FYP FBKT

UNIVERSITI
MALAYSIA
KELANTAN

Phytochemical Screening and Antioxidant Activity of Curcuma Xanthorrhiza crude plant extract

Nur Baiti Jannati Binti Sanusi
J20A0564

A thesis submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UMK

2024

UNIVERSITI MALAYSIA KELANTAN

FYP FBKT

DECLARATION

I declare that this thesis entitled "Phythochemical Screening and Antioixidant Activity of Curcuma Xanthorrhiza Crude Plant Extract" is the results of my own research except as cited in the references.

Signature : _____

Student's Name : NUR BAITI JANNATI BINTI SANUSI

Date : 1/ 03/ 2024

Verified by:

Signature : _____

Supervisor's Name : DR ROSMAWANI BINTI MOHAMMAD

Stamp : _____

Date : _____

UNIVERSITI MALAYSIA KELANTAN

FYP FBKT

ACKNOWLEDGEMENT

I would like to express my greatest gratitude to my supervisor Dr. Rosmawani Binti Mohammad, for her supervision, encouragement and precious advices regarding this final year project. Her support and guidance have brought me to complete this project in time given. I also want to thank to my supervisor for sharing her knowledge which allowed me to improve my acknowledge about this project.

A special thanks goes to my family for their continuous support since from beginning of this project. They have become my inspiration and make me stronger to carry out this project. With their moral and also financial support, I am able to finish this project. Besides, I also want to thank my housemate who helped and accompany me during my project works. I also want to thank to my teammate and all my fellow friends who support and give the strength to complete this project.

Last but not least, I would like to acknowledge the crucial role of the laboratory assistants especially Mr. Mohammad and Mrs. Syahirah who assisted me in the laboratory throughout the project. They also taught and shared lots of laboratory skills regarding my project.

UNIVERSITI MALAYSIA KELANTAN

Phytochemical Screening and Antioxidant Activity of Curcuma Xanthorrhiza Crude Plant Extract

ABSTRACT

Curcuma Xanthorrhiza contains a variety of phytochemical components, including terpenoids and cucuminoids, it is thought to have considerable promise as a herbal medicine for both people and animals. In this work, two distinct solvents which is distilled water and methanol were utilised in the maceration process to extract the phytochemical components from the plant. Based on phytochemical screening, the presence of bioactive compounds in the the crude extract of *Curcuma Xanthorrhiza* was influenced by the solvents used. Tannins were only present in methanol extracts, while saponins and steroids were only present in distilled water extract. Next, DPPH radical inhibition and IC_{50} data show that ascorbic acid has better antioxidant activity than plant extract. Distilled water extract has a higher antioxidant activity with an IC_{50} value of 1.5021 mg/mL. This is followed by ascorbic acid (1.3671 mg/mL) and methanol extract (1.4837 mg/mL). Based on the phytochemical screening, distilled water yielded better results for the extraction of *Curcuma Xanthorrhiza* rhizome compared to the methanol extract. Meanwhile, the antioxidant activity study showed both extract and ascorbic acid standard have a very strong antioxidant activity.

Keywords: *Curcuma Xanthorrhiza*, Phytochemical Screening, Antioxidant Activity, Effect of solvent, Effect of plant size.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

Saringan Fitokimia dan Aktiviti Antioksidan Ekstrak Tumbuhan Daripada

Curcuma Xanthorrhiza

ABSTRAK

Curcuma Xanthorrhiza mengandungi pelbagai komponen fitokimia, termasuk terpenoid dan cucuminoid, ia dianggap mempunyai janji yang besar sebagai ubat herba untuk manusia dan haiwan. Dalam kerja ini, dua pelarut berbeza iaitu air suling dan metanol telah digunakan dalam proses pemekatan untuk mengekstrak komponen fitokimia daripada loji. Berdasarkan saringan fitokimia, kehadiran sebatian bioaktif dalam ekstrak mentah *Curcuma Xanthorrhiza* dipengaruhi oleh pelarut yang digunakan. Tannin hanya terdapat dalam ekstrak metanol, manakala saponin dan steroid hanya terdapat dalam ekstrak air suling. Seterusnya, perencatan radikal DPPH dan data IC_{50} menunjukkan bahawa asid askorbik mempunyai aktiviti antioksidan yang lebih baik daripada ekstrak tumbuhan. Ekstrak air suling mempunyai aktiviti antioksidan yang lebih tinggi dengan nilai IC_{50} 1.5021 mg/mL. Ini diikuti oleh asid askorbik (1.3671 mg/mL) dan ekstrak metanol (1.4837 mg/mL). Berdasarkan saringan fitokimia, air suling menghasilkan keputusan yang lebih baik untuk pengekstrakan rizom Curcuma Xanthorrhiza berbanding dengan ekstrak metanol. Sementara itu, kajian aktiviti antioksidan menunjukkan kedua-dua ekstrak dan piawaian asid askorbik mempunyai aktiviti antioksidan yang sangat kuat.

Kata kunci: *Curcuma Xanthorrhiza*, Saringan Fitokimia, Aktiviti Antioksidan, Kesan pelarut, Kesan saiz tumbuhan.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

TABLE OF CONTENTS

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

FYP FBKT
UNIVERSITI
MALAYSIA
KELANTAN

FYP FBKT

UNIVERSITI MALAYSIA KELANTAN

FYP FBKT

LIST OF TABLES

UNIVERSITI
MALAYSIA
KELANTAN

LIST OF FIGURES

FYP FBKT

UNIVERSITI MALAYSIA KELANTAN

CHAPTER 1

INTRODUCTION

1.1 Background of study

Since ancient times, medicinal plants have been utilised extensively to treat a variety of ailments. They possess several healing qualities, including the ability to relieve pain and have antibacterial, anti-inflammatory, and anti-diabetic effects (Rosario & Josephine, 2015). The main source of basic healthcare for individuals in underdeveloped nations, according to the World Health Organisation, is herbal medicine. Additionally, a significant portion of contemporary medications are either developed from or based on medicinal plants. Any plant that includes compounds with therapeutic properties or compounds that can be utilised as building blocks for semi-synthetic pharmaceuticals is considered a medical plant (Siju et al., 2014).

Furthermore, plant compounds are isolated and evaluated to see if they are physiologically active in a process known as phytochemical screening. Because of medicinal plants are frequently used to treat a wide range of ailments, several studies have evaluated the effectiveness of phytochemical screening to access the bioactive chemicals in such plants. As in the previous study by Maruthupandian and Mohan (2011) observed the presence of phytochemicals such as alkaloids, coumarins, flavonoids, glycosides, terpenoids, tannins, phenols, saponins, and steroids in the ethanol extract of wood and bark that had anti-diabetic, anti-hyperlipidemic, and antioxidant properties. Phenolic and flavonoid molecules are regarded as the most significant groups of phytochemicals due to their extensive health benefits (Roopashree et al., 2008).

Any agent that prevents or reduces oxidative damage to a target molecule is an antioxidant, according to a wide definition. A key role in the fight against oxidative stress is played by molecules originating from secondary metabolism, particularly phenolic compounds. These substances have a reputation for acting as antioxidants due to both their

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

stability as intermediate radicals and their capacity to transfer electrons or hydrogen (Nićiforović et al., 2010). When the plants are eaten as food, phenolic chemicals also have protective benefits on people. In general, the anti-oxidant properties of phenols in plant extracts are effective at low concentrations, and in people, they are linked to the prevention of cancer and cardiovascular disease (Balmus et al., 2016).

In this study, *Curcuma Xanthorrhiza* rhizome were chosen for phytochemical screening and antioxidant of crude plant extract. An extremely valued native Indonesian plant known locally as "*Temulawak*" or Java turmeric is *Curcuma Xanthorrhiza Roxb*., which is a member of the Zingiberaceae family. It is mostly grown in Southeast Asian nations including Indonesia, Malaysia, Thailand, Vietnam, and the Philippines (Salleh et al., 2016). In Malaysia, mostly people also commonly call it as '*Kunyit Temulawak'*. *Curcuma Xanthorrhiza* was grown in Indonesia on a large scale in 2019, with yields of 29,637,119 kg produced from a destroyed harvested area of more than 13,042,873 m2.

1.2 Problem Statement

Curcuma Xanthorrhiza are claimed to have many benefits for human diseases and has been used in folk medicine for several treatment including lack of appetite, stomach disorder, liver illness, constipation, bloody diarrhea, etc. A recent study had shown many benefit of this plant and identification of secondary metabolites. However, there is no proper research and comprehensive review for these studies. Therefore, to determine the phytochemical of *Curcuma Xanthorrhiza*, more study is required. Moreover, the goal of the study is to determine whether *Curcuma Xanthorrhiza* can replace synthetic drugs in medical settings.

1.3 Objectives

The objectives of this study are:

1. To determine the effect of solvents to the presence of phytochemical constituents.
2. To identify the antioxidant activity in rhizome of *Curcuma Xanthorrhiza*.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

1.4 Scope of Study

In this study, *Curcuma Xanthorrhiza* had been evaluated for its phytochemical analysis and antioxidant activity. Phytochemical analysis is a test for flavonoid, tannins, saponnins, etc. were carried out using qualitative phytochemical test. For example, *Liebermann Test*, alkaline reagent test and others chemical test. Meanwhile, antioxidant activity was assessed using the DPPH free radical scavenging test.

The *Curcuma Xanthorrhiza* plant were processed using traditional extraction methods which are maceration, filtering, and vacuum rotary evaporation to extract the bioactive components. On plant rhizome, phytochemical analyses and antioxidant activity were conducted. The parameter such as extraction time and concentration had been included in this current study.

Furthermore, the size and weight of this *Curcuma Xanthorrhiza* rhizome also had been taken into account in this research study. In this study the rhizome was used for phytochemical screening and antioxidant activity.

1.5 Significance of study

Since this current study plant is said to have therapeutic benefits for humans and has the potential to be developed in the future, it is crucial to conduct this study to ascertain its useful components. In addition, this study can support any legal claims on the value and quality of its findings. Finding out and comprehending the potential phytochemical and antioxidant activities of *Curcuma Xanthorrhiza* depends on this research. People may learn about this plant and the outcomes from this study, avoiding misunderstandings about its usage and potential effects. After all, this research may aid the industry of medical plants in creating innovative products or useful pharmaceuticals.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

FYP FBKT

CHAPTER 2

2 LITERATURE REVIEW

2.1 Herbal Medicine Plant

Herbal medicine, commonly referred to as herbalism or phytotherapy, is an ancient medical technique that makes use of plants and plant extracts to promote health. It entails using a variety of plant components, including leaves, blossoms, roots, and bark, to cure or prevent disease. These plant-based products include physiologically active ingredients that interact with the body to improve physical and mental wellbeing.

Many civilizations have used herbal medicine throughout history, including Traditional Chinese Medicine (TCM), Ayurveda, and Native American medicine. The inclusion of phytochemicals, such as alkaloids, flavonoids, terpenes, and phenolic compounds, which have therapeutic qualities, is frequently credited with the efficacy of herbal treatments. Teas, infusions, decoctions, tinctures, capsules, and topical treatments are a few of the preparation techniques used in herbal therapy. The particular condition being treated and the person's distinctive qualities are taken into account when choosing which herbs to use and in what combinations.

Numerous plants have therapeutic characteristics that have been the subject of scientific study, demonstrating their efficacy in treating a range of illnesses. For instance, St. John's wort (Hypericum perforatum) has been the subject of in-depth research about its putative antidepressant properties (Sarris et al., 2016). Echinacea (Echinacea purpurea) has also been researched for its potential to boost the immune system (Hudson, 2012)

While herbal medication may have certain advantages, it should nevertheless be taken with caution. When taken incorrectly, herbs might interfere with pharmaceuticals, trigger allergic responses, or have negative consequences. It is essential to get the advice of a trained healthcare provider, such as a naturopathic physician or a herbalist, before utilising herbal therapies.

2.2 Herbal Medicine Plant in Malaysia

In Malaysia, the term "herbal medicine" refers to the conventional method of treating illnesses by using plants and products obtained from plants. It is commonly practised alongside modern medicine and has a strong cultural foundation in the nation.

"Tongkat Ali," or traditional Malay medicine, uses a number of herbal treatments and cures. In addition to using decoctions, infusions, and poultices, it makes use of plant components such leaves, stems, roots, and bark. These treatments are used to treat a variety of illnesses, including digestive disorders, respiratory issues, skin concerns, and more. They are frequently based on information that has been passed down through centuries. The plant "Tongkat Ali" (Eurycoma longifolia) is frequently used as herbal medicine in Malaysia. It is widely prized for its possible advantages in increasing male sexual performance, raising energy levels, and enhancing general wellbeing. Scientific research has been done on Tongkat Ali to determine its therapeutic effects and it has acquired international reputation.

Centella asiatica, sometimes referred to as "Pegaga" or "Gotu Kola," is another well-known herbal treatment. It is frequently utilised because of its conceivable cognitive-improving qualities and is said to enhance memory and focus. The Traditional and Complementary Medicine (T&CM) Act 2016, which offers standards for the registration, licencing, and quality control of traditional medicines, regulates herbal medicine in Malaysia. Through stringent testing and regulation, the Malaysian Ministry of Health contributes significantly to guaranteeing the security and effectiveness of herbal products. While Malaysian traditional herbal medical practises have a significant cultural impact, it's vital to remember that the scientific data demonstrating the effectiveness and security of certain herbal medicines might differ. When contemplating herbal remedies, it is advised to speak with medical specialists and depend on evidence-based research.

2.3 *Curcuma Xanthorrhiza*

The Zingiberaceae family includes the Indonesian native *Curcuma Xanthorrhiza* (Figure 2.1), which grows in many tropical areas. Temulawak and Javanese turmeric are only two of the regional names for *Curcuma Xanthorrhiza*, which also goes by the names koneng gede (Sundanese), temu labak (Madurese), tommo (Bali), tommon (South Sulawesi), and karbanga (Ternate). In the lowlands, this plant may reach a height of 2500 m above sea level.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

This plant is widely grown across Indonesia, including on practically all of the country's major islands, including Java, Sumatra, Kalimantan, Sulawesi, and Maluku. In addition, some Southeast Asian nations including Malaysia, Thailand, the Philippines, and Vietnam have also grown *Curcuma Xanthorrhiza* (de Padua et al., 1999). In addition, China, India, Japan, and Korea may also have cultivars.

An annual plant with clump-forming growth and a pseudostem (2–2.5 m in height) is called *Curcuma Xanthorrhiza*. Each colony comprises between three and nine plants, each of which has two to nine leaves. The leaves of *Curcuma Xanthorrhiza* measure 50–55 cm in length and 18–20 cm in breadth (Rukmana, 1995). The rhizome of *Curcuma Xanthorrhiza* releases blooms alternately throughout the year. The inflorescentia, or flower arrangement, measures 1.5 cm in height and has a flower stalk that is about 3 cm long. Three or four blooms grow from one armpit. Flowers have thin, hairy stems that range in length from 4 to 37 cm. The 23 cm long, flower-shaped grain has an extended spherical form. Many protecting leaves, some of which are proportionate to the length of the flower crown on *Curcuma Xanthorrhiza* blooms, are present. In the morning, flowers blossom, and in the afternoon, they wither (Dalimartha, 2000). The parent rhizome of *Curcuma Xanthorrhiza* has an oval, egg-like structure, but the branch rhizome on the side has an extended shape. There are around 3–4 branch rhizomes on each plant. The fibrous root structure of *Curcuma Xanthorrhiza* comprises irregularly spaced roots that are around 2.5 cm in length.

In Indonesia and Malaysia, the plant *Curcuma Xanthorrhiza* is well-known and frequently used by different tribes to cure a wide range of illnesses. Traditional applications of *Curcuma Xanthorrhiza* include treating thrush and vaginal discharge, as well as overcoming anorexia, constipation, haemorrhoids, acne, diarrhoea, and seizure medicine. It is also used to dissolve gallstones, cure kidney and liver problems, rheumatic pain, rheumatism, and arthritis, and to treat haemorrhoids (Syamsudin et al., 2019). Since at least 1963, *Curcuma Xanthorrhiza* (Temulawak) has also been shipped to and used in Europe, mostly for the treatment of dyspepsia, infections, skin conditions, and liver disorders.

Traditional uses for *Curcuma Xanthorrhiza* rhizome include decoctions, steepings, powders, and even as food. Simplicia, starch, essential oil, and extract are the semifinished industrial goods from the rhizome of *Curcuma Xanthorrhiza*, whereas food and drink, cosmetics, syrup, instant powder, tablets, and capsules are the finished industrial products. Along with other therapeutic herbs, *Curcuma Xanthorrhiza* is frequently mixed with others to create jamu (Indonesian herbal medicine) products, which are widely manufactured commercially in Indonesia.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

For instance, the Javanese believe that a combination of *Curcuma Xanthorrhiza*, *Curcuma longa Linn*. syn., and Zingiber officinale may boost endurance, maintain the immune system, and preserve digestive system health. Additionally, the people of Sulawesi frequently utilise *Curcuma Xanthorrhiza* together with Tinospora cordifolia and Andrographis paniculata as a treatment for gastrointestinal sickness (Sari et al., 2017).

Figure 2.1 : *Curcuma Xanthorrhiza* rhizome

(Source: Hindawi, 2021)

2.4 Phytochemical Screening

Phytochemical screening benefits plants by performing a number of ancillary tasks for them, such as promoting plant growth, protecting them by triggering defence mechanisms, and giving them flavour, colour, and aroma. When compared to other synthetic alternatives, natural chemicals and their derivatives have fewer adverse effects and greater effectiveness. These phytochemicals, such as flavonoid, glycosides, steroids, tannins, and saponins, carry out certain biological processes that improve therapeutic actions including anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant characteristics (Batiha et al., 2020).

In order to identify different classes of phytoconstituents present in different parts of the base for the discovery of drugs, a scientific process known as phytochemical screening involves analysing, inspecting, extracting, and experimenting. The active components are then taken for further study and research. The procedure, known as phytochemical screening, was

FYP FBKT

qualitative. The study's findings may help in the development of effective medications for a number of disorders.

Table 2.1 Shows list of medicinal plant and their usage

Name	Part taken	Local name	Local uses
Allium cepa (ACB)	Bulb	Onion	Vegetable.
Curcuma longa (CLR)	Rhizome	Turmeric	Antiseptic, anti-diabetic and antibacterial agent. (Maithalikarpagaselvi *et al*. 2020)
Ocimum sanctum (OSL)	Leaves	Tulsi	Antioxidant, Anti-inflammatory (Chaudhary *et al.* 2020)
Mentha arvensis (MAL*)*	Leaves	Mint	As an antibacterial, and an antiseptic agent. (Patil *et al.* 2016)
Allium sativum (ASB)	Bulb	Garlic	Antioxidant. (Melania *et al*. 2019)
Zingiber officinale (ZOR)	Rhizome	Ginger	Treats cold, cough, in gastric problems (Arwande *et al*. 2018)
Acorus calamus (ACR)	Rhizome	Calamus	To treat throat problems and stomach problems. (Nath & Yadav, 2016)
Zanthoxyllum armatum (ZAS)	Seeds	Timur	Used in intestinal problems. (Bharti & Bhushan 2015)
Nyctanthes arbortristis (NAL)	Leaves	Parijat	Anti-diabetic. (Haque *et al*. 2015)
Nyctanthes arbortristis (NAF)	Flowers	Parijat	Anti-diabetic, treats hypertension. (Haque *et al*. 2015)

2.4.1 Flavonoids

Secondary metabolites called flavonoids are mostly made up of a benzopyrone ring with phenolic or polyphenolic groups arranged at various locations as Figure 2.2 shown. Fruits, herbs, stalks, grains, nuts, vegetables, flowers, and seeds are the most common places to find them. These various plant parts possess biological activity and therapeutic potential due to the presence of bioactive phytochemical ingredients. More than 10,000 flavonoid compounds have been discovered and isolated thus far. The majority of flavonoids are recognised as effective medicinal agents (Shkondrov et al., 2017). These are produced spontaneously via the phenylpropanoid pathway, and their bioactivity is reliant on their bioavailability and mode of absorption.

FYP FBKT
UNIVERSITI
MALAYSIA
KELANTAN

Flavonoids have been utilised as anti-wrinkle skin agents, natural colours, and cosmetics and skin care products. Nonetheless, the medical area is where these polyphenols are most prominently used. As anticancer, antibacterial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative medicines, flavonoids have been widely used. Flavonoid-rich apple peel extracts are an efficient antihypertensive agent and inhibit acetylcholinesterase (ACE) in vitro (Balasuriya & Rupasinghe, 2012). Additionally, it has been shown to better preserve cognitive function with ageing and avoid cardio-metabolic problems.

Depending on their degree of unsaturation, oxidation of the carbon ring, and chemical structure, they are divided into several categories. The many subclasses of flavonoids include anthoxanthins (flavanone and flavanol), flavanones, flavanonols, flavans, chalchones, anthocyanidins, and isoflavonoids. In nature, each of these flavonoids is extensively dispersed. Increased consumption of foods high in flavonoids has several health advantages. There is a growing push to separate these natural substances from different plants because of their beneficial benefits on human health. Citrus fruits, for example, are a great source of flavonoids. Oranges, lemons, and grapes contain two flavonoids: narigenin and hesperetin. Mulberries are rich in flavonoids called anthocyanins and quercetin glycosides.

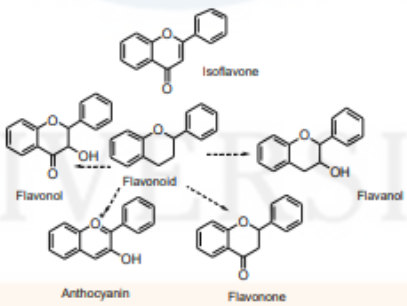


Figure 2.2 : Structure of Flavanoids

(Source: Ullah et al., 2020)

2.4.2 Glycosides

Any of a broad range of naturally occurring compounds known as glycosides are formed when a hydroxy molecule combines with a component of carbohydrates, which can be one or more sugars or uronic acid, also known as a sugar acid. The hydroxy molecule is often a non-sugar entity (aglycon), such an alcohol or phenol derivative. However, it can also be another carbohydrate, like the numerous glucose units found in cellulose, glycogen, and starch.

In plants, glycosides are abundant and frequently found in fruit and floral colours, such as anthocyanins. Glycosides are found in many medications, sauces, and plant-based colours. Of particular importance are the heart-stimulating glycosides found in Digitalis and Strophanthus, which belong to the cardiac glycosides group. Glycosides include several antibiotics (such as streptomycin). Plants contain glycosides called saponins, which reduce water's surface tension. Saponin solutions have been employed as cleaning agents.

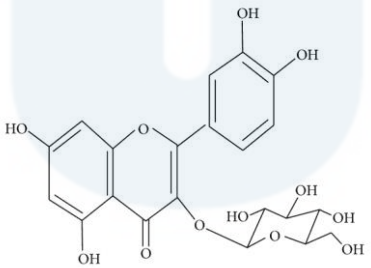


Figure 2.3: Structure of Glycosides

2.4.3 Tannins

Tannin is any of a class of phenolic chemicals found in woody flowering plants that have several industrial uses and serve as significant herbivore deterrents. Tannins are confined in plant cells' vacuoles as secondary metabolites, shielding the other constituents of the cell. They are often found in numerous plant tissues, including the roots, wood, bark, leaves, and fruit of the genus Quercus; they are especially abundant in the bark of Quercus oaks, Rhus

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

sumacs, and Terminalia chebula oaks. They can also be found in galls, which are diseased growths brought on by insect bites (Britannica, 2024).

Commercial tannins are often amorphous, pale yellow to light brown, and can take the shape of flakes, powder, or a spongy mass. Their main uses include tanning leather, dying textiles, creating ink, and a number of medicinal uses. Solutions containing tannins taste astringent and are acidic. The astringency, colour, and mild taste of black and green teas are all attributed to tannins.

Apart from their primary uses in the production of leather and dyes, tannins are also employed in the clarity of wine and beer, as a component to lower the viscosity of drilling mud for oil wells, and as a preventive measure against scale development in boiler water. Tonsillitis, pharyngitis, haemorrhoids, and skin eruptions have all been treated with tannin due to its styptic and astringent qualities. It has also been taken internally to control diarrhoea and intestinal bleeding and as an antidote for metallic, alkaloidal, and glycosidic poisons, with which it forms insoluble precipitates. Tannins are soluble in water and combine with iron salts to generate dark blue or dark green solutions, a feature that is used to make ink.

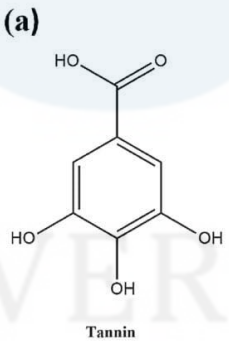


Figure 2.4 : Structure of Tannins

(Source: Kavitha, 2020)

FYP FBKT
UNIVERSITI
MALAYSIA
KELANTAN

2.5 Antioxidant Activity

A chemical known as an antioxidant is one that can stop a substrate from oxidising. Hydrogen atom transfer (HAT) is one of the chemical processes used by antioxidant chemicals to react. One of their functions is as a microvascular system to maintain the levels of oxygen in the tissues. Antioxidant protection can be enzymatic or nonenzymatic and also functions as a biochemical mechanism for mending molecules. By controlling the released radicals and reducing oxidative damage, antioxidants assist humans. For instance, Rakshamani Tripathi, H. Mohan, and Kamat (2007) used rat liver membrane as a model system for pharmacological research to evaluate the oxidative damage brought on by radiation exposure. Utilising the anti-oxidant properties of two different medicinal herbs, Andrographis paniculata (Ap) and Swertia chirata (Sc). Because of this, extracts of Andrographis paniculata and Swertia chirata have significant antioxidant activity, boosting the quenching of reactive oxygen and enhancing the termination of free radical reactions, protecting the liver from deteriorating tissues.

2.5.1 DPPH scavenging assay

DPPH stands for 2,2-diphenyl-1-picrylhydrazyl as shown as in Figure 2.5. It is a chemical compound commonly used in antioxidant assays to evaluate the radical scavenging activity of various substances. DPPH is a stable free radical with a deep purple color in its radical form. When an antioxidant compound is added to a solution containing DPPH radicals, it can donate hydrogen atoms or electrons to the DPPH radical, neutralizing it and causing the color to fade from purple to yellow. The extent of discoloration is used to measure the antioxidant capacity of the tested substance. DPPH assay is widely used in the field of pharmacology, food science, and cosmetics to assess the antioxidant properties of natural and synthetic compounds.

Blois (1958) devised this approach with the goal of employing a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M = 394.33) to measure the antioxidant activity in a similar manner. The assay's foundation is the assessment of antioxidants' ability to scavenge it. By transferring a hydrogen atom from antioxidants to the matching hydrazine, the odd electron of the nitrogen atom in DPPH is decreased.

Because the spare electron delocalizes throughout the whole molecule rather than dimerizing like most other free radicals do, DPPH is classified as a stable free radical. Along

FYP FBKT

with the deep violet hue, delocalization is responsible for the absorption in ethanol solution at about 520 nm. The reduced form of DPPH solution is produced when it is mixed with a material that may donate a hydrogen atom, losing its violet hue. Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z^* + AH = ZH + A^*$$

Where A• is the free radical created in the first step and ZH is the reduced form. Subsequent reactions using the latter radical will regulate the total stoichiometry. The DPPH molecule Z• is therefore meant to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH. As a result, the reaction is intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance.

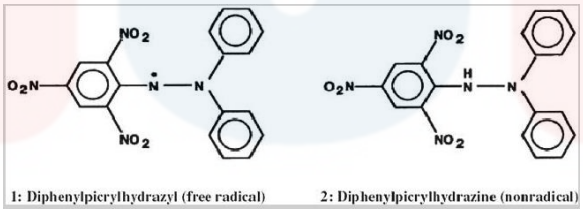


Figure 2.5: Structure of DPPH

(Source: Kedare et al., 2011)

2.6 Method of extraction

The method of extracting medicinal plants involves using the proper solvent and following a conventional extraction procedure to separate the active plant material (such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides) from the inert or inactive material. Plant materials containing high levels of flavonoids and phenolic compounds have

been shown to have antioxidant qualities; as a result, they are utilised to treat age-related conditions such depression, anxiety, Parkinson's disease, and Alzheimer's disease (Azwanida,2015).

A variety of techniques, including maceration, infusion, decoction, percolation, digesting, and Soxhlet extraction, as well as superficial extraction, ultrasound-assisted and microwave-assisted extraction, were employed in the extraction of medicinal plants. The secondary metabolites were also separated and purified using gas chromatography (GC), paper chromatography (PC), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). The kind of plant material, the solvent employed, the solvent's pH, temperature, and the solvent to sample ratio all play a role in selecting the best extraction technique. It also relies on how the finished goods are going to be used (Doughari, 2012).

2.7 Effect of Solvent

The choice of solvent is essential to the outcome of the extraction of active substances. The two types of extraction solvents include polar solvents like water, methanol, and ethanol and nonpolar organic solvents like chloroform, n-butanol, and ethyl acetate. When extracting bioactive compounds from plants, ethanol or water solvents have a substantial influence on the antioxidant and phenolic production of those compounds. The extraction process may be affected by factors including species, raw material kinds, particle size, plant sample components, and phytochemical makeup.

In an earlier study by Sun, Zhengshuang Wu, Ziyan Wang, and Zhang (2015), they extracted phenolics from propolis plants using a 75 weight percent ethanol and water solvent. The similarity between these two classes of solvents is that they may extract bioactive chemicals with high polarities that are often present in plants. In comparison to pure water or aqueous ethanol extraction, pure ethanol extraction produced reduced total phenolic levels (Nguyen et al., 2019). This could be due to the stronger compounds' bonds with phenolics, which make ethanol extraction and dissolution more difficult.

Based on a prior work, Truong et al. (2019) reported utilising similar methodologies while employing a different solvent to assess the phytochemical component extraction yield and antioxidant activity of the medicinal plant Severinia buxifolia (Rutaceae). These findings coincide with those of earlier research. In comparison to methanol, acetone, and chloroform, the results revealed that methanol, ethanol, and distilled water had the highest extraction yields.

FYP FBKT

FYP FBKT

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Materials

In this study, the material that used is methanol for the preparation of plant extraction. Next, bromine water, acetic acid, chloroform, NaOH and H_2SO_4 were used for the test of phytochemical screening. For antioxidants activity, 2,2-diphenyl-1-picryl-hydrezyl (DPPH) was used in this current study. Apparatus like filter paper, glass funnel, test tube, beaker, brown glass, glass rod and volumetric flask also used in this project.

3.2 Methods

3.2.1 Sample Collection

Fresh *Curcuma Xanthorrhiza* rhizome was picked up from garden at Kelantan. Rhizome had been picked up and dusted to get rid of soil and dirt. The plants were collected, shielded from pollutants and light, and allowed to dry completely at room temperature and humidity. Firstly, the *Curcuma Xanthorrhiza* rhizome was cut into small pieces and then dried. The procedure then continued ground the small pieces using a standard grinder to create a powdered sample with a larger surface (C' ujic' et al., 2016).

3.2.2 Preparation of Plant Extraction

The sample powdered then continued for maceration. The maceration was ready for the effect of the solvent. Methanol and distilled water were used as a solvent in this preparation. The solvents were prepared is about 200 mL each. The effective plant materials were weight 20g and was putted in a brown glass bottle. It had been macerated for five days in two different

solvents, which is methanol and distilled water. After that, the bottle was covered with aluminium foil (Azwanida, 2015) and kept in the lab at room temperature before being shaken. The filtrate was then coarsely filtered through many layers of muslin fabric. The crude filtrate was filtered using Whatman No. 1 filter paper. The filtrate was then reduced to one-third of its original volume by being evaporated in a vacuum rotary evaporator at 50–60°C bath temperature under reduced pressure. The extracts were obtained and kept in sealed glass bottles between 0°C and 4°C.

3.3 Phytochemical Qualitative Analysis

The following standard methods were used to determine the presence of phytochemical analysis in plant extracts. The plant extraction was carried out using two different solvents which is methanol and distilled water.

3.3.1 Test for Flavonoids

Alkaline Reagent Test : Two to three drops of sodium hydroxide (NaOH) were added to 2 mL of extract. Initially, a deep yellow colour were appeared but it gradually became colourless by adding 2 drops of dilute HCL, revealing the presence of flavonoids (Pandey and Tripathi, 2014).

3.3.2 Test for Tannins

A 10 mL of bromine water was added to 5 mL of an aqueous plant extract. The tannins are present bromine water has a discoloured appearance (Gul et al., 2017).

3.3.3 Test for Saponins

1 mL solution of the extract were diluted with distilled water to a volume of 20 mL and stirred for 15 minutes on the magnetic hotplate using a magnetic stirrer. Saponins were present if layer-stable foam is present (Devmurari, 2010).

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

3.3.4 Test for Glycosides

Liebermann Test : The entire aqueous plant crude extract were mixed with 2.0 mL of acetic acid and 2.0 mL of chloroform. After cooling the mixture, concentrated H_2SO_4 were added. The aglycone, the steroidal component of glycosides, were shown in green (Gul et al., 2017)

3.3.5 Test for Steroids

Salkowski Test : The 5 mL of aqueous plant crude extract were combined with 2 mL of chloroform and concentrated H_2SO_4. Red colour was develop in the bottom chloroform layer, indicating the presence of steroids (Gul et al., 2017).

3.4 Analysis of Antioxidant Activity

The plant's antioxidant activity was evaluated using the DPPH free radical scavenging test technique. 2,2-diphenyl-1-picryl-hydrezyl (DPPH) was used as a free radical to assess antioxidant activity. To determine the IC_{50}, serial dilutions were performed. To create serial dilutions of 1, 2, 3, 4, and 5 mg/mL, the extract sample were diluted in each solvent which is methanol and distilled water separately. After that putted 1 mL of each dilution in volumetric flask and 3 mL of the DPPH solution were added. For the DPPH solution, 4 mg DPPH were diluted in 100 mL methanol. The mixture and preservation were last for 30 minutes in a dimly lit environment. Using a UV-vis spectrophotometer, the absorbance at 517 nm were determined.

Additionally, ascorbic acid was utilised as a common antioxidant. 2.5g of ascorbic acid were weight and dilute in 25 mL methanol. After that, repeat the same steps as for DPPH. Every reading had been taken three times. The percentage of DPPH radical inhibition used to test antioxidant activity was shown on a graph, and the IC_{50} was determined to compare the results. When reading at 517 nm, colour variations from purple to yellow had been used to determine the reduction capacity of DPPH (Tamizhazhagan et al., 2017).

A decrease in absorbance indicated increased radical scavenging activity, which was determined by the following formula:

FYP FBKT

$$\% \text{ Inhibition} = \frac{(\ Absorbance\ of\ control - Absorbance\ of\ test\ sampel)}{Absorbance\ of\ control} \text{ X } 100$$

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

FYP FBKT

UNIVERSITI
MALAYSIA
KELANTAN

CHAPTER 4

4 RESULT AND DISCUSSION

4.1 Introduction

In order to ascertain whether *Curcuma Xanthorrhiza* contains the bioactive ingredient, the effect of plant size and the effect of extraction solvents were conducted. This chapter discusses phytochemical screening experiments and antioxidant activity of the plant extract using DPPH scavenging test technique.

4.2 Phythochemical's Screening of *Curcuma Xanthorrhiza* rhizome

To determine the bioactive component of the *Curcuma Xanthorrhiza* plant extract, a number of chemical experiments were conducted. The extract from the two distinct solvents which is methanol and distilled water were used in every test. The most well-known phytochemicals, including tannins, steroid, flavonoids, saponins, and others, are the focus of this section. The summary of *Curcuma Xanthorrhiza's* phytochemical test results is shown in Table 4.1.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

Table 4.1: Phytochemical Screening Test of the two extract of *Curcuma Xanthorrhiza*

Phytochemical Test	Type of extracts	
	Methanol	Distilled Water
Flavonoids	-	-
Tannins	+	-
Saponins	-	+
Glycosides	-	-
Steroids	-	+

*(+) present, (-) absent

Preliminary phytochemical screening revealed that the *Curcuma Xanthorrhiza* plant extract absence the majority of predicted beneficial components. These variations in the phytochemical elements' appearance might be caused by a number of variables, including the kind of solvents used for extraction, the technique used, or the specific portion of the plant under study. The *Curcuma Xanthorrhiza* plant's abundance of phytochemicals, such as terpenoids and curcuminoids, is primarily responsible for the plant's therapeutic qualities (Rahmat, 2021).

4.2.1 Flavonoids

Tests using alkaline reagents were used to identify the flavonoid component of the plant extract. Following their reaction with sodium hydroxide, both extracts' colours changed. A few drops of hydrochloric acid were added, and the extract's hue gradually diminished until it was colourless. Test for flavonoids showed same results for both extracts, as presented in Figures 4.1 and 4.2. Flavonoids are totally absence in distilled water and methanol extracts of *Curcuma Xanthorrhiza.*

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

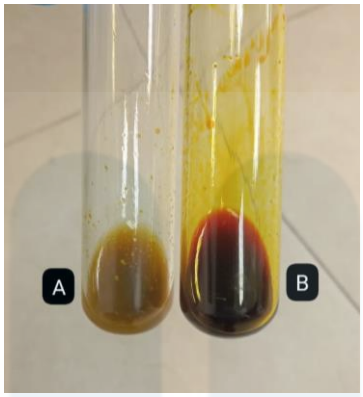


Figure 4.1: Phytochemical screening of flavonoids (before reaction)

* A= distilled water, B= methanol

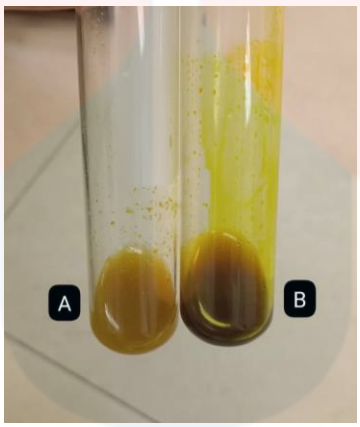


Figure 4.2: Phytochemical screening of flavonoids (after reaction)

* A=distilled water, B=methanol

The degree of polymerization, the interaction between the phenolic content and other phytochemicals, vitamins, and minerals, as well as the solvent used, all affect how soluble flavonoids are. According to Singh and Kumar (2017), flavonoids are hydroxylated polyphenolic compounds that have a number of functions in plants, including interacting with pollinators, fending off environmental stresses including microbial invasion, and regulating cell development.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

4.2.2 Tannins

This study's results on the tannin content of *Curcuma Xanthorrhiza* distilled water extracts were negative based on the interaction with bromine water. To verify the existence of tannins, the interaction between plant extract and bromine water has to cause the bromine water to become less coloured. Methanol extracts seemed rather yellowish in comparison to distilled water extracts. Limmatvapirat et al. (2020) state that the presence of yellow precipitates indicates the presence of tannins.

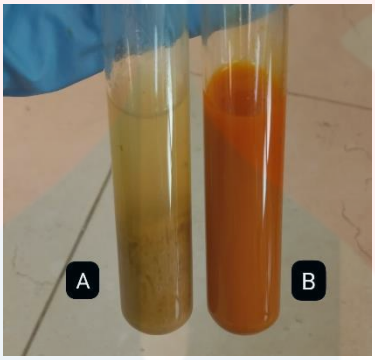


Figure 4.3: Phytochemical screening of tannins

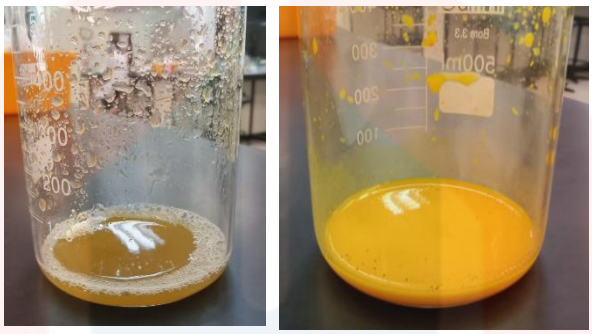
* A= distilled water, B=methanol

In summary, the analysis showed that methanol extracts had a favourable result in the reaction with bromine water, in distilled water tannins were absent. Compared to aqueous extracts, organic extracts often have a higher concentration of bioactive chemicals (Hayat et al., 2020). Furthermore, the interactions between tannic substances and solvents are probably connected to chemical compositions and designs. Because of an increase in hydroxyl groups (-OH), tannin solubility is correlated with polymerization degree.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

4.2.3 Saponins

Devmurari (2010) states that the saponins were tested qualitatively. The distilled water extract shows that the creation of a stable layer foam showed favourable findings on saponins. Nevertheless, the methanol extract of *Curcuma Xanthorrhiza* yielded unfavourable outcomes due to inadequate foam formation, even following the agitation stage using a magnetic stirrer. It was thought to have an extraction solvent's impacts on the existence of bioactive chemicals. Because saponins contain a hydrophobic aglycone base and hydrophilic glucose molecules, they can foam and emulsify substances (Kregiel et al., 2017). The results of the test for both extracts showed in Figures 4.4.



(A) (B)

Figure 4.4: Phytochemical screening of saponins

* A= distilled water, B=methanol

Plants manufacture saponins to fight against parasite illnesses and support human immunity by protecting against germs and viruses. The non-sugar portion of saponins is directly responsible for their antioxidant effect, which may help reduce the risk of cancer and heart disease.

4.2.4 Glycosides

Liebermann's test was employed to determine the entity of aglycone in steroidal components of glycosides, drawing on a prior research by Gul et al. (2017). Glycosides were denoted by the green hue. With reference to Figure 4.5, neither of the extracts is depicted in green. According to the study, methanol and distilled water extracts showed no evidence of steroidal glycosides. The outcome demonstrated that a number of variables, including the polarity of the solvent, may affect the existence of phytochemical elements.

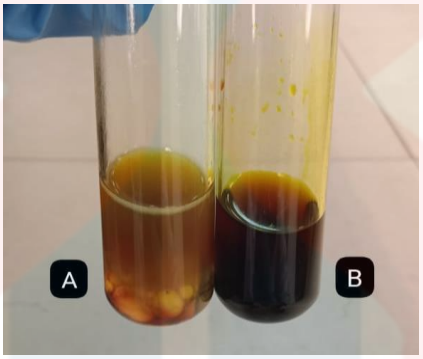


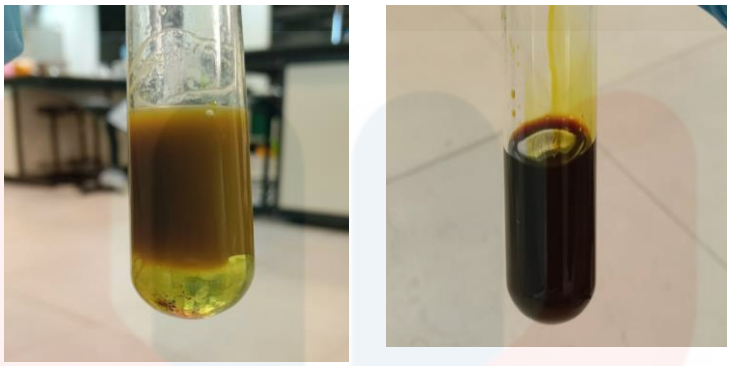
Figure 4.5: Phytochemical screening of glycosides

*A=distilled water, B=methanol

4.2.5 Steroids

Salkowski's test, which uses the response test's red hue to indicate the presence of steroids. The outcomes of both extracts' reactions with hydrochloric acid were shown in Figure 4.6. The deep crimson layer representing the distilled water extract was poor visible at the bottom. The presence of steroids in plant extract is indicated by the development of a red colour in the bottom layer. Both the methanol and the distilled water extracts tested differently for the presence of steroids, with the colour change appearing.

FYP FBKT



(A) (B)

Figure 4.6: Phytochemical screening of steroids

*A=distilled water, B=methanol

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

Based on the findings displayed in Table 4.1, it was observed that the plant extracts exhibited both the presence and lack of the bioactive component. Furthermore, distilled water extract and methanol both displayed precisely the same negative and positive outcomes, but with distinct bioactive components. It can conclude that the presence of phytochemicals in plant extracts can be impacted by solvent extraction.

In methanol extracts, flavonoids, saponins, glycosides and steroid are absent. When compared to the extract made from distilled water, saponins were more visible in the foam that was produced. According to this study's qualitative test, distilled water worked well as a solvent for saponin extraction. On the other hand, as Figures 4.3 show, methanol extract yields superior outcomes for the tannins.

Furthermore, distilled water extract did not show any presence of tannins, flavonoids and glycoside. Both extract showed the same result for flavonoids and glycoside test except for tannins. For the tannins test, it was observed that tannins compound might be low in this distilled water extract compared to methanol extract. In the case of the both test, which flavonoids and glycosides, it was observed that flavonoids and glycoside compound might be

low in this plant or the part of the plant that has been studied because the result for both extract is same which no appearance of both compound.

In addition, the most suitable solvent that can concluded to use as solvent in phytochemical experiment is distilled water solvent. Based on the result in Table 4.1, distilled water extract showed most positive appearance of the bioactive compound compared to the methanol extract. It is because distilled water is pure and doesn't change the solute's chemical makeup, it makes observations and measurements more precise. However, there are another factor can be influenced for the appearance of bioactive compound in different solvent.

4.4 Analysis Of Antioxidant Activity in *Curcuma Xanthorrhiza* Extract

The *Curcuma Xanthorrhiza* plant's antioxidant activity was assessed in this study using its capacity to scavenge free radicals (DPPH). To determine the scavenging action of DPPH towards the extracts, the DPPH inhibition percentage of each plant extract was determined (Syed Salleh et al., 2021). A shift in colour from purple to yellow indicates a reduction in the absorption of DPPH radicals. This demonstrates the good interaction between an antioxidant and free radicals in a mixed solution. The value of IC_{50} is an important parameter to assess when measuring antioxidant activity. It is defined as the amount of antioxidant concentration needed to lower the initial DPPH content by roughly 50%. Therefore, when the IC_{50} value is smaller, the antioxidant is regarded as strong.

The percentage of radical scavenging activity and IC_{50} values of ascorbic acid (Table 4.2), methanol extract (Table 4.3), and distilled water (Table 4.4) showed with concentrations between 0.1 to 0.5 g/mL. The results demonstrated that the percentage of DPPH scavenging activity of *Curcuma Xanthorrhiza* based on ascorbic acid (94.44%) specifically at the concentration of 5 mg/mL was higher than distilled water (73.18%) and methanol (78.62%). Besides, the IC_{50} value of distilled water (1.5021 mg/mL) was higher than that of methanol extract (1.4837 mg/mL) and ascorbic acid (1.3671 mg/mL).

According to the evaluation of antioxidant activity, distilled water and *Curcuma Xanthorrhiza* methanol extract are both less active than ascorbic acid. With reference to the IC_{50} results shown in tables 4.2, 4.3, and 4.4, a lower IC_{50} number indicates a higher level of antioxidant activity. According to Jadid et al. (2017), extracts having IC_{50} values between 50 and 100 mg/mL are considered to have medium antioxidant activity. On the other hand, extracts

FYP FBKT

with an IC_{50} value between 10 and 50 mg/mL are assumed to have significant antioxidant activity.

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

Concentration (g/mL)	Absorbance	% RSA	IC_{50} (mg/mL)
0.1	0.071	94.54	1.3671
0.2	0.070	94.60	
0.3	0.071	94.51	
0.4	0.071	94.51	
0.5	0.072	94.44	

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

Table 4.3: The DPPH free radical scavenging activity of *Curcuma Xanthorrhiza* plant based on methanol extract

Concentration (g/mL)	Absorbance	% RSA	IC_{50} (mg/mL)
0.1	0.111	91.40	1.4837
0.2	0.155	88.04	
0.3	0.194	85.00	
0.4	0.236	81.79	
0.5	0.277	78.62	

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

Table 4.4: The DPPH free radical scavenging activity of *Curcuma Xanthorrhiza* plant based on distilled water extract

Concentration (g/mL)	Absorbance	% RSA	IC_{50} (mg/mL)
0.1	0.128	90.08	1.5021
0.2	0.177	86.35	
0.3	0.195	84.96	
0.4	0.222	82.82	
0.5	0.347	73.18	

From the results, the results of the DPPH radical scavenging activity percentage of standard ascorbic acid might be affected by the various factor. The control may be too concentrated, which might be the source of the issue. The sample colour's intensity may change depending on the concentration. In addition, the outcome showed how crucial solvent volume and extract concentration to the synthesis of antioxidant qualities. The higher concentration of extract, the lower measured level of antioxidant activity.

Furthermore, the variations in the antioxidant activity that the *Curcuma Xanthorrhiza* plant shown might be attributed to the kinds of solvents that were employed during the extraction procedure. The extraction solvent had a significant impact on the antioxidant extracts as solvent polarity affects the recovery of antioxidant components from herbals.

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

CHAPTER 5

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on phythochemical screening, the presence of bioactive compounds in the crude extract of *Curcuma Xanthorrhiza* was influenced by the solvents used. Tannins were only present in methanol extracts, while saponins and steroids were only present in distilled water extracts. Furthermore, the antioxidant activity of the plant extract was also influenced by the solvent used for extraction. The findings of IC_{50} show that the ascorbic acid standard plant has a great level of antioxidant activity than the plant extract in both solvents. The IC_{50} findings indicate that the *Curcuma Xanthorrhiza* plant, when extracted with distilled water, exhibits a lower level of antioxidant activity compared to both the methanol extract and the ascorbic acid standard. According to Phongpaichit et al. (2007), the standard IC_{50} value can be marked when IC_{50} was <10 mean they have very strong antioxidant activity. So, this means distilled water extraction, methanol extraction and ascorbic acid standard have a very strong antioxidant activity.

5.2 Recommendation

Further research on the remaining plant components can be done with various extraction solvents. To increase the study's effectiveness, the investigation of how solvents affect extraction yield can be added. Aside from that, incorporating other extraction techniques, like the standard procedure, can improve the outcome and help the study reach its goal. Furthermore, a research institute might be a useful resource for accurately identifying plant species.

To improve the comparability with the prior study, more distinct testing for each bioactive molecule might be performed for phytochemical screening. Tests on the terpenoids

FYP FBKT
UNIVERSITI
MALAYSIA
KELANTAN

and curcuminoids of different species of turmeric, for instance, can be performed for preliminary phytochemical screening. Furthermore, it is advised to use a lower concentration value in dilution to calculate the absorbance value when analysing antioxidant activity using the DPPH free radical scavenging test technique. In addition, an alternative test such as the ferric reducing (FRAP) assay can be employed to evaluate the plant's antioxidant capacity. It is advisable to conduct tests on the anticancer and antibacterial activities of herbal plants in order to get recognition for their advantages and functionalities.

FYP FBKT

UNIVERSITI
MALAYSIA
KELANTAN

FYP FBKT

REFERENCES

Azwanida, N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants, 04 (03), 3–8.

Balasuriya, N., & Rupasinghe, H. V. (2012). Antihypertensive properties of flavonoid-rich apple peel extract. Food Chemistry, 135(4), 2320-2325.

Balmus, I. M., Ciobica, A., Trifan, A., & Stanciu, C. (2016). The implications of oxidative stress and antioxidant therapies in Inflammatory Bowel Disease: Clinical aspects and animal models. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association, 22(1), 3.

Batiha, G.E. & Beshbishy, A.M. (2020). Gas chromatography-mass spectrometry analysis, phytochemical screening and anti-protozoal effects of the methanolic Viola tricolor and acetonic Laurus nobilis extracts, BMC Complementary Medicine and Therapies, 20(87). https://doi.org/10.1186/s12906-020-2848-2, Accessed: 01.11.2020.

Belscak-Cvitanovic, A., Benkovic, M., Komes, D., Bauman, I., Horzic, D., Dujmic, F., & Matijasec, M. (2010). Physical properties and bioactive constituents of powdered mixtures and drinks prepared with cocoa and various sweeteners. Journal of agricultural and food chemistry, 58(12), 7187-7195.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199-1200.

Britannica, T. Editors of Encyclopaedia (2024, January 25). tannin. Encyclopedia Britannica. https://www.britannica.com/science/tannin

C´ ujic´, N., Katarina Šavikin, Teodora Jankovic´, Dejan Pljevljakušic´, Gordana Zdunic´, & Ibric´, S. (2016). Optimization of polyphenols extraction from dried chokeberry using

maceration as traditional technique. Food Chem, 194, 135-142. doi:10.1016/j.foodchem.2015.08.008

Dalimartha S. Atlas Tumbuhan Obat Indonesia. Jakarta, Indonesia: Trubus Agriwidya; 2000.

de Padua L. S., Bunyapraphatsara N., Lemmens R. H. M. J. Medicinal and Pousionous Plants 1. Vol. 12. Leiden, Netherlands: Backhuys Publishers; 1999. Plants resources of south east asia; p. p. 711.

Devmurari. (2010). Phytochemical screening study and antibacterial evaluation of Symplocos racemosa Roxb Archives of Applied Science Research, 2, 354-359.

Doughari, J. H. (2012). Chapter 1: Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals (Ed. Rao, V.). A Global Perspective of Their Role in Nutrition and Health. A Global Perspective of Their Role in Nutrition and Health: London, 1-32.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. ScientificWorldJournal, 2017, 5873648. doi:10.1155/2017/5873648

Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S., & Belmalha, S. (2020). Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. Heliyon, 6(11), e05609. doi:10.1016/j.heliyon.2020.e05609

Hudson, J. (2012). Applications of the phytomedicine Echinacea purpurea (Purple Coneflower) in infectious diseases. Journal of Biomedicine and Biotechnology, 2012, 769896. doi: 10.1155/2012/769896

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., & Wikanta, W. (2017). Antioxidant activities of different solvent extracts of Piper retrofractum Vahl. using DPPH assay.

Kavitha, V.U., Kandasubramanian, B. Tannins for wastewater treatment. SN Appl. Sci. 2, 1081 (2020). https://doi.org/10.1007/s42452-020-2879-9

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology, 48(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., . . . Zhang, B. (2017). Saponin-Based, Biological-Active Surfactants from Plants. In Application and Characterization of Surfactants.

Limmatvapirat, C., Nateesathittarn, C., Dechasathian, K., Moohummad, T., Chinajitphan, P., & Limmatvapirat, S. (2020). Phytochemical analysis of baby corn silk extracts. J Ayurveda Integr Med, 11(3), 344-351. doi:10.1016/j.jaim.2019.10.005

Maruthupandian, A., & Mohan, V. R. (2011). Antidiabetic, antihyperlipidaemic and antioxidant activity of Pterocarpus marsupium Roxb. in alloxan induced diabetic rats. Int J Pharm Tech Res, 3(3), 1681-1687.

Molyneux., R.J., Lee, S.T., Gardener, L.E.,& Panter K.E. (2007). Phytochemicals: The good, the bad, and the ugly? Phytochem, 68(22- 24): 2973-2985. https://doi.org/10.1016/j.phytochem.2007.09.004, Accessed: 05.05.2018.

Nguyen, T. M. T., Cho, E. J., Song, Y., Oh, C. H., Funada, R., & Bae, H. J. (2019). Use of coffee flower as a novel resource for the production of bioactive compounds, melanoidins, and bio-sugars. Food Chem, 299, 125120. doi:10.1016/j.foodchem.2019.125120

FYP FBKT
UNIVERSITI MALAYSIA KELANTAN

Nićiforović, N., Mihailović, V., Mašković, P., Solujić, S., Stojković, A., & Muratspahić, D. P. (2010). Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food and Chemical Toxicology, 48(11), 3125-3130.

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry, 2(5), 115-119.

Phongpaichit, S., Nikom, J., Rungjindamai, N., Sakayaroj, J., Hutadilok-Towatana, N., Rukachaisirikul, V., & Kirtikara, K. (2007). Biological activities of extracts from endophytic fungi isolated from Garcinia plants. FEMS Immunol Med Microbiol, 51(3), 517-525. doi:10.1111/j.1574-695X.2007.00331.x

Rahmat, E., Lee, J., & Kang, Y. (2021). Javanese Turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, Phytochemistry, Biotechnology, and Pharmacological Activities. Evidence-based complementary and alternative medicine : eCAM, 2021, 9960813. https://doi.org/10.1155/2021/9960813

Rakshamani Tripathi, H. Mohan, & Kamat, J. P. (2007). Modulation of oxidative damage by natural products. Food Chemistry, 100(1), 81-90. doi:10.1016/j.foodchem.2005.09.012

Roopashree, T. S., Dang, R., Rani, S. R. H., & Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. International Journal of Applied research in Natural products, 1(3), 20-28

Rosario, J. C. J., & Josephine, R. M. (2015). A review on traditional medicinal plants for anti-cancerous activity. Int J Recent Sci Res, 6(8), 5634-5637.

Rukmana, I. H. R. (1995). Temulawak, Tanaman Rempah dan Obat. Kanisius.

Salleh, N. A. M., Ismail, S., & Ab Halim, M. R. (2016). Effects of Curcuma xanthorrhiza extracts and their constituents on phase II drug-metabolizing enzymes activity. Pharmacognosy Research, 8(4), 309.

FYP FBKT

Sari, N., Wahidah, B. F., & Gaffar, N. A. (2017). Etnobotani tumbuhan yang digunakan dalam pengobatan tradisional di kecamatan Sinjai Selatan Kabupaten Sinjai Sulawesi Selatan. In Prosiding Seminar Nasional Biologi (Vol. 3, No. 1).

Sarris, J., Panossian, A., Schweitzer, I., Stough, C., & Scholey, A. (2016). Herbal medicine for depression, anxiety, and insomnia: a review of psychopharmacology and clinical evidence. European Neuropsychopharmacology, 26(6), 823-841. doi: 10.1016/j.euroneuro.2016.02.002

Shkondrov, A., Krasteva, I., Pavlova, D., & Zdraveva, P. (2017). Determination of flavonoids in related Astragalus species (Sect. Incani) occurring in Bulgaria. Comptes rendus de l'Académie Bulg. des Sci, 70, 363-366.

Siju, E. N., Rajalakshmi, G. R., Hariraj, N., Anusha, K. V., Kuttoor, D. S., & Shirwaikar, A. (2014). Elementary analysis of Thespesia populnea fruits. Int J Phytopharmacol, 5(2), 139-42.

Singh, V., & Kumar, R. (2017). Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. International Journal of LifeSciences Scientific Research, 3(6), 1451-1458. doi:10.21276/ijlssr.2017.3.6.

Sun, C., Zhengshuang Wu, Ziyan Wang, & Zhang, H. (2015). Effect of Ethanol/Water Solvents on Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts. Evid Based Complement Alternat Med, 2015, 595393. doi:10.1155/2015/595393

Syamsudin, R. A., Perdana, F., Mutiaz, F. S., Galuh, V., Rina, A. P. A., Cahyani, N. D., ... & Khendri, F. (2019). Temulawak plant (Curcuma xanthorrhiza Roxb.) as traditional medicine. Farmako Bahari, 10, 51-65.

Syed Salleh, S. N. A., Mohd Hanapiah, N. A., Ahmad, H., Wan Johari, W. L., Osman, N. H., & Mamat, M. R. (2021). Determination of Total Phenolics, Flavonoids, and

FYP FBKT

Antioxidant Activity and GC-MS Analysis of Malaysian Stingless Bee Propolis Water Extracts. Scientifica (Cairo), 2021, 3789351. doi:10.1155/2021/3789351

Trease, G. E., & Evans, W. C. (1983). Pharmacognosy.

Truong, D.-H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality, 2019, 1-9. doi:10.1155/2019/8178294

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. Molecules (Basel, Switzerland), 25(22), 5243. https://doi.org/10.3390/molecules25225243

USDA Plants Database. (n.d.). Lantana camara L. Retrieved from https://plants.usda.gov/core/profile?symbol=LACA12

FYP FBKT

UNIVERSITI MALAYSIA KELANTAN