Chemical Profiling and Biological Activities of Methanol Extracts and Essential Oils from *Ferulago platycarpa* Boiss. & Balansa (Endemic): Enzyme Inhibition, Antioxidant, and Antimicrobial Properties

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This study aimed to evaluate the phytochemical composition and biological activities of *Ferulago platycarpa* methanol extract (ME) and essential oil (EO). The EO was obtained *via* hydrodistillation and analyzed by GC-MS, revealing sesquiterpene hydrocarbons as the dominant class, with caryophyllene (65.8%) and *α*-pinene (9.65%) as the major constituents. The methanol extract was subjected to LC-MS/MS analysis, which identified eleven phenolic compounds, with chlorogenic acid as the most abundant. The extract showed significantly higher total phenolic (1196.22±11.64 mg GAE/100g) and antioxidant (1870.00±17.69 mg QEE/100g) contents compared to the ME. *In vitro* enzyme inhibition assays demonstrated that the methanol extract exhibited potent inhibitory activity against carbonic anhydrase II (CA-II, IC_{50}=0.023 µg/mL), acetylcholinesterase (AChE, IC_{50}=110 µg/mL), and butyrylcholinesterase (BChE, IC_{50}=390 µg/mL). In contrast, the EO showed higher inhibition against α-amylase (IC_{50}=5920±10.45 µg/mL) and BChE (IC_{50}=1.32±0.65 µg/mL), while its *α*-glucosidase showed no inhibition. Antioxidant assays indicated superior activity for the methanol extract compared to the EO. Furthermore, antimicrobial testing revealed that the EO demonstrated broader and more effective antimicrobial action, exhibiting lower MIC and MBC values against several bacterial and fungal strains. Collectively, these results highlight *F. platycarpa* as a valuable source of bioactive compounds with promising applications in antidiabetic, neuroprotective, antioxidant, and antimicrobial therapies.

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INTRODUCTION

Plants have continued to be a natural resource for humans throughout human history (Mohammed *et al.* 2019, 2020). In the early days, plants were used in a variety of ways, either instinctively or by following animals' patterns. As time went on, it became more

reasonable to use because of new discoveries made from various experiences (Saxena *et al.* 2013; Mohammed *et al.* 2018; Mohammed *et al.* 2020). All these discoveries, combined with beliefs and practices and made through trial and error, have been passed down from generation to generation for centuries, creating a vast amount of knowledge (Ji *et al.* 2009; Ekor 2014). It has been estimated that 75% of the natural herbal compounds used in health treatment today have been discovered as a result of traditional folk medicine studies (Pan *et al.* 2013).

Apiaceae is one of the families known to contain essential oils (EOs) and is found worldwide. It is stated that most of the species belonging to this family are collected in the Asian continent (Harborne 1982; Bakar *et al.* 2016). *Ferulago* W. Koch., which has about 40 species, is a perennial genus of the Apiaceae family. Thirty-two of these species, 17 of which are endemic, grow naturally in the flora of Türkiye. This is strong evidence that the gene center of the *Ferulago* genus is Anatolia (Davis 1972; Davis *et al.* 1988; Akalın 1999; Özhatay and Akalın 2000; Akalın and Pimenov 2004; Kandemir and Hedge 2007; Erdemoğlu *et al.* 2008; Kılıç *et al.* 2010). Türkiye, Kazakhstan, Iran, China, and Russia, located in the Asian continent, are the countries with the highest biodiversity of the *Ferulago* species. In different regions of Türkiye, species of this genus are known by the names *çelebi kişnişi*, *kuzukişnişi*, *kişniş* (*coriander*), *kuzubaşı*, *kuzukemirdi*, *asaotu*, *cağşır*, and *çakşırotu* (Mohammed *et al.* 2020).

The *Ferulago* genus contains many bioactive components. In previous studies, it was stated that *Ferulago* species contain aromatic compounds, flavonoids, coumarins, furanocoumarins, coumarin esters, sesquiterpenes and quinones (Miski *et al.* 1990; Doğanca *et al.* 1991; Doğanca *et al.* 1992; Giuseppe *et al.* 1994; Jiménez *et al.* 2000; Erdurak Kılıç and Coşkun 2006; Erdurak Kılıç *et al.* 2006; Kılıç *et al.* 2010). The main components of the oils of species belonging to the mentioned genus, which are rich in essential oil (EO), were limonene, *α*- and *β*-phellandrene, *β*-ocimene, *p*-cymene, *α*-pinene and myrcene (Gençler Özkan *et al.* 2008; Kılıç *et al.* 2010).

The main components EO of aerial parts of *F. platycarpa* were determined to be 2,3,6-trimethylbenzaldehyde (29.8%), *cis*-chrysanthenyl acetate (24.2%), nonacosan (7.7%), and *α*-pinene (4.2%). The main components in *F. pachyloba* oil were identified as (*Z*)-*β*-ocimene (25.7%) and *α*-pinene (9.8%). The main components *F. isaurica* oil were exhibited as nonacosan (25.5%) and hexadecanoic acid (14.8%). The main components in *F. longistylis* oil were shown as 2,3,6-trimethylbenzaldehyde (32.7%), and bornyl acetate (12.6%) (Kılıç *et al.* 2010).

Ferulago species are known to be consumed as food in the form of pickles and also as a flavouring and preservative in dairy products, especially cheese (Miski *et al.* 1990; Mohammed *et al.* 2020).

Ferulago species have been used since ancient times in the treatment of diseases such as ulcers, headaches, spleen diseases, hemorrhoids, intestinal worms, snake bites (Demetzos *et al.* 2000; El-Thaher *et al.* 2001; Kılıç *et al.* 2010; Ameen 2014; Shahbazi *et al.* 2015; Mohammed *et al.* 2020). Due to these wide areas of use, studies on the biological activities of the mentioned genus have accelerated in recent years. The results attained from these studies revealed that *Ferulago* has anti-apoptotic, anti-cancer, anti-proliferative, immunomodulatory, anti-fungal, anti-bacterial, anti-oxidant, anti-coagulant, and anti-viral effects (Harborne 1982; Kılıç *et al.* 2010; Shahbazi *et al.* 2015; Mohammed *et a*l. 2020).

According to the literature, very few studies have been found in the world regarding the detailed analysis of *F. platycarpa* essential oils. Therefore, the present work aimed to examine ME, and EOs, including their chemical composition, enzyme inhibitory activities,

antimicrobial and antioxidant activities obtained from aerial parts of *F. platycarpa* growing as an endemic species in the world.

EXPERIMENTAL

Plant Material

In this study, plant samples of *F. platycarpa* were collected from Bayburt to İspir near the Arslandede Village (40°23'08"N, 40°27'26"E, Altitude: 1474 m, 17 July 2024, Sandy places) in Bayburt Province, Türkiye. The work titled "FLORA OF TURKEY and the East Aegean Islands" was used to identify the plant (Peşmen 1972), by using a Leica E4Z stereo microscope with mm measurements. The plant's aerial parts are depicted in Fig. 1. A total of 500 g of plant samples were gathered. The plant's taxonomic identification was confirmed by Assoc. Prof. Abdurrahman Sefalı from the Bayburt University, Faculty of Education, Department of Primary Education, Bayburt, Türkiye. The sample was stored in the Herbarium of Bingöl University, with the reference number BIN *Sefalı* 1250.

Fig. 1. Habitus of *F. platycarpa* Boiss. & Balansa

Extraction Procedure

Extraction of essential oil

A 500 g plant sample was dried in shade, ground, and sieved through a 250-micron mesh. From this, a 100 g sample was added to a 2000 mL glass flask containing 1000 mL of distilled water. The plant samples were heated on a jacketed heater at 100 ºC for about 4 hours. The essential oil was allowed to accumulate in the modified Clevenger type device with internal and external cooling. The collected essential oil was taken with 1 mL of hexane in a brown vial of GC quality, and the remaining water was dried with anhydrous Na_2SO_4 (sodium sulfate). The liquid was filtered through a 0.45 micron filter and stored in amber bottles.

Extraction with methanol solvent

The methanol extraction was carried out using an ultrasonic bath (3 L, 320 W, Bandelin Ultrasonic Bath). After grinding the plant parts, 10 g were weighed, and 50 mL of 30% aqueous methanol (MeOH) was added. The mixture was then subjected to ultrasound-assisted extraction for 60 min at 40 °C. Following the extraction, the solution was filtered twice through Whatman No. 1 filter paper and centrifuged at 4000 rpm for 10 min to obtain the plant extracts. After centrifugation, the supernatant liquid was transferred to a beaker and the methanol-water mixture was completely evaporated in a Heidolp rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 40 °C for 40 min to yield the final extracts.

Analysis of Essential Oil Components by GC-MS/FID

For GC-MS/FID analysis, a 1.0 µL injection of the oil solution (1:5 split ratio) was made, using helium (> 99.999%) as the carrier gas at a flow rate of 1.0 mL/min. The analysis was performed with an HP-5 apolar capillary column (30 m × 0.32 mm, film thickness 0.25 µm) on an Agilent 7890A GC and Agilent 5975C MS system (GC-FID Agilent-7890A model, MS Agilent 5975C, Agilent Technologies Inc, Santa Clara, CA, USA). The injector, ion source, and quadrupole temperatures were set to 250, 230, and 150 °C, respectively. The GC oven temperature was programmed to start at 60 °C for 2 min, then raised to 240 °C at a rate of 3 °C/min. The FID detector was set to 250 °C with hydrogen and air flows of 35 and 350 mL/min, respectively. Mass spectra were acquired from *m*/*z* 45 to 450 after a 3.8-min solvent delay.

The compounds of the essential oil were identified by comparing their mass spectra with reference data from the Adams (Adams 2007), Willey and NIST libraries and validated by comparing Kovats indices and their retention times with both authentic compounds and literature values (Öz *et al.* 2023).

Determination of Enzyme Inhibitory Activities

Activity assay for butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) enzymes

The activity of BChE and AChE enzymes was determined spectrophotometrically using the method of Ellman. This method used acetylthiocholine iodide as the substrate. In regard to the tenet of the Ellman method, acetylthiocholine iodide is hydrolyzed by acetylcholinesterase, and the resulting thiocholine reacts with the Ellman reagent, DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid)). At the end of this reaction, a yellow-colored chromophore, TNB (5-thio-2-nitrobenzoic acid), is formed. The rate of formation of this yellow compound (the intensity of the color) was measured by absorbance at 412 nm using a UV-Spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan) (Ellman *et al.* 1961).

The procedure for butyrylcholinesterase enzyme was the same as for AChE, with the only difference being the use of butyrylthiocholine iodide as the substrate for BChE instead of acetylthiocholine iodide, which is used in AChE assays. IC_{50} graphs were drawn by measuring enzyme activity at 5 different inhibitor concentrations and IC_{50} values were determined from the graph equation. The graph was plotted by writing 5 different substrate concentrations on the horizontal axis (X axis) and % activity values on the vertical axis (Y axis) and IC_{50} values were calculated from the equation obtained from the graph. The standard substance used for AChE and BChE is Tacrine (Lineweaver and Burk 1934).

The inhibitory activities of the samples against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were detected spectrophotometrically based on the Ellman method. The assay was performed in 0.1 M phosphate buffer (pH 8.0) using Ellman's

reagent (DTNB), prepared by dissolving 4 mg of DTNB in 1 mL of buffer. For each reaction, 1 mL of sample solution was mixed with 50 µL of DTNB and pre-incubated at room temperature for 10 to 15 min. Then, 30 µL of enzyme (AChE or BChE) and 75 µL of the appropriate substrate (acetylthiocholine iodide for AChE; butyrylthiocholine iodide for BChE) were added. The absorbance was measured at 412 nm. A blank without DTNB was used to correct for background absorbance.

Enzyme activities were measured at five different inhibitor concentrations, and IC_{50} values were shown from the regression equation of inhibition curves. Graphs were plotted with inhibitor concentrations on the X-axis and % enzyme activity on the Y-axis. Tacrine was used as the reference standard (Lineweaver and Burk 1934).

Carbonic anhydrase enzyme activity assay

The basis of this method lies in the esterase activity of carbonic anhydrase. According to this method, carbonic anhydrase hydrolyzes *p*-nitrophenyl acetate, which is utilized as the substrate, into *p*-nitrophenol or *p*-nitrophenolate that absorbs at 348 nm. Both *p*-nitrophenol and *p*-nitrophenolate exhibit the same absorbance at 348 nm. Therefore, the dissociation of the H^+ ion from the phenolic OH group does not affect the measurement (Armstrong *et al.* 1966; Kandel *et al.* 1970; Landolfi *et al.* 1997).

α-Glucosidase enzyme activity assay

The *α*-glucosidase enzyme activity was realized considering the procedure previously established by Tao *et al*. (2013). 650 µL of phosphate buffer (pH: 6.8 and 0.1 M) was added to the test tubes. Then, 20 µL of sample and 30 µL of α-glucosidase enzyme (*Saccharomyces cerevisiae*) prepared in phosphate buffer were added. After the mixture was incubated at 37 °C for 10 min, 75 µL of substrate (*p*-Nitrophenyl-*α*-*D*-glucopyranoside) was added. The mixture was kept at 37 °C for 20 min; then 650 µL of 1 M Na_2CO_3 was added to all tubes and the reaction was stopped. Absorbance was measured spectrophotometrically at 405 nm. The activities of the samples were measured at different inhibitor concentrations, and % activity *vs*. graphs were drawn. IC_{50} values were determined from the equation of the curve (Lineweaver and Burk 1934).

α-Amylase enzyme activity assay

The *α*-amylase inhibitor activity was evaluated according to the Caraway-Somogyi iodide/potassium iodide (IKI) method with some adaptations, based on the procedure of Yang *et al.* (2012). In this study, 25 µL of each sample was pipetted into the wells of a microplate using an automatic pipette. Next, 50 µL of *α*-amylase solution prepared with phosphate buffer (pH 6.9, 6 mM sodium chloride) was included to each well. The mixtures were incubated in an oven at 37 °C for 10 min. After this pre-incubation, 50 µL of 0.05% starch solution was pipetted into each well. Simultaneously, a blank solution without enzyme was also prepared. The 96-well microplate was incubated at 37 °C for 10 min, and at the end of this period, the reaction was stopped by adding 25 µL of 1 M HCl to each well using an automatic pipette. Following this, 100 µL of iodine-potassium iodide solution was added to each well, and the formation of color was allowed to occur. The absorbance of the samples was read using a microplate reader spectrophotometer, set to 630 nm UV-Spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan). Acarbose was used as the standard. The *α*-amylase inhibitor activity results for the samples were calculated as IC_{50} values.

Determination of Antioxidant Activities and Bioactive Components

The antioxidant activities of the methanol extract and EO of *F. platycarpa* were assessed based on its Fe (III) ion reducing antioxidant power (FRAP), and its free radical scavenging abilities using ABTS and DPPH assays. Additionally, the amounts of bioactive components were determined through total antioxidant capacity (TAC), total flavonoid content (TFC), and total phenolic content (TPC) measurements.

Fe (III) ion reducing antioxidant power (FRAP)

The FRAP analysis of the methanol extracts was performed following the method described by Fidan *et al.* (2023) using a FRAP solution. 2750 µL of FRAP solution was added to 250 µL of sample. The mixture was vortexed, followed by a 30 min waiting period. Distilled water (500 µL) was used as the blank, and 250 µL of standard solutions were prepared and treated in the same manner. The FRAP values in the samples were calculated using the calibration curve derived from $FeSO_4$ solutions, with the total iron-reducing capacity expressed as mg $FeSO_4$ equivalent/100 g.

Free radical scavenging abilities using ABTS and DPPH assays

The ABTS (2,2′-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) activity was analyzed according to the method outlined by Kobya *et al.* (2024) using an ABTS solution. 2850 µL of ABTS working solution was added to as 150 µL of sample. The mixture was vortexed and left in the dark for 120 min. Methanol (150 µL) was used as the blank, and 150 µL of standard ascorbic acid solutions and sample extract were treated in the same way. The absorbance of the resulting solution was determined at 734 nm using a spectrophotometer. The ABTS cation radical scavenging activity in the samples was calculated based on a calibration curve derived from Trolox standards. The results are presented as mg TRE/100 g, mg Trolox equivalent/100 g, and the percentage of free radical scavenging, as shown in Eq. 1.

$$\text{Inhibition (\%)} = (\text{Control Absorbance} - \text{Example / Control Absorbance}) \times 100 \quad (1)$$

The DPPH (2,2-diphenyl-1-picrylhydrazyl) activity of the methanol extract and essential oil obtained from the plant was assessed using DPPH according to the method by Yılmaz *et al.* (2023). In this method, the methanol extract and DPPH solutions at specific concentrations were mixed by vortexing and incubated at room temperature in the dark for 30 min. After incubation, the absorbance of the samples was measured at 517 nm, and the DPPH % inhibition was calculated using Eq. 1. The results are expressed as mg AA equivalent/100 g, and the percentage of free radical scavenging.

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of the methanol extract and essential oil from the plant was measured using a molybdate reagent, following the method described by Yilmaz *et al.* (2023). 2500 µL deionized water was added to as 500 µL of essential oil sample and then 1000 µL of molybdate was added to the mixture. This mixture was vortexed and incubated in a 95 °C water bath with closed caps for 90 min. It was taken from the water bath, followed by waiting 20 to 30 min to reach room temperature. Pure water (250 µL) was used as the blank instead of the sample. The absorbance of the resulting reaction mixtures was measured at 695 nm using a spectrophotometer. For the standards, 500 µL of solutions were prepared and treated in the same manner. The TAC content in the methanol extract and EO samples was calculated as mg gallic acid equivalent (GAE)

per 100 grams, using the calibration curve derived from ascorbic acid solutions (Yilmaz *et al*. 2023).

Total flavonoid content (TFC)

The total flavonoid content (TFC) of the methanol extracts and essential oil (EO) was determined by a colorimetric method, as described by Yilmaz *et al.* (2023), with minor modifications to improve clarity and reproducibility. Briefly, 500 µL of the sample or standard solution (quercetin, 10 to 100 µg/mL in ethanol) was mixed with 3200 µL of 30% methanol (v/v, HPLC grade). Then, 150 µL of 0.5 M sodium nitrite ($NaNO_2$) solution was added, followed by 150 µL of 0.3 M aluminum chloride ($AlCl_3$) solution. After standing at room temperature for 5 min, 1 mL of 1 M sodium hydroxide (NaOH) was added. The mixture was vortexed and allowed to react for 10 min at room temperature. The absorbance of the resulting solution was measured at 506 nm using a UV–Vis spectrophotometer. Distilled water was used as the blank. TFC values were expressed as milligrams of quercetin equivalent per 100 grams of dry sample (mg QEE/100 g), using a calibration curve generated from quercetin standards ($R^2 > 0.999$)

Total phenolic content (TPC)

The total phenolic content (TPC), a bioactive component of the methanol extracts and EO was determined according to the method outlined by Yilmaz *et al.* (2023) using Folin-Ciocalteu reagent. After preparing the mixture, it was vortexed and incubated in the dark at room temperature for 120 min. Following the incubation, the absorbance of the mixture was measured at 760 nm. A blank was prepared using a mixture of 3.7 mL of water, 500 µL of methanol, 100 µL of Folin-Ciocalteu reagent, and 600 µL of 10% Na_2CO_3 solution. The phenolic content in the samples was expressed as mg GAE/100 g, using the calibration curve derived from the gallic acid solution.

Identification and Quantitation of Phenolic Compounds Using LC-MS/MS

The LC-MS/MS analyses were conducted using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled with a TSQ Quantum Access Max tandem mass spectrometer. The liquid chromatography system was equipped with an autosampler, degasser, dual pump, and column chamber. Chromatographic separation was achieved using a C18 reversed-phase Inertsil ODS Hypersil analytical column (250 mm × 4.6 mm, 5 µm), maintained at a constant temperature of 30 °C. The mobile phase consisted of two components: mobile phase A (water containing 0.1% formic acid) and mobile phase B (methanol). The gradient elution program was set as follows: 0 to 1 min, 0% B; 1 to 22 min, 95% B; 22 to 25 min, 95% B; 25 to 30 min, 100% B. The total run time, including conditioning, was 34 min. The injection volume and flow rate were set at 20 µL and 0.7 mL/min, respectively. After extensive optimization trials to achieve optimal ionization and separation of the target molecules, the described mobile phase and gradient were selected for this study. The phenolic compounds listed in Table 2 were analyzed using this LC-MS/MS method, and a chromatogram of the standard phenolic compounds is provided. The analytical method described here was adapted from the methodology developed by Kayir *et al.* (2023).

Determination of Antimicrobial Activities

Disk diffusion test

The antimicrobial activities of methanolic extracts and EOs of *F. platycarpa* were investigated using the disc diffusion test (DDT) method with modifications based on CLSI (2017) guidelines. The test organisms included gram-positive bacteria (*Bacillus cereus* ATCC 9634, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923), gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853), and the yeast *Candida albicans* ATCC 18804.

Fresh cultures of pathogens were prepared on Müller-Hinton agar (MHA) and suspended in 0.9% sterile saline to achieve a 0.5 McFarland turbidity (1.5×10^8 microorganisms/mL). The microorganisms were spread on the MHA plates using a sterile swab. Sterile disks (5.5 mm) were placed on the inoculated agar, and 15 µL of the samples (prepared in DMSO at 25 mg/mL) were applied to the disks. The plates were incubated at 4 °C for 2 h to allow diffusion.

Chloramphenicol and nalidixic acid were utilized as positive controls, and DMSO served as the negative control. The solutions of positive controls were prepared at a concentration of 512 µg/mL. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for yeast. After incubation, the clear zones of inhibition around the disks were measured using a digital caliper. The experiment was performed three times, and the results were recorded for statistical analysis.

The MIC and MBC values

The minimum bactericidal concentration (MBC) and Minimum inhibitory concentration (MIC) values of *F. platycarpa* extracts and essential oils against selected pathogens were determined according to CLSI (2017) guidelines. The MIC value was detected using the broth dilution method with sterile 96-well microplates. Müller-Hinton broth (MHB) was used as the medium. The first wells were filled with 2×concentrated MHB, and the remaining wells with standard MHB. Sample solutions were prepared at a concentration of 25 mg/mL, and 100 µL was pipetted into the first wells to achieve a starting concentration of 12.5 mg/mL. Nalidixic acid and chloramphenicol were dissolved in ethanol and used as positive controls, with concentrations adjusted to 256 µg/mL. About 10 µL of microbial suspension at 0.5 McFarland turbidity was added to each well. Negative controls included the extracts and medium.

The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for yeast. After incubation, the wells with no microbial growth were recorded as MIC values. For MBC determination, after the MIC test, 50 µL from wells corresponding to MIC, 2×MIC, and 4×MIC were transferred to Müller-Hinton agar plates and spread using a Drigalski spatula. The plates were incubated at 37 °C for a period of 24 h for bacteria and at 28 °C for 48 h for yeast. After incubation, colony counts were performed, and the lowest concentration that killed 99.9% of the initial microorganisms was recorded as the MBC. The determined concentrations represent the MBC values of the extracts against the pathogens.

RESULTS AND DISCUSSION

Essential Oil Analysis

The results from GC-MS/FID analysis of the EO obtained from the aerial parts of the *F. platycarpa* plant and the structures of a total of 85 chemical compounds analyzed were determined, but the structures of 3 compounds could not be identified. The identified compounds accounted for 98.86% of the essential oil. It was determined that the highest compounds in percentage in the EO isolated from the plant were caryophyllene (65.8%), *α*-pinene (9.65%) and *cis*-crysanthenyl acetate (3.71%). It was observed that the most abundant main constituent in the aerial part of *F. platycarpa* EO was caryophyllene (Table 1).

The predominance of *β*-caryophyllene (65.8%) and *α*-pinene (9.65%) in the essential oil composition of *F. platycarpa* suggests a considerable potential for pharmacological and industrial applications. *β*-caryophyllene is a bicyclic sesquiterpene known for its anti-inflammatory, analgesic, antimicrobial, and cytoprotective properties (Gertsch *et al.* 2008). Moreover, it selectively binds to the cannabinoid receptor type 2 (CB2), making it a promising non-psychoactive therapeutic agent in the treatment of inflammatory and neurodegenerative diseases. *α*-pinene, a monoterpene commonly found in coniferous trees, has demonstrated bronchodilator, anti-inflammatory, and cognitive-enhancing effects (Miguel 2010). The high content of these compounds may allow for their direct use in pharmaceutical formulations, or they could serve as precursors in semi-synthetic derivatization, leading to structurally modified analogs with enhanced bioactivity or pharmacokinetics. Thus, the essential oil of *F. platycarpa* not only holds promise for direct therapeutic application but also as a raw material for bio-based fine chemical synthesis.

The chemical classification of 85 compounds detected in the EO was determined as 12 groups. The percentage value of the classes were determined. The percentages of compounds they contained were detected as sesquiterpene hydrocarbons 66.38% (10 compounds), monoterpene hydrocarbons 14.19% (17 compounds), oxygenated monoterpenes 9.55% (20 compounds), hydrocarbons 4.41% (9 compounds), aldehydes 1.74% (6 compounds), oxygenated sesquiterpenes 0.96% (6 compounds), others 0.92% (4 compounds), esters 0.58% (9 compounds), ketones 0.07% (1 compound), alcohols 0.05% (2 compounds), and unidentified compounds 1.14% (3 compounds), respectively. The chemical class with the highest percentage of chemical compounds was sesquiterpene hydrocarbons and the highest number of compounds was oxygenated monoterpenes (Table 1).

The EOs of *F. platycarpa*, *F. pachyloba*, *F. longistylis*, and *F. isaurica* (Apiaceae) were extracted by hydrodistillation from the aerial parts and analyzed by GC and GC-MS. The highest oil yield (1.50%) was attained from *F. pachyloba*, followed by *F. longistylis* (0.16%), *F. isaurica* (0.08%), and *F. platycarpa* (0.07%). A total of sixty-seven volatile compounds were identified in the EO of aerial parts of *F. platycarpa*, representing 94.10% of the oil. 2,3,6-trimethylbenzaldehyde, *cis*-chrysanthenyl acetate, nonacosan, and *α*-pinene were the main components with 29.8%, 24.2%, 7.7% and 4.2%, respectively. Fifty-three compounds, including (*Z*)-*β*-ocimene (25.7%) and *α*-pinene (9.8%), were identified as the main components in *F. pachyloba* oil. Seventy-eight compounds were identified in *F. isaurica* oil, the main components of which were nonacosan (25.5%) and hexadecanoic acid (14.8%). Fifty-nine compounds were found in *F. longistylis* oil, the main components

of which were 2,3,6-trimethylbenzaldehyde (32.7%) and bornyl acetate (12.6%) (Kılıç *et al.* 2010).

The differences in the composition of EOs can be attributed to the different plant species and plant parts, as described in the literature. In addition, each plant can produce different chemical compounds. El-Hawary *et al*. (2018) reported that differences in the composition of volatile substances may vary depending on environmental conditions, preparation methods, and different cultivation techniques.

Table 1. The Essential Oil Components of Aerial Parts in *F. platycarpa*

No	RT (min)	Concentration (%)	Compounds	RI	LRI
1	4.533	0.02	Heptane	699	700
2	4.733	0.24	1,5,5-Trimethyl-cyclopentadien	708	708
3	5.992	0.01	Toluene	763	763
4	6.849	0.11	Hexanal	801	800
5	8.614	0.02	(*E*)-2-Hexenal	850	850
6	9.212	0.01	1-Hexanol	867	867
7	10.437	0.00	Heptanal	902	902
8	12.111	9.65	*α*-Pinene	942	942
9	12.442	0.17	Camphene	950	950
10	12.648	0.13	Verbenene	955	956
11	13.593	2.38	Sabinene	978	978
12	13.670	0.27	*β*-Pinene	980	980
13	13.815	0.01	*β*-Myrcene	984	984
14	14.369	3.04	Mesitylene	997	996
15	14.761	0.05	*p*-Cymene	1006	1006
16	14.990	0.01	*α*-Phellandrene	1012	1012
17	15.264	0.10	*α*-Terpinene	1018	1018
18	15.564	1.03	Pseudocumene	1025	1025
19	15.807	0.35	Limonene	1031	1031
20	16.168	0.13	*cis*-*β*-Ocimene	1040	1040
21	16.391	0.01	Benzeneacetaldehyde	1045	1045
22	16.600	0.01	*trans*-*β*-Ocimene	1050	1050
23	16.948	0.23	*γ*-Terpinene	1058	1058
24	17.262	0.15	*α*-Ocimene	1065	1056
25	17.397	0.04	Benzyl alcohol	1069	1065
26	17.589	0.01	*p*-Mentha-3,8-diene	1073	1073
27	18.068	0.03	(*E*)-*p*-Menth-2,8-dien-1-ol	1084	1102
28	18.372	0.52	α-Terpinolene	1092	1092
29	18.639	0.37	Linalool	1098	1098
30	19.256	0.01	*p*-Mentha-1,5,8-triene	1113	1113
31	19.672	0.05	Thujol	1123	1123

32	19.925	0.72	*α*-Campholenal	1129	1129
33	20.455	0.19	Sabinol	1142	1142
34	20.549	0.18	Pinocarveol	1144	1143
35	20.769	0.59	Verbenol	1150	1148
36	20.890	0.60	*p*-Mentha-1,5-dien-8-ol	1153	1153
37	21.305	0.22	2-Ethyl-phenol	1163	1160
38	21.434	0.17	Myrtenal	1166	1166
39	21.612	0.44	Safranal	1170	1171
40	22.061	0.46	Terpinen-4-ol	1181	1181
41	22.311	0.33	4-Ethylbenzaldehyde	1187	1194
42	22.801	0.11	2-Pinen-10-ol (Myrtenol)	1199	1199
43	23.327	0.12	Verbenone	1212	1212
44	23.709	0.07	*cis*-Carveol	1222	1222
45	24.087	0.34	*trans*-Carveol	1232	1232
46	25.022	0.09	*cis*-*p*-Mentha-1(7),8-dien-2-ol	1255	1235
47	25.616	3.71	*cis*-Crysanthenyl acetate	1271	1267
48	25.764	0.31	3,4,5-Trimethyl-phenol	1274	1288
49	26.378	0.28	Bornyl acetate	1290	1290
50	26.601	0.50	Sabinyl acetate	1296	1295
51	26.925	0.55	Myrtenyl acetate	1304	1305
52	28.026	1.27	Mesitaldehyde	1334	1335
53	31.273	65.88	Caryophyllene	1422	1422
54	31.445	0.18	Methyleugenol	1427	1428
55	31.637	0.07	3-Hydroxy-4-phenyl-3-buten-2-one	1432	1433
56	32.282	0.64	*γ*-Elemene	1451	1451
57	32.424	0.10	Seychellene	1455	1456
58	32.714	0.09	(-)-Aristolene	1463	1455
59	32.977	0.21	Methylisoeugenol	1470	1468
60	33.058	0.06	Neryl isobutanoate	1473	1474
61	33.264	0.02	*γ*-Decalactone	1479	1478
62	33.561	0.14	*γ*-Cadinene	1487	1487
63	33.770	0.24	Germacrene D	1493	1493
64	34.027	0.10	Phenethyl isovalerate	1500	1499
65	34.266	0.11	Bicyclogermacrene	1507	1507
66	34.560	0.09	*β*-Himachalene	1516	1516
67	34.904	0.07	(+)-Ledene	1527	1523
68	35.093	0.16	Eremophylene	1532	1527
69	36.174	0.19	Geranyl butyrate	1565	1565
70	36.437	0.22	Caryophyllene oxide	1573	1573
71	36.980	0.52	(+)-Spathulenol	1589	1589
72	37.439	0.05	Salvial-4(14)-en-1-one	1603	1603

73	37.946	0.08	Santalol	1619	1617
74	38.800	0.12	2-Phenethyl hexanoate	1646	1645
75	38.907	0.05	α-Cadinol	1650	1650
76	42.131	0.04	Geranyl hexanoate	1754	1756
77	42.560	0.03	Benzyl Benzoate	1769	1769
78	42.951	0.05	cis-Lanceol	1781	1781
79	44.575	0.03	Neophytadiene	1837	1837
80	45.560	0.01	Benzyl salicylate	1871	1870
81	46.272	0.01	Nonadecane	1896	1900
82	48.024	0.01	Hexadecanoic acid	1956	1957
83	49.001	0.01	Ethyl hexadecanoate	1990	1990
84	49.152	0.01	Eicosane	1995	2000
85	52.858	0.05	Heneicosane	2095	2100
		14.19	Monoterpene hydrocarbons (No: 8-13, 15-17, 19, 20, 22-24, 26, 28, 30)		
		9.55	Oxygenated monoterpenes (No: 27, 29, 31-36, 38-40, 42-47, 49-51)		
		67.52	Sesquiterpene hydrocarbons (No: 53, 56-58, 62, 63, 65-68)		
		0.96	Oxygenated sesquiterpenes (No: 70-73, 75, 78)		
		1.74	Aldehydes (No: 4, 5, 7, 21, 41, 52)		
		0.05	Alcohols (No: 6, 25)		
		0.58	Esters (No: 60, 61, 64, 69, 74, 76, 77, 80, 83)		
		4.41	Hydrocarbons (No: 1-3, 14, 18, 79, 81, 84, 85)		
		0.07	Ketones (No: 55)		
		0.01	Oil acids (No: 82)		
		0.92	Others (No: 37, 48, 54, 59)		
Total		100.00			

RT: Retention time, RI: Retention indices calculated against, LRI: Literature retention indices based on Adams (2007), NIST and WILLEY

Results of Enzyme Inhibitory Activities

The inhibition potency of the methanol extract and essential oil on various enzymes was assessed by determining the IC_{50} values, which represent the concentration of a compound required to inhibit 50% of enzyme activity. A lower IC_{50} value indicates stronger inhibition and greater potency at lower concentrations. The IC_{50} values for different enzymes are summarized in Table 2. The bar chart of enzyme activity results is also presented in Fig. 2. The bar chart demonstrates the enzyme inhibitory activities of *F. platycarpa* methanol extract (ME) and essential oil (EO), based on the mean logarithmic IC_{50} values. Overall, the methanol extract exhibited stronger inhibition against BChE, α-glucosidase, and α-amylase compared to the essential oil (Fig. 2.). In contrast, both samples showed similar inhibition profiles for CA-II and AChE. Notably, the essential oil had minimal activity against α-glucosidase, as reflected by highly negative log IC_{50} values.

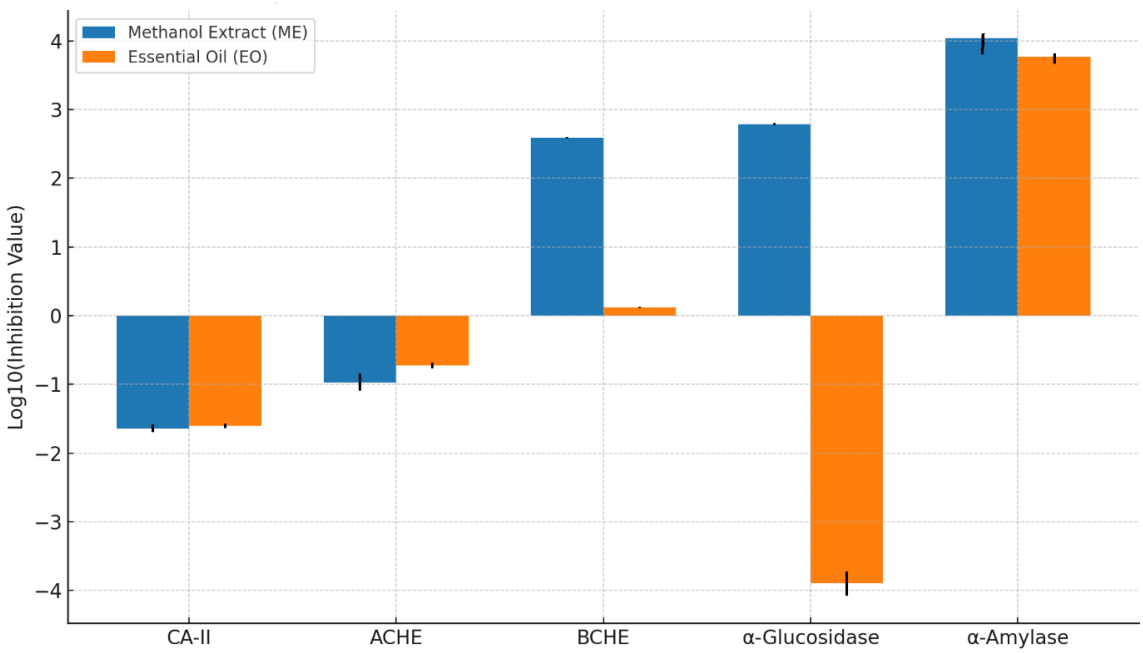

Fig. 2. Mean log (IC_{50}) values of enzyme inhibition by *F. platycarpa* methanol extract and essential oil

CA-II (Carbonic Anhydrase II) IC_{50}

The methanol extract demonstrated a low IC_{50} value of 0.023 ± 0.0005 µg/mL, indicating a strong inhibitory effect at very low concentrations. This suggests that the methanol extract is highly effective in inhibiting CA-II activity. The EO exhibited a slightly higher IC_{50} value of 0.025 ± 0.0004 µg/mL, still showing strong inhibition. However, both were less potent compared to the standard inhibitor Acetazolamide, which had an IC_{50} value of 0.0029 ± 0.0002 µg/mL, highlighting its superior efficacy as a CA-II inhibitor.

AChE (Acetylcholinesterase) IC_{50}

The IC_{50} value for acetylcholinesterase inhibition by the methanol extract was 0.11 ± 0.006 mg/mL, suggesting moderate yet effective inhibition. The essential oil had a lower IC_{50} value of 0.19 ± 0.008 µg/mL, implying it is more effective at inhibiting AChE than the methanol extract. However, both extracts were significantly less potent than the standard inhibitor Tacrine, which exhibited an IC_{50} value of 0.016 ± 0.001 µg/mL, demonstrating its exceptional inhibitory activity against AChE.

BChE (Butyrylcholinesterase) IC_{50}

The methanol extract exhibited an IC_{50} value of 0.39 ± 0.01 mg/mL for BChE inhibition, indicating moderate inhibition. The EO showed a significantly lower IC_{50} value of 1.32 ± 0.14 µg/mL, suggesting much stronger inhibition of BChE. Despite this, both extracts were less effective than Tacrine, which had an IC_{50} value of 0.0019 ± 0.0003 µg/mL, underscoring its remarkable potency as a BChE inhibitor.

α-Glucosidase IC_{50}

The methanol extract exhibited an IC_{50} value of 0.61 ± 0.19 mg/mL for α-glucosidase inhibition, showing moderate activity. In contrast, the EO showed no inhibition

on α-glucosidase, indicating that it has no significant effect on this enzyme at the tested concentrations. The standard inhibitor Acarbose outperformed both extracts with an IC_{50} value of 0.065 ± 0.002 µg/mL, demonstrating its strong inhibitory effect on *α*-glucosidase.

α-Amylase IC_{50}

The methanol extract exhibited an IC_{50} value of 10.89 ± 0.05 mg/mL for *α*-amylase inhibition, suggesting relatively low potency compared to other enzymes. The EO demonstrated a lower IC_{50} value of 5.92 ± 0.19 mg/mL, indicating that it is more effective than the methanol extract at inhibiting *α*-amylase. However, both extracts showed weaker inhibition against this enzyme compared to Acarbose, which had an IC_{50} value of 3.23 ± 0.12 µg/mL, highlighting its superior efficacy as an *α*-amylase inhibitor.

The results clearly demonstrate that lower IC_{50} values are indicative of stronger inhibition and greater enzyme potency. The methanol extract generally showed more potent inhibitory activity, particularly for CA-II, AChE, and BChE, with the lowest IC_{50} value observed for CA-II, highlighting its significant potential as an enzyme inhibitor. On the other hand, the EO was also more effective on CA-II with the lowest IC_{50} value, while its effect on AChE and BChE was less than CA-II, but stronger than *α*-amylase. This indicates lower activity at the tested concentrations. The absence of inhibition for α-glucosidase by the EO further indicates its relatively limited effect compared to the methanol extract. When compared to the standard inhibitors (acetazolamide**,** tacrine**,** and acarbose), both the methanol extract and EO were less potent, emphasizing the exceptional efficacy of these standards in inhibiting their respective enzymes.

Çakar (2010) conducted a study using *Ferulago* species and their bioactive compounds, in which he used methanol and dichloromethane for extraction from *Ferulago idea* and *Ferulago trojana* plants and isolated secondary metabolites. He investigated their anticholinesterase activities, finding that both methanol and dichloromethane extracts exhibited moderate activity compared to the standard galantamine. The isolated compounds, including isomaltol-3*β*-*O*-glucoside,3,6-dimethoxy-7-isopropylcoumarin-4-tetradeca-13-one, isoimperatorin, and 3'-epidecursin, showed good to moderate AChE and BChE inhibition. Of these, 3'-epidecursin was the most active compound, while bergapten showed no activity against AChE but exhibited significant inhibition against BChE. This suggests the potential of coumarins in anticholinesterase activity, as noted in recent studies highlighting their antioxidant and anticholinesterase potentials (Çakar 2010).

Furthermore, a study by Satır *et al.* (2009) identified prantschimgin (a furanocoumarin) in the methanol extract of *F. platycarpa* collected from Nevşehir, indicating the presence of bioactive compounds with potential medicinal properties. Karakaya *et al.* (2019) also isolated coumarins such as peucedanol, suberosin, and grandivitinol from the roots of *Ferulago cassia* Boiss and evaluated their AChE and BChE inhibitory effects. Among these, umbelliferon showed strong inhibition against AChE, while suberosin and peucedanol demonstrated potent inhibition against BChE. This study further emphasizes the significant role of coumarins in inhibiting cholinesterase enzymes.

In another study by Golfakhrabadi *et al.* (2016), coumarins such as *β*-sitosterol, suberosin, bergapten, isopimpinellin, suberenol, xanthotoxin, and prantschimgin were isolated from *Ferulago carduchorum* and assessed for their AChE inhibitory activity. The compound xanthotoxin exhibited the strongest inhibitory effect with an IC_{50} value of 39.64 µM, reinforcing the potential of these bioactive coumarins for therapeutic use.

Additional studies, such as those by Kızıltaş *et al.* (2021), reported on the *Ferulago stellata* ethanol extract, which showed inhibition against AChE, *α*-glucosidase, and *α*-

amylase enzymes with IC_{50} values of 1.772 µg/mL, 33.56±2.96 µg/mL, and 0.639 µg/mL, respectively. Moreover, *F. trachycarpa* extracts prepared by supercritical fluid extraction and maceration showed effective inhibition against cholinesterases, tyrosinase, *α*-amylase, and *α*-glucosidase, suggesting their potential for treating enzyme-related diseases (Nilofar *et al.* 2024).

The findings from the studies of *Ferulago bracteata* (Karakaya *et al.* 2018) and *F. pauciradiata* (Karakaya *et al.* 2021) further support the idea that these plants contain bioactive compounds with potent inhibitory activities against various enzymes such as *α*-amylase, *α*-glucosidase, and carbonic anhydrases, providing valuable insights into their therapeutic applications. In conclusion, the results from this study and previous research highlight the promising anticholinesterase, antioxidant, and enzyme-inhibitory activities of *Ferulago* species and their potential as natural sources for developing new therapeutic agents for enzyme-related disorders.

Table 2. The Enzyme Activity Results of *F. platycarpa* Methanol Extract and Essential Oil

	CA-II IC_{50}	AChE IC_{50}	BChE IC_{50}	*α*-Glucosidase IC_{50}	*α*-Amylase IC_{50}
Methanol extract	0.023 ± 0.0005^{b} µg/mL	0.11 ± 0.006^{b} mg/mL	0.39 ± 0.01^{b} mg/mL	0.61 ± 0.19^{b} mg/mL	10.89 ± 0.05^{c} mg/mL
Essential Oil	0.025 ± 0.0004^{b} µg/mL	0.19 ± 0.008^{c} µg/mL	1.32 ± 0.14^{c} µg/mL	No inhibition observed	5.92 ± 0.19^{b} mg/mL
Asetazolamid	0.0029 ±0.0002^{a} µg/mL	-	-	-	-
Tacrine	-	0.016 ±0.001^{a} µg/mL	0.0019±0.0003^{a} µg/mL	-	-
Acarbose	-	-	-	0.065 ±0.002^{a} µg/mL	3.23±0.12^{a} µg/mL

Different letters within the same column in the table indicate significant statistical differences in pairwise comparisons ($p<0.05$). n:3. Duncan's test was applied.

Results of Antioxidant Activity and Bioactive Components

This study investigates the antioxidant activity and bioactive components of the methanol extract and EO of *F. platycarpa*, a plant known for its potential health benefits (Rahimpour *et al.* 2021). Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays, while bioactive components were quantified through total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant content (TAC). The DPPH assay, which measures free radical scavenging, revealed that the methanol extract (286 mg AAE/100 g) had significantly stronger activity compared to the essential oil (83.8 mg AAE/100 g), with a higher inhibition percentage (65.3% *vs*. 23.6%).

The ABTS assay showed that the methanol extract was also more effective in scavenging ABTS radicals (71.9 mg TRE/100 g) than the EO (30.3 mg TRE/100 g). Similarly, the FRAP assay, which measures ferric ion reduction, indicated that the methanol extract had a much higher reducing power (975 mg $FeSO_4$/100 g) compared to the EO (113 mg $FeSO_4$/100 g), suggesting stronger antioxidant potential in the methanol extract. In terms of bioactive components, the methanol extract contained significantly higher levels of total phenolic content (1196 mg GAE/100 g vs. 63.1 mg GAE/100 g), total flavonoid content (165 mg QEE/100 g vs. 26.6 mg QEE/100 g), and total antioxidant content (1870 mg AAE/100 g *vs.* 753 mg AAE/100 g) compared to the EO. These higher

concentrations of phenolics, flavonoids, and antioxidant likely contribute to the superior antioxidant activity observed in the methanol extract (Table 3).

In general, the methanol extract of *F. platycarpa* demonstrated importantly higher levels of antioxidant activity and bioactive components compared to the EO. This could be attributed to the higher extraction efficiency of polar compounds such as phenolics, flavonoids, and alkaloids in the methanol solvent. These compounds are typically more soluble in methanol, which explains the greater concentrations observed in the methanol extract. The EO, while still containing bioactive components, showed lower antioxidant potential across all assays. Essential oils are often dominated by volatile compounds that may not possess the same level of antioxidant properties as the polar compounds found in methanol extracts. However, essential oils are still valuable in other applications, including as antimicrobial agents or in aromatherapy, due to their volatile, aromatic compounds (Ali *et al.* 2015; Baptista-Silva *et al.* 2020).

Table 3. Antioxidant Activity and Bioactive Component Profile of *F. platycarpa* Methanol Extract and Essential Oil

Antioxidant Activity Levels						
	Essential Oil and Methanol extract Yield	**DPPH**			**ABTS**	**FRAP**
	g/100 g	mg AAE/ 100 g	Inhibition %	IC_{50} (mg/mL)	mg TRE/ 100 g	mg $FeSO_4$/100 g
Methanol extract	16.42±0.21[a]	286.49±0.01[a]	65.34±0.63[b]	43.73±0.42[c]	71.93±0.24[a]	974.78±5.73[a]
Essential Oil	0.53±0.06[b]	83.85±1.49[b]	23.64±0.42[c]	747.40±13.28[b]	30.30±0.05[b]	113.41±7.75[b]
Ascorbic Acid			98.32±0.01[a]	92.65±0.11[a] µg/mL		
Bioactive Components						
	TPC mg GAE/100 g		**TFC** mg QEE/100 g		**TAC** mg AAE/100 g	
Methanol extract	1196.22	±11.64[a]	165.42	±2.18[a]	1870.00	±17.69[a]
Essential Oil	63.14	±2.44[b]	26.65	±2.18[b]	752.70	±2.75[b]

Different letters within the same column in the table indicate significant statistical differences in pairwise comparisons ($p < 0.05$). n:3. Duncan's test was applied. AAE: Ascorbic Acid Equivalent, TRE: Trolox Equivalent, GAE: Gallic Acid Equivalent, QEE: Quercetin Equivalent

Result of Phenolic Compounds

In the methanol extract of *F. platycarpa*, a total of 11 phenolic compounds were identified (Table 4). Among these, chlorogenic acid was found to be the most abundant, highlighting its significance as a key compound contributing to the plant's antioxidant and antidiabetic potential. Other important compounds, such as caffeic acid and rutin, were also detected in high concentrations. These compounds are well-documented for their antioxidant, anti-inflammatory, and antidiabetic properties. Additionally, polyphenolic compounds such as ellagic acid and rosmarinic acid were identified as significant components supporting the plant's therapeutic potential. Furthermore, compounds such as protocatechuic acid, gentisic acid, vanillin, *p*-coumaric acid, and apigenin were also detected. These compounds are known for their antioxidant, anti-inflammatory, and antidiabetic effects, further emphasizing the importance of *F. platycarpa* as a source of

bioactive compounds with traditional medicinal uses. These findings demonstrate that the plant is rich in phenolic compounds and holds potential for therapeutic applications in modern medicine. Moreover, the chromatograms of the standards used in the LC-MS/MS analysis of the methanol extract, as well as the chromatogram of the extract itself, are presented in Figs. 3 to 4, respectively. These chromatograms serve as a reference for the identification and quantification of the analyzed phenolic compounds.

The methanol extract of *F. platycarpa* contains a diverse profile of phenolic compounds, including chlorogenic acid, caffeic acid, rutin, ellagic acid, and rosmarinic acid, as well as smaller amounts of protocatechuic acid, gentisic acid, vanillin, *p*-coumaric acid, and apigenin. The detailed quantitative analysis of these phenolic compounds is presented in Table 4. The analyses were performed on the dry methanol extract. The phenolic profile of F. *platycarpa* is consistent with previous studies on other *Ferulago* species, which have also reported the presence of protocatechuic acid, rutin, quercetin, apigenin, and *p*-coumaric acid (Gözcü *et al.* 2024). However, the high concentration of chlorogenic acid and the presence of rutin and ellagic acid in *F. platycarpa* are particularly noteworthy, as these compounds have been extensively studied for their health benefits. Chlorogenic acid is widely recognized for its strong antioxidant activity and its role in reducing oxidative stress. It has also been reported to exhibit antidiabetic properties by inhibiting glucose-6-phosphatase and reducing hepatic glucose output, which aligns with the traditional use of Ferulago species in managing metabolic disorders (Clifford 1999; Tundis *et al.* 2010). Caffeic acid further supports the medicinal value of this plant by modulating glucose metabolism and improving insulin sensitivity (Muhammad Abdul Kadar *et al.* 2021).

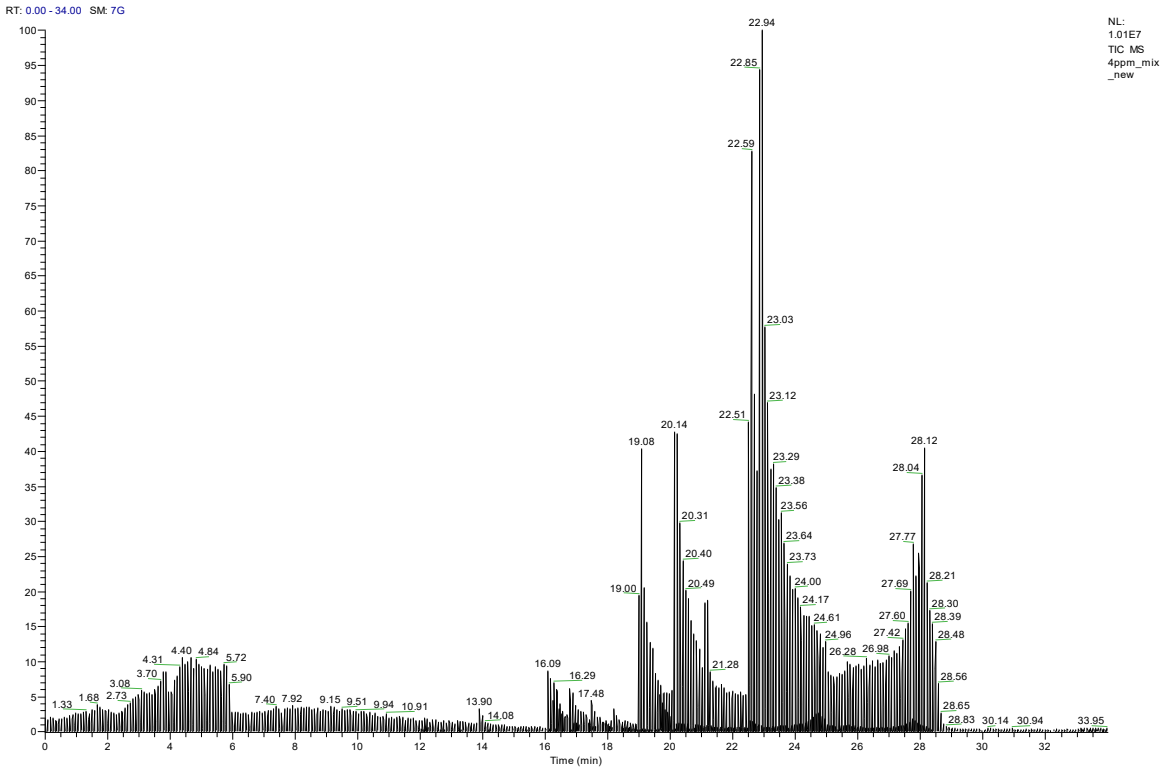

Fig. 3. Chromatogram of standard phenolic compounds analyzed by LC-MS/MS

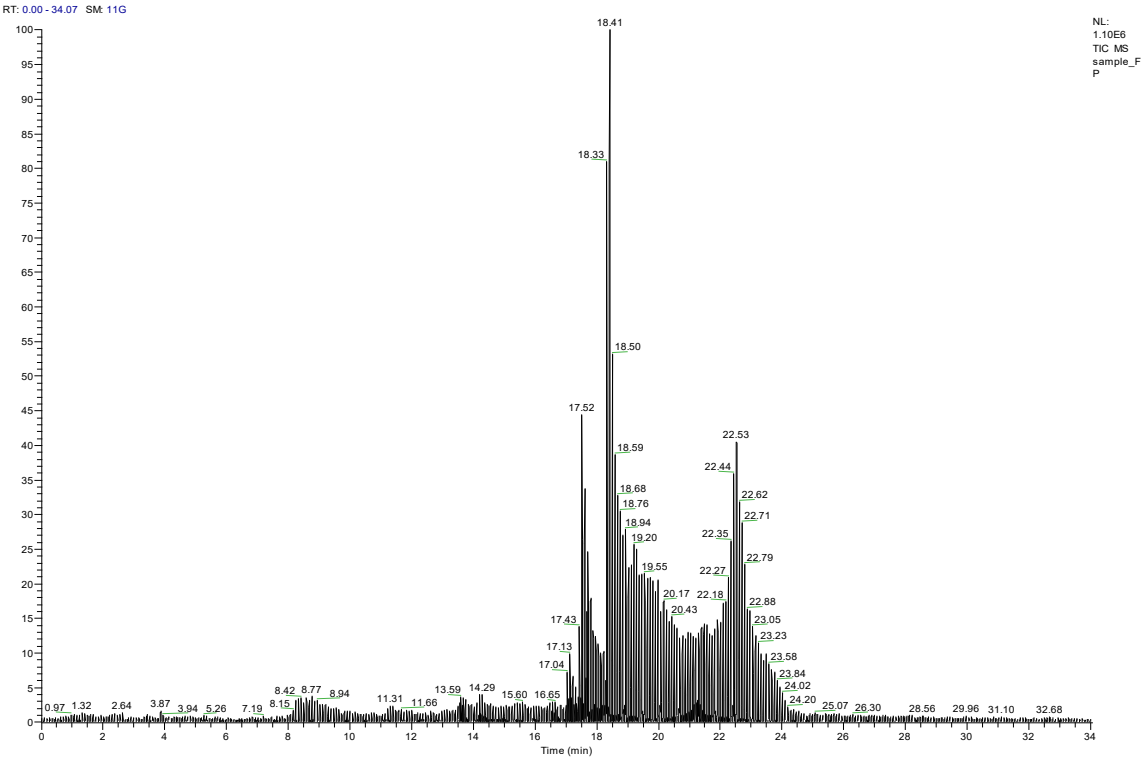

Fig. 4. Chromatogram of extracts of aerial parts of *F. platycarpa* by LC-MS/MS

Rutin, a flavonoid glycoside, is known for its strong antioxidant and anti-inflammatory effects. It has been shown to protect against oxidative stress-induced damage and improve vascular health, which is particularly relevant in the context of diabetes and cardiovascular diseases (Gullón *et al.* 2017). The presence of rutin in *F. platycarpa* suggests that this species could be beneficial in managing chronic diseases associated with oxidative stress.

Ellagic acid is a polyphenolic compound with notable antioxidant and anticancer properties. It has been reported to inhibit *α*-glucosidase and α-amylase enzymes, which are key targets in the management of type 2 diabetes (Khan *et al.* 2015). Rosmarinic acid is another bioactive compound with antioxidant, anti-inflammatory, and neuroprotective properties. It has also been reported to exhibit antidiabetic effects by improving insulin sensitivity and reducing blood glucose levels (Petersen and Simmonds 2003).

Quercetin, a well-studied flavonoid, is known for its strong antioxidant and anti-inflammatory properties. It enhances pancreatic *β*-cell function and insulin secretion, supporting the antidiabetic potential of *F. platycarpa* (Li *et al.* 2016). Protocatechuic acid, a dihydroxybenzoic acid derivative, exhibits hepatoprotective, neuroprotective, and cardioprotective effects. It has also been shown to inhibit α-glucosidase and α-amylase enzymes (Semaming *et al.* 2015). Gentisic acid is a phenolic acid with antioxidant, anti-inflammatory, and analgesic properties. It is known to provide protective effects against oxidative stress and modulate inflammatory pathways (Razliqi *et al.* 2023; Mîrza *et al.* 2024).

Vanillin, a well-known flavoring agent, also possesses antioxidant, anti-inflammatory, and neuroprotective properties. It has been reported to exhibit antidiabetic effects by improving insulin sensitivity and reducing blood glucose levels (Bezerra *et al.*

2017). *p*-coumaric acid, a hydroxycinnamic acid derivative, shows antidiabetic effects by inhibiting *α*-glucosidase and *α*-amylase enzymes. It also protects against oxidative stress-induced damage and improves lipid metabolism (Pei *et al.* 2016; Güzel *et al.* 2018). Apigenin, a flavonoid, is known for its antioxidant, anti-inflammatory, and anticancer properties. It has been reported to exhibit antidiabetic effects by improving insulin sensitivity and reducing blood glucose levels (Ren *et al.* 2016).

Overall, the diverse phenolic profile of *F. platycarpa* underscores its potential as a natural source of antioxidants and antidiabetic agents. The presence of these compounds aligns with the traditional use of *Ferulago* species in managing metabolic disorders and highlights the plant's therapeutic potential in modern medicine. Further research is warranted to fully elucidate the mechanisms of action and therapeutic applications of these bioactive compounds.

Among the standard phenolic compounds screened during the analysis, catechin, epicatechin, ferulic acid, gallic acid, hesperidin, kaempferol, naringenin, sinapic acid, and syringic acid were not detected under the applied experimental conditions.

The diverse phenolic profile of *F. platycarpa*, including high concentrations of chlorogenic acid, caffeic acid, rutin, and other bioactive compounds, underscores its potential as a natural source of antioxidants and antidiabetic agents. The presence of these compounds aligns with the traditional use of *Ferulago* species in managing metabolic disorders and highlights the plant's therapeutic potential in modern medicine. Further research is warranted to fully elucidate the mechanisms of action and therapeutic applications of these bioactive compounds.

Table 4. The Amount of Phenolic Compound in the Extracts of Aerial Parts of *F. platycarpa*

No	Phenolic Compound	Amount (µg/g extract)	Biological Activity Summary
1	Chlorogenic acid	30936.46±24.12	Antioxidant, antidiabetic, metabolic regulation
2	Caffeic acid	1138.93±15.76	Antioxidant, anti-inflammatory, insulin sensitivity enhancer
3	Rutin	931.68±12.78	Antioxidant, anti-inflammatory, vascular protection
4	Ellagic acid	213.01±9.65	Antioxidant, α-amylase/α-glucosidase inhibitor
5	Rosmarinic acid	133.08±8.11	Neuroprotective, antioxidant, antidiabetic
6	p-Coumaric acid	119.47±7.54	Antioxidant, lipid metabolism regulation
7	Protocatechuic acid	82.38±7.25	Antioxidant, hepatoprotective, antidiabetic
8	Gentisic acid	44.95±2.21	Antioxidant, analgesic, anti-inflammatory
9	Vanillin	37.82±3.76	Antioxidant, neuroprotective, insulin sensitivity enhancer
10	Quercetin	12.56±0.98	Antioxidant, anti-inflammatory, insulin secretion modulator
11	Apigenin	2.69±0.23	Antioxidant, anti-inflammatory, anticancer, antidiabetic

Results of Antimicrobial Activity Evaluations

From the data, essential oil stood out with the highest DDT (disc diffusion test) value of 9.25 mm against *P. aeruginosa*. This large inhibition zone indicates that essential oil has a strong antibacterial effect on this particular bacterium, suggesting its potential as a potent antimicrobial agent. Chloramphenicol, another highly effective agent, showed a DDT value of 10.5 mm against *P. aeruginosa*. This also indicates a significant inhibitory effect, highlighting chloramphenicol's strong antibacterial properties (Table 5).

On the other hand, MeOH 30% exhibited its highest DDT values against *Bacillus cereus* (8.47 mm) and *E. faecalis* (8.00 mm). Although these values are notable, they were somewhat lower compared to essential oil and chloramphenicol. This indicates that while MeOH 30% was effective against certain bacteria, its overall antibacterial activity was not as pronounced as the other two agents (Table 5).

Lastly, nalidixic acid showed its highest DDT value of 23.0 mm against *Escherichia coli*. This value suggests that nalidixic acid is particularly effective at inhibiting the growth of *E. coli*, providing a solid level of antibacterial activity. Nalidixic acid and chloramphenicol demonstrate the strongest antibacterial effects in terms of DDT values, indicating their high potency in inhibiting bacterial growth. While MeOH 30% and essential oil also showed effectiveness, their DDT values were relatively lower, suggesting that their antibacterial activity is somewhat less powerful compared to nalidixic acid and chloramphenicol (Table 5).

According to the data, chloramphenicol demonstrated the lowest MIC value of all the agents tested, with a value of 0.002 mg/mL against *Staphylococcus aureus*. Essential oil also showed a very low MIC value, with 0.12 mg/mL against *Pseudomonas aeruginosa*. In contrast**,** MeOH 30% exhibited a higher MIC value of 1.63 mg/mL against *Pseudomonas aeruginosa*. Finally, nalidixic acid showed an MIC value of 0.02 mg/mL against *Staphylococcus aureus* (Table 5).

In this dataset, essential oil exhibited a very low MBC value of 0.23 mg/mL against *P. aeruginosa*. Chloramphenicol also demonstrated impressive bactericidal properties, with an MBC value of 0.004 mg/mL against *S. aureus*. Nalidixic acid also showed high bactericidal activity. It achieved an MBC of 0.04 mg/mL against *Pseudomonas aeruginosa* and 0.02 mg/mL against *E. faecalis.* In comparison, MeOH 30% had relatively higher MBC values. It showed an MBC of 0.85 mg/mL against *C. albicans* and 1.70 mg/mL against *Bacillus cereus* (Table 5).

These data compare the antimicrobial effects of various agents, including MeOH 30%, essential oil, chloramphenicol, and nalidixic acid on different microorganisms. The effectiveness of these agents was assessed through three key parameters: disk diffusion test (DDT), minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC). Each of these parameters plays a crucial role in evaluating the antibacterial activity of the substances. Specifically, DDT (measured in millimeters) indicates the degree of inhibition of bacterial growth by the antimicrobial agent, MIC represents the lowest concentration of the agent required to prevent microbial growth, and MBC refers to the minimum concentration needed to completely kill the microorganism. Lower MIC and MBC values indicate higher efficacy, making them important benchmarks in evaluating antimicrobial agents (Gajic *et al.* 2022). The disk diffusion test (DDT) is a commonly used method to evaluate the antimicrobial activity of various agents (Benkova *et al.* 2020). In this test, a microorganism is cultured on a petri dish, and small discs soaked with antimicrobial agents are placed on the surface. The area around the disc where

bacterial growth is inhibited is measured in millimeters, known as the zone of inhibition. A larger zone indicates a more effective antimicrobial agent (Lewis *et al.* 2022).

Minimum inhibitory concentration (MIC) represents the lowest concentration of an antimicrobial agent required to prevent the growth of microorganisms. Essentially, the MIC value indicates the potency of the antimicrobial agent—lower MIC values signify greater effectiveness, as the agent can inhibit microbial growth at much lower concentrations. This parameter is crucial in determining the efficiency of an antimicrobial substance in controlling bacterial proliferation (Lewis *et al.* 2022).

According to the data, chloramphenicol demonstrated the lowest MIC value of all the agents tested, with a value of 0.002 mg/mL against *Staphylococcus aureus*. This extremely low MIC indicates that chloramphenicol was highly effective in inhibiting the growth of *S. aureus*, even at very low concentrations. Such a low value is indicative of the potent antibacterial properties of chloramphenicol, suggesting that it is an exceptionally efficient antimicrobial agent when used in minimal amounts.

Essential oil also showed a very low MIC value, with 0.03 mg/mL against *Pseudomonas aeruginosa*. This value signifies that essential oil was effective at inhibiting the growth of *P. aeruginosa* at relatively low concentrations, further highlighting its strong antimicrobial potential. The fact that essential oil achieved such low MIC values reinforces its status as a potent natural antimicrobial agent.

In contrast**,** MeOH 30% exhibited a higher MIC value of 0.12 mg/mL against *Pseudomonas aeruginosa*. While this still indicated effective inhibition, it was notably higher than the MIC values seen for chloramphenicol and essential oil, suggesting that MeOH 30% is somewhat less efficient at lower concentrations. Nevertheless, it still demonstrates considerable antimicrobial activity, particularly against *P. aeruginosa*. On the other hand, in the study conducted by Mohammed *et al.* (2020) using *F. platycarpa*, the MIC value of methanol extract against *P. aeruginosa* was 0.2 mg/ml.

Finally, nalidixic acid showed an MIC value of 0.02 mg/mL against *Staphylococcus aureus*. This value suggests that nalidixic acid is quite effective at inhibiting the growth of *S. aureus*, although its MIC was higher than that of chloramphenicol. Nevertheless, nalidixic acid remains a potent antimicrobial agent against this particular bacterium.

Chloramphenicol and nalidixic acid stood out with the lowest MIC values, demonstrating their exceptional potency in inhibiting bacterial growth even at minimal concentrations. These findings highlight their strong antimicrobial efficacy. Essential oil also exhibited notable antimicrobial activity with a relatively low MIC value, while MeOH 30%**,** although effective, required higher concentrations to inhibit bacterial growth compared to the other agents.

Minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial agent required to kill a microorganism. The MBC is a critical measure, as it not only assesses the ability of an agent to inhibit bacterial growth, but also its effectiveness in eliminating the bacteria entirely. A lower MBC value indicates that the agent is capable of killing bacteria at a lower concentration, reflecting a more potent bactericidal activity.

In this dataset, essential oil exhibited a very low MBC value of 0.06 mg/mL against *B. cereus*. This indicates that essential oil has a strong bactericidal effect, as it can kill *Bacillus cereus* at a very low concentration. The low MBC value underscores the efficiency of essential oil as a bactericidal agent, making it particularly effective in eradicating bacteria at minimal doses.

Table 5. Antimicrobial Activities of *F. platycarpa* Methanol Extract and Essential Oil

	MeOH 30%			Essential Oil			Chloramphenicol			Nalidixic Acid		
	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL	DDT (mm)	MIC mg/mL	MBC mg/mL
Bacillus cereus ATCC 9634	8.47	0.85	1.70	8.97	0.46	0.92	21.27	0.03	0.06	16.73	0.07	0.14
	±0.15	±0.03	±0.07	±0.05	±0.02	±0.04	±0.15	±0.01	±0.01	±0.06	±0.01	±0.01
Escherichia coli ATCC 25922	7.67	0.85	3.39	8.24	0.46	0.92	19.67	0.03	0.06	22.97	0.07	0.14
	±0.06	±0.03	±0.13	±0.14	±0.02	±0.04	±0.15	±0.01	±0.01	±0.12	±0.01	±0.01
Klebsiella pneumoniae ATCC 13883	6.87	3.39	6.78	8.46	0.92	1.84	29.73	0.03	0.06	20.83	0.03	0.06
	±0.06	±0.13	±0.26	±0.09	±0.04	±0.07	±0.15	±0.01	±0.01	±0.15	±0.01	±0.01
Pseudomonas aeruginosa ATCC 27853	7.63	1.63	3.25	9.25	0.12	0.23	10.47	0.03	0.06	26.47	0.02	0.04
	±0.12	±0.13	±0.02	±0.11	±0.01	±0.01	±0.12	±0.01	±0.01	±0.29	±0.01	±0.01
Staphylococcus aureus ATCC 25923	7.63	1.70	3.39	8.62	0.92	1.84	18.73	0.002	0.004	20.77	0.02	0.04
	±0.06	±0.07	±0.13	±0.14	±0.04	±0.07	±0.12	±0.001	±0.001	±0.12	±0.01	±0.01
Enterococcus faecalis ATCC 29212	8.00	1.70	3.39	9.03	0.46	0.92	11.17	0.017	0.034	21.97	0.01	0.02
	±0.10	±0.07	±0.13	±0.09	±0.02	±0.04	±0.12	±0.001	±0.001	±0.15	±0.01	±0.01
Candida albicans ATCC 18804	7.47	0.43	0.85	9.75	0.15	0.30	21.43	0.033	0.066	18.67	0.07	0.14
	±0.06	±0.02	±0.03	±0.05	±0.01	±0.01	±0.12	±0.001	±0.001	±0.12	±0.01	±0.01

The discs have a diameter of 5.5 mm. Samples: 25 mg/mL, Antibiotic: ±0.51 mg/mL.
15 µL were pipetted onto each disc, n = 3. DDT: Disk Diffusion Test

Chloramphenicol also demonstrated impressive bactericidal properties, with an MBC value of 0.02 mg/mL against *S. aureus*. This extremely low MBC value shows that chloramphenicol can be highly effective at killing *S. aureus*, even at very low concentrations. The strong bactericidal effect of chloramphenicol, coupled with its low MIC and MBC values, confirms its potency as a broad-spectrum antimicrobial agent. Nalidixic acid also showed high bactericidal activity. It achieved an MBC of 0.04 mg/mL against *Pseudomonas aeruginosa* and 0.02 mg/mL against *S. aureus*, both of which are indicative of its strong ability to kill these bacteria at low concentrations. Nalidixic acid's MBC values highlight its potent bactericidal effect against these two pathogens, reinforcing its effectiveness in therapeutic applications.

In comparison, MeOH 30% had relatively higher MBC values. It showed an MBC of 0.23 mg/mL against *P. aeruginosa* and 1.70 mg/mL against *Bacillus cereus*. While these values were still effective, they were significantly higher than the MBC values observed for essential oil, chloramphenicol, and nalidixic acid. This suggests that, although MeOH 30% was still able to kill these bacteria, it required higher concentrations to do so, making it less efficient as a bactericidal agent compared to the other substances.

When considering MBC values across the board, chloramphenicol and nalidixic acid stood out as the most potent bactericidal agents. Their extremely low MBC values demonstrate their powerful ability to kill bacteria, even at very low concentrations. Essential oil also proved to be highly effective, particularly against *B. cereus*, where its low MBC value underscored its efficient bactericidal activity.

On the other hand, MeOH 30%, while still effective, exhibited less pronounced bactericidal properties compared to the other agents, requiring higher concentrations to achieve bacterial elimination.

Based on the MBC data, chloramphenicol and nalidixic acid emerged as the most potent bactericidal agents, with very low MBC values that confirm their high effectiveness in killing bacteria. Essential oil, with its low MBC value against *B. cereus*, was also a highly potent bactericidal agent, especially against specific pathogens like *P. aeruginosa*. MeOH 30%, although effective, demonstrated lower bactericidal efficiency compared to the other agents, as reflected in its higher MBC values. Nevertheless, it still showed potential in combating bacterial infections, especially with higher concentrations.

CONCLUSIONS

1. Chemical classes with the largest number of compounds in the essential oils of *F. platycarpa* were identified as oxygenated monoterpenes in the aerial parts. The chemical class with the largest constituent percentage in the EOs of plant parts was determined to be sesquiterpene hydrocarbons. The main chemical compound in the EOs was identified as caryophyllene.
2. The enzyme inhibitory activities in the aerial parts of *F. platycarpa* were evaluated for CA-II, AChE, and BChE. The methanol extract exhibited low IC_{50} values, indicating strong inhibitory effects at very low concentrations. The EO showed slightly higher IC_{50} values but still demonstrated strong inhibition. For α-glucosidase, the methanol extract displayed moderate activity, while the essential oil showed no inhibition. In contrast, the essential oil was more effective in inhibiting α-amylase, with a lower IC_{50}

value compared to the methanol extract. These results clearly demonstrate that lower IC_{50} values indicate stronger inhibition and greater enzyme potency.

3. The antioxidant activity and bioactive components of the methanol extract and EO of *F. platycarpa* were investigated. The methanol extract demonstrated stronger antioxidant properties than that in EO for all assays (DPPH, ABTS, and FRAP). Additionally, the methanol extract showed significantly higher levels of total phenolic content, total flavonoid content, and total antioxidant capacity compared to the essential oil.
4. The methanol extract of *F. platycarpa* was found to contain a diverse profile of phenolic compounds, including chlorogenic acid (30,900 µg/g), caffeic acid (1140 µg/g), rutin (932 µg/g), ellagic acid (213 µg/g), and rosmarinic acid (133 µg/g), among others. These compounds contribute to the plant's antioxidant, anti-inflammatory, and antidiabetic properties, further supporting its therapeutic potential.
5. The results of antimicrobial activity demonstrated that essential oils commonly exhibit stronger antimicrobial activity compared to the 30% methanol extracts across most fungal and bacterial strains, as evidenced by lower MBC and MIC values and larger inhibition zones. However, chloramphenicol consistently detected the highest antimicrobial efficacy, with the lowest MBC and MIC values and the largest inhibition zones.
6. In conclusion, although having important moderate quantities compared to some other *Ferulago* species, it appears that these caryophyllene and *α*–pinene are the major constituents present in the aerial parts. It was also shown that the plant could be a rich natural source for these identified constituents. It was demonstrated for the first time that the plant could be used as the source of antioxidant, biological, and pharmacological agents.

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