microbial species (Table 3), where the ranged MIC values were from 7.8 to 500 µg/mL. Besides MIC, the MBC values of the extract at 60 ºC of SFE condition were less than that at 40 and 80 ºC. The earlier investigation proposed that the MIC quantity if ranged among 156 and 625 µg/mL, the tested compound can be regarded as a good antibacterial agent. Similarly, Kuete (2010) reported significant values of MIC *via* reviewing several investigations indicating that MIC is significant, good, and low if its values were fewer than 100 µg/mL, among 100 and 625 µg/mL, and more than 625 µg/mL, correspondingly. In the present study, the values of MIC are considered significant and good antibacterial agent. Fruit and stem extracts of *D. repens* reflected MIC ranging from 32 to 128 µg/mL against different species of *Shigella* including *S. boydii*, *S. flexneri*, *S. dysenteriae*, besides *S. sonnei* (Nikkon *et al*. 2008). According to Ogbuagu *et al*. (2015), the antibacterial potential of *D. erecta* leaves extract was documented for inhibiting the growth of *Bacillus subtilis*, *Proteus mirabilis*, *Boletus aereus*, and *Salmonella typhi* with MIC 141, 129, 100, and 81 mg/mL, respectively.

Table 3. Provided Values of MIC, MBC, Besides Index of MIC/MBC or MIC/MFC of *D. erecta* Fruits Extract at Different Temperatures of SFE

Tested Microorganisms	MIC at			MBC at			MBC/MIC or MIC/MFC Index at		
	40 ºC	60 ºC	80 ºC	40 ºC	60 ºC	80 ºC	40 ºC	60 ºC	80 ºC
S. aureus	31.25	15.62	62.5	62.5	15.62	125	2	1	2
S. typhi	-	15.62	31.25	-	31.25	62.5	-	2	2
B. subtilis	31.25	7.8	15.62	62.5	15.62	31.25	2	2	2
E. coli	62.5	15.62	31.25	125	15.62	62.5	4	1	2
C. albicans	62.5	7.8	125	125	15.62	500	2	2	4

Ultrastructures of treated *B. subtilis* by the *D. erecta* fruits extract at 60 and 80 °C of SFE conditions are shown in Fig. 5 and are compared with the untreated bacteria. A TEM analysis of untreated cells of *B. subtilis* exhibited typical continuous thin cell wall, cell membrane, and nuclear material. While in treated cells with the extract at 60 °C showed the split-up of *B. subtilis* cell wall from the cell membrane with the presence of several vacuoles besides the presence of dense cytoplasm. This reveals high severe influences of the extract on cell wall, leading to its rupture.

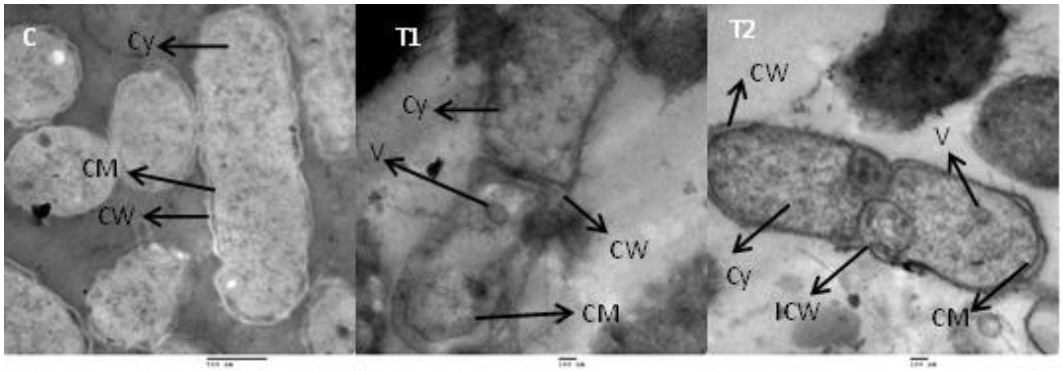

Fig. 5. Ultrastructures of treated *B. subtilis* cells *via* TEM under the effect *D. erecta* fruits extract obtained at different operating temperatures of SFE (C: control; T1: at 60 ºC; T2: at 80 ºC; CW: cell wall; CM: cytoplasmic membrane; Cy: cytoplasm; V: vacuole; ICW: irregular cell wall).

Ultrastructures of treated *B. subtilis* by the extract at 80 °C of SFE conditions illustrated that the cell wall became irregular with the appearance of budding scars inside the cell and underwent degenerative alterations showing shrinking of the membrane layer a far from cell wall. According to other studies, ultrastructural changes were observed in some bacteria such as *Campylobacter jejuni*, *Bacillus cereus*, *E. coli*, as an effect to exposure to plant extracts (Dholvitayakhun *et al*. 2017; Famuyide *et al*. 2020).

Antioxidant Properties of *D. erecta* Fruits Extract

The DPPH test was employed to measure the antioxidant properties of the *D. erecta* fruit extracts. Figure 6 illustrates the ability of *D. erecta* fruit extracts obtained at different SFE temperatures (40, 60, and 80 °C), to scavenge DPPH free radicals, in comparison to ascorbic acid at doses between 1.95 and 1000 µg/mL. The scavenging activity of DPPH for the tested extracts depended on the concentration, and it rose as the extract concentration was increased. Out of all the extracts, the sample extracted at 60 °C exhibited the maximum antioxidant activity across all concentrations tested. Moreover, sample extracted at 80 °C exhibited higher DPPH scavenging activity than that at 40 °C. All outcomes were compared to standard ascorbic acid at the same used doses. The obtained IC_{50} confirmed the different levels of antioxidant potentials of tested extracts, where its values were in following order: 9.66, 15.01, and 18.65 µg/mL for the extracts at 60, 80, and 40 °C compared with the IC_{50} value 3.44 µg/mL of ascorbic acid.

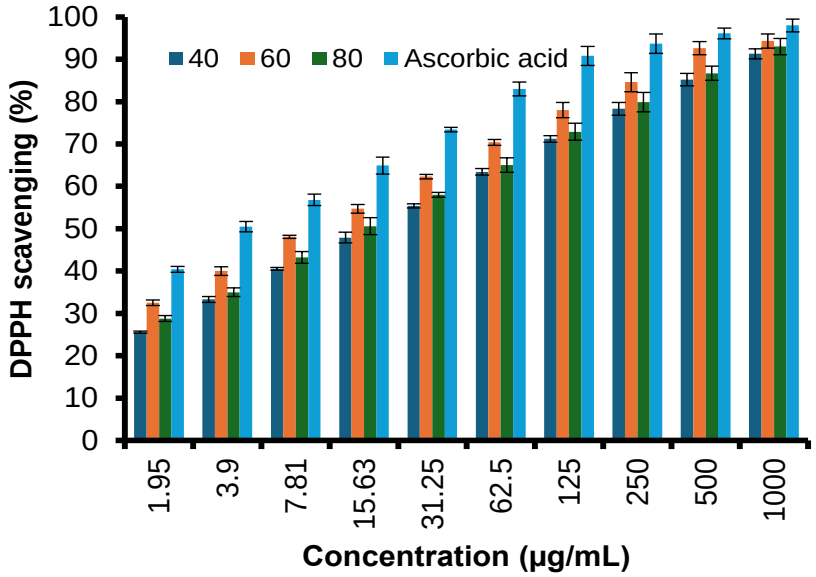

Fig. 6. DPPH scavenging percentage of different concentrations of the *D. erecta* fruit extract obtained at different temperatures (40, 60, and 80 ºC) of SFE compared with ascorbic acid

Another approach, FRAP confirmed the antioxidant properties of the used extracts. Also, the sample extracted at 60 ℃ exhibited the greatest antioxidant activity if compared to sample extracted at 80 ℃ and 40 ℃ with 355.0, 183.9, and 132.2 equivalent (AAE) µg/mg, respectively (Fig. 7). The importance of traditional medicine is demonstrated by the high scavenging activity of the used plant fruits. Antioxidants inhibit lipid peroxidation through free radical scavenging, which is a well-known mechanism. The administration of oxidative stress in individuals benefits from the antioxidant property of such plants. Great scavenging activity accompanying with the used plant fruits explains their application in traditional treatment. One of the known mechanisms, which is known as free radical scavenging, explains how antioxidants prevent peroxidation of lipids. The antioxidant assets mean that such plant extracts can be suitable in the management of oxidative stress in humans. Donkor *et al*. (2019) informed the antioxidant potential of *D. erecta* fruits (including both ripe and unripe) where DPPH scavenging was 77.0 and 71.5%, respectively.

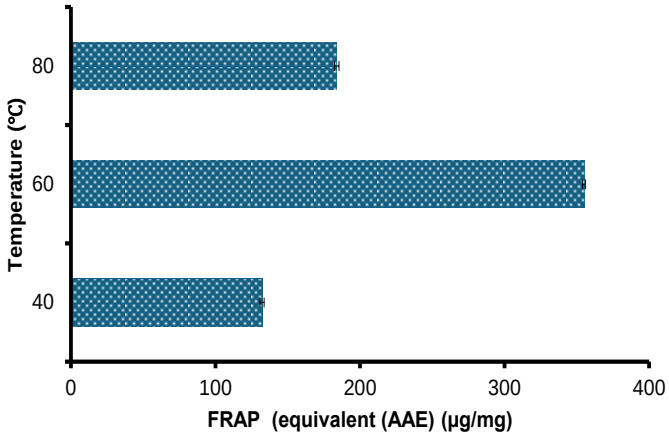

Fig. 7. FRAP (equivalent AAE) in µg/mg of the extracted *D. erecta* fruits at different temperatures of SFE

Wound Healing Properties of *D. erecta* Fruits Extract

Many plants that have been traditionally utilized in folk medicine have had their wound-healing abilities confirmed by ethnopharmacology, indicating that wound healing is a complex process. To learn more about the extended use of crude plant extracts or isolated secondary metabolites in wound healing, several *in vitro* investigations have been carried out (Bakri *et al*. 2024). Presently, the extract of *D. erecta* fruits obtained at 60 °C of SFE was used to assess their activity in wound healing by way of an *in vivo* approach using human skin melanocytes (HFB4) cells (Table 4 and Fig. 8). Various measurements indicated the efficacy of *D. erecta* fruits extract particularly at 250 µg/mL of the extract, where the wound closure %, and rate of migration, besides area difference % were 43.3 μm^2, 8.57 µm, and 312000% compared to its levels at control (untreated cells) 80.1 μm^2, 15.8 µm, and 576000%. *D. erecta* fruits exhibited wound healings that could be attributed to its antioxidant and anti-inflammatory activities. Previous research indicated that extract from *D. erecta* leaves has been utilized for the management of wound healing (Ogbuagu *et al*. 2015).

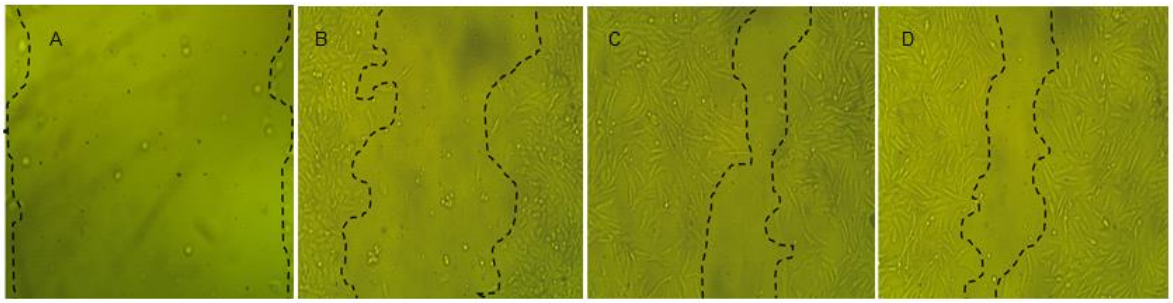

Fig. 8. Healing properties of different doses of the extracted *D. erecta* fruits at 60 ºC of SFE. A: Untreated cells at 0 h; B: Untreated cells at 48 h; C: Treated cells at 48 h by 125 µg/mL of the extract; D: Treated cells at 48 h by 250 µg/mL of the extract.

Table 4. Healing Properties of Different Doses of the Extracted *D. erecta* Fruits at 60 ºC of SFE

Treatment	Period (h)				*RM (µm)	Wound Closure (%)	Area Difference (%)
	0.0		48				
	Area	Width	Area	Width			
Untreated cells	970	733000	196	148000			
	996	753000	128	96800			
	948	719000	220	167000			
	962	731000	224	170000			
	878	664000	226	171000			
	946	719000	142	108000			
	Average						
	950	720000	189	143000	15.85	80.067	576000
Treated cells at 125 µg/mL	970	733000	218	165000			
	996	753000	206	156000			
	948	719000	118	89500			
	962	731000	218	166000			
	878	664000	318	241000			
	946	719000	246	187000	15.19	76.767	553000

	Average						
	950	720000	221	167000			
Treated cells at 250 µg/mL	970	733000	594	449000			
	996	753000	474	358000			
	948	719000	498	378000			
	962	731000	510	388000			
	878	664000	600	454000			
	946	719000	556	423000			
	Average						
	950	720000	539	408000	8.57	43.3	312000

***RM, rate of migration**

CONCLUSIONS

1. The finding of high-performance liquid chromatography (HPLC) analysis exhibited variations in phenols and flavonoids contents in *Duranta erecta* fruits extract depending on the operator conditions of supercritical fluid extraction (SFE) temperature.
2. Antimicrobial activity showed that *D. erecta* fruits extract exhibited good result particularly the extract obtained at 60 ℃ of the SFE condition against *S. aureus*, *S. typhi*, *B. subtilis*, *E. coli*, and *C. albicans*. Moreover, ultrastructure changes were observed in the treated *B. subtilis* by *D. erecta* fruits extract at 60 ℃ of the SFE condition
3. *Duranta erecta* fruits extract displayed a promising radical scavenging potential *via* 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) evaluates.
4. The recorded measurements indicated the ability of *D. erecta* fruits extract for wound healing, particularly the extract at 60 ℃ of the SFE condition.

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