A Review on Pharmacological Properties of *Marsilea quadrifolia*

By

Pritom Kumer Das
Student ID: 17146021

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Department of Pharmacy
Brac University
January, 2022

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Pritom Kumer Das
17146021

Approval

The thesis/project titled "A review on pharmacological properties of *Marsilea quadrifolia* " submitted by Pritom Kumer Das (17146021) of Spring, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 24th January, 2022.

Examining Committee:

Supervisor:
(Member)

_____

Dr. Farhana Alam Ripa
Assistant Professor, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

_____

Namara Mariam Chowdhury,
Lecturer, Department of Pharmacy
Brac University.

Deputy Chairperson:
(Member)

_____

Dr. Hasina Yasmin,
Professor, Department of Pharmacy
Brac University.

Departmental Head:
(Chair)

_____

Dr. Eva Rahman Kabir,
Professor, Department of Pharmacy
Brac University.

Ethics Statement

This study does not involve any kind of animal or human trial.

Abstract

Medicinal plants have been used to treat different diseases throughout the history of humans. They are potential sources of many medicines and traditional medicines greatly depend on medicinal plants. *Marsilea quadrifolia* is a very common medicinal plant in Indian sub-continent and it has a big role in traditional medicines. This plant belongs to Marsileaceae family. This plant contains a wide range of chemicals including Phenolic compounds, Tannins, Saponins, Flavonoids, Steroids, Terpinoids, Alkaloids, Carbohydrates and etc. Plant extract of this plant has different therapeutic activities such as cytotoxic activity, anti-inflammatory activity, diuretic activity, anti-venom activity, hypo glycemic activity and anti-bacterial activity. This review article is a comprehensive review of different pharmacological activities of *M. quadrifolia*.

Keywords: *Marsilea quadrifolia*; Cytotoxicity; Anti-inflammatory; Diuretics; Anti-venom

Dedication

Dedicated To My Parents

Acknowledgement

This research could not have been completed without the help of numerous individuals who are gratefully acknowledged here.

I would like to express my fullest gratitude to my supervisor, **Dr. Farhana Alam Ripa** Ma'am, Assistant Professor, Department of Pharmacy, Brac University. She always supported and motivated me to finish this thesis paper. It would have been impossible for me to complete this thesis paper without her guidance. I am also thankful to **Prof. Dr. Eva Rahman Kabir** Ma'am, Honorary Chairperson, Department of Pharmacy and Brac University, **Dr. Hasina Yasmin** Ma'am, Professor and Deputy Chair, Department of Pharmacy, Brac University, academic coordinator **Namara Mariam Chowdhury** Ma'am, Lecturer, Department of Pharmacy, Brac University and all the faculty members of Department of Pharmacy, Brac University for helping me with my work and providing me with the opportunity to complete my B. Pharm program.

Table of Contents

Declaration ..... **ii**

Approval ..... **iii**

Ethics Statement ..... **iv**

Abstract ..... **v**

Dedication ..... **vi**

Acknowledgement ..... **vii**

List of Tables ..... **x**

List of Figures ..... **xi**

List of Acronyms ..... **xii**

Chapter 1 Introduction **1**

1.1 Medicinal Plants 1

1.2 *M. quadrifolia* 3

Chapter 2 Plant Description **6**

2.1 Natural Habitat 6

2.2 Morphology 6

2.2.1 Rhizome 7

2.2.2 Leaves 7

2.2.3 Roots 8

2.2.4 Interior Structure of *M. quadrifolia* 9

Chapter 3 ..... **15**

Phytochemicals **15**

3.1 Phenolic Compounds 15

3.2 Flavonoids 16

3.3 Tannins 17

3.4 Saponins 18

Chapter 4 Pharmacological Properties of *M. quadrifolia* 21

4.1 Cytotoxic Effect 21

4.2 Anti Inflammatory Effect 24

4.3 Diuretic Effect 28

4.4 Anti-venom Activity 29

4.5 Hypoglycemic Effect 31

4.6 Antibacterial Activity 31

Chapter 5 Discussion 33

Chapter 6 Conclusion 35

Reference 36

List of Tables

Table 1 Taxonomic Classification of *M. quadrifolia*.....4

Table 2Name of *M. quadrifolia* in different language/geographical location 5

Table 3 Presence of Different chemicals in Leaf and Stem of *M. quadrifolia* 19

Table 4 Amount of different compounds in leaf and stem of *M. quadrifolia*.....20

Table 5 Assay on the effect of *M. quadrifolia* on Cytotoxicity of Cells. 23

Table 6 Evaluation of anti-inflammatory effect of *M. quadrifolia*.....27

Table 7 Evaluation of diuretic activity of *M. quadrifolia*.....29

List of Figures

Figure 1:(a) leaves (b) Aerial parts of *M. quadrifolia* 4

Figure 2: Leaves of *M. quadrifolia* 7

Figure 3: M. quadrifolia Internal structure of rhizome 9

Figure 4: M. quadrifoliaT. S of petiole 11

Figure 5: V.S of leaflet or pinna 13

Figure 6: Internal structure of Root 13

Figure 7: Common Phenolic Compounds 15

Figure 8: Some common Flavonoids 16

Figure 9: Tannin and its classification 17

Figure 10: Structure of Saponin 21 18

Figure 11: Neutralization of Hemorrhagic activity of Russell's viper venom by *M. quadrifolia*.....30

.

List of Acronyms

M. quadrifolia	*Marsilea quadrifolia*
APEMQ	Aerial part extract of *Marsilea quadrifolia*
WHO	World Health Organization
API	Active Pharmaceutical Ingredient
TS	Transverse section
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
LC_{50}	Lethal Concentration 50%
HRBC	Human Red Blood Cell

Chapter 1

Introduction

1.1 Medicinal Plants

We are part of nature and we depend on nature for our existence. In recent years due to the vast development of science and technology, we can produce a lot of daily using products synthetically. However, we hardly can avoid the things we can get from nature. In ancient days, our ancestors used to rely on medicinal plants for medicines. There were no alternative sources of medicines. Even in modern days, a lot of people use medicinal plants for treatment. Medicinal plants are the most important part of traditional medicines. Plant medicines are being used for a lot of diseases. For example, skin diseases, common ailments, microbial infection, inflammation etc. The human being is using medicinal plants since the beginning of mankind (Petrovska, 2012). Man has a strong connection with natural medicinal sources, especially with medicinal plants. Ancient human being used to doing experiments with different plants and their knowledge used to be passed to the next generations (Shakil et al., 2021). All ancient communities including the Mayans, Indians, Egyptians, Chinese and Romans knew about medicinal plants and their usage. They build their own medicines from medicinal plants. The Indians made Ayurveda which is one of the oldest medical sciences (around 5000 years old) and it is maintaining a prominent position all over the world even today with the recognition of WHO (Javalgekar, 1982). According to the WHO, 60% of the world population still depends on medicinal plants for primary treatment. Herbal medicines were also used in ancient Egypt and their medicinal knowledge was quite rich. They knew how to mummify dead bodies, they had knowledge about the functions of some organs and they knew how to use medicinal plants (Aboelsoud, 2010). Previously, we know that medicinal plants played a great role in Egyptian medicinal science. Use of cannabis, garlic,

opium, myrrh, henna, aloe etc. Onion and garlic were an important part of their culture and they used to believe these are holy plants due to their medicinal function. The ancient people used different leaves such as willow, acacia etc. They used to use pomegranate root to deal with tape worm. The Mayans also used to use medicinal plants for treatment. All of the ancient civilizations knew how to use medicinal plants. Mankind have been using medicinal plants since the beginning and their knowledge of medicinal plants have been transferred from one generation to another which varies from one civilization to another due to the time, geographical location and the plants available in that area (Aboelsoud, 2010). The modern pharmaceutical industry depends a lot on these medicinal plants. Active pharmaceutical ingredient which is the key component of a medicine, a lot of them is found in medicinal plants such as cannabis-based medicines have API extracted from cannabis (Nyakudya et al., 2020). API of aspirin is also found in plants. Few plants have been known for antiemetic function while some plants are known for their neurological effect. Neurological diseases include Alzheimer's disease, Parkinson's disease, brain tumor, traumatic diseases etc. Over one billion people all over the world are suffering from any kind of neurological diseases. Also, In Ghana, twenty eight families of plants were reviewed among which 50% plants had analgesic, 18.8% anxiolytic, 15.6% anticonvulsant properties (Amoateng et al., 2018). Botanical survey of India estimated that India is home to more than 8000 species of medicinal plants. Even in Andaman and Nicobar Island, the indigenous people use 39 medicinal plants and among those plants 17 plants can be used for multiple diseases. A study shows that 55 plants which are available in Bangladesh have allopathic properties belong to 32 families (Mominul Islam et al., 2018). Around 5000 species of plants are found in Bangladesh among which 1000 are known to have medicinal properties. 250 medicinal plants are being used regularly in our country. These medicinal plants are very important part of traditional medicines in Bangladesh. Medicinal plants have great role in modern medical

science. Some modern medicines were extracted from medicinal plants such as paracetamol, aspirin etc. Chemotherapeutic agents are also found in plants. There are some plants which help to deal with nephrotoxicity (Khajavi Rad et al., 2017).

1.2 *M. quadrifolia*

M. quadrifolia is a very common medicinal plant and it is being used for various medicinal purposes from the ancient period of time. It is basically an aquatic fern (Uma & Pravin, 2013). It has different names based on its geographical location. In this sub-continent alone, it has multiple names. In Bangladesh it is known as Shushni saak, in Tamil known as Aaraikeerai, in Malayalam known as Neeraral, in Hindi known as Sunsuniya. In Europe, *M. quadrifolia* is known as European water clove. In a recent study by (Uma & Pravin, 2013) claimed that it has four parted leaf which forms 4- leaf clover. This plant is normally found near water sources like pond, lakes, paddy fields etc. Swamps, marshes, and riverbanks, streams, ponds, and lakes in Asia and Europe are native habitats for this plant (B.-L. L. Liu, 1984). It is also found in America but it is known as alien species over there.

Figure 1:(a) leaves (b) Aerial parts of M. quadrifolia (Subramanian & Balakrishnan, 2018)

It has two parts where one part (leaf) remains floating on water surface and other part remains submerged and erected in shallow water or land. It is also seen in wet farm lands and in farm lands it is considered as weed. It belongs to Marsileaceae family.

Table 1 Taxonomic Classification of M. quadrifolia

Kingdom	Plantae
Division	Pteridophyta
Class	Pteridopsida
Order	Salviniales
Family	Marsileaceae
Genus	Marsilea

M. quadrifolia has different pharmaceutical activities. Leaf juice of this plant works as diuretic and also used to treat snake bite. This plant is well known for its action on

inflammation. This plant is also known for its cytotoxic activity. In rural areas people use this plant to treat cough, skin diseases, diarrhoea, diabetes, psychiatric diseases and etc. Some studies have found its antioxidant activity which has created a chance of its use in cancer treatment. In rural areas of Indian subcontinent this plant is used to treat snake bite. Snake bite is a very common problem in rural areas and people hardly get proper treatment of snake bite. Rather people mostly relay on traditional treatments. In such areas this plant plays vital role. This plant has created a strong position in herbal medicines. People of rural area are using this plant to treat inflammation from ancient period. On the other hand, *M. quadrifolia* is known to have diuretic effect. It is claimed that juice of this plant can increase frequency of urination.

Table 2 Name of M. quadrifolia in different language/geographical location (Subramanian & Balakrishnan, 2018)

Language/Geographical Location	Name
Bangla	Shushni saak
Hindi	Sunsuniya
Tamil	Aaraikeerai
Europe	European Water Clove

Chapter 2

Plant Description

2.1 Natural Habitat

The species are either hydrophytic or amphibious, meaning they grow rooted in mud, marshes, and small pools, or they are totally immersed, partially immersed, or totally out of water in moist settings. *Mairia hirsuta* and *M. quadrifolia* are two of the most widespread Indian species, typically found in marshy areas, damp soil, or near muddy pond borders (Kumar Agarwal et al., 2018)

Swamps, marshes, and riverbanks, streams, ponds, and lakes in Asia and Europe are native habitats for this aquatic moss species. This plant was intentionally introduced to the United States (Bruni et al., 2013)

2.2 Morphology

M. quadrifolia is an aquatic fern of the Marsileaceae family that is known in Bengali as Sushni Saak. It's a fern that grows in water. Figure 2 shows a four-part leaf that resembles four-leaves. The leaves float in deep water or even on the surface of the river. In a shallow watery environment, the plant is observed standing upright. A long-stalked petiole is present in the plant. Besides, this plant is seen in wet soil (X et al., 2014)

Figure 2: Leaves of M. quadrifolia (Subramanian & Balakrishnan, 2018)

Rhizome, leaves, and roots are separate parts of the plant body.

2.2.1 Rhizome

Rhizome of *M. quadrifolia* can be seen on or just beneath the soil surface. It is thin, dichotomously branched with distinct nodes and internodes, and capable of unbounded growth in all directions, occupying an area of 25 meters or greater in diameter. Internodes in aquatic species are longer, whereas those in subterranean species are short. The leaves are usually given out from the top side of nodes, while the roots are given out from the bottom side (Ramya Juliet et al., 2020).

2.2.2 Leaves

They are borne in two rows on the top side of the rhizome at nodes. Young leaves have a circinate vernation pattern (like ferns). Multicellular hairs cover the immature leaves of several plants. The leaves are complex, with a petiole at the base and a lamina at the end. The petiole of a leaf in submerged plants is a long and flexible structure, and the lamina floats

above the water's surface, but the petiole of a leaf in muddy or marshy plants is short and inflexible, with short lamina spreading in the air (B.-L. Lin & Yang, 1999).

At the apex of the petiole, the lamina consists of four leaflets (pinnae). The four leaflets appear as a result of three lamina dichotomies occurring in close succession, two leaflets arise a little higher than the other two. A reticulum is formed when veinlets near the border are joined by loops. Shapes of pinnae can vary between obovate to obcuneate, and their margins range from entire to crenate or crenate to lobed (Kumar Agarwal et al., 2018).

The pinnas are sometimes profoundly dichotomously lobed or toothed. The pinna is folded upwards at night. This is referred to as pinna sleeping movement. The stalked bean-shaped sporocarps are produced toward the base of the petiole (Kao & Lin, 2010).

2.2.3 Roots

The roots are adventitious, growing individually or in bunches from the underside of the rhizome node. The roots are given out even from the internodes in some circumstances (B. Liu, 1984)

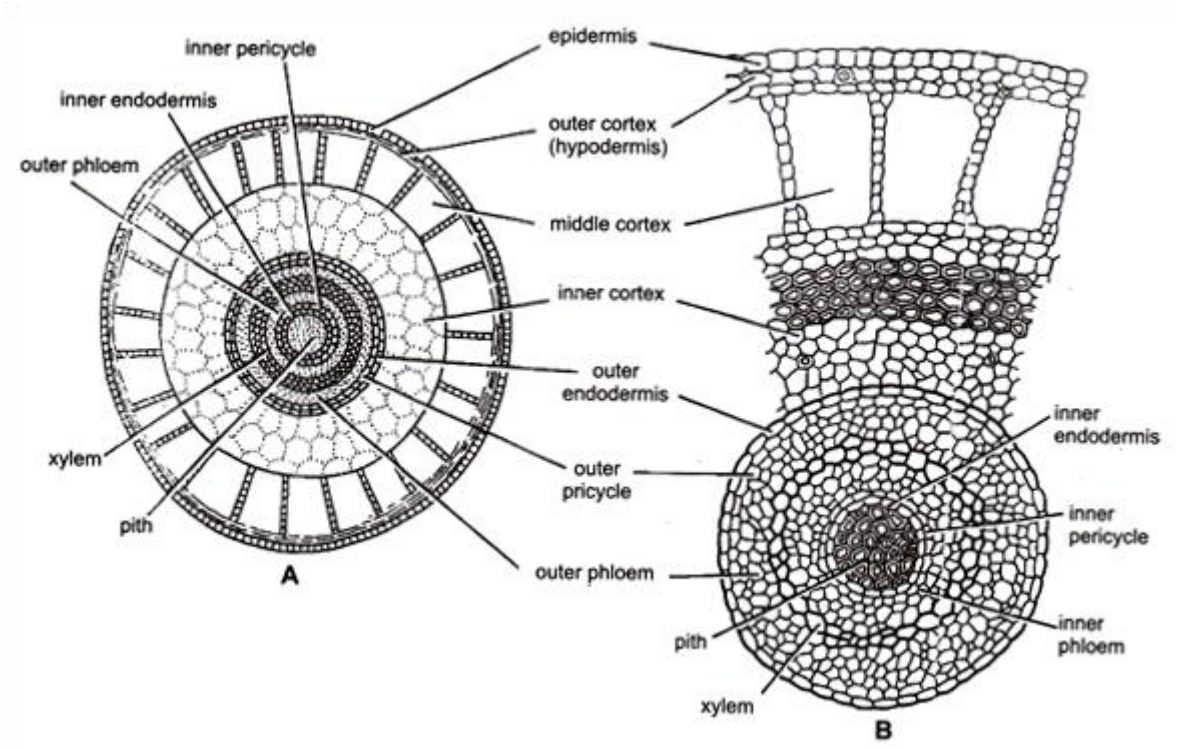

Figure 3: M. quadrifolia Internal structure of rhizome (R Vishnupriya, 2015)

2.2.4 Interior Structure of *M. quadrifolia*

1. Rhizome (stem)

A T. S. of an immature rhizome reveals a protostelic structure, in which pith is empty and xylem is fully surrounded by phloem, but in an old stem, pith develops in the center and the stele is amphiphloic siphonostelic type.

The old stem's A. T. S. has a fairly round form and has the following structures:

(i) Epidermis:

It's the single-celled thick parenchymatous cells' outermost limiting layer. Stomata are missing (Kumar Agarwal et al., 2018).

(ii) Cortex:

The outer cortex, middle cortex, and inner cortex are the three sections that make up the cortex.

(a) Outer cortex:

It can be found just underneath the epidermis (also called hypodermis). It is parenchymatous and can range in thickness from one to many cells. Tannin is found in certain of its cells (Ramya Juliet et al., 2020).

(b) Middle cortex:

Aerenchyma is another name for it. It is located under the hypodermis. It is made up of huge air gaps (chambers) divided by parenchymatous septa that are one cell thick (Juliet et al., 2020). The air chambers are eliminated in xerophytic species such as aegyptiaca.

(c) Inner cortex:

It is a solid tissue with a thickness of several cells. The outer layers of cells have thick walls (sclerenchymatous), but the interior layers have thin walls (parenchymatous) and are compactly organized. Some of these cells are starch or tannin-filled (Ahmad et al., 2011).

(iii) Stele:

The stele is amphiphloic-siphonostele, which means it has a pith in the center that might be parenchymatous (aquatic species) or sclerenchymatous (sclerenchymatous species) (terrestrial muddy species). The xylem consists of a full ring encircled on both sides by a complete ring of inner and outer phloem, pericycle, and endodermis (Rechard, 2014).

Outer endodermis, outer pericycle, outer phloem, xylem, inner phloem, inner pericycle, and inner endodermis are the tissues that continue in the form of a full ring in the stele (R Vishnupriya, 2015).

2. T. S. of Petiole:

The petiole's T. S. has a fairly round contour and is divided into epidermis, cortex, and stele.

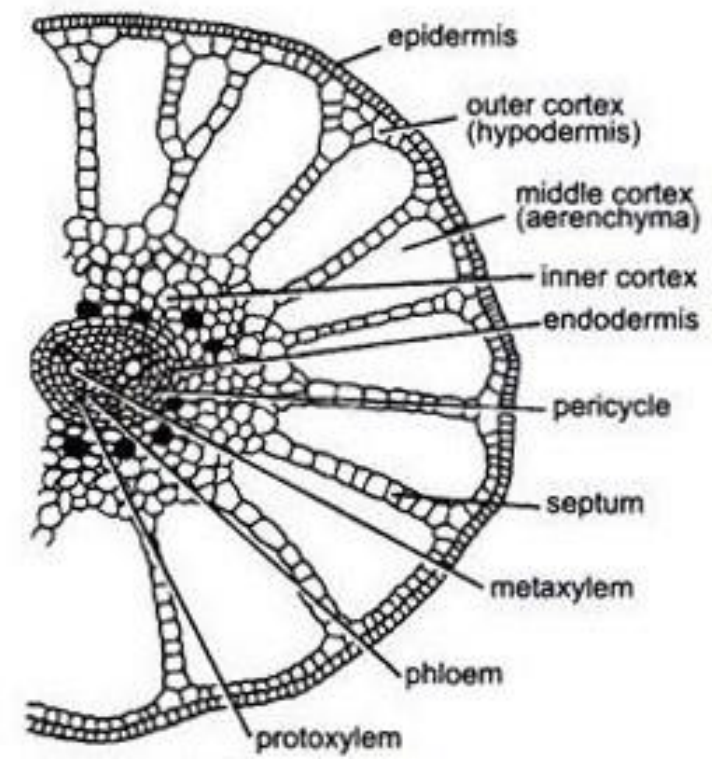

Figure 4: M. quadrifoliaT. S of petiole (R Vishnupriya, 2015)

(i) Epidermis:

It is the one cell thickness outermost layer. The cells are somewhat elongated and parenchymatous (G Karikalan et al., 2008).

(ii) Cortex:

It is differentiated into three regions: The outer cortex, the middle cortex and the inner cortex. The outer cortex is found just beneath the epidermis which is made of cells with thin cell

wall. The middle cortex is also known as aerenchyma and it is present below the hypodermis. The inner cortex is a solid tissue that has thickness of several cells. The cell layers contain cells filled with starch and tannin.

(iii) Stele:

Stele is triangular in shape and pith is not present here. The xylem is "V" in shape which has two arms. Phloem surrounds the xylem on all sides and phloem is surrounded by a single layer of parenchymatouspericycle from outside.

3. Transverse Section of Leaflet:

The epidermis, mesophyll, and vascular bundles are found in the A. T. S. of the leaflet.

(i) Epidermis:

The epidermis is the outer layer of the skin.

It is only one cell thick and is the outermost surrounding layer. Upper and lower epidermis are two types of epidermis. Stomata are only found on the upper epidermis of floating leaflets, while they are found on both the top and lower epidermis of plants growing in mud or damp soil with airborne leaves (Gopalakrishnan et al., 2017).

(ii) Mesophyll:

It takes up a lot of room between the upper and lower epidermis. Upper palisade tissue and lower spongy parenchyma are frequently distinguished. Palisade tissue is made up of chloroplast-equipped elongated cells. The spongy tissue is made up of loosely packed parenchymatous cells separated by single layered septa with huge air gaps. The mesophyll of submerged species, on the other hand, is not divided into palisade and spongy parenchyma (Gopalakrishnan Karikalan & Rajangam, 2018).

(iii)

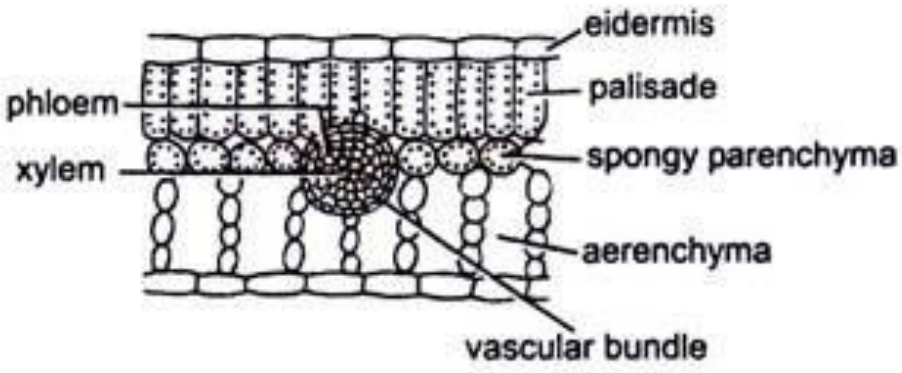

Figure 5: V.S of leaflet or pinna (R Vishnupriya, 2015)

Vascular bundles:

Several vascular bundles can be found in between the mesophyll tissue. Each vascular bundle is amphicribal in nature, consisting of a centrally located xylem encircled on all sides by phloem. A single layered thick endodermis surrounds the phloem (Bordonneau & Tourte, 1994).

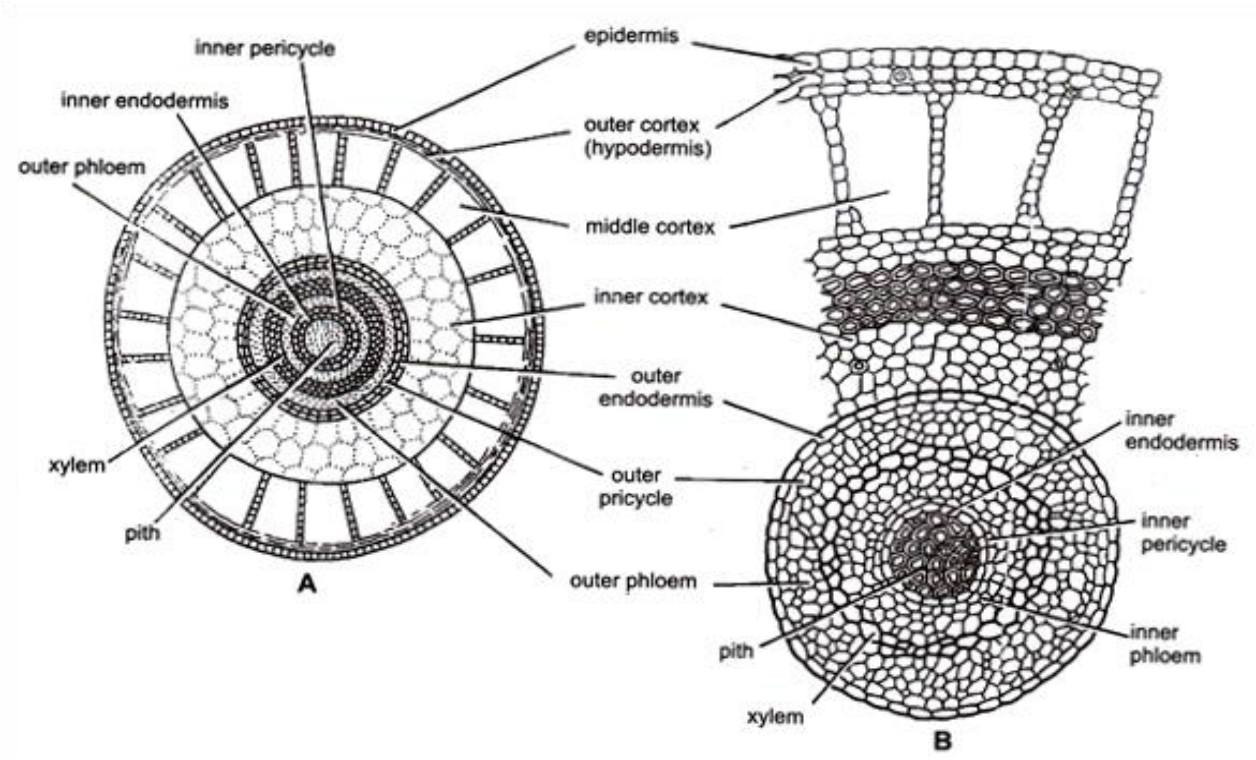

Figure 6: Internal structure of Root (R Vishnupriya, 2015)

4. T. S. Root:

A T. S. of root has a circular form and is divided into three layers: epidermis, piliferous layer, cortex, and stele.

(i) Epidermis:

It is the single-layered, parenchymatous outermost layer.

(ii) Cortex:

It is divided into two parts: the outer cortex and the inner cortex. The outer cortex is made up of huge air chambers that are organized in a ring (parenchymatous). Longitudinal septa divide these chambers from one another. The outer parenchymatous and inner sclerenchymatous areas of the inner cortex are distinguished.

(iii) Stele:

It is protostelic in shape and sits in the center of the room. It's completely bereft of pith. The diarch and exarch Xylem is located in the center. It is encased in the phloem. A single layer of pericycle surrounds the phloem on the outside.

Chapter 3

Phytochemicals

M. quadrifolia contains a wide range of chemicals including Phenolic compounds, Tannins, Saponins, Flavonoids, Steroids, Terpinoids, Alkaloids, Carbohydrates and etc.

3.1 Phenolic Compounds

Plants are a large source of phenolic compounds and a large amount of plant metabolite is phenolic compounds. These compounds have different therapeutic effect in our body such as anti-inflammatory effect, anticarcinogen, cardiovascular protection and etc. They have anti-aging activity too. Researchers have found that plant phenolic compounds are widely distributed and sometimes those are found in high concentration. These compounds can play important role in lipid oxidation stabilization which helps in taking anti oxidative action. Some researchers have found that daily consumption of phenolic compounds through fruits can reduce the risk of cancer and can inhibit mutagenesis (Gopalakrishnan et al., 2017).

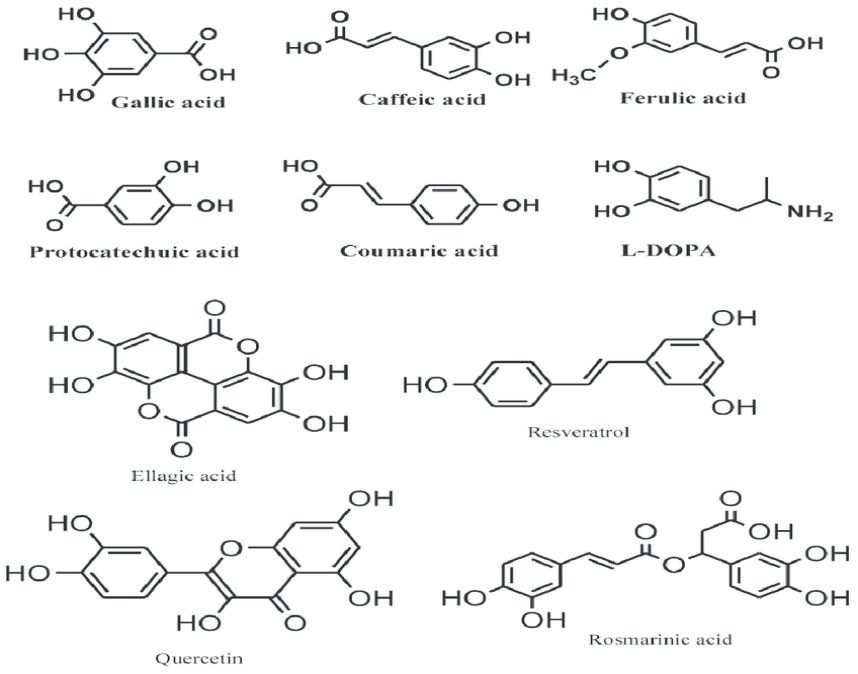

Figure 7: Common Phenolic Compounds (D. Lin et al., 2016)

3.2 Flavonoids

Flavonoids are another group of plant metabolites and this group is very diverse and large numbers of compounds belong to this group. These compounds are known to have scavenging properties. As a result, flavonoids are known to have anti-oxidant activities. Beside this, flavonoids have other therapeutic activities too such as vasoprotective, anti-inflammatory, antiviral, antibacterial and antitumor. Flavonoids and flavones are basically secondary metabolites. Flavonoids extracted from medicinal plants shows activities against host mediated diseases such as antimalarial activity, antibacterial activity, antiviral activity, anti-inflammatory and etc. (Gopalakrishnan et al., 2017).

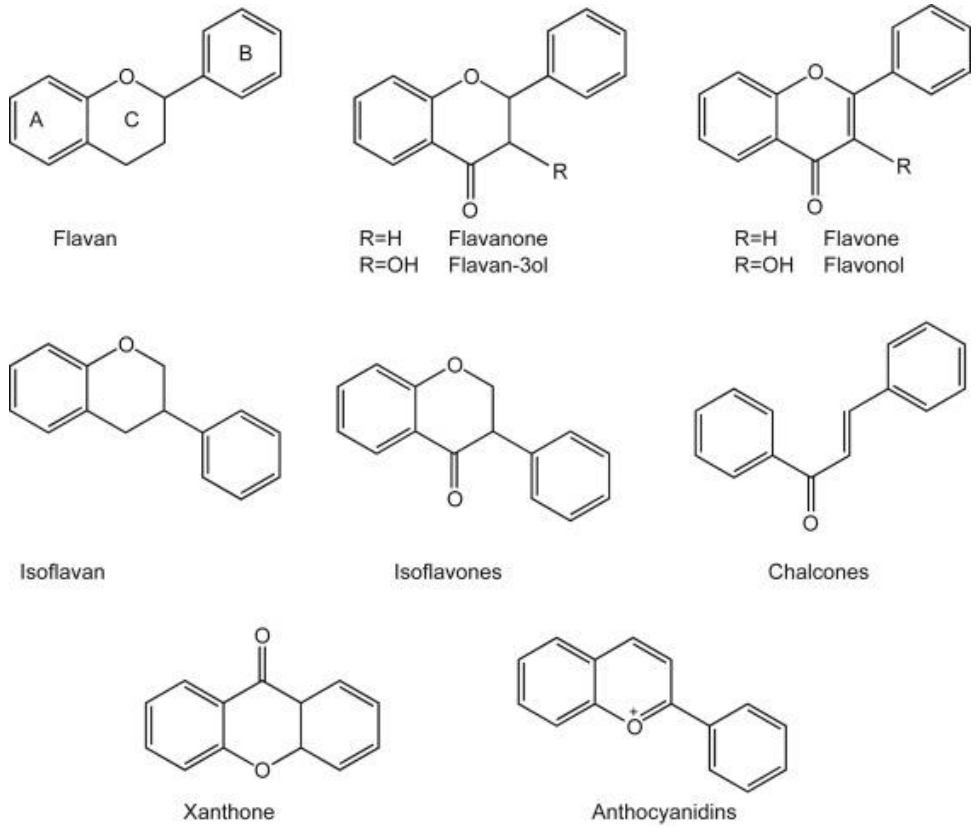

Figure 8: Some common Flavonoids (Gopalakrishnan et al., 2017)

3.3 Tannins

Tannins are plant polyphenols which are produced naturally. Main characteristic of tannins is these compounds bind with proteins and precipitate those. Animals can consume tannins through foods such as fruits, seeds, roots etc. Tannins are very common in fruits, tea, grasses, coffee, chocolates and etc. Concentration of tannins is highest in unripen fruits. Tannins are basically different tannic acids and this is why unripen fruits taste sour. These compounds have a great nutritive value in our daily diet and this is why we are suggested to eat fruits and vegetables. Researchers have found that tannins help to treat diarrhea and bacterial infections. Tannins are also used in skin care products (Gopalakrishnan et al., 2017).

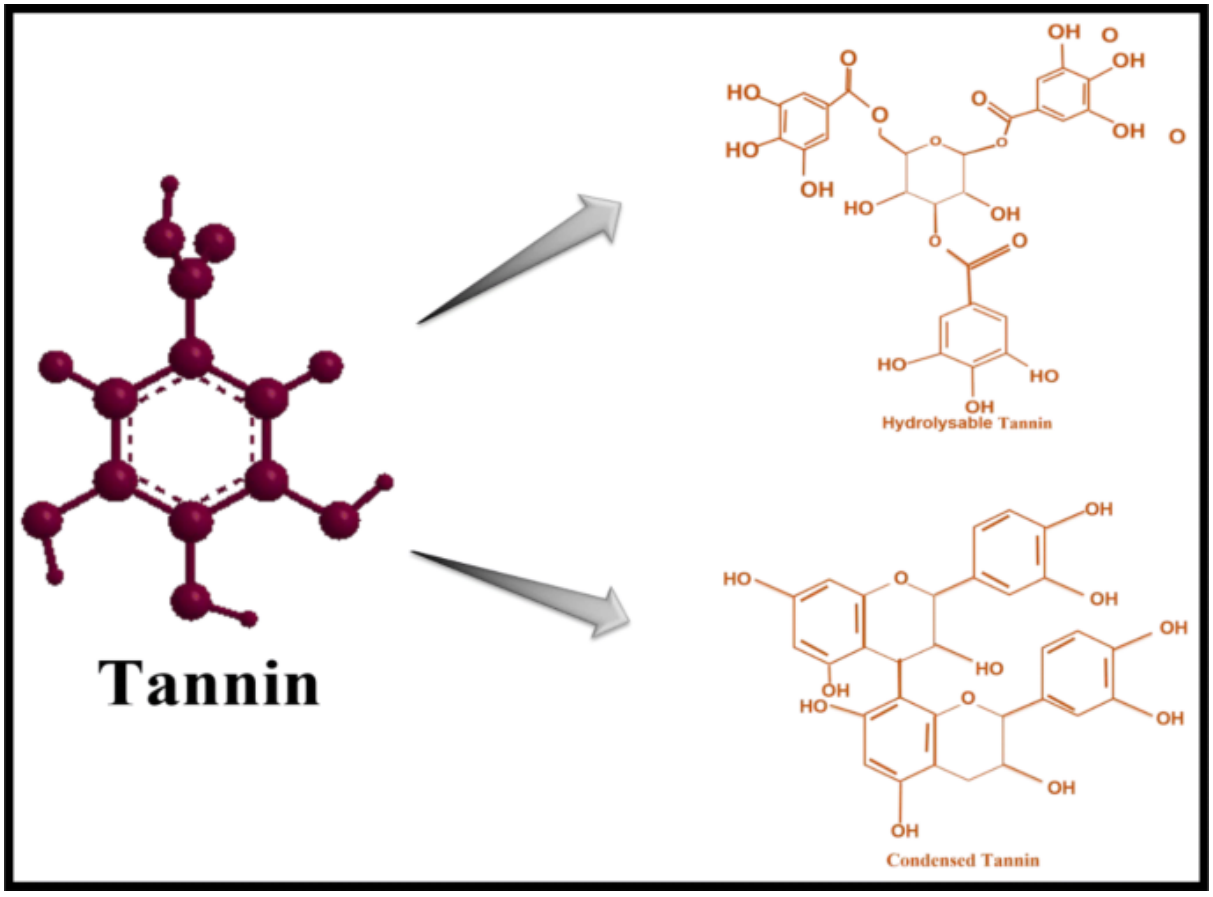

Figure 9: Tannin and its classification (Kavitha & Kandasubramanian, 2020)

3.4 Saponins

Saponins are known to have various pharmacological activities. Saponins can affect our immune system. These compounds help to improve our immune system by adjuvant activity. They can improve the ability of orally administrated drugs and vaccines by enhancing absorption of molecules and which in combined give immune stimulatory effect. Saponins are found in many different plants and commonly found in foodstuffs and plant medicines. Saponins can be used specifically for its anti-inflammatory effect, hypocholesterolemic activity and immunity stimulating activity (Gopalakrishnan et al., 2017).

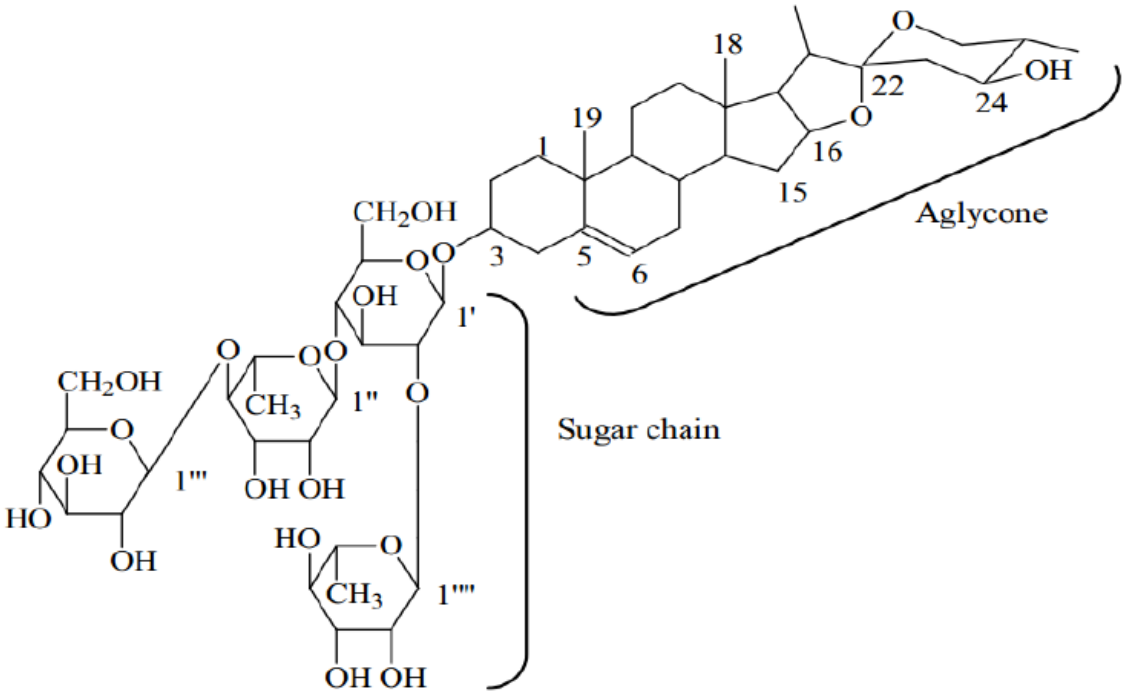

Figure 10: Structure of Saponin 21 (Moghimipour & Handali, 2015)

There are other phytochemicals present in *M. quadrifolia*also. Presence of different phytochemicals in different types of extract of leaf and stem is given below.

Table 3 Presence of Different chemicals in Leaf and Stem of M.quadrifolia (Gopalakrishnan et al., 2017)

Name of the Phytocompounds	**Methanolic Extracts (Leaf)**	**Aqueous Extracts (Leaf)**	**Methanolic Extracts (Stem)**	**Aqueous Extracts (Stem)**
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Terpinoids	-	-	+	+
Treterpinoids	-	-	+	-
Alkaloids	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Anthroquinones	-	-	-	-
Phenolic Compounds	+	+	+	+
Phytosterols	+	-	+	-
+ present; - absent				

Table 4 Amount of different compounds in leaf and stem of M. quadrifolia (Gopalakrishnan et al., 2017)

Name of Phytocompounds	**Leaf**	**Stem**
Phenolic Compounds (mg/g plant extract in gallic acid equivalents)	8.34±0.92	7.31±0.46
Flavonoids (mg/g plant extract in quercetin equivalents)	7.46±0.64	6.45±0.68
Alkaloids (mg/g)	6.12±0.51	5.89±0.61
Tannins (mg/g)	6.58±0.72	6.07±0.56
Saponins (mg/g)	5.32±0.48	6.30±0.58

Chapter 4

Pharmacological Properties of *M. quadrifolia*

4.1 Cytotoxic Effect

The impact of toxic substances being toxic to cells is known as cytotoxicity (Ruano et al., 2016). When cells are exposed to a cytotoxic chemical, they might have a variety of reactions. The cells may be actively progressing toward the dying phase at this stage. Additionally, the cells might initiate a mediated cellular dying (apoptosis) process, or necrosis, in which the loss of membrane integrity occurs and uncontrolled death occurs as a result of cell lysis. Necrosis causes cells to expand quickly, lose membrane integrity, shut down metabolism, and discharge entire material into the extracellular space. Moreover, cells that undergo rapid necrosis *in vitro* lack the energy or time to activate apoptotic pathways, and hence would not exhibit apoptotic indicators. Apoptosis is distinguished by cytoplasmic shrinkage, nuclear condensation, and regulated DNA breakage by enzymes, among other logical and molecular phenomena. Apoptosis-inducing cells in culture eventually succumb to secondary necrosis. The cell's metabolism pauses at this point, and the structure of its wall is damaged. Cytotoxic compounds are defined as any components which are toxic to cells, including substances that impede cell development and, in certain cases, lead to death, and are used to cure a variety of illnesses. Chemical, biological, and physical factors can all produce cytotoxicity by impacting cells in different ways. Chemical substances that operate by limiting cellular synthesis (including protein and dna synthesis), influencing cellular energy generation systems (mitochondrial action), or weakening the cell's membrane integrity are among these agents.

To measure cytotoxicity, different tests are done. Those are known as assay. There are different assay techniques through which we can determine the change in cell viability. Assay

can be done through both *in vitro* and *in vivo* tests (Bahadar et al., 2016). Brine shrimp lethality bioassay is one of those assay techniques. It is a widely used method for bioassay of bioactive compounds (Ripa et al., 2009). Mayer's method is used to perform cytotoxicity assay on brine shrimp. With this technique, we can determine the LC_{50} value at µg/ml scale (Meyer et al., 1982). This assay technique is very easy, convenient, fast and safe to perform. This assay method works by dissolving the test samples in DMSO. The lethality of the crude extracts of *M. quadrifolia* was determined. For vincristine sulphate, the LC_{50} was found 6.628µg/ml. For petroleum ether extract, it was 9.543µg/ml. For chloroform extract, it was found 7.820µg/ml. For Ethyl acetate extract, the value was found 8.589µg/ml. From this data, we can see that *M. quadrifolia* has cytotoxic effect (Ripa et al., 2009).

There are some *in vitro* tests also which are used to determine cytotoxic effect of any drug or molecule. MTT assay is one of them and it is widely used to check cytotoxic action of any substance. MTT assay is based on evaluating the cell metabolites and it is a colorimetric assay technique (Bahuguna et al., 2017). Evaluation of cytotoxic activity of *M. quadrifolia* through MTT assay was performed by Uma and Parvin. They used (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) to carry out the assay. Mitochondrial enzyme dehydrogenase of functioning cells cut MTT which produced formazan. Formazan is purple in color. Production of formazan is related to functioning cells. More production of formazan means higher number of functioning cells. So it means production of formazan is directly proportionate to number of functioning cells and inversely proportionate to level of cytotoxicity (Uma & Pravin, 2013). They prepared working herbal extracts by dissolving 0.5 ml of stock herbal extract with 4.5 ml of DMSO where the concentration of stock was 100mg/ml and concentration of DMSO was 10mg/ml. Then it was filtered with membrane filter. Then it was further diluted and transferred to 10 wells culture plate. A 12 wells culture plate was used. Then MFC cells were added at a concentration of 105cell/ml. In 2 control

cells, they didn't add plant extract. Then the culture plate was incubated at 37ºc for 5-6 hours. After that, medium was separated from the wells and those wells were cleaned with EMEM. 200µl MTT solution was added. Then the plate was incubated again and after that 1 ml DMSO was added. After 45 seconds, formazan was formed which is purple in color. Opacity density of those suspensions was measured at 540nm wave length using DMSO as blank. IC 50 Concentration (µg/ml) was measured.

This assay showed that plant extract of *M. quadrifolia* has anti-proliferative effect. This study found that IC_{50} for methanolic extract is least. So, we can determine that methanolic compound of *M. quadrifolia* has cytotoxic activity (Uma & Pravin, 2013).

.

Table 5 Assay on the effect of M. quadrifolia on Cytotoxicity of Cells.

Reference	*In-vivo/In-vitro*	Method	Extracted Compounds	Findings
Test done by (Ripa et al., 2009)	*In-vivo*	Brine shrimp lethality bioassay	Petroleum ether extract Chloroform extract Ethyl acetate extract	Cytotoxic activity of plant extract was found
Test done by (Uma & Pravin, 2013)	*In-vitro*	MTT assay of MCF cells of human breast Cancer	Methanol extract Ethyl acetate extract	Cytotoxic activity of plant extracts was found

4.2 Anti Inflammatory Effect

When a tissue is injured then the tissues show some reaction which is known as inflammation. Inflammation is basically a protective action which leads to healing of the tissues and refurbishes the normal activity of tissues. This procedure includes the structural modification of micro vessels, which results in fluids and leucocyte retention in extravascular tissues as well as local pain. Chemical mediators of inflammation mediate inflammatory reaction. These chemical mediators are active substances which are mobilized individually. These mediators grow either from plasma or cells. Eicosanoids have a role in almost every stage of inflammation (Subramanian & Balakrishnan, 2018a).

There are few methods through which we can determine anti-inflammatory effect of *M. quadrifolia.* Human red blood cell (HRBC) membrane stabilization method is one of them (Umukoro & Ashorobi, 2009). This method was applied by Subramanian and Balakrishnan. At first, they collected blood sample from a healthy human who didn't have any record of falling sick in recent past and no record of taking NSAIDS in 2 weeks prior to this experiment. Same amount of Alsever solution was mixed with the blood sample. Then this mixture was centrifuged at 3000 rpm. Separated red blood cells were washed with isosaline. Then they prepared a 10% suspension. Then they prepared extracts with concentration of 50µg/ml, 100µg/ml, 200µg/ml and 400µg/ml. 1ml phosphate buffer, 0.5 ml of HRBC suspension and 2ml of hyposaline were added. Then they incubated it for 30 minutes at 37ºC and after that it was centrifuged for 20 minutes at 3000rpm. Then they examined the hemoglobin content spectrophotometrically at 560nm wavelength. Then they calculated the inhibition of red blood cell at different concentrations. Plant extracts at 50µg/ml concentration showed least inhibition of human red blood cells. On the other hand, at 400µg/ml concentration showed highest value. Then they compared the results with standard

diclofenac at 100µg/ml concentration, 200µg/ml concentration and 400µg/ml concentration. This also showed inhibition of human red blood cells (Subramanian & Balakrishnan, 2018a).

Inhibition of protein denaturation method is another way through which we can determine anti-inflammatory activity of any substance (Subramanian & Balakrishnan, 2018a). Subramanian and Balakrishnan determined anti-inflammatory effect of *M. quadrifolia* by using inhibition of protein denaturation method. They made the reaction mixture by adding 0.2ml of egg albumin, 2.8ml of phosphate buffered saline and aerial part extract of *M. quadrifolia* at various concentration so that the final concentration of mixture can be 50µg/ml, 100µg/ml, 200µg/ml and 400µg/ml respectively. They used distilled water as control and took exact at the same volume. Then they incubated those mixtures at 37ºC and 70ºC for 15 minutes and 5 minutes respectively. After that, they cooled the mixture and measured the absorbance at 660nm. They also measured viscosity with Ostwald viscometer. Diclofenac sodium was used as reference drug and it went through the same procedures. Then they calculated the percentage inhibition of protein denaturation and compared those at different concentrations.

It was found that when the plant extract concentration is higher, percent inhibition of protein denaturation was higher too which indicates that plant extract of *M. quadrifolia* has anti-inflammatory effect (Subramanian & Balakrishnan, 2018a).

Some *in-vivo* methods have been used to determine anti-inflammatory effect of *M. quadrifolia.* Using carrangeenan and histamine induced paw edema is one of those (X et al., 2014). This method was used to evaluate anti-inflammatory effect of *M. quadrifolia*. Both male and female albino winstar rats were used for the experiment and those rats were kept in the same environment for last ten days. Then they injected 0.1ml of 1%w/v carrangeenan in left hind paw of every rat. They injected carrangeenan to induce edema in rats. They measured swelling of paw after every one hour. However, they injected plant extract in the

treated group of rats one hour before they applied carrangeenan on rats. Then they compared the percent increase of paw edema and inhibition of inflamation in treated group and controlled group. The amount of injected plant extract was 200 and 400mg/kg body weight of the rats. They found that plant extract with higher dose has greater inhibition of edema.

On the other hand, they also evaluated anti-inflammatory activity of *M. quadrifolia* by inducing edema in rats with histamine. At first, they took 24 rats and divided them is four groups where each group contained 6 rats. Then they injected plant extract and diclofenac sodium where the dose of plant extract was 200mg/kg and 400mg/kg and diclofenac sodium concentration was 25mg/kg. After one hour of injecting the drugs, histamine 0.1% was injected in right hind paw of rats. Then after every one hour the paw volume was measured and % inhibition of edema was calculated. Here, they also found that methanolic extract of *M. quadrifolia* has shown significant effect.

They compared between the edemas induced by carrageenan and histamine and it was found that methanolic extract of *M. quadrifolia* has shown greater inhibition of edema which was induced by carrageenan than the edema enduced by histamine. Some reports found that methanolic extracts of *M. quadrifolia* contains linolenic acid, a-3 fatty acid which is metabolized to 6, 9, 12, 15octadecatetraenoic acid (18:4, n-3), stearadonic acid (20:4, n-3) and eicosapentaenoic acid. Eicosapentaenoic acid can prohibit the production of prostaglandins which caused anti-inflammatory effect (X et al., 2014).

Table 6 Evaluation of anti-inflammatory effect of M. quadrifolia

Reference	*In-vivo*/*In-vitro*	Method	Extracted Compound	Findings
Test done by (Subramanian & Balakrishnan, 2018a)	*In-vitro*	Human red blood cell (HRBC) membrane stabilization method	Methanolic Compound	Inhibition of inflammation was found
Test done by (Subramanian & Balakrishnan, 2018a)	*In-vitro*	inhibition of protein denaturation method	Methanolic Compound	Inhibition of protein denaturation was found
Test done by (X et al., 2014)	*In-vivo*	Inhibition of carrageenan induced paw edema	Methanolic Compound	Inhibition of edema was found
Test done by (X et al., 2014)	*In-vivo*	Inhibition of histamine induced paw edema	Methanolic Compound	Inhibition of edema was found

4.3 Diuretic Effect

The effect of increased removal of fluid is known as diuretic effect. Any drug or substances which cause increase in urination is known as diuretics. It increases removal of body fluid, salts and waste products such as urea, poisons, extra water etc. It helps to control edema by excreting excess body fluid. Diuretics help to treat pulmonary congestion. Diuretics also reduce blood pressure that's why they are often used as medicine of hypertension (Venkatachalam et al., 2017). Diuretics are often known as water pills. *M. quadrifolia* is a medicinal plant and it has many medicinal activities. It is also being used by hypertension patients to reduce blood pressure. Here we will review its diuretic effect. There are some *in-vivo* and *in-vitro* tests through which we can evaluate diuretic activity of *M. quadrifolia.* Lipschitz method is one of those. This method is *in-vivo* method and diuretic activity of *M. quadrifolia* through this method was evaluated (Venkatachalam et al., 2017). They did this *in-vivo* test on winstar albino rats. They took 20 rats and divided them into 4 groups. Here, one group served as control group, another group was treated with standard solution and rests of the groups were treated with plant extracts of different concentrations. All the groups were starved for 5 hours and then they were given normal saline at a dose of 10ml/kg. Here group 1 was treated as control group and they were administrated with only normal saline. Group 2 was standard and they were administrated with frusemide at dose of 100mg/kg. Group 3 and 4 received plant extracts at dose of 200mg/kg and 400mg/kg respectively. Then they placed those rats in metabolic cages. Those rats were kept in room temperature. Five hours later, amount of collected urine was measured. Concentrations of electrolytes were measured too for individual rats (Venkatachalam et al., 2017). They used digital flame photometer to measure the concentration of sodium and potassium ions. Concentration of chloride ion was measured by schales and schales method. Then they compared the values and found that where the plant extract dose was higher, more volume of urination was found and the

concentration of ions was higher too. Which proves that plant extract of *M. quadrifolia* has significant diuretic effect (Venkatachalam et al., 2017).

Table 7 Evaluation of diuretic activity of M. quadrifolia

Reference	*In-vivo*/*In-vitro*	Method	Extracted Compound	Findings
Test done by (Venkatachalam et al., 2017)	*In-vivo*	Lipschitz Method	Ethyle Extracts	Diuretic activity was found

4.4 Anti-venom Activity

According to WHO, snake bite is one of the main reason of occupational deaths and injury in Indian subcontinent among the people who work in farmlands and most of the cases they are bitten either by cobras or vipers (Hansiya & Geetha, 2021). There are around 3000 snake species in this world but only few hundreds of them are venomous. There are mainly three types of snake venoms. They are cytotoxic venoms, neurotoxic venoms and haemo-toxic venoms. Cytotoxic venoms primarily target the cells which lead to necrosis. On the other hand, neurotoxic venoms target our nervous system. The neurotransmitters are disrupted by neurotoxic venoms which lead to paralysis and breathing difficulties. Haemo-toxic venoms are known as blood poisons. It can lead to tissue injury and damage of organs (Goswami et al., 2014). Snake venoms consist of different types of proteins, enzymes, neurotoxins, coagulants, anti-coagulants and etc. Snake venom is acidic in nature.

M. quadrifolia is known to have anti-venom activity. Subramanian and Balakrishnan evaluated anti-venom activity of *M. quadrifolia.* They tested neutralization of lethality,

neutralization of hemorrhagic activity, neutralization of necrotizing activity, and neutralization of defibrinogenating activity of Russell's viper venom. Russell's viper venom has different effects on human body such as hemorrhagic activity, necrotizing activity, and defibrinogenating activity. At first they calculated the value if LD_{50} which was 4µg/20g body weight of mice. Then they used methanol extract of *M. quadrifolia* to measure neutralization of lethality where they found that dose of 400mg/kg can neutralize 70% lethality. They also found that methanol extract of this plant neutralizes hemorrhagic activity, necrotizing activity, and defibrinogenating activity of Russell's viper venom (Subramanian & Balakrishnan, 2018b). This study proves that methanol extract of *M. quadrifolia* has anti-venom activity.

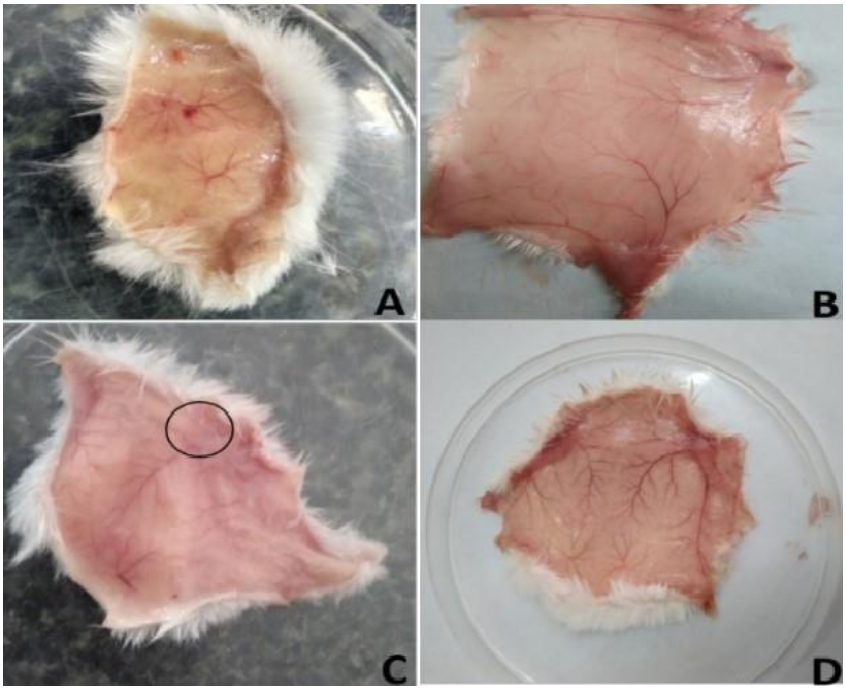

Figure 11: Neutralization of Hemorrhagic activity of Russell's viper venom by M. quadrifolia (Subramanian & Balakrishnan, 2018b)

4.5 Hypoglycemic Effect

Diabetes or hyperglycemia is a chronic disease which is characterized by disturbance in metabolism of carbohydrates. This disease can be occurred due to two main reasons. Those reasons are either by insufficient production of insulin hormone or by resistant of cells to insulin and sometimes both of these can cause diabetes. Insulin is produced in the islets of Langerhans of pancreas and any problem of those cells can induce diabetes. Insulin helps to restore glucose inside our cells and hyperglycemic situation is triggered when glucose is not stored inside the cells and blood glucose level is increased. Diabetes can trigger different diseases later and sometimes those diseases can be lethal. There are some oral-synthetic anti-diabetic drugs which are effective but those drugs gradually become ineffective due to resistant to those drugs. Some drugs have adverse effects as well. So, medicinal plants can be a good alternative of those medicines (Mia et al., 2019).

M. quadrifolia has potentiality to have hypoglycemic activity. Some tests have been conducted to find out this activity. One test was performed on mice where diabetic condition was developed by applying Alloxan. Mice were divided in four groups where one group was normal group; one group was diabetic group which was not treated with any drug substances. The third group was treated with anti-diabetic drug and last group was treated with plant extract of *M. quadrifolia*. By comparing blood glucose level in each group, anti-diabetic activity of this plant was found. Blood glucose level was found significantly low in the group which was treated with plant extract of *M. quadrifolia.* So it proves that *M. quadrifolia* has hypoglycemic effect (Zahan et al., 2011).

4.6 Antibacterial Activity

Activity of killing bacteria or inhibit grow of bacteria is known as antibacterial activity. Antibiotics are prescribed to prevent bacterial infections but in the long run some bacteria

become resistant to those antibiotics. Some plants are known to have antibacterial activities. A test was performed to evaluate antibacterial activity of plant extracts of *M. quadrifolia* where ethyl acetate, hexane, methanol and ethanol extracts were used. They used disc diffusion method. It was found that each of the extracts created a zone of inhibition which means plant extract of *M. quadrifolia* has antibacterial activity. Among all the extracts, ethyl acetate was most effective against bacteria and the zone of inhibition created by ethyl acetate extract was greater than others. So, it proves that *M. quadrifolia* has antibacterial activity (Gini & Jothi, 2016).

Chapter 5

Discussion

Recent researches have proved that *M. quadrifolia* has different pharmacological activities. Its cytotoxic activity, anti-inflammatory activity, diuretic activity, anti-venom activity, hypo glycemic activity and antibacterial activities have been discussed in chapter 4. Through brine shrimp lethality bioassay and MTT assay of MCF cells of human breast cancer cytotoxic activity of *M. quadrifolia* has been proved. Methanol extract of this plant has been found to have cytotoxic effect of cells. By using human red blood cell (HRBC) membrane stabilization method it was found that methanol extract of this plant has anti-inflammatory effect. It was also found that methanolic extract of *M. quadrifolia* inhibits the protein denaturation which supports that this plant has anti-inflammatory effect. Protein denaturation is an important part of inflammation. Inhibition in protein denaturation means inhibition in inflammation. Some *in-vivo* tests were done for confirmation of anti-inflammatory effect. In those tests it was found that plant extract of *M. quadrifolia* can inhibit edema induced by carrageenan and histamine. Those tests have confirmed that plant extract of *M. quadrifolia* has anti-inflammatory effect. Diuretic activity of *M. quadrifolia* was also evaluated through Lipschitz method where proof of diuretic activity was found. Increased volume of urine was found which indicated that plant extract of *M. quadrifolia* has diuretic effect (Uma & Pravin, 2013). On the other hand, this plant is also used as anti-venom of snake bite. There are multiple types of venoms and each type of venoms have different effect on human body. Some snake venoms damage tissues and have hemorrhagic activity and necrotizing activity. It was found that plant extract of *M. quadrifolia* can neutralize effects of venom. This plant has hypo glycemic activity also. When diabetic mice were treated with plant extract of *M. quadrifolia*, significant reduction in blood glucose level was found which indicates that plant extracts of this plant has ability to reduce blood glucose level. In another study it has been found that *M.*

quadrifolia has antibacterial activity too. Plant extracts of *M. quadrifolia* created areas with no bacterial growth on agar media and ethyl acetate extract created the largest zone of inhibition among all extracts which means plant extracts of *M. quadrifolia* has antibacterial effect too. So, it is confirmed that plant extract of *M. quadrifolia* has cytotoxic effect, anti-inflammatory effect, diuretic effect, anti-venom effect, hypo glycemic effect and antibacterial effect. So plant extract can be used to treat different diseases and especially in rural areas people can use it in emergency situation where medicine or medical services can't be reached or afforded.

Chapter 6

Conclusion

Medicinal plants played important role in ancient medical science and even in modern medical science plants have great impact. Plant medicine can be a good alternative to some medicines. This plant has potentiality to play a big role in modern pharmaceutical industries where plant extracts can be a good source of pharmaceutical ingredients. This plant contains different chemical constituents which can be extracted from plant extract. Now-a-days, natural habitat of this plant is being destroyed due to excessive use of chemical fertilizers and pollution which can lead to extinction of this plant. So, we should be careful to preserve the natural habitats of *M. quadrifolia.*

Reference

Aboelsoud, N. H. (2010). Herbal medicine in ancient Egypt. *Journal of Medicinal Plants Research*, *4*(2), 082–086. https://doi.org/10.5897/JMPR09.013

Ahmad, A., Ghufran, R., Water, A. Z.-, Air, U., Pollution, & S., & 2011, U. (2011). Phytosequestration of metals in selected plants growing on a contaminated Okhla industrial areas, Okhla, New Delhi, India. *Springer*.

Amoateng, P., Quansah, E., Karikari, T. K., Asase, A., Osei-Safo, D., Kukuia, K. K. E., Amponsah, I. K., & Nyarko, A. K. (2018). Medicinal plants used in the treatment of mental and neurological disorders in Ghana. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/8590381

Bahadar, H., Maqbool, F., Niaz, K., & Abdollahi, M. (2016). Toxicity of Nanoparticles and an Overview of Current Experimental Models. *Iranian Biomedical Journal*, *20*(1), 1–11. https://doi.org/10.7508/ibj.2016.01.001

Bahuguna, A., Khan, I., Bajpai, V. K., & Kang, S. C. (2017). MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh Journal of Pharmacology*, *12*(2), 115–118. https://doi.org/10.3329/bjp.v12i2.30892

Bordonneau, M., & Tourte, Y. (1994). Differential expression of parental genomes during formation of embryonic organs in the early development of the fern Marsilea quadrifolia. *Plant Systematics and Evolution*, *189*(3–4), 149–164. https://doi.org/10.1007/BF00939723

Bruni, I., Gentili, R., De Mattia, F., Cortis, P., Rossi, G., & Labra, M. (2013). A multi-level analysis to evaluate the extinction risk of and conservation strategy for the aquatic fern

M. quadrifoliaL. in Europe. *Aquatic Botany*, *111*, 35–42. https://doi.org/10.1016/J.AQUABOT.2013.08.005

Gini, T. G., & Jothi, G. J. (2016). *In vitro Screening of Antibacterial and Antifungal Activity of M. quadrifolia(Marsileaceae) Linn. Extract*. *January 2015*. www.ajpct.org

Gopalakrishnan, K., and, R. U.-J. of P. S., & 2017, undefined. (2017). Phytochemical content of leaf and stem of M. quadrifolia(L.). *Core.Ac.Uk*. https://doi.org/10.29328/journal.jpsp.1001003

Goswami, P. K., Samant, M., & Srivastava, R. S. (2014). Snake venom, anti-snake venom & potential of snake venom. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(5), 4–7.

Hansiya, V. S., & Geetha, N. (2021). *In vitro* anti-venom potential of various solvent based leaf extracts of Andrographis serpyllifolia (Rottler ex Vahl) Wight against Naja naja and Daboia russelli. *Journal of Ethnopharmacology*, *269*, 113687. https://doi.org/10.1016/j.jep.2020.113687

Javalgekar, R. R. (1982). Utility of ayurvedic dietetics in the modern world. In *Ancient Science of Life* (Vol. 2, Issue 2, pp. 84–87).

Juliet, M. R., … G. P. B.-I. J. of, & 2020, U. (2020). Biogenic synthesis of Copper nanoparticles using aquatic pteridophyte M. quadrifoliaLinn. rhizome and its antibacterial activity. *Ijnd.Ir*.

Kao, W.-Y., & Lin, B.-L. (2010). Phototropic leaf movements and photosynthetic performance in an amphibious fern, Marsilea quadrifolia. *Journal of Plant Research 2010 123:5*, *123*(5), 645–653. https://doi.org/10.1007/S10265-009-0300-2

Karikalan, G, Investigation, U. R.-J. of P., & 2018, U. (2008). Effect of M. quadrifolia(L.) on carbohydrate metabolic enzymes in alloxan induced diabetic rats. *Springer*.

Karikalan, Gopalakrishnan, & Rajangam, U. (2018). Effect of M. quadrifolia(L.) on carbohydrate metabolic enzymes in alloxan induced diabetic rats. *Journal of Pharmaceutical Investigation*, *48*(4), 477–486. https://doi.org/10.1007/S40005-017-0347-3

Kavitha, V. U., & Kandasubramanian, B. (2020). Tannins for wastewater treatment. *SN Applied Sciences*, *2*(6), 1–21. https://doi.org/10.1007/s42452-020-2879-9

Khajavi Rad, A., Mohebbati, R., & Hosseinian, S. (2017). Drug-induced nephrotoxicity and medicinal plants. *Iranian Journal of Kidney Diseases*, *11*(3), 169–179.

Kumar Agarwal, S., Roy, S., Pramanick, P., Mitra, P., Gobato, R., & Mitra, A. (2018). Marsilea quadrifolia: A floral species with unique medicinal properties. *Parana Journal of Science and Education*, *4*(5), 15–20.

Lin, B.-L., & Yang, W.-J. (1999). Blue Light and Abscisic Acid Independently Induce Heterophyllous Switch in Marsilea quadrifolia. *Plant Physiology*, *119*(2), 429–434. https://doi.org/10.1104/PP.119.2.429

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules*, *21*(10). https://doi.org/10.3390/molecules21101374

Liu, B.-L. L. (1984). ABSCISIC ACID INDUCES LAND FORM CHARACTERISTICS IN M. QUADRIFOLIAL. *American Journal of Botany*, *71*(5), 638–644.

https://doi.org/10.1002/J.1537-2197.1984.TB14170.X

Liu, B. (1984). *STUDIES OF LATERAL ROOT INITIATION IN M. QUADRIFOLIAL.(FERN)*.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, *45*(1), 31–34. https://doi.org/10.1055/s-2007-971236

Mia, S., Roy, R., Akter, A., Barek, M. A., & Basher, M. A. (2019). Hypoglycemic effect of leaf extract of Fimbristylis miliacea in mice model. *Clinical Phytoscience*, *5*(1), 25–27. https://doi.org/10.1186/s40816-019-0119-x

Moghimipour, E., & Handali, S. (2015). Saponin: Properties, Methods of Evaluation and Applications. *Annual Research & Review in Biology*, *5*(3), 207–220. https://doi.org/10.9734/arrb/2015/11674

Mominul Islam, A. K. M., Hasan, M., Musha, M. M. H., Uddin, M. K., Juraimi, A. S., & Anwar, M. P. (2018). Exploring 55 tropical medicinal plant species available in Bangladesh for their possible allelopathic potentiality. *Annals of Agricultural Sciences*, *63*(1), 99–107. https://doi.org/10.1016/j.aoas.2018.05.005

Nyakudya, T. T., Tshabalala, T., Dangarembizi, R., Erlwanger, K. H., Ndhlala, A. R., De Martino, L., & Mcphee, D. J. (2020). molecules The Potential Therapeutic Value of Medicinal Plants in the Management of Metabolic Disorders. *Molecules*, *25*. https://doi.org/10.3390/molecules25112669

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, *6*(11), 1–5. https://doi.org/10.4103/0973-7847.95849

Quadrifolia Linn, M., Venkatachalam, G., & Balasundaram, J. (2017). Evaluation of Diuretic Activity of Ethanolic Extract of Leaves of. *European Journal of Pharmaceutical and Medical Research*, *4*(11), 458–461. www.ejpmr.com

Ramya Juliet, M., Priyanka Beulah, G. R. G., Tresina, P. S., Doss, A., & Mohan, V. R. (2020). Biogenic synthesis of Copper nanoparticles using aquatic pteridophyte M. quadrifoliaLinn. rhizome and its antibacterial activity. *International Journal of Nano Dimension*, *11*(4), 337–345.

Research, P. S.-A. J. of P. S. and, & 2014, undefined. (2014). Antimicrobial activities of Turbinaria conoides (J. Agardh) Kutzing and M. quadrifoliaLinn. *Researchgate.Net*, *4*(6), 36–40.

Ripa, F. A., Nahar, L., Haque, M., & Islam, M. M. (2009). Antibacterial, cytotoxic and antioxidant activity of crude extract of Marsilea Quadrifolia. *European Journal of Scientific Research*, *33*(1), 123–129.

Ruano, P., Delgado, L. L., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., & Masuelli, M. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *tourism*, 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Shakil, S. S. M., Gowan, M., Hughes, K., Azam, M. N. K., & Ahmed, M. N. (2021). A narrative review of the ethnomedicinal usage of Cannabis sativa Linnaeus as traditional phytomedicine by folk medicine practitioners of Bangladesh. *Journal of Cannabis Research*, *3*(1). https://doi.org/10.1186/s42238-021-00063-3

Subramanian, M., & Balakrishnan, S. (2018a). *In vitro* anti-inflammatory and anti-venom

activities of aerial parts of M. quadrifoliaLinn. *Asian Journal of Pharmacy and Pharmacology*, *5*(1), 73–77. https://doi.org/10.31024/ajpp.2019.5.1.10

Subramanian, M., & Balakrishnan, S. (2018b). INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES Anti-venom activities of methanol extract of M. quadrifoliaLinn (Marsileaceae) against Russell's Viper venom Production and Hosted by. *International Journal of Research in Pharmaceutical Sciences*, *10*(2), 1540–1546. https://doi.org/10.26452/ijrps.v10i2.873

Uma, R., & Pravin, B. (2013). Invitro cytotoxic activity of M. quadrifoliaLinn of MCF-7 cells of human breast cancer. *International Research Journal of Medical Sciences*, *1*(1), 10–13.

Umukoro, S., & Ashorobi, R. (2009). Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of Momordica charantia in rats. *African Journal of Biomedical Research*, *9*(2), 119–124. https://doi.org/10.4314/ajbr.v9i2.48892

X, J. B., X, A. J., & Kp, S. K. (2014). *EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF THE PLANT M. QUADRIFOLIAON ALBINO RATS USING CARRAGEENAN AND HISTAMINE INDUCED PAW EDEMA*. *5*(2), 1640–1644.

Zahan, R., Ripa, F. A., Alam, M. B., Haque, M. A., Mosaddik, M. A., & Nahar, L. (2011). Hypoglycemic and *in vitro* antioxidant effects of methanolic extract of M. quadrifoliaPlant. *Pharmacognosy Journal*, *3*(26), 86–92. https://doi.org/10.5530/pj.2011.26.14

8_19364041.docx

ORIGINALITY REPORT

8%	8%	4%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	gacbe.ac.in Internet Source	3%
2	"Phytochemical content of leaf and stem of Marsilea quadrifolia (L.)", 'Heighten Science Publications Corporation', 2017 Internet Source	1%
3	en.wikipedia.org Internet Source	1%
4	www.yourarticlelibrary.com Internet Source	1%
5	www.ijppsjournal.com Internet Source	<1%
6	www.heighpubs.org Internet Source	<1%
7	www.ijcmas.com Internet Source	<1%
8	Submitted to Higher Education Commission Pakistan Student Paper	<1%

9	www.authorstream.com Internet Source	<1 %
10	Submitted to VIT University Student Paper	<1 %
11	link.springer.com Internet Source	<1 %
12	Amina Bougandoura, Brigida D'Abrosca, Souad Ameddah, Monica Scognamiglio et al. "Chemical constituents and in vitro anti-inflammatory activity of Cistanche violacea Desf. (Orobanchaceae) extract", Fitoterapia, 2016 Publication	<1 %
13	Sun Wenke, Zhang Shuping. "Inhibition of Active Substances of Vibrio Parahaemolyticus and its Application Research", E3S Web of Conferences, 2019 Publication	<1 %
14	gradesfixer.com Internet Source	<1 %
15	innovareacademics.in Internet Source	<1 %
16	medcraveonline.com Internet Source	<1 %
17	www.coursehero.com Internet Source	<1 %

18 Yuh-Fung Chen, Huei-Yann Tsai, Tian-Shung Wu. " Anti-Inflammatory and Analgesic Activities from Roots of ", Planta Medica, 2007 <1%
Publication

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		