SECOND METABOLITES FROM *Musa paradisiaca* BLOSSOMS EXTRACTS AND THEIR BIOLOGICAL ACTIVITIES

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FEBRUARY 2023

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Final Year Project Report Submitted in Partial Fulfilment of The Requirements for the Degree of Bachelor of Science (Hons.) Chemistry with Management in The Faculty of Applied Sciences Universiti Teknologi MARA

FEBRUARY 2023

This Final Year Report Project entitled **"Second Metabolites from *Musa paradisiaca* Blossoms Extracts and Their Biological Activities"** was submitted by Aina Zalikha binti Zulkipli, in partial fulfillment of the requirement for Degree of Bachelor of Science (Hons.) Chemistry with Management, in the Faculty of Applied Science, and was approved by

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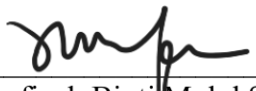

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Date: 27 FEBRUARY 2023

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LIST OF ABBREVIATIONS

^{13}C NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H NMR	:	Proton Nuclear Magnetic Resonance
$AlCl_3$	:	Aluminum Chloride
$CDCl_3$	:	Deuterated chloroform
$CHCl_3$	:	Chloroform
CH_2Cl_2	:	Dichloromethane
DPPH	:	2,2-Diphenyl-1-picrylhydrazyl
DMEM	:	Dulbecco's Modified Eagle's Medium
DMSO	:	Dimethyl sulfoxide
EtOAc	:	Ethyl Acetate
EC	:	*Escherichia coli*
FTIR	:	Fourier Transform Infrared
GC-MS	:	Gas Chromatography-Mass Spectroscopy
Hex	:	Hexane
MBC	:	Minimum Bactericidal Concentration
MIC	:	Minimum Inhibition Concentration
NA	:	Nutrient Agar
NB	:	Nutrient Broth
NMR	:	Nuclear Magnetic Resonance
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

PA	:	*Pseudomonas aeruginosa*
PE	:	Petroleum ether
SA	:	*Staphylococcus aureus*
SP	:	*Streptococcus pyogenes*
TLC	:	Thin Layer Chromatography
TMS	:	Tetramethylsilane
UV	:	Ultraviolet
µg	:	microgram
g	:	gram
mg	:	milligram
mL	:	milliliter
°C	:	degree celsius
%	:	percentage

ABSTRACT

SECOND METABOLITES FROM *Musa paradisiaca* BLOSSOMS EXTRACTS AND THEIR BIOLOGICAL ACTIVITIES

Musa paradisiaca, locally known as "Pisang sekaki", belongs to the Musa family. Previous research revealed many ethnomedical uses for the *Musa paradisiaca* blossoms. However, due to a lack of exposure and knowledge, the blossoms are only used as organic fertilisers on the plantation and are not used for medicinal benefits. There needs to be more research on *Musaceae* species, particularly *Musa paradisiaca* blossoms, in biological and chemical evaluations. As such, this study aims to establish the chemical and biological activities of *M. paradisiaca* blossom from Sarawak to prove the medicinal claims of the old folks. The isolation and identification of the chemical compounds of *M. paradisiaca* blossoms extract were carried out based on various chromatographic and spectroscopic methods. Then, the pure compound will determine its structure using spectroscopic techniques: Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance Spectrometry (NMR), and Fourier-Transform Infrared Spectroscopy (FT-IR). In addition, the antioxidant assay of the pure compound isolated from *M. paradisiaca* blossoms was performed by using radical scavenging assay 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the results exhibited strong activity with 0.376 μg mL^{-1}. The antimicrobial activity of the pure compound exhibited strong activities with MIC and MBC values against four bacteria, *Staphylococcus aureus* (SA), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA) which ranged from 225 to 450 μg/mL. Meanwhile, anticancer activity, which was performed by using an MTT assay, showed that the ethyl acetate pure compound of *M. paradisiaca* was reported to be selectively cytotoxic *in vitro* to DU-145, HeLa, and HaCat cell lines, with IC_{50} values of 1170 μg/mL, 1040 μg/mL, and 1270 μg/mL, respectively. Yet, compared to fluorouracil, a positive control chemotherapeutic medication, the pure compound was more potent than fluorouracil, a positive control chemotherapeutic medication the pure blossoms compound had stronger action. The data from this finding provides further knowledge to the local community and serve as new scientific evidence for further drug development. Furthermore, the data acquired will aid in increasing the crop's commercialisation values, enhancing farmers' income and the future development of the technology-based agro-food sector, and indirectly contributing to the country's economic growth.

ABSTRAK

METABOLIT KEDUA DARIPADA EKSTRAK BUNGA JANTUNG PISANG *Musa paradisiaca* DAN AKTIVITI-AKTIVITI BIOLOGINYA

Musa paradisiaca, yang dikenali sebagai "Pisang sekaki", tergolong dalam keluarga *Musa*. Kajian terdahulu mendedahkan banyak kegunaan etnoperubatan untuk bunga jantung pisang *Musa paradisiaca*. Namun, disebabkan kurangnya pendedahan dan pengetahuan, bunga-bunga itu hanya digunakan sebagai baja organik di ladang dan tidak digunakan untuk faedah perubatan. Perlu ada lebih banyak penyelidikan tentang spesies *Musaceae*, terutamanya bunga *Musa paradisiaca*, dalam penilaian biologi dan kimia. Oleh itu, kajian ini bertujuan untuk mewujudkan aktiviti kimia dan biologi bunga jantung pisang *M. paradisiaca* dari Sarawak untuk membuktikan tuntutan perubatan orang tua. Pengasingan dan pengenalpastian sebatian kimia ekstrak bunga jantung pisang *M. paradisiaca* telah dijalankan berdasarkan pelbagai kaedah kromatografi dan spektroskopi. Kemudian, sebatian tulen akan menentukan strukturnya menggunakan teknik spektroskopi: Gas Spektroskopi Jisim Spektroskopi (GC-MS), Resonan Magnetik Nuklear (NMR), dan Spektroskopi Inframerah Transformasi Fourier (FT-IR). Di samping itu, ujian antioksida bagi sebatian tulen yang diasingkan daripada bunga jantung pisang *M. paradisiaca* dilakukan dengan menggunakan ujian penangkapan radikal 2,2-diphenil-1-picrylhidrazil (DPPH), dan keputusan menunjukkan aktiviti yang kuat dengan 0.376 μg mL^{-1}. Aktiviti antimikrob sebatian tulen menunjukkan aktiviti kuat terhadap empat bakteria, *Staphylococcus aureus* (SA), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC), dan *Pseudomonas aeruginosa* (PA) dengan nilai MIC dan MBC dalam lingkungan antara 225 hingga 450 μg/mL. Sementara itu, aktiviti antikanser, yang dilakukan dengan menggunakan ujian MTT, menunjukkan bahawa sebatian tulen etil asetat *M. paradisiaca* dilaporkan sitotoksik *in vitro* secara terpilih kepada garisan sel DU-145, HeLa, dan HaCat, dengan nilai IC_{50} 1170 μg/mL, 1040 μg/mL, dan 1270 μg/mL, masing-masing. Namun, berbanding dengan fluorourasil, ubat kemoterapi kawalan positif, sebatian tulen adalah lebih kuat daripada fluorourasil, ubat kemoterapi kawalan positif sebatian bunga tulen mempunyai tindakan yang lebih kuat. Data daripada penemuan ini memberikan pengetahuan lanjut kepada masyarakat setempat dan berfungsi sebagai bukti saintifik baharu untuk pembangunan dadah selanjutnya. Tambahan pula, data yang diperoleh akan membantu dalam meningkatkan nilai pengkomersilan tanaman, meningkatkan pendapatan petani dan pembangunan sektor agromakanan masa depan berasaskan teknologi dan secara tidak langsung menyumbang kepada pertumbuhan ekonomi negara.

ACKNOWLEDGEMENTS

First and foremost, Alhamdulillah, thank you to Allah SWT for my good health and well-being and for providing me with the knowledge to accomplish my final year assignment. I would not have been able to undertake this investigation and report if I had been unwell and deprived of information.

I express my sincerest gratitude to my supervisor, Professor Ts. Dr. Khong Heng Yen, for her invaluable expertise, advice, support, and understanding during the project. She is more than a supervisor; she is an inspiration to keep me motivated and believes in my ability to accomplish this project.

I express my sincerest gratitude to my co-supervisor, Associate Professor Korawinwich Boonpisuttinant, and Dr. Siriphatr Chamutpong, the Dean of the Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi for accepting us during the academic attachment programme at Rajamangala University of Technology Thanyaburi Rangsit Center in Thailand. I also thanked Miss Sarinporn Udompong, the researcher assistant who assisted us in doing anticancer research during the 4-weeks academic attachment. The sharing of information will be remembered the most, and the memories will last forever.

I also want to record my heartfelt gratitude to my senior for his direction, fascinating talks, and support during my lab work. My deepest thanks to my seniors, Siti Suhana, Siti Khuzaifah and Mas Atikah for their guidance and encouragement during my lab works. My sincere gratitude goes to laboratory officers, Madam Fathymah Tukijo and Miss Siti Hajijah Binti Ismail, who provided me with technical support.

Not to mention my encouraging friends, Mohd Izzat Arif Bin Nordin, Nurashmeeda Bin Mohd Subari, and Asla Marleena Binti Nazerie, who have shared their expertise, infinite support, and memories with me this year.

Finally, I am grateful to my family and parents for their love, patience, and moral and financial support during my studies.

Aina Zalikha Binti Zulkipli

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nature has been probing chemical space throughout evolution, as evidenced by secondary metabolites or natural products. Natural products (NPs) are the chemicals discovered in microorganisms that play a vital role in metabolic reactions. They are assemblages of small-molecule metabolites produced by organisms through primary and secondary metabolisms (Shen and Hao, 2020). Secondary metabolism can be defined as the molecules that are not required for the host's survival in the laboratory but provides a specific benefit to the host in its natural environment (Katz and Baltz, 2016).

According to (Grothaus *et al.,* 2010), humankind has relied on Nature, particularly plants, for medicines to heal various ailments for most of history. Biologists started to use natural products as new technologies and methods to intrude with cellular functions in the mid-twentieth century. Nowadays, many professions, from chemistry to ecology, clearly assert that natural products are a part of their discipline. Many chemicals produced from plant sources have long been known to have interesting pharmacological properties. These plants have historically yielded many effective medications for human use. Natural products remain a substantial

and viable source of lead compounds in many drug discoveries and development.

1.1.1 Genus of *Musa*

The banana (*Musa spp*.) is one of the most important tropical fruits. Because of their high nutritional values, bananas can serve as a complete meal for people from all socioeconomic backgrounds. The *Musaceae* family comprises three genera: *Ensete*, *Musa,* and *Musella*, which can be found in damp tropical lowlands, while some species have lately been discovered at higher latitudes. *Musa* is the largest genus in the *Musaceae* family (Sulistyaningsih, 2016). The genus of the wet tropical world is the world's fourth most cultivated food crop. They vary in size and colour of the fruit and are elongated and curved, with soft flesh full of starch covered by a peel that can be green, yellow, red, purple, or brown when ripe. Almost all edible-fruit cultivars are descended from two diploid species, *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Emaga *et al.,* 2007). Elayabalan *et al.* (2017) stated that Carl Linnaeus was the first to divide bananas into two species based on their food uses: *M. sapientum* for dessert bananas and *M. paradisiaca* for plantains.

1.1.2 Characterisations of banana plant

Musa species are perennial herbaceous plants, attributable to their rigorous, hard, fibrous pseudo stems made of overlapping bases of the big, spirally arranged leaves (8 to 20 per plant). The prime stem generates a single

massive terminal inflorescence in the form of a spike. The spike is composed of pistillate (female) flowers at the bottom and staminate (male) flowers at the top. This spike later matures into a cluster of bananas, usually 6 to 9 clusters of 10 to 25 bananas each. A single flowering procedure results in the finalisation of the main stem, which dies, allowing new stems to emerge from the rhizome/corm beneath the soil (Robinson and Saúco, 2010).

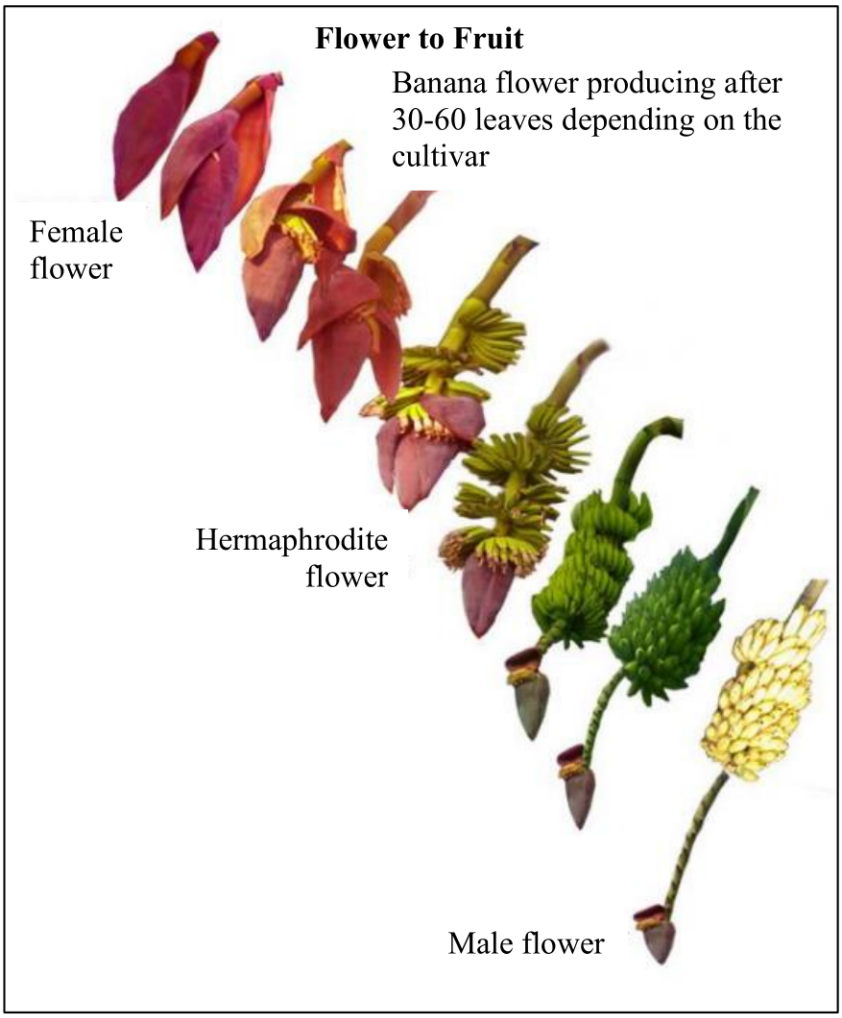

Figure 1.1 Banana development stage from inflorescence to fruit
Source: Elayabalan *et al.* (2017)

Banana inflorescence sprouts from the heart of the pseudostem, where it starts as a bulky, long-oval, narrowing purple bud, and later the slender, tube-like, white flower opens slowly. Double rows of whorls are grouped around the floral stalk. Each cluster sheathes a chunky, hood-like bract with purple on the outside and dark red on the inside. Figure 1.1 shows the development stage from inflorescence to fruit. The inflorescence grows as the flower's rachis fills with sterile flowers with abortive male and female parts. On elongation, normal staminate ones with abortive ovaries form later. The two newly formed flower types eventually dry and die almost in all edible cultivars. Parthenocarpic (self-pollinated) fruits are formed from the ovaries found in the first female flowers of the fruit clusters known as the hands.

1.2 Ethnobotany uses of *Musa paradisiaca*

Musa paradisiaca, locally known as "Pisang sekaki", shows various benefits for consumers, particularly their health. Different parts of *Musa spp.*, including roots, fruits, and flowers, have been employed in African, American, Indian, and Asian folk medicine traditions. Potassium is an essential element of cells and body fluids that helps support muscles and nerves. It regulates heart rate and blood pressure and mitigates the adverse effects of sodium. Magnesium is necessary for strong bones and plays a protective role in the heart. The body uses manganese as a cofactor for the antioxidant enzyme superoxide dismutase. Copper is needed to form red blood cells (Rajesh, 2017). Other advantages include fastened wound healing, lower blood glucose levels, and treatment for heavy menstrual

bleeding. *M. paradisiaca* spread worldwide after travelling from its native home in the Southwestern Pacific to India around 600 BC. Different parts of *M. paradisiaca* L., such as fruits, leaves, peels, roots, and stalks, have been consumed orally or applied topically to treat various diseases (Jawla *et al.*, 2012). Jyothirmayi *et al.* (2014) highlight that those young leaves are used as poultices for burns and other skin diseases, while blossom extracts are used to cure bronchitis, diarrhoea, and ulcers. The astringent ashes of the unripe peel and leaves are used to treat dysentery and malignant ulcers; roots have long been used to treat digestive problems, diarrhoea and other diseases; and seed mucilage is used to treat ocular cataracts and diarrhoea. Stem juice can also treat nerve disorders such as epilepsy, hysteria and dysentery. The root extract prevents pregnancy, activates labour during childbirth, and treats sexually transmitted infections such as HIV/AIDS, genital ulcers, vaginitis, and leucorrhoea (Mathew and Negi, 2017). The fruit provides enough folic acid during pregnancy to prevent nutritional megaloblastic anaemia (Loganayaki *et al.,* 2010).

Previous studies revealed that *M. paradisiaca* has antimicrobial and antioxidant properties. The phenolic compounds in Musaceae species, such as flavonoids and anthocyanins, are abundant. There are many ethnomedical uses for the blossoms. Traditionally, banana blossoms have been used by the old generation to treat disease problems such as diabetes or asthma. Because of the presence of phenols and abundant fibres, *Musa* flower buds are grown and consumed in folk medicine (Lau *et al.,* 2020). However, due

to a lack of exposure and knowledge, the blossoms are only used as organic fertilisers on the plantation and are not used for medicinal benefits. Hence, banana farms generate tons of underutilised by-products and waste because most edible bananas are grown primarily for their fruits. The waste produced by a single banana plant can account for up to 80% of the total plant mass (Padam *et al.,* 2014). Recycling agricultural waste and massive by-products provide an excellent supply of highly valued raw materials, avoiding losing untapped biomass and environmental difficulties.

In addition, there is insufficient research on *Musaceae* species, particularly *Musa paradisiaca* blossoms, in biological and chemical evaluations. Sarawak also has a scarcity of scientific data on the banana floret on *Musa spp.* It is due to previous research focusing solely on plant parts such as peels, pulps, and leaves. Thus, this research aims to determine the phytochemical and biological studies of *M. paradisiaca* on their blossoms to provide additional scientific data about this plant.

1.3 Problem statement

Previous studies revealed that *M. paradisiaca* has antimicrobial and antioxidant properties. The phenolic compounds in Musaceae species, such as flavonoids and anthocyanins, are abundant. There are many ethnomedical uses for the blossoms. Traditionally, banana blossoms have been used by the old generation to treat disease problems such as diabetes or asthma. Because of the presence of phenols and abundant fibres, *Musa* flower buds

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1.4 Objectives of study

This study aims to establish the chemical and biological activities of *M. paradisiaca* blossoms from Sarawak to prove the medicinal claims of the old folks. The specific objectives of the study are

1. To isolate the chemical compound(s) of *M. paradisiaca* blossoms extracts based on various chromatographic methods.
2. To elucidate the structure of the isolated pure compound(s) based on

spectroscopic techniques.

3. To determine the antioxidant, antimicrobial, and anticancer activities of the isolates from *M. paradisiaca.*

1.5 Significance of study

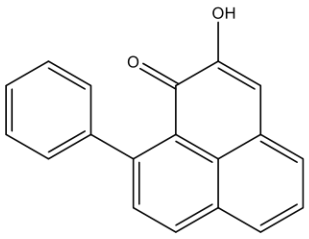
This study provides new scientific evidence for further investigation, which is critical for the plant's potential usage as a medication. This additional data is essential to establish medicinal plants' therapeutic characteristics, traditional knowledge, and applications. Furthermore, the data acquired will aid in increasing the crop's commercialisation values, which will enhance farmers' income and the future development of the technology-based agro-food sector, which is in line with the National Agrofood Policy 2030 and indirectly contribute to the country's economic growth.

CHAPTER 2

LITERATURE REVIEW

2.1 Phytochemical constituents of the *Musa* genus

A review of the *Musa* genus has been reported by Kumar *et al.* (2014) on the *Musa* genus. Still, more focus is on the phytochemical and biological properties of *Musa acuminata* pseudostem, whereas Deka *et al.* (2018) reviewed the *Musa Balbisiana Colla.* Previously, the *Musa* genus has shown the presence of a rich diversity of phytochemicals such as saponins, flavonoids, terpenoids, steroids, anthocyanins, fatty acids, tannins, phenols, and alkaloids. Several *Musa* species have revealed different bioactive compounds, namely, anigorufone **(1)**, apigenin glycosides **(2)**, β-sitosterol **(3)**, cycloartenol **(4)**, campesteryl glucoside **(5)**, delphinidin-3-rutinoside **(6)**, quercetin (**7**), stigmasterol **(8)**, catechin **(9)** and gallocatechin **(10)**.



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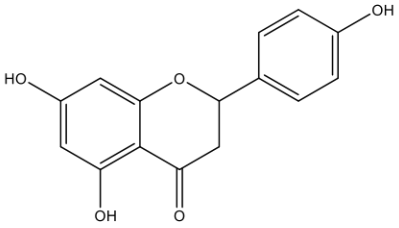

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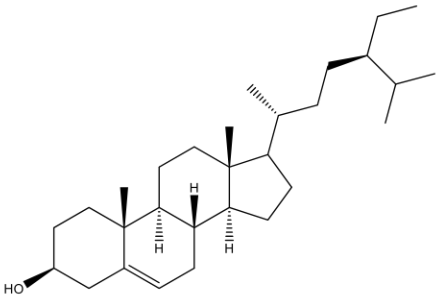
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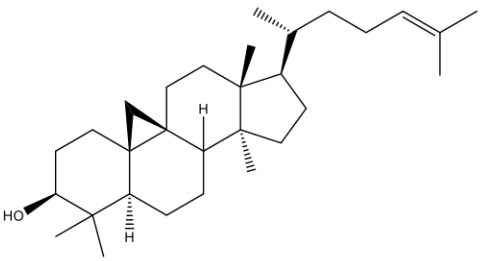
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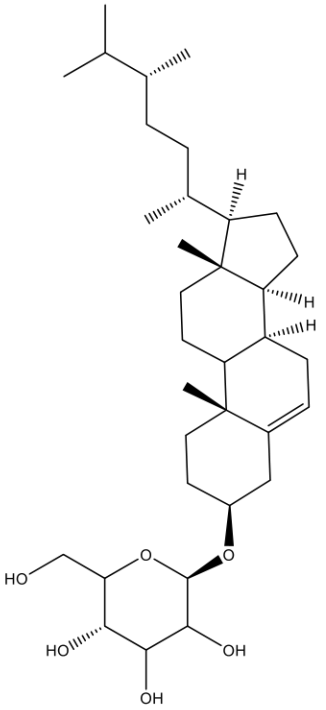
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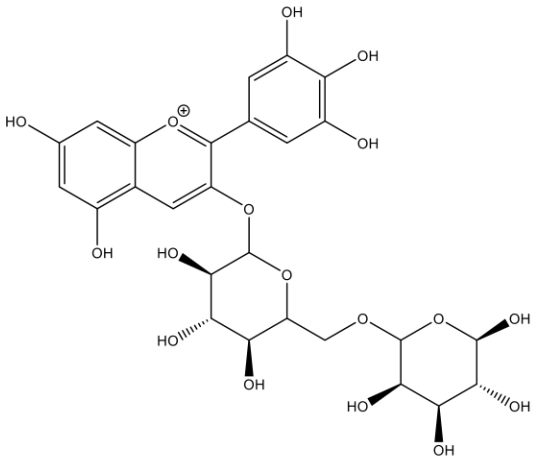
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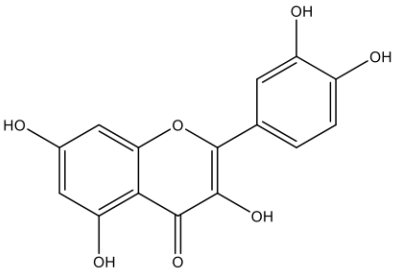
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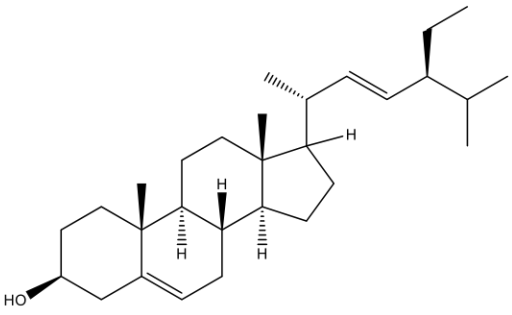
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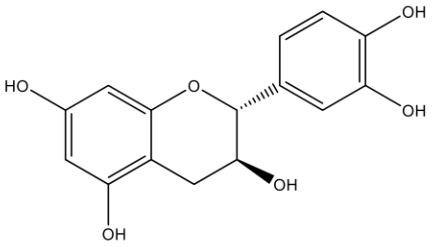

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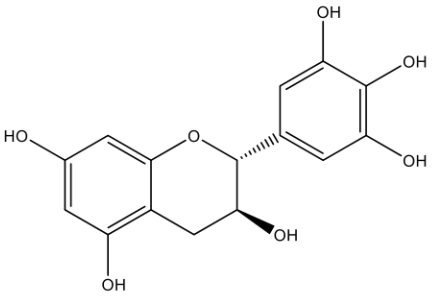
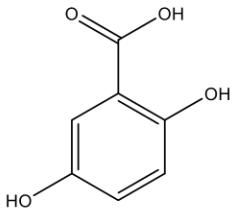


(9)

From the pseudostem of various banana cultivars, the phenolic acids gentisic acid **(11)**, protocatechuic acid **(12)**, (+)-catechin (2R-3S) **(13)**, ferulic acid **(14)**, cinnamic acid **(15)**, and caffeic acid **(16)** were isolated.



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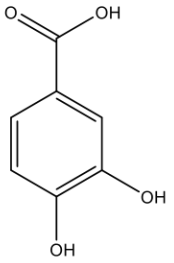

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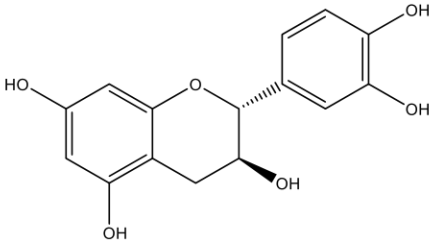

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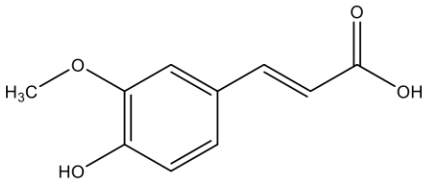

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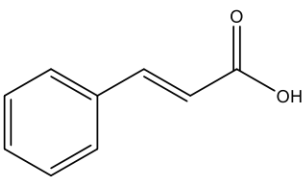

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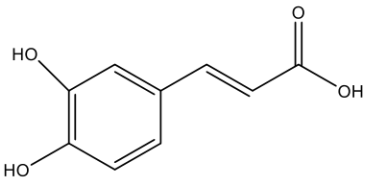


(16)

Elayabalan *et al.* (2017) highlight that pectin, a hydrocolloid, can relieve constipation by stabilising and lubricating intestine movement. The presence of higher levels of fructooligosaccharide, which acts as a prebiotic, nourishes the intestinal flora, allowing it to produce valuable vitamins and enzymes. The fruit's carotenoid content has significant antioxidant effects to defend against vitamin A deficiency, which causes night blindness and other diseases. It is also an excellent source of vitamin B6 (pyridoxine). Pyridoxine is an essential B-complex vitamin. It is effective for the treatment of neuritis and anaemia. Furthermore, it aids in the reduction of homocysteine (one of the potential causes of coronary artery disease (CHD) and stroke episodes) levels within the body (Rajesh, 2017).

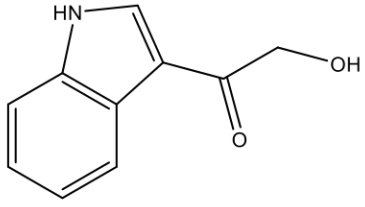
Banana contains a variety of aliphatic and aromatic amines. Active amines such as dopamine and serotonin are derived from tyramine and tryptophan. Their presence in these fruits may directly impact their concentration levels in human serum (Elayabalan *et al.,* 2017). In terms of aromatic compounds, isopentyl acetate is the distinctive aromatic compound of bananas. Pentanol esters, like propionic, acetic, and butyric acid esters, have been believed to lead to the different aromas of bananas. Simultaneously, the esters of butanol and hexanol with acetic and butyric acids have a fruitier flavour. The scent of bananas may change when heated due to the release of glycosidic precursors, water addition, oxidation, and cyclisation of chemical components. The carbohydrate content of bananas has been

investigated. Monosaccharides occur only in sporadic amounts in addition to glucose (3.5% of the edible portion) and fructose (5.7% of the edible part). Maltose appears in tiny quantities in bananas and saccharose, where sucrose is 2.4% of the edible portion, the dominant oligosaccharide. Ripe bananas have been found to contain 6-kestose.

Isoamyl acetate, also known as banana oil, is the main chemical component responsible for the distinctive fragrance of fresh fruits. Butyl alcohol and isobutyl alcohol also play a vital role in the pleasant flavour of banana cultivars/varieties. (Idachaba and Onyezili, 1994). Ripening stimulates the formation of ethylene gas, a plant hormone that indirectly influences the fruit's flavour. Ethylene stimulates the enzyme amylase, breaking starch into sugar, and giving the pulp a sweet taste. Ethylene production also starts the synthesis of the enzyme that softens the tissues. (Elayabalan *et al.,* 2017).

Carotenoids are naturally present in significant amounts in all fruits and are the primary factors responsible for fruit colour. Bananas are categorised as fruits that contain little carotenoid content. HPLC could quickly analyse the pattern of distribution of carotenoids. Carotenoids are classified according to their structures, and banana consists of beta-carotene (VII) and lutein (IX). Malic acids are prevalent among organic acids in bananas. They are present in 4 milli-equivalents per 100 g fresh weight of the banana pulp. The edible portion of the banana contains 7-21 mg/100 g of vitamin C.

A study revealed that 17 significant compounds were detected in petroleum ether and (PE) and ethyl acetate (EtOAc) extract of *Musa nana* flower buds. The results revealed that 3-(hydroxyacetyl)-indole **(17)**, bi-indol-3-yl **(18)**, 5-[(1R)-1-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]-3-methyl-(2Z, 4E)-2,4-penta dienoic acid (Valdes) **(19)**, 5,6(S),7,7a(R)-tetrahydro-6-hydroxy-4,4-dimethyl-2(4H)-benzofuranone **(20)**, 2R,4R)-3,4-dihydro-4-methoxy-2-methyl-2H-1-benzopyran-5-ol **(21)**, alternariol 4, 10-dimethyl ether **(22)**, 1,1′-oxybis[2,5-bis(1,1-dimethylethyl)-(9CI)-benzene **(23)**, 2,4-dichloro-benzoic acid **(24)**, 3-(4-hydroxyphenyl) -2-propenoic acid **(25)**, 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid **(26)**, 4-hydroxy-3-methoxy-benzaldehyde **(27)**, 2-hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one **(28)**, 2-methoxy-9-phenyl-1H-phenalen-1-one **(29)**, 2-methoxy-9-(4-methoxyphenyl)-1H-phenalen-1-one **(30)**, 9-(Z)-eicosenoic acid, 2,3-dihydroxypropyl ester **(31)**, 5-hydroxy-3,4-dimethyl-5-pentyl-(5S)-2(5H)-furanone **(32)**, 9,12,15-octadecatrienoic acid **(33)** were found as the major compounds in the PE and EtAOc extract of the flowers of *M. nana* (Tang *et al.* , 2021). Table 2 lists the known bioactive compounds isolated from banana by-products and their respective nutraceutical properties (Padam *et al.*, 2014).



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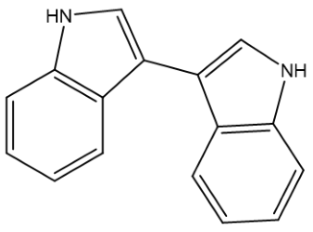

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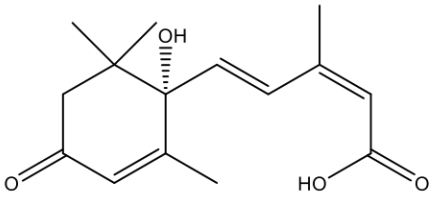

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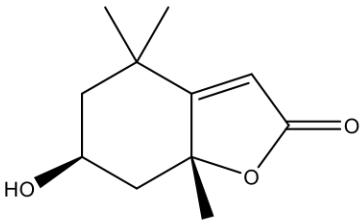

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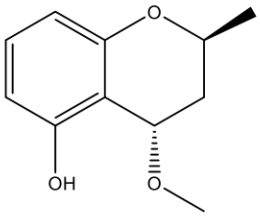

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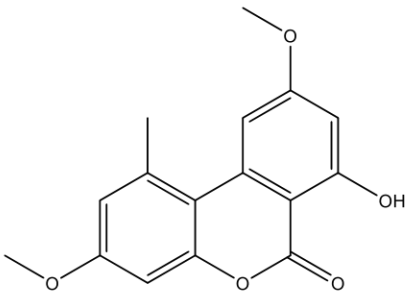

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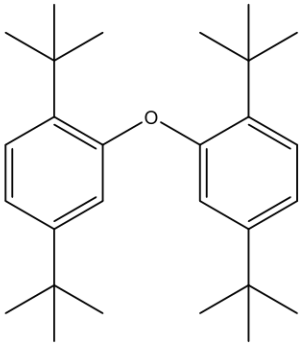

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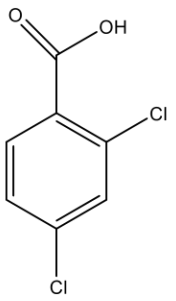

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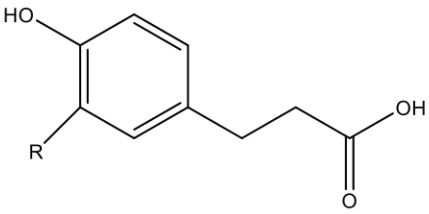

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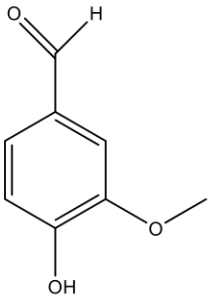
(26) R=OCH_3



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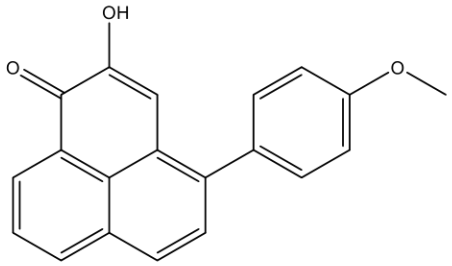

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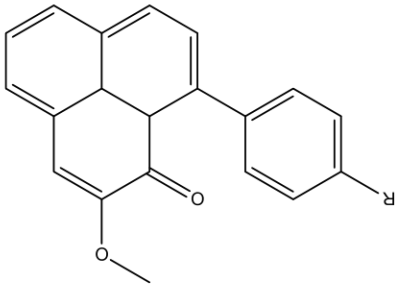
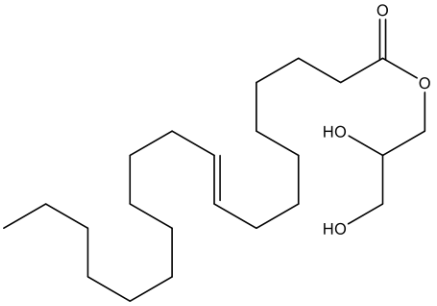


(29) R=H

(30) R=OCH_3



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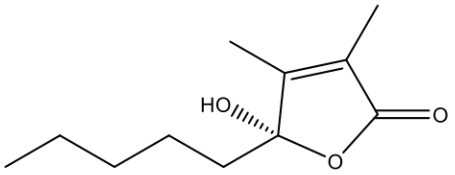

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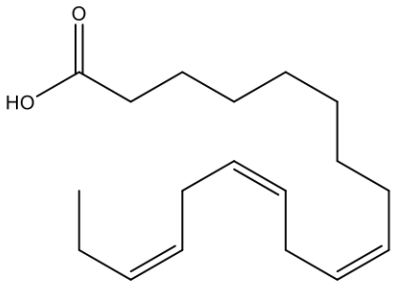

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Table 2.1 Bioactive compounds derived from banana/plantain by-products as potential nutraceuticals

Bioactive compounds	Banana by-products (Species/variety)	Bioactivity	References
Cyanidin-3-rutinoside	Bracts (*M. paradisiaca*)	Antioxidant, Anticancer	Roobha *et al.* (2011)
Epigallocatechin and derivatives	Male flower (*M. paradisiaca*)	Antibacterial, Antioxidant	Tin *et al.* (2008); Tin *et al.* (2010)
ß-sitosterol, malic acid, succinic acid, palmitic acid, 12-hydroxy stearic acid, glycoside	Peels (*M. acuminata* cv. Cavendish)	Anti-inflammatory, Anti-cholesterol, Antioxidants, Antibacterial	Mokbel and Hashinaga (2005)
Campesteryl 3-β-d-glucopyranoside, stigmasteryl 3-β-d-glucopyranoside and sitosteryl 3-β-d-glucopyranoside	Petioles, leaves, floral stalk (*M. acuminata* cv. Cavendish)	Anti-inflammatory, Anti-cholesterol	Oliveira *et al.* (2005)
Sterols, steryl glucosides, steril esters, tocopherols, phenolic compounds	Peels and pulp (*M. acuminata* cv. Dwarf Cavendish)	Anti-inflammatory, Anti-cholesterol, Antioxidants	Oliveira *et al.* (2008)
Putrescine, spermidine, serotonin, dopamine, tyramine, spermine	Pulp (*M. acuminata* x *M. balbisiana*)	Stimulants	Adão and Gloria (2005); Lima *et al.* (2008)
Entisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid	Pseudostem (*M. acuminate* x balbisiana cv. Nanjanagudu Rasabale)	Antioxidants	Saravanan and Aradhya (2011)
Anthocyanins, catecholamines, tocopherols, phytosterols, ascorbic acid	Peel (*M. acuminata* Colla AAA)	Antioxidants	González-Montelongo *et al.* (2010)

Source: Padam *et al.* (2014)

2.2 Phytochemical constituents of *Musa paradisiaca*

Sujithra and Manikkandan (2019) discovered alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, cyclogylcosides, total phenols, and steroids in the preliminary qualitative analysis of phytochemicals of *M. paradisiaca*. Alkaloids and glycosides were present in the petroleum ether extract bract but not in the chloroform extract. The ethanol extract contained alkaloids, saponins, flavonoids, terpenoids, coumarins, glycosides, phenols, and steroids. Only coumarins and phenols were present in the aqueous extract. All extracts were absent from other phytoconstituents, such as quinones, steroids, and phlobotannins. Table 3 shows the elemental composition of the different parts of banana plant fraction ashes (Rajesh, 2017). Table 4 depicts the qualitative analysis of *Musa paradisiaca* L. flower extract bract, and Table 5 of its quantitative analysis, including alkaloid, total phenolic, saponin, tannin, and flavonoid (Sujithra and Manikkandan, 2019).

Table 2.2 The elemental composition of banana plant fraction ashes (% of ash content)

Element	Petioles/midrib	Leaf blades	Floral stalk	Leaf-sheaths	Rachis
Si	7.0	24.9	7.8	2.7	1.2
Ca	32.3	8.0	0.6	5.5	0.6
K	9.4	11.6	23.1	21.4	28.0
P	0.7	0.7	0.7	0.9	1.7
Mg	2.9	1.1	0.5	1.9	0.3

Source: Rajesh (2017)

Table 2.3 Phytochemicals content present in *M. paradisiaca* flower bract extracts

Phytochemicals content	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Carbohydrates	-	-	-	-
Reducing sugar	-	-	-	-
Alkaloids	+	-	+	-
Saponins	-	-	+	-
Tannins	-	-	++	-
Flavonoids	-	-	++	+
Terpenoids	-	-	+	-
Phlobotannins	-	-	-	-
Coumarins	-	-	+	+
Cycloglycosides	+	-	+	-
Total phenols	-	-	+	+
Quinones	-	-	-	-
Anthraquinones	-	-	-	-
Steroids	-	-	+	-

Source: Sujithra and Manikkandan (2019)

Table 2.4 Phytochemicals content of *M. paradisiaca* flower bract

Phytochemicals Content	Mean Concentration
Alkaloid (g/100 g)	1.16 ± 0.22
Saponin (g/100 g)	1.13 ± 0.14
Total phenolic (g/100g)	4.62 ± 0.78
Tannin (mg/100 g)	68.37 ± 4.53
Flavanoid (mg/100 g)	4.08 ± 0.15

Source: Sujithra and Manikkandan (2019)

2.3 Biological activities of *Musa paradisiaca* species

M. paradisiaca exhibits various biological activities, such as antimicrobial and antioxidant activity.

2.3.1 Antimicrobial activity

Several studies have found that flavonoids are responsible for some ethnomedicinal plants' antimicrobial activity (Table 2.5). Hexane extract of *M. paradisiaca* and *M. acuminata* leaves showed antimicrobial activity was effective against *S. aureus*, *Citrobacter sp.*, *E. aerogenes*, *P. aeruginosa* and *P. mirabilis* with MIC values of 15.63 to 250.00 µg/mL (Karuppiah and Mustaffa, 2013). The highest inhibition zone was observed on the ethyl acetate extract of *M. paradisiaca* against *E. coli* (18.6±0.5 mm) and *P. aeruginosa* (16.4±0.6 mm). Another study by Jawla *et al.* (2012) showed that ethanol extract of *M. paradisiaca* flower showed antimicrobial activity against *Bacillus species* and *P. aeruginosa,* where the MIC values are 5.93, 6.82, and 7.95 g/mL. The MICs of EtOH and EtOH: water (1:1) extracts were 5.62-25.81 and 7.60-31.58 g/mL, respectively, as shown in Table 2. With the MICs values obtained, EtOH extract was highly effective against *P. aeruginosa, B. subtilis,* and *B. cereus*. Both extracts inhibited *C. albicans* and *Cryptococcus albidus* effectively. The EtOH and EtOH: water (1:1) extracts demonstrated antimicrobial activity against Gram-positive and Gram-negative organisms and fungi.

Table 2.5 The antimicrobial activity of extracts of *M. paradisiaca* flowers using disc diffusion assay (n=3).

Microorganism	Zone of inhibition (mm)			
	A	C	**Amik**	**Clotr**
B. cereus (MTCC-430)	13.7±0.8	10.3±0.5	16.9± 0.6	NC
B. subtilis (MTCC-121)	11.9 ± 0.4	13.2±0.8	13.7± 1.4	NC
E. coli (MTCC- 443)	3.6±0.5	6.7±1.3	2.7±0.8	NC
K. pneumonia (MTCC-109	5.2±0.7	5.9±1.3	6.4±0.5	NC
P. mirabilis (MTCC-1429)	3.4±0.9	5.6±1.2	3.2±1.4	NC
P. aeruginosa (ATCC-9027)	11.1±0.9	9.4±1.7	12.8± 1.1	NC
S. typhimurium (MTCC-98)	1.3±1.6	1.4±0.6	13.3± 0.2	NC
S. aureus (ATCC-25923)	7.3±1.8	5.1±0.8	13.8± 0.3	NC
S. pneumonia (MTCC-2672)	4.7±1.3	1.6±1.2	4.7±0.9	NC
C. albicans (MTCC-183)	8.3±0.7	1.5±0.8	NC	12.9± 1.2
C. albidus (MTCC-2661)	5.5±1.2	3.8±2.6	NC	13.5± 0.8

Each value represents mean ± SEM; A - EtOH extract; B - Ethanol: water (1:1) extract; Amik - Amikacin; Clotr – Clotrimazole and as NC - Not Carried.
Source: Jawla *et al.* (2012)

A previous study by Sivasamugham *et al.* (2021) reported that *M. paradisiaca* ethyl acetate extract demonstrated antibacterial activity against multidrug-resistant nosocomial pathogen isolates of MRSA and MSSA, with MSSA serving as the control group. Methicillin-resistant *Staphylococcus aureus* (MRSA) and MSSA are two strains of *Staphylococcus aureus* (or staph). MSSA isolates had substantially lower antibiotic resistance rates and a slightly lower prevalence of virulence genes than MRSA isolates. If the 30-day mortality rate of patients with MSSA BSI remains high, MSSA infections occur. MRSA strains are far more dangerous than MSSA strains. Compared to the negative control,

80% ethanol, the plant's ethanolic leaf extract had substantially bigger inhibition zones ($p \leq 0.05$) against the three strains of MRSA. The diameters of the inhibition zones show that the extracts had the same antibacterial effect as clindamycin, an antibiotic used to treat MRSA-related diseases. Although the phytochemical assays were qualitative, identifying various compounds such as sanguinarine, myricetin, datiscetin, kaempferol, quercetin, and flavones could be the cause of the MRSA inhibitory effects. Sanguinarine is an alkaloid that can disrupt MRSA's cytoplasmic membrane (Kumar and Chopra, 2013). One of the primary reasons for the antibacterial activity against MRSA isolates could be the collective mode of the effect of various phytochemicals extracted using 80% ethanol. Using a different solvent, such as ethyl acetate, methanol, or hexane, may improve the extraction of the phytochemicals needed to inhibit MRSA.

2.3.2 Antioxidant activity

The antioxidant capacity of *M. paradisiaca* could play a significant role in wound healing due to tannins, saponins, and alkaloids (Padilla-Camberos *et al.*, 2016). Ghany *et al.* (2019) revealed that the antioxidant activity of exudate was determined in triplicate using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. With increasing exudate concentrations, the DPPH scavenging % increased from 14.52 at one µL to 89.19 at 128 µL. The lower the value of IC_{50} (2.2 µL), the greater the antioxidant activity since this yields a significant concentration of antioxidants needed to minimise the free radical DPPH by

half. The antioxidant activity reported by Ghany *et al.* (2019) was high for *M. paradisiaca* flowers, which had IC_{50} values of 0.77 mg/mL (Table 2.6).

Table 2.6 Antioxidant activity of *M. paradisiaca* L. pseudostem exudate using DPPH scavenging

Exudate concentration (µL)	DPPH scavenging %
128	89.19
64	84.19
32	79.52
16	75.48
8	71.13
4	60.32
2	48.71
1	14.52
0	0
IC_{50}	2.2 µL

Source: Ghany *et al.* (2019)

2.3.3 Anticancer activity

One of the most feared diseases of the 20th century, cancer is still spreading and becoming more common in the 21st. As the top cause of death in the country, it is currently responsible for 25% of all human deaths (Talaviya, 2011). Although this disease is the subject of numerous interdisciplinary scientific studies, there is yet no surefire, flawless treatment that has been introduced into mainstream medicine. Numerous studies on herbs have been done for a variety of ethnobotanical reasons. The potential offered by natural products may help fight against cancer, the second leading cause of death worldwide, as many organic products have been linked to anticancer properties.

M. paradisiaca blossoms can be an excellent natural source for developing an anticancer lead chemical with few side effects. According to

Ghany *et al.* (2019), the antitumor activity of the exudate demonstrates that it is an excellent anticancer source, with IC_{50} values of 29.4 µl and 29.4 µl for HepG-2 and HCT-116 cell lines, respectively. Exudate at 100 µl had the most significant cytotoxicity against HCT-116 and HepG-2 cell lines, inhibiting them by 86.53% and 79.06%, respectively. On the cervical cancer cell line HeLa, banana flower extract was tested for anticancer efficacy, according to Timsina and Nadumane (2014). The percentage viability dropped as the extract's exposure duration grew from 24 to 48 to 72 hours. The percentage vitality of 24-hour treated HeLa cells was 91% at a concentration of 5 µg/mL of the extract. However, it dropped to 50.0% after 72 hours. The percentage viability of HeLa cells reduced to 38% as the concentration increased from 5 to 20 µg/mL. When CHO cells were treated with an ethanol extract from the banana flower, we observed a drop in percentage viability with increasing concentration. After 72 hours of treatment at a concentration of 20 µg/mL, the percentage viability was 60.0%, compared to 86% after 24 hours. Thus, the ethanol extract had significant cytotoxicity to HeLa cells with an IC_{50} of 20 µg/mL.

Furthermore, another finding by Deep *et al.* (2020) showed that the extract's inhibitory activity by MTT assay was concentration-dependent and that increasing the concentration of extract results in greater inhibition efficiency. At 320 µg/ml, the most significant suppression of cell growth was seen which was 54.35 and 55.97 for the HeLa and A375 cell lines, respectively. As a result, a 320 µg/ml concentration of the extract was used

for further research, and the IC_{50} values were discovered to be 249.1 and 224.4, respectively. Ariffin *et al.* (2021) also discovered that the ethyl acetate fraction demonstrated considerable cytotoxicity against DU-145 malignant cells, with an inhibitory concentration IC_{50} value of 37.94 µg/ml, comparable to the commercial chemotherapeutic medication, 5-fluorouracil (IC_{50} = 32.50 µg/ml).

CHAPTER 3

METHODOLOGY

3.1 Experimental procedures

The phytochemical study on *M. paradisiaca* blossoms extract was performed using various chromatographic and spectroscopic methods. The biological activities such as antimicrobial, antioxidant, and anticancer were assayed based on standard protocols as described in Figure 3.1.

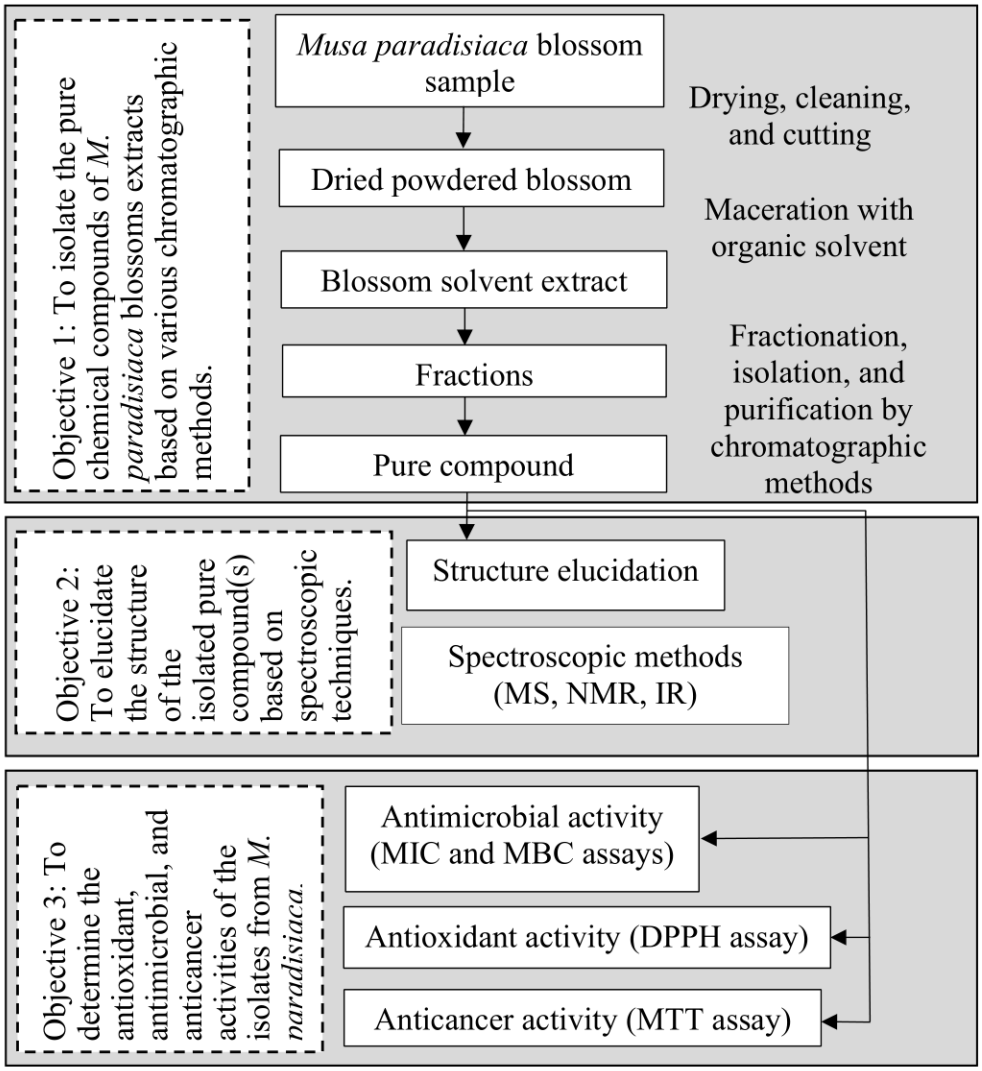


Figure 3.1 Flowchart of experimental procedures

3.2 Plant materials

The blossoms of *M. paradisiaca* were collected from Banana Tree Sdn Bhd, Kuching, Sarawak, Malaysia. The plant specimen was deposited at the Faculty of Applied Science, UiTM Sarawak Branch, Samarahan 2 Campus.

3.3 Preparation of crude extracts

The preparation of crude extracts was determined, as reported initially by Ariffin *et al.* (2021). The blossoms of *M. paradisiaca* were air-dried at room temperature at 27°C to a constant weight. The dried plant materials were grinded using a commercial blender. After grinding, the powdered *M. paradisiaca* florets sample was macerated in hexane for 24 hours at room temperature. To remove the solvent, the filtrate was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated in vacuo using rotavapor (BUCHI, R-200, Switzerland). The extraction procedure was repeated with different solvents, including chloroform, ethyl acetate, and methanol. All extracts were adequately sealed in an airtight container and stored at room temperature before being tested chemically and biologically.

3.4 Fractionation, isolation and purification

The fractionation, isolation, and purification processes used chromatographic methods such as thin layer chromatography (TLC), radial chromatography (Chromatotron), and liquid vacuum chromatography subjected to the TLC profile of fraction. TLC was used to determine the best solvent system for these processes. Meanwhile, a chromatotron was used to

perform the fractionation, isolation, and purification of the *M. paradisiaca* leaves extract. Different solvent polarities were used as the eluents, for example, hexane: ethyl acetate.

3.4.1 Thin layer chromatography (TLC)

TLC was used to examine the purity of chemical compounds on each fraction collected from the isolation. TLC plates with dimensions of 40 mm × 80 mm × 0.25 mm aluminium sheets coated with silica gel 60 F254 (Merck 1.05735) were used. TLC plates were measured with 5 cm × 5 cm lines as a base and a front line, and the bottom and upper were marked with 0.5 cm lines as a base and a front line. The sample dissolved in acetone was spotted onto the TLC plate using a microcapillary tube. The plate was placed in a solvent mixture chamber and pre-saturated with solvent vapour.

Because of capillary action, the mobile phase (solvent) migrated up the plate through the stationary phase (silica sorbent). A non-polar compound in a less polar solvent system had a higher retention factor, R_f. This is because non-polar compounds migrate faster to the front line. After all, they have a lower affinity for the stationary phase. In contrast, the polar compound was firmly held on the stationary phase and slowly migrated to the front line as a solvent migrated. As a result, the compound had a lower R_f. To detect the presence of natural compounds, the spots were used to examine under a UV lamp with both short (254 nm) and long wave (365 nm) wavelengths. Rf, the retention factor value, was computed as follows:

$$\text{Retention factor, } R_f = \frac{\text{Distance travelled by the compound (cm)}}{\text{Distance of the solvent front (cm)}}$$

The spots were also sprayed with an H_2SO_4-MeOH solution (1:9) and then burned on a hot plate (80 °C) to verify the presence of additional compounds that were unable to be observed under the UV lamp, both short (254 nm) and long wave (365 nm) wavelengths.

3.4.2 Liquid vacuum chromatography

As Oluah *et al.* (2020) described, liquid vacuum chromatography was performed for fractionation. Silica gel was added to 30 g of methanolic extract and thoroughly mixed as a material adsorbent for the column. A sintered glass funnel was filled with 130 g of TLC-grade silica gel and then dispersed evenly to a height of 4.5 cm. To safeguard the column bed, treated sand was uniformly distributed on top of the packed silica gel. The produced extract was then added and evenly spread to a height of 1.5 cm on top of the loaded silica gel in the sintered glass funnel. Binary mixes of n-hexane (n-Hex), dichloromethane (DCM), and methanol (MeOH) were used for gradient elution. TLC was used to gather and examine the fractions containing about 20 to 50 mL. The R_f values of these spots were calculated. Combined fractions were created by combining the fractions with spots with comparable R_f values. These fractions were removed from the solvent using a rotary vapour and air-dried, weighed, and put into airtight glass vials for additional analysis.

3.4.3 Centrifugal thin-layer chromatography (Chromatotron)

In addition to column chromatography and TLC plates, centrifugal thin-layer chromatography or radial chromatography (Chromatotron) isolated and purified the chemical compounds. A chromatotron plate was prepared using silica gel 60 PF254 containing gypsum (Merck 1.07749.1). The chromatotron plate was coated with the appropriate amount of silica gel subjected to the thickness of the plate prepared. For example, 35 g silica gel was used to prepare a 0.5 mm thickness plate. The silica gel slurry was prepared using a sufficient volume of cold distilled water. After that, the slurry was stirred, poured onto the chromatotron plate surface and left at room temperature for 24 hours. The silica plate was then scrapped to the desired thickness and placed in the oven. It was activated in an oven at 60 °C for about an hour before use. This procedure can ensure the silica gel is thoroughly dried and activated.

The sample (fraction) was dissolved and filtered using cotton wool. Afterwards, it was applied to the plate through the opening. UV light (shortwave and longwave) was used to examine the compound partitioning on the plate. Different organic solvent systems in increasing polarities were used as the mobile phase. Each partition or eluent was collected using a vial separately based on the band.

3.5 Structure elucidation

To elucidate the structures and to identify the isolated pure compounds of the *M. paradisiaca* extract, various spectroscopic techniques were employed, including Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance Spectrometry (NMR), Infrared Spectroscopy (IR), and Ultraviolet-Visible Spectroscopy (UV-Vis).

3.5.1 Gas-chromatography-mass spectrometry (GC-MS)

The Perkin Elmer Clarus 680 with MS was used to determine the mass of a molecular or an isolate. MS scan range was estimated to be 50-550 atomic mass units (AMU). The chromatographic column for the analysis was fused with a silica wall-coated open tubular capillary column (WCOT) where the size is 30 m long, 0.25 mm inner diameter, and 0.25 μm thickness. The carrier gas used was helium gas which was run at a flow rate of 1.0 mL/min. Temperature programming was used to examine samples with the column, with the initial temperature set at 50°C for 1 minute and increasing to 200°C at a rate of 10°C/min. The temperature was raised to 250°C and held for 20 minutes. The injection was carried out in a split mode. The mass fragments and e/Z values of each component, as well as a comparison with the reported data, were used to determine the single component using the Wiley and NIST mass spectral libraries.

3.5.2 Nuclear magnetic resonance spectrometry (NMR)

The ^{13}C spectra and ^{1}H spectra of the pure compounds were identified using Bruker Ascend 400 MHz NMR. Tetramethylsilane (TMS) was used as the internal standard for 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR, respectively. The NMR spectral data was obtained on the number and types of protons and carbons present in the molecule.

3.5.3 Infra-red Spectroscopy (IR)

The functional groups of the sample substance were identified via IR spectroscopy. The IR was obtained from 4000 to 400 cm^{-1} using the synergy between a molecule and radiation from the IR part of the electromagnetic spectrum handled by an IR Perkin Elmer FTIR spectrometer. The apparatus was then run to produce IR spectra after a small sample was placed on the plate.

3.5.4 Ultraviolet-visible Spectroscopy (UV-Vis)

The UV spectrum of the sample was acquired by utilising a UV-Vis spectrometer (Lambda 25, Perkin Elmer). The organic compound absorbed the light of ultra-violet (UV) at the region of 200-400 nm, in which absorption spectroscopy occurs in the UV spectroscopy. The UV radiation absorbed caused the electron to be excited from the ground state to the higher state.

3.6 Biological activities

The biological activities, which are antioxidant and antimicrobial were conducted on the isolate (s) of *M. paradisiaca* blossoms.

3.6.1 Antioxidant activity

The antioxidant activity was determined by measuring free radical scavenging activity with 2,2-diphenyl-2-pycrylhydrazal (DPPH). The DPPH is a free radical that is stable. Scavenging of DPPH denotes the antioxidant activity's free radical reducing activity based on one-electron reduction. This method was used to determine the antioxidant potential of the test sample, demonstrating its effectiveness, prevention, interception, and repair mechanism in a biological system. The absorbance at 517 nm was measured to determine the amount of DPPH that remains (Farag *et al.*, 2020). The colour was transitioned from purple to yellow.

Ascorbic acid and absolute ethanol were used as the standard and working solutions, respectively, as described by Ariffin *et al.* (2021) and Stanković *et al.* (2016), with minor modifications. The DPPH solution was prepared in absolute ethanol, and then DPPH was added. The DPPH was dissolved in absolute ethanol to make the control. Before analysis, all samples were shaken vigorously and incubated in the dark for at least 60 to 90 minutes at room temperature. A UV spectrometer was used to measure the absorbance at 517 nm. The free radical scavenging activity was determined using the following equation:

$$\text{Inhibition (\%)} = \frac{A_O - A}{A_O} \times 100$$

A_0 denotes the absorbance of the negative control, which contains all reagents except the test compound, and A denotes the absorbance of the tested sample.

A graph of per cent inhibition of DPPH radicals (per cent) against sample concentrations was established and used to obtain 50% inhibitory concentration (IC_{50}) values. The IC_{50} value for DPPH indicates the minimum concentration of substance required to scavenge 50% of DPPH free radicals. Following the experimental design, these assays were performed with three independent replicates, and each sample was measured in triplicate. The data were presented as a mean and standard deviation. The DPPH stock solution was kept at four °C before being used. The lower the absorbance of the reaction mixture, the greater the DPPH free radical scavenging activity.

3.6.2 Antimicrobial activity

The Minimum Inhibitory Concentration (MIC) and the antibacterial Concentration (MBC) evaluated the antibacterial activity. The MIC assay determined the lowest dose of a drug that prevents bacteria from growing microscopically visible. On the other hand, the MBC assay, which represents a concentration capable of killing up to 99% of bacteria, was used to revalidate the MIC results by detecting bacterial growth in agar plates (Ariffin *et al.*, 2021). The minimum antimicrobial concentration that

prevents an organism from growing after subculture onto antibiotic-free media was used to determine MBC.

Preparation of nutrient agar (NA)

The nutrient agar was dissolved in distilled water. After stirring, the mixture was autoclaved for 15 minutes at 121°C. After the solution had cooled, the media was poured aseptically into a petri dish.

Preparation of nutrient broth (NB)

The agar was prepared by dissolving the NB powders in distilled water and thoroughly mixing them. The mixture was autoclaved at 121°C for 15 minutes. NB was used as a diluent, and each organism was individually inoculated into the diluent before being stored overnight at 37°C.

Bacterial strain

The sample was tested with four bacteria: two Gram-positive bacteria, *Streptococcus pyogenes* (SP) and *Staphylococcus aureus* (SA), and two Gram-negative bacteria, *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA). All the bacterial strains were cultured in nutrient agar overnight at 37°C.

Culture bacteria

Four vials of NB were prepared and filled. Each bottle was labelled with the desired bacteria's initials and the set date. Each vial received four bacteria

stock solutions, and the bottles' lids were sealed with parafilm. The vials were then kept in an incubator at 37°C.

Minimum inhibitory concentration (MIC) assay

The sample concentrations served as the foundation for interpreting the in vitro data. The nutrient broth, agar solutions, pipettes, pipette tips, and beakers were prepared and sterilised in an autoclave. The stock solutions were prepared at 1,800 g/mL concentration by dissolving 3.6 mg of each extract in 2 mL dimethyl sulfoxide (DMSO). Laminar flow was used to prepare the agar on the petri dish. Before the preparation, the laminar flow surface was cleaned with ethanol to kill any unwanted bacteria that could contaminate the agar. The agar that solidified was stored in the refrigerator. The bacteria were cultured by placing them in bottles with warm NB. The bottles were incubated at 37°C for 24 hours.

Rows A and B of the 96-well plate were filled with the 100 g/mL stock solution, while rows B–H was filled with 100 g/mL NB. The NB and sample were mixed well in row B, and the mixture was shifted downward into each well from C to H to achieve a serial dilution by a factor of two with a concentration range of 1,800 to 14.07 g/mL. Each well received 100 g/mL of the inoculated bacteria. Streptomycin sulphate was used as a positive control for bacteria, while media was used as a negative control. The 96-well plate was sealed, covered, and incubated at 37°C for at least 24 hours. The bottom of the wells was later analysed for pellets' presence

along with turbidity changes, indicating microbe growth.

Minimum bacterial concentration (MBC) assay

This assay extends the evaluation of MIC results by deciding the number of surviving organisms from bacterial growth. The sample at the clearest stage/concentration in the 96-well plate (during MIC analysis) was removed using a sterilised cotton bud and gently spread on the agar plate. The plate was sealed and incubated for 24 hours at 37°C. When the agar in the plate became clear during observation, the bacteria's growth was inhibited and stopped growing. MBC produced the same results as MIC. If the agar becomes cloudy, the MBC result indicates that the concentration was one step higher than the MIC. The MBC of the extracts were compared to that of the positive control, streptomycin sulphate, a water-soluble antibiotic that is commercially available. Figure 3.1 depicts the 96 wells-plate.

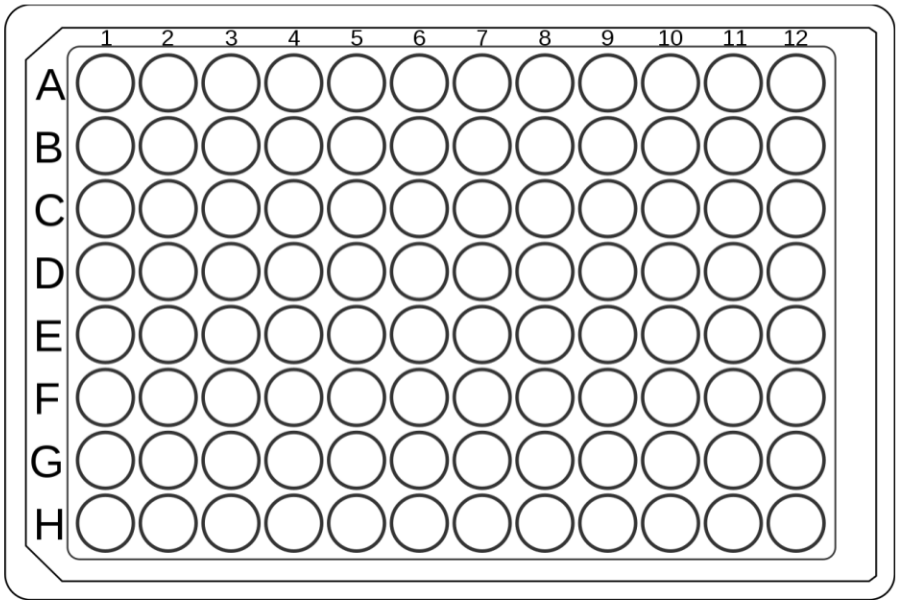


Figure 3.2 96-wells plate

3.6.3 Anticancer activity

On three cancer cell lines—human skin cancer (HaCat), cervical cancer (HeLa), and human prostate cancer cell—four pure chemical samples were examined for their antiproliferative potential (DU-145). The Thai Traditional Medicine College at the Rajamangala University of Technology in Thanyaburi Pathumthani, Thailand, is where all cancer cell lines were acquired. All cell lines were grown and maintained at 37 °C in an incubator in a 5 % CO_2 environment. According to Ariffin *et al.* (2021), the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the antiproliferation activity for these compounds.

3.6.4 Preparation of media (DMEM)

Distilled water and the 500 mL Duran bottle were autoclaved. 3.7 g of sodium bicarbonate was combined with one packet of Dulbecco's Modified Eagle's Medium (DMEM). Once the powder was dissolved, 900 mL of sterile, distilled water was added and agitated with a magnetic stirrer. To bring the pH of the medium to between 7 and 7.2, 10 drops of 1M hydrochloric acid were added. After that, the medium was filtered in a laminar flow. Before use, the medium was chilled and then warmed in a water bath.

3.6.5 Cell lines culture

The three cell lines were sub-cultured aseptically in a laminar flow into a fresh flask. Before beginning the treatment, the laminar flow was held under UV light for 30 minutes. To eliminate any microorganisms that can cause contamination, the laminar flow surface was thoroughly cleaned with ethanol. Alcohol was sprayed upon every piece of equipment. The medium was taken out using a suction pump, and any leftover media was taken out with 5 mL of phosphate-buffer saline (PBS). To get the PBS to cover the flask's surface, the flask was gently shaken. After removing the PBS, 2 mL of trypsin was added. After giving the liquids in the flask a gentle shake to cover all the cell lines, they were incubated for 5 minutes at 37 °C with 5% CO_2. Under an inverted microscope, it was checked to make sure the cell lines were completely separated. The solution was poured into a 50 mL centrifuge tube, and it was spun at 3000 rpm for 5 minutes. After removing

the supernatant, 20 mL of fresh culture media with 10% fetal bovine serum (FBS) was added. This was then divided between two fresh flasks holding 10 mL each. The flask was then incubated for a week at 37 °C with 3% CO_2. Every day, the flasks were checked, and the medium was replaced as necessary until all the cell lines had grown completely inside the flask.

3.6.6 MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to test the crude extract's anticancer efficacy according to Ariffin *et al.* (2021). Cell counting was required for the MTT test, which was performed to determine the vitality of the cells using 96-well plates. HaCat, HeLa, and DU-145 cancer cell lines in DMEM solution were specifically planted at a density of 5×10^4 cells/well in 96-well plates before 180 µL of DMEM was added. They underwent a 24-hour incubation period at 37 °C in a 5% CO_2 environment. The sample stock solutions were prepared in serial dilution at 1, 0.1, 0.01, 0.001, and 0.0001 mg/mL. Using a multi-channel micropipette, 20 µL of *M. paradisiaca* pure compounds were added to the 96-well plates after 24 hours. To distribute the sample throughout the cell line medium uniformly, the plates were gently shaken. The cancer cells were then cultured for 24 hours at 37 °C in a 5% CO_2 environment. Each well plate's medium was taken out after 24 hours of incubation. Each well-plate received 100 L of a 0.5 mg/mL MTT solution, which was then incubated for two hours at 37 °C with 5% CO_2 atmosphere. The MTT solution and 100 µL of dimethyl sulfoxide (DMSO) were

withdrawn to dissolve the formazan crystals. The plate was then read for the absorbance at 570 nm using a microplate reader. The percentage of toxicity was calculated by using the following formula:

$$\% \text{ toxicity} = 100 - \frac{A_s \times 100}{A_c}$$

A_s : Sample absorbance

A_c : Control absorbance

The IC_{50} was obtained by plotting the graph of % toxicity against concentrations.

3.7 Physical, chemicals and spectroscopy data of pure compounds

The results from various spectroscopic analyses were used to elucidate the structure of pure compounds. The spectroscopic techniques used were FTIR, GC-MS, and NMR. Perkin Elmer Spectrum One was used to calculate FTIR spectra.

The samples were produced as plain liquid samples and KBr disc solid samples. High-resolution mass spectra were acquired with an ESI-TOF Waters LCT Premier XE mass spectrometer in positive ion mode, while GC-MS was evaluated using a Perkin Elmer Clarus 600 or 680 spectrometer. The ^{1}H NMR spectra were obtained using Bruker 500 or 400 spectrometers that operate at around 500 or 400 MHz, respectively, while the ^{13}C NMR spectra were obtained using the same equipment and conditions that operate at approximately 125 or 100 MHz. Chemical

changes in ppm were calculated using internal $CDCl_3$. The melting points of the pure compounds were determined using the HINOTEX X-4 melting point instrument and a microscope. For extraction, ethyl acetate was employed, and for chromatography, n-hexane, chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), ethyl acetate, acetone, and methanol (MeOH) were utilised.

3.7.1 Compound EA2.6.1 (2'-hydroxy-4-methylchalcone)

Yellow crystals. $C_{16}H_{14}O_2$. Melting point: 109-110°C. IR ν_{max} cm^{-1} (KBr): 3590.09 (OH), 3005.02 (C-H), 1709.48 (C=O), 1420.64 (C=C), 1220.74 (C-O), 1092.58, 902.68 cm^{-1} (=C-H); EI-MS m/z: 238 (M^+, $C_{16}H_{14}O_2^+$, 105), 223 ($C_{16}H_{13}O^+$, 334.5), 147 ($C_{15}H_{11}O^+$, 83.16), 118 ($C_9H_7O^+$, 2006.66), 105 ($C_7H_5O^+$, 114.58), 91 ($^+C_7H_5$, 1758). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 2.43 (3H, *s*, H-7), 6.97 (1H, *m*, H-3'), 7.06 (2H, *d*, *J* = 8, H-5'), 7.28 (1H, *d*, *J* = 8, H-3), 7.51 (1H, *m*, H-5), 7.59 (2H, *d*, *J* = 8, H-4'), 7.67 (1H, *d*, *J* = 15.4, H-α), 7.96 (1H, *d*, *J* = 15.8, H-β), 7.94 (1H, *d*, *J* = 8, H-6'), 12.88 (1H, s, 8-OH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 119.1 (C-α), 145.6 (C-β), 141.6 (C-1), 129.6 (C-2), 129.8 (C-3), 136.3 (C-4), 129.8 (C-5), 128.7 (C-6), 21.6 (C-7), 193.8 (C-9), 128.7 (C-1'), 163.6 (C-2'), 118.6 (C-3'), 136.3 (C-4'), 118.8 (C-5'), 131.9 (C-6').

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Isolation and purification of *M. paradisiaca*

A Thin Layer Chromatography (TLC) profile was used to determine the solvent system for isolation and the purification of the chemical obtained from *M. paradisiaca* blossoms ethyl acetate extract. Various solvent systems with increasing polarity, such as 9:1, 8:2, 7:3, and 6:4 mixtures of hexane and ethyl acetate, hexane and chloroform, and hexane and methanol, were used in this study to find the best-suited solvent system for purification processes. A hexane-ethyl acetate solvent solution was chosen to perform the isolation procedure based on the TLC profile. TLC was applied to each pure compound to confirm the purity of the isolated pure compound or the TLC profile of mixtures. In a saturated chromatographic chamber exposed to vapour test on the TLC plate, a 9:1 solvent system of hexane-ethyl acetate was utilised. One dark spot was shown under shortwave UV (254 nm), and no spot under longwave UV (360 nm).

4.2 Structure elucidation of a pure compound from *M. paradisiaca*

One pure compound was isolated from *M. paradisiaca* blossoms hexane extract, and it was indicated by a spot on the TLC profile. It was labelled as **EA2.6.1**. The pure compound was further analysed using various spectroscopic methods, including Gas-Chromatography Mass Spectroscopy

(GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), ^{1}H Nuclear Magnetic Resonance (NMR) and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy. The obtained data were also compared with the published data.

4.2.1 Characterisation of EA2.6.1

Pure compounds **EA2.6.1** have been isolated as yellow crystalline needles with a melting point of 112–115°C (Lima *et al.*, 2017). The mass spectrum revealed the molecular ion peak at m/z 238, indicating the molecular formula of this compound was $C_{16}H_{14}O_2$. In addition, the base peak for this compound was at m/z = 105, giving 100% natural abundance, while the other peaks observed were m/z 223, 147, 118 and 91. The mass fragmentation pattern of **EA2.6.1** is shown in Figure 4.1.

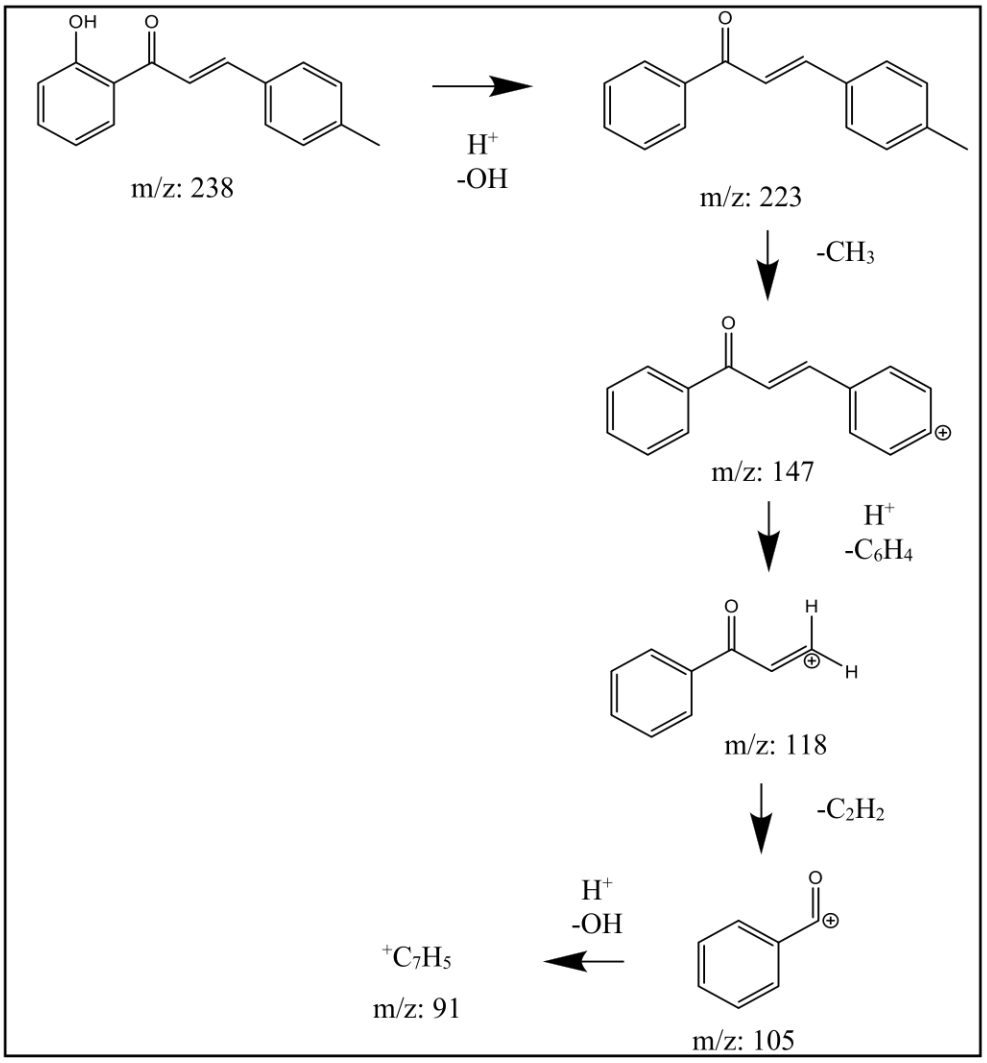


Figure 4.1 Mass fragmentation pattern of **EA2.6.1** (2'-hydroxy-4-methylchalcone)

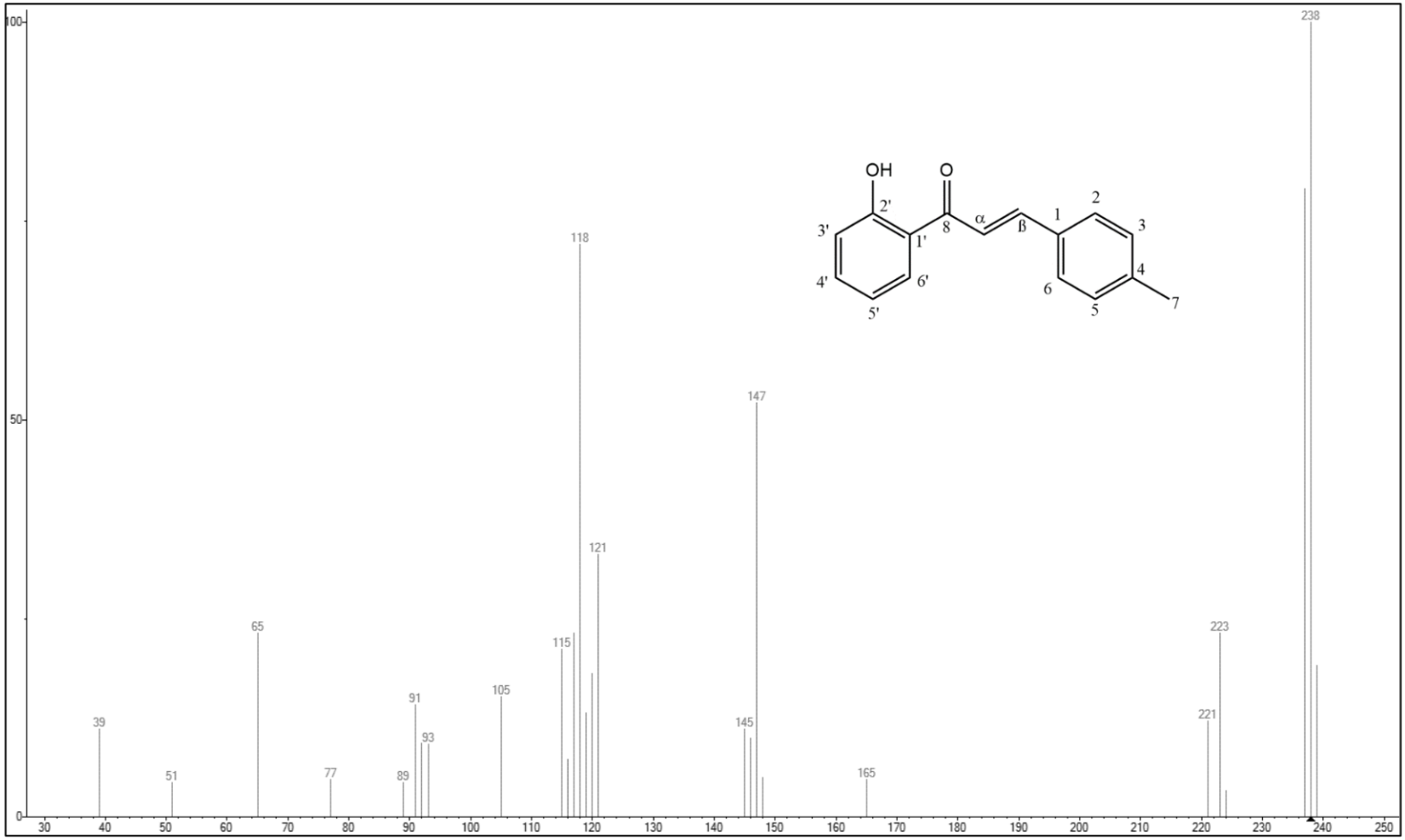


Figure 4.2 Mass spectrum **EA2.6.1** (2'-hydroxy-4-methylchalcone)

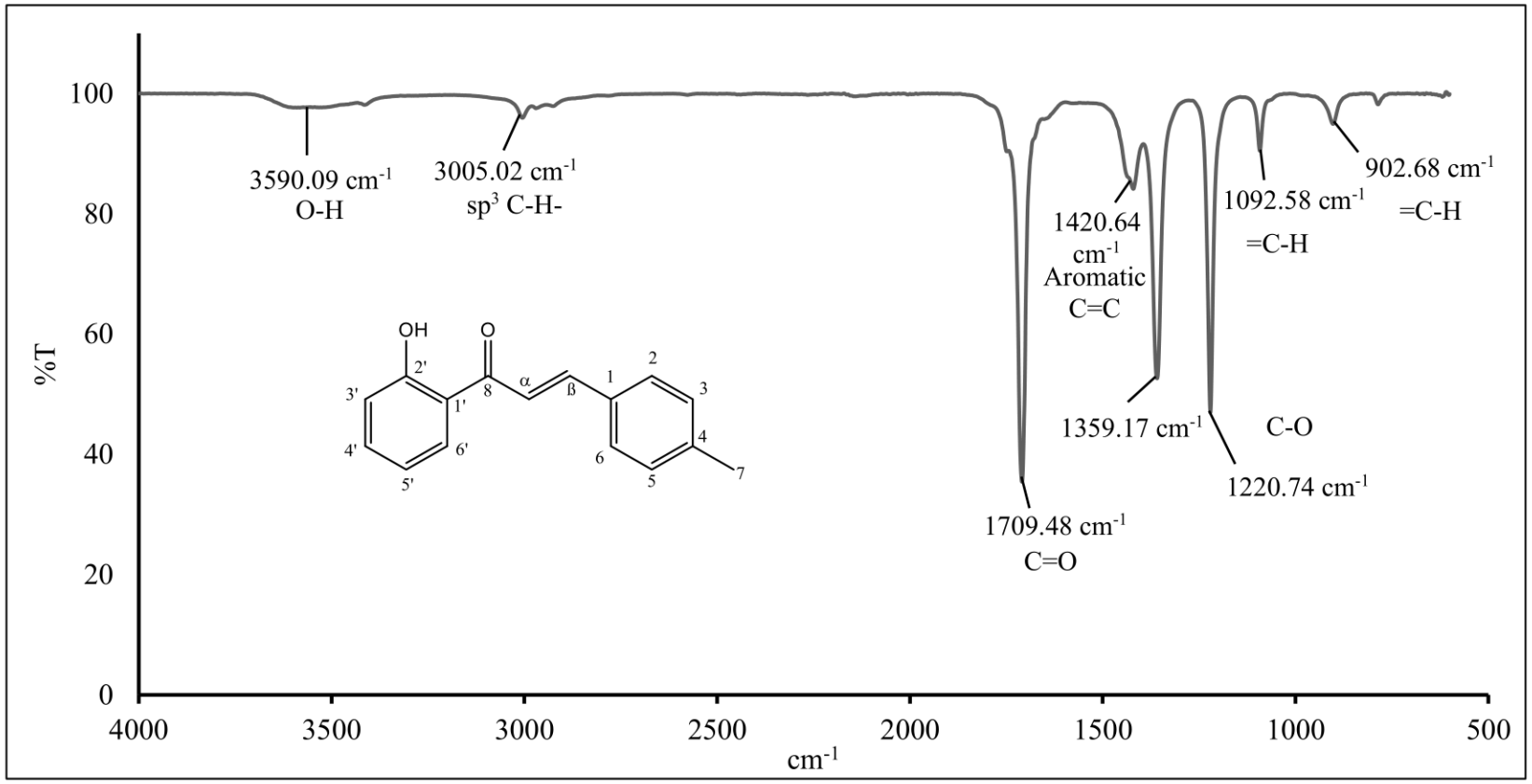


Figure 4.3 IR Spectrum of **EA2.6.1** (2'-hydroxy-4-methylchalcone)

The IR spectrum (Figure 4.3) revealed the presence of an absorption band at 3590.09 cm^{-1} that displays characteristics of O-H stretching, as well as an olefin functional group (C=C) at 1420.64 cm^{-1} and (C-O) at 1220.74 cm^{-1}. Furthermore, the absorption at 3005.02 cm^{-1} was due to C-H stretching. A strong absorption peak at 1709.48 cm^{-1} showed the presence of the C=O carbonyl group of the ketone, and at 1092.58 cm^{-1} and 902.68 cm^{-1} stretching (=C-H).

The ^{1}H-NMR spectrum (Figure 4.4) showed one singlet signal at δ 2.43 attributed to the presence of one methyl group (-CH_3) for H-7. A doublet signal resonated at δ 7.06 (J = 8 Hz), corresponding to one aromatic proton H-5'. The proton coupled with H-4' produced a multiplet signal at δ 7.59. The two two-proton doublet signals resonated at δ 7.91 and δ 7.28 with a J value of 8.0 Hz, designated to H-3; H-5, and H-2; H-6, respectively. The olefinic proton of an α, β-unsaturated ketone was observed at δ 7.94 (J = 15.2 Hz) and δ 7.96 (J = 15.2 Hz), corresponding to H-α and H-β, respectively. The greater the coupling value, the more trans-positional the two olefinic protons are. The two doublet signals resonated at a lower field where δ 7.96 was attributed to one aromatic proton, H-6'. The ^{1}H NMR spectrum displayed a singlet signal at δ 12.88, attributed to the presence of one (=OH) group located at H-2’.

The ^{13}C-NMR spectrum (Figure 4.5) gave a total of 16 carbon signals, which included one methyl (CH_3) group, 11 methane (CH) groups, and four quaternary (C) carbons. One methyl group was located at δ 21.6, whereas the four quaternary carbons were at δ 128.7, 141.6, 163.6, and 193.8. The assignments of the NMR spectra for **EA2.6.1** were summarised in Table 4.1

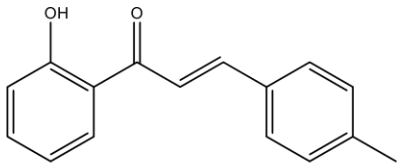
Table 4.1 Comparison ^{1}H NMR and ^{13}C NMR Spectral Data of **EA2.6.1**

No.	δ ^{13}C (ppm)$^{+}$	δ ^{13}C (ppm)*	δ ^{1}H (ppm) Multiplicity (*J* Hz)	δ ^{1}H (ppm)** Multiplicity (*J* Hz)
α	119.1	120.4	7.67 (*d*, 15.4, 1H)	7.67 (*d*, 15.6, 1H)
β	145.6	146.4	7.96 (*d*, 15.8, 1H)	7.94 (*d*, 15.6, 1H)
1	141.6	142.4		
2	129.6	130.4	7.92 (*s*, 8)	7.79 (*d*, 8)
3	129.8	130.6	7.28 (*d*, 8, 1H)	7.27 (*d*, 8, 1H)
4	136.3	133.0		
5	129.8	130.6	7.51 (*m*, 1H)	7.50 (*m*, 1H)
6	128.7	130.0		
7	21.6	21.5	2.43 (*s*, 3H)	2.43 (*s*, 3H)
8	193.8	195.1		
1'	128.7	130.0		
2'	163.6	164.5	12.88 (*s,* 1H)	12.89 (*s*, 1H)
3’	118.6	118.9	6.97 (*m*, 1H)	6.95 (*m*, 1H)
4'	136.3	137.4	7.59 (*d,* 8, 2H)	7.59 (*d*, 8, 2H)
5’	118.8	119.8	7.06 (*d*, 8, 2H)	7.06 (*d*, 8, 2H)
6’	131.9	131.3	7.94 (*d*, 8, 1H)	7.96 (*d*, 8, 1H)

Note: $^{+}$ ^{13}C NMR in $CDCl_3$, 100 MHz; *^{13}C NMR in Acetone-d_6, 150 MHz
Source: *Krawczyk-łebek *et al.,* 2022, **Uddin *et al.* , 2020

Due to the unavailable (decomposition) of the pure compound, further spectroscopy analysis such as Two-dimensional nuclear magnetic resonance (2D-NMR), Distortionless Enhancement by Polarization Transfer (DEPT), and Attached Proton Test (APT) is not able to perform for the conformation of structure elucidation.

Nonetheless, based on the available spectral data of GC, MS, IR, ^{1}H NMR, and ^{13}C NMR discussed above, the pure compound *M. paradisiaca* is provisionally identified as 2'-hydroxy-4-methylchalcone.



EA2.6.1

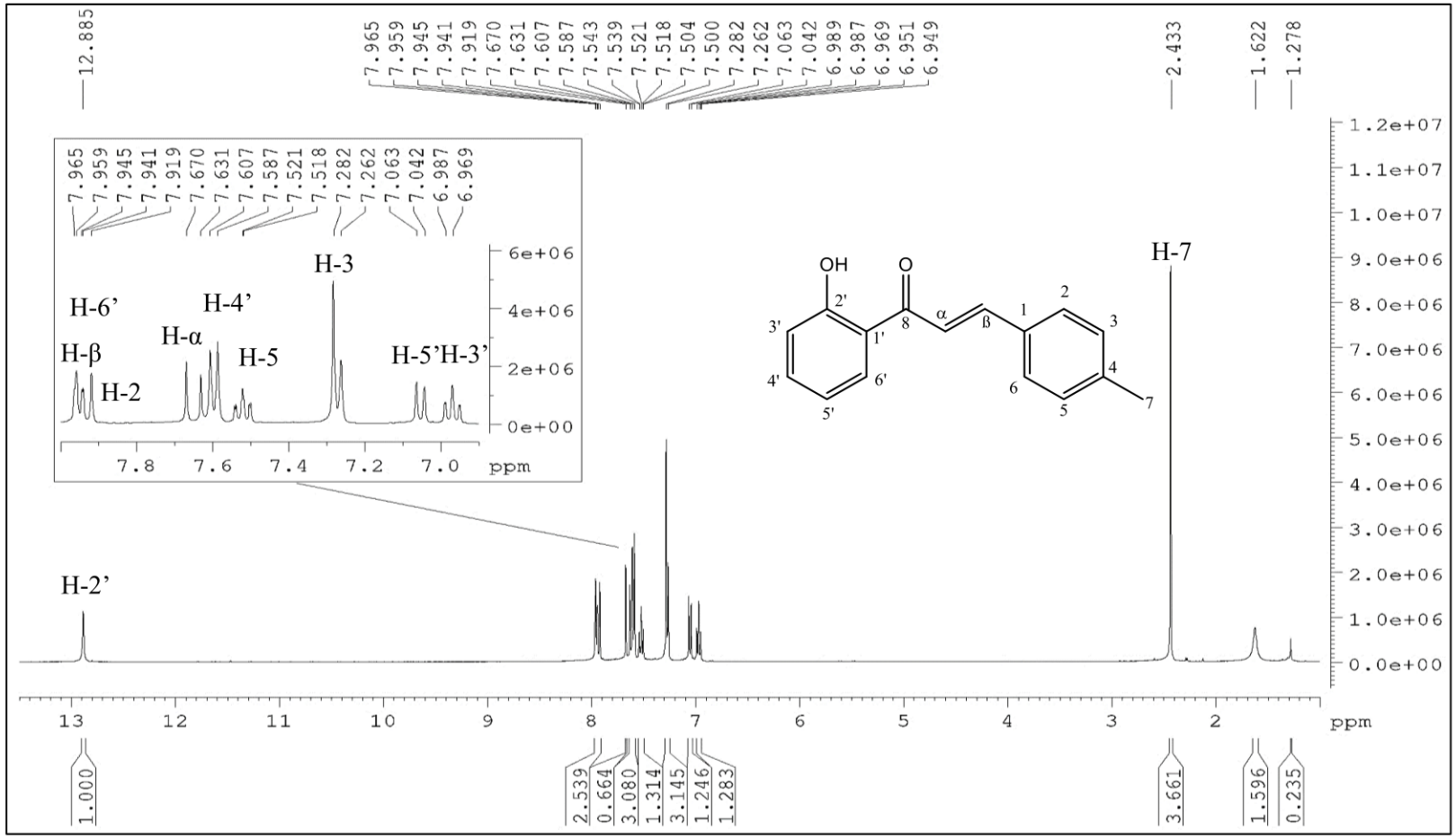


Figure 4.4 ^{1}H NMR Spectrum of **EA2.6.1** (2'-hydroxy-4-methylchalcone) ($CDCl_3$, 400 MHz)

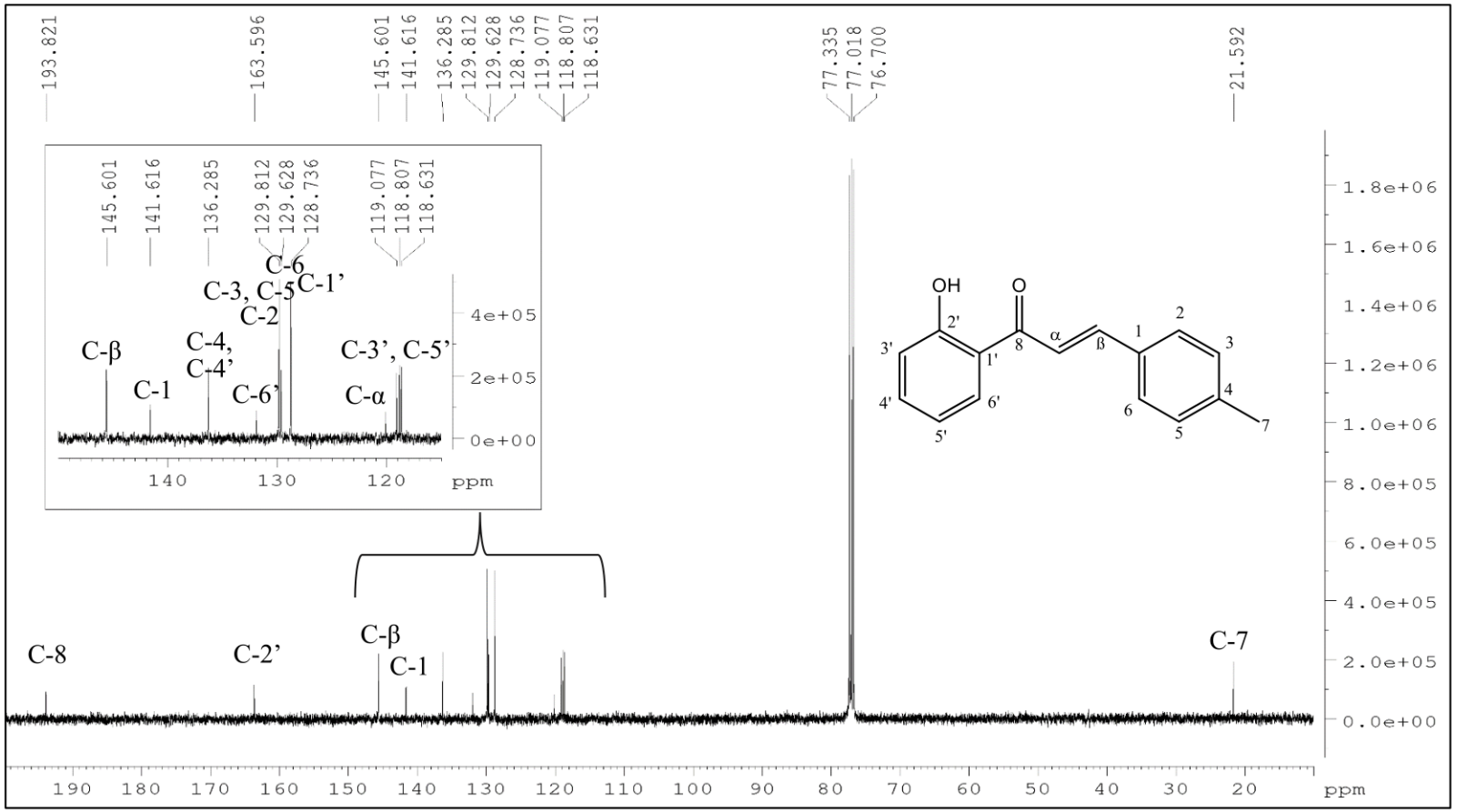


Figure 4.5 ^{13}C NMR Spectrum of **EA2.6.1 (**2'-hydroxy-4-methylchalcone) ($CDCl_3$, 100 MHz)

4.3 Antioxidant activity

Antioxidants are vital in the prevention of illnesses induced by oxidant damage. The antioxidant evaluation demonstrates a biological system's efficacy, prevention, interception, and repair process against harm. The radical scavenging assay 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to investigate the free radical capacity of *M. paradisiaca* banana blossoms pure compound. The antioxidant activity was assessed using inhibition concentration (IC_{50}). Many studies employed this radical scavenging test to examine the antioxidant properties of various substances. The radical DPPH is scavenged by donating a proton, which lowers the DPPH. The actions of antioxidants may be easily seen by the colour of the combined solution, where DPPH reduction occurs when the colour of the mixture solution changes from dark purple to yellow. The decreasing value of absorbance may measure this at 517 nm.

Using a UV spectrophotometer, the pure compound isolated from *M. paradisiaca* blossoms **(EA2.6.1) (**2'-hydroxy-4-methylchalcone) was evaluated for free radical scavenging activity based on DPPH. The standard reference used was quercetin. The three samples chosen were run in triplicate, and the results are shown in Table 4.2. All tested materials' DPPH free radical scavenging activities increased with increasing concentrations ranging from 1.95 to 3000 µg/mL.

Compared to quercetin (positive control), this finding shows that the pure compound has a high ability to scavenge free radicals with an IC_{50} value of 0.376 µg/mL.

Table 4.2 DPPH radical scavenging activity of *M. paradisiaca*

Samples	**DPPH Free Radical Scavenging Activity (IC_{50}) (µg mL^{-1})**
EA.2.6.1	0.376
Quercetin	2.150

4.4 Antimicrobial activities

The isolated pure compound, **EA2.6.1 (**2'-hydroxy-4-methylchalcone) was investigated for antimicrobial activity against two Gram-positive bacteria and two Gram-negative bacteria, namely *Staphylococcus aureus* (SA), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA). The concentration that inhibited bacterial/yeast growth completely (the first clear well) was taken as the MIC value. MIC values were determined at least in duplicate and repeated to confirm activity (Mogana *et al.*, 2020). A negative control experiment was conducted using only dimethyl sulfoxide (DMSO). The presence of turbidity and a pellet on the well bottom determines the evaluation of the MIC values. The last-clear well is taken as a MIC result. The proposed classification of antibacterial activity for the pure compound.

Table 4.3 The inhibitory concentration of the isolated pure compound (**EA2.6.1)** against different types of bacteria (µg/mL).

Sample	Test organism							
	SA		SP		EC		PA	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EA2.6.1	450	450	225	225	450	450	450	450
Streptomycin sulfate	14.01	14.01	14.01	14.01	14.01	14.01	14.01	14.01

Note: EA2.6.1: the isolated pure compound from *M. paradisiaca* ethyl acetate extract.
Less than 500 µg/mL: strong; 501 µg/mL to 1500 µg/mL: moderate; more than 1500 µg/mL: weak. Test organism: SA=*Staphylococcus aureus*, SP=*Streptococcus pyogenes*, EC : *Escherichia coli*, and PA =: *Pseudomonas aeruginosa*.

The findings revealed that the pure compounds inhibited strong activity against all the tested Gram-positive and Gram-negative bacteria with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranging from 225 µg/mL to 450 µg/mL. The results are summarised in Table 4.3. The isolated pure compound of *M. paradisiaca* inhibited the activity of all microorganisms. This observation supports the notion that *M. paradisiaca* pure compounds have high antimicrobial activity. The results recorded are similar to the studies reported by Jawla *et al.* (2012). Ariffin *et al.* (2021) stated that the ethyl acetate extract of *M. paradisiaca* demonstrated an active inhibitory effect toward all the tested pathogens with values ranging from 14.07 to 450 µg/ml. Nonetheless, more pharmacological, and toxicological research is needed to corroborate it. According to Uddin *et al.* (2020), the compound 2'-hydroxy-4-methylchalcone showed bacterial growth in sample solutions was greater than in controls. The growth of all

examined organisms is severely hindered as the concentration increases.

4.5 Anticancer activities

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazololium bromide] assay was used to conduct a preliminary investigation on the possible cytotoxic action of pure compounds isolated from *M. paradisiaca* blossoms. Following purification, several anticancer medicines obtained from plant materials are evaluated on cells (including various cancer cell lines) and experimental animals before being submitted to clinical trials. Cancer is a complicated collection of disorders characterised by aberrant cell proliferation that can infiltrate other parts of the human body. The cytotoxicity of the human prostate cancer (DU-145) cell lines, human cervix (HeLa) cell lines, and human skin (HaCat) cell lines were determined using a microculture test based on MTT metabolic reduction (Table 4.4). Three drugs, namely Cisplatin, Doxorubicin, and Fluorouracil were utilised as positive controls. All these drugs were frequently used in chemotherapy to treat cancer cells. The values of very active anticancer activity should be lower than 20 µg/mL, moderate active is the IC_{50} values between 20 to 100 µg/mL, and low activity is the IC_{50} values between 100 to 1000 µg/mL (Ariffin *et al.*, 2021).

Table 4.4 Anticancer activity of the isolated pure compound (**EA2.6.1**)

Sample	IC_{50} (µg/mL) of the extracts on cancer cell lines		
	DU-145	**HeLa**	**HaCat**
EA2.6.1	1170	1040	1270
Cisplatin	181	7	70
Doxorubicin	649	210	140
Fluorouracil	11691	6520	7640

Note: EA2.6.1: The isolated pure compound from *M. paradisiaca* ethyl acetate extract, Strong: <20 µg/mL, Moderate: 20 µg/mL to100 µg/mL, Weak: 100 µg/mL to 1000 µ g/mL

Compared to cisplatin and doxorubicin, the pure compound EA 2.6.1 inhibited the three cancer cell lines rather weakly. Nonetheless, *M. paradisiaca* is a more effective anticancer drug than fluorouracil. *M. paradisiaca* has shown positive action against breast cancer cell lines. The antiproliferative activity of these extracts against all cancer cell lines might be attributed to the phytochemical composition of the medicinal herbs, which included flavonoids, alkaloids, tannins, and xanthones. These data suggested that *M. paradisiaca* was not a viable natural source of antiproliferative and cytotoxic activity. These findings are supported by Ariffin *et al.* (2021), where the anticancer drugs at low concentrations showed higher cytotoxicity on human dermal fibroblasts.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

One pure compound was isolated from the *M. paradisiaca* blossoms ethyl acetate extract. The purity of the isolates was validated by a single spot on the Thin Layer Chromatography (TLC) profile which was employed tested with various solvents system and different ratios. It was also confirmed by one single peak displayed on a GC chromatogram.

This pure isolate was identified as 2'-hydroxy-4-methylchalcone with a molecular weight of 238 based on the spectral data of Gas-Chromatography Mass Spectroscopy (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), ^{1}H Nuclear Magnetic Resonance (NMR), and ^{13}C Nuclear Magnetic Resonance (NMR) Spectrometry. This chemical compound is reported for the first time in this plant.

This pure isolate, 2'-hydroxy-4-methylchalcone revealed several biological activities. It exhibited high inhibitory activities against four bacteria: *Staphylococcus aureus* (SA), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA), with MIC values ranging from 225 to 450 µg/mL. The results showed that this plant has strong antibacterial activities. As such, this study suggested this plant blossom extract could have

great potential to be used as a food poisoning control and natural preservative in preserving food for replacing chemical preservatives.

Furthermore, 2'-hydroxy-4-methylchalcone was reported to be selectively cytotoxic *in vitro* to anticancer activity against DU-145 (human prostate cancer cell line), HeLa (human cervix cell line), and HaCat (human skin cell line), with IC_{50} values of 1170 µg/mL, 1040 µg/mL, and 1270 µg/mL, respectively. Yet, compared to fluorouracil, a positive control chemotherapeutic medication, the pure compound was more potent than fluorouracil, a positive control chemotherapeutic medication the pure blossoms compound had stronger action.

In addition, this isolate had significant DPPH free radical scavenging action, with an IC_{50} value of 0.376 µg/mL, indicating 2'-hydroxy-4-methylchalcone is a potent antioxidant agent. In summary, the study proves the assertions of local folks about *M. paradisiaca*'s ethnomedicinal characteristics as a traditional herb.

Further research on *M. paradisiaca* blossoms may be conducted using comparable chemical analyses and biological activity on various extraction techniques and solvent extraction. The outcomes of this study are encouraging for the future development of a medicinal product. Furthermore, the therapeutic characteristics of this plant can be researched and developed

for creative goods that can benefit higher-income countries. As a result, this discovery gave helpful information and added to the significance of the chemotaxonomic research of the *Musa* genus from the Sarawak region.

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B. Hobbies and interests

I enjoy travelling and exploring new places all around the world. Meeting new people and studying different cultures and histories are my favourite aspects of travelling.

I am fluent in written and spoken Malay and English and have basic knowledge of Bahasa Melanau and Spanish.

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Degree	Area	Institution	Year awarded
B.Sc. (Hons.)	Chemistry with Management	Universiti Teknologi MARA, Malaysia	2020 - Present
Diploma	Science	Universiti Teknologi MARA, Malaysia	2019
S.P.M	Science	SMK St. Teresa, Kuching, Sarawak	2016

D. Related experience

Post	Place	Year
Member	CHEMISTS UiTM Cawangan Sarawak	2021-2022
Master of Ceremonies	9th Undergraduates Colloquium of Applied Sciences Faculty, UiTM, Cawangan Sarawak	2021
Committee	10th Undergraduates Colloquium of Applied Sciences Faculty, UiTM, Cawangan Sarawak	2022
Committee	Outbound-Inbound Virtual Mobility Programme, Japan	2022
Exchange student	Academic Attachment Program at Thai Traditional Medicine College, Rajamangala University of Technology, Thanyaburi (RMUTT), Thailand	2022
Member	Sukarelawan Polis Siswa Siswi (SUKSIS), UiTM, Sarawak	2017-2019

E. Awards

Type	Name of award / awarding organisation	Date
Certificate	Inspektor Sukarelawan Simpanan Polis Siswa / Siswi IPTA (KOR SUKSIS)	15 Feb 2020
Certificate	Dean's List Award 2020, Universiti Teknologi MARA, 94300 Sarawak, Malaysia	9 Sep 2020
Certificate	Dean's List Award 2021, Universiti Teknologi MARA, 94300 Sarawak, Malaysia	10 Sep 2021
Certificate	Dean's List Award 2021, Universiti Teknologi MARA, 94300 Sarawak, Malaysia	15 Mar 2022
Certificate	Dean's List Award 2022, Universiti Teknologi MARA, 94300 Sarawak, Malaysia	9 Sep 2022