

## COMPARATIVE ANALYSIS OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY IN THE MOTHER TREE AND IN VITRO REGENERATED FEGRA FIG (*FICUS PALMATA* FORSSK.)

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**Abstract.** The fegra fig (*Ficus palmata* Forssk.), is a significant medicinal plant that produces fruit and is utilized in tropical climates worldwide. In traditional medicine, it is used to cure a variety of conditions including ulcers, gonorrhea, diabetes, asthma, diarrhea, liver, and kidney problems. The natural stand of this plant is being exhausted due to the growing demand for it. It is also a rare kind of tree in the Arabian region with slow growth. For these reasons, we have previously developed an in vitro regeneration technique for the large-scale proliferation of this plant. The mother plant and the in vitro-regenerated plant were subjected to phytochemical and antioxidant analyses in the current investigation. When compared to extracts of maternal plants, phytochemical analysis showed that the extract of in vitro regenerated plants had higher levels of phenolic (70.78 mg GAE/g), flavonoid (45.04 mg QE/g), and tannin (28.00 mg TAE/g) contents. These findings may be related to the higher antioxidant potential of the extract. The analysis using gas chromatography-mass spectroscopy (GC-MS) revealed the existence of several bioactive compounds. In conclusion, the herbal industry may be able to exploit the in vitro regenerated material since it contains significant amounts of bioactive compounds.

**Keywords:** *bioactive compounds, DPPH, flavonoids, GC-MS, micropropagation, phenolics*

### Introduction

*Ficus* is a significant fruit-bearing genus that its distribution comprises the tropics and subtropics (Badgujar et al., 2014). One species, *Ficus plamata* Forssk, often known as 'Fegra fig' or 'Wild fig,' produces edible fruit that is said to be high in flavonoids, phenolics, and other bioactive substances (Tewari et al., 2021; Al-Qahatani et al., 2023). This plant produces edible, nutrient-rich leaves (Al-Aizari et al., 2024a, b). Furthermore, scabies, gonorrhea, diabetes, asthma, diarrhea, and antiseptic are among the ailments for which the aerial portions of the fegra fig are utilized in traditional medicine (Khan et al., 2022). Additionally, gastrointestinal, fungal, and cancer disorders are treated with fegra fig plants (Sati et al., 2020). According to reports, latex and stem extracts of these plants have anti-ulcer, hepatoprotective, nephroprotective, anticancer, and antidiabetic properties (Khajuria et al., 2018; Al-Qahatani et al., 2023). Bioactive compounds, which are regarded as dietary supplements, are abundant in plants. In higher plants, phenolic compounds are second in quantity only to carbohydrates. They exhibit a wide range of structural variations, ranging from simple phenols to complex polymeric compounds like lignin (Sun and Shahrajabian, 2023). Many biological actions of phenolic compounds are well-known, such as immunomodulatory, anti-inflammatory, antioxidant, and antibacterial properties (Sun and Shahrajabian, 2023). Flavonoids, on the other hand, have been shown to have immune system-promoting, antioxidant, antibacterial, antiviral,

anticancer, and cardioprotective properties. These include anthocyanins, flavones, flavanones, and isoflavones (Roy et al., 2022). Tannins are polyphenolic substances with a variety of pharmaceutical and medical uses. They are categorized as hydrolyzable, condensed, and complicated tannins (Pizzi, 2021). It is crucial to measure these compounds in both naturally occurring plants and in vitro regenerated plants to determine their biological potential.

Wild figs are used to make herbal medicines, and plant material is currently harvested from their natural state. It is a slow-growing tree whose natural population has decreased due to overexploitation, particularly in the Arabian region. Several studies have shown that in vitro regenerated plants have accumulated larger quantities of plant secondary metabolites compared to mother plants, making in vitro propagation an excellent technique for getting plant biomass (Colin, 2001; Matkowski, 2008). We developed in vitro propagation techniques for the large-scale propagation of the fegra fig because the plant can only be propagated through cuttings and grafting (Al-Aizari et al., 2024a, b). The aim of the present study was to explore potential applications of in vitro regenerated fegra fig plant biomass for use in the herbal industry. In this study, we examined the phenolic, flavonoid, tannin contents, and antioxidant activities of leaf material from both in vivo and in vitro plants of fegra fig. Furthermore, we investigated the phytochemical composition of leaf material using gas chromatography (GC-MS) and mass spectrometry.

## Materials and methods

### *Plant material*

Fresh leaves that were collected from the mother plant of *Ficus palmata* growing in the wild ( $\approx 10$  years old; *Fig. 1a*) in Huraymial district, Saudi Arabia ( $20^{\circ} 7' 36''$  North,  $46^{\circ} 7' 21''$  East), and from in vitro regenerated plants following 9 weeks of culture (*Fig. 1b*) were utilized for phytochemical analysis. In vitro regeneration of plants was conducted in accordance with the established methodology reported by Al-Alzari et al. (2024a, b), utilizing nodal explants as the starting material. The axillary shoots of *F. palmata* were propagated in Magenta vessels containing 60 mL of Murashige and Skoog (1962) medium, which was supplemented with 3% sucrose and the optimal concentration of 2 mg/L benzylaminopurine (BAP) as the cytokinin, with the medium gelled using 0.8% agar. The cultures were maintained under controlled conditions for a duration of 5 weeks at a temperature of  $25 \pm 2^{\circ}\text{C}$ , subjected to a 16-hour photoperiod provided by cool-white fluorescent lamps, achieving a photosynthetic photon flux density (PPFD) of  $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ , followed by an additional 4 weeks of culturing on MS medium devoid of plant growth regulators (PGRs), while kept under the same conditions.

### *Phytochemical analysis*

#### *Extraction*

The leaf samples were washed thoroughly in distilled water and then dried in an oven at  $50^{\circ}\text{C}$  for 48 h, then ground into a fine powder using a laboratory grinder. Then one g of the powdered samples was macerated with 50 ml of absolute methanol and the samples were kept on gyratory shaker for 24 h. After that, the samples were filtered through the Whatman No.1 filter paper. The solvent was dried using a rotary evaporator at  $40^{\circ}\text{C}$  (IKA<sup>®</sup>-Werke GmbH & Co. KG, Stufen, Germany).



**Figure 1.** Plant materials of *Ficus palmata* used for phytochemical analysis. (a) Mother plant in its natural habitat. (b) In vitro regenerated shoots

#### *Quantification of phenolics*

The total phenolic content within the samples was quantified in accordance with the methodology established by Murthy et al. (2022). A volume of one hundred  $\mu\text{l}$  of methanolic extract was combined with 2.5 ml of deionized water, subsequent to which 0.1 ml of 2N Folin-Ciocalteu reagent was incorporated. The resultant solution was thoroughly mixed and permitted to stand for a duration of six minutes prior to the introduction of 0.5 ml of 20% sodium carbonate solution. Thereafter, the solution was maintained at ambient temperature for thirty minutes to facilitate color development. The absorbance of the solutions was assessed utilizing a UV-visible spectrophotometer (Hitachi U-3310, Ibaraki, Japan) at a wavelength of 765 nm, and the total phenolic content in the leaf samples was calculated employing gallic acid as the reference standard. The outcomes were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of the extract.

#### *Quantification of flavonoids*

The total flavonoid content within the samples was assessed through the colorimetric method delineated by Murthy et al. (2023, 2024). A volume of one ml of the sample (1 mg/ml) and one ml of 2% aluminum chloride solution were placed into test tubes, and following a thirty-minute incubation period at room temperature, the absorbance was measured at 420 nm using a UV-visible spectrophotometer (Hitachi U-3310, Ibaraki, Japan). Quercetin served as the standard for this analysis, and the results are conveyed as milligrams of quercetin equivalent (QE) per gram of dry weight of the extract.

#### *Quantification of tannins*

The estimation of total tannins within the samples was conducted utilizing the technique according to Makkar (2003). A hundred ml of the sample (1 mg/ml) was mixed with 200  $\mu\text{l}$  of Folin-Ciocalteu reagent, 1.5 ml of ultrapure water, and 200  $\mu\text{l}$  of 35% sodium carbonate solution; the mixture was subsequently agitated thoroughly and allowed to incubate for thirty minutes at room temperature in the absence of light. The absorbance of the samples was measured at a wavelength of 700 nm by means of a UV-visible

spectrophotometer (Hitachi U-3310, Ibaraki, Japan). Tannic acid was employed as the standard for this assessment, and the results are presented as milligrams of tannic acid equivalent (TAE) per gram of dry weight of the extract.

#### ***Analysis of antioxidant activity by 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay***

The antioxidant activity of samples was determined by DPPH radical scavenging activity by following the method of Yadav et al. (2022). 1 ml of extract was combined with 1 ml of 0.135 mM DPPH at different concentrations (31-100 µg/ml) in test tubes. The mixture was incubated at room temperature in the dark for 30 min. Ascorbic acid was used as the positive control and the absorbance of samples and control solution was measured using a UV-visible spectrophotometer (Hitachi U-3310, Ibaraki, Japan) at 517 nm. The percentage of scavenging activity is expressed in percentage compared to standard.

#### ***Gas chromatography-mass spectroscopy (GC-MS) analysis***

The specimens underwent analysis utilizing GC-MS (Thermo Scientific, Austin, Texas, USA), employing a capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The temperature within the column oven was initially maintained at 50°C, subsequently increased at a rate of 5°C/min to reach 250°C, where it was held for 2 minutes before further escalating to 300°C at a rate of 30°C/min. The injector temperature was maintained at 270°C throughout the analysis. Helium gas served as the carrier gas, maintained at a consistent flow rate of 1 ml/min. The solvent delay was established at 4 minutes, and diluted samples of 1 µl were injected automatically utilizing an autosampler AS3000 in conjunction with the GC in split mode. Electron Ionization (EI) mass spectra were acquired at ionization voltages of 70 eV across the mass-to-charge ratio (m/z) range of 50-650 in full scan mode. The ion source and transfer line temperatures were calibrated to 200°C and 280°C, respectively. The identification of chemical compounds was achieved through comparison with WILEY 09 and NIST14 library data, and the percentage peak area was computed from the total peak area of the chromatogram.

#### ***Statistical analysis***

The data were analyzed using analysis of variance (ANOVA). Students unpaired t-test was used to compare total phenolic, flavonoid, and tannin content in the in vitro leaves and the mother plant. Tukey's multiple range test was used for analysis of DPPH radical scavenging activity. The mean values were compared at  $p \leq 0.05$  in SAS (version 9.4; SAS Institute Inc., Cary, North Carolina, USA).

## **Results and discussion**

### ***Phenolic, flavonoid and tannin content***

In the current investigation, we determined the concentrations of tannin, flavonoids, and phenolics in fegra fig leaves. The findings are shown in *Table 1*. Leaves of in vivo plants contained a total of 48.53 mg GAE/g DW, 40.48 mg/g QE DW, and 20.09 mg TAE/g DW of phenolic, flavonoid, and tannin content, respectively. In comparison, the leaves of in vitro regenerated plants had their values quantified as follows: 70.78 mg GAE/g DW, 45.04 mg/g QE DW, and 28.00 mg TAE/g DW, respectively.

**Table 1.** Total phenolic, flavonoid, and tannin content of the *Ficus palmata* plants growing in the mother, and in vitro propagated plants

Phytochemicals	Total phenolics (mg GAE/g DW)	Total flavonoids (mg QE/g DW)	Total tannins (mg TAE/g DW)
Wild/mother plant	48.53 ± 0.322	40.38 ± 0.065	20.09 ± 0.030
In vitro plants	70.78 ± 0.052	45.04 ± 0.568	28.00 ± 0.010
<i>P</i> -value	< 0.0001*	0.0006*	< 0.0001*
Difference score calculation	Wild/mother plant: $N_1: 3$ $df_1 = N - 1 = 3 - 1 = 2$ $M_1: 48.53$ $SS_1: 0.62$ $s^2_1 = SS_1/(N - 1) = 0.62/(3-1) = 0.31$  In vitro plant: $N_2: 3$ $df_2 = N - 1 = 3 - 1 = 2$ $M_2: 70.78$ $SS_2: 0.02$ $s^2_2 = SS_2/(N - 1) = 0.02/(3-1) = 0.01$	$N_1: 3$ $df_1 = N - 1 = 3 - 1 = 2$ $M_1: 40.38$ $SS_1: 0.03$ $s^2_1 = SS_1/(N - 1) = 0.03/(3-1) = 0.01$  $N_2: 3$ $df_2 = N - 1 = 3 - 1 = 2$ $M_2: 45.04$ $SS_2: 1.93$ $s^2_2 = SS_2/(N - 1) = 1.93/(3-1) = 0.97$	$N_1: 3$ $df_1 = N - 1 = 3 - 1 = 2$ $M_1: 20.09$ $SS_1: 0.01$ $s^2_1 = SS_1/(N - 1) = 0.01/(3-1) = 0$  $N_2: 3$ $df_2 = N - 1 = 3 - 1 = 2$ $M_2: 28$ $SS_2: 0$ $s^2_2 = SS_2/(N - 1) = 0/(3-1) = 0$

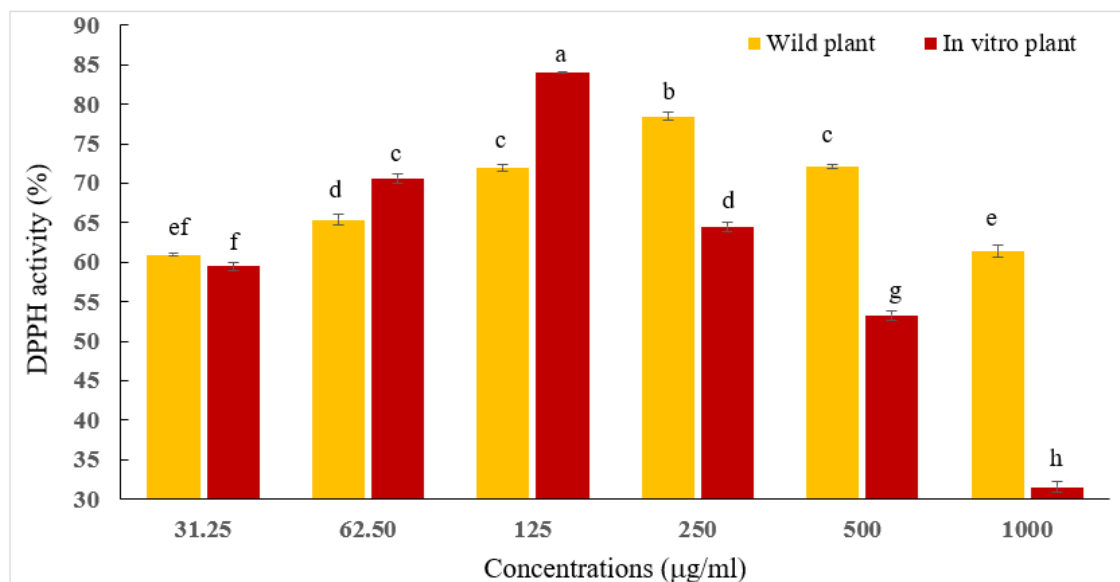
Data presented are means ± standard error (n = 3). \* = significant at  $P \leq 0.05$  according to Students unpaired *t*-test

Al-Qahtani et al. (2023) revealed that the amounts of flavonoids and phenolics in *Ficus palmata* leaves were 29.9 mg QE/g and 49.24 mg GAE/g, respectively. Utilizing methanol maceration, water infusion, and Soxhlet extraction using methanol as the solvent, Tewari et al. (2021) isolated the phenolics and flavonoids from fruits of *Ficus palmata* that were collected from the Himalayan region of India. While the total flavonoid content was calculated to be 1.25, 4.86, and 10.65 mg/rutin equivalent/g DW, the total phenolic content was found to be 29.43, 45.04, and 36.09 mg GAE/g DW. The total phenolic content and total flavonoid content of an aqueous extract of *Ficus palmata* were evaluated by Abbasi et al. (2015) in a different study. The total phenolic content and total flavonoid content values were 9.2 mg GAE/g and 14.2 mg rutin equivalent/g (on a fresh weight basis), respectively. Variations in the concentrations of total phenolic content and total flavonoid content in various plant organs may result from differences in the geographic locations from which plants were taken, as well as from the solvent and extraction technique employed to extract the bioactive compounds. When compared to in vivo plants, the leaves of in vitro regenerated plants had the greatest total phenolic and total flavonoid contents in the current investigations (Table 1). Similarly, compared to in vivo-grown plants of other medicinal species as *Ceropegia thwaitesii* (Muthukrishnan et al., 2018) and *Codonopsis pilosula* (Gang et al., 2023), micropropagated plants were found to have a higher amount of total phenolics and flavonoids.

### Antioxidant capacity

Assessments of the in vitro antioxidant activity and the nutraceutical qualities of bioactive substances are frequently employed, particularly when evaluating the capacity to scavenge free radicals. The DPPH radical scavenging method was used to assess the

free radical scavenging ability of several febra fig extracts. The findings are shown in *Figure 2*. Extracts from the leaves of in vitro regenerated plants had better DPPH scavenging activity findings than extracts from the leaves of in vivo-produced plants, according to a comparative investigation. The extract of in vitro regenerated plants showed 65.4 and 71.9% DPPH radical scavenging at 62.20 and 125 µg/ml DPPH concentrations, compared to 61.8 and 66.2% for leaf extracts of in vivo grown plants.



**Figure 2.** DPPH radical scavenging activity (%) of *Ficus palmata* leaves of mother plant and in vitro propagated plants. Different letters show significant differences at ( $p \leq 0.05$ ) according to Tukey's multiple range test ( $n = 3$ )

The research conducted by Alqasoumi et al. (2014) on the leaves of *Ficus palmata* revealed that even at low concentrations (50 and 100 µg/ml), the ethyl acetate fractions exhibited antioxidant activity and decreased the stable free radical DPPH. Nonetheless, low concentrations were found to have a modest antioxidant effect, while chloroform fractions 500 and 1000 µg/ml also showed strong antioxidant activity. Iqbal et al. (2014) discovered in another investigation that the methanol extract of febra pig leaves and the aqueous extract of bark both showed noticeably greater levels of concentration-dependent free radical scavenging activity. Our findings corroborate the findings of earlier research (Alqasoumi et al., 2014; Iqbal et al., 2014) regarding the antioxidant properties of febra fig leaf extract.

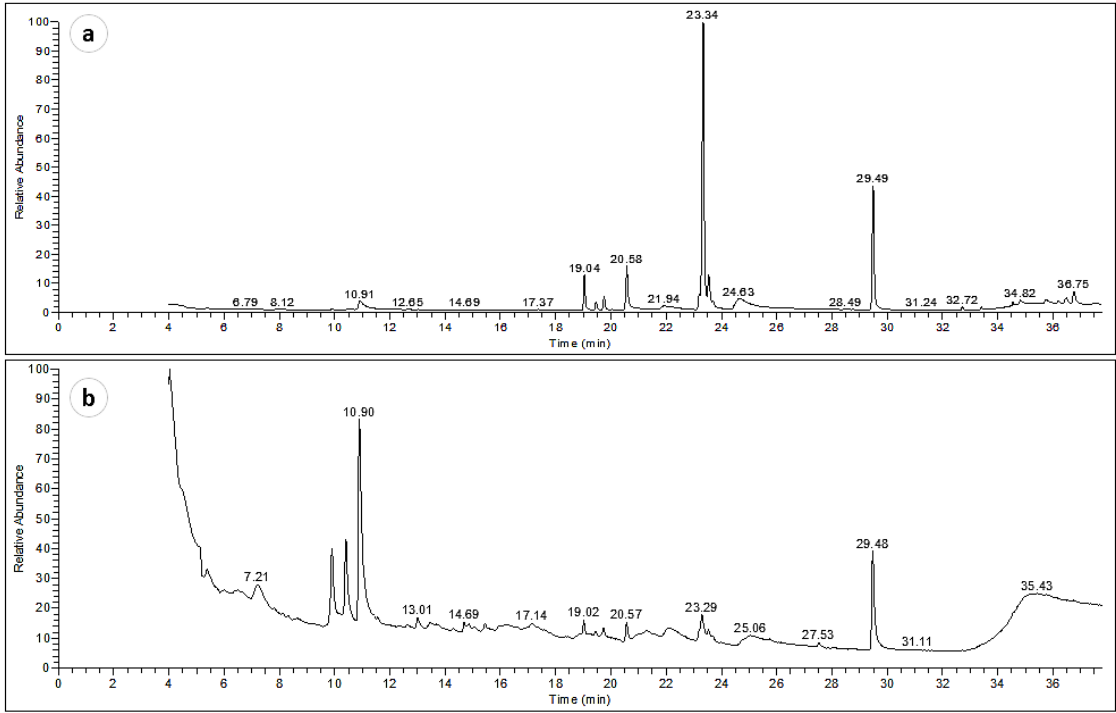
### Analysis of phytochemicals by GC-MS

GC-MS chromatogram is presented in *Figure 3* and major phytochemicals analyzed are given in *Table 2*. Totally 30 compounds were identified altogether in the mother plant and in vitro regenerated plants. It was quite interesting that only two compounds 9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]- (3 $\alpha$ ,5 $\alpha$ ,7 $\alpha$ )-, and 1,25-Dihydroxyvitamin D<sub>3</sub>, TMS derivative were common in both wild and in vitro regenerated plants. The content of those two compounds was higher in the wild plant than the in vitro regenerated febra fig plants. The major compounds that were identified in mother plants of febra fig were phytol (acyclic diterpene), neophytadiene (diterpene), lup-

20(29)-ene-3,28-diol, (3á)-, and 4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy- (flavanone). Others were fatty acid methyl esters and vitamins. Similarly, the major compounds which were identified with in vitro regenerated plants of fegra fig were 1-heptatriacotanol, 7-methyl-Z-tetradecen-1-ol acetate, 12-methyl-e,e-2,13-octadecadien-1-ol, estra-1,3,5(10)-trien-17á-ol, 9,12,15-octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (z,z,z)-. The heterogeneity observed between wild or mother plants with that of in vitro regenerated plants may be attributed to the age differences since age affects the chemical composition of plants (Achakzai et al., 2009; Morshedloo et al., 2018). Variations in bioactive substances have also been shown by phytochemical evaluation of the mother and regenerated plants of *Ceropegia thwaitesii* (Muthkrishnan et al., 2018) and *Codonopsis pilosula* (Gang et al., 2023).

**Table 2.** GC-MS analysis of the leaves of the *Ficus palmata*

No.	Retention time (min)	Name of compound	Area %		Molecular weight (g/mol)	Structural formula
			Mother plant	Regenerated plant		
1	17.36	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxymethyl]ethyl ester, (z,z,z)-	0.51	-	496	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>
2	19.02	Neophytadiene	3.86	-	278	C <sub>20</sub> H <sub>38</sub>
3	19.44	9,12-Octadecadienoic acid (Z,Z)-	1.22	-	280	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
4	19.74	13-Heptadecyn-1-ol	1.95	-	252	C <sub>17</sub> H <sub>32</sub> O
5	20.05	4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	0.50	-	344	C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>
6	20.56	Hexadecanoic acid, methyl ester	4.19	-	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
7	23.30	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	8.85	-	292	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>
8	23.52	Phytol	11.42	-	296	C <sub>20</sub> H <sub>40</sub> O
9	23.69	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	0.66	-	310	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>
10	32.71	Cholestan-3-ol, 2-methylene-, (3á,5á)-	0.67	-	400	C <sub>28</sub> H <sub>48</sub> O
11	33.40	Docosane	1.17	-	310	C <sub>22</sub> H <sub>46</sub>
12	34.54	Dotriacontane	1.74	-	450	C <sub>32</sub> H <sub>66</sub>
13	34.81	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5z,7e)-	0.66	0.49	488	C <sub>30</sub> H <sub>52</sub> O <sub>3</sub> Si
14	36.18	1,25-Dihydroxyvitamin D3, TMS derivative	0.86	0.75	488	C <sub>30</sub> H <sub>52</sub> O <sub>3</sub> Si
15	36.45	Methyl commate c	3.35	-	486	C <sub>31</sub> H <sub>50</sub> O <sub>4</sub>
16	36.74	Lup-20(29)-ene-3,28-diol, (3á)-	6.62	-	442	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>
17	36.48	1-Heptatriacotanol	-	2.02	536	C <sub>37</sub> H <sub>76</sub> O
18	7.22	Desulphosinigrin	-	5.43	279	C <sub>10</sub> H <sub>17</sub> NO <sub>6</sub> S
19	10.41	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	-	10.86	216	C <sub>12</sub> H <sub>24</sub> O <sub>3</sub>
20	13.00	7-Methyl-Z-tetradecen-1-ol acetate	-	1.60	268	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>
21	13.45	Aspidospermidin-17-ol, 1-acetyl-16-methoxy-	-	1.20	370	C <sub>22</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub>
22	14.85	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-à-methylene-	-	1.03	166	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub>
23	15.45	1-(Hydroxymethyl)-2,5,5,8A-tetramethyldecahydro-2-naphthalenol	-	1.05	240	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>
24	19.44	12-Methyl-e,e-2,13-octadecadien-1-ol	-	0.73	280	C <sub>19</sub> H <sub>36</sub> O
25	20.56	Methyl-9,9,10,10-D4-octadecanoate	-	2.79	302	C <sub>19</sub> H <sub>34</sub> D <sub>4</sub> O <sub>2</sub>
26	22.09	Estra-1,3,5(10)-trien-17á-ol	-	2.02	256	C <sub>18</sub> H <sub>24</sub> O
27	22.14	Tetraacetyl-d-xylonic nitrile	-	3.07	343	C <sub>14</sub> H <sub>17</sub> NO <sub>9</sub>
28	23.19	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (z,z,z)-	-	0.92	436	C <sub>25</sub> H <sub>40</sub> O <sub>6</sub>
29	23.29	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-z)-	-	4.08	318	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>
30	34.99	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy] methyl] ethyl ester, (z,z,z)-	-	1.53	496	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>



**Figure 3.** GC-MS analysis of *Ficus palmata* leaf extracts. (a) Mother plants. (b) In vitro propagated plants

Among the many phytochemicals evaluated by GC-MS, a number of them have strong biological activity; this data is shown in *Table 3*. Antioxidant, antibacterial, antioxidant, anti-inflammatory, anticancer, and hepatoprotective actions are mostly attributed to them. For example, phytol an acyclic diterpene was reported to possess antimicrobial, anticancer, anti-inflammatory, antioxidant, anti-diabetic, anti-diuretic, neuroprotective, antidepressant, anti-hypercholesterolemic, and hepatoprotective activities (Costa et al., 2016; Lee et al., 2016; and Phukan et al., 2017). Consistent with the aforementioned discoveries, *Ficus palmata* extracts have been shown to have antibacterial, antidiabetic, thrombolytic, and anticancer properties by Al-Qahatani et al. (2023). On the other hand, Tewari and colleagues (2021) showed strong antioxidant and in vitro inhibitory abilities on the enzymes  $\alpha$ -glucosidase,  $\alpha$ -amylase, cholinesterase, and tyrosinase.

**Table 3.** Biological activity of some identified compounds

Compound name	Biological activity	Reference
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Antimicrobial, antioxidant, anticancer	Albergoni et al. (1980)
Phytol	Antimicrobial, anticancer, antiinflammatory antioxidant, anti-diabetic, anti-diuretic, neuroprotective, antidepressant, anticonvulsant	Costa et al. (2016), Lee et al. (2016), Phukan et al. (2017)
9,12-Octadecadienoic acid (z,z)-	Anti-inflammatory, anti-arthritis, antiacne, anti-histaminic, anti-eczemic, anti-androgenic, anti-coronary, anti-cancer, antihypercholesterolemic, hepatoprotective	Jones (2002)
Hexadecanoic acid, methyl ester	Antioxidant, antimicrobial, hemolytic	Albergoni et al. (1980)
4 h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5- dihydroxy-7-methoxy-	Antioxidant, antimicrobial	Albergoni et al. (1980)
Dotriacontane	Antimicrobial, antifungal, anti-inflammatory, cytotoxic	Harris (1992)



Neophytadiene	Antioxidant, antibacterial activity	Sosa et al. (2016)
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5z,7e)-	Anticancer, regulate calcium in human blood	Elgorban et al. (2019)
13-Heptadecyn-1-ol	Antimicrobial, anti-inflammatory, antifungal, anticancer, antioxidant, cytotoxic	Al-Garawi et al. (2019)
Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	Antiviral, antifungal, Anti-HIV	Manjamalai et al. (2011)
Cholestan-3-ol, 2-methylene-, (3á,5à)-	Anti-inflammatory and cytotoxic	Hameed et al. (2016)
Tetraacetyl-d-xylic nitrile	Anti-viral	Al-Marzoqi et al. (2016)
Methyl commate c	Antidiabetic, antihyperlipidemic	Duke (2014)
Desulphosinigrin	Antibacterial	Sosa et al. (2016)
12-Methyl-e,e-2,13-octadecadien-1-ol	Antihistamine, antioxidant, analgesic, anesthetic, allergic, antibacterial, anticonvulsant	Lakshmi et al. (2018)
1-Heptatriacotanol	Anti-microbial	Kalairasan et al. (2011)
9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (z,z,z)-	Antiinflammatory, anticancer, hepatoprotective, nematocidal, antihistaminic, antieczemic, antiacne, antiandrogens, antiarthritic, anti-coronary	Dhayabaran and Thangarathinam (2016)
7-Methyl-Z-tetradecen-1-ol acetate	Anti-cancer, anti-inflammatory, hepatoprotective	Hameed et al. (2015)

## Conclusions

The in vitro regenerated plants displayed larger quantities of phenolics, flavonoids, and tannins than the mother fegra fig plants, according to the phytochemical analysis of the two. When compared to extracts of mother plants, the antioxidant experiment showed that the in vitro regenerated plant extracts had superior DPPH scavenging activity. Numerous bioactive phytochemicals were shown to be present in both the mother plant and the in vitro regenerated plants, according to the GC-MS results. Significant amounts of bioactive compounds were found only in in vitro regenerated plants and could be further explored for their therapeutic activities. Considering that the fegra fig is a woody, slow-growing species that are relatively rare in the Arabian region, the micropropagated plants may provide an alternative supply for the herbal business.

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