INSTITUTE OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION OF *LAMIUM MOSCHATUM* SUBSP. *MICRANTHUM*

SALEM EDAWDI

PHARMACOGNOSY

MASTER THESIS

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SUPERVISOR

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APPROVAL

A thesis submitted to the Instuitute of Graduate Studies of Near East University in partial fulfillment of the requirement for the degree of Master of Science in Pharmacognosy.

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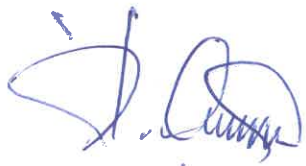

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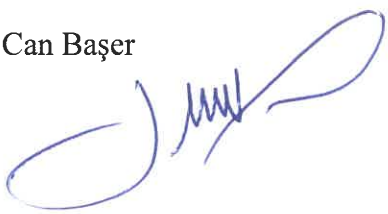

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Etnofarmakolojik Uygunluk: *Lamium moschatum* ve *L. amplexicaule* türleri Kıbrıs halk hekimliğinde gastrointestinal ve solunum yolu hastalıklarının tedavisinde kullanılmaktadır.

Çalışmanın amacı: *Lamium moschatum* subsp. *micranthum* (%80 EtOH özütü).

Gereç ve Yöntemler: Polar bileşiklerin uygun kromatografik teknikler kullanılarak izolasyonu için, toprak üstü kısımlarından %80 etanol ekstraktları fraksiyonlanarak hazırlandı ve bileşikler izole edildi. Daha sonra izole edilen bu bileşiklerin yapıları spektroskopik (NMR: 1D ve 2D-NMR: 1H, 13C, COSY, HSQC, HMBC) yöntemlerle aydınlatılmış ve literatür verileri ile karşılaştırılmıştır.

Sonuçlar: İki flavonoid izole edildi ve aydınlatıldı LM-2: Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside and LM-4: Kaempferol 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside.

Anahtar Kelimeler: Fitokimya, *Lamium moschatum,* Flavonoidler

ABSTRACT

Ethnopharmacological Relevance: *Lamium moschatum* and *L. amplexicaule* species are used for the treatment of gastrointestinal and respiratory tract diseases in Cyprus folk medicine.

Aim of the study: Isolation and structure elucidation of flavonoids from the whole aerial parts of *Lamium moschatum* subsp. *micranthum* (80% EtOH extract).

Material and Methods: For the isolation of polar compounds by using proper chromatographic techniques, 80% ethanol extracts of aerial parts were prepared fractioned and the compounds were isolated. Then, the structures of these isolated compounds were elucidated by means of spectroscopic (NMR: 1D and 2D-NMR: ^{1}H, ^{13}C, COSY, HSQC, HMBC) methods and compared with literature data.

Results: Two flavonoids were isolated and elucidated LM-2: Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside and LM-4: Kaempferol 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside respectively.

Keywords: Phytochemistry, *Lamium moschatum*, Flavonoids

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Many ailments may be treated with less adverse effects by using plants as a traditional medicine source (Bako et al., 2015). Plants that contain pharmaceutically active compounds can be used in the commercial manufacture of medicines. Since antiquity, the importance of plant diversification in health treatment has been widely recognised. According to a literature review, more than 50,000 plant species are effectively utilised for medical reasons across the world, with flowering plants accounting for approximately 13 percent of those (Sanni, 2017). These phytochemicals have been isolated from diverse plant components such as the root, stem and leaves as well as the bark and seeds, and they have been shown to have a positive physiological response in the treatment of a wide range of human and animal diseases (Bianco et al., 2019). As local, national, and worldwide interest in medicinal and aromatic plants has grown in recent years, plant wealth is seen as a source of revenue by those working in the pharmaceutical business. Both in developing and developed nations, the desire for medicinal plants is on the rise due to their simple availability, few side effects, and often only source of health treatment (Kupeli, et al., 2008).

A connection has existed between life, illness, and plants ever since the beginning of time. Diseases and remedies were first studied by prehistoric man (Lyons & Petrucelli, 2001). No one can deny that ancient people were interested in synthetic medications for their ailments, but they did try to make the most of what they could easily obtain. The saplings and animals in the area provided them with the most basic clothing. It was discovered that majority of these medicinal plants could be used as food, while some were toxic or therapeutic (Tandon et al., 2016). They passed on their knowledge of herbal treatments as family medicine to succeeding generations based on their own personal experience. The history of herbal medicine is therefore as old as the history of mankind. Despite great advancements in synthetic chemistry, most of these plant-derived medicines were discovered through traditional treatments

and folk knowledge of indigenous people. A plant's active ingredients can be extracted and administered in standardised dose form, but it can also be used as a crude medication for the general population. There is a complementary usage of modern medications and herbal remedies in different developing nations, including the Turkish Republic of Northern Cyprus (TRNC). Plants have played a key role in the treatment of human and animal illnesses for centuries, and continue to do so today. The usage of therapeutic and aromatic herbs is becoming more popular across the world (Kupeli et al., 2008). Although modern medicine has made enormous strides in recent decades, plants are still a vital contributor to health care. We have found the best success using natural items as a source of medications. Unlike a factory, every plant can combine an endless number of very complex and rare chemical compounds (Deng et al., 2020).

The genus *Lamium* belonging to the Lamiaceae family represented by more than 40 annual or perennial herbaceous species distributed throughout Europe, Asia and Africa (Willis and Shaw 1973). In the Flora of Cyprus, *Lamium* genus is represented by 3 species which are *L. amplexicaule* L., *L. garganicum* L. and *L. moschatum* Mill. (Meikle, 1985). Ethnobotanically, *Lamium moschatum* and *Lamium amplexicaule* are used for the treatment of gastrointestinal and respiratory tract diseases (González-Tejero et al., 2008).

The present study focuses on phytochemical study on *Lamium moschatum* subsp. *micranthum* focussing on polar compounds.

1.2 Taxonomy and Ecology of *Lamium* Species

Sharma & Singh (1981) recognised five *Lamium* species and one *Lamium* variation under the Species section. Three genera, *Lamium* (a*lbum, album var. maculatum, amplexicule, purpureum), Galeopsis (galeobdolon),* and *Orvala*, had these six taxa (*garganica*). *Lamium multifidum*, which Linnaeus assigned to *Lamium*, which Fischer & C.Meyer first named in 1836 (Bentham, 1836). As shown in Figure 1.1, Linnaeus distinguished eight species of *Lamium* in his second edition of Species, namely the three species mentioned in the first edition as well as the *Lamium multifidum, Lamium moschatum, Lamium garganicum, Lamium laevigatum* and *Lamium orvala*. Linnaeus incorrectly placed *Orvala garganica* in the genus *Lamium orvala (Mill*, 1982).

Lamium's distribution may be described as temperate Eurasiatic (holarctic). It extends from Western Europe to Eastern Asia, encompassing Northern Africa north of the Atlas Mountains and Macaronesia (the Azores, Madeira, and Canary Islands), roughly between 65° and 30° latitudes. In Macaronesia, the genus *Lamium* is found in both natural and artificial habitats. Small numbers of taxa are imported (and occasionally naturalised) beyond the native region, including in Greenland and Iceland, the Americas, Australia, and tropical and South Africa. This species has the greatest distribution region of any *Lamium* taxon. There are records of *Lamium* taxa being introduced throughout the entire temperate zone of Eurasia, as well as to the complete area of the world. These species, both indigenous to Asia Minor, have the lowest distribution ranges of all the *Lamium* species. Portugal's isolated position in the *Lamium bifidum* and *Lamium confertum* regions is notable. As the original Mediterranean woods were cut down, *Lamium flexuosum's* distribution range was probably split in two (Bentham, 1836).

Figure 1. 1: Diagram of *Lamium moschatum (Matkowsı & Pıotrowska, 2018)*

1.2.1 Taxonomy of *Lamium moschatum* subsp. *micranthum*

Herbaceous plant growing up to 50 cm high has an erect stem which is unbranched and angled. Leaves are decussate, simple, entire, ovate, hairy on both sides, margin crenate irregularly toothed, teeth rounded. Flowers aromatic, hermaphrodite, zygomorphic, in multi-flowered whorls on interrupted spikes (verticillasters); bracts leaf-like, smaller, often with white or purplish blotch at base; corolla white 2-lipped; upper (adaxial) lip entire, bending forward, hood-like, densely hairy externally; lateral lobes triangular, glabrous, lower lip obovate suborbicular, 2-parted, sometimes emarginated, glabrous; calyx campanulate, 5-toothed, teeth spreading, pointed, margin purplish, hairy externally; stamens 4 (2 pairs), of which 2 with short filaments (didynamous); anthers 2-thecous, hairy; ovary superior, style single, stigma 2-lobed. Flowering time Feb-May. This plant is native to Mediterranean region (Meikle, 1985). Known for its ability to grow and adapt in moist and shady environments, it is cultivated on land without stony topography, roadside waste, or

slopes. A large part of its distribution is found in the eastern part of Turkey as well as Cyprus, Lebanon, and western Jordan (Mennema, 1989).

1.3 Ethnobotanical Uses and Pharmacological Activities of *Lamium* Species

Traditional plant medicine is a key element of Ethnopharmacology (established in 1967). Ethnopharmacology is the study of ingredients used by ethnic groups as elixirs.

Lamiaceae plants have been utilised as medicine for thousands of years. Flavonoids and phenolic chemicals, in particular, are responsible for the physiological effects of these plants. These indigenous medicinal herbs are utilised as spices and food plants by many cultures across the world. Infusions of aerial parts or powder combined with honey or olive oil have been used for Lamiaceae taxa. Traditional folk medicine is also practised by the indigenous population (Deng et al., 2020).

Lamium has been used medicinally for centuries. *L. album* is regarded as the most popular. To cure menorrhagia, bleeding, vaginal and cervical irritation as well as leukorrhea, dried flowers from this plant were used (Bisset, 2014). Matkows & Potrowska (2018) also reported that *Lamium maculatum* is utilised in Chinese traditional medicine to treat trauma, fracture, paralysis and hypertension. Antioxidant, free radical scavenging and antiproliferative activities have been found for *Lamium album* flowers. Antioxidant and free radical scavenging properties were also observed in *Lamium purpureum* flowers. Bacteriostatic activity against Gram-positive and negative bacteria was found for the essential oil of *Lamium garganicum* L. subsp. *laevigatum Arcangeli* in Western Anatolia, entire plants of *Lamium album* and other *Lamium* species are used to ease pain in rheumatism and other rheumatic illnesses, while *Lamium album*, *Lamium maculatum*, and *Lamium purpureum* have been recorded to be used as tonics and home treatments for constipation (Bianco et al., 2019). In addition to anti-inflammatory, antinociceptive, antimicrobial, and free radical scavenging activities, different extracts prepared from the over ground parts of *Lamium eriocephalum* subsp. *erocephalum, Lamium garganicum* subsp.

laevigatum, Lamium garganicum subsp. *pulchrum and Lamium purpureum* exhibited anti-oxidative properties (Kupeli, et al., 2008).

1.4 Phytochemistry of *Lamium* Species

As a result of widespread interest in *Lamium* species and their therapeutic qualities, much phytochemical research has been conducted (Flamini et al., 2018). It has been researched extensively since 1967 into how *Lamium*'s phytochemistry affects its physiology. The *Lamium* genus has been studied for over 40 years, and iridoids and secoiridoids, flavonoids, anthocyanins and phytoecdysteroids, betaines, benzoxazinoids, terpenes, megastigmane compounds and essential oils have been identified. Herbal remedies use *Lamium* species, sometimes known as dead nettles, to treat trauma, fracture, paralysis, and uterine bleeding. *Lamium* species have been utilised in folk medicine for thousands of years. These plants have been the subject of extensive phytochemical research due to their therapeutic qualities. Some of the chemicals identified as a consequence of these phytochemical research includes iridoids, seco-iridoids, phenylpropanoids, flavonoids, anthocyanins (phytoecdysteroids), betaine- and benzoxazinoide-containing compounds as well as terpenes and megastigmane compounds (Bisset, 2014).

1.5 Traditional Medicinal Uses of *Lamium* Species

For ages, plants of the *Lamium* genus have been widely used in folk medicine to treat a wide range of ailments. The countries of the Mediterranean basin (Europe and North Africa) and Western Asia are considered as having the most widespread applications. Branches, leaves, and flowers are also commonly employed in many Mediterranean nations to prepare native cuisines. A vast number of chemical compounds found in plants of the *Lamium* genus have found extensive use in folk medicine due to their health advantages.

As a folk medicine remedy, *Lamium* species, often known as dead nettles, have been used to treat a variety of ailments, including injuries, fractures, paralysis,

hypertension, menorrhagia, and uterine bleeding. The therapeutic properties of *Lamium* species have sparked intense phytochemical research. This research has led to the discovery of various iridoids, secoiridoids, flavonoids, anthocyanins and phytoecdysteroids, as well as terpenes, megastigmane compounds and essential oils in *Lamium* species. The iridoid glucosides, which have a C10 or C9 structure, are the most prominent chemicals in *Lamium* species. *Lamium* glucosides were also considered to be chemical taxonomy markers. Since prehistoric times, humans have used plants as food and medicine. Here are some examples of traditional medical uses of *Lamium* plants. In Europe, China, and Japan, the white dead-nettle (*Lamium* album) has been employed for decades during times of famine. Many of the plant's aerial portions are edible and have been consumed as raw or cooked food in some cultures for centuries now. Most notably in various cuisine from the Mediterranean region and its neighbour's (Fathi & Mohammadi, 2013). White dead Nettle is also used in numerous well-known vegetarian recipes and salads. Adding L. album to food supplements can prevent menstrual, musculoskeletal, and fat metabolic diseases, among other benefits. "Seven Spring Herbs" rice porridge in Japan is made with *Lamium amplexicaule* (Luhata & Luhata, 2017).

Various *Lamium* species are used in traditional and folk medicine around the world to treat fractures, hypertension, leucorrhoea, paralysis, putrescence, and trauma, as well as vaginal and cervical inflammation, bleeding after childbirth, and believed to be a contraceptive, among other things. As a result of ethnobotanical investigations, *Lamium album*'s aerial parts and flowering branches have been used to treat renal ailments, such as removing stones. Infusions and decoctions of the leaves are used to treat respiratory tract issues. Plants have been the source for medicinal treatments for thousands of years. Traditional medicine uses plants for both their curative and their preventive properties. When used for preventive purposes, i.e. for the maintenance of overall good health, medicinal plants can be classified as functional foods and/or nutraceuticals. A good example is the use of spices that besides adding the flavour to foods can improve digestion or help in prevention of diseases. Plants have been used by a human as food and medicine since ancient times. Examples of the traditional medicinal uses of *Lamium* plants are here described. White dead-nettle (*Lamium album* L.) used for decades in Europe, China, and Japan during times of famine.

Different aerial parts of this plant are edible and traditionally used as raw or cooked food in some countries. Addition, non-stinging nettle is considered a base component of some well-known vegetarian dishes and salads. *L. album*, when added to food supplements, can prevent menstrual, musculoskeletal disorders and ameliorate fat metabolism. *L. amplexicaule* is used in the preparation of Japanese traditional rice porridge which called "seven spring herbs".

Moreover, there are many types of activities, e.g., antipyretic, astringent, bronchitis, diuretic, emollient, expectorant, insomnia, pains, sciatica, vasodilator, homeostatic, wound healing, antihypertensive, anti-inflammatory which recorded by literature. *L. amplexicaule* is also used as anti-rheumatic, laxative and diaphoretic. Sometimes fresh leaves of *L. amplexicaule* are crushed a paste formed used topically to joints swelling. Aerial parts decoction of *L. galeobdolon* traditionally used for fever, malaria, warts, constipation, hair loss, rheumatism, dandruff, haemorrhage, depression, nerve tonic. Many people have relied on medicinal plants to cure their illnesses. This is due to the presence of chemical compounds in these plants which cause physiological changes in the human body. As a result, plants produce a wide variety of bioactive chemicals. These indigenous medicinal herbs are utilised as spices and food plants by a large number of people around the world. Such a display, it is now abundantly evident, has no bearing whatsoever on the power of plants and their constituent elements Culture and tradition of the Turkish Republic of Northern Cyprus include the usage of plants for medical purposes. Primary healthcare is therefore provided directly by traditional medicine for around 80 percent of the population (Kupeli, et al., 2008). The significant goal of this study is to assess the potential phytochemicals present in *Lamium moschatum* and quantify using different analytical techniques. The phytochemical screening of *Lamium moschatum* subsp. *micranthum* has never been done before which makes it a novelty for this present study.

1.6 Scope of the Study

In comparison to traditional medicine, herbal products have less adverse effects, are more readily available, and are less expensive. According to indigenous traditional medicine, *Lamium moschatum* contains bioactive components like cucurbitacin, triterpenes and alkaloids, as well as vitamins and minerals.

1.6.1 Study Aims

The objective of the study is to:

1. Investigate potential medicinal constituents of *Lamium moschatum* subsp. *micranthum* by screening for the presence of phytochemicals.
2. Qualitatively determine the presence of some secondary metabolites found in the plant using Chromatographic and Spectroscopic Techniques.

1.7 Thesis Outline

The outline of this study covers five chapters. The chapter one is the introduction which assesses the background of the study with the research problems. The second chapter reviews literature on previous studies conducted on the study with other necessary information pertaining to the study. Chapter three reveals the research methodology utilized for the study, while chapter four is designated for the results and discussions of the analysis conducted. Chapter five which is the last chapter concludes the study and gives some future recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Phytochemical Properties of *Lamium* Species

Known as the mint family, Lamiaceae is a large and diverse family of flowering plants (Raja, 2019). As a general rule, plants in this family are shrubs or herbs that include fragrant chemicals such as essential oils in their leaves or blossoms. Aromatherapy, gastronomy, smell and flavour are all utilised to benefit from the therapeutic qualities of several varieties of geranium (Tamokou et al., 2017). An herbaceous genus of about 40 species found in temperate to subtropical regions of Africa, Asia and Europe, *Lamium* is part of the Lamiaceae family. The leaves are cordate or reniform, ovate to lanceolate, with an acute apex and cordate base, petiolate on the lower nodes, and sessile or seldom amplexicaule on the higher nodes, according to the botanical features. There are verticillasters in the leaf axils (2-12 flowered). A campanulate or tubular calyx with subequal teeth is seen in the genus. Dark purple or yellowish green, white, yellow or other colours can be seen on the corolla (Baran & Ozdemir, 2013). *Lamium album*, *Lamium purpureum*, and *Lamium maculatum* are among the well-studied species in the genus. In spite of their superficial resemblance to Stinging Nettles, Dead Nettle is not equipped with trichomes that may produce poisonous chemicals. Entomophilous pollination is a key feature of this genus from an ecological standpoint. Specifically, this review aims to provide an updated snapshot of *Lamium* plants' main beneficial properties, with a focus on antimicrobial, antiviral, anti-inflammatory, anti-nociceptive, and pain therapy properties, as well as cytotoxicity and cytoprotective activity, in order to better address nutraceutical uses and formulations and applications.

The presence of iridoids was determined by spectroscopy in research done by Ersoz et al (2016) on *lamium garganicum* subsp. *laevigatum*. Lamium species and *Lamiastrum galeobdolon* were studied using GC/MS to determine their volatile constituents. A total of 49 compounds were discovered, 43 of which were novel for *Lamium* and *Lamiastrum*. They revealed comparable volatile profiles and a site-

dependent oil-composition. Squalene was detected in all samples. C12 to C31 straight chain alkanes were shown to exist in homological sequence. Only Lamium maculatum contained phenethyl alcohol (Alipieva et al., 2018). Bouasia et al. (2021) investigated the effects of solvent extraction on lamium species for phytochemical research where various solvent systems and in vitro assessment were used to produce the plant extracts. As a consequence of their research, it was shown that distinct extracts included a wide variety of biologically active chemicals in significant levels (high content of flavonoids, medium content of orthodiphenols, anthocyanins as well as flavanols, and low content of total phenols as well as tannins).

One of the edible Lamiaceae species found in Iran is *Lamium garganicum* subsp. *pictum*. According to Ghalkhani et al. (2021), this plant has antibacterial, cytotoxic, and antioxidant properties. It was determined that 55 constituents of essential oils could be identified using GC–MS/FID. Trans-phytol acetate, trans-beta farnesene, and trans-caryophyllene were the three main components. According to the study of antibacterial activity, Bacillus cereus was the most resistant to the essential oil. According to the DPPH test, hydroalcoholic extract had the strongest scavenging activity of all the extracts.

Lamium galeobdolon contains benzoxazinoids, a novel class of chemicals for the Lamiaceae family. Among *Lamium amplexicaule, Lamium purpureum,* and *Lamium garganicum's* glucosides, 24-epi-pterosterone and verbascoside have been identified. According to Soni and Naved (2010), the *Lamium* iridoids 5-deoxylamiol and sesamoside have never been discovered before in the genus, an investigation of iridoids, phytochemical components of a subspecies of *lamium*, was done by Ahmed et al. in 2021. According to their data, three iridoid-glucoside derivatives were obtained via infrared imaging and chromatographic methods. The existence of phytochemicals in 62 distinct Lamiaceae species was examined by Mehrnia et al. (2021).

Around the world, medicinal plants have a significant role in affecting human health and well-being. Saudi Arabia has performed a limited number of phytochemical

experiments on medicinal plants, particularly in the Albaha area in the south-western part of the country. *Lamium* species of all kinds were studied. In addition to evaluating the antioxidants and anti-cancer properties of these plants' extracts, it was determined from the data that the examined plant extracts were rich in phenols, flavonoids, saponins, and glycosides, which were all phytochemical components. *Solanum incanum, Aerva javanica and Dodonaea viscosa* were the plants with the greatest levels of phytochemical components and antioxidant capability, respectively. In comparison to the standard (ascorbic acid), their antioxidant capacities increased by 38, 32, 22 and 14 percent, respectively (Alzandi, et al., 2021).

2.2 Biological Active Components of *Lamium* Species (Review of Studies)

There are several types of chemical components in *Lamium*, including hydroxycinnamic acids, flavonoids, phytoecdysteroids, benzoxazinides and betaine (Budzianowski & Skrzypczak, 2005). It is therefore possible to test for biological activities such as antioxidants, anti-inflammatory, antimicrobials, antischistosomal pain alleviation in rheumatism and arthritis, constipation tonics, antinociceptive, or anticancer in the presence of these compounds. Phenolics and essential oils are responsible for the majority of *Lamium* species' bioactivities. *Lamium* species contains polyphenols, flavonoids, terpenes, steroidal derivatives, and ecdysteroids, which contribute to its biological activity (Cao et al., 2019). The review of studies of various chemical components of these phytochemicals found in *Lamium* species are shown in Table 2.1.

Table 2. 1: Review studies of phytochemical constituents in *Lamium* species

Phytochemical	Specie	Compound	Analytical method	Reference
Flavonoid	*Lamium album*	Luteolin-7-p-coumaroyl glucoside	Colorimetric	(Flamini, Cioni, & Morelli, 2015)
	Lamium album	Luteolin	Thin-layer chromatography	(Marino, Bersani, & Comi, 2001)
	Lamium hypericum perforatum	Glucoside	Thin-layer and sephadex chromatography	(Fathi & Mohammadi, 2013)
Anthocyanins	*Lamium labiatae*	monomalonyl esters of peonidin 3,5-diglucoside	Spectrophotometry	(Arora, 2016)
	Lamium Salvia	dimalonyl esters of delphinidin and malvidin 3-(6″-p-coumarylglucoside)-5-glucoside.	Colorimetric	(Bianco et al., 2019)
	Lamium amplexicaule	peonidin glycosides	-	(Yalcin & Kaya, 2007)
Hydroxycinnamic	*Lamium purpureum*	allantoin	HPLC	(Raja, 2019)

Acids	*Lamium Ocimum basilicum*	uridine	HPLC	(Soni & Naved, 2010)
Terpenoids	*Lamium maculatum*	Daucosterol	TLC	(Yalcin & Kaya, 2007)
	Lamium purpureum	β-sitosterol	Reverse Phase Liquid Chromatography	(Yalcin & Kaya, 2007)
Iridoids	*Lamium amplexicaule*	Lamiol and lamioside	TLC and HPLC	(Umar & Sekar, 2014)
	Lamium garganicum subsp. *laevigatum*	6-*O*-syringyl-8-*O*-acetylshanzhiside methyl ester	Reverse phase chromatography	(Yalcin & Kaya, 2007)
	Lamium eriocephalum	Lamerioside	Spectrophotometric	(Yalcin & Kaya, 2007)
Benzoxazinoids	*Lamium galeobdolon* subsp. *galeobdolon*	4-hydroxyl-2H-1-4 benzoxanin-3(4H)	HPLC	(Soni & Naved, 2010)
Betaine	*Lamium maculatum*	pipecolic acid derivative betaines	HPLC	(Yalcin & Kaya, 2007)
	Lamium galeobdolon	pipecolic acid derivative betaines	TLC	(Yalcin & Kaya, 2007)
	Lamium purpureum, Lamium galeobdolon	proline betaines	HPLC and Reverse phase liquid chromatography	(Rao & Anna, 2009)

2.2.1 Flavonoids

These phenolic molecules contain antiviral, anti-allergic, anti-inflammatory, and antioxidant properties, according to research. They are polyphenolic chemicals found in nature and are classified into flavonols and other chemically structured compounds (catechins, anthocyanidin and chalcones) based on their chemical structure. Fruits, vegetables, and drinks contain many of the flavonoids that have been discovered. As antioxidants, flavonoids' molecular structure determines their effectiveness. According to Figure 2.1, the location of hydroxyl groups and other features of flavonoids' chemical structures are crucial for their antioxidant effects.

Flavonoids provide protection against allergies, inflammation, platelet aggregation, germs, ulcers, and cancers, among other biological functions. In terms of plant phenolics, flavonoids are the most prevalent and extensively dispersed category. As free radical scavengers, superantioxidants, and water-soluble potentiators, flavonoids protect cells from oxidative damage and have powerful anti-cancer properties. *Spondias mombin L.'s* anti-inflammatory flavonoids may explain its usage in herbal therapy to treat digestive problems (Arora, 2016).

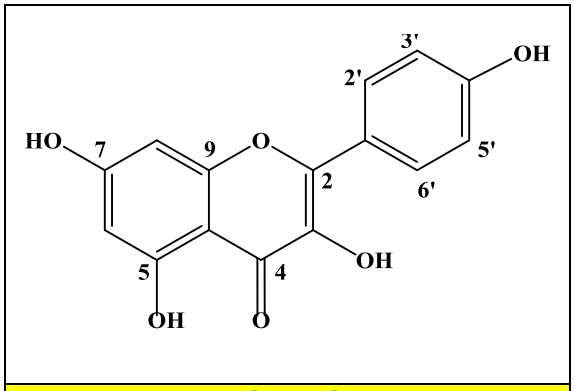


Figure 2. 1: Structure of Flavonoids *(Arora, 2016)*

2.2.2 Anthocyanins

As illustrated in Figure 2.2, anthocyanins are water-soluble pigments that are coloured. As a result, the pigments are glycosylated. Fruits and vegetables contain anthocyanins, which are responsible for the red, purple, and blue hues. Anthocyanins

are found in berries, currants, grapes, and other tropical fruits. Leafy vegetables, cereals, roots, and tubers that are red to purple blue in hue have high levels of anthocyanins. Anthocyanin pigments are present in plants in large quantities, with cyanidin-3-glucoside being the most common. A natural food colourant, anthocyanin pigments have been utilised for centuries as a result of their vibrant colours. pH, light, temperature, and structure all impact the colour and stability of these pigments. If the pH is low, anthocyanins look red. If the pH is raised, they turn blue. (Matkows & Potrowska, 2018) Chromatography has been extensively used in the extraction, separation, and measurement of anthocyanins.

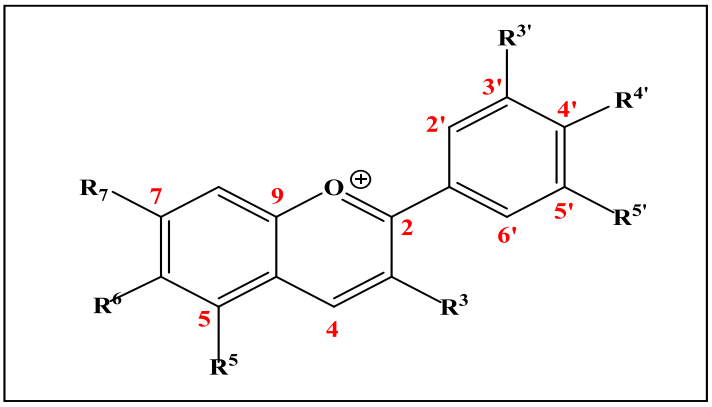


Figure 2. 2: Structure of Anthocyanins *(Bentham, 1836)*

2.2.3 Hydroxycinnamic Acids

These vital natural phenolic chemicals are found in significant levels in our food items, including hydroxycinnamic acids (HCAs). A brief description of HCA dietary consumption and nutritional relevance is provided. Pharmacokinetic characteristics, which have a strong influence on HCAs' ability to reach their target tissues, are also discussed. HCAs and, in recent years, their metabolites produced in the gastrointestinal system, liver, and kidneys, have been shown to provide a variety of health benefits (Clifford, 2000) It is important to understand that HCAs' metabolic activity can be retained, enhanced, or lost depending on their metabolite composition. Chemically, HCAs consist of phenylpropanoid C6-C3 scaffolds, which are distinguished by the presence of hydroxyl groups on aromatic rings and carboxyl

groups on the side chains. Diversity of HCAs is influenced by the amount and location of hydroxyl groups and other substituents. Caffeic, sinapic and para-coumaric acids are the four most common HCAs in nature (Figure 2.3). Natural occurrences of all four acids are rare; they are generally esterified with quinic and tartaric acids or different carbohydrate compounds.

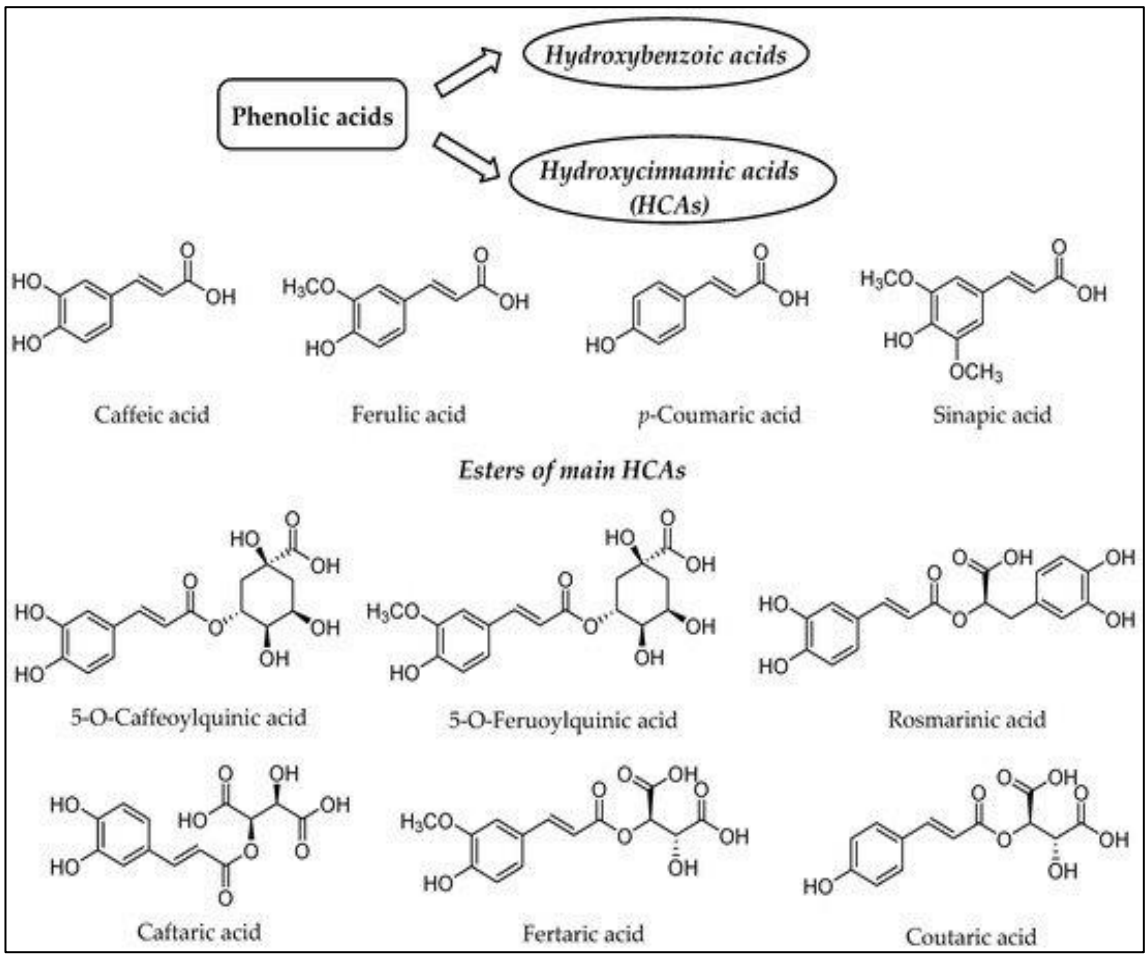


Figure 2. 3: Structure of Hydroxycinnamic acids and its derivatives *(Clifford, 2000)*

2.2.4 Terpenoids

This family of naturally occurring organic molecules, also known as isoprenoids, includes a wide range of compounds, including isoprene and its polymer derivatives termed terpenes, as illustrated in figure 2.4. They are frequently confused with terpenes, but they include extra functional groups, generally including oxygen, and

are hence more complicated. It is estimated that 60 percent of all known natural compounds are terpenoids. Medicinal chemists are interested in several terpenoids because of their bioactivity. In traditional herbal treatments, plant terpenoids are utilised for their fragrant properties. Sunflowers' golden hue and tomatoes' red colour are due to terpenoids (Baran & Ozdemir, 2013).

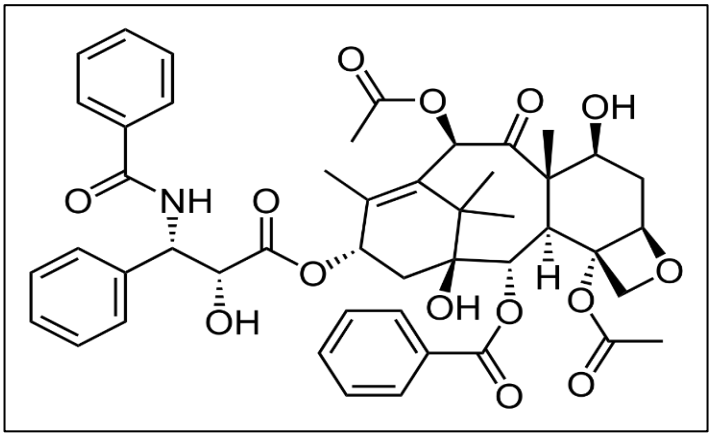


Figure 2. 4: Structure of a Terpenoid *(Baran & Ozdemdr, 2013)*

2.2.5 Iridoids

This family of active chemicals is found in plants in abundance. In recent years, breakthroughs in phytochemical research have led to the identification of a large number of substances with new structures and exceptional activity. The prototype and aglycone, as well as I and II metabolites, of iridoid molecules have been demonstrated to exist primarily in biological transformation. As well as neuroprotective, hepatoprotective, antiinflammatory, anticancer, hypoglycemic, and hypolipidemic properties, these metabolites have also been demonstrated to inhibit tumour growth. Many plants produce iridoids as secondary metabolites, including those in the Apocynaceae, Lamiaceae, Loganiaceae, Rubiaceae, Scrophulariaceae, and Verbenaceae families, among others. There is a long history of traditional usage of several of these ethnobotanicals in the treatment of inflammation. However, this review will focus on their anti-inflammatory properties. Iridoids have a variety of pharmacological activities, including cardiovascular, hepatoprotective and

hypoglycaemic effects, as well as mutagenic and spasmodic inhibitors, anti-tumor, antiviral, immunomodulatory and purgative effects. Iridoids, on the other hand, have not been systematically arranged and summarised in recent years. By analysing and comparing the structure and function of known iridoids, this review paper seeks to characterise iridoids based on their phytochemistry, biological activity, pharmacokinetics (Cao et al., 2019).

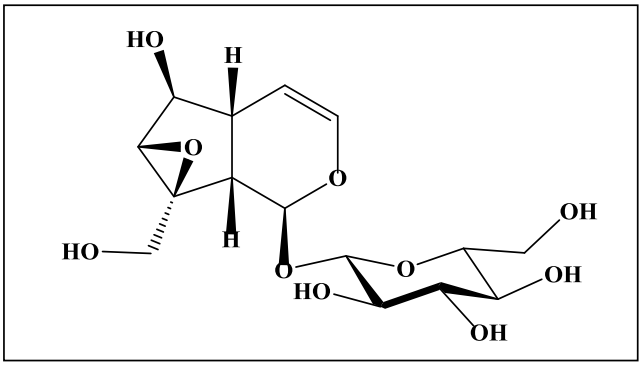


Figure 2. 5: Structure of Iridoid (Cao et al., 2019)

2.2.6 Phenylpropanoids

The phenylpropanoids are present throughout the plant world, where they are used as structural polymers, UV protection, defence against herbivores and diseases, and as floral colours and smell molecules that facilitate plant-pollinator interactions. fruits, vegetables, cereal grains, drinks, spices and herbs all contain secondary plant metabolites known as phenylpropanoids and their derivatives. In addition, they are known to have a wide range of beneficial benefits including those relating to antibacterial, antioxidant, anti-inflammation, diabetes, and cancer prevention. They are used in food preservation, packaging films, and edible coatings as well as in the pharmaceutical, cosmetic, and other sectors, including textiles (colourants), biofuels (antioxidant additives), and sensors (sensing biologically relevant molecules). Commercially accessible nutritional supplements and skin care products include phenylpropanoids. Phenylpropanoids and their derivatives are discussed in this study, along with the mechanisms of action and prospective uses in a wide range of sectors

(Lev & Amar, 2002). Salidroside (Jensen & Nielsen, 1974) and Liriodendrin have been reported from *Lamium galeobdolon* and *Lamium maculatum* respectively (Deng et al., 2005).

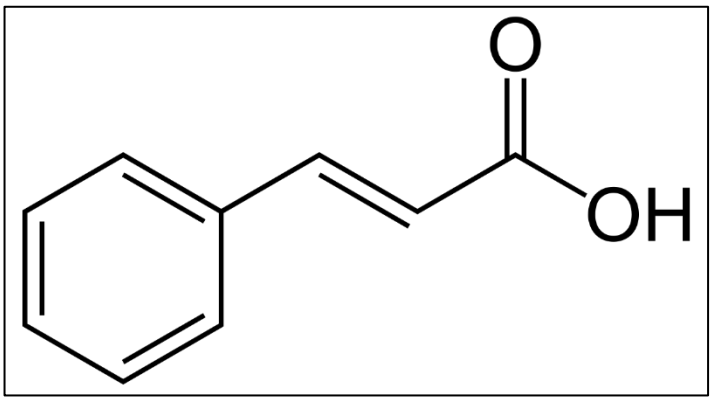


Figure 2. 6: Structure of a cinnamic acid (Phenylpropanoid) *(Lev & Amar, 2002)*

2.2.7 Benzoxazinoids

The bioactive benzoxazinoids, as shown in Figure 2.7, are secondary metabolites of many Poaceae, and certain benzoxazinoids and their downstream metabolites serve as allelochemicals and natural insecticides. The complete breakdown of chemicals by microbes is of extreme relevance since a short lifespan of the compounds is essential to minimise long-term environmental impacts. In addition to the crops maize, rye, and wheat, some dicotyledonous plants contain benzoxazinones. When immature roots and shoots create them, they undergo glycosylation before being retained in vacuoles or extruded by roots. Benzoxazinones have been studied extensively for decades as potential natural herbicides, lead structures for novel herbicides, and anti-herbivory compounds (Erhirhie & Ekene, 2017). Benzoxazinoides have been extensively investigated for their allopathic effects. Seedling age, dose, and species all affect weeds and crops' sensitivity to the chemicals. Plants and microorganisms must be able to successfully detoxify benzoxazinoids in order to recover from and survive damage induced by these chemicals (Raja, 2019).

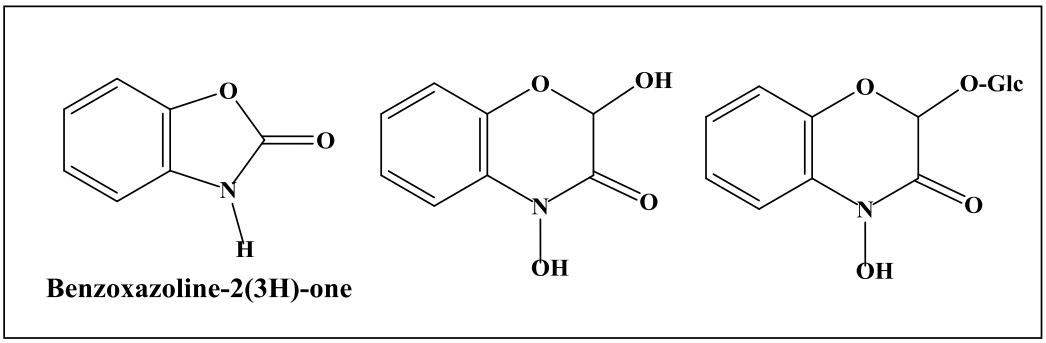


Figure 2. 7: Structure of Benzoxaninoid (Raja, 2019).

2.2.8 Betaine

In addition to positively charged functional groups, betaine may also include negative charges such as a carboxylate group that is not always next to the positively charged functional group quaternary ammonium cation or phosphonium cation. It is an example of one form of Zwitterion (see Figure 2.8). In addition to being used to treat homocystinuria, betaine is an amino acid that has been modified to include three methyl groups on glycine. A small number of cases of blood enzyme increases or clinically evident liver damage have not been related to betaine's usage in the clinic (Arora, 2016). Uses for betaine include the treatment of homocystinuria (an inherited condition in which the body cannot break down a certain protein, causing build-up of homocysteine in the blood). An excess of homocysteine can cause symptoms such as fatigue, seizures, dislocation of the lens of the eye and osteoporosis (weak bones), blood clots, or a decrease in weight or pace of weight increase in children, as well as delayed development. There is a family of medicines called nutrients that includes betaine. Homocysteine is reduced in the blood as a result of the drug's action (Soni & Naved, 2010).

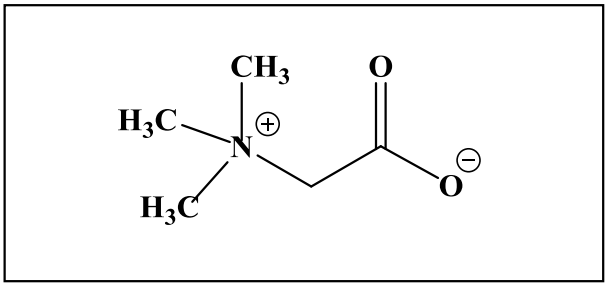


Figure 2. 8: Structure of Betaine (Cao et al., 2019) (Trimethylglycine)

2.2.9. Phenylethanoid glycosides

Phenylethanoid glycosides (PhGs) are generally water-soluble phenolic compounds that occur in many medicinal plants. In addition to antibacterial, anticancer, antidiabetic, anti-inflammatory, antiobesity, antioxidant, antiviral and neuroprotective capabilities, PhGs also have antiviral, antiviral, and neuroprotective effects as well as other qualities (Wu, et al., 2020). Due to limited bioavailability, PhGs have been unable to achieve their therapeutic potential despite their promised effects. For example, lamioside has been obtained by acid hydrolysis and chiral identification by HPLC analysis from the *Lamium purpureum* species and is an important phenylethanoid. A polar organic solvent (methanol or, less commonly, ethanol or Ethyl Acetate) is used to extract PhGs from the plant, which is then dissolved in water and washed with organic solvents. However, the water-soluble part is either dried in an oven or extracted into water saturated butanes, and any solids or pigment are removed by filtration (celite, charcoal) before purification by chromatography is carried out (Ito et al., 2006).

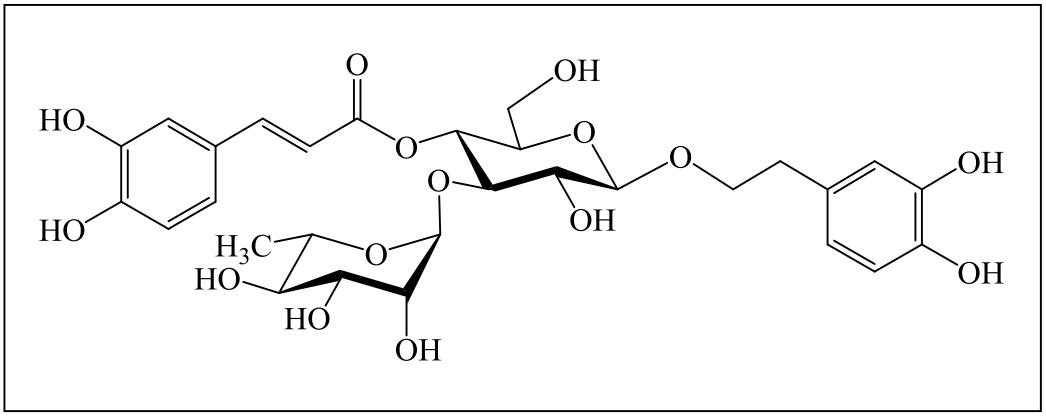


Figure 2. 9: Structure of a Phenylethanoid Glycoside (Verbascoside = Acteoside)

CHAPTER THREE

METHODOLOGY

3.1 Introduction

This research methodology entails the preliminary phytochemical screening of *Lamium moschatum* subsp. *micranthum*. The chapter reveals the various sample preparation and the final quantifications for phytochemicals investigations.

3.2 Materials and methods

3.2.1 Plant material collection

The whole aerial parts of *Lamium moschatum* subsp. *micranthum* were collected during the month of March 2020. The aerial parts were washed thoroughly and dried in shade as seen in Figure 3.1. The shade dried plant extracts were powdered and used for further studies.

3.2.2 Chemicals

All chemicals were purchased from sigma Aldrich and Merck chemical company.

The chemicals include ethanol, n-butanol, distilled water, methanol, dichloromethane, 10% vanillin/methanol, 10% sulfuric acid and silica gel.

3.3 Plant Extraction

Extraction of the plant extracts was carried out by washing the plants and drying at room temperature in 14 days. After that, they were filtered with sieve analyser to get homogeneous particles and defatted with 2.5L of petroleum ether (60-80˚C) by cold maceration method for 72h. The solvent was then removed by filtration and the marc was dried. The dried marc was re-soaked with 2.5L of methanol.

Figure 3. 1: Dried aerial parts of *Lamium moschatum* subsp. *micranthum*

3.3.1 Ethanolic Extraction

The plants extracts were air dried and grinded and after which they were dried in a drying oven at a temperature of 40oC for 12 hrs. Ethanol and distilled water was used as the dissolving solvent in the ratio (80:20 % v/v). Serial dilutions of 1 litre, 500 ml and 250 ml were made with the same solvent system before commencing the filtration process. After collection of the extracted liquid, the used dissolving solvent was evaporated by using rotary evaporator. Starting with ethanolic evaporation at vacuum set in 175 mbar for boiling point at 40^{o}C. Liquid-liquid extraction involving an extraction principle of two immiscible solvents where the liquids are mixed and the solutes are allowed to distribute between the phases until equilibrium is attained. 150 ml of the first solvent (Dichloromethane) and 90 ml of distilled water were added to the dried extracts in a separatory funnel as seen in Figure 3.2. After the dichloromethane extraction, it was partitioned with another solvent butanol and the collected butanolic layer were collected after each extraction as seen in Figure 3.3.

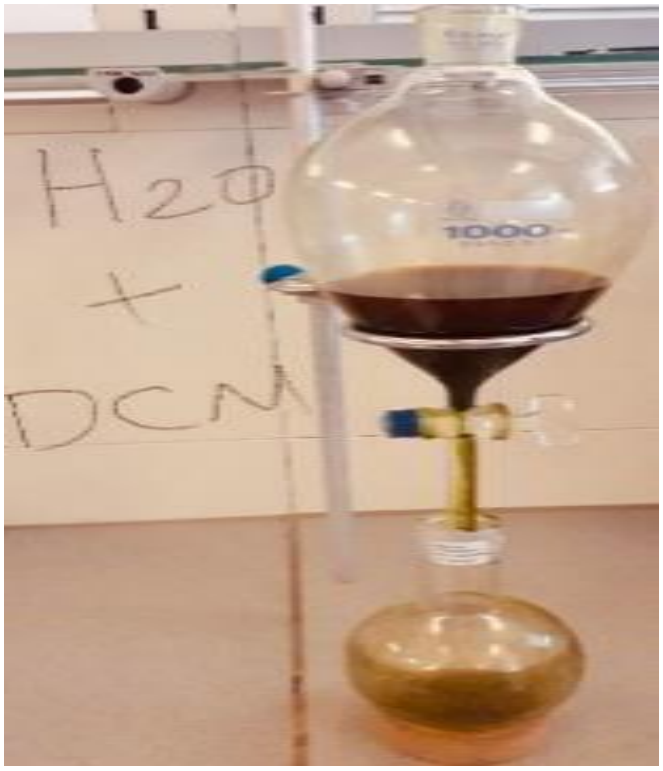


Figure 3. 2: Plant Extract extraction with dichloromethane

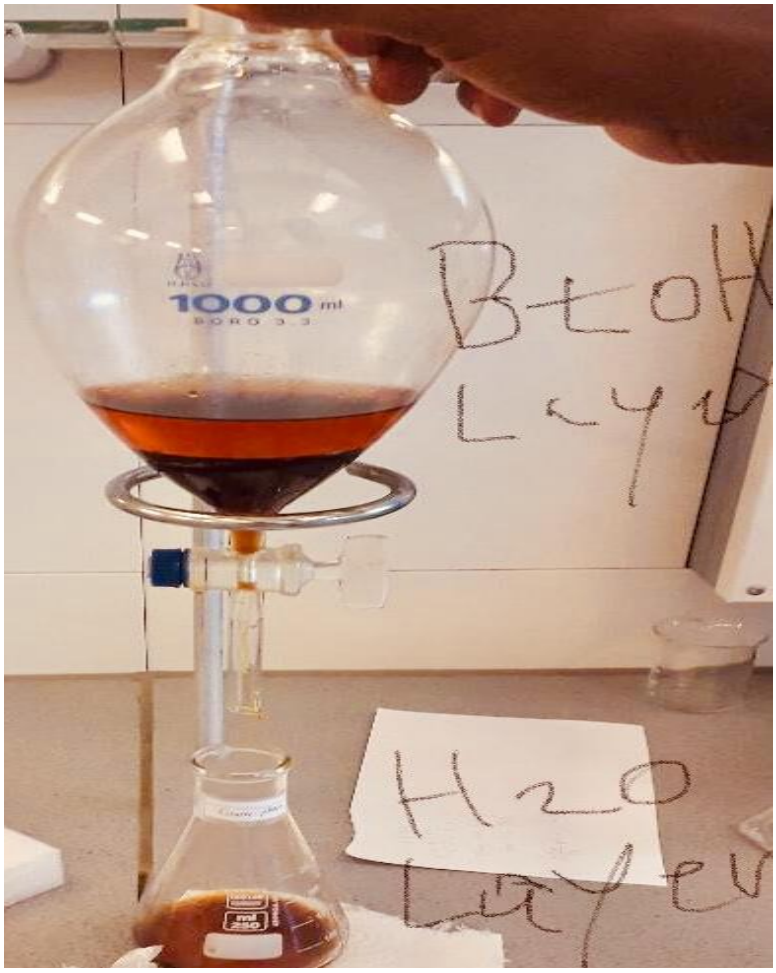


Figure 3. 3: Liquid-liquid extraction of plant extracts with Butanol

3.3.2 Methanolic Extraction

100 grams of the plants extracts *Lamium moschatum* subsp. *micranthum* were grinded and dissolved in 500 ml of methanol. Thereafter, it was filtered using Whatman Filter and the dissolving solvent was evaporated till dryness. 30 ml of distilled water were added to the plant extracts. The plant extracts were washed thrice using 100:150:200 ml of dichloromethane. The upper phases were evaporated until dryness under vacuum with 100, 150, 200 ml of butanol for the diluted plant extracts.

3.4 Preparation of TLC Plates

Adsorbent (silica gel G) suspension was produced in water using the following proportions: (1: 2). Each plate was cleaned and put out in line as a template, then the suspension was added to the Stahl TLC spreader that had been adjusted to 0.25mm thickness. The plates were then covered in a single pass with the spreader. For 30 minutes at 105oC, the plates were dried by air and activated in a hot air oven. They were then stored in a desiccator. To analyze the samples, pre-coated aluminum plates covered with silica gel G F254 (Merck) were employed as stationary phases.

3.4.1 Sample Application

A spray bottle was used to apply spots on TLC plates after extracts had been dissolved in mobile phase.

3.4.2 Development of the Chromatographic plates

After drying of the spot, the plates were developed in a chromatographic tank containing the solvent system. After one third of the plate was developed the plates were taken outside and dried. The TLC plate was examined visually or under UV light.

3.5 Chromatographic methods

3.5.1 Reversed Phase Vacuum Liquid Chromatography (RP-VLC)

The fractionation variables of the chromatographic technique are shown in Table 3.1. The solvent system entails methanol: water and the mobile phase uses dichloromethane: Methanol: water in ratio 80:20:2 as seen in Figure 3.4.

Table 3. 1: Table showing fractionation parameters for Reverse phase liquid chromatography

Fraction No	Water Percentage	Methanol Percentage
1-4	100	0
5-8	100	0
9-12	90	10
13-16	80	20

17-20	70	30
21-24	60	40
25-26	50	50
27-28	40	60
29-30	30	70
31-32	20	80
33-34	10	90
35-38	0	100

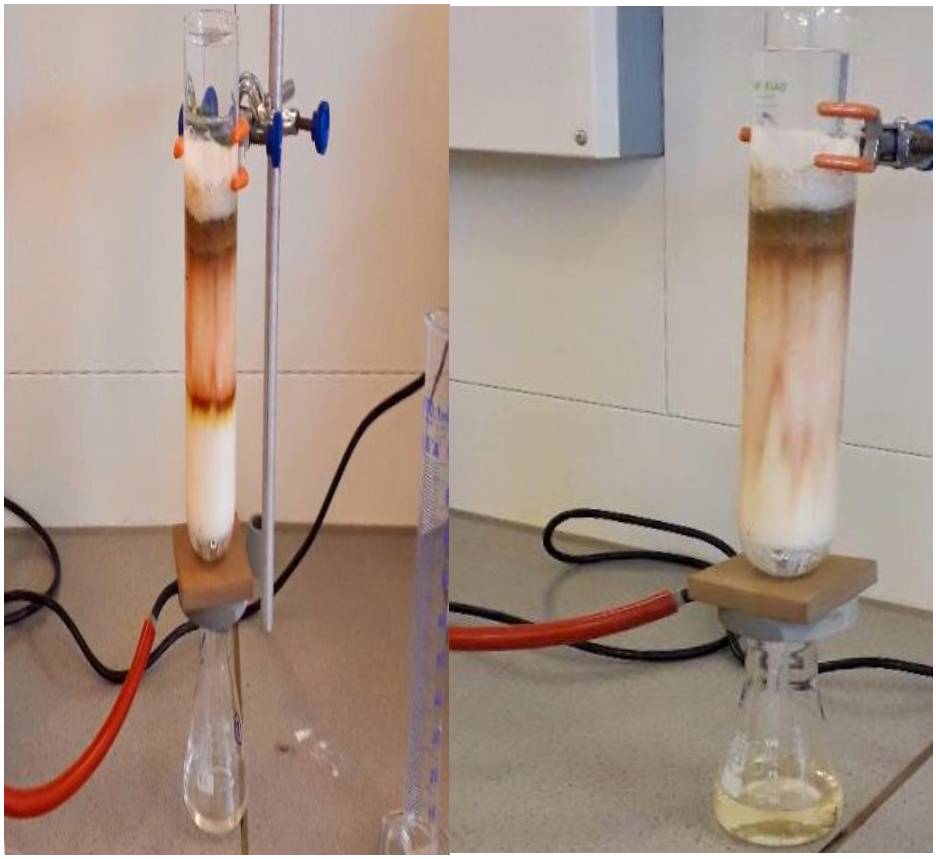


Figure 3. 4: Reverse Phase Vacuum Liquid Chromatography

3.5.2 Fractionation procedure using Sephadex LH-20

The fractionation procedure of the Sephadex gel chromatography is shown in Table 3.2. Fractionation procedure was performed using silica as stationary phase and DCM-MeOH-H_2O mixtures (80:20:2; 70:30:3; 61:32:7) as seen in Figure 3.5.

Table 3. 2: Table showing fractionation parameter for Sephadex gel chromatography

Methanol (ml)	Water (ml)	Total (ml)
75	75	150
50	50	100
50	50	100
50	50	100
150	0	150
100	0	100
200	0	200

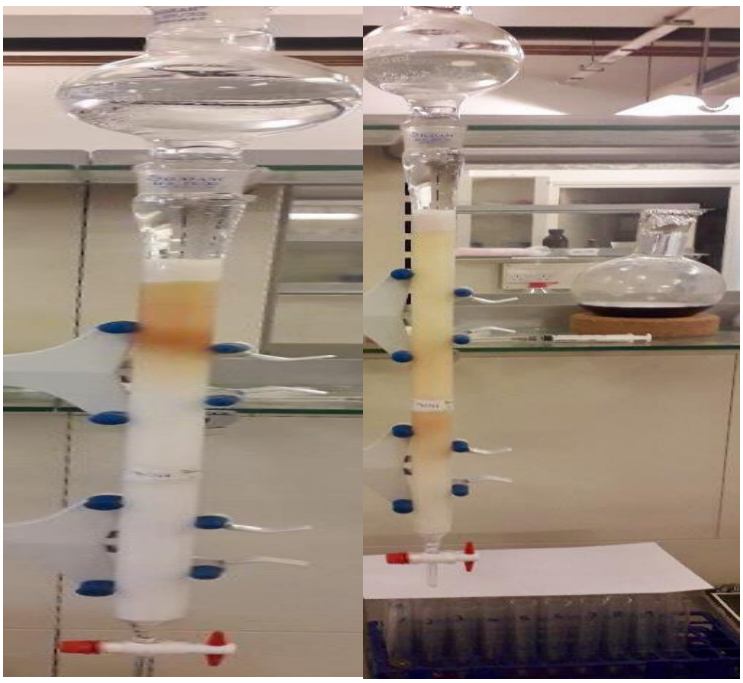


Figure 3. 5: Sephadex Gel Chromatography of Plant extracts

3.5.3 Silica Gel Chromatography

The adsorbent material used was silica gel. 23 grams of the plant extracts were added to 2 gr of silica gel and this was placed into the column as seen in Figure 3.8. The solvent system parameter is shown in Table 3.3.

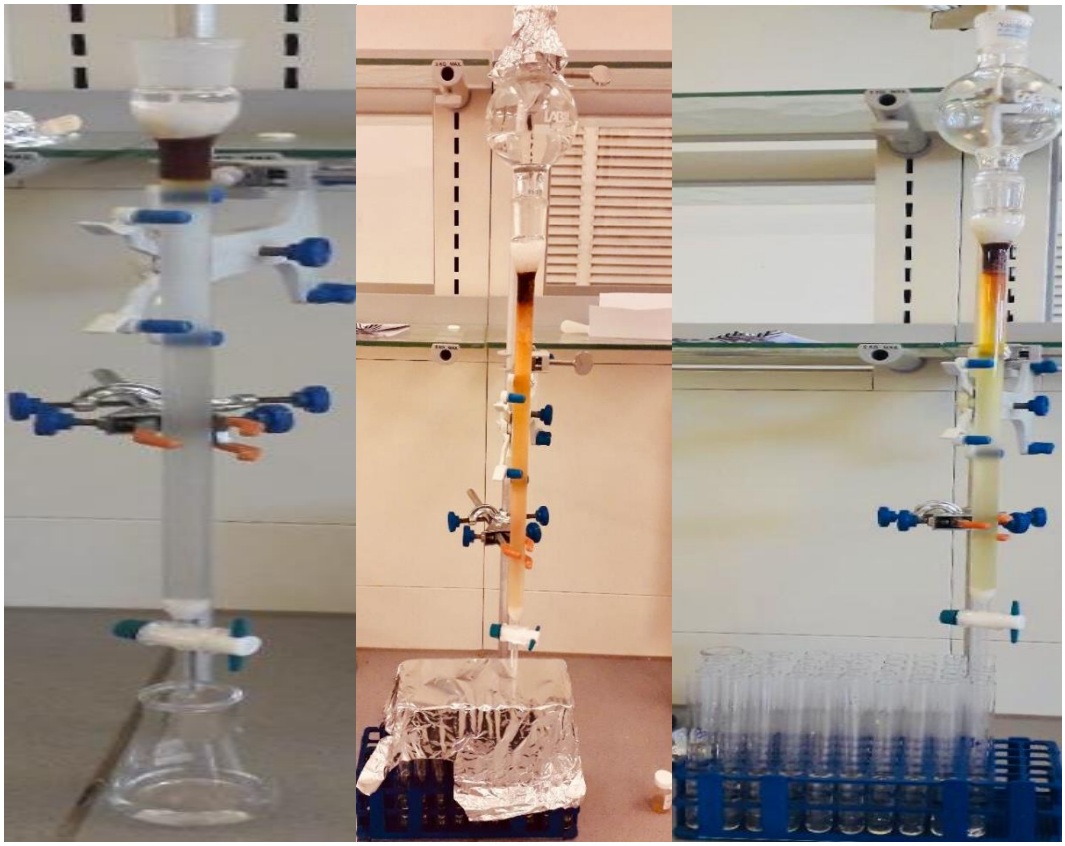


Figure 3. 6: Silica Gel Chromatography of Plant extracts

Table 3. 3: Table showing the silica gel chromatography solvent parameters

Dichloromethane (%)	Methanol (%)	Water (%)	Total (ml)
90	10	1	200
80	20	1	200
80	20	2	200

3.6 Instruments

Lyophilizator: CHRIST Alpha 1-4 LD Plus

NMR: Bruker (^{1}H-NMR; 500 MHz ; ^{13}C -NMR: 125 MHz)

UV Lamp: Camag

Rotary Evaporator: Büchi R-210 and Heidolph 4001

Vaccum : Rockk vacuum

Electrical Grinder : Retsch SK 100

Weight : Mettler Toledo PB 1502-S/FACT

Plate Heater : Camag TLC Plate Heater III

Separatory funnel 100ml

3.7 Phytochemical Components Identification

For the identification of the specific bioactive compounds present in the *Lamium moschatum*, a Nuclear Magnetic Resonance technique was used to detect the various spectrums and peaks of the spectroscopic data.

3.7.1 Isolation of LM-2 and LM-4

The flavonoid fractions were extracted from the plant extracts where methanolic extracts of the samples were partitioned with dichloromethane and butanol. The fractions were thereafter separated by silica gel column and eluted step wisely. After

the chromatographic purifications of the plant extracts, the fractions were isolated via Sephadex LH-20 before spectra analysis and characterization was carried out.

3.8 Analytical Techniques

Chromatographic fingerprint of an herbal medicine is a pattern formed by extracting specific chemical elements that are pharmacologically active or chemical constituents that are prevalent in herbal medicines. In order to chemically describe the herbal medication studied, this chromatographic contour should be highlighted by the fundamental attributes of dependability and fuzziness or similarity and difference. According to the proposed method, herbal medicines may be reliably confirmed and identified by using chromatographic fingerprints, even if the amount and/or concentration of chemically characteristic components are not exactly the same in different samples (thus, fuzziness). The quality of herbal medicine extracts should thus be evaluated by considering all of the components, not just one or two. Many of the components in herbal medicine and its extracts, on the other hand, are nameless and present in little quantities. These variations are also common within the same botanical substance. Determining the pharmacologically active and chemically different components of the herbal medicine using accurate chromatographic fingerprints is therefore extremely important (Bisset, 2014).

3.8.1 Thin-Layer Chromatography (TLC)

When it comes to separating chemicals components, TLC is one of the most accepted methods. To determine the phytochemical composition of herbal medicines A high-performance TLC (HPTLC) scanner may capture the chromatogram, retardation factor (Rf) values, the colour of the separated bands, their absorption spectra, and the max of all the resolved bands in a TLC fingerprinting process. With the addition of derivatization with various reagents, these reflect the TLC fingerprint profile for this sample. The data obtained in this way may be used to determine whether a medication is legitimate, to exclude adulterants, and to maintain the drug's tone and consistency. With various mobile phases, HPLC fingerprinting comprises recording of chromatograms and retention time of individual peak (Bako et al., 2015).

3.8.2 High Performance Thin Layer Chromatography (HPTLC)

To identify pesticides, mycotoxins, and quality control of herbaceous plants and health foods, the HPTLC technique is widely utilized by the pharmaceutical sector (Soni & Naved, 2010). Multiple samples can be run simultaneously by using less mobile phase than in HPLC (Jianga et al., 2018). The use of HPTLC mobile phases of pH 8 and higher has been reported. Another advantage of HPTLC is that the chromatogram can be exposed to the same or different conditions several times. HPTLC has since been used for the simultaneous analysis of many components in a multi-component formulation. Using this approach, it is also feasible to authenticate different plant species. Herbal substances can be separated and purified using HPLC, both analytically and preoperatively. Two main forms of preparative HPLC may be distinguished from each other: low pressure and high pressure HPLC (usually under 5 bar and over 20 bars, respectively) (Chimezie et al., 2008). It's crucial to examine analytical HPLC's resolution, sensitivity and rapid analysis time, but preparative HPLC also has to take into account solute purity and throughput or recovery. The use of bigger stainless-steel columns and packing materials (particle size 10-30 m) is necessary for preparative HPLC (pressure >20 bar) (Rao & Anna, 2009).

3.8.3 Reverse Phase Liquid Chromatography (RPLC)

Reverse-phase chromatography (RPC) is a liquid chromatography method that separates molecules based on hydrophobic interactions between the solute molecules in the mobile phase and the ligands linked to the stationary phase. Hydrophobic surfaces are created by covalently attaching alkyl or aromatic ligands to the stationary phase (Soni & Naved, 2010). Hydrophobic stationary phase is passed over by aqueous solvent containing solutes. Due to its chemical and structural stability, the chemical composition of the base matrix is crucial in RPLC. Due to their compatibility with these criteria, Silica and synthetic polystyrene are frequently utilized as RPLC matrix materials. The particle size of the beads is determined by the separation process. In general, larger bead sizes imply greater capabilities and maybe less stress on the system. Using beads with a diameter larger than 10 m is beneficial for large-scale preparative processes, whereas smaller-scale preparative and analytical separations benefit from beads in the 3–5 m range (Wood, et al., 2018).

CHAPTER FOUR

RESULTS AND DISCUSSION

The solvents chosen for extraction were ethanol and methanol. Initially ethanol was used for dissolving the extracts but low yield was obtained. Thereafter, methanol was used for subsequent dissolving of the plant extracts. The results of the solvent extracts yield are discussed below.

4.1 Ethanolic Extraction

After fractional distillation, the results are as follows:

Mass of empty flask = 208.72 g

Mass of flask after drying = 211.21 g

n-butanolic extract yield = (211.21 – 208.72) = 2.49 g.

4.2 Methanolic Extraction

After the plant extracts were grinded and evaporated to dryness using dichloromethane and distilled with butanol, the results of the experiment yield are as follows:

Mass of empty flask = 164.02 g

Mass of flask after drying = 165.19 g

n-butanolic extract yield = (165.19 – 164.02) g = 1.17 g.

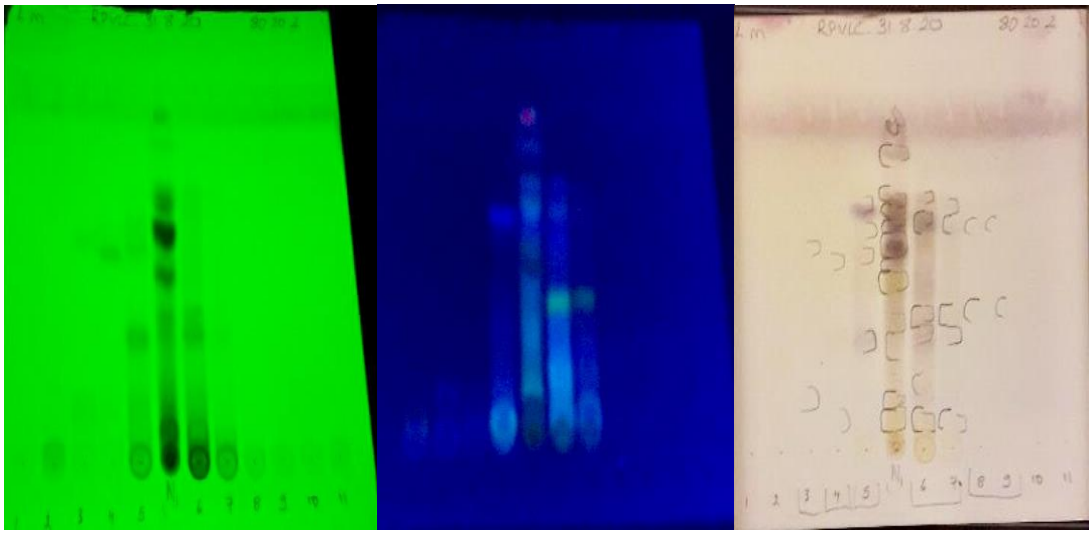
4.3 Reverse phase vacuum liquid chromatography (RPVLC)

Fractionation procedure was performed using LiChroprep C18 as stationary phase and MeOH-H_2O mixtures (with increasing amount of MeOH in H_2O) as mobile phase (RP-VLC= Vacuum Liquid Chromatography) (Table 4.1). Fractions were monitored by TLC (Thin Layer Chromatography) using or Silica gel 60 as stationary phase and DCM-MeOH-H_2O mixtures (80:20:2; 70:30:3; 61:32:7) with increasing

the polarity. Chromatograms were checked by daylight, under UV (254 and 366 nm). For the visualization of the spots on TLC plates, 10% vanillin/methanol (Reagent A) and 10% H_2SO_4 (Reagent B) were used successively.

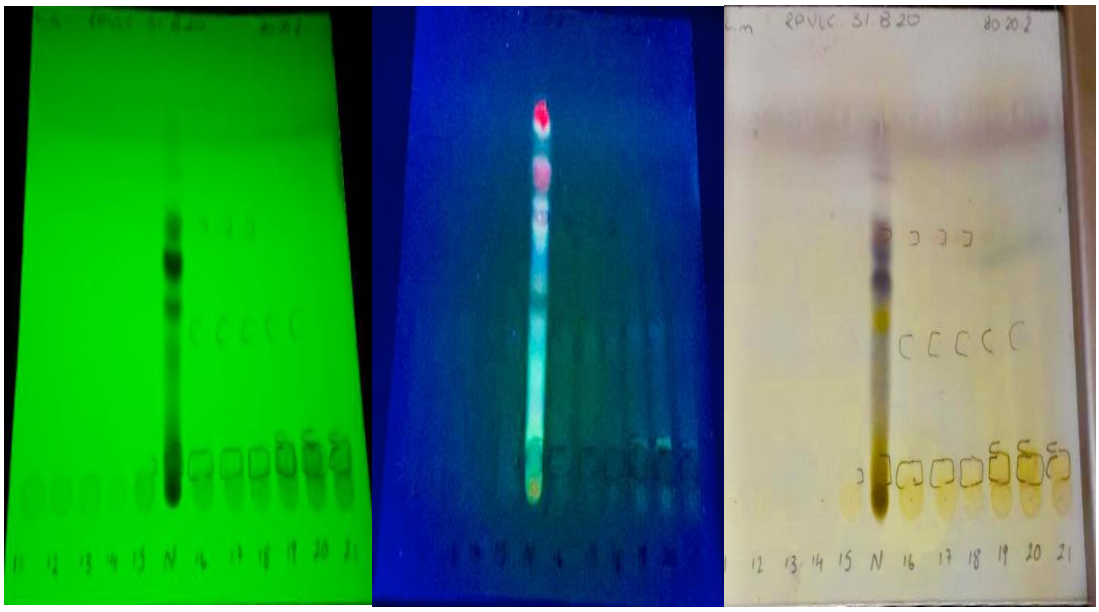
Table 4. 1: Fractionation Procedure by RP-VLC

Fraction No	Water Percentage	Methanol Percentage
1-4	100	0
5-8	100	0
9-12	90	10
13-16	80	20
17-20	70	30
21-24	60	40
25-26	50	50
27-28	40	60
29-30	30	70
31-32	20	80
33-34	10	90
35-38	0	100



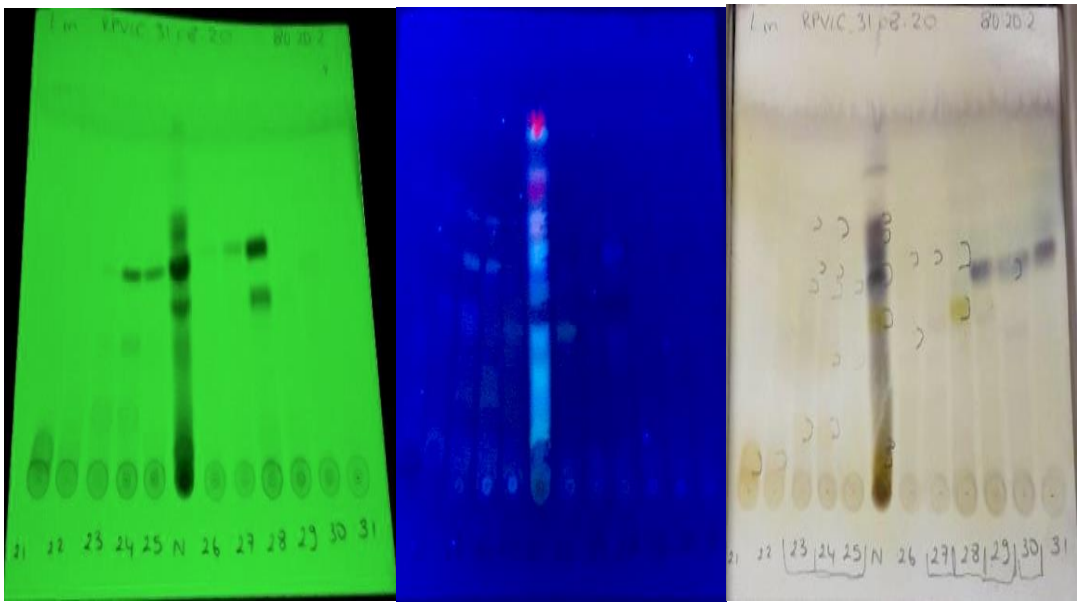
(a) (b) (c)

Figure 4. 1: TLC plates of fraction no 1-11 (a) under 254 nm, (b) under 366 nm, (c) daylight



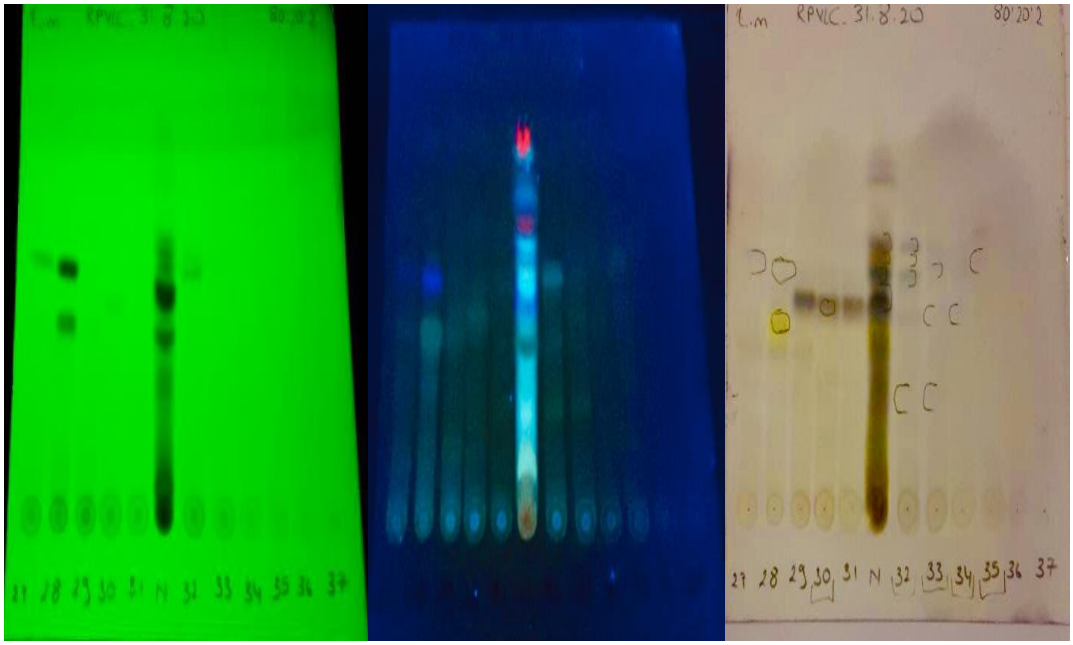
(a) (b) (c)

Figure 4. 2: TLC plates of fraction no 11-21 (a) under 254 nm, (b) under 366 nm, (c) daylight



(a) (b) (c)

Figure 4. 3: TLC plates of fraction no 21-31 (a) under 254nm, (b) under 366nm, (c) daylight



(a) (b) (c)

Figure 4. 4: TLC plates of fraction no 27 -37 (a) under 254nm, (b) under 366nm, (c) daylight

After the TLC plates were visualized, all the fractions were concentrated by rotary evaporator and lyophilized by lyophilized machine. The results are shown in Table 4.2.

Table 4. 2: Results of fractionation after fractions lyophilizedation

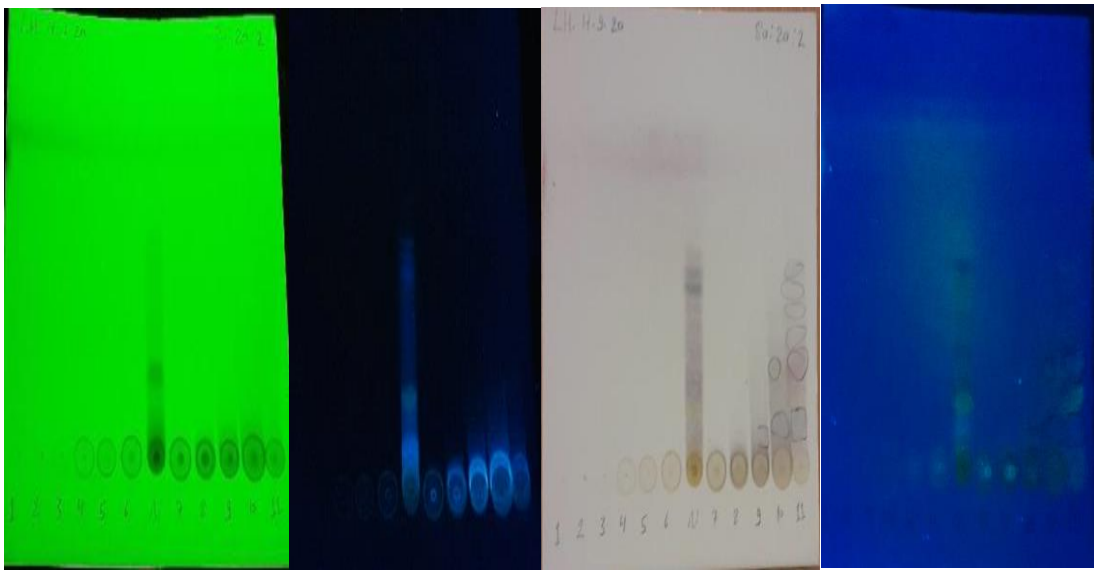
Fraction number	Weight of empty bottle (g)	Yield (mg)
3	14.2323	45.7
4	14.4728	72.9
5	14.0848	253.5
6-7	14.3093	525.5
8-9	14.3108	21.2
10-14	14.3084	36.7
15-18	7.1997	72.9
19-21	14.3180	129.7
23-25	7.2397	141.9
26-27	7.1488	38.5
28	14.3136	136.8
29-31	7.1404	108.7
33	14.1834	28
34	14.5334	14.5
35	14.0909	21.4
36-38	14.5573	62.3
		Total: 1710.2 mg

4.4 Sephadex Gel Chromatography (SGC)

For Sephadex Gel Chromatography, silica gel was used as the stationary phase and 500ml methanol was used as the mobile phase. And after lyophilization with the use of a freeze drier, the final yields of the fractions are shown in Table 4.3 and the visualized plates are seen in Figure 4.5-4.8.

Table 4. 3: Fractionation for Sephadex gel chromatography

Fractions number	Weight of empty bottles (g)	Yield (mg)
1-5	14.2566	5.1
6	14.1643	1.1
7	14.0914	1.6
8-11	14.2355	2.3
12-14	14.0375	2
15-50	14.2998	10.2
51-53	7.0156	4.8
54-55	14.1287	60.7
56-57	14.4577	5.1
58-59	14.2415	0.7
60	6.9774	3.9
61-66	7.1206	77.3
67-72	14.5950	4.4
		Total= 179.2 mg



(a) (b) (c) (d)

Figure 4. 5: TLC plates of fraction 1-11 for SGC (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366 after spraying and heating

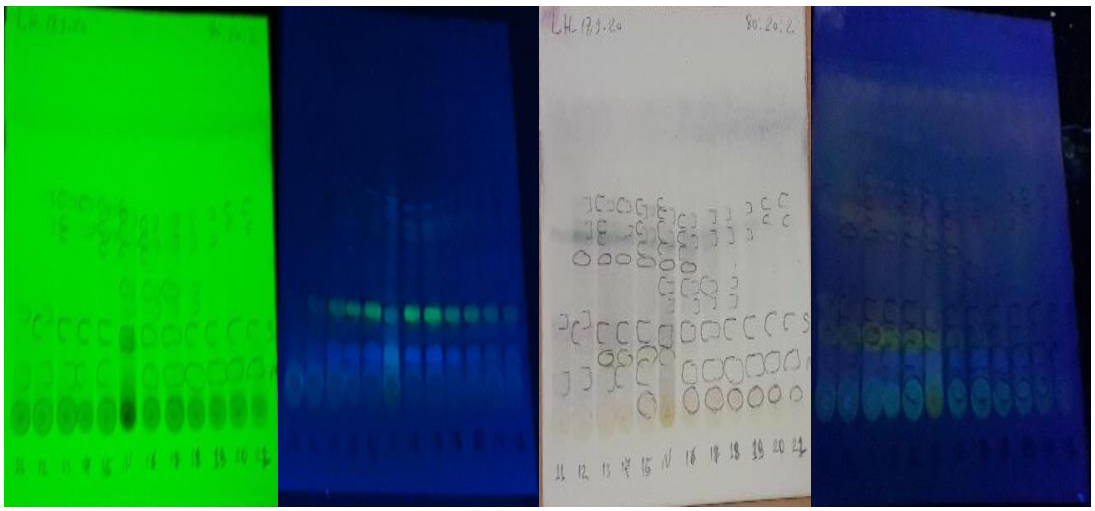
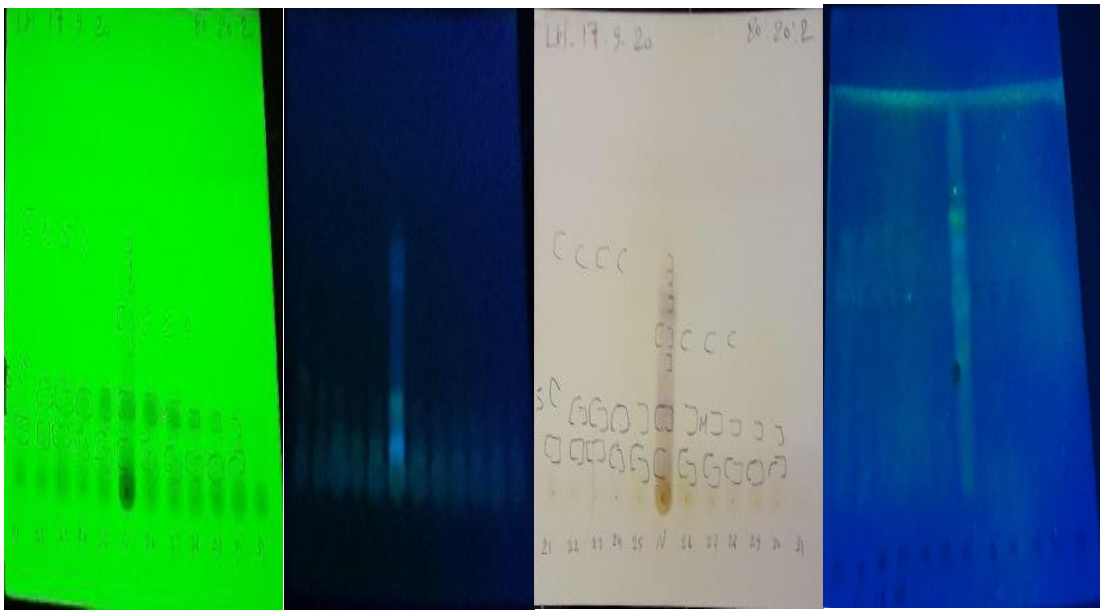
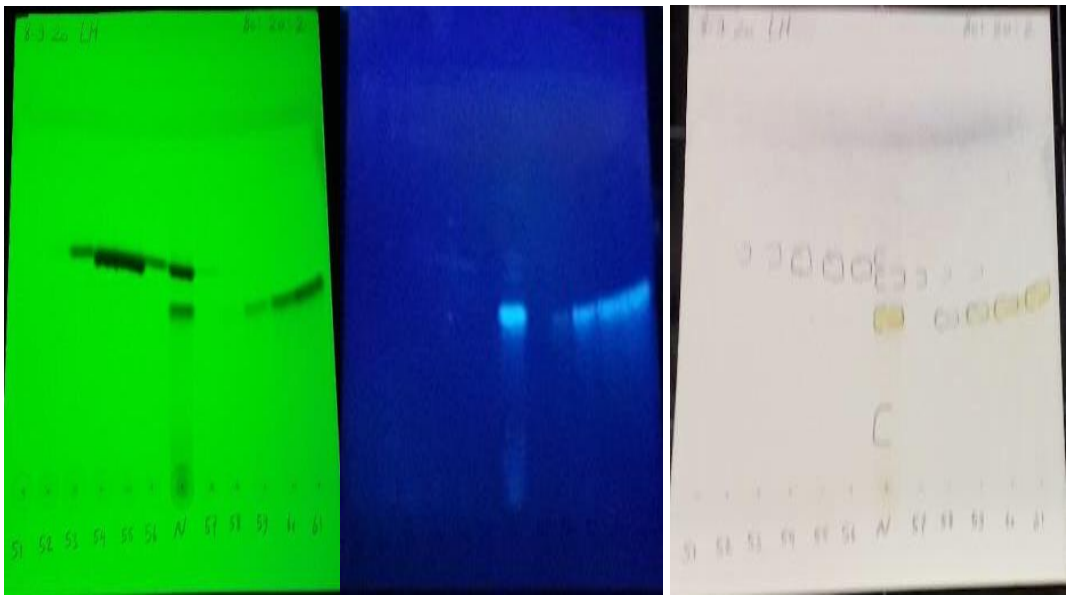


Figure 4. 6: TLC plates of fraction 11-21 for SGC (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366 after spraying and heating



(a) (b) (c) (d)

Figure 4. 7: TLC plates of fraction 21-31 for SGC (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366 after spraying and heating



(a) (b) (c)

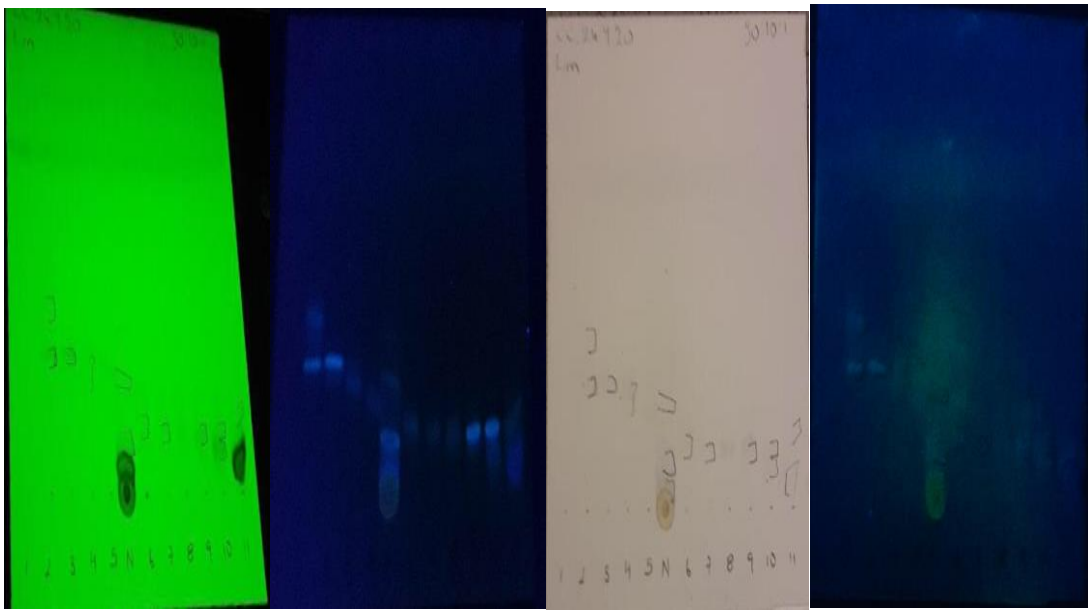
Figure 4. 8: TLC plates of fraction 51-61 for SGC (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying

4.5 Silica Gel Chromatography

For the silica gel chromatography, the stationary phase was silica gel which is common stationary phase for column chromatography. The solvent chosen usually depends on the polarity of the molecules to facilitate movement through the stationary phase dichloromethane/methanol/water solvent system was used as the mobile phase. According to the results of TLC studies, all the fractions were combined and concentrated under vacuum and then lyophilized. The fractionation data before visualization are shown in Table 4.4 and the TLC plates after spraying were visualized as seen in Figure 4.9-4.12 for 141 mg dry application, for 72.9 mg dry application are shown in Figure 4.13-4.14 and for 139 mg dry application as seen in Figure 4.15-4.16.

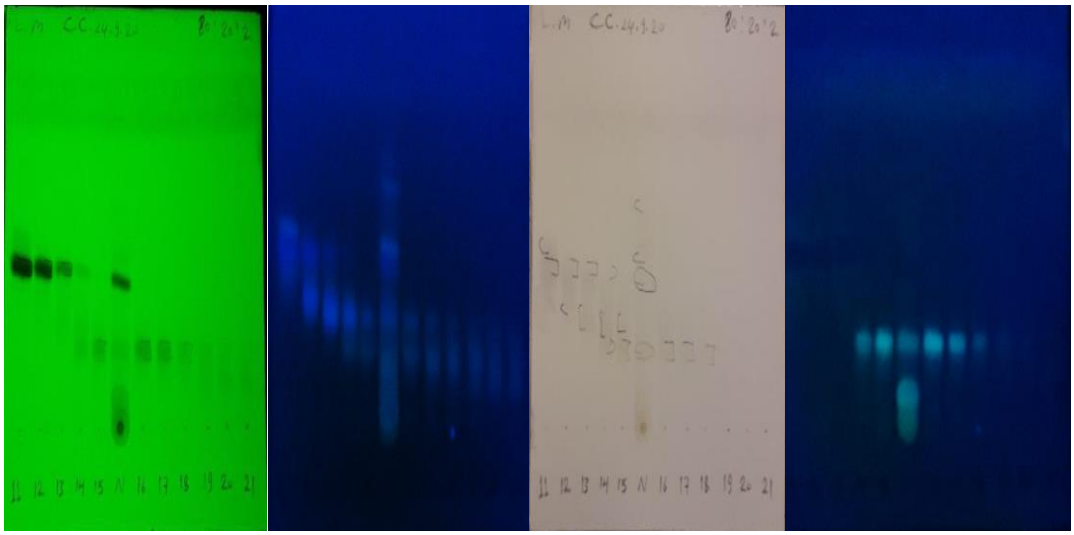
Table 4. 4: Fractionation for Silica Gel chromatography

Fractions number	Weight of empty bottles (g)	Yield (mg)
1-5	14.6028	2.4
6-8	14.1377	-
9-10	14.0701	-
12-13	14.1524	-
14-17	14.4837	13.4
18-20	14.0007	19.4
21-25	14.5744	5.5
26-41	14.0923	4.8
21-51	14.3595	5.5
		Total = 51mg



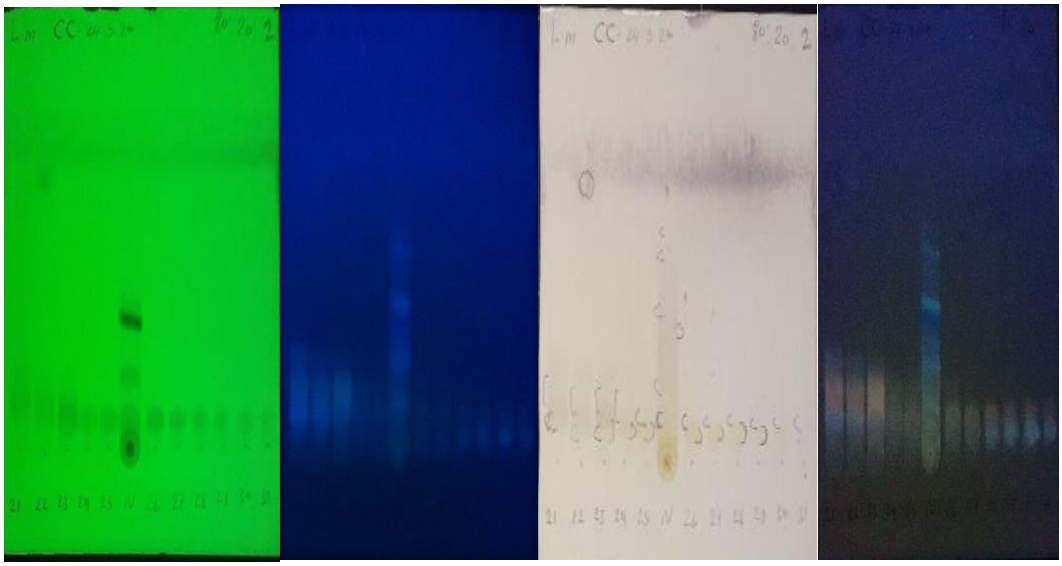
(a) (b) (c) (d)

Figure 4. 9: TLC plates of fraction 1-11 for SGC (141.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (1-2: 1.1mg, 3:0.8 mg, 4-5: 2.4mg, 6-10: 3.9 mg, 11:18mg)



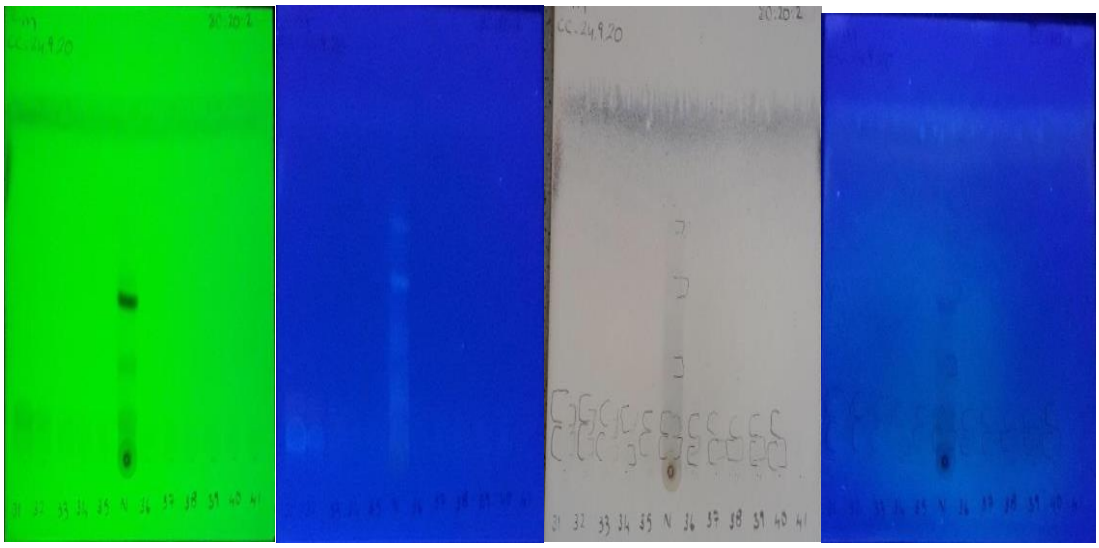
(a) (b) (c) (d)

Figure 4. 10: TLC plates of fraction 11-21 for SGC (141.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (12: 18 mg, 13: 5.1 mg, 14-15: 3.9 mg, 16-18: 11.4 mg)



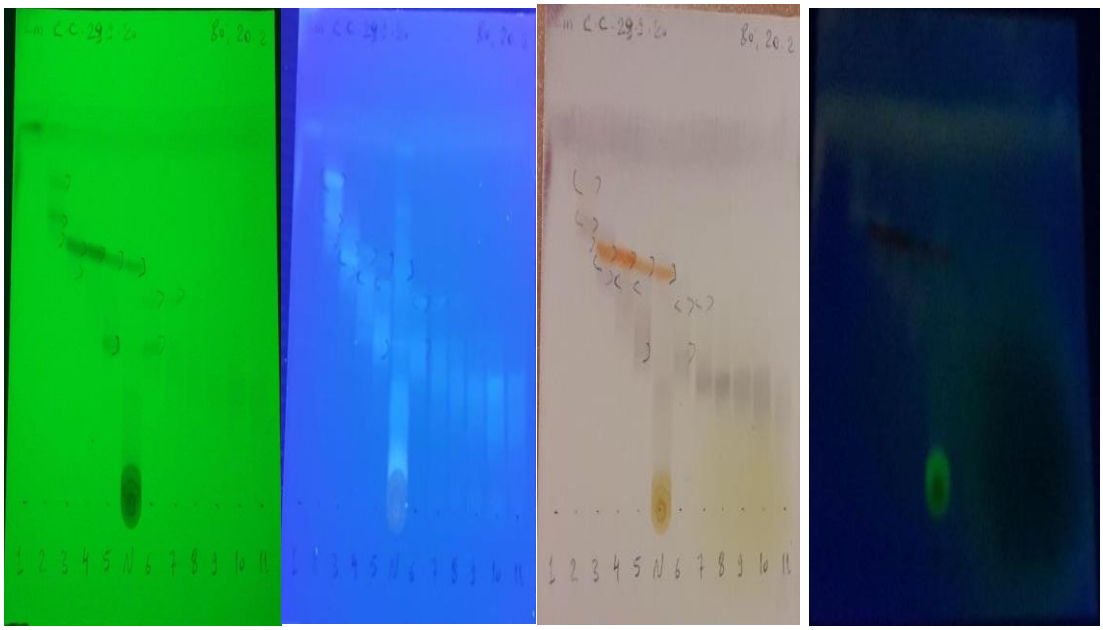
(a) (b) (c) (d)

Figure 4. 11: TLC plates of fraction 21-31 for SGC (141.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (19-24: 11.6 mg, 25: 1.7 mg, 26-28: 3.4 mg)



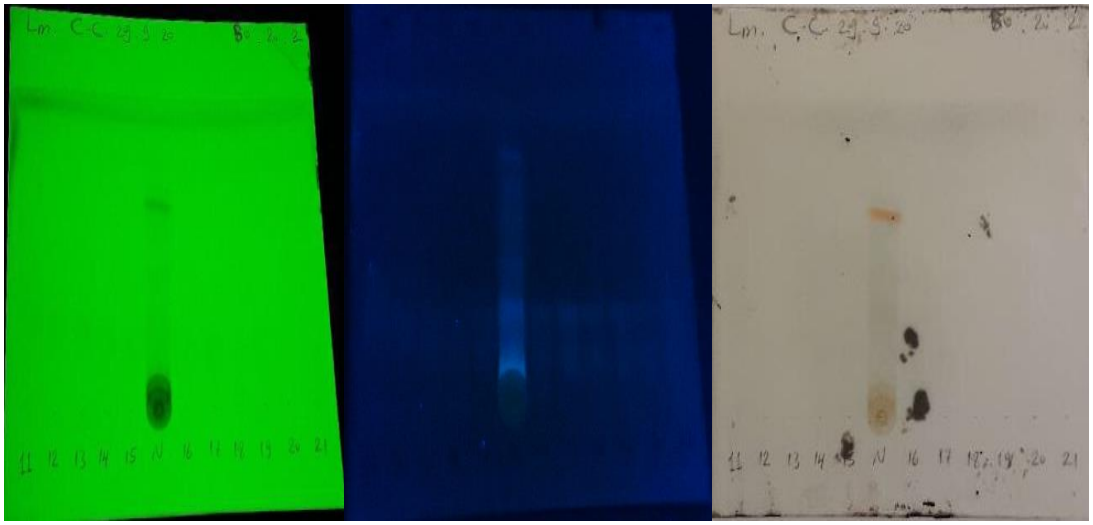
(a) (b) (c) (d)

Figure 4. 12: TLC plates of fraction 31-41 for SGC (141.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (29-35: 6.5mg, 36-44: 1.5mg)



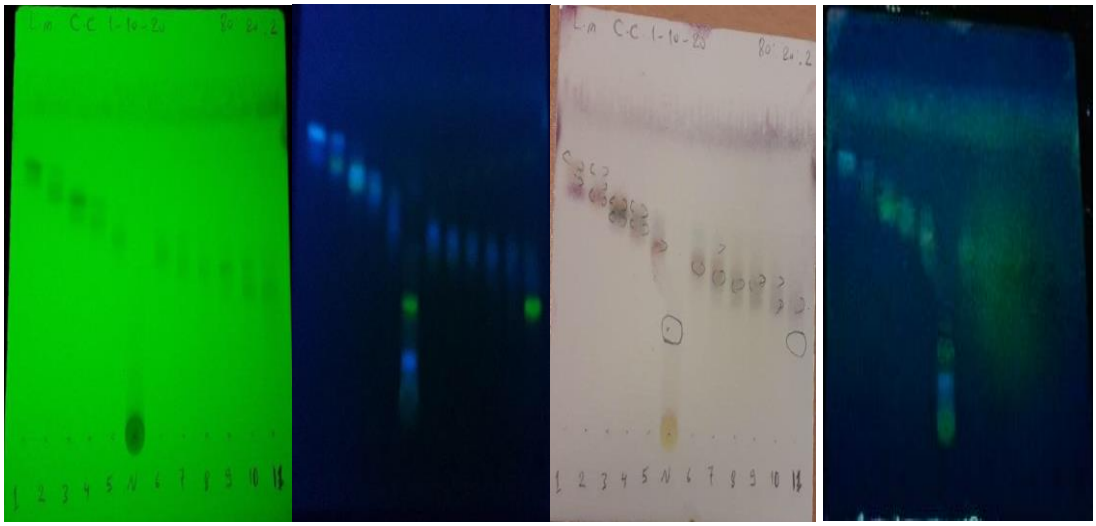
(a) (b) (c) (d)

Figure 4. 13: TLC plates of fraction 1-11 for SGC (72.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (2: 0.5mg, 3-5: 5.3mg, 6-7:2.1mg, 8-11: 20.3mg)



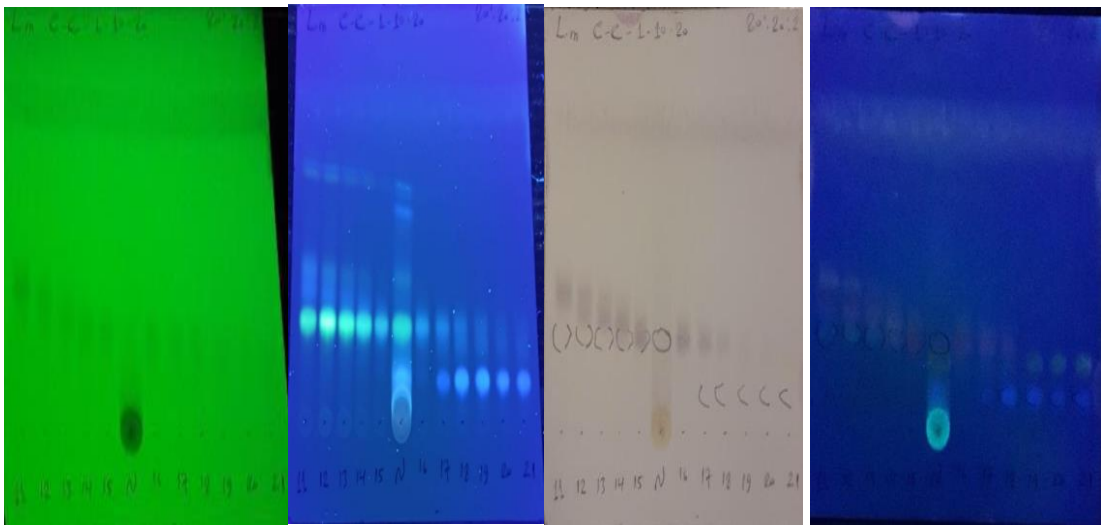
(a) (b) (c)

Figure 4. 14: TLC plates of fraction 11-21 for SGC (72.9 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) under 366nm after spraying and heating. (11-21: 5.8 mg)



(a) (b) (c) (d)

Figure 4. 15: TLC plates of fraction 1-11 for SGC (139 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (1-2: 3.7mg, 3-5: 9mg, 6-7: 2.5mg, 8-10: 7.8mg)



(a) (b) (c) (d)

Figure 4. 16: TLC plates of fraction 11-21 for SGC (139 mg dry application) (a) under 254nm, (b) under 366nm before spraying, (c) daylight after spraying, (d) under 366nm after spraying and heating. (11-14: 10.4mg, 15: 3.9mg, 16-18: 8.9mg)

4.6.1 Nuclear Magnetic Resonance (NMR)

NMR measurements were done after the fractionation and dry freezing of the plants extracts and the best spots were selected from each chromatographic technique as shown in Table 4.5 to determine the structures of isolated compounds.

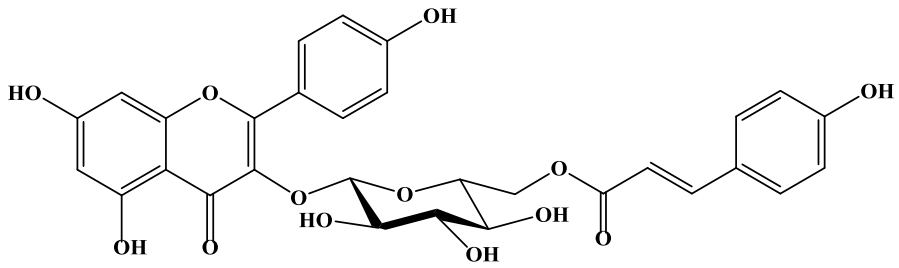
Table 4. 5: Samples for NMR Measurements

Name of Column	Number of Fraction	Whole Quantity (mg)	Used Solvent	Quantity for NMR (mg)
SGC (Sephadex Gel)	LM I 54-55	60.7	DMSO- d_6	9.8
SGC (Sephadex Gel)	LM II 61-66	77.3	DMSO- d_6	9.1
Silica Gel	LM III 12	18	DMSO- d_6	7.3
Silica Gel	LM IV 26-28	3.4	DMSO- d_6	2.2
Silica Gel	LM V 8-11	20.3	DMSO- d_6	7.6

The ^{1}H-NMR spectra of the compounds isolated (Table 4.5) showed that only two compounds, LM-2 and LM-4 were pure for further analysis. Additional NMR measurements (1D-NMR: ^{13}C -NMR and DEPT-135; 2D-NMR: COSY, HSQC and HMBC) for LM-2 and LM-4 helped us to determine the structures.

High resolution mass spectrometry (HRMS) was used in analyzing the unknown isolated compounds. As a strong instrument for the analysis and quantification of compounds, the determination of elemental compositions, and the identification of unknowns, HRMS combines high resolution with high mass accuracy. The structure of LM-2 is shown in Figure 4.17. Figure 4.18 and 4.19 shows the positive and negative HRMS of LM-2 respectively.

LM-2: Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside (Tiliroside)



Chemical Formula: $C_{30}H_{26}O_{13}$

Exact Mass: 594,1373

Figure 4. 17: Structure of LM-2

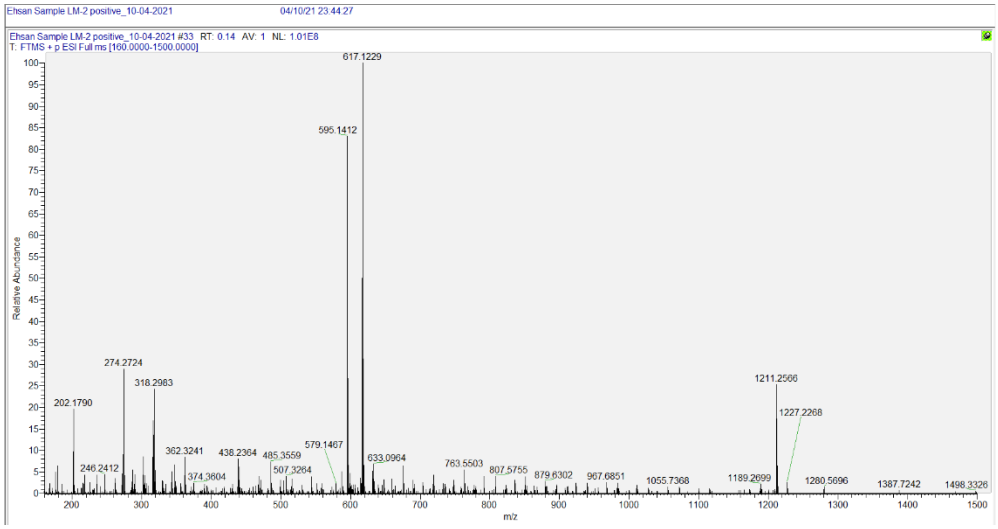


Figure 4. 18:The Positive ion HRMS of LM-2.

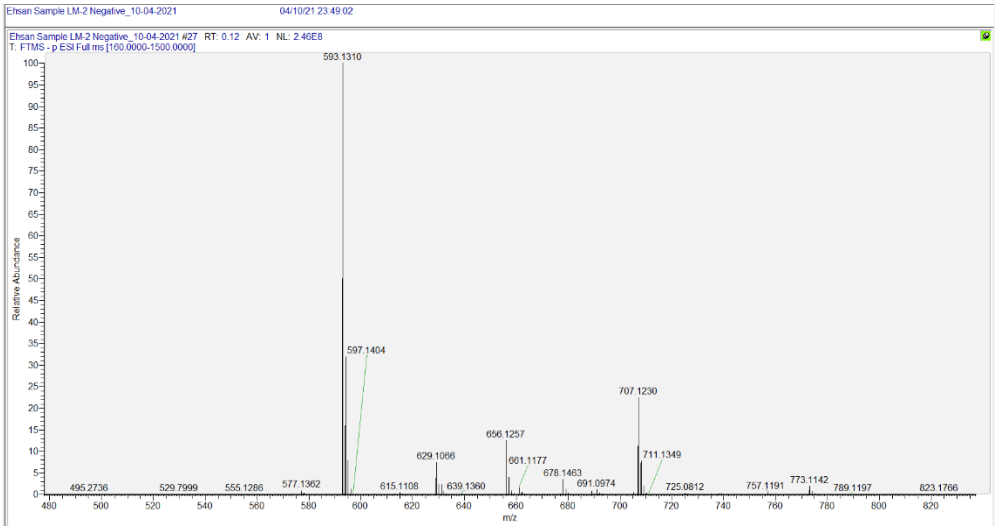


Figure 4. 19:The Negative ion HRMS of LM-2.

Table 4. 6: HRMS and Calculated molecular weight of LM-2

LM-2: $C_{30}H_{26}O_{13}$; Calculated Mol. Wt.: 594.1373	
Unsaturation degree:	**18**
Calculated	**Found**
$[M+H]^+$ Exact Mass: **595,1452** (calc.)	m/z 595,1412
$[M+Na]^+$ Exact Mass: **617,1271** (calc.)	m/z 617,1229
$[M-Na]^-$ Exact Mass: **593,1295** (calc.)	m/z 593,1310

According to the NMR and HRMS results, it was observed that LM-2 is a flavonol glycosides named Tiliroside and it can be concluded that there exist a monoglycosidic structure. This is evident in the calculated molecular weights of the both the HRMS of LM-2 as shown in Table 4.6.

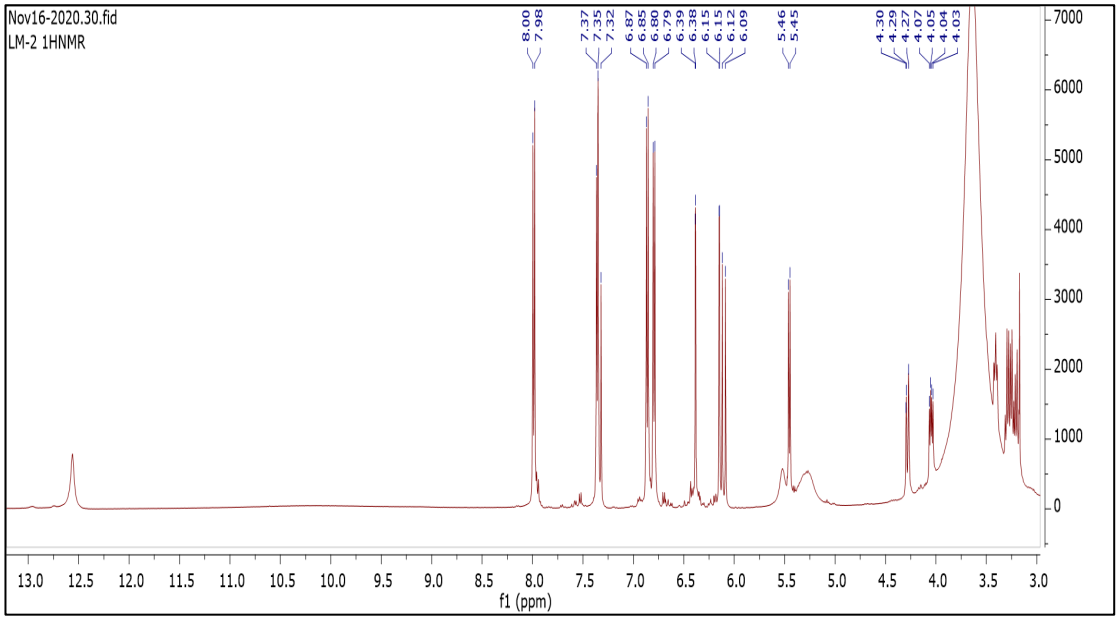


Figure 4. 20: ^{1}H -NMR Spectrum of LM-2 (DMSO-d_6 , 500 MHz)

As it can be seen from the spectrum (Fig. 4.20; Table 4.7), the ^{1}H NMR spectrum revealed the signals arising from the aromatic moieties and one sugar unit.

The signals at δ_H 6.15 (1H; *J* = 2.0 Hz, H-8), δ_H 6.39 (1H; *J* = 2.0 Hz, H-6), δ_H 7.99 (2H; *J* = 8.8 Hz, H-2' and 6'), and δ_H 6.89 (2H, *J* = 8.8 Hz, H-3' and 5') were consistent for a flavonoid structure as aglycone belonging to kaempferol moiety. Additional aromatic signals showed the presence of a hexose and a *p*-coumaroyl units (Fig. 4.21).

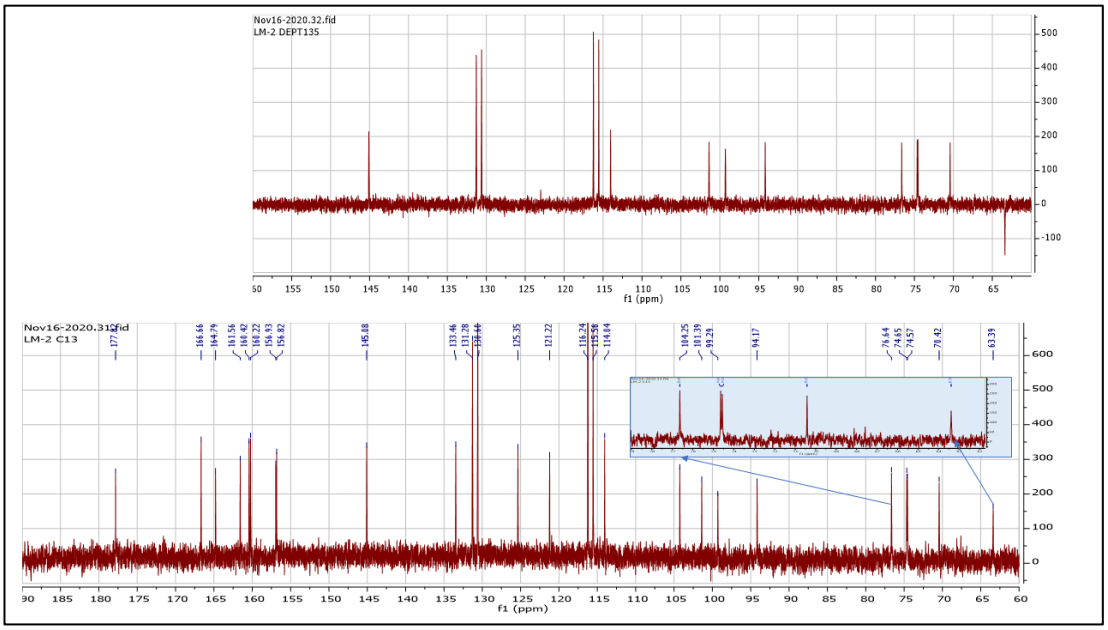
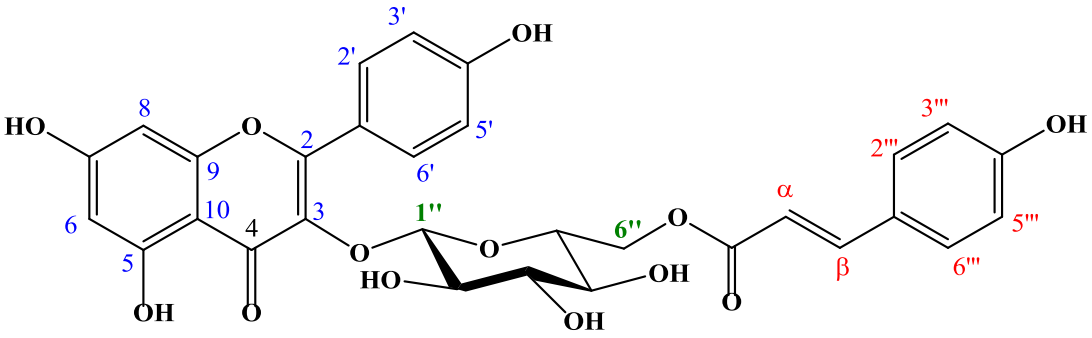


Figure 4. 21: ^{13}C-NMR and DEPT-135 Spectra of LM-2 (DMSO-d_6, 125 MHz)

The ^{13}C-NMR Spectrum of LM-2 revealed 26 carbon signals of which four were doubled (30 carbon). The chemical shift values of the signals with double intensity indicated the presence of a 4'-*O*-substituted flavonol skeleton and a *p*-coumaroyl unit. The lack of the H-3 signal of the flavonoid structure together with *trans*-olefinic protons at δ 6.10 and 7.34 (each 1H; both d, J_{AX} = 15.9 Hz, H-α and H-β, resp.) confirmed this proposal.

The presence of only one anomeric proton at δ 6.10 (^{1}H; d, J = 15.9 Hz, H-1") in the ^{1}H-NMR spectrum indicated the presence of a monoglycosidic structure. These observations were indicative for a monoglycosidic kaempferol esterified by a *p*-coumaric acid. All proton and carbon resonances were assigned using 2D-NMR (COSY and HSQC) (Figs. 4.22 and 4.23).



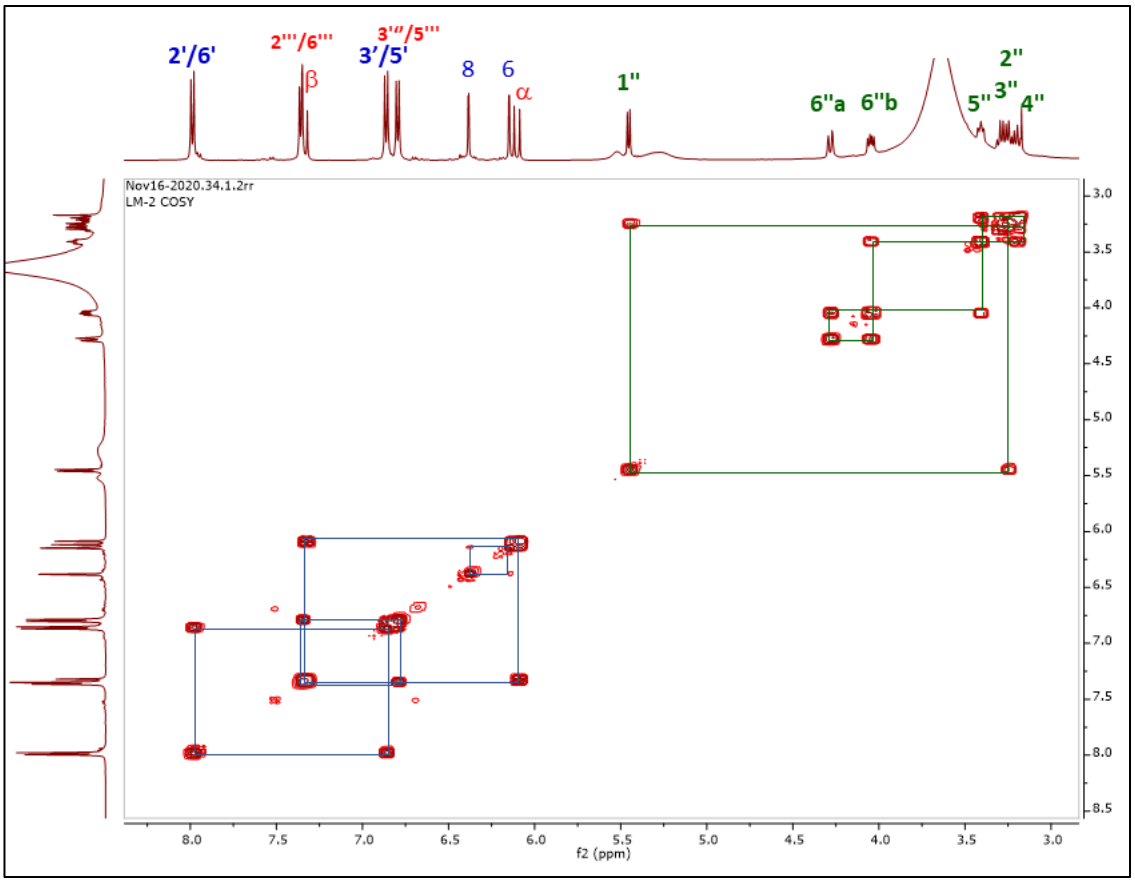
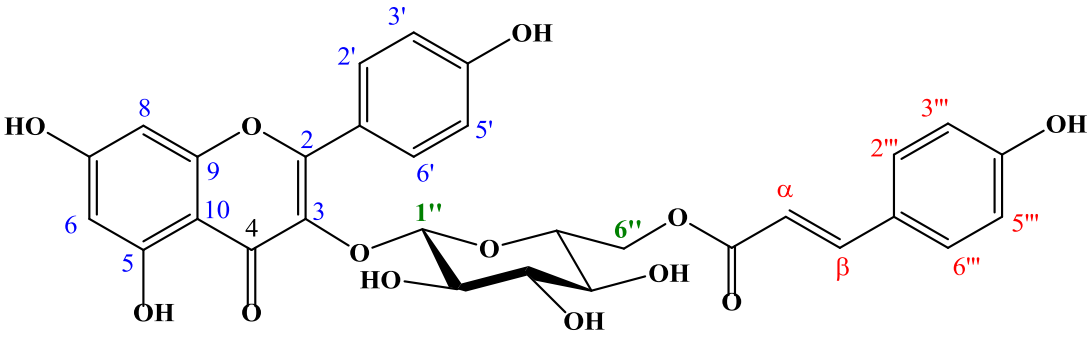


Figure 4. 22: COSY (^{1}H , ^{1}H- Homonuclear Correlated SpectroscopY) of LM-2



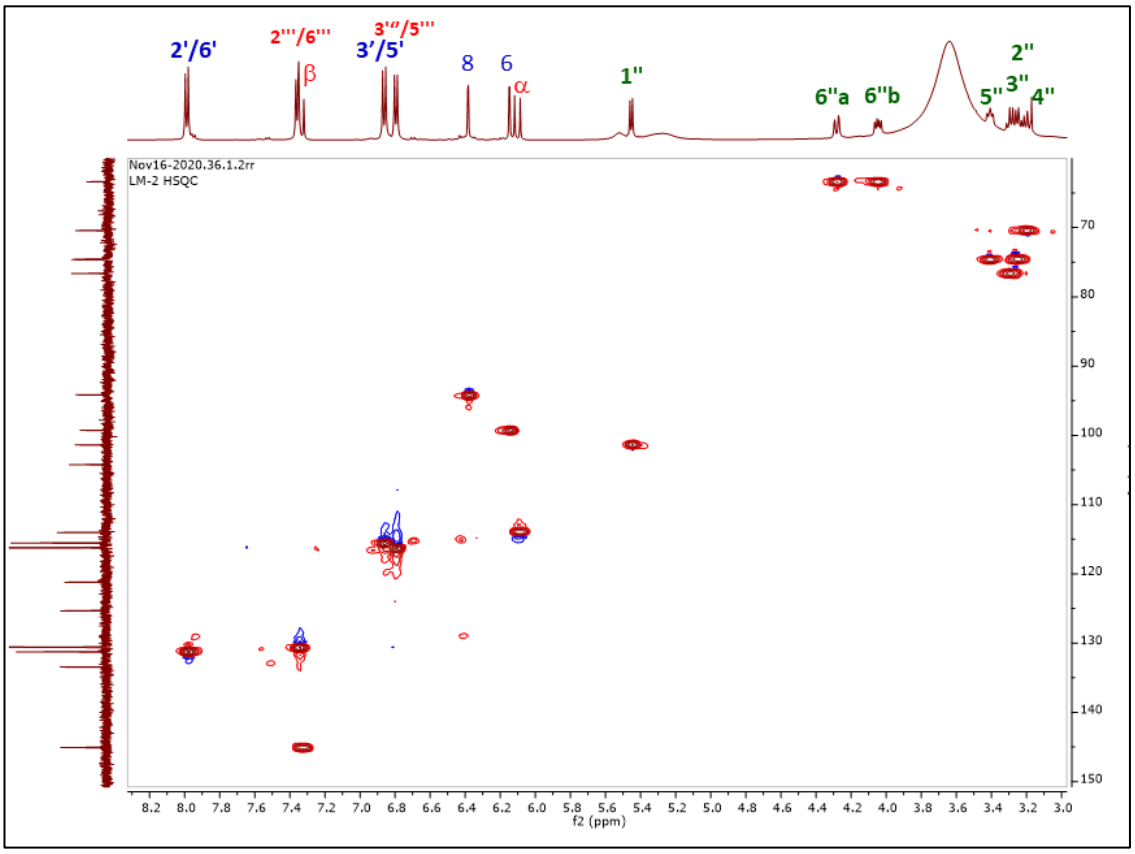


Figure 4. 23: HSQC (^{1}H , ^{13}C-Heteronuclear Single-Quantum Coherence) of LM-2

Apart from the anomeric proton signal, the proton resonances in the same spin system and the corresponding carbon resonances determined by the help of HSQC experiment determined the sugar unit as a β-D-glucopyranose (Table 4.6, Figures 4.22 and 4.23).

Finally, intermolecular connectivities were established by the help of HMBC experiment (Figs. 4.24 and 4.25). The carbon signal assigned as H-3 of the kaempferol moiety at δ 133.46 showed the long-range correlation to the anomeric proton (δ 5.45, d = 7.5 Hz) of the glucose unit indicating the site of glucosidation. The coupling constant shows the trans diaxial position of the anomeric proton suggesting the β-configuration.

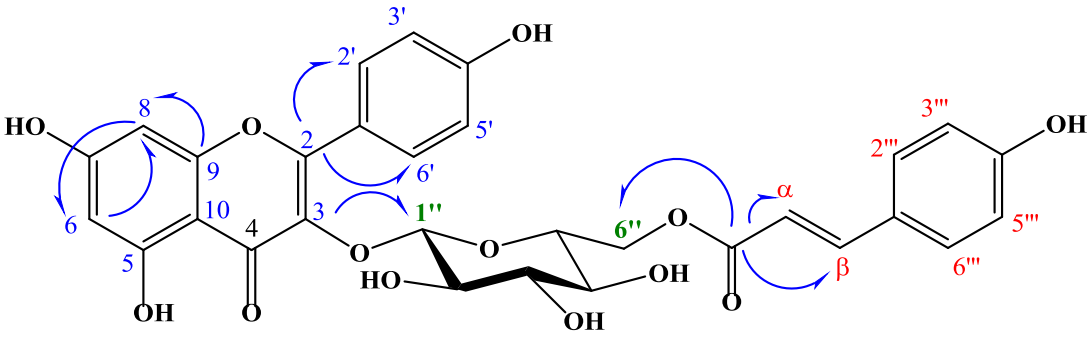


Figure 4. 24: The significant $^{1}H,^{13}C$ long-range correlations (see Fig. 4.25)

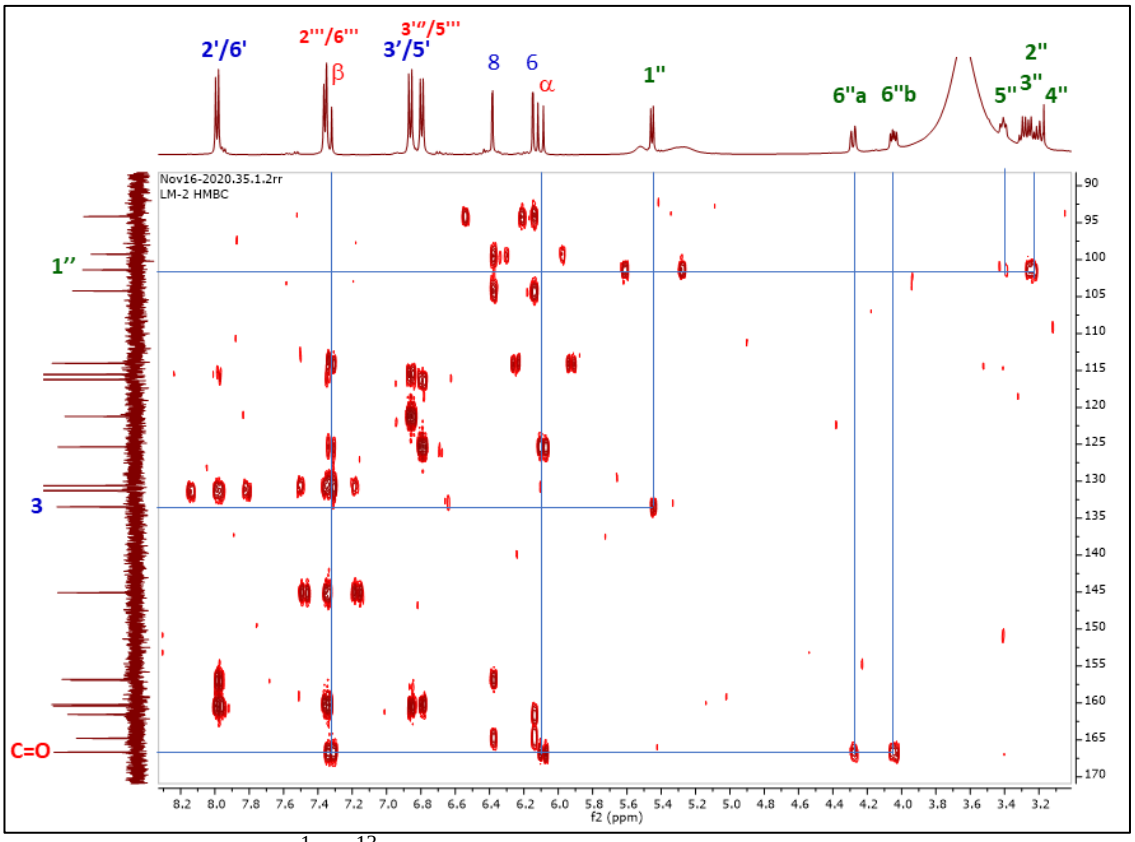


Figure 4. 25: HMBC (^{1}H , ^{13}C -Heteronuclear Multiple Bond Correlation) of LM-2.

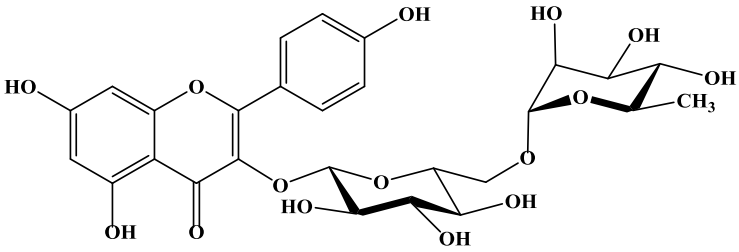
The site of esterification was determined by the long-range correlation observed between the carbonyl resonance (δ_C 166.66, C=O) of *p*-coumaroyl unit and the hydroxymethylene protons of the glucose unit (δ_H 4.05 dd and 4.28 brd; H_2-6"). Moreover, carbonyl resonance showed also long-range correlations with the olefinic protons at δ 6.10 and 7.34 (each 1H and d, J_{AX} = 15.9 Hz; H-α and H-β, resp.) (Figs. 4.24 and 4.25). These results showed the structure of LM-2 as Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside. The spectroscopic data was also in good agreement with those reported for tiliroside (Luhata & Luhata, 2017). For the characterization of LM-4, the structure of LM-4 is shown in Figure 4.26 along with the positive and negative HRMS as seen in Figure 4.27 and 4.28 respectively.

Table 4. 7: The ^{1}H- and ^{13}C-NMR data of Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside (**LM-2**) and ^{13}C, ^{1}H-Heteronuclear long-range correlations (HMBC) (^{1}H: 500 MHz, ^{13}C: 125 MHz, DMSO-d_6)

C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
Kaempferol 2	C	156.93	-	H-2', H-6'
3	C	133.46	-	H-1"
4	C	177.82	-	
5	C	161.56	-	H-6
6	CH	99.29	6.15 d (2.0)	H-8
7	C	164.79	-	H-6, H-8
8	CH	94.17	6.39 d (2.0)	H-6
9	C	156.82	-	H-8
10	C	104.25	-	H-6, H-8
1'	C	121.22	-	H-3', H-5'
2'/6'	CH	131.28	7.99 d (8.8)	
3'/5′	CH	115.56	6.86 d (8.8)	
4′	C	160.42	-	H-2', H-6'
5-OH	-	-	12.57 s	
Glucose 1"	CH	101.39	5.45 d (7.5)	H-2"
2"	CH	74.57	3.25 dd (7.5/9.0)	H-3"
3"	CH	76.64	3.29 t (9.0)	H-2", H-4", H-5"
4"	CH	70.42	3.20 t (9.0)	H-3"
5"	CH	74.65	3.41 m	
6"	CH_2	63.39	4.05 dd (12.0/6.5) 4.28 brd (12.0)	H-4"
p-coumaroyl 1"'	C	125.35	-	H-α, H-β, H-3"', H-5"'
2"'/6"'	CH	130.60	7.36 d (8.8)	
3"'/5"'	CH	116.24	6.80 d (8.8)	
4"'	C	160.22	-	H-2"', H-6"'
α	CH	114.04	6.10 d (15.9)	H-β
β	CH	145.08	7.34 d (15.9)	H-2"', H-6"'
C=O	C	166.66	-	H-α, H-β, H-6"a, H-6"b

*The assignments of ^{1}H and ^{13}C NMR signals are based on 2D NMR experiments (COSY, HSQC, HMBC) †) *J* values are not clear due to overlapping.)

LM-4



Chemical Formula: $C_{27}H_{30}O_{15}$
Exact Mass: 594,1585

Figure 4. 26:: Structure of LM-4

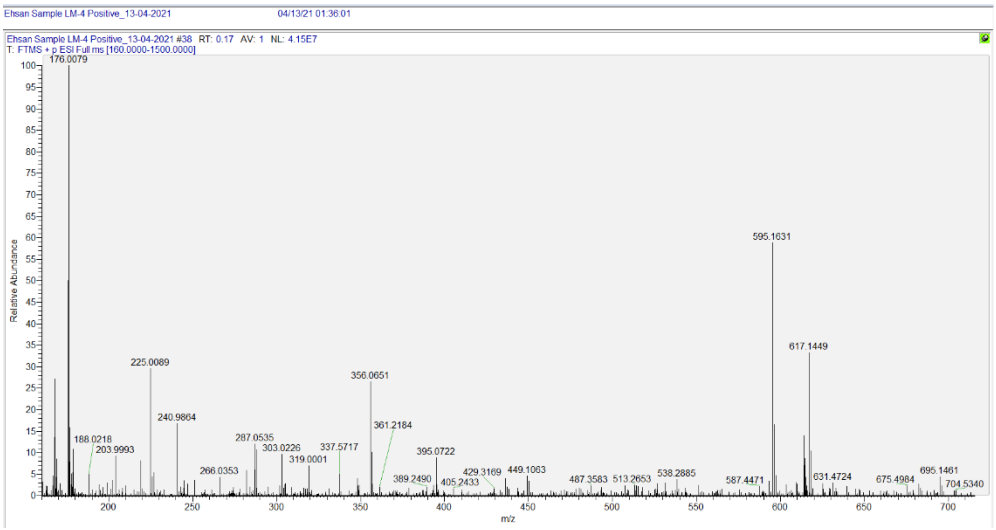


Figure 4. 27:The Positive ion HRMS of LM-4.

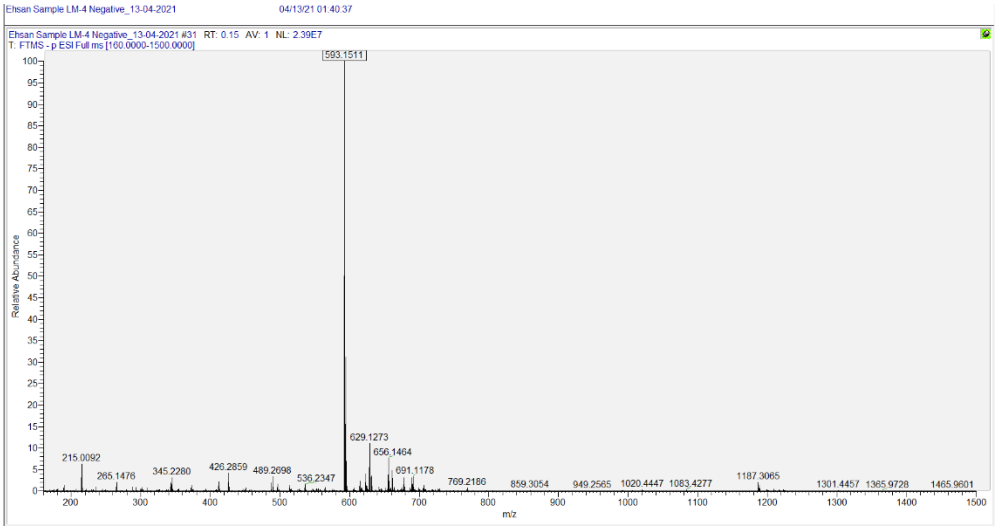
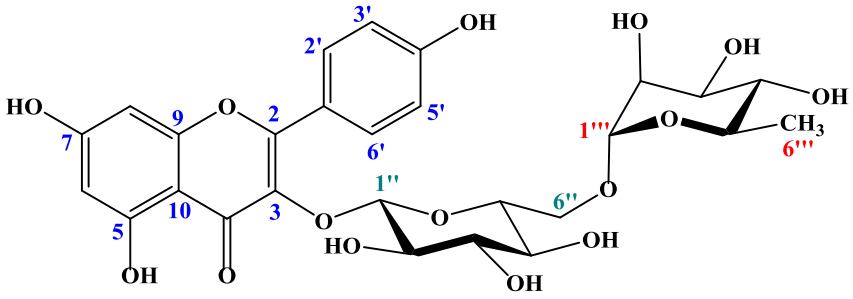


Figure 4. 28: The Negative ion HRMS of LM-4.

Table 4. 8: HRMS and Calculated molecular weight of LM-4

LM-4: $C_{27}H_{30}O_{15}$; Calculated Mol. Wt.: 594.1585	
Unsaturation degree:	**13**
Calculated	**Found**
$[M+H]^+$ Exact Mass: **595,1663** (calc.)	m/z 595,1631
$[M+Na]^+$ Exact Mass: **617,1482** (calc.)	m/z 617,1449
$[M-H]^-$ Exact Mass: **593,1506** (calc.)	m/z 593,1511

The positive- and negative-ion High Resolution Mass Spectra of LM-4 indicated same molecular weight with those of LM-2 but different unsaturation degree (UN = 13) as seen in Table 4.8.



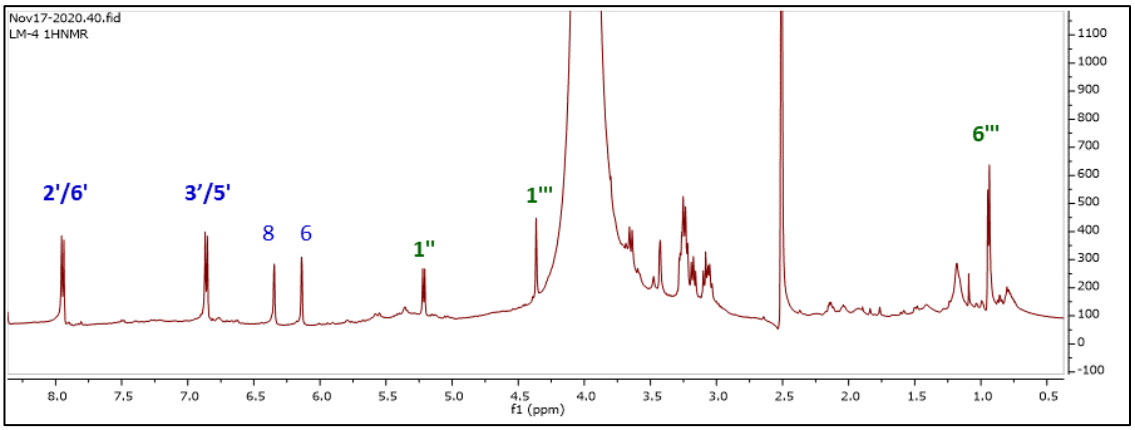


Figure 4. 29: Fig. 4.A. ^{1}H-NMR Spectrum of LM-4 (DMSO-d_6, 500 MHz)

The ^{1}H-NMR Spectrum of LM-4 showed the signals arising from a 5,7,4'-trisubstituted flavonol (kaempferol) as an aglycone such as LM-2 and two anomeric

protons (Fig. 4.29, Table 4.9). The presence of a secondary methyl resonance at δ 0.94 (3H, d, J = 6.3 Hz) suggested that one of the sugar units is a 6-deoxy-hexose (or a methylpentose). The coupling constant of the anomeric proton at δ 4.36 (1H, br s) was consistent for a α-L-rhamnopyranosyl moiety (Table 4.7). Because of insufficient amount of LM-4, the ^{13}C-NMR and DEPT-135 spectra were not informative. However, by the help of 2D-NMR experiments, it was possible to make full assignments of the proton and carbon signals of the sugar moiety. Unfortunately, most of the coupling constants could not be measured. However, the chemical shift values were in good agreement with those of rutinose (6-*O*-α-L-rhamnopyranosyl-glucopyranoside). COSY experiment (Fig. 4.30) helped to assign two spin systems arising one hexose and on methyl-pentose units. HSQC experiments (Figs. 4.31 and 4.32) showed the carbon proton correlations providing all assignments arising from the diglycosidic oligosaccharide chain. The chemical shifts assigned to sugar units proved the presence of β-D-glucopyranose and α-L-rhamnopyranose units.

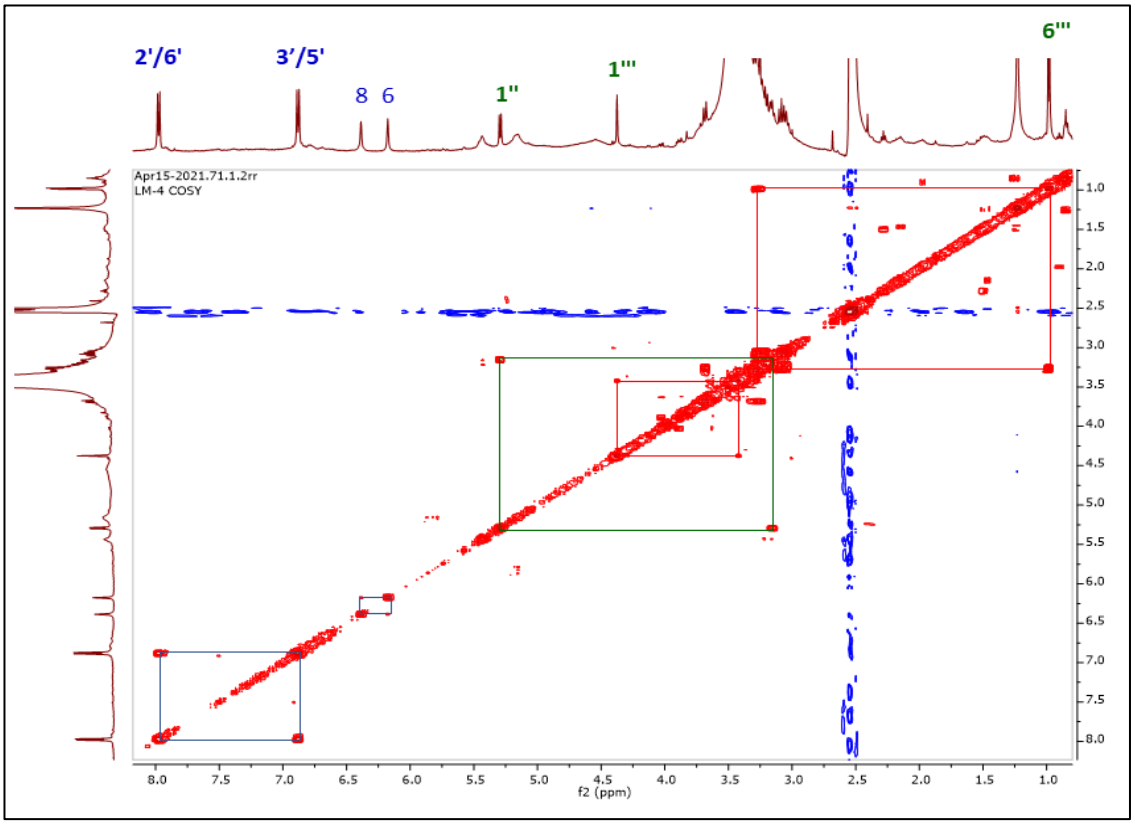
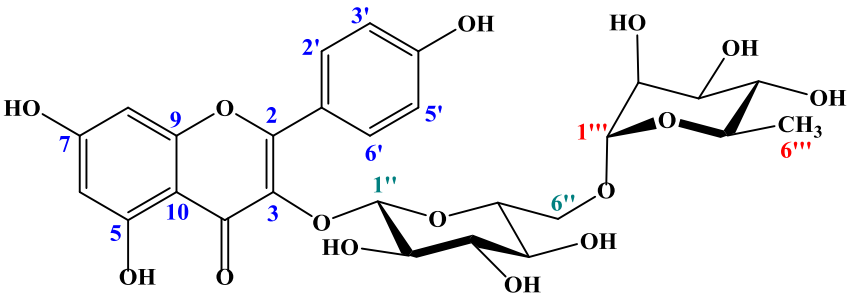


Figure 4. 30: COSY (^{1}H,^{1}H-**H**omonuclear **C**orrelated **S**pectroscop**Y**) of LM-4

Table 4. 9: The ^{1}H and ^{13}C -NMR data of Kaempferol 3- *O* -(6''- *O* -α-L-rhamnopyranosyl)- β-D-glucopyranoside (LM-4) and ^{13}C, ^{1}H -Heteronuclear long-range correlations (HMBC); ^{1}H: 500 MHz, ^{13}C : 125 MHz, DMSO-d_6.

C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
Kaempferol 2	C	no	-	
3	C	133.5	-	
4	C	no	-	
5	C	no	-	
6	CH	99.3	6.14 brs	
7	C	no	-	
8	CH	94.4	6.35 brs	
9	C	no	-	
10	C	no	-	
1'	C	121.5	-	H-3', H-5'
2′/6'	CH	131.3	7.94 d (8.8)	
3'/5′	CH	115.6	6.86 d (8.8)	
4′	C	160.2	-	H-2', H-6'
5-OH	-	-	12.50 s	
Glucose 1''	CH	101.5	5.21 d (7.6)	
2''	CH	74.7	3.15 dd (7.5/9.0)	
3''	CH	76.6	3.29 t (9.0)	
4''	CH	70.3	3.20 t (9.0)	
5''	CH	76.4	3.41 m	
6''	CH_2	67.8	3.69†, 3.25†	H-1'''
Rhamnose				
1'''	C	101.3	4.36 brs	
2'''	CH	70.7	3.42†	
3'''	CH	70.6	3.42†	
4'''	C	72.2	3.08 t (9.0)	H-2''', H-6'''
5'''	CH	69.9	3.27†	H-1''', H-6'''
6'''	C	18.4	0.94 d (6.3)	

* The assignments of ^{1}H and ^{13}C NMR signals are based on 2D NMR experiments (COSY, HSQC, HMBC) (*J* values are not clear due to overlapping.) Not observed



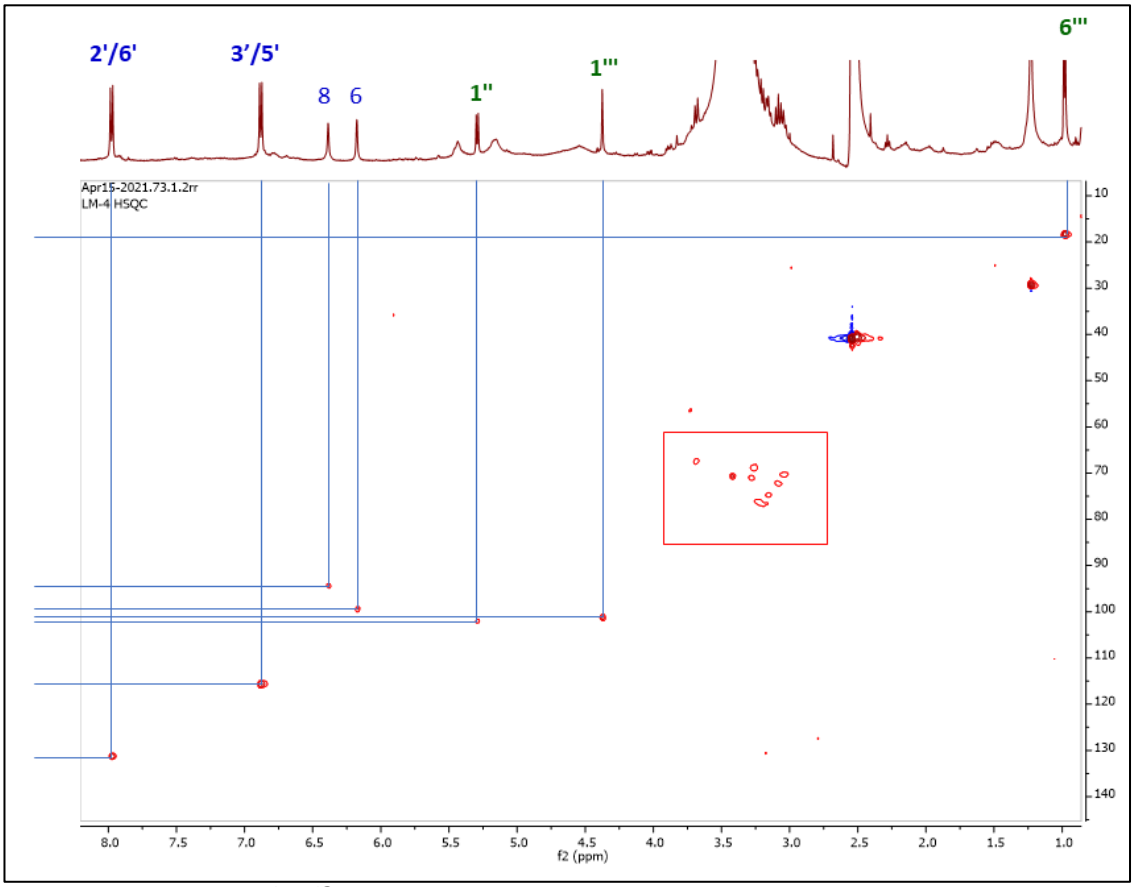
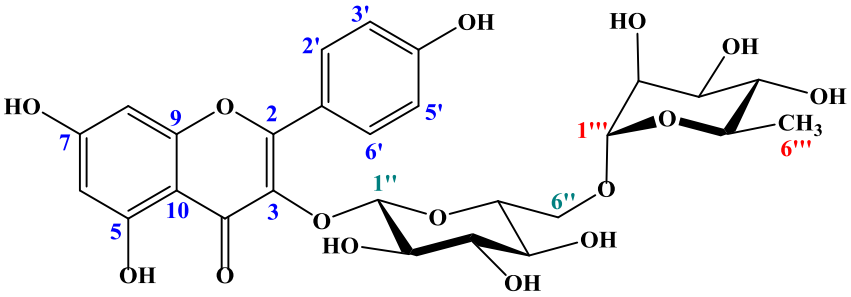


Figure 4. 31:HSQC (^{1}H,^{13}C-Heteronuclear Single-Quantum Coherence) of LM-4

The HMBC experiment showed that the rhamnose unit was found to be glycosylated at the C-6"(OH) of the glucose unit which was confirmed by the long-range correlation between C-6" (δ 67.8) and the anomeric proton of the rhamnose unit (H-1'''; δ 4.36) (Fig. 4.33). Based on these results, the structure of LM-4 was determined

as a **Nicotiflorin** which is a synonym for kaempferol 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside.



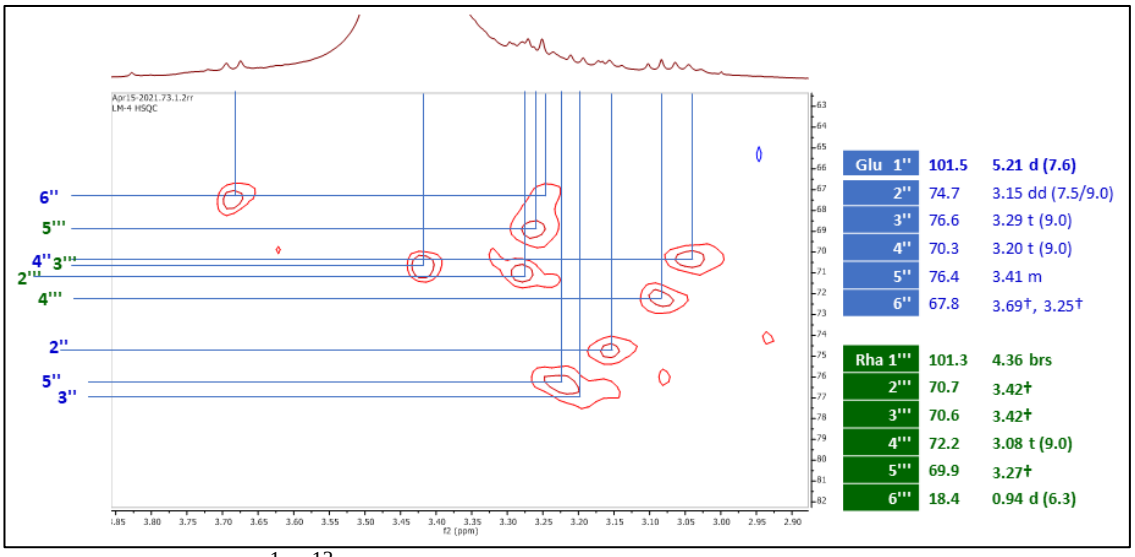


Figure 4. 32:HSQC (^{1}H,^{13}C-**H**eteronuclear **S**ingle-**Q**uantum **C**oherence) of LM-4 (δ_C: 63 – 82; δ_H: 2.90 – 3.85; Sugar signals)

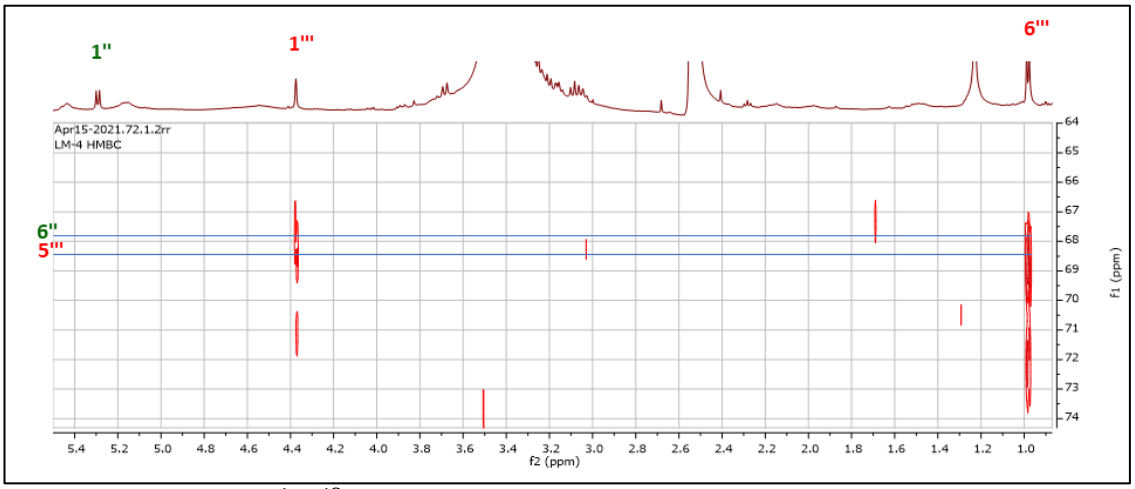


Figure 4. 33:HMBC (^{1}H,^{13}C-**H**eteronuclear **M**ultiple **B**ond **C**orrelation) of LM-4.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Around the world, the Lamiaceae family has a large number of medicinal plants that have been utilized for centuries. In addition to phytochemicals, many plants contain other chemical compounds that have a particular physiological effect on the human body. Pharmacological screens, chemical analyses, and bioactive activity testing are required for ethnomedicinal plant species reports. This study was aimed to analyse the presence of these phytochemicals from *Lamium moschantum* subsp. *micranthum* frequently used in folk medicine in TRNC. The plants extracts were collected and were prepared for preliminary phytochemical testing before being analysed for the presence of specific chemical compositions.

The plants extracts were collected and air dried before further analysis was carried out. Plants extractions were carried out at room temperature and homogenised before soaking in methanol. Both ethanolic and methanolic extraction were carried out before fractional distillation of the plant extracts with butanol before washing with dichloromethane. Preliminary phytochemical screening was also carried on the prepared TLC plates (silica gel as adsorbent mobile phase). A reddish-brown coloration confirms the presence of terpenoids. After applying the various chemical compounds on the TLC plates from the chromatographic tanks, the plates were visualized under UV light with different spectrum. The different coloration was observed under daylight, 254nm, and 366nm before and after spraying and heating. The chromatographic techniques utilized were Reverse phase vacuum Liquid, Silica Gel, and Sephadex Gel Chromatography. Using different solvent systems with dichloromethane and methanol as mobile phase, the samples were prepared for NMR measurements. The NMR data revealed the presence of ^{1}H-NMR which was good enough to predict the presence of Kaempferol 3-*O*-(6"-*O*-*p*-coumaroyl) glucopyranoside as LM-2 and kaempferol 3-*O*-(6"-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside as LM-4. As a result, the NMR data reveals the presence of two flavonol derivatives for *Lamium moschatum* subsp. *micranthum*.

Plants have been used as medicine by humans throughout history. Herbs are still used by people all over the world to ease pain and heal illnesses. Because a vast number of current pharmaceuticals are simple copies or synthetic modifications of natural chemical compounds found in plants, medicinal plants play a vital part in the creation of modern medicine. Currently, a large portion of research funding is devoted to finding and describing novel therapeutic plants. Around 80% of the world's population is entirely reliant on plants for their health and healing. In the industrialised world, surgery and pharmaceutical medication are more common, but in recent years, an increasing number of people have begun to supplement their treatment with natural supplements. Furthermore, people are becoming more interested in herbs as a result of their concerns about the adverse effects of pharmaceuticals, particularly those made from synthetic elements.

Phytochemicals are biologically active, naturally occurring chemical substances that protect plant cells from environmental risks such as pollution, stress, dehydration, UV exposure, and pathogenic attack. Phytochemicals contribute to the colour, aroma, and flavour of plants in addition to safeguarding them from illness and injury. Surprisingly, phytochemicals have been found to play a major effect in human health protection when consumed in large amounts. Preliminary phytochemical screening is an important stage in detecting the bioactive principles found in medicinal plants, which can lead to drug discovery and development. Essentially, the pharmacological screening of plant extracts will give insight into both the medicinal and harmful properties of these plants' extracts. Numerous plant species' therapeutic efficacy in ethnomedicine is determined by the presence of phytochemicals with diverse pharmacological and biological characteristics. Plants include phytochemicals, which are the active elements that make them helpful for both medical and medicinal purposes. Other species are not as helpful to scientists and the pharmaceutical industry as the Lamiaceae. Both of these can enable us to recognize therapeutic plants, while also helping us to remove activities which could damage us. In addition, phytochemicals, such as alkaloids, flavonoids, sterols, saponins, tannins, terpenoids, and glycosides, have been shown to have curative effects against pathogens, supporting their historic use in treating a wide range of ailments (Okach et al., 2013; Bouasia et al., 2021). In addition to anti-inflammatory and antioxidant

properties, flavonoids also have hepatoprotective, antithrombotic, antiviral, antidiarrheal and anti-carcinogenic properties (Umar & Sekar, 2014). *Laminum moschatum* includes two flavonoids that have been shown to be effective against several diseases, including renal pain relief and analgesics; seizures; dyspepsia; abdominal pain; joint pain; and anticancer. Flavonoids found in thymus species have been utilized in folk medicine as antibacterial, antispasmolytic, and analgesic medicines (Okach et al., 2013).

Conclusively, medical plants possess a wide range of bioactive secondary metabolites that have antimicrobial characteristics, including those against fungal and bacterium infections as well as those that reduce inflammation in the body. So, the findings of the current investigation showed that these extracts include a variety of bioactive chemicals that are responsible for their antibacterial activities. The presence of phytochemicals such as terpenoids, alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides also contributes to the hypoglycemic potential of the plant. The results of present study are helpful for the discovery of potent remedies on various diseases. Medicinal plants are main source for potent bioactive compounds.

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