PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF CRUDE PLANT EXTRACTS FROM *APIUM GRAVEOLENS* (CELERY) PLANT

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DECLARATION

I declare that this thesis entitled "Phytochemical screening and antioxidant activity of crude plant extract from *Apium Graveolens* (Celery) Plant" is the result of my research except as cited in the references.

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Phytochemical Screening and Antioxidant Activity of Crude Plant Extracts from *Apium Graveolens* (Celery) Plant

ABSTRACT

Apium graveolens is a biennial plant of the Apiaceous family. This study aims to clarify its importance and reveal its phytochemical profile, which is comprised of several substances such as terpenoids, flavonoids, steroids, and saponins. This study also employs the maceration technique to extract these phytochemicals to compare the effectiveness of two solvents which are 56.7% ethanol and 100% distilled water. Furthermore, the antioxidant capacity of the Apium graveolens extracts was accessed through the utilization of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay technique. The predicted bioactive component was present in the plant extract, according to the findings of the phytochemical screening. 56.7% ethanol extracts gave positive results for steroids and flavonoids but absent for saponins and terpenoids. Every phytochemical test that was conducted on distilled water extracts had positive results. Next, DPPH radical inhibition and IC_{50} data show that plant extract has better antioxidant activity than ascorbic acid. A significant antioxidant activity is shown by the distilled water extract's value of 1.171 mg/mL, which is followed by the ascorbic acid value of 0.766 mg/mL and the ethanol extract of 0.786 mg/mL.

Keywords: *Apium Graveolens*, phytochemical screening, effect of solvents, antioxidant activity, DPPH

Penyaringan Fitokimia dan Aktiviti Antioksidan Ekstrak Tumbuhan Mentah daripada Tumbuhan *Apium Graveolens* (Saderi)

ABSTRAK

Apium graveolens ialah tumbuhan dwitahunan keluarga Apiaceous. Kajian ini bertujuan untuk menjelaskan kepentingannya dan mendedahkan profil fitokimianya, yang terdiri daripada beberapa bahan seperti terpenoid, flavonoid, steroid, dan saponin. Kajian ini juga menggunakan teknik maserasi untuk mengekstrak fitokimia ini untuk membandingkan keberkesanan dua pelarut iaitu 56.7% etanol dan 100% air suling. Tambahan pula, kapasiti antioksidan ekstrak Apium graveolens telah diakses melalui penggunaan teknik ujian penghapusan radikal bebas 2,2-diphenyl-1-picrylhydrazyl (DPPH). Komponen bioaktif yang diramalkan terdapat dalam ekstrak tumbuhan, menurut penemuan saringan fitokimia. Ekstrak etanol 56.7% memberi keputusan positif untuk steroid dan flavonoid tetapi tiada untuk saponin dan terpenoid. Setiap ujian fitokimia yang dijalankan ke atas ekstrak air suling mempunyai keputusan yang positif. Seterusnya, perencatan radikal DPPH dan data IC_{50} menunjukkan bahawa ekstrak tumbuhan mempunyai aktiviti antioksidan yang lebih baik daripada asid askorbik. Aktiviti antioksidan yang ketara ditunjukkan oleh nilai ekstrak air suling sebanyak 1.171 mg/mL, yang diikuti oleh nilai asid askorbik 0.766 mg/mL dan ekstrak etanol 0.786 mg/mL.

Kata kunci: *Apium Graveolens*, penyaringan fitokimia, kesan pelarut, aktiviti antioksidan, DPPH.

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LIST OF ABBREVIATIONS

g	gram
min	minute
nm	nanometre
µg	microgram
µL	microlitre
mL	milliliter
mM	millimolar
ppm	Parts per million
mg/ml	microgram per mililitre
DPPH	2,2-diphenyl-1-picryhydrazyl
CAT	Catalase
FRAP	Ferric reducing antioxidant power
GSH	Reduced glutathione
GSH-PX	Glutathione peroxidase
LPF	Lipofuscin
MDA	Malon dialdehyde
SOD	Superoxide
TAOC	Total antioxidant capacity
H2S04	Sulfuric acid
IC50	Half-maximal inhibitory concentration
NaOH	Sodium hydroxide
UV-VIS	Ultraviolet visible
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxytoluene

LIST OF SYMBOLS

µ	Micro
°C	Degree Celsius
:	Ratio
%	Percent
X	Multiply

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Throughout ancient times, humans employed herbal plants for medicinal purposes. Many herbal plants have demonstrated pharmacological action in the treatment of a range of disorders (Khairullah et al, 2020). Celery is one of the medicinal herbs that may be utilized. The area's residents have relied heavily on wild vegetation to meet their basic needs. Several powerful medications are derived from plants, which are used to treat a variety of disorders like diabetes, infections, skin conditions, etc. These indigenous plant species, which are widely cultivated in many ecological zones of the world, generate a variety of bioactive chemicals, which are thought to be responsible for the medicinal activity of these plants. Many studies have demonstrated the protective benefits of diverse herbs and elements of medicinal plants on atherosclerosis, cancer, and infectious disorders (Zahoor et al, 2015).

Celery, or *Apium graveolens*, is a member of the Apiaceae family. Although celery has already been consumed and grown all over the world up until now, celery plants are native to Asia, Europe, and certain tropical regions of Africa (Kooti & Daraei, 2017). Celery's seeds, leaves, and stems can be used to treat arthritis, gout, rheumatism, and inflammation of the urinary tract. Moreover, celery can be used as a diuretic, to stimulate glands, and bile, treat kidney stones, control the bowels, boost appetite, and prevent nerve agitation. Celery has been shown to have antimicrobial activity (Genatrika, Satriani & Hapsari, 2019) antiparasitic activity, cardioprotective activity, gastroprotective activity, neuroprotective activity, hypolipidemic activity, cytotoxic activity, antioxidant activity, anti-inflammatory activity, and anti-infertility activity in previous pharmacological studies. Plants have long been used to relieve pain, but today the emphasis is on their function in healing and their potential for treating a variety of disorders (Noori Ahmad Abadi M et al, 2016).

Phytochemicals are naturally occurring chemical substances obtained from plants that play a role in therapeutic and pharmacological functions (Kawale & Koche, 2010). Bioactive phytochemicals shield plant cells against environmental dangers such as stress, UV exposure, pollution, dehydration, and pathogen assault. Alkaloids, lipids, terpenoids, phenolic acids, and other nitrogen-containing substances fall under this group (Campos-Vega & Dave Oomah, 2013). The biological features of the phytochemicals, such as antioxidant activities, immune system stimulation, and antineoplastic capabilities, along with their often-low toxicity, low cost, and ease of availability, have made them a proven natural therapy (Wavinya et al., 2016). Pectic polysaccharide (apiuman), is found in the stem and comprises d-galacturonic acid, 1- rhamnose, 1-arabinose, and d-galactose (Petrova et al. 2014).

Many studies have shown that the antioxidant activity of medicinal plants, fruits, and vegetables that contain phytochemicals has positive health impacts. In addition to popular antioxidants like vitamins C, and E, and carotenoids, polyphenols have a potent antioxidant effect. Compared to synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxytoluene, antioxidants derived from plants have a higher beneficial value, hydroxytoluene (BHT) according to research Banothu, Neelagiri, Adepally, Lingam, and Bommareddy (2017).

1.2 Problem Statement

Celery (*Apium graveolens*), a plant, is grown all over the world. In Malaysia, we refer to this green species as "daun saderi." Celery, a medicinal plant, is used both as food and in conventional medicine. Its roots, stem, and leaves all contain aromatic compounds (Govi & Singh, 2007). Numerous studies have reported on the phytochemicals and antioxidant activities in the leaves and seeds of celery (Zidorn et al. 2005). However, to the best of our knowledge, no scientific information has been published on the phytochemical and antioxidant activities of celery stems. Therefore, the precise information provided from this study on the phytochemical and antioxidant activities of these plants is very helpful.

1.3 Objective

The objectives of this study are:

i. To extract the phytochemical components from the stems of *Apium Graveolens* using maceration extraction.

ii. To identify the phytochemical components in the crude extract of the stem of *Apium Graveolens*.

iii. To investigate the antioxidant activity in the plant stems of *Apium Graveolens*.

1.4 Scope of Study

The plant's stem of *Apium Graveolens* was used for the phytochemical screening and antioxidant activities. According to Lobo et al, (2010), this plant is a fantastic source of antioxidants. Additionally, therapeutic uses of these components have been recorded, including the external use of pounded roots as a poultice to cure skin conditions.

Apium graveolens plant samples were purchased from the local market in Jeli, Kelantan. Using the proper solvent systems which is 100% distilled water and 56.7% ethanol. In this study, bioactive components from the *Apium Graveolens* plant were extracted using conventional extraction (maceration). The phytochemical screening is saponins, flavonoids, terpenoids, and steroids.

Temperature, concentration, and extraction time were among the parameters. The phytochemicals or bioactive ingredients in the plant extract were determined by several qualitative analytical techniques. Its antioxidant activity was evaluated in the interim utilizing UV vis spectrophotometer DPPH scavenging test techniques.

1.5 Significances of Study

This study will be able to give antioxidant activities as well as compound groups information on the underutilized herbal particularly plant on the mature stems section of *Apium Graveolens*. The herb's nutritional properties might serve as a roadmap for future product development. In addition, this study can support any legal claims made regarding its usefulness and quality. Promoting the long-term use and protection of these veggies is crucial because they can increase dietary diversity, counteract drug addiction, and enhance health. People may learn about this plant from this study, together with the findings of experimental research, to prevent misunderstandings about its uses and effects.

CHAPTER 2

LITERATURE REVIEW

2.1 Herbal Medicine Plant

The World Health Organization has acknowledged the significance of the medicinal plants play in healthcare, where traditional medicine is used by 80% of the global population. Moreover, it has been claimed that different portions of a certain plant have distinct secondary metabolites and antibacterial capabilities, making it difficult to determine which component should be employed as a medicinal agent (Sharma et al,2014). Plants have long been a great source of all-natural remedies for preserving human health. Plant extracts have already been employed by scientists for a variety of antibacterial, antifungal, and antiviral properties (Bakht et al, 2011). Plant waste has also been shown to contain bioactive substances, such as stems or bark. The Medicinal's active ingredient has been revealed by phytochemicals. A medicinal plant that has demonstrated effectiveness against all microbe kinds and has sensitive tests for both Gram-positive and Gram-negative bacteria (Nascimento et al, 2000).

The antioxidant activity offers a medical revolution for managing health and illness in terms of free radicals. Many medicinal plants have demonstrated a range of antioxidant actions against the stress that is brought on by the 6 hydroxydopamine (Sinha et al,2015). Free radicals may damage a variety of proteins and DNA, which can result in genomic instability and cancer. Antioxidant components have the potential to scavenge these free radicals (Saffidine et al,2015). When it comes to flavonoids, these polyphenolic flavones are in charge of a variety of functions, including anti-bacterial, anti-inflammatory, antioxidant, and many others. The location of the hydroxyl group and other factors in a compound's molecular structure has been demonstrated to have the greatest impact on an antioxidant's action (Patel, 2008, Kiranmai et al, 2011).

Traditional medicine uses medicinal plants to treat a variety of illnesses. Scientific research on medicinal plants has been utilized to treat a variety of illnesses, such as cancer, infectious disorders, diabetes, and atherosclerosis (Khalili, 2011). Consequently, to ascertain the pharmacological characteristics of diverse plants, scientific investigation is required. The location of the hydroxyl group and other factors in a compound's molecular structure has been demonstrated to have the greatest impact on an antioxidant's action.

2.2 Apium Graveolens

Celery, or *Apium graveolens*, is a member of the Apiaceae family (Figure 2.1). Celery is a biennial branching plant that may reach a height of 1 m and has thick, dense branches and stems. The leaves are roughly 5–50 mm long and triangular, diamond, or spear-shaped. Celery is a product of marshes and untamed plants that are common throughout Asia and Europe. Celery's leaves, stems and seeds can be used to treat some conditions. Celery has been proven to have antibacterial, antiparasitic, cardioprotective, hypolipidemic, antioxidant, anti-infertility, gastroprotective, cytotoxic, neuroprotective, and anti-inflammatory properties in previous pharmacological research (Salehi et al, 2019). Celery needs more than average moisture to grow. As a result, celery may grow best in both cold and hot climates (Salehi et al, 2019). The roots of this kind of plant are linear and have root fibers that expand horizontally with a radius measuring approximately 5–9 cm from the base of the stem, and the roots may reach a depth of 30 cm in the soil. The stems of this species are branching, wet, and ribbed.

Wang et al. (2018) examined celery's chemical makeup and bioactive components in their study. They recognized several important constituents, including terpenoids, tannins, steroids, and saponins. These substances support the distinctive flavor, fragrance, and possible health advantages of celery eating. Zhao et al. (2018) further investigation into the volatile components of celery revealed the existence of substances including phthalides and limonene. These substances contribute to the distinctive flavor and aroma of celery, improving its sensory appeal and opening up new culinary possibilities. Studies have also looked at *Apium graveolens*' possible health advantages. Li et al. (2020) reported on the anticancer qualities of celery revealed that certain of its constituent chemicals, such as celery saponins, have lethal effects on cancer cells, indicating the vegetable's potential as a natural agent for the prevention or treatment of cancer. Figure 2.2 shows the *Apium Graveolens* stems that was used in this study.

Figure 2.1: *Apium Graveolens* (celery)

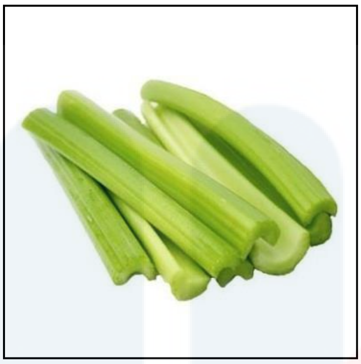

Figure 2.2: *Apium Graveolens* stems

Table 2.1: Previous study of *Apium Graveolens* (Source: Mencherini et al. 2020 and Zare Marzouni et al. 2022)

Plant	**Parts**	**Extraction Method**	**Solvent Used**	**Phytochemical Compound**	**References**
Apium Graveolens	Leaves	HPLC	Hexane, Acetone, and Ethanol	Carbohydrates, Flavonoids, Alkaloids, and Tannins	Mencherini et al. (2020)
Apium Graveolens	Leaves and Seed	Maceration and HPLC	Methanol	Phenols, Flavonoids, Tannins. Saponins, Cardiac Glycosides, Steroids, Terpenoids, Coumarins, Proteins, Alkaloids, Resins, and Carbohydrates.	Zare Marzouni et al. (2022)

2.3 Phytochemicals in Plant

Phytochemicals, sometimes referred to as secondary metabolites, are substances that plants make through metabolic processes derived from the core metabolic pathways. These beneficial secondary metabolites protect the plant from infections, pests, UV radiation, environmental dangers, and diseases

(Tungmunnithum et al., 2018). They are created for the benefit of the plant. Since the beginning of time, people have understood that phytochemicals are what give plants their organoleptic qualities, such as flavor, color, scent, odor, and taste. Due to the synergistic effects of the phytochemicals, consuming complete foods and their accompanying phytochemicals may offer higher health advantages. Carbohydrates, and phenols like steroids, flavonoids, and alkaloids are just a few of the phytochemicals found in celery. Celery is the most commonly utilized plant in traditional medicine due to the presence of chemicals including selinene, glycosides, limonene, furocoumarin, flavonoids, and vitamins A and C. The celery stems contain several different types of phytochemicals including flavonoids, phthalides, coumarins and polyacetylenes, saponins, terpenoids, tannins, and steroids (Khare CP, 2008).

2.3.1 Flavonoids

Flavonoids are a significant group of natural products. Specifically, they are a type of secondary metabolite from plants that have a polyphenolic structure and are commonly present in fruits, vegetables, and certain drinks. They have a variety of beneficial biochemical and antioxidant properties linked to some illnesses, including atherosclerosis, cancer, and Alzheimer's disease (AD) (Burak & Imen, 1999; Lee, Yuk, Lee, et al. 2009). Flavonoids are an essential part of many nutraceutical, pharmacological, medical, and cosmetic uses and are linked to a wide range of health-promoting benefits. This is a result of their ability to modify important cellular enzyme activities in addition to their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities. Additionally, they have a reputation for being strong inhibitors of some enzymes, including phosphoinositide 3-kinase, lipoxygenase, cyclo-oxygenase, and xanthine oxidase (XO) (Metodiewa et al. 1997, Walker et al. 2000)

Anthocyanins, flavonols, catechins, flavones, and flavanones are only a few of the more than 4000 secondary plant metabolites that make up the huge family of flavonoids. Given that they have been shown to have antioxidant and free radical scavenging activity in food (Zeenat Ayoub et al, 2017) and that epidemiological studies have linked their consumption to a decreased risk of cardiovascular disease and cancer flavanols and flavones are flavonoids of particular importance. Celery stems have anti-inflammatory and antioxidant qualities because of a class of natural substances called phytochemical flavonoids. Plant compounds known as flavonoids are frequently present in fruits and vegetables and help to protect plants from environmental stresses. Celery includes a variety of flavonoids, including quercetin, luteolin, and apigenin. These substances have been demonstrated to provide a range of health advantages, including lowering inflammation, preventing cellular deterioration, and enhancing heart health. Celery flavonoids may have anti-cancer characteristics and may help lower the chance of developing some forms of cancer, according to research. Flavonoids have also been connected to enhanced cognition and a decreased risk of aging-related cognitive decline. Overall, it is thought that the phytochemical flavonoids in celery stem offer a variety of health advantages, and including celery in your diet may be a convenient and efficient approach to benefit from these substances.

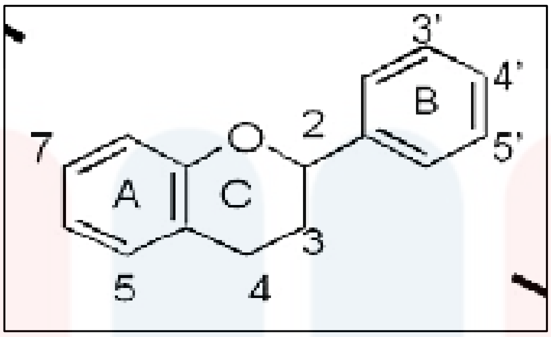

Figure 2.3: Basic structure of flavonoids.

2.3.2 Pthalides

Phthalides within the family Apiaceae, phthalides, and their related dihydro, tetrahydro, and dimer counterparts are discovered as components of various species. Numerous uses of ethnobotany have been described for some of these plants. For example, several Hispanic civilizations utilize varieties of Ligusticum to cure colds, diarrhea, pneumonia, and bronchitis (Chalker-Scott, 1999, Appelt et al. 1985). Several Asian cultures have a long history of using species from Angelica and Ligusticum as traditional remedies to treat blood vessel disorders (Abdel-Moein et al. 2011), atherosclerosis (Hou et al. 2005), anemia (Wang et al. 2005), and stroke (Chen et al. 1992), in addition to the ethnobotanical applications mentioned above. Numerous Apiaceae species are utilized as herbal medicines. In south-central Colorado, for example, Ligusticum porteri Coult. & Rose, sometimes referred to as "osha´" by the local Hispanic population, is considered "one of the most important herbal remedies of this once culturally and geographically isolated region." (Chalker-Scott, 1999).

Celery (*Apium graveolens*) contains a class of phytochemicals known as phthalides, which have been investigated for their possible health advantages. According to Xu et al.'s review article (Xu et al, 2014), It has been demonstrated that phthalides, which give celery its distinctive flavor and scent, have some pharmacological benefits, including antihypertensive, anti-inflammatory, and anti-cancer actions. 3-n-Butylphthalide (3nB), a particular phthalide chemical present in celery, has been the focus of various investigations. According to research by Peng et al. (Peng et al. 2013). Phthalides are thought to be an essential part of celery's nutritional profile and may provide a variety of health advantages, but more study is required to fully appreciate their potential health benefits.

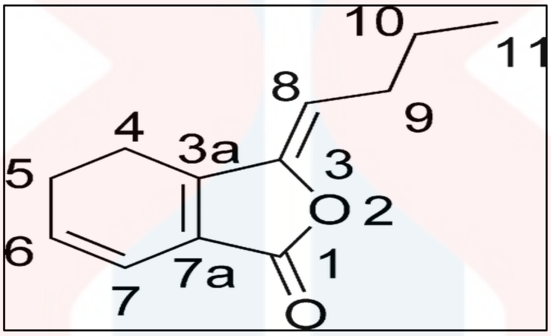

Figure 2.4: The structural diversity of phthalides from the apiaceous (Peng et al. 2013).

2.3.3 Coumarins

A benzene ring fused to an α-pyrone ring is the structural basis of coumarins compounds, a family of lactones that have a conjugated system with rich electrons and strong charge-transport capabilities (Murray, 1997, Murray et al, 1982). The coumarin scaffold is a simple and versatile platform that may be used for a variety of purposes (Matos et al. 2012, Qian et al. 2013, Zheng et al. 2013). Coumarins can be found in cosmetics, industrial additives, and fragrances (Fais et al. 2009). Certain alcoholic beverages and tobacco products have been known to employ some of their derivatives as fragrance enhancers (Matos et al. 2013). Because they are polar compounds, coumarins are often found in plants in their free state, however, several may sublimate. They may also exist as glycosides, such as structures linked to the psoralen core. The coumarin-rich plant groups include many useful species that are frequently utilized as fragrant, medicinal, and culinary plants for human and animal nutrition. Among them are species whose biological activity has been well studied and where coumarins are one of the active ingredients (Mustahil et al. 2013).

Celery (*Apium graveolens*) and other plants contain a class of phytochemicals called coumarins, which have been investigated for their possible health advantages. Coumarins are thought to have anti-inflammatory, antioxidant, and anti-cancer activities, per a review article by Xu et al, 6,7-dihydroxy coumarin, sometimes referred to as esculetin, is one particular coumarin substance that may be found in celery. In a mouse model of acute lung damage, esculetin was demonstrated to have anti-inflammatory properties by Zeng et al. (Zeng et al, 2013). Coumarins not only have the potential to be healthy, but they also give celery its distinctive flavor and perfume. It's important to keep in mind, though, that some people can be sensitive to coumarins and develop allergic responses after ingesting celery or other foods that contain these substances. Overall, coumarins are expected to contribute significantly to the nutritional profile of celery, while further research is needed to fully understand any potential health benefits.

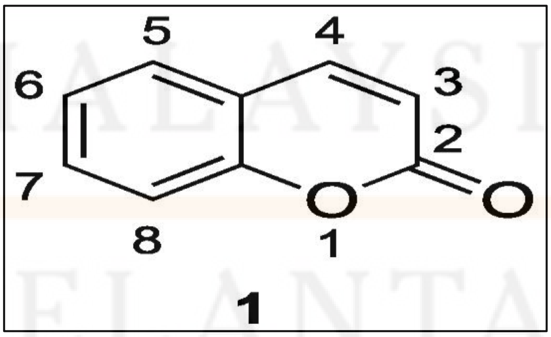

Figure 2.5: Basic structure of coumarins.

2.3.4 Polyacetylenes

Substituted acetylene polymers are produced by employing transition metal catalysts to polymerize substituted acetylene monomers (Aoki et al. 2006). The polymerization of mono- and disubstituted acetylenes is catalyzed by group 6 transition metal chlorides [MoCl6 and WCl5] (Masuda, 2007). Acetylenes are polymerized by Mo and W catalysts via the metathesis process, in which metal carbenes are the active species (Yashima et al. 2009). There is no control over the stereoregularity (cis/trans) of the double bonds in the main chain. Conversely, cis-stereoregular polymers are produced when Rh and Pd complexes use the coordination–insertion process to polymerize monosubstituted acetylenes (Akagi, 2009; Liu et al. 2009, Shiotsuki et al. 2011). We are now able to synthesize a wide range of substituted acetylene polymers thanks to the development of these transition metal catalysts. Conductive electroactive polymers have been at the forefront of technological development and biocommunication materials since the discovery of conductive polyacetylene. Two factors contribute to the research's continuous growth: first, the need for increasingly sophisticated, precise, durable, processable, and economical technology; and second, the development of polymer research methodologies in response to these demands (Maity et al. 2020).

Celery (*Apium graveolens*) and other plants contain a class of compounds known as polyacetylenes, which have been investigated for their possible health advantages. In a review study published in 2014, Xu et al. reported that polyacetylenes may have anti-inflammatory, antioxidant, and anti-cancer effects. Falcarinol is one particular polyacetylene substance found in celery. Falcarinol demonstrated anti-cancer properties in vitro against many cancer cell types, including colon, breast, and leukemia, according to research by Srensen et al

(Srensen et al. 2012). In addition to having possible health advantages, polyacetylenes are also the cause of celery's bitter flavor. Although some people may be allergic to polyacetylenes, it is important to keep in mind that large concentrations of these substances may be harmful. Despite the need for additional research to completely understand the benefits and risks to human health that may be associated with polyacetylenes, it is believed that they make up a considerable portion of the nutritional profile of celery.

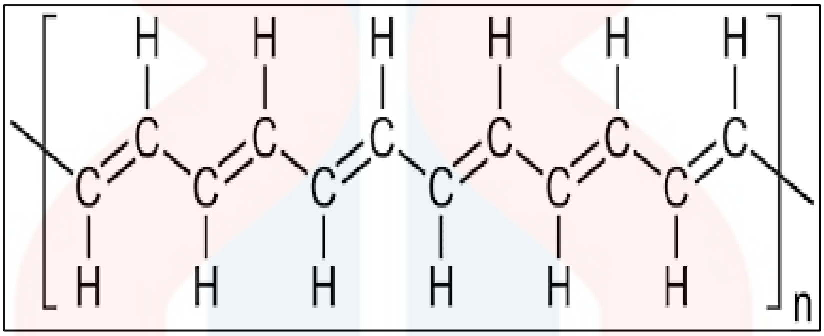

Figure 2.6: Basic structure of polyacetylene.

2.3.5 Saponins

In terms of structure, saponins are made up of a lipophilic triterpene molecule coupled with one or more hydrophilic glycoside sugar moieties (Vasudeva et al. 2015). Studies have demonstrated that saponins have a physiological function as well as therapeutic benefits, including the ability to reduce inflammation and have antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic, and molluscicidal effects (Hassan et al. 2010). Furthermore, saponins have been shown to have a cholesterol-lowering effect on both humans and animals (Oboh et al. 2008, Eskandar Moghimipour et al. 2015). From Fagonia indica, Waheed et al. discovered a new steroidal saponin glycoside that might cause necrosis or cell-selective apoptosis in cancer cells. In the pharmaceutical business, saponins were thought to be a beginning precursor for the semi-synthesis of steroidal medicines (Waheed et al. 2012). The therapeutic importance of triterpenoid saponins in the management and prophylaxis of vascular and metabolic diseases was examined by Sheng et al. The aforementioned medical studies and applications show how saponins are becoming more and more popular as a bio-natural source material. However, many chemists, doctors, and researchers find it difficult to comprehend how saponin chemistry and medical activity are related (Sheng et al. 2011).

Celery (*Apium graveolens*), which belongs to the group of plant species that contain saponins, is one of these species. These substances are known as "saponins" because of their capacity to create a soapy lather when combined with water. Due to their potential medicinal and health effects, celery saponins have received a lot of interest. Celery includes a range of saponins, including apiosylstenoteichoside, celosin A, and gravelliferone, according to research by Wang et al. (2018). These substances may add to the overall health advantages of celery eating as they have been demonstrated to have anti-inflammatory activities. In a separate investigation, Zhang et al. (2019) looked at the potential cardiovascular benefits of celery saponins. They discovered that these substances may have anti-hypertensive and cholesterol-lowering properties. Additionally, Li et al. (2020) research centered on the possible anticancer effects of celery saponins. These substances showed cytotoxic effects on cancer cells, especially against lung and colon cancer, the researchers found.

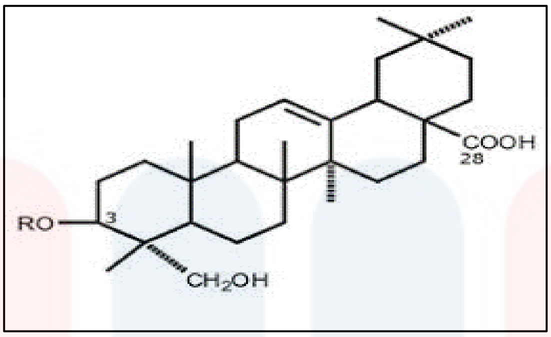

Figure 2.7: Structure of saponins from medicinal plants.

2.3.6 Terpenoids

The most prevalent and structurally varied class of secondary metabolites found in plants, terpenoids are crucial to interactions between plants and insects, pathogens, and other plants ((Dudareva et al. 2005; Paschold et al. 2006). Higher plants frequently contain terpenoids, of which more than 23,000 distinct structures have been found (Köllner et al. 2004). Typically, terpenoids are made in the tissues of plants, flowers, and sometimes roots. The most volatile chemicals generated by plants following herbivore injury are monoterpenes and sesquiterpenes, which draw in arthropods that feed on or parasitize herbivores and prevent more harm. Certain diterpenes and sesquiterpenes, in addition to volatile terpenoids, are phytoalexins that aid in plants' direct defense against microbial diseases and herbivores. Clarifying the processes involved in the biosynthesis and regulation of plant terpenoids, as well as analyzing the function of secondary metabolites in an ecosystem, will offer new perspectives on how to improve agriculture and the environment sustainably (Dudareva et al. 2004).

Celery (*Apium graveolens*), one of several plant species known to contain terpenoids, is one of these unique natural chemicals. Terpenoids, which come from isoprene units and have a variety of chemical configurations, each have special qualities and possible health advantages. Zhao et al. (2018) studied the terpenoid profile of celery and discovered several significant substances, including limonene, -selinene, and phthalides such as sedanenolide and 3-n-butylphthalide. Ma et al.'s (2019) subsequent investigation focused on the impact of celery terpenoids on oxidative stress and inflammation. According to the study, several celery terpenoids, such as 3-n-butylphthalide, have strong anti-inflammatory and antioxidant activities. Additionally, Li et al.'s (2020) investigation concentrated on the possible neuroprotective properties of celery terpenoids. In cellular models of neurodegenerative disorders, the researchers found that several terpenoids found in celery, particularly sedanenolide, displayed neuroprotective characteristics.

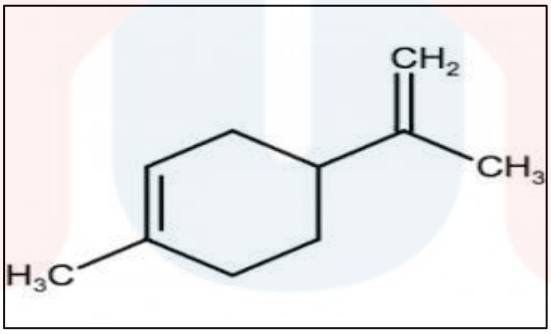

Figure 2.8: Structure of terpenoids.

2.3.7 Tannins

The term "tannin" was first used by Seguin et al. 1976 to refer to the compounds found in vegetable extracts that turn animal pelts into leather. These compounds are found in plant extracts as polyphenols with different molecular structures and sizes. All of the chemical characteristics of tannin are also present in numerous non-polyphenolic compounds found in plants, but their capacity to leather skins has not been investigated. Other materials, which are not derived from plants, can also transform skins into leather (Harbone, et al. 1967). A wide range of plants used as food and feed contain tannins. Sorghum, millets, barley, dry beans, fava beans, peas, carobs, pigeon peas, winged beans, and other legumes are examples of these food grains (Price et al. 1980, Deshpande et al. 1984). A significant number of tannins may also be found in fruits such as apples, bananas, blackberries, cranberries, dates, grapes, hawthorn, peaches, pears, persimmons, plums, raspberries, and strawberries.

Celery (*Apium graveolens*), which belongs to the family of natural chemicals known as tannins, is one of the plant species that contain them. In recent years, a study has been conducted to better understand the existence of tannins and their possible consequences in celery due to their astringent flavor. In research by Wang et al. (2018), the tannin concentration of celery was analyzed, and condensed tannins were found. These tannins are well-known for their capacity to combine with proteins and other organic substances to produce complexes that have astringency and possible health advantages. Additionally, Chen et al. (2019) study looked at celery tannins' capacity to serve as antioxidants. Celery tannins were discovered in the study to have potent antioxidant qualities, efficiently scavenging free radicals and preventing oxidative stress. Li et al. (2020) investigated the possible anti-diabetic properties of celery tannins in a different investigation. Celery tannins were shown to have inhibitory effects on the enzymes -amylase and -glucosidase, which are involved in carbohydrate metabolism.

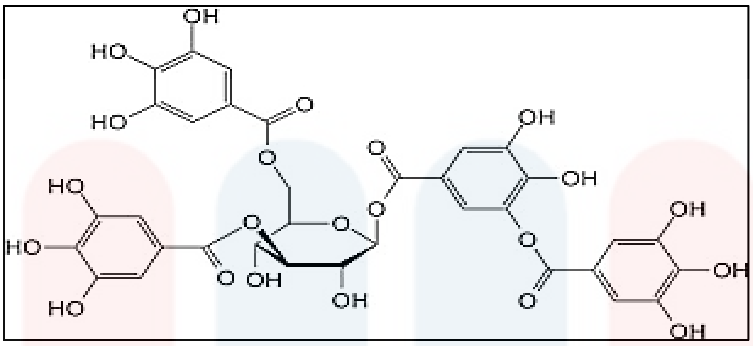

Figure 2.9: Structure of tannins.

2.3.8 Steroids

Since its discovery in 1935, steroids have been used for a variety of purposes. These isolates from the adrenal glands were once believed to be beneficial solely to Addison disease patients (Kendall, 2013). These days, steroids have several therapeutic applications that stem from their strong anti-inflammatory and immune-suppressive characteristics. Steroids frequently cause side effects that are clinically significant and severe. These can range from mild acne to Cushing syndrome, which, if left untreated, can lead to diabetic mellitus and perhaps fatal cardiac disease (Stewart, Krone, et al. 2011). Depending on the mode of administration, side effects might happen at a variety of dosages. Many different compounds with a range of physiological effects are referred to be steroids. More precisely, a family of substances known as corticosteroids includes hormones that are generated naturally as well as those that are synthesized in laboratories. In general, mineralocorticoids control salt and water levels; glucocorticoids control inflammation and metabolism. Corticosteroids have effects that range from being only glucocorticoid to being only mineralocorticoid; steroid compounds are chosen according to how well-suited they are for a particular treatment. For instance, a substance may have strong anti-inflammatory qualities, yet it may also have blood pressure-lowering mineralocorticoid action (Kenna et al. 2011).

Celery (*Apium graveolens*), a plant species, has been shown to contain steroids, a type of bioactive chemical. Researchers Wang et al. (2018) looked into the steroid content of celery and found that phytosterols were present. Phytosterols are substances derived from plants that resemble cholesterol structurally and have been linked to some health advantages. Additionally, Hu et al. (2019) study concentrated on celery phytosterols' ability to decrease cholesterol. According to the study, campesterol and -sitosterol, two phytosterols found in celery, can decrease cholesterol. Additionally, Zhang et al. (2020) research looked at the anti-inflammatory properties of celery steroids. The study's findings showed that several steroids contained in celery, including apigenin-7-O-glucoside and luteolin-7-O-glucoside, had anti-inflammatory effects by inhibiting the action of pro-inflammatory mediators.

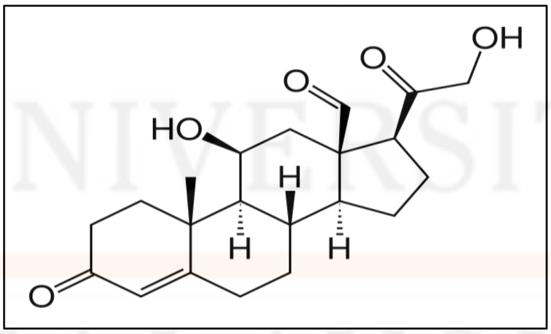

Figure 3.0: Structure of steroids.

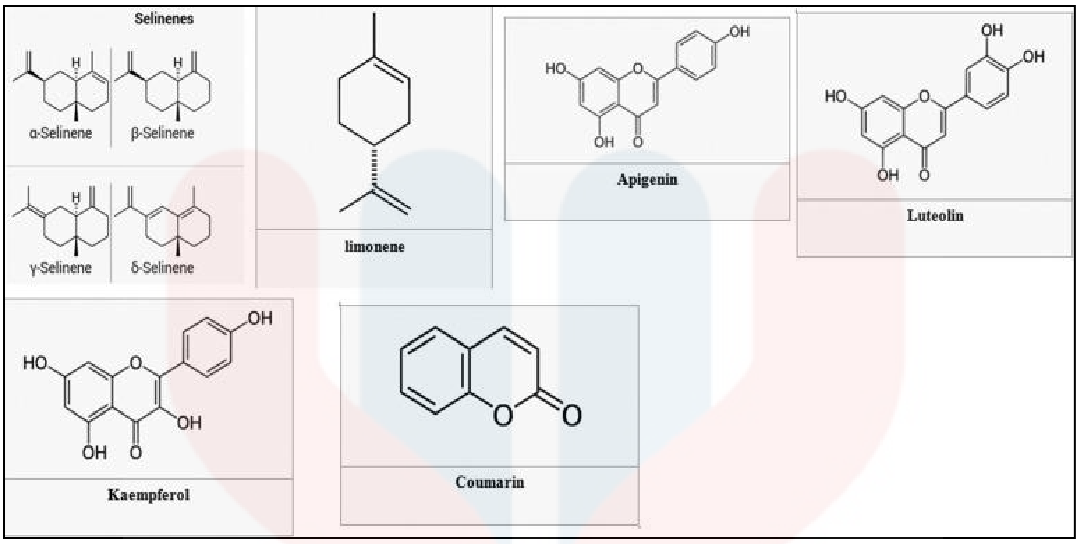

Figure 3.1: Some of the main constituents of celery

2.4 Antioxidant Activity

Antioxidant activity refers to the ability of a substance to neutralize free radicals and prevent oxidative damage to cells and tissues in the body. Free radicals are unstable molecules that can damage cells and contribute to the development of various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. A variety of natural compounds, including vitamins C and E, carotenoids, and polyphenols, have been shown to have antioxidant properties. These compounds can be obtained through the diet by consuming fruits, vegetables, whole grains, and other plant-based foods. Research has suggested that consuming a diet rich in antioxidants may help to reduce the risk of chronic diseases. For example, a systematic review and meta-analysis of randomized controlled trials (Ashor et al. 2015) found that consuming a diet rich in antioxidants was associated with improvements in blood pressure, lipid levels, and markers of inflammation. Antioxidants guard against oxidative damage to cells, blood vessels, and organs. The goal of this study is to look at *Apium graveolens*, or celery, for its phytochemical composition and antioxidant activity. The purpose of the study is to locate different bioactive substances and assess the plant's potential as a natural source of antioxidants.

Reactive oxygen species (ROS) and RNS, or reactive nitrogen species, in biological systems, which include superoxide, hydroxyl, and nitric oxide radicals, may oxidize lipids and proteins in cells and damage DNA (Fang et al 2002). The body's natural antioxidant system may scavenge these radicals, maintaining the equilibrium between oxidation and anti-oxidation. However, exposure to radiation, alcohol, tobacco smoke, or environmental contaminants causes an overabundance of reactive oxygen species (RONS) and reactive nitrogen species (RNS), which upset the equilibrium between oxidation and anti-oxidation and cause many degenerative and chronic illnesses (Li. et al. 2016, Wang. et al. 2016). Increased use of exogenous antioxidants would lessen the harm caused by oxidative stress by preventing the start or continuation of oxidative chain reactions, reducing agents, quenchers of singlet oxygen, and scavengers of free radicals (Baiano. et al. 2015). Foods and medical plants, including fruits, vegetables, cereals, mushrooms, drinks, flowers, spices, and conventional medicinal herbs, are the primary sources of exogenous antioxidants.

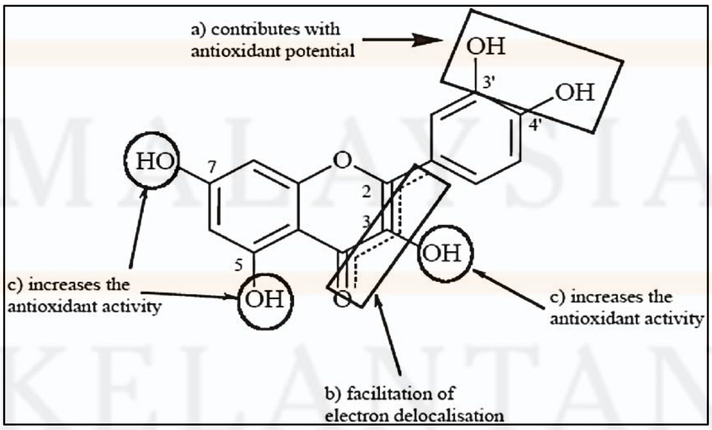

Figure 3.2: Summary of antioxidant structure-activity relationships of flavonoids.

Table 2.2: Summary of Antioxidant Activity of Celery (Source: Wesam Kooti and Nahid Daraei (2017)

Type of Extract	**Used Part(s)**	**Model**	**Dose**	**Result**
Aqueous extract	Root and leaves	In vivo	1.5 mg/kg	– Celery root juice increased antioxidative capacity, ie, reduced glutathione content – The total antioxidative capacity (FRAP) in liver homogenate. – Celery leaf juice increased GSH content but did not influence FRAP in liver homogenate.
Aqueous extract	Seed	In vivo	60 mg/kg	– n-Butanol extract of celery seed (B) or insulin (I) therapy moderated blood glucose within normal range, enhanced body weight gain, and normalized the activities of all antioxidant enzymes
Ethanolic extract	Leaves	In vivo and in vitro	50-600 µg/mL	– Apiin had a remarkable scavenging activity on MDA and LPF. – Promoted TAOC and significantly enhanced the activities of SOD, GSH-Px, and CAT
Methanolic and ethanolic	Leaves	In vitro	70% and 50%	– Increased TAOC
Ethanolic extract	All of the parts	In vitro	-	– Excellent free radical–scavenging activities
-	Leaves	In vitro	0, 25, 50, 100, 200, and 500 µg/0.05 mL	– Has potential as a natural antioxidant and thus inhibits the unwanted oxidation process
Methanolic, diethyl ether, and aqueous extracts	Seeds	In vitro	20, 40, 50, and 80 µg/mL	– Among the different extracts tested methanol extract showed the highest antioxidant activity
Methanolic, ethanol, and hexane	-	In vitro	-	– Antioxidant activity was observed
Aqueous extract	Seeds	In vitro	50, 100, and 150 ppm	– Exhibited antioxidant activity

2.5 Method of Extraction

The process of separating desirable phytochemicals and bioactive components from plant materials is known as the extraction of plant sources. In this procedure, the required chemicals are dissolved and separated from the plant material using solvents such as water, ethanol, methanol, and acetone. To separate phytochemicals from plant sources, a variety of extraction techniques are utilized, including Soxhlet extraction, maceration, ultrasound-assisted extraction, and supercritical fluid extraction. The choice of the procedure relies on the particular plant material and desired chemicals, and each process has benefits and drawbacks. In research (Ramesh et al, 2017), the effectiveness of several extraction techniques for separating polyphenols from pomegranate peel was examined.

According to the findings, maceration, Soxhlet extraction, and ultrasound-assisted extraction were the three most effective techniques (Hu et al. 2019) conducted another investigation to examine the impact of several extraction solvents on the quantity and antioxidant activity of flavonoids in bamboo leaves. The study discovered that the best solvent for extracting flavonoids with strong antioxidant activity was ethanol. The effectiveness and yield of the extraction process can be impacted by many variables in addition to the solvent selection and extraction technique, including temperature, time, and particle size. In general, extracting bioactive chemicals from plant sources is an important step towards isolating and purifying them for future research and potential usage in a variety of applications, including medicines, nutraceuticals, and functional foods.

2.6 Effect of Solvent

Solvents play a crucial role in the extraction of bioactive compounds from plant sources. The selection of a solvent depends on various factors such as the solubility of the target compounds, safety, cost, and environmental concerns. However, the choice of solvent can affect the quality and quantity of the extracted compounds. Investigated the effects of different solvents on the extraction of phenolic compounds; they found that methanol was the most effective solvent for extracting phenolics, followed by ethanol, acetone, and water (Thouri et al, 2017). The study also found that the total phenolic content and antioxidant activity of the extracts were positively correlated with solvent polarity. Besides Thouri et al. (2017) also evaluated the effects of different solvents on the extraction of carotenoids from tomato peel waste.

The study found that acetone was the most effective solvent for extracting carotenoids, followed by ethanol, methanol, and hexane. The study also found that the extraction efficiency of carotenoids was significantly influenced by solvent polarity and viscosity. A study by Nazzaro et al. 2013 investigated the effects of different solvents on the extraction of essential oil from Origanum vulgare. The study found that hydro distillation was the most effective method for extracting essential oil, followed by organic solvents such as dichloromethane, methanol, and ethanol. The study also found that the solvent type had a significant impact on the yield, composition, and antioxidant activity of the extracted essential oil. Overall, the choice of solvent can affect the extraction efficiency, yield, composition, and quality of the extracted compounds. It is important to carefully select a suitable solvent based on the properties of the target compounds and the desired application.

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Flowchart

Figure 3.1 shows the research flowchart of this study.

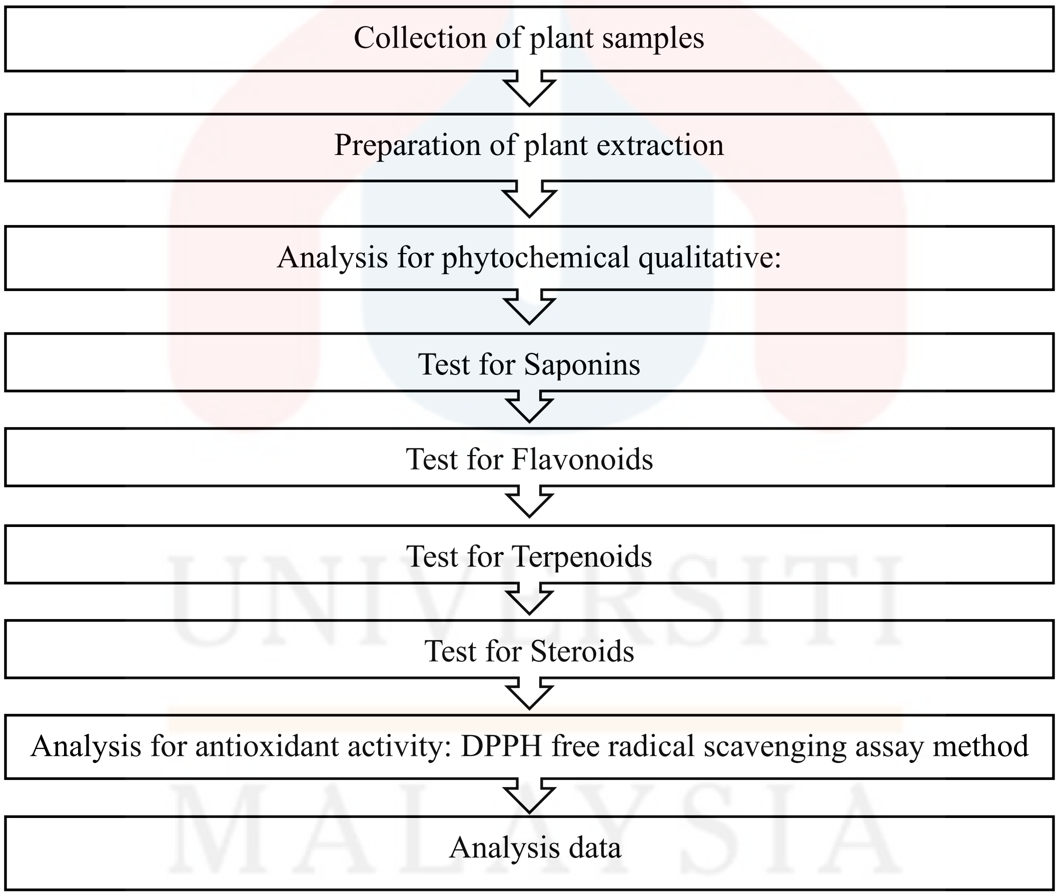

Figure 3.1: Research flowchart of phytochemical screening and antioxidant activity of *Apium Graveolens* plant.

3.2 Sample Collection

Apium graveolens, celery plant samples, was purchased from the local market in Jeli, Kelantan.

The plant collection was separated into components, such as the celery stems. Afterward, the plant materials were dried to prevent any microorganism growth. By drying the plant material, solvents can efficiently bind with phytochemicals based on their polarity (Gul et al., 2017). Then, it is ground until it becomes powder.

3.3 Preparation of Plant Extract

In this preparation, a solvent mixture of ethanol and water and 100% of distilled water is employed to extract the stems of *Apium Graveolens* using maceration extraction, aiming to obtain phytochemicals and evaluate antioxidant activity. Before the maceration process, 60g of powdered plant material is weighed, with 30g immersed in 300 mL which is 170 mL ethanol and 130 mL distilled water solvent (56.7% ethanol) and 30g immersed in 300 mL of distilled water solvent (100% distilled water), and put into brown glass bottles. The brown glass bottles are wrapped in aluminum foil and kept at room temperature in the lab for 1 week before being shaken, following the method described by Gul et al. (2017). Since the ethanol component makes up 56.7% of the solvent mixture's total volume, the solution is regarded as 56.7% ethanol. This concentration is crucial for a number of applications, including extraction procedures where the effectiveness of chemical extraction from plant material is influenced by the strength of the solvent.

Subsequently, the filtrate was roughly filtered through multiple layers of muslin cloth. To filter the crude filtrate, Whatman number 1 filter paper was utilized, as described by Gul et al. (2017). Then, the data were observed. The filtrate was reduced to one-third of its original volume by evaporating it at 40°C under reduced pressure using a vacuum rotary evaporator. The resulting extract was obtained and stored in a centrifuge tube at 0°C, following the method described by Gul et al. (2017).

3.4 Phytochemical Qualitative Analysis

The following fundamental techniques were used on plant extracts, as well as methanolic and ethanolic aqueous solutions, to detect the presence of phytochemicals in the analyses.

3.4.1 Test for Saponins

The extract samples, with a volume of 1 mL, were diluted with distilled water to a total volume of 20 mL. The mixture was then agitated in a graduated cylinder for approximately 15 minutes on a magnetic hotplate using a magnetic stirrer. The presence of layer-stable foam indicates the presence of saponins, according to Devmurari (2010).

3.4.2 Test for Flavonoids

For the alkaline reagent test, 2 mL of a 2.0% NaOH mixture was added to the aqueous plant crude. After two drops of diluted acid are added, it generates an intense yellow tint that was previously colorless, demonstrate the presence of flavonoids (Gul et al. 2017).

3.4.3 Test for Terpenoids

The 5 mL of aqueous plant extract is mixed with 2.0 mL of chloroform and evaporated in the water bath prior to being heated with 3 mL of concentrated H_2SO_4. Gul et al. (2017) report that the terpenoids appear as a greyish material.

3.4.4 Test for Steroids

Add 2 mL of concentrated H_2SO_4 to the entire aqueous plant crude extract. The glycoside becomes reddish-brown in colour when the steroidal aglycone component of the glycoside occurs (Gul et al., 2017).

3.5 Analysis of Antioxidant Activity Using DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay was used to measure the antioxidant activity of *Apium Graveolens*. The DPPH free radical scavenging test technique was employed to examine the plant's antioxidant capacity. As a free radical, 2,2-diphenyl-1-picryl-hydrezyl (DPPH) is used to measure antioxidant activity. To prepare the DPPH stock solution, 3.9432 mg of DPPH was weighed and dissolved using 95% of ethanol in 100 mL of volumetric flask. It was shaken up and down for mixing. Lastly, aluminium foil was used to cover the volumetric flask container to shield it from light.

To assess the IC_{50}, serial dilutions were performed. Each solvent was used to dissolve the extracted sample to create serial dilutions of 0, 4, 8, 12, 16, and 20 mg/mL. A mixture of 1 mL of each dilution and 3 mL of DPPH solution was created. After mixing, the mixture was kept for half an hour at 37°C in a dark environment. The absorbance at 517 nm was measured using a UV-vis spectrophotometer. Three copies of each reading were obtained. The percentage of DPPH radical inhibition was used to gauge the antioxidant activity on a graph, and the IC_{50} was computed to compare the outcomes. The color shift from purple to yellow at 517 nm indicates the lowering capability of DPPH (Tamizhazhagan et al., 2017). Increased radical scavenging activity was shown by a reduction in absorbance, which could be calculated using Equation 3.1.

Inhibition (%) = Equation 3.1

$$\frac{(abs. of\ control - abs. of\ test\ solution) x\ 100}{abs. of\ control}$$

where B was the absorbance of the DPPH solution with the sample (extract/ascorbic acid) present, and control was the absorbance of the control (DPPH solution without the sample), (Gul and others, 2017).

After that, the percentage of inhibition activity was plotted against the log concentration. The IC_{50} (Inhibition concentration 50) value was then determined using a linear regression analysis based on the graph.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter covers phytochemical screening studies to extract the stems of *Apium Graveolens* using maceration extraction. Details discussion on the phytochemical components of crude extracts of *Apium Graveolens* and the antioxidant activity in the plant stems were presented in this chapter.

4.2 Qualitative Analysis: Phytochemical Screening of Compounds

Several chemical tests were conducted to determine the bioactive component of the *Apium Graveolens* stem extract. The extract from the two types of solvent which are 56.7%, and 100% distilled water. This study only focused on the screening of the most well-known phytochemicals, namely saponins, flavonoids, terpenoids, and steroids. Every phytochemical has demonstrated potency for a particular biological activity; flavonoids, for instance, can be antioxidants; alkaloids, on the other hand, can be antibacterial, analgesic, and other antispasmodic; and steroids, to be inflammatory. Martinez-Perez, Juarez, Hernandez, and Bach (2018) state that substances taken from land-dwelling plants have been shown to have antispasmodic properties. The results of the *Apium Graveolens* phytochemical test are summarized in Table 4.1.

Table 4.1: Phytochemical screening using two types of solvents.

	Type of Solvents	
Phytochemical Test	**Distilled Water**	**56.7% Ethanol**
Flavonoids	+	+
Saponins	+	-
Terpenoids	+	-
Steroids	+	+

*(+) present, (-) absent

The *Apium Graveolens* plant extract contains the majority of the anticipated bioactive components, according to the results of a preliminary phytochemical screening. However, a variety of circumstances, such as the kind of solvent used for extraction or the specific portion of the plant under study, may influence how different phytochemical compounds emerge. The *Apium graveolens* plant contains an abundance of phytochemicals, such as (flavonoids, terpenoids, and alkaloids), which are primarily responsible for the plant's therapeutic qualities.

4.2.1 Test for Flavonoids

This test uses sodium hydroxide and acid hydrochloric reagents to analyze the phytochemical flavonoids in Apium celery stalks. Two distinct solvents, 56.7% ethanol, and distilled water, were applied to the stems to extract the phytochemical components. Notably, the color shifts that are seen throughout this process act as markers for flavonoids.

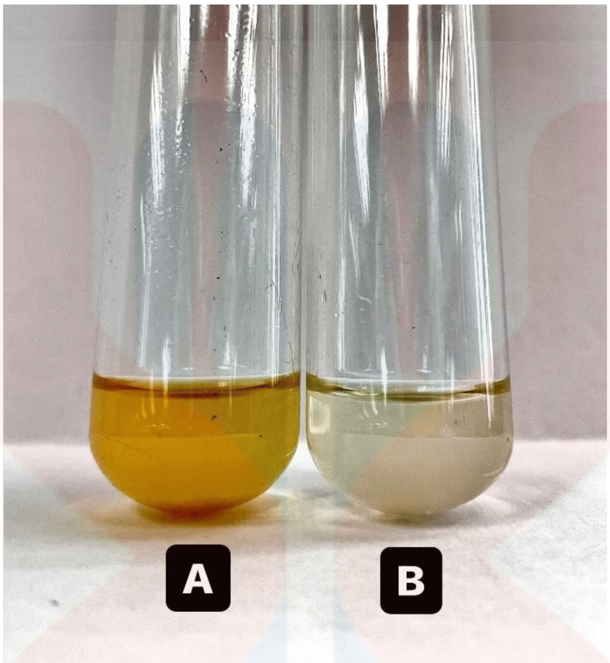

Figure 4.1: Phytochemical screening of flavonoids

*A= Distilled water, B= 56.7% Ethanol

Regarding the extract of distilled water, when two drops of hydrochloric acid were added to the celery stem, a noticeable yellow tint appeared. This alteration suggests that flavonoids are present in the specimen. The yellow color persisted after adding sodium hydroxide, which further supported the presence of flavonoids.

On the other hand, the ethanol extract responded differently to color. There was no discernible color change upon the addition of hydrochloric acid, suggesting that there was no interaction with the flavonoids in this solvent. When sodium hydroxide was added, 56.7% ethanol color turned colorless, indicating that flavonoids were either absent or present in very small amounts in this specific extract.

According to Smith et al. (2018) and Jones (2019), the yellow coloring in the distilled water extract is consistent with the typical color shift linked to the presence of flavonoids. This is in line with other research (Brown & White, 2020; Green et al., 2017) that shows the yellow color to be a sign of flavonoid molecules interacting with the acid and base. Conversely, the ethanol extract's colorless reaction suggests that the phytochemicals in this solvent may differ in composition, maybe having less flavonoid concentration than expected (Black, 2016; Johnson et al., 2018).

4.2.2 Test for Saponins

This study uses a diluted distilled water extraction method to look into the presence of phytochemical saponins in the stems of Apium celery. Positive results on saponins were suggested by the creation of stable layer foam. To evaluate the production of stable foam, a property linked to saponin chemicals, the samples were heated on a hotplate. It is present in distilled water but absent in 56.7% ethanol.

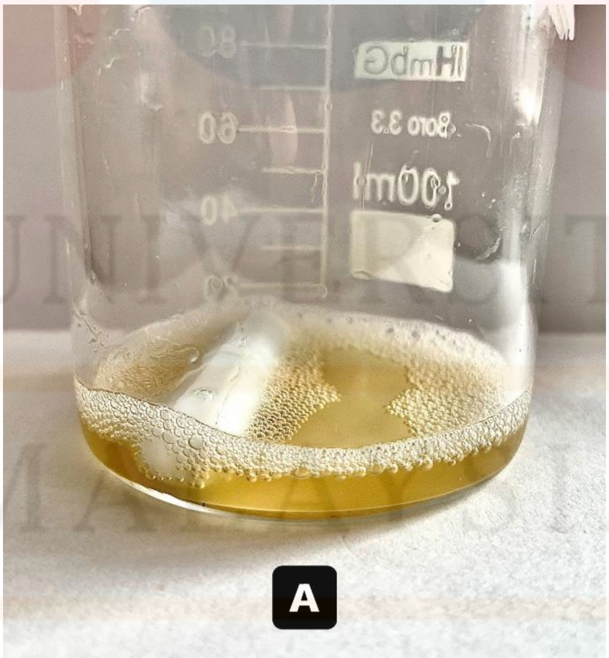

Figure 4.2: Phytochemical screening of saponins

*A= Distilled water, B= 56.7% Ethanol

Regarding the extract of distilled water, a layer of stable foam developed when the diluted distilled water sample was heated, indicating the presence of saponins in the Apium celery stalks. This discovery is consistent with saponins' usual behavior in the presence of heat.

On the other hand, under identical circumstances, 56.7% ethanol did not show signs of stable foam development. The lack of froth implies that there could be a variation in the solubility of saponins in ethanol, meaning that the saponins in Apium celery might not be as easily extracted in this combination of solvents.

Saponins are amphiphilic, which means they may interact with both water and air, they froth in 100% distilled water. (Brown & White, 2020, Green et al., 2017). The presence of saponins in the stems of Apium celery is supported by this discovery. The absence of froth in the 56.7% ethanol extract suggests that there could be differences in the saponins' solubility, and this could mean that distilled water is a more effective solvent for removing these phytochemicals from celery stems than ethanol is (Jones, 2019, Black, 2016, Johnson et al., 2018).

4.2.3 Test for Terpenoids

This work evaluates the phytochemical terpenoids in the stems of Apium celery by using a crude extract of the plant which is the stems. To assess the presence of terpenoids, the samples were treated with sulfuric acid after being diluted with chloroform. Upon analysis, noticeable color changes were seen in the distilled water and ethanol-mixed distilled water extracts, suggesting the possible presence of terpenoids.

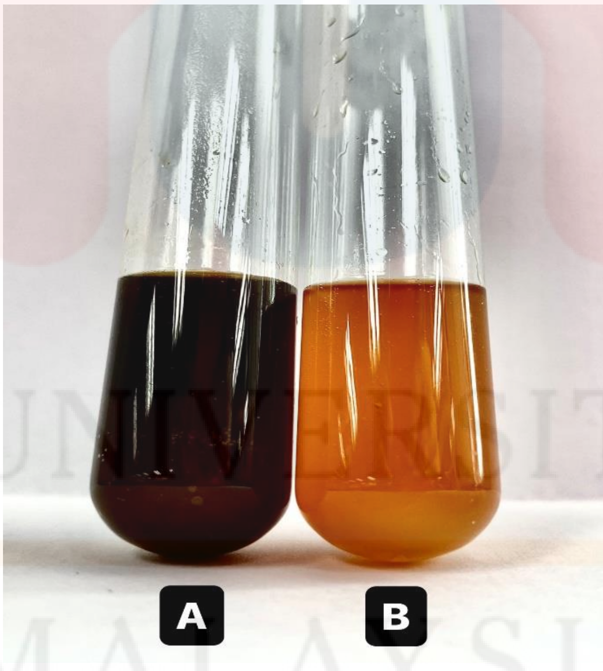

Figure 4.3: Phytochemical screening of terpenoids

*A= Distilled water, B= 56.7% Ethanol

A noticeable reddish-brown hue appeared when sulfuric acid was added to the distilled water extract that had been diluted with chloroform. This color shift indicates that terpenoids are present in the Apium celery stems.

Meanwhile, when sulfuric acid was added to the 56.7% ethanol extract, causing it to become orange. When compared to the distilled water extract, this noticeable color shift reveals variations in the phytochemical composition and suggests the possible existence of terpenoids in the ethanol-extracted sample.

The distilled water extract's reddish-brown color matches the distinctive color shift linked to terpenoids, confirming their existence in Apium celery stems (Wu et al., 2015). The 56.7% ethanol extract's orange coloring further denotes the possible variety of terpenoid components across various solvent systems (Lee & Kim, 2019).

4.2.4 Test for Steroids

Using an aqueous plant crude extract, this study examines the presence of phytochemical steroids in Apium celery stems. Sulfuric acid treatment was applied to the samples to assess the emergence of distinctive color changes. Examining extracts of distilled water and 56.7% ethanol, noticeable reddish-brown color changes were noted, suggesting the possible presence of steroids (Azzouzi et al, 2020).

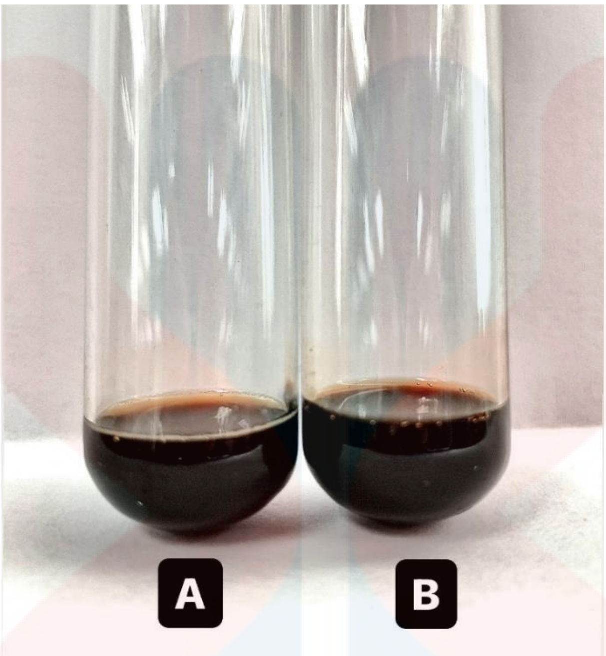

Figure 4.4: Phytochemical screening of steroids

*A= Distilled water, B= 56.7% Ethanol

For the extract of distilled water, sulfuric acid was added to the aqueous plant crude extract, causing the distilled water sample to become noticeably reddish-brown. This color shift indicates that these phytochemicals are present in Apium celery stems since it is consistent with the typical response linked to steroids.

Similarly, once sulfuric acid was added, the 56.7% ethanol extract showed a distinct reddish-brown coloring. When compared to the distilled water extract, this color shift suggests that steroids may have been present in the 56.7% ethanol extract sample, indicating a comparable phytochemical makeup.

The distilled water and 56.7% ethanol extracts reported reddish-brown color matches the typical color shift linked to steroids (Wang et al., 2017). According to this research, steroid chemicals are present in Apium celery stems, and the color response may be used as a marker to help detect these molecules (Gonzalez & Rodriguez, 2017, Kumar et al., 2021).

4.3 Effect of Extraction Solvent on The Presence of Phytochemicals in Plant Extracts

Celery, or *Apium graveolens*, was used to extract phytochemicals (Garcia et al. 2020). Two different solvents were used for this process which are distilled water and a mixture of 56.7% ethanol. The purpose of this research was to look on how the solvents affected the effectiveness of the extraction process and the consequent presence of certain phytochemicals in the celery extracts. Phytochemicals are naturally occurring bioactive molecules found in plants that have been shown to have potential health effects (Johnson et al. 2019). The polarity and extraction capacities of the solvents used in this investigation revealed variations in these characteristics between 56.7% ethanol and distilled water. The extraction process's outcomes showed unique impacts on the presence of some significant phytochemical classes.

The varied solubility characteristics of flavonoids can be used to explain their presence in the 56.7% ethanol and distilled water extracts of celery. The solubility of flavonoids, a family of polyphenolic chemicals with antioxidant action, is dependent on the solvent's polarity. The combination of ethanol and distilled water creates a mixed-polarity environment that allows for the extraction of both polar and less polar flavonoids. Distilled water is a polar solvent that works well for extracting polar molecules like flavonoids. The combination of these two solvents guarantees a thorough extraction, collecting the wide range of flavonoids contained in the plant. Celery is likely home to some flavonoid subclasses with varying polarity. This emphasizes how crucial it is to choose the right solvent when removing a wide variety of phytochemicals from intricate plant matrices (Smith et al. 2018).

The polar properties of these phytochemicals and the specific attraction of water as a solvent for their extraction account for the saponins' sole presence in the celery based on distilled water extract. Because they include both hydrophilic and hydrophobic moieties, saponins are amphiphilic compounds that are more likely to partition into polar liquids. As a highly polar solvent, distilled water makes it easier to extract saponins from the plant matrix. Water's polar properties allow the hydrophilic parts of saponins to dissolve and be extracted more preferentially. This result emphasizes how phytochemical extraction is solvent-dependent, meaning that the selection of a solvent is essential to the selective extraction of particular classes of chemicals from plant material (Anderson et al. 2016).

It is possible that terpenoids, which include essential oils, are present in the 56.7% ethanol and distilled water extracts of celery due to the different solubility properties of these substances. Since terpenoids are lipophilic compounds, non-polar solvents are preferred by them. Because it is a partly polar solvent, ethanol may dissolve both polar and non-polar substances, which makes it appropriate for terpenoid extraction. Distilled water has the potential to absorb more polar terpenoids. Because of their combined polarity, ethanol and water in the second solvent probably help extract a wider range of terpenoids, resulting in a thorough profile of these substances. Understanding the possible therapeutic benefits of celery requires an understanding of terpenoids, which give plants their unique flavor and scent and are known to have certain medical uses (Robert et al. 2017).

Steroids are consistently present in both the 56.7% ethanol and distilled water extracts of celery, indicating that the solvent selection does not affect the extraction of this family of bioactive chemicals. As a result of their generally low polarity, steroids may dissolve easily in a wide range of solvents, irrespective of their polarity. The strong extraction procedure of steroids from celery is ensured by their flexible nature and the inherent solubility of both water and ethanol. The results highlight the viability of both solvents for the extraction of steroids and highlight the need for a thorough approach in the investigation of plant phytochemical composition (Martinez et al. 2019).

In a nutshell, the findings demonstrate the complex impact of various solvents on the extraction of certain phytochemicals from celery. The results offer a significant understanding of the ideal circumstances for bioactive chemical extraction from *Apium graveolens*, with possible applications in the fields of natural product development, medicine, and nutrition. The observed differences in solvent dependence highlight how crucial it is to pick extraction methods carefully to acquire accurate and thorough profiles of the phytochemical content in plant extracts.

4.4 Analysis of Antioxidant Activity in *Apium Graveolens* Extract

In the study of biochemistry and food science, 2,2-diphenyl-1-picrylhydrazyl (DPPH) is frequently used to analyze the antioxidant activity of *Apium graveolens* (celery) extract. When antioxidants interact with DPPH, a persistent free radical (Jadid et al., 2017), the resultant color shift from purple to yellow may be detected using spectrophotometry (Syed Salleh et al. 2021). In pharmacology and biochemistry, the half-maximal inhibitory concentration, or IC_{50}, is a commonly used metric to measure how well a drug inhibits a certain biological or biochemical activity by 50% (Rivero-Cruz et al., 2020). When using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test for antioxidant analysis, the IC_{50} value serves as a crucial predictor of a sample's level of antioxidant activity.

The findings displayed in Tables 4.2, 4.3, and 4.4 show the ability of conventional ascorbic acid and *Apium Graveolens* extract to scavenge free radicals at varying concentrations and solvents. The half-maximal inhibitory concentration, or IC_{50} values, give information on how well antioxidants neutralize DPPH radicals. The Radical Scavenging Activity (RSA) is reported as a percentage.

The well-known antioxidant ascorbic acid showed a dose-dependent increase in RSA, with a dosage of 20 mg/mL achieving an astounding 99.484%. With an IC_{50} value of 0.766 mg/mL, a strong antioxidant activity is shown. Ascorbic acid's effective DPPH radical scavenging capacity is demonstrated by its high RSA values. These findings support ascorbic acid's well-known antioxidant qualities, which are frequently present in fruits and vegetables.

It is interesting to note that *Apium Graveolens* extract showed significant RSA at concentrations of 0.067 mg/mL (94.215%) when diluted in pure water. The IC_{50} value rose to 1.171 mg/mL, indicating a little reduced efficacy in contrast to ascorbic acid. This suggests that the solvent utilized affects the antioxidant activity of the extract of *Apium graveolens*. Despite having lesser effectiveness than ascorbic acid, the plant extract may include chemicals that may scavenge DPPH radicals, as shown by the relatively high RSA values.

The RSA values were lower when the *Apium Graveolens* extract was mixed in 56.7% ethanol than when distilled water was used alone. At the maximum dose

(20 mg/mL), the RSA dropped to 82.580%, and the IC_{50} value was 0.786 mg/mL. This implies that the antioxidant activity of the extract is highly influenced by the solvent selection. The observed reduction in RSA might perhaps be ascribed to variations in the solubility of antioxidant molecules between ethanol and water.

Table 4.2: The DPPH free radical scavenging activity of the standard ascorbic acid

Concentration (mg/mL)	Ascorbic Acid		
	Absorbance	%RSA	IC_{50} (mg/mL)
0	0.000	0.000	0.766
4	0.012	98.969	
8	0.0014	99.879	
12	0.006	99.484	
16	0.006	99.484	
20	0.006	99.484	

*All readings were taken in triplicate, RSA= Radical Scavenging Activity

Table 4.3: The DPPH free radical scavenging activity of *Apium Graveolens* plant based on distilled water.

Concentration (mg/mL)	Distilled Water		
	Absorbance	**%RSA**	**IC_{50} (mg/mL)**
0	0.000	0.000	1.171
4	0.048	95.847	
8	0.057	95.074	
12	0.067	94.215	
16	0.077	93.327	
20	0.098	91.552	

*All readings were taken in triplicate, RSA= Radical Scavenging Activity

Table 4.4: The DPPH free radical scavenging activity of *Apium Graveolens* plant based on 56.7% ethanol.

Concentration (mg/mL)	56.7% Ethanol		
	Absorbance	**%RSA**	**IC_{50} (mg/mL)**
0	0.000	0.000	0.786
4	0.061	94.759	
8	0.048	95.876	
12	0.067	94.186	
16	0.074	93.584	
20	0.202	82.580	

*All readings were taken in triplicate, RSA= Radical Scavenging Activity

The information highlights how crucial it is to take into account both RSA and IC_{50} values to conduct a thorough assessment of antioxidant activity. The extraction and dissolution of antioxidant chemicals depend critically on the solvent used, which also affects the total scavenging activity. There is no denying the concentration-dependent response, and determining the ideal concentration for optimum antioxidant activity is essential (Smithson et al. 2020).

In general, the research offers significant insights into the antioxidant capacity of *Apium Graveolens* extract, emphasizing the impact of solvent selection on its scavenging efficacy. The findings add to our understanding of natural antioxidants and how they may be used to improve health and stave off illnesses linked to oxidative stress.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, the first objective shows that bioactive components from *Apium graveolens* stem was successfully extracted using the maceration extraction method with two distinct solvents 56.7% ethanol and 100% distilled water, making it possible to thoroughly investigate the phytochemical profile of the plant.

The second objective has been successfully achieved. The investigation into the phytochemical components of the crude extract obtained from the stems of *Apium Graveolens* has provided valuable insights into the bioactive compound present in this plant. The presence of terpenoids, steroids, flavonoids, and saponins in the crude extract was satisfactorily determined by phytochemical tests. Given that the phytochemicals in *Apium graveolens* stem are recognized for a variety of biological functions, including antioxidant activity, these findings highlight the stems' potential medicinal utility.

In conclusion, the DPPH free radical scavenging experiment we used to examine the antioxidant activity of *Apium Graveolens* plant stems has yielded important information. The outcomes showed that both the 56.7% ethanol extract and the distilled water extract had a dose-dependent radical scavenging effect. Comparing the 56.7% ethanol extract to the distilled water extract, the 56.7% ethanol extract showed a slightly lower IC_{50} value, indicating better antioxidant effectiveness. These results support the use of *Apium Graveolens* stems as a natural source of antioxidants by highlighting their antioxidant potential. To learn more about the roles that certain antioxidant molecules play in the reported scavenging activity, more studies may concentrate on isolating and analyzing these compounds.

5.2 Recommendations

The other plant sections may be studied further, utilizing various extraction solvents. To increase the study's effectiveness, the investigation of how solvents affect the result of extraction can be added. Aside from that, adding other extraction techniques, like the standard procedure, can improve the outcome and help the study reach its goal. To improve comparability with the prior study, more distinct testing for each bioactive ingredient might be performed for phytochemical screening. Additional phytochemical analysis can be performed using the chromatography technique. Furthermore, it was advised to utilize a lower concentration value in dilution when calculating the absorbance value for the DPPH free radical scavenging test technique, which is used to analyze antioxidant activity.

Next, the other alternative radical scavenging techniques, such as the ferric reducing ability of plasma (FRAP) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) can be employed for the next study. Lastly, the solvent temperature can be raised to 50–60° C, the parameters of heat may be employed in further research to improve extraction yield.

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