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Comparative Studies of Two Indonesian Medicinal Plants, Bidara Upas (*Merremia mammosa* Lour. Hall.f) and Adas (*Foeniculum vulgare* Miller): Antioxidant, Antidiabetic, and Antimicrobial Activities

Mohamad Fajar[1], Wahyu Safriansyah[1], Muhamad Imam Muhajir[1], Rani Maharani[1] and Unang Supratman[1,2,*]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[2]Central Laboratory, Universitas Padjadjaran, Sumedang 45363, Indonesia

([*]Corresponding author's e-mail: unang.supratman@unpad.ac.id)

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Abstract

Bidara upas (*Merremia mammosa*) and adas (*Foeniculum vulgare* Miller) are 2 medicinal plants that grow abundantly in Indonesia. This study investigated the chemical composition and biological activity of *M. mammosa* and *F. vulgare*. Phytochemical screening was conducted on both plants. However, only the essential oil of *F. vulgare* was analyzed for GC spectra. A total of 23 compounds were identified from the essential oil of *F. vulgare*, with anethole being the main constituent (79.02 %). The extract of *M. mammosa* had a higher total phenolic content (TPC) and total flavonoid content (TFC) of 50.241 ± 0.012 mg gallic acid equivalent (GAE) per gram extract and 53.968 ± 0.742 mg quercetin equivalent (QRE) per gram extract, respectively. In the DPPH antioxidant test, the IC_{50} value indicated that *M. mammosa* extract had better activity than *F. vulgare*, with an IC_{50} of 89.139 ± 0.189 μgmL^{-1}. This is supported by the TAC (Total Antoxidant Capacity) and Reducing Power Hexacyanoferrate (III) values, which also show a better antioxidant potential in *M. Mammosa* than in *F. vulgare*. In addition, *M. mammosa* has been shown to have an IC_{50} value of 2.215 ± 0.015 $mgmL^{-1}$ against α-amylase inhibition, which is slightly different from *F. vulgare* with an IC_{50} value of 2.467 ± 0.025 $mgmL^{-1}$. Overall, the antimicrobial activity of *F. vulgare* and *M. mammosa* was found to be resistant against *S. mutans* bacteria. The ethanol extract of *F. vulgare* was more potent against gram-positive bacteria and the essential oil was only effective against fungi. On the other hand, the ethanol extract of *M. mammosa* and the combination of their ethanol extracts showed no significant activity against bacteria and fungi. The combination of water extracts is most effective against 2 microbes, namely *S. epidermis* and *E. faecalis* with MIC 7.81 and 3.91 $mgmL^{-1}$.

Keywords: Antimicrobial, Antioxidant, α-amylase inhibitory, *Merremia mammosa*, *Foeniculum vulgare*

Introduction

In the age of modern drug development, medicinal plants are the fundamental subject for future research. An estimated 60 - 80 % of the world's population still uses herbal medicines, according to the World Health Organisation (WHO) [1,2]. The term "medicinal plants" refers to plants which have therapeutic or pharmacological properties. Apart from certain plant characteristics that indicate medicinal use, there is no morphological difference between medicinal plants and other plants [3]. Indonesia is a tropical, archipelago nation in the equatorial zone. It ranks second in megabiodiversity with over 25,000 plant species due to its location. About 30 % are used medicinally [4,5]. Indonesian culture, especially Javanese culture, values medicinal plants and their use in traditional health care. Traditional herbal cure "jamu" is made from these plants [6].

There are numerous medicinal plants located in Indonesia, such as bidara upas (*Merremia mammosa*) and adas (*Foeniculum vulgare* Miller) are promising herbal remedies due to their high activity [7]. *M. mammosa* (Lour.) Hall.f., commonly known as bidara upas, blanar or widara upas in Java, hailale in Ambon, jujube or Chinese date in English, widara upas in Malaysia, and angcoa in the Philippines [8,9]. The plant grows naturally in forests and in tropical climates up to 250 m above sea level. The plant grows as a vine or climber and has thin stems that are usually between 3 and 6 m long. The stems are purplish-red, and the heart-shaped leaves are 5 - 12 cm long and 4 - 15 cm wide. The umbrella-shaped blooms have 1 - 4 flowers. These purplish-white blossoms resemble trumpets or bells. Like sweet potatoes, the tubers grow in clusters in the soil [8,10]. The pharmacological activities of *M. mammosa* tuber are extensive, including its anti-

inflammatory, analgesic, antioxidant, antidiabetic, anti-edematous, laxative and antidotal characteristics. Some of the many health benefits that *M. mammosa* tubers provide include treating whooping cough, bloody and slimy stools, diphtheria, snake bites, diabetes, skin cuts, vomiting blood, typhoid, colitis and syphilis [8,11].

Futhermore, some regional names for *F. vulgare* include, Adas (Indonesia-Malaysia), Adase (Bugis), Hades (Sunda) and Londa (Java). It is alternatively referred to as Fennel in English, Bisbas in Arabic, Fenouille in French and Fenchel in German [11]. While *M. mammosa* prefers lower elevations, *F. vulgare* can be seen growing as high as 1,800 m above sea level. The plant clumps and has grooved, fibrous, hollow bluish-green stems. The plant has complex, alternating leaves with 2 needle-shaped segments and a pointed base. The yellow corolla blooms occur in compound umbels with 6 - 40 peduncles. The round fruit is ribbed and bright green to dark green-brown. Chamfer-like and aromatic [11-13]. In line with *M. mammosa*, *F. vulgare* is a high-potential plant. The biological activities of *F. vulgare* fruit include anti-inflammatory, antiallergic, hepatoprotective, anxiolytic, estrogenic, galactogenic, expectorant, anticolitic, antinociceptive, diuretic, cardiovascular, antimutagenic, gastrointestinal and chemomodulator effects [14]. Traditional and modern uses of *F. vulgare* include treatment of mouth ulcers, insomnia, constipation, conjunctivitis, colds, hair growth, diarrhoea, as a laxative, for antihypertensive and anti-cholesterol purposes, gum sores, cough and stomach pain [15,16].

In this research, we examined the content of compounds in *M. mammosa* and *F. vulgare* plants. Additionally, various biological activities were carried out, including 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free scavenging activity assay, phosphomolybdate (Total Antioxidant Capacity) assay, reducing power assay, α-amylase inhibition assay and antimicrobial assays. Furthermore, a comparison of the biological activities of the 2 plants was conducted.

Materials and methods

Plant material

The tuber of Bidara upas was gathered from Pangandaran Botanical Garden, Pangandaran, West Java, in January 2022. The adas fruit was gathered from Manoko Experimental Garden, Installation for Research and Experimentation of Agricultural Technology (IP2TP), Lembang, Bandung, West Java Province, Indonesia, in December 2021. Both plants were determined at Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java, and the voucher specimen was deposited at the Laboratory.

Chemicals and reagents

Chemical: Aluminum chloride, $AlCl_3$ (Sigma-Aldrich); Sodium acetate trihydrate, $CH_3COONa \cdot 3H_2O$ (Merck); Folin-Ciocalteau (Sigma-Aldrich); 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) and $K_3[Fe(CN)_6]$ potassium hexacyanoferrate(III) (Sigma-Aldrich); Trichloroacetic acid, CCl_3COOH (Loba Chemie); Ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$ (Merck); Ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, (Loba Chemie); Monosodium dihydrogen phosphate dihydrate, $NaH_2PO_4 \cdot 2H_2O$ and disodium hydrogen phosphate dihydrate, $Na_2HPO_4 \cdot 2H_2O$ (Sigma-Aldrich); Trisodium phosphate anhydrate (Merck); Iodine, I_2 and potassium iodide, KI, (Sigma-Aldrich); Sodium chloride, NaCl (Sigma-Aldrich); Sodium Carbonate, Na_2CO_3 (Sigma-Aldrich); and Dragendroff's reagent (Sigma-Aldrich); α-Amylase (Chatson Jaya Lab).

Drugs: Ciprofloxacin supplied from Fluka Analytical, an Indonesian pharmaceutical industry. Vancomycin came from Indonesian pharmaceutical industry Pratapa Nirmala. Nystatin, supplied from Indonesian pharmaceutical industry, Metiska Farma under the brand Kandistatin. Ketoconazole came from Indonesian pharmaceutical industry, Hexapharm Jaya. Chlorhexidine came from Indonesian pharmaceutical company Minorock supplied under the brand MinoSep®. Acarbose, L(+)-ascorbic acid and Gallic acid were from sigma. Quercetin were purchased from Indonesian chemical companies, Nitrakimia. Solvents and media: Organic solvents, such as ethanol, methanol and dimethyl sulfoxide (DMSO), were obtained from Merck, while purified water was acquired through the Milli-Qplus185 system from Millipore in Billerica, MA in the USA. Müeller-Hinton Broth (MHB), Brain-Heart Infusion Broth (BHIB), Nutrient Broth (NB), and Potato Dextrose Broth (PDB) were supplied by HiMedia.

Extracts preparations

First, *M. mammosa* tubers and *F. vulgare* fruits were dried, then ground to powder using a blender to obtain a weight of 1.12 kg and 530 g, respectively. Then, 800 g of *M. mammosa* tubers were macerated in 20 L of 96 % ethanol at room temperature for 3×24 h. A rotary evaporator evaporated the extract at 40 °C

for a long time under decreasing pressure, leaving 124 g of concentrated ethanol extract of *M. mammosa*. In the same way, 250 g of dry powder of *F. vulgare* fruit was macerated with ethanol, and concentrated to give 82 g of concentrated ethanol extract of *F. vulgare*.

Essential oil was extracted according to the method described in [12,13] with slight modifications. Briefly, dried of *F. vulgare* fruits (280 g) were ground and distilled with water for 12 h using a Dean-stark apparatus. The layer of oil that formed on top of the distilled water was separated and collected. A total of 3.8 mL of essential oil was obtained and stored in a dark, tightly closed bottle at 4 °C until further analysis. Furthermore, the essential oil was analysed by GC-MS and tested for antimicrobial properties.

A total of 50 g of dried powder from *F. vulgare* fruits and 50 g from *M. mammosa* tubers were mixed equally. It was then divided into 2 parts to be extracted with ethanol and the other part was extracted with hot water. The ethanol-extracted parts were soaked in 96 % ethanol at room temperature for 3×24 h, then evaporated to dry and yielded 12 g of concentrated extract. For the other part, the sample was decocted with water for 12 h, then cooled to room temperature and concentrated in a rotary evaporator at 55 °C under reduced pressure to give 7 g of concentrated extract.

Antioxidant, antidiabetic and antimicrobial assays of ethanolic extracts of *M. mammosa* tubers and *F. vulgare* fruits were investigated along with phytochemical screening. The essential oil of *F. vulgare* fruit, a combination of ethanolic extract and aqueous extract of *M. mammosa* tubers and *F. vulgare* fruit, was investigated for antimicrobial screening only.

GC-MS for essential oil of *F. vulgare* Mill

The analysis of the essential oil of *F. vulgare* was performed using a Agilent 7890A gas chromatography (Santa Clara, CA, United States) equipped column: DB-5MS 5 % phenylmethylsiloxane, 30 m, Ø 0.25 mm, film 0.25 μm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 $mLmin^{-1}$ equipped with an Agilent 5977B GC/MSD operating in EI mode. Injector and MS transfer line temperatures were set at 200 °C, respectively. Column temperature was initially kept at 50 °C for 2 min to 70 °C, then gradually increased from 70 to 300 °C at a 5 °C min^{-1} rate. Diluted samples (1/100 in chloroform, v/v) of 1.0 μL were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of GC-MS database.

Phytochemical screening

Phytochemical screening was conducted following established protocols [17]. Ethanolic extracts of *M. mammosa* tuber and *F. vulgare* fruit were analysed to identify a range of phytoconstituents such as alkaloids, saponins, terpenoids, flavonoids, coumarins, tannins and phenolics based on **Table 1**.

Table 1 Phytochemical screening.

No.	Chemical constituent	Test
1	Tanin	1 % $FeCl_3$ solution
2	Phenolic	5 % $FeCl_3$ solution
3	Flavonoid	Conc. HCl + Mg
4	Coumarin	2N NaOH solution
5	Triterpenoid/Steroid	Liebermann-Burchard
6	Saponin	Foam test
7	Alkaloid	Dragendorff's test

Total Phenolic Content (TPC)

Total phenolic compound contents were determined by the Folin-Ciocalteau method [18]. Firstly, 0.5 mL extract samples (0.01 g diluted in 10 mL water) were mixed with 2.5 mL Folin-Ciocalteau reagent (10 %) and 2 mL aqueous sodium carbonate (7.5 %) were then added. This mixture was incubated at 45 ° for 15 min and cooled to room temperature. The phenols were determined by colorimetry at 765 nm using spectrophotometer UV-VIS (PerkinElmer Lambda 35). The standard curve was prepared by 0, 50, 100, 150, 200 and 250 $mgmL^{-1}$ solutions of gallic acid in water. Total phenol values are expressed in terms of gallic acid equivalent (mg per g of dry mass), which is a common reference compound.

Total Flavonoid Content (TFC)

The total flavonoid content was determined according to the previous method [19], with minor modifications. Firstly, 0.2 mL of crude extracts of *F. vulgare* and *M. mammosa* were prepared (0.1 g of each crude extract per fraction in 100 mL of methanol) and mixed with 0.2 mL of aluminium chloride hexahydrate solution (10 % $AlCl_3$ solution), 0.2 mL of 1.0 M sodium acetate and 4.4 mL of deionised water. The solution was shaken and incubated at room temperature (25 °C) for 20 min and the absorbance was read at 415 nm using a UV-Vis spectrophotometer. By using a quercetin solution, a standard curve was constructed from 0.0125 to 0.15 $mgmL^{-1}$. The resultant total flavonoid content (TFC) was denoted in milligrams of Quercetin equivalent (QRE) per gram of dry extract.

Antioxidant test of extracts

DPPH (1,1-diphenyl-2-picrylhydrazyl) free scavenging activity assay

The assay was conducted using the DPPH assay method [20,21], with significant alterations. Briefly, 0.6 mL of 0.4 mM DPPH solution (Sigma Aldrich, India) in methanol was mixed with 2.4 mL of methanol of *M. mammosa* and *F. vulgare* ethanol extract at different concentrations (50 to 500 μgmL^{-1}). Then, the mixture was placed in the dark at room temperature for 30 min. The control sample was prepared by mixing 0.6 mL of DPPH solution with 2.4 mL of methanol. Measurements were made of the absorbance against a blank using a UV-VIS spectrophotometer at 517 nm. A lower absorbance indicates increased DPPH free radical scavenging activity. Ascorbic acid (Merck, India) at different concentrations varying from 1.0 to 5.0 μgmL^{-1} was used as standard. The inhibitory activity of each extract on the DPPH radical was calculated as the % inhibition of DPPH (I %) using the following equation:

$$I\ \% = [(Ao - As)/Ao] \times 100$$

here, Ao denotes the absorbance of the control sample and As denotes the absorbance of the tested extract solution.

Reducing power assay

For the determination of the reducing power of *M. mammosa* and *F. vulgare* ethanolic extracts and ascorbic acid, the method described in [22,23] was used with significant modifications. Essentially, a mixture of 1.0 mL of sample extract in methanol and 2.5 mL of 1 % potassium hexacyanoferate (III) was incubated at 50 °C for 20 min. After that, 2.5 mL of 10 % trichloroacetic acid was added to the mixture, and the upper layer of 2.5 mL was collected. It was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % $FeCl_3$ solution. Finally, the mixed solution was measured spectrophotometrically at 700 nm using a UV-VIS spectrophotometer and a blank. The controls consisted of all reagents except the extract fractions, with methanol as blank. Ascorbic acid was employed as a positive control. Increasing absorbance values indicated greater reducing power.

Phosphomolybdate assay (total antioxidant capacity)

The total antioxidant capacity of the ethanolic extracts of *M. mammosa* and *F. vulgaris* was determined according to the reported method [24], with minor modifications. This assay is based on the reduction of phosphomolybdate ions in the presence of an antioxidant, resulting in the formation of a bluish-green phosphate/molybdenum (V) complex, which is measured spectrophotometrically at 695 nm. The total antioxidant capacity of the ethanolic extracts *M. mammosa* and *F. vulgare* was assessed using the reported method. For this purpose, 500 µL sample prepared in methanol at different concentrations (500 - 2,500 μgmL^{-1}) was mixed in a test tube with 5 mL phosphomolybdate reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), the test tube was covered with aluminium foil and incubated in a water bath at 95 °C for 90 min. The mixture was cooled to ambient temperature. The absorbance was measured using a UV-Vis spectrophotometer at 695 nm against a blank. For the blank sample, 500 µL of methanol was added. Ascorbic acid served as a standard and the findings were presented as µg/mL of ascorbic acid equivalents.

α-amylase inhibition assay

Stock solutions of plant extracts and Acarbose were prepared using water, then filtered. The α-amylase activity was performed according to the previous method [25], which was based on starch-iodine discoloration, with some modifications. Test tubes containing 300 µL of plant extracts at different concentrations which ranged from 0.1 to 5.0 $mgmL^{-1}$ were mixed with 300 µL of α-amylase enzyme (100 UmL^{-1}) in 0.02 M pH 6.9 sodium phosphate buffer (containing 0.006 M sodium chloride) and incubated at

37 °C for 10 min. Subsequently, 300 µL of 1 % starch solution was added and incubated again at 37 °C for 10 min. Thereafter, the tubes were filled with 150 µL of 1.0 M HCl and 150 µL of iodine solution (containing 25.4 mg I_2 and 400 mg KI in 100 mL of distilled water), followed by the addition of 3.8 mL of distilled water. The absorbance of the solution was measured at 680 nm with the use of a UV-Vis spectrophotometer. As the positive control, acarbose (0.01 to 1.0 $mgmL^{-1}$) was used. The % inhibition of α-amylase was calculated as follows:

$$\% I: [1 - ((A_0 - A_s)/A_0)] \times 100$$

Antimicrobial assay plant of extracts

Preparation of bacterial and fungal culture

In this study, a total of 8 bacterial and 3 fungal strains were employed. The bacterial strains include 5 gram-positive bacteria: *Streptococcus mutans* ATCC 25175, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778, *Staphylococcus epidermidis* ATCC 12228 and *Enterococcus faecalis* ATCC 29212, as well as 3 gram-negative bacteria: *Escherichia coli* ATCC 11229, *Salmonella typhimurium* ATCC 14028, and *Klebsiella pneumoniae*. Additionally, 3 fungal strains were used, which were *Candida albicans* ATCC 10231, *Malassezia furfur* and *Trichophyton mentagrophytes*. Microbial strains without ATCC are clinical microbial strains.

The equipment and materials were sterilised for 120 min at 121 °C in an autoclave. Subsequent to incubating bacterial and fungal cultures on agar media, various bacterial and fungal colonies were selected using an inoculation loop. The selected colonies were suspended into Müeller-Hinton Broth (MHB), Brain-Heart Infusion Broth (BHIB), Nutrient Broth (NB) or Potato Dextrose Broth (PDB), then incubated for a duration of 18 - 24 h at 37 °C. The broth medium MHB was utilised for all gram-positive bacteria, aside from *S. mutans*, then BHIB was solely used for *S. mutans*. For all gram-negative bacteria, NB was employed, while PDB was used to cultivate all fungal cultures. After incubation, the suspended bacterial and fungal cultures were standardized to a 0.5 McFarland (2×10^8 $CFUmL^{-1}$) standard, and subsequently diluted to a 5×10^5 $CFUmL^{-1}$ concentration [26-28].

MIC determination of plant extracts

Five extracts were dissolved in 2 % dimethyl sulfoxide (DMSO) at a concentration of 1.0 gmL^{-1}. The following extracts were obtained: The ethanol extract of the tuber of *M. mammosa*, the ethanol extract of the fruit of *F. vulgare*, and the essential oil of *F. vulgare* fruit, as well as the ethanol and water extracts of the combined *M. mammosa* and *F. vulgare*. In addition, a positive control was used in this study: Ciprofloxacin, nystatin, chlorhexidine and ketoconazole, while employing 2 % DMSO as a negative control. Antimicrobial concentrations between 0.12 to 500 $mgmL^{-1}$ (for 5 extracts) and 0.24 to 500 μgmL^{-1} (for the positive control) were incubated in a 96-well microplate at 37 °C for 24 h. Following incubation, each microbe's growth was visually observed at various sample concentrations. Samples demonstrating antimicrobial activity are identified by the absence of visible microbial growth on the turbidity at the lowest concentration tested, defined as the minimum inhibitory concentration (MIC). For validation of the test results, spectrophotometric measurements were performed at a wavelength of 600 nm with a multimode reader (Infinite 200 PRO NanoQuant) [29-33].

Statistical analysis

The experiments were conducted in triplicate, and the results were expressed as the mean ± standard deviation (SD). The IC_{50} value was calculated by linear regression analysis using Ms Excel 2021 software. A 1-way analysis of variance (ANOVA) followed by a Tukey test was conducted using GraphPad Prism 9.5.1. The results were considered significant at $p < 0.05$. Minitab® Statistical Software version 21.4.1 was used to determine the Pearson correlation coefficient (r) between the inhibition of α-amylase, DPPH, TAC, TPC and TFC.

Results and discussion

Phytochemical screening and GC-MS for essential oil of *F. vulgare*

Tannins, phenolics, flavonoids, coumarins, saponins and terpenoids were found in the ethanolic extract of *M. mammosa* tuber. On the other hand, the ethanolic extract of *F. vulgare* contained tannins, phenolic compounds, flavonoids, coumarins and terpenoids without saponins and alkaloids (**Table 2**).

Table 2 Phytochemical screening.

No	Test	*M. mammosa*	*F. vulgare*
1	Tannin	+	+
2	Phenol	+	+
3	Flavonoid	+	+
4	Coumarins	+	+
5	Saponin	+	−
6	Triterpenoid/steroid	+	+
7	Alkaloid	−	−

(+): Present and (−): Absent

The essential oil derived from *F. vulgare* fruit was analysed using GC-MS, resulting in a chromatogram displaying numerous peaks. The molecular formulas, retention times and molecular weights of the compounds are listed in **Table 3**, along wih chemical structures in **Figure 1**. The GC-MS analysis results show that anethole (**9**) comprises 79.02 % of the composition of fennel fruit. Benzaldehyde (**8**) is the second most abundant component at 5.94 %, followed by estragole (**7**) at 4.89 %, fenchone (**4**) at 3.43 %, 1-(4-methoxyphenyl) 2-propanone (**11**) at 3.26 % and *n*-hexadecanoic acid (**16**) at 0.96 %, (*Z,Z*)-9,12-octadecadienoic acid (**17**) (0.55 %), 1-(4-methoxyphenyl)propane-1,2-diol (**15**) (0.37 %); pregna-3,5-dien-20-one (**18**) (0.29 %); 1-methoxy-4-(1-methylpropyl)benzene (**10**) (0.17 %) and other compounds with an abundance lower than 0.17 % are present. These results are in contrast to the observations of *F. vulgare* essential oil in a previously published study [34], which only obtained the content of trans-anethole (**9**) (36.8 %), 4-methoxybenzaldehyde (p-anisaldehyde) (**8**) (7.7 %) and other compounds not found in the essential oil observed here, such as α-ethyl-p-methoxybenzyl alcohol (9.1 %) and fenchyl butanoate (4.2 %).

Table 3 Characterisation of the essential oil from the fruit of *F. vulgare* was performed using GC-MS.

No	Compound	Molecular formula	RI	Percentage	Molecular weight
1	p-cymene	$C_{10}H_{14}$	9,910	0.02 %	134.22
2	D-limonene	$C_{10}H_{16}$	10,031	0.06 %	136.24
3	γ-terpinene	$C_{10}H_{16}$	10,908	0.03 %	136.24
4	Fenchone	$C_{10}H_{16}O$	11,892	3.43 %	152.24
5	Camphor	$C_{10}H_{16}O$	13,637	0.15 %	152.24
6	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	$C_{10}H_{18}O$	14,596	0.06 %	152.25
7	Estragole	$C_{10}H_{12}O$	15,144	4.89 %	148.21
8	4-methoxybenzaldehyde	$C_8H_8O_2$	16,988	5.94 %	136.15
9	*Trans*-anethole	$C_{10}H_{12}O$	17,999	79.02 %	148.21
10	1-methoxy-4-(1-methylpropyl) benzene	$C_{11}H_{16}O$	19,605	0.17 %	164.25
11	1-(4-methoxyphenyl) 2-propan-one	$C_{10}H_{12}O_2$	20,240	3.26 %	164.20
12	α-guaiene	$C_{15}H_{24}$	21,511	0.02 %	204.36
13	2,6-dimethyl-4-(2,2,2-trifluoro-1-hydroxy-1-trifluoromethyl-ethyl) phenyl 4-ethoxybenzoic acid	$C_{19}H_{16}F_6O_4$	21,939	0.07 %	422.32
14	p-Anisic acid, 4-cyanophenyl ester	$C_{15}H_{11}NO_3$	22,180	0.09 %	253.26
15	1-(4-Methoxyphenyl) propane-1,2-diol	$C_{10}H_{14}O_3$	25,137	0.37 %	182.22
16	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	33,307	0.96 %	256.43
17	(*Z,Z*)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	37,368	0.55 %	280.45
18	Pregna-3,5-dien-20-one	$C_{21}H_{30}O$	42,628	0.29 %	298.23
19	Nonadecane	$C_{19}H_{40}$	43,562	0.09 %	268.53
20	2-butyl-1-Octanol	$C_{12}H_{26}O$	45,986	0.16 %	186.34
21	N-[4-bromo-*n*-butyl]-2-piperidinone	$C_9H_{16}BrNO$	47,532	0.15 %	234.14
22	Di-*n*-decylsulfone	$C_{20}H_{42}O_2S$	49,012	0.12 %	346.61
23	2,4-Bis(4-methoxyphenyl) -3,5-dimethyltetrahydrofuran	$C_{120}H_{24}O_3$	49,012	0.09 %	312.41

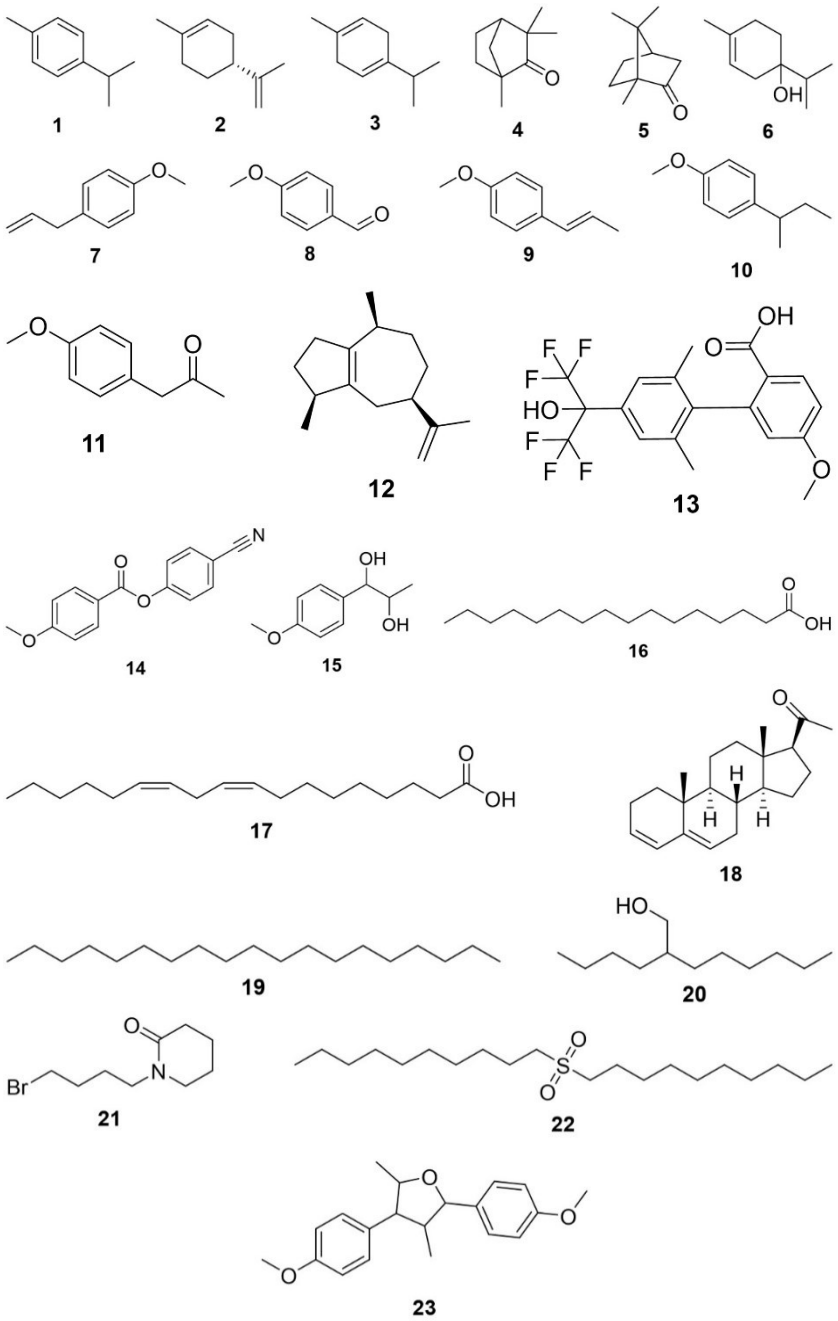


Figure 1 Chemical structure of essential oil from *F. vulgare* fruit characterized by GC-MS.

Total Phenolic Content (TPC) & Total Flavonoid Content (TFC)

The total phenolic content of *M. mammosa* and *F. vulgare* was assessed using the Folin-Ciocalteu colorimetric method. A gallic acid standard curve regression equation ($Y = 0.0082X + 0.1015$) with an R^2 of 0.9952 was employed. Within the limits of detection, the results showed a good linear relationship. According to **Table 4**, *M. mammosa* tubers had the highest amount of Total Phenolic Content (50.241 ± 0.012 mg GAE per gram extract compared to *F. vulgare* fruits (18.898 ± 0.030 mg GAE per gram extract).

Table 4 Total Phenolic Content (TPC) & Total Flavonoid Content (TFC).

No.	Sample	TPC mg GAE per g	TFC mg QRE per g
1	*F. vulgare*	18.898 ± 0.030	22.823 ± 0.435
2	*M. mammosa*	50.241 ± 0.012	53.968 ± 0.742

*All values are expressed as mean ± SD (n = 3); GAE: Galic Acid Equivalent, QRE: Quercetin Equivalent.

In agreement with TPC results, the total flavonoid content of *F. vulgare* fruit extract (22.823 ± 0.435 mg QRE per gram extract) is lower than the flavonoid content of *M. mammosa* tubers extract (53.968 ± 0.742 mg QRE per grams extract) (**Table 4**). This value was derived by using the quercetin standard curve regression (Y = 0.0031X + 0.0299), which yielded an R^2 value of 0.9939. The implication of these findings is that the *M. mammosa* tuber has a greater antioxidant potential than the *F. vulgare* fruit extract.

Antioxidant assay

Scavanger DPPH assay

DPPH is a stable radical with maximum absorption at 517 nm [35], which is easily scavenged by antioxidants. Therefore, it has become widely used as an instrument to assess the free radical-capturing capabilities of natural products.

Table 5 DPPH free radical scavenging activity.

No.	Samples	DPPH IC_{50} (μgmL^{-1})
1	*F. vulgare*	192.901 ± 0.426
2	*M. mammosa*	89.139 ± 0.189
3	L(+)-ascorbic acid	4.085 ± 0.050

*All values are expressed as mean ± SD (n = 3); All tests showed statistically significant differences ($p < 0.0001$) between the different treatments according to Tukey's HSD post hoc test.

The study discovered that the IC_{50} values for DPPH radical scavenging in ethanol extracts of *M. mammosa* tuber and *F. vulgare* fruit were 89.139 ± 0.189 and 192.901 ± 0.426 μgmL^{-1}, respectively (**Table 5**). The IC_{50} values of the plant extracts were very significantly different with $p > 0.0001$. Despite the fact that the antioxidant potential of these extracts were lower than that of ascorbic acid it was found that *M. mammosa* has free radical scavenging or scavenger properties, acting as a primary antioxidant. On the other hand, the extract of *F. vulgare* had a poor free radical scavenging capacity. The relationship between the phenolic and flavonoid content of *F. vulgare* fruits and *M. mammosa* tubers and their antioxidant activity was analysed using Pearson's correlation coefficient (r). The results showed a strong negative correlation between IC_{50} DPPH and TPC (r = −1.000) as well as IC_{50} DPPH and TFC (r = −0.995). This indicates that the lower the phenolic and flavonoid content in the plant extract, the higher the IC_{50} DPPH of the plant extract, resulting in a decrease in antioxidant activity.

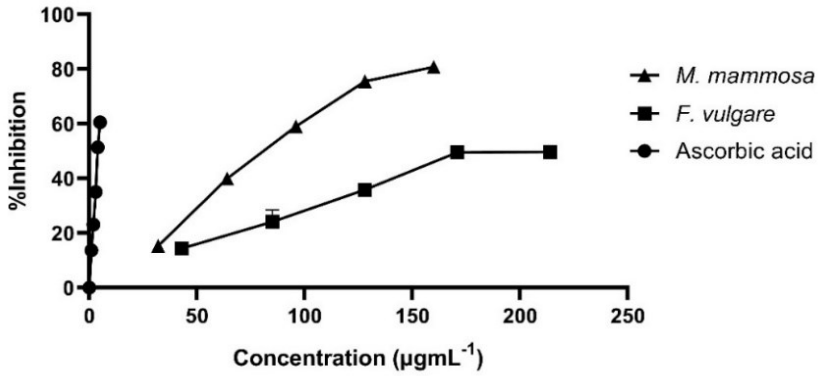


Figure 2 Free radical scavenging activity DPPH of ascorbic acid, *F. vulgare* and *M. mammosa* ethanol extracts.

Total Antioxidant Capacity (TAC) phosphomolybdate assay

The total antioxidant capacity of plant extracts was determined using the phosphomolybdate assay, measured as mg ascorbic acid equivalents per gram of extract weight. It is based on the reduction of molybdenum (IV) to molybdenum (V) by plant samples and the subsequent formation of a green phosphomolybdate complex under acidic conditions [36]. The ascorbic acid standard curve regression equation (Y = 0.005X + 0.0577) was utilised, achieving an R^2 value of 0.9937.

Table 6 Total Antioxidant Capacity (TAC) phosphomolybdate assay.

No.	Sample	Phosphomolybdate (TAC) mg AAE per gram extract			
		500 µgmL^{-1}	**1,000 µgmL^{-1}**	**1,500 µgmL^{-1}**	**2,500 µgmL^{-1}**
1	*F. vulgare*	51.064 ± 0.624	103.513 ± 0.047	148.350 ± 0.060	203.337 ± 1.643
2	*M. mammosa*	68.542 ± 0.883	125.138 ± 0.318	176.590 ± 0.990	256.300 ± 0.060

*All values are expressed as mean ± SD (n = 3); AAE: Ascorbic Acid Equivalent; All tests showed statistically significant differences ($p < 0.0001$) between the different treatments according to Tukey's HSD post hoc test.

The study findings revealed that the ethanolic extract of *M. mammosa* tuber had a better total antioxidant capacity for phosphomolybdate reduction compared to the ethanolic extract of *F. vulgare* fruit. The values for the total antioxidant capacity are given in **Table 6** and are illustrated in **Figure 3**. The gap between the TAC values of the 2 plant extracts widens as the concentration used increases. Commencing at a concentration of 500 µgmL^{-1}, there is a discrepancy of approximately 17.478 gap between the 2 extracts. At 1,000 µgmL^{-1}, the gap increases to around 21.625, which further rises to 28.240 at 1,500 µgmL^{-1}. Subsequently, at a concentration of 2,500 µgmL^{-1}, the gap widens significantly with a difference of about 52.963. It has been demonstrated that the higher the concentration of plant extracts used, the greater the antioxidant potential seen. The higher phenolic and flavonoid content of the *M. mammosa* extract compared to the *F. vulgare* extract is responsible for the significant difference in results. This was confirmed by a strong positive Pearson's correlation with r = 0.988 to 0.999.

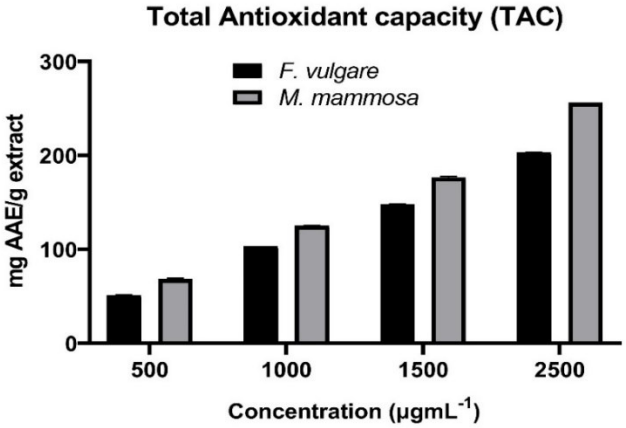


Figure 3 Total Antioxidant Capacity (TAC) phosphomolybdate assay *F. vulgare* and *M. mammosa* ethanol extract.

Reducing power hexacyanoferrate (III)

Reducing Power is often used to assess the effectiveness of an antioxidant in donating electrons. The assay is carried out using a spectrophotometric method at a wavelength of 695 nm to measure the reduction of potassium ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) [37].

Table 7 Reducing power antioxidant.

No.	Samples	Reducing power $K_3Fe(CN)_6$ absorbance at 695 nm (A)	
		At 10 µgmL^{-1}	**At 200 µgmL^{-1}**
1	*F. vulgare*	0.0015 ± 0.001	0.0351 ± 0.001
2	*M. mammosa*	0.0146 ± 0.008	0.2130 ± 0.003
3	L(+)-ascorbic acid	0.2099 ± 0.001	-

*All values are expressed as mean ± SD (n = 3).

The reducing capacity of the ethanolic extracts of *M. mammosa* and *F. vulgare* is measured by absorbance at a wavelength of 695 nm are given in **Table 7** and are illustrated in **Figure 4**. At a concentration of 10 μgmL^{-1}, neither extract exhibit a considerable ability to reduce antioxidants (less than 0.02 A) when compared to ascorbic acid (0.2099 ± 0.001 A). However, at a concentration of 200 μgmL^{-1}, the ethanolic extract of *M. mammosa* demonstrated a reduction power of 0.2130 ± 0.003 A, similar to ascorbic acid (vitamin C) at 10 μgmL^{-1}. This revealed that the extract possesses antioxidant properties to halt radical chain reactions and convert free radicals into more stable products. Conversely, the reducing power of the ethanolic extract of *F. vulgare* is only 0.0351 ± 0.001 A at 200 μgmL^{-1}, which means that it lacks the electron-donating capacity compared to the extract of *M. mammosa*. This discrepancy may be related to the higher phenolic and flavonoid content of the *M. mammosa* extract compared to the *F. vulgare* extract.

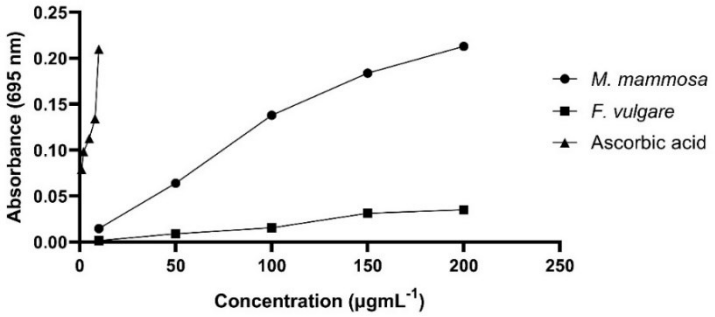


Figure 4 Reducing power hexacyanoferrate (III) of standard, *F. vulgare* and *M. mammosa* ethanol extract.

α-amylase inhibitory assay

The enzyme α-amylase catalyses the hydrolysis of α-1,4-glucosidic linkages of starch to induce starch digestion and promote glucose uptake. Inhibition of this enzyme has been shown to be effective in regulating diabetes. In the current study, we used an *in vitro* model of α-amylase inhibition to evaluate the potential hyperglycaemic effects of ethanolic extracts of *M. mammosa* and *F. vulgare* tested by the iodine-starch reaction method [38].

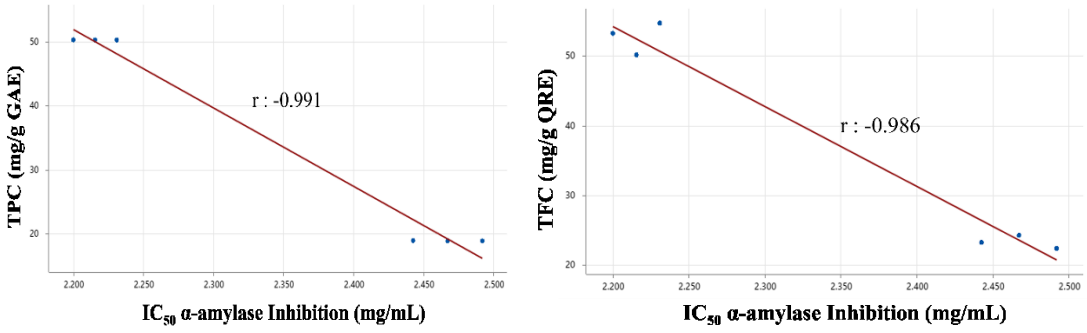
Table 8 α-amylase inhibitory assay.

No.	Samples	α-amylase inhibition IC_{50} ($mgmL^{-1}$)
1	*F. vulgare*	2.467 ± 0.025
2	*M. mammosa*	2.215 ± 0.015
3	Acarbose	0.930 ± 0.011

*All values are expressed as mean ± SD (n = 3); There was no significant difference ($p > 0.05$) between the 2 extracts and the positive control.

The inhibition of α-amylase by the ethanolic extracts of *M. mammosa* and *F. vulgare* is shown in **Table 8**. Concerning the IC_{50} values, the inhibitory activity of *M. mammosa* was significantly better than that of *F. vulgare*, with IC_{50} values of 2.215 ± 0.015 and 2.467 ± 0.025 $mgmL^{-1}$, respectively. In comparison, it was still less active than acarbose with an IC_{50} of 0.930 ± 0.011 $mgmL^{-1}$.

Based on the above results, it was found that the difference between the IC_{50} of *M. mammosa* extract and *F. vulgare* extract was non-significant ($p > 0.05$). Relationship between IC_{50} of α-amylase inhibition with total phenolic content (TPC) and total flavonoid content (TFC) was described by Pearson's correlation as shown in **Figure 5**. A strongly negative correlation was found with r = −0.991 (IC_{50} α-amylase inhibition vs. TPC) and r = −0.986 (IC_{50} α-amylase inhibition vs. TFC). The strongly negative correlation between TPC and TFC with the IC_{50} of α-amylase inhibition implies that significant TPC and TFC will further increase the anti-diabetic activity, and higher anti-diabetic activity will be expressed as lower IC_{50} values.



(a) (b)

Figure 5 Pearson's correlation TPC vs IC_{50} α-amylase inhibition (a) and TFC vs IC_{50} α-amylase inhibition (b).

Antimicrobial activity

The antimicrobial activity can be seen in **Tables 9** and **10** as the antimicrobial activity of the positive control used. This study observed that *S. mutans* exhibited the highest resistance among Indonesian medicinal plants, namely *F. vulgare* and *M. mammosa*, against their ethanol extracts, essential oils and combinations. Conversely, *K. pneumoniae* and *T. mentagrophytes* were the most susceptible microbes in all the preparations tested.

In comparison with almost all positive controls shown in **Table 10** (ciprofloxacin, vancomysin, chlorhexidine and nystatin), the antimicrobial activity of the extracts of both plants was shown to be less active. However, they could potentially replace ketoconazole, which has shown resistance to *T. mentagrophytes* (MIC: 312.5 mg/mL). Both plants (ethanol extract, essential oil and combination) had MICs of 15.62 and 62.5 mg/mL.

The ethanol extract of *F. vulgare* proved more potent against gram-positive bacteria, excluding *S. mutans*, where its minimum inhibitory concentration (MIC) was 125 $mgmL^{-1}$. It had moderate activity (MIC: 62.5 $mgmL^{-1}$) against *S. aureus* and *S. epidermidis*, then high activity against *B. cereus* (MIC: 7.81 $mgmL^{-1}$). Additionally, the treatment exhibited significant effectiveness against gram-negative bacteria *K. pneumoniae* (MIC: 1.95 $mgmL^{-1}$) and fungus *T. mentagrophytes* (MIC: 15.62 $mgmL^{-1}$).

Table 9 Antimicrobial activity.

No.	Microbes	MIC ($mgmL^{-1}$)				
		F. vulgare		*M. mammosa*	Combination	
		Ethanol[a]	Essential oil[b]	Ethanol[a]	Ethanol[a]	Water[c]
1	*S. mutans*	125	500	250	250	> 500
2	*S. aureus*	62.5	250	125	250	> 500
3	*B. cereus*	7.81	125	125	250	> 500
4	*S. epidermidis*	62.5	> 500	125	125	7.81
5	*E. faecalis*	NT	NT	> 500	> 500	3.91
6	*E. coli*	125	125	250	125	31.25
7	*S. typhimurium*	125	250	62.5	> 500	15.62
8	*K. pneumoniae*	1.95	62.5	15.62	62.5	31.25
9	*C. albicans*	500	3.91	> 500	> 500	62.5
10	*M. furfur*	> 500	1.95	> 500	125	31.25
11	*T. entagrophytes*	15.62	15.62	15.62	62.5	62.5

[a]Ethanol: Sample was extracted with ethanolic solvent.
[b]Essential Oil: Sample was obtained using the hydrodistillation method.
[c]Water: Sample was decocted with boiling water.
NT: Not Tested.

Futhermore, *F. vulgare* essential oil had antifungal properties with minimum inhibitory concentrations (MICs) of 3.91, 1.95 and 15.62 $mgmL^{-1}$ against *C. albicans*, *M. furfur* and *T. mentagrophytes*, respectively. As the MICs ranged from 125 to 500 $mgmL^{-1}$ against bacterial strains, *F. vulgare* essential oil was unsuitable for antibacterial use. There was an antimicrobial agent with a MIC of

62.5 $mgmL^{-1}$, indicating moderate efficacy against a single strain of *K. pneumoniae* bacteria. Different extraction techniques yield varying contributions of compounds with antimicrobial activity. Phytochemical screening studies showed that the ethanolic extract of *F. vulgare* is rich in tannins, phenolics, flavonoids and terpenoids, which have significant antibacterial activity and comparatively weaker antifungal activity. Whilst the main constituents of *F. vulgare* essential oil are simple monoterpene volatiles which confer significant antifungal properties, they lack the potency of antibacterial agents.

Table 10 Antimicrobial activity of positive control.

No.	Microbes	MIC control positive ($mgmL^{-1}$)				
		Ciprofloxacin	**Vancomysin**	**Chlorhexidine**	**Nystatin**	**Ketoconazole**
1	*S. mutans*	-	-	3.906	-	-
2	*S. aureus*	0.098	6.25	-	-	-
3	*B. cereus*	0.195	0.781	-	-	-
4	*S. epidermidis*	0.195	-	-	-	-
5	*E. coli*	1.560	50	-	-	-
6	*S. typhimurium*	0.098	-	-	-	-
7	*K. pneumoniae*	0.391	3.125	-	-	-
8	*E. faecalis*	0.781	12.5	-	-	-
9	*C. albicans*	-	-	-	4.880	-
10	*M. furfur*	-	-	-	-	0.049
11	*T. mentagrophytes*	-	-	-	-	312.5

(-): Not tested.

The ethanolic extract of *M. mammosa* exerted poor antimicrobial activity against several microorganisms with an average MIC of 125 - 500 $mgmL^{-1}$. Based on the study's findings, the extract shows effectiveness solely against *K. pneumoniae* and *T. mentagrophytes* with an MIC of 15.62 $mgmL^{-1}$. Additionally, it displayed a moderate level of activity against *S. typhimurium*, with a MIC of 62.5 $mgmL^{-1}$. The phenolic and flavonoid compounds discovered in the extract of *M. mammosa*, which were of a greater amount than those found in *F. vulgare* extract, did not exhibit any antimicrobial activity. This stands in opposition to its notable impact on antioxidant activity, which is primarily governed by the existence of these 2 compounds.

The combination ethanol extract of *F. vulgare* and *M. mammosa* had the poorest antimicrobial activity, with a MIC of $\geq$ 62.5 $mgmL^{-1}$. The antimicrobial activity of the combination of ethanol extracts of the 2 plants is very low, since the antimicrobial activity of the *F. vulgare* extract alone is not particularly potent, and the activity of the ethanol extract of *M. mammosa* alone as an antimicrobial agent is extremely weak.

Combination water extract of *M. mammosa* and *F. vulgare* was prepared by decocting the sample in a reflux apparatus. The use of hot water mimics the community's long-established practice of steeping a mixture of medicinal herbs. The hot water 2 extract combination outperformed ethanol 2 extract combination in antimicrobial activity. **Table 8** shows that these extracts were most effective against 2 gram-positive bacteria, *S. epidermidis* and *E. faecalis*, with MICs of 7.81 and 3.91 $mgmL^{-1}$. MICs for gram-negative bacteria and fungi except *S. typhimurium* ranged from 31.25 to 62.5 $mgmL^{-1}$, indicating moderate activity.

Conclusions

In this study, we examined the characteristics and bioactivity of the ethanol extracts from *M. mammosa* tubers and *F. vulgare* fruits. These extracts have similar phytochemical composition, although the saponin content was found exclusively in the *M. mammosa* extract. The GC-MS analysis of the fennel fruit extract showed anethole **(9)** as the major compound amongst other minor compounds. Both the total phenolic content (TPC) and total flavonoid content (TFC) of the *M. mammosa* extract were greater than that of the *F. vulgare* extract.

We also found that *M. mammosa* extract was more active than *F. vulgare* extract in antioxidant activity; thus, the higher TPC and TFC content of *M. mammosa* extract affects the antioxidant activity. This is unlike the α-amylase enzyme inhibitory activity where both extracts have the same activity, which means that the TPC and TFC of both extracts do not significantly affect the antidiabetic activity. In addition, MIC value of *F. vulgare* ethanol extract was more active against gram-positive bacteria, while essential oil is

only active against fungi. The combination of water extracts had the highest activity against 2 microbes, specifically *S. epidermis* and *E. faecalis*.

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