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PHYTOCHEMICAL CONSTITUENTS ANALYSIS OF ETHANOLIC EXTRACT OF MUSA PARADISIACA L. FLOWER USING GC-MS AND LC-MS

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Abstract

The investigation of natural sources for bioactive compounds with potential therapeutic applications has gained significant attention in recent years. Banana, or Musa paradisiaca L., is highly regarded for its nutritional content as well as for the variety of phytochemical components that contribute to its therapeutic benefits. This study aimed to analyze the phytochemical constituents of the ethanolic extract from M. paradisiaca flowers, with a focus on identifying bioactive compounds containing antimicrobial activities. The complex chemical composition of the ethanolic extract of M. paradisiaca flowers has been determined by analyses using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). The GC-MS analysis was conducted using the QP2010 Ultra gas chromatography-mass spectrometer, coupled with a BP5MS column to facilitate precise separation of compounds. For LC-MS analysis, the Agilent 1290 Infinity LC system, coupled with the Agilent 6520 Accurate-Mass Q-TOF mass spectrometer featuring dual electrospray ionization (ESI) modes, was

employed. The GC-MS analysis results showed that the ethanolic extract contains a variety of bioactive compounds, including hexadecenoic acid, 1-heptacosanol, 1-heneicosanol, 17-Pentatriacontene, diacetone alcohol, diisooctyl phthalate, fucosterol, heptadecanol, octadecane, octadecanoic acid methyl ester, phenol, 2,4-bis(1,1-dimethylethyl)-, squalene, and triacontane. Additionally, the LC-MS analysis identifies new bioactive compounds from M. paradisiaca flower ethanolic extract, such as hippeastrine, L-(-)-carvone, 4-hydroxybenzaldehyde, and vanillin. These compounds are believed to have antimicrobial properties, which is consistent with what has been found in extracts from other plants in previous studies. Thus, this study contributes to the understanding of the phytochemical composition of the ethanolic extract from M. paradisiaca flowers, which can be used for further exploration and applications in the fields of medicine and natural product-based antimicrobial approaches.

1. Introduction

The use of medicinal plants and their derivatives in traditional cultures across the globe is rising and becoming more popular in contemporary society as natural remedies or supplements to toxic chemicals (Van Wyk & Wink, 2018). Among all medicinal plants, various parts of the M. paradisiaca are used for medicinal purposes, particularly leaves, roots, and flowers. The leaf juice has long been used to cure wounds, cuts, and bug bites (Onyenekwe et al., 2013). The sap of the plant (fluid excretion) has been successfully utilized to treat epilepsy, hysteria, dysentery, and diarrhea; the root parts have been used as an anthelmintic, while the roots' cold infusion has been utilized for treating anemia and venereal diseases; the lower part of the plant has been used as an astringent; and the fruits have been used as mild laxatives (Okareh et al., 2015). The fungicidal activities of M. paradisiaca peel and stalk extracts have been reported by Okorondu et al. (2012).

While the antibacterial activities of the M. paradisiaca leave extract have been reported against E. coli, S. aureus, P. aeruginosa, S. Typhi, S. dysenteriae as well as B. cereus (Karadi et al., 2011; Sahaa et al., 2013; Karuppiah & Mustafa, 2013). Several flavonoids and their related compounds were extracted from the unripe pulp of M. paradisiaca, such as quercetin leucocyanidin, and its derivatives, including 3-O-glucoside, 3-Ogalactoside, and 3-O-rhamnosyl glucoside (Lewis et al., 1999; Lewis & Shaw, 2001; Ragasa et al., 2007). In contrast, other phytochemical substances discovered in M. paradisiaca fruit pulp include tannin, tryptophan, indole compounds, nor-epinephrine, serotonin, starch, crystallizable and non-crystallizable sugars, vitamins B, and C, iron, lipids, and mineral salts (Ghani, 1998). On the other hand, several compounds were isolated from M. paradisiaca flower, such as syringin, (6S, 9R)-rose oxide, hemiterpenoid glucoside (1,1-dimethylallyl alcohol), benzyl alcohol glucoside, (24R)-4α, l4 α, 24-trimethyl-Sacholesta-8, and 25(27)-dien-3β-o1 (Dutta et al., 1983; Martin et al., 2000).

Several separation methods, such as gas chromatography (GC), liquid chromatography (LG), high-performance liquid chromatography (HPLC), and capillary electrophoresis, have been used to detect the different chemicals contained in crude plant extracts and their fractions (Shui et al., 2005). LC-MS and GC-MS are commonly employed for MS's ability to prevent some overlap

and for structure verification of the different compounds in an extract (Sánchez-Rabaneda et al., 2004). Moreover, gas chromatography has been widely employed in the field of applications, with its principal use being the isolation and examination of composite mixtures, including hydrocarbons, essential oils, and solvents (Al-Rubaye et al., 2017), while LC-MS is frequently used to identify phenolic compounds in plant extracts (Maulidiani et al., 2012). The existence of active chemicals in plant extracts in conjugation with other phytochemicals with different polarities, which makes it difficult to separate them, is a significant defiance of identifying these compounds (Wolfender et al., 2003). In recent years LC-MS and GC-MS have been used more frequently in the examination of natural items under the aptitude of the LC-MS as well as GC-MS devices to separate and identify the components present in an extract (Wu et al., 2013). Therefore, this study aims to analyze phytochemicals in ethanolic M. paradisiaca flower extract that could be responsible for antimicrobial activities employing GC-MS and LC-MS analyses.

2. Materials and methods

2.1 Samples collection

M. paradisiaca flower was purchased from Taman Pertanian university UPM (University Agricultural Park. Plant taxonomic identification was done by Mohd Firdaus Botanist plant in Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) under the voucher specimen number of MFI 0207/21. The sample was analyzed in the Laboratory of Natural Products, Institute of Bioscience (IBS), UPM. The samples were washed and shade-dried for 10 days at room temperature. The dried flowers were kept at room temperature in sealable plastic bags before further processing.

2.2 Preparation of crude Extract

The M. paradisiaca flowers extraction has been carried out using the methodology provided by Rukayadi et al. (2008), with little modifications. 100 g of dried M. paradisiaca flowers were ground using a Panasonic dry blender MK-5087M (Panasonic Corporation, Osaka, Japan). The grounded flowers were then saturated in 400 mL of 99.8% ethanol (R and M Marketing, Essex, UK), and placed in a shaker water bath (Saintifik Maju, Selangor, Malaysia) at 30°C for overnight. Afterwards, the soaked flowers powder was vacuum-filtered using Whatman filter paper No. 2 (Whatman International Ltd., Middlesex, England) by EYELA A-1000S aspirator pump (Tokyo Rikakikai Co., Tokyo, Japan). The filtrate was then concentrated for 3 hrs at 40°C and 150 rpm, using a rotating vacuum evaporator Heidolph laborota (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). The crude ethanolic extract has been kept at 4°C before commence to use.

2.3 Gas chromatography-mass spectrometry

The ethanolic M. paradisiaca flower extract sample has been prepared for GC-MS following the technique proposed by Javadi et al. (2014) with slight modifications. GC-MS analysis has been conducted to establish the volatile compounds in ethanolic M. paradisiaca flower

extract. To get 5 mg/mL, 5 mg of the material was dissolved in 1 mL of ethanol HPLC analytical grade (R & M Marketing Essex, UK). The extract was analyzed using the QP2010 Ultra gas chromatography-mass spectrometer (Shimadzu Corporation, Kyoto, Japan), which it coupled with a BP5MS column for compounds separation, and the column's dimensions were: 0.25 mm for internal diameter, 30 m for length and 0.25 µm for film thickness. Furthermore, helium was employed as a carrier gas at a flow rate of 0.8 mL/min. Alternatively, the oven's operational parameters were: 50°C for the initial temperature, increase rate at 3°C/min up to 300°C, and hold time of 10 mins. In contrast, the injection and ion-source temperatures were 200°C, respectively. The peaks for the ethanolic M. paradisiaca flower extract was obtained by comparing their similarity index, retention indices (RI), and molecular weight using standard spectra accessible in Shimadzu GC-MS NIST/ Wiley library and with literature published previously.

2.4 Liquid chromatography-mass spectrometry

The raw samples of ethanolic M. paradisiaca flower extract was prepared for LC-MS following Ado et al. (2015) method, with minor modifications. 5 mg of the extract was diluted in 1 mL of HPLC grade ethanol, vortexed, and filtered using a 0.45 m Nylon syringe filter. The Agilent 1290 Infinity LC system was used in the study, together with an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer (Agilent Technologies Inc., California, United States) with dual ESI; positive and negative ionization mode. Agilent Zorbax Eclipse XDB-C18 column has been used for separation, where the analyte (injection volume was 1.0 µL) and column dimensions were 2.1 mm internal diameter, 150 mm length, and the temperature was adjusted at 25°C. On the other hand, the auto-sampler temperature was 23°C, with a linear gradient of mobile phase containing solvent A (0.1% formic acid in water), and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 0.5 mL/min. The gradient has been adjusted with a time interval of 30 mins, commencing from 5% B at 0 minutes to 100% at 30 mins, while the flow rate has been adjusted at 0.5 mL/min. The operating conditions were gas temperature of 300°C, nebulizer pressure of 40 psi, dry gas of 10 mL/min, and fragment voltage of 125V. The mass ranges for screening were between 100 - 1000 m/z, and the polarity was placed in negative and positive modes. The control and processed data (obtained compounds) were analyzed using the XcaliburTM version 2.2 (Thermo Fisher Scientific, San Jose, CA, USA).

2.5 Statistical Analysis

All experiments have been conducted three times, each of which has three replicates (n = 3 × 3). MINITAB software was used to analyze the collected data by analysis of variance (two-way ANOVA). The Turkey's test was used to determine the significance of difference ($P < 0.05$) between the treatments. The outcomes of the replicate analysis have been reported as means ± standard deviation (SD).

3. Results and discussion

3.1 Bioactive compound detected by GC-MS

Sixty peaks have been recognized in the GC-MS chromatogram of ethanolic M. paradisiaca flower extract. Comparing their mass-spectral databases with Wiley and NIST libraries, the phytochemical compounds were identified and characterized as listed in Table 1. Seven significant compounds were identified from the ethanolic M. paradisiaca flower extract. The most abundant compounds were 9,19-Cyclolanostan-3-ol, 24- methylene-, (3. beta.)- (8.10% and 6.19, peak No. 54 and 55) followed by Hexadecanoic acid (7.83%, peak No. 12), (Z)-18-Octadec-9-enolide (6.99%, peak No. 17), Stigmasterol (6.24%, peak No. 47), Stigmast-5-en-3-ol, (3. beta.)- (5.48%, peak No. 50), and 1-Heptacosanol (5.07%, peak No. 32). The other identified compounds were present in a quantity of less than 4%. Based on the GC-MS results, hexadecanoic acid is a fatty acid and was the major component in the ethanolic M. paradisiaca flower extract. According to Gomathi et al. (2015), hexadecanoic acid from the ethanolic extract of Evolvulus alsinoides possessed antimicrobial activity in addition to the antioxidant, anticancer, antidiabetic, anti-inflammatory, 5-alpha-reductase inhibitor and antifibrinolytic activities. Dubal et al. (2013) and Adeoye-Isijola et al. (2018) demonstrated that the hexadecanoic acid from Lentinus squarrosulus and Tectaria coadunata (J. Smith) C. chr. has some biological activities, including antibacterial, antioxidant, anti-inflammatory, hypolipidemic, insecticide, oils, anticancer, flavor, immunostimulant, chemopreventive, hemolytic 5-α reductase, and lipo-oxygenase suppressors. 1-heptacosanol also had been detected in ethanolic M. paradisiaca flower extract. 1-heptacosanol is known to possess antimicrobial properties. Kalsum et al. (2016) reported that the 1-heptacosanol from the ethanolic extracts of propolis Trigona spp. of the South Sulawesi region, Indonesia possessed antimicrobial, anticancer, antioxidant, and nematicidal. The same activities of 1-heptacosanol have been demonstrated by Vajjiram et al. (2017) from Nicotiana tobacum (Solanaceae). Begum et al. (2016) reported the antimicrobic action of 1-heptacosanol from Paracoccus pantotrophus FMR19 against Proteus spp., Salmonella spp., P. aeruginosa, S. paratyphi-B, S. aureus and S. paratyphi-A.

On the other hand, eleven out of the rest compounds with a peak quality of less than 4%, such as 1-heneicosanol, 17-pentatriacontene, diacetone alcohol, diisooctyl phthalate, fucosterol, n-heptadecanol, octadecane, methyl stearate, 2,4-di-tert-butylphenol, squalene, and triacontane were believed to have antimicrobic activities based on the previous studies. These phytochemical compounds are characterized as listed in Table 2. 1-Heneicosanol is another compound of the components found in ethanolic M. paradisiaca flower extract, which belongs to the phenol group. According to Nautiyal and Dubey (2021), 1-Heneicosanol from Badri cow urine was found to exhibit antibacterial activity against L. monocytogenes (MTCC657), S. aureus (MTCC7443), P. aeruginosa (MTCC424), K. pneumoniae (MTCC432) and S. Typhi (MTCC733). Thekkangil et al. (2021) demonstrated that this compound from Streptomycetes albidoflavus STV1572a possesses antifungal properties against Trichophyton mentagrophytes. Goda et al. (2020) reported the antimicrobic action of 1-Heneicosanol from Seagrass Thalassodendron ciliatum (Forsk.) against C. albicans, C. krusei, P. aeruginosa, and S. aureus. Moreover, Amudha et al. (2018) discovered that this chemical from Enhalus acoroides seagrass extract has antifungal action. In the same context, Heng et al. (2020) established the antibacterial, antifungal, and antioxidant activities of

this chemical derived from Cibotium barometz rhizome hairs (Cibotiaceae). 17-Pentatriacontene is another bioactive compound also detected in ethanolic M. paradisiaca flower extract. Kumar et al. (2018) demonstrated many biological activities, including the antibacterial, anti-inflammatory, anticancer, and antiarthritic of 17-Pentatriacontene from Eichhornia crassipes (Mart) Solms. Kumar et al. (2018) reported the 17-Pentatriacontene compound from Eichhornia crassipes (Mart) Solms. Exhibited potential medicinal values with antibacterial activity against P. fluorescens. This phytocompound might be the potential inhibitory source against the microbial protein. Rajkumar and Bhavan (2017) reported this bioactive compound's antibacterial and antiviral properties from the marine brown alga Turbinaria ornate.

Diacetone alcohol was detected in ethanolic M. paradisiaca flower extract. Based on El Bouchti et al. (2021), diacetone alcohol from Stipa tenacissima L. extract showed antifungal, antibacterial, anticancer, and antioxidant activities and is also used to treat dysentery and diarrhea. This bioactive compound possessed pharmacological activity, including antiproliferative properties. In the same trend, Seddek et al. (2019) demonstrated that the diacetone alcohol isolated from Anabaena oryzae, Oscillatoria spp., and Stigonema ocellatum possessed antimicrobial (against K. pneumoniae, P. aeruginosa, Serratia marcescens, Micrococcus luteus, C. albicans, C. glabrata, and C. krusei), antioxidant as well as cytotoxic activities. Maligan et al. (2016) report the antimicrobial property of this bioactive compound isolated from the Microalgae Tetraselmis chuii. Diisooctyl phthalate, a plasticizer compound, was detected in ethanolic M. paradisiaca flower extract. Tyagi and Agarwal (2017) demonstrated that the diisooctyl phthalate isolated from Pistia stratiotes possessed antimicrobial and antifouling properties. This was in agreement with Mary and Giri (2017). They also reported the antimicrobial property of this compound from Achyranthes aspera and other activities such as solvent, plastilixer, pesticide, and repellent. Sayed-Ahmad et al. (2014) reported that the diisooctyl phthalate Lebanese Urtica dioica possessed antibacterial, antioxidant, anti-inflammatory, analgesic, and sedative activities.

Fucosterol is one of the bioactive compounds detected in ethanolic M. paradisiaca flower extract. According to Jung et al. (2016), fucosterol was the most abundant sterol in brown seaweeds, accounting for 83-97% of the total sterol content. The isolated fucosterol from Ecklonia stolonifera was the most copious phytosterol and had many biotic actions, including antifungal, anticancer, cholesterol-reducing, antidiabetic, antioxidant, antiadipogenic, antihistaminic, anticholinergic, anti-inflammatory, and butyrylcholinesterase inhibitory activities. This report was in line with Abdul et al. (2016) study, which verified the biological activities of fucosterol isolated from Seaweeds (marine Algae), including fungicidal, antitumor, antioxidant, antimutagenic, antidiabetic, antidepressant, antihyperlipidemic, anticholinergic, antiosteoporotic, antiphotodamaging, liver-preventive, blood cholesterol lowering, and butyrylcholinesterase suppressing properties. n-Heptadecanol is a fatty acid where it was detected in ethanolic M. paradisiaca flower extract. Nautiyal and Dubey (2021) demonstrated that n-heptadecanol might be responsible for the antibacterial activity of cow urine's bioactive component. According to Pradhan and Dubey (2021), n-heptadecanol from Camellia sinensis and Camellia assamica has antibacterial

and anti-inflammatory properties. In contrast, Balamurugan et al. (2012) reported the antimicrobial activity of n-heptadecanol from Polycarpaea corymbosa. Octadecane, belonging to alkaloids, was also detected in ethanolic M. paradisiaca L. flower extract. Sasikumar et al. (2020) reported the antimicrobial activity of octadecane isolated from blood fruit (Haematocarpus validus Bakh. F. Ex Forman) of North-East India. Naragani et al. (2016) discovered that this compound of Streptomyces cheonanensis VUK-a from mangrove origin recorded moderate to significant antimicrobial properties against clinically and agriculturally essential bacteria and fungi, namely B. subtilis, B. megaterium, B. cereus, P. vulgaris, S. aureus, E. coli, P. aeruginosa, Serratia marcescens, S. Typhi, Streptococcus mutans, S. epidermis, Xanthomonas campestris, and X. malvacearum.

The methyl stearate is a fatty acid methyl ester detected in ethanolic M. paradisiaca flower extract. Belakhdar et al. (2015) demonstrated that the methyl stearate from Thesium humile Vahl possessed antibacterial and antifungal activities (antimicrobial activity). This was in agreement with the study by Hameed et al. (2016), who also reported the fungicidal action of methyl stearate from Callosobruchus maculutus, where it was highly effective to suppress the growth of Asp. niger. Alternatively, Othman et al. (2015) described that this compound possessed anti-inflammatory activity when isolated from Jatropha curcas L. plant root. Another bioactive compound detected in ethanolic M. paradisiaca flower extract was 2,4-di-tert-butylphenol. According to Elgorban et al. (2019), 2,4-di-tert-butylphenol was the primary compound in Salvadora persica and is a naturally occurring chemical found in medicinal plants. Streptomyces spp. generated this drug, which has antibacterial activity against methicillin-resistant S. aureus (MRSA) with a method of action against bacterial cell wall production and modest cytotoxic activity. Similarly, 2,4-di-tert-butylphenol was reported to exhibit herbicidal properties. Ghaly et al. (2020) demonstrated that this bioactive compound from different sources possessed antibacterial, antifungal, anticancer, and antioxidant properties. According to Zhao et al. (2020), this bioactive compound can modulate the secreted extracellular polymeric substances (EPS) of Serratia marcescens (which play critical roles in biofilm formation and biocorrosion), which could facilitate biofilm disruption while also favoring antimicrobial diffusion into cell aggregates, resulting in the eradication of persistent biofilms. It has also been shown to have antifungal activity against the agriculturally essential root-rot fungus Fusarium oxysporum by suppressing spore germination and hyphal development.

Squalene also had been detected in ethanolic M. paradisiaca flower extract, and this compound is a natural lipid (polyunsaturated) belonging to the triterpene family. It is a precursor in the phytosterol biosynthesis in plants or cholesterol biosynthesis in humans, where it could be attained from the diet (for example, olive oil contains 0.2-0.7% squalene) (Smith, 2000; Reddy & Couvreur, 2009; Lou-Bonafonte et al., 2018). This compound is known to possess antimicrobial properties. According to Biswas and Chakraborty (2013), squalene from Artocarpus leaves showed an antifungal inhibitory effect against Asp. fumigatus, Asp. niger and Asp. tamari. On the other hand, it showed an antibacterial inhibitory effect against each E. coli and S. lutea, while S. aureus

remained unaffected. In this contest, Rameshkumar et al. (2018) demonstrated that Nilgirianthus ciliatus (one of the commercially medicinal plants) contains various phytochemicals, including squalene. The presence of this phytoconstituent is responsible for various biological potentialities, including antibacterial, antifungal, antioxidant, and anticancer. According to Rency et al. (2015), squalene from ethanolic leaf extracts of Premna serratifolia had antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemopreventative, Lipoxygenase- inhibitor, and pesticide properties. Triacontane belonging to alkane was detected in ethanolic M. paradisiaca flower extract. Based on Ibnouf (2021) where demonstrated certain biological activities of triacontane isolated from O-7 Actinomycete, including antimicrobial and cytotoxic properties. Kumari et al. (2019) reported the potential antibacterial (S. aureus, P. aeruginosa, K. pneumoniae, P. vulgaris, and B. subtilis), antidiabetic and antitumor activities of triacontane from Actinomycete isolated from soil. Casuga et al. (2016) found the same activities of triacontane isolated from the Broussonetia luzonica (Blanco) (Moraceae) leaves.

3.2 Bioactive compound detected by LC-MS

In biomedical research, LC-MS has become the most extensively utilized equipment for critical analysis. LC-MS can provide qualitative and quantitative analysis of a wide range of biomolecules in a high-throughput method, making significant advances in biological research (Tsai et al., 2016). Positive ion mode is generally utilized to identify saponins and aldehyde groups, whereas negative ion mode is used to estimate organic acids and OH-containing organic bioactive components (Biswas & Chakraborty, 2013). The present study's data were analyzed using XcaliburTM version 2.2 (Thermo Fisher Scientific, San Jose, CA, USA). The LC-MS results showed that the M. paradisiaca flower ethanolic extract demonstrated the presence of almost 25 compounds and 15 undefined compounds (no name, no molecular mass) based on their retention time, as shown in Table 3. Because most of the extract's contents responded better in negative than in positive mode, the negative ion mode was employed further.

Natural products may possess a broad spectrum of biological activities, including anti-inflammatory, antibacterial, antifungal, and antiviral properties, antiviral, antiallergic, anticarcinogenic, antithrombotic, and vasodilator actions (Soobrattee et al., 2005). Out of 25 detected compounds in M. paradisiaca flower ethanolic extract, four compounds were assumed to exhibit antimicrobial properties based on previous studies. These compounds are hippeastrine, L-(-)-carvone, 4-hydroxybenzaldehyde, and vanillin, as shown in Table 4. Plant-derived chemicals are typically secondary metabolites, with most of those molecules being phenols or their oxygen-substituted counterparts. These secondary metabolites provide a variety of advantages, including antibacterial activity against pathogenic and spoilage microbes in food (Hayek et al., 2013). Plants' antimicrobial action is attributed to numerous chemicals, including phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Ciocan & Băra, 2007). Variations in certain chemicals' structure and chemical makeup cause variances in their antibacterial action (Savoia, 2012). Furthermore, there have been few studies on the structure-function link of these molecules.

Consequently, the significance of the chemical makeup of plant-derived chemicals in terms of antibacterial action has yet to be discovered. Like many other plant-derived chemicals, polyphenolic compounds have a wide range of structural and chemical composition changes and consequently vary in their antibacterial activity against pathogenic microbes (Stojkovi et al., 2013). These phenolic chemicals and other hydrophobic components in essential oils of plant extracts may thus be responsible for antimicrobial activity (Gyawali & Ibrahim, 2014). The structural diversity of plant-derived chemicals is enormous, and the antibacterial properties they generate against microbes are influenced by their structural arrangement. The hydroxyl (-OH) groups of phenolic compounds are thought to have an inhibitory effect (Lai & Roy, 2004) since these groups may interact with microorganism cell membranes, disrupting membrane structures and causing cellular component leakage (Xue et al., 2013). The hydroxyl (-OH) group is one of the active groups that promotes electron delocalization, which subsequently acts as a proton exchanger and reduces the gradient across the cytoplasmic membrane of microbial cells. This will result in the depletion of the ATP pool and the collapse of the proton motive force, eventually leading to cell death (Ultee et al., 2002). Farag et al. (1989) observed in the same context that -OH groups may easily attach to the active site of enzymes by modifying the cell metabolism of microorganisms. This action demonstrates the significance of -OH groups in antibacterial activity.

According to Dorman and Deans (2000), the location of the -OH group determines the antibacterial efficacy of the components. Alcaraz et al. (2000) have established that the -OH group at position 5 of flavanones and flavones is essential for antibacterial action against methicillin-resistant S. aureus strains. A specific mechanism contributed to the instability of the cytoplasmic membrane by bioactive chemicals containing phenolic -OH groups, which function as proton exchangers, lowering the pH gradient across the cytoplasmic membrane and ultimately leading to cell death. Some bioactive compounds, although having a -OH group, do not have a high antibacterial activity owing to the lack of a system of delocalized electrons (double bonds). As a result, the -OH group is prevented from releasing its proton. According to Gochev et al. (2010) the amount of double bonds is essential in antibacterial efficacy. Citronellol was shown to have less activity than geraniol and nerol because of the existence of just one double bond. In comparison, geraniol and nerol with two double bonds revealed more robust antibacterial activity against tested bacteria (B. cereus, E. coli, and S. aureus) and yeast (C. albicans). The antibacterial activity of two isomeric phenolic compounds, eugenol and isoeugenol, has also been shown to vary purely owing to the location of the double bond in the aliphatic side chain. Friedman et al. (2002) found eugenol to be about 13-fold more potent than isoeugenol against Campylobacter jejuni and Listeria.

Griffin et al. (2005) studied the structure-function correlations of terpenes. They discovered that tenuous structural alterations changing only in the location of the -OH group might substantially affect the antibacterial activity of terpenes. Terpinen-4-ol, for example, has been demonstrated to promote K+ leakage from E. coli cells at lower doses than -terpineol. The antibacterial activity of oxygenated terpenes is attributed to their general characteristics and

capacity to produce membrane permeability and K+ leakage. Sokovic et al. (2010) also revealed that oxygenated terpenes found in particle phenols, such as thymol and carvacrol, had more antibacterial activity than hydrocarbon monoterpenes. Stojkovi et al. (2013) found that caffeic acid has better antibacterial activity than p-coumaric acid owing to the presence of one extra -OH group at the phenolic ring of caffeic acid. This discovery was verified by Figueiredo et al. (2008), who showed that adding two or more -OH groups to benzaldehyde derivatives might boost their antibacterial efficacy. Benzaldehydes containing two or more adjacent -OH groups are more active than aldehydes since benzaldehydes are expected to function mainly on the cell's exterior surface, interacting with protein sulphydryl groups (Ramos-Nino et al., 1998; Friedman et al., 2003).

Based on Table 4, hippeastrine had been identified in ethanolic extract of M. paradisiaca flower, where this compound belongs to the alkaloid class of organic compounds. So far, there has been no previous report regarding the detection of hippeastrine in M. paradisiaca flower extract. According to Evidente et al. (2004), this bioactive compound isolated from the bulb of Amaryllis belladonna were reported to possess antifungal property against C. albicans with an inhibition zone diameter of 25 mm using the agar diffusion method; at the same time, it did not show any action against the examined bacteria (E. coli, S. aureus, and P. aeruginosa). While according to Bastida et al. (2006) found that hippeastrine isolated from Narcissus displayed antiviral properties against Herpes simplex type 1. Based on Evidente and Kornienko (2009), hippeastrine was found to possess antiproliferative properties and also apoptosis inducer. In this context, Hippeastrine was moderately efficacious in reducing the in vivo and in vitro proliferation of a range of tumor cells, including Molt 4 lymphoma, HepG2 human hepatoma, LNCaP human prostate cancer, and HT (Ding et al., 2017).

L- (-)-Carvone had also been identified in M. paradisiaca flower ethanolic extract, which belongs to a family of terpenoids. However, there was no previous report regarding detecting L-(-)-Carvone in M. paradisiaca flower extract. According to Aggarwal et al. (2002), L- (-)-Carvone extracted from the oils of Mentha spicata L. and Anethum sowa Roxb. was discovered to have antibacterial activities against human pathogenic bacteria (Enterococcus faecalis, Enterobacter aerogenes, P. aeruginosa, S. Typhimurium, S. mutans, Mycobacterium smegmatis, S. aureus, Y. enterocolitica, K. pneumoniae and B. subtilis) and human pathogenic fungi (Asp. niger, C. albicans, Microsporum gypseum, Sporothrix schenckii, and Trichophyton rubrum). Gharib and Da Silva (2013) demonstrated that the L- (-)- Carvone extracted from the essential oils of marjoram displayed antioxidant activity. Another research carried out by Mannan et al. (2014) found the antidiabetic property of the L- (-)-Carvone isolated from Carum carvi L.

This study also identified the presence of 4-hydroxybenzaldehyde in M. paradisiaca flower ethanolic extract. This molecule belongs to the organic compound class known as hydroxybenzaldehydes. There was no previous report regarding the isolation of 4-hydroxybenzaldehyde from M. paradisiaca flower extract. According to Abbas et al. (2011), 4-hydroxybenzaldehyde derived from a saprophytic perennial plant Gastrodia elata was shown to have antibacterial action against several bacterial and yeast species, as well as anticonvulsive and

antiepileptic characteristics in an in vivo experiment using rats. Based on Tang and Eisenbrand (2013), 4-Hydroxybenzaldehyde was isolated from Gastrodia elata (Tianma) as an active compound used for centuries as a Chinese herbal medication to treat headaches, migraines, neuralgias, and neurological problems. Several types of research have shown that 4-hydroxybenzaldehyde is a viable choice for reducing insulin resistance and lowering cholinesterase (Yu et al., 2010; Park et al., 2011). Vanillin is another compound detected in ethanolic M. paradisiaca flower extract. This compound belongs to phenolic compounds and has not been reported in the M. paradisiaca flower. Vanillin has been studied for its antioxidant, antibacterial, and antimutagenic properties (Peng et al., 2010; Kayaci & Uyar, 2012; Kumar et al., 2012). Monu et al. (2016) also demonstrated the antimicrobial activity of vanillin. According to Widowati et al. (2016), vanillin isolated from Oryza sativa extract has antioxidant activity that can inhibit aging processes. Vanillin has various pharmacological activities, including anticancer, neuroprotective, antibiotic potentiation, and anti-quorum sensing (Bezerra et al., 2016; Li et al., 2018; Arya et al., 2019).

4. Conclusion

The identification of bioactive compounds present in ethanolic extracts of M. paradisiaca flower was carried out using GC-MS and LC-MS. The GC-MS analysis identified 60 compounds and showed the presence of common compounds such as 9,19-Cyclolanostan-3-ol, 24-methylene-, hexadecanoic acid, (Z)-18-octadec-9-enolide, stigmasterol, stigmast-5-en-3-ol, and 1-Heptacosanol. Some of these compounds have previously been found in other plants and are believed to have antimicrobial properties. Meanwhile, the LC-MS analysis revealed 25 compounds in the ethanolic extract of M. paradisiaca flowers. Among these, four compounds were found to possess antimicrobial abilities: hippeastrine, L-(-)-carvone, 4-hydroxybenzaldehyde, and vanillin. These findings provide valuable insights into the potential utilisation of M. paradisiaca flower extracts, particularly their promising role in antimicrobial applications.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Identification of phytochemical compounds in ethanolic M. paradisiaca flower extract by using GC-MS

Sample	Peak No	Compounds name	Molecular formula	Molecular Weight	Area (%)	tR (Min)	SI	RI
Ethanolic crude extract	1	2-Propyn-1-ol (CAS) Propargyl Alcohol	C_3H_4O	56	0.15	3.81	93	773
	2	Propanoic acid, 2-oxo-, methyl ester (CAS) Methyl pyruvate	$C_4H_6O_3$	102	0.06	4.36	95	794
	3	Diethoxymethyl acetate	$C_7H_{14}O_4$	162	0.35	4.71	82	972
	4	Diacetone alcohol	$C_6H_{12}O_2$	116	0.14	5.53	97	838
	5	Phenol, 2,4-bis (1,1-dimethylethyl)- (CAS) 2,4-Di- tert-butylphenol	$C_{14}H_{22}O$	206	0.10	34.15	92	1513
	6	n- Heptadecanol	$C_{17}H_{36}O$	256	0.14	37.40	94	1592
	7	Octadecane (CAS) n-Octadecane	$C_{18}H_{38}$	254	0.09	37.70	91	1599
	8	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.13	43.93	90	1763
	9	1-Heneicosanol	$C_{21}H_{44}O$	312	0.14	45.03	90	1792
	10	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.11	47.51	93	1862
	11	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	0.95	49.73	96	1927
	12	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.45	51.70	93	1985
	13	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.20	52.02	91	1995
	14	9,12-Octadecadienoic acid (Z, Z)-	$C_{19}H_{34}O_2$	294	1.15	55.28	94	2096

Table 1. Continued

		, methyl ester (CAS) Methyl Linoleate						
	15	Methyl linolenate	$C_{19}H_{32}O_2$	292	0.70	55.48	94	2102
	16	Octadecanoic acid, methyl ester (CAS) Methyl stearate	$C_{19}H_{38}O_2$	298	0.13	56.27	93	2128
	17	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	280	17.51	57.15	94	2156
	18	Tricos-(9Z)-ene	$C_{23}H_{46}$	322	0.35	60.68	97	2274
	19	Tricos-(9Z)-ene	$C_{23}H_{46}$	322	0.13	60.87	95	2281
	20	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	$C_{10}H_{21}NO_3$	203	0.16	61.05	89	2287
Ethanolic crude extract	21	Triacontane	$C_{30}H_{50}$	422	0.56	61.44	97	2300
	22	methyl heptadecanoate	$C_{18}H_{36}O_2$	284	0.08	62.27	87	2329
	23	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	0.09	63.07	78	2358
	24	n-Nonacosane	$C_{29}H_{60}$	408	0.07	64.26	90	2399
	25	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213	0.12	65.82	86	2457
	26	1-Heptacosanol	$C_{27}H_{56}O$	396	1.49	66.34	96	2476
	27	Tricos-(9Z)-ene	$C_{23}H_{46}$	322	0.29	66.52	95	2482
	28	Triacontane	$C_{30}H_{62}$	422	1.64	67.03	97	2501
	29	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	0.43	67.31	91	2512
	30	Eicosanoic acid, methyl ester (CAS) arachidic acid methyl ester	$C_{21}H_{42}O_2$	326	0.11	67.81	91	2531

Table 1. Continued

	31	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	0.12	68.36	94	2552
	32	1-Heptacosanol	$C_{27}H_{56}O$	396	2.46	71.60	95	2678
	33	1-Heptacosanol	$C_{27}H_{56}O$	396	0.43	71.78	95	2685
	34	Ethyl linoleate	$C_{20}H_{36}O_2$	308	0.61	71.96	85	2692
	35	Triacontane	$C_{30}H_{62}$	422	1.07	72.16	95	2700
	36	cis-10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	310	0.16	72.36	90	2709
	37	Methyl tetracosanoate	$C_{25}H_{50}O_2$	382	0.11	72.94	90	2732
	38	Squalene	$C_{30}H_{50}$	410	0.14	75.37	93	2833
	39	1-Heptacosanol	$C_{27}H_{56}O$	396	1.59	76.45	95	2878
Ethanolic	40	17-Pentatriacontene	$C_{35}H_{70}$	490	0.52	76.61	94	2885
crude	41	Tetratriacontyl Heptafluorobutyrate	$C_{38}H_{69}F_7O_2$	690	0.47	76.94	94	2899
extract	42	17-Pentatriacontene	$C_{35}H_{70}$	490	115	81.00	94	3080
	43	17-Pentatriacontene	$C_{35}H_{70}$	490	0.18	81.17	86	3087
	44	Cholest-5-en-3-ol (3. beta.)-	$C_{27}H_{46}O$	386	0.35	82.21	86	3135
	45	Cholesta-5,22-dien-3-ol, (3. beta.)	$C_{27}H_{44}O$	384	0.14	84.29	78	3230
	46	Stigmast-5-en-3-ol, (3. beta.)-	$C_{29}H_{50}O$	414	2.38	84.73	86	3250

		(CAS) 24. beta. -ethyl-5. delta. - cholesten-3. beta. -ol						
	47	Stigmasterol	$C_{29}H_{48}O$	412	6.74	85.63	90	3289
	48	Obtusifoliol	$C_{30}H_{50}O$	426	1.19	86.11	78	3310
	49	24,28-methylene-fucosterol;2nd 24,28-diastereomer	$C_{30}H_{50}O$	426	2.23	86.75	74	3338

Table 1. Continued

Ethanolic crude extract	50	Stigmast-5-en-3-ol, (3. beta.)-(CAS) 24. beta. -ethyl-5. delta. - cholesten-3. beta. -ol	$C_{29}H_{50}O$	414	6.71	87.16	87	3356
	51	Fucosterol	$C_{29}H_{48}O$	412	0.94	87.55	86	3373
	52	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	0.77	87.74	80	3381
	53	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	0.86	88.11	85	3397
	54	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	13.62	88.78	84	3426
	55	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	5.82	89.06	85	3439
	56	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	2.34	89.41	85	3454
	57	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	0.86	89.72	84	3467
	58	2,5-cyclohexadiene-1-one, 2,6- bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-	$C_{15}H_{24}O_2$	236	0.43	90.10	71	3484
	59	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	0.45	90.53	89	3503
	60	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-	$C_{31}H_{52}O$	440	1.13	90.94	87	3521

Table 2. Bioactive compounds, which had antimicrobial properties in ethanolic M. paradisiaca flower extract

Peak No.	Peak concentration (%)	Compound name	Similarity index	Molecular weight (g/mol)	Molecular formula	References
12	7.83	hexadecanoic acid	93	256	$C_{16}H_{32}O_2$	Gomathi *et al.* (2015)
32	3.58	1-heptacosanol	96	396	$C_{27}H_{56}O$	Kalsum *et al.* (2016)
9	0.40	1-Heneicosanol	90	312	$C_{21}H_{44}O$	Goda *et al.* (2020)

40	0.93	17-Pentatriacontene	94	490	$C_{35}H_{70}$	Kumar *et al*. (2018)
4	0.67	diacetone alcohol	97	116	$C_6H_{12}O_2$	El Bouchti *et al*. (2021)
31	0.28	diisooctyl phthalate	94	390	$C_{24}H_{38}O_4$	Tyagi and Agarwal (2017)
51	0.98	Fucosterol	86	412	$C_{29}H_{48}O$	Jung *et al*. (2016)
6	0.42	n-heptadecanol	94	256	$C_{17}H_{36}O$	Nautiyal and Dubey (2021)
7	0.28	Octadecane	91	254	$C_{18}H_{38}$	Sasikumar *et al*. (2020)
16	0.36	Methyl Stearate	93	298	$C_{19}H_{38}O_2$	Belakhdar *et al*. (2015)
5	0.31	2,4-di-tert-butylphenol	92	206	$C_{14}H_{22}O$	Elgorban *et al*. (2019)
38	0.35	Squalene	93	410	$C_{30}H_{50}$	Rameshkumar *et al.* (2018)
21	1.38	Triacontane	97	422	$C_{30}H_{50}$	Ibnouf (2021)

Table 3. Identification of phytochemical compounds in ethanolic M. paradisiaca flower extract by using LC-MS

Peak No.	Compound name	Molecular Mass	Molecular Formula	tR (min)	*m/z* value
1	tert-Butyl 3-amino-1-methyl-2,3-dioxopropylcarbamate	216.11	$C_9H_{16}N_2O_4$	0.73	215.11
2	Myristyl sulfate	582.05	$C_{26}H_{19}N_2O_{10}PS$	1.15	581.05
3	Hippeastrine	315.11	$C_{17}H_{17}NO_5$	1.55	314.11
4	L- (-)-Carvone	150.10	$C_{10}H_{14}O$	3.02	149.10
5	Caffeic acid	180.04	$C_9H_8O_4$	2.93	179.04
6	4-Hydroxybenzaldehyde	122.04	$C_7H_6O_2$	3.78	121.04
7	Vanillin	152.05	$C_8H_8O_3$	5.25	151.05
8	Mdmb-Chmica	406.22	$C_{23}H_{32}N_2O_3$	7.04	405.22
9	Ethyl caprate	472.25	$C_{16}H_{41}N_8O_2PS_2$	7.21	471.25
10	(3aR,4S,6R,11aR)-6,9-Dihydroxy-6,10-dimethyl- 3-methylene-2,7-dioxo-2,3,3a,4,5,6,7,8,9,11a-decahydrocyclodeca[b]furan-4-yl methacrylate	346.14	$C_{19}H_{24}O_7$	7.44	345.14
11	Furaneol	128.05	$C_6H_8O_3$	7.75	127.05
12	Tetralin	132.09	$C_{10}H_{12}$	7.79	131.09
13	3,4-Dihydrocadalene	200.16	$C_{15}H_{20}$	7.88	199.16
14	Monobenzone	200.08	$C_{13}H_{12}O_2$	9.34	199.08
15	Apocarotenal	416.31	$C_{30}H_{40}O$	9.61	415.31
16	3-(2-Hydroxy-4-methoxyphenyl)-8,8-dimethyl- 2H,8H-pyrano[2,3-f] chromen-2-one	350.11	$C_{21}H_{18}O_5$	10.01	349.11
17	(+/-)12(13)-Dihome	296.23	$C_{18}H_{34}O_4$	10.72	295.23
18	2-(12-Tridecyn-1-yl) furan	246.20	$C_{17}H_{26}O$	11.07	245.20
19	15-Deoxy-δ12,14 -Prostaglandin J2	338.19	$C_{20}H_{28}O_3$	11.52	337.19
20	4,4'-Diaponeurosporene	402.33	$C_{30}H_{42}$	11.6	401.33
21	1,2,3,4-Tetramethyl-1,3-cyclopentadiene	122.11	C_9H_{14}	13.20	121.11
22	(5c,9c,16c)-17-Hydroxykauran-19-oic acid	458.41	$C_{25}H_{55}N_4OP$	14.09	457.41

23	15-cis-4,4'-diapophytoene	408.38	$C_{30}H_{48}$	15.73	407.38
24	10-[4-(2,4,4-Trimethyl-2-pentenyl) phenoxy]-1-decanol	362.32	$C_{24}H_{42}O_2$	16.04	361.32
25	N-acetyl-L-2-aminoadipic acid	203.08	$C_8H_{13}NO_5$	17.15	202.08

Table 4. Identification of bioactive compounds that have antimicrobial properties in ethanolic M. paradisiaca flower extract using LC-MS

Compound	Retention Time (RT)	Molecular mass	*m/z*	Molecular formula	Activity	References
Hippeastrine	1.55	315.11	1.55	$C_{17}H_{17}NO_5$	Antifungal	Evidente *et al.* 2004; Elgorashi & van Staden, 2009
L- (-)-Carvone	3.02	150.10	3.02	$C_{10}H_{14}O$	Antimicrobial	Aggarwal *et al.*, 2002
4-Hydroxybenzaldehyde	3.78	122.037	3.78	C7H6O2	Antimicrobial	Andersen *et al.*, 1974; Abbas *et al.*, 2011
Vanillin	5.25	152.05	5.25	C8H8O3	Antimicrobial	Shakeel *et al.*, 2015; Ribes *et al.*, 2019