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HPLC PROFILES COMPARISON OF LEAF SKIN AND LEAF GEL OF ALOE GENUS PLANT

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DECLARATION

I declare that this dissertation is the result of my own research except as cited in reference. The dissertation had not been accepted for any degree and is concurrently submitted in candidature of any degree.

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VERIFICATION

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ABSRACT

Aloe vera is a popular traditional medicinal plant around the world. Extracts from both parts of the aloe leaf gel and leaf skin were obtained by using methanol as the extract solvent. These extracts were then partitioned by water and chloroform to separate polar and non-polar compounds. Four extracts were analysed under HPLC using Sulpercosil LC-18 column (25cm x 4.6 mm I.D.). According to the analysis, the best elution programme needs to include both isocratic and linear gradient steps. Further more, while in the isocratic steps, lower flow rate, at 0.5ml/minute, showed better resolution. Based on the parameters mobile phase solvent, acetonitrile showed better result than methanol. After the profiles optimizations, the best profiles for leaf gel and leaf skin were compared. The results showed most of the compounds in leaf gel and leaf skin were the same.

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ABSTRAK

Aloe vera merupakan tumbuhan ubat traditional yang popular di antarabangsa. Ekstrak-ekstrak telah diperolehi daripada bahagian gel daun dan kulit daun dengan menggunakan metanol sebagai pengekstrak. Ekstrak-ektrak ini kemudian dipisahkan kepada sebatian-sebatian polar dan tidak polar dengan teknik pemisahan menggunakan air dan kloroform. Ke-empat-empat ekstrak ini telah dianalisa melalui kromatografi cecair pretasi tinggi (HPLC) dengan menggunakan kolum Sulpercosil LC-18 (25cm x 4.6 mm I.D.). Didapati, program elusi yang baik perlu meliputi kedua-dua langkah isokratik dan cerunan. Tambahan pula, semasa langkah isokratik, kadar alir yang lebih rendah menunjikkan keputusan yang lebih baik. Berdasarkan parameter jenis pelarut fasa gerak, acetonitrile memberi resolusi yang lebih baik daripada metanol. Selapas pengoptimuman, kramatogram-kramatogram yang didapat telah dibandingkan. Keputusan menunjukkan bahawa kandungan sebatian dalam kedua-dua bahagian daun *Aloe vera* ini mempunyai kesamaan yang tinggi.

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CONTENT

UMS
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UMS
UNIVERSITI MALAYSIA SABA

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF TABLE

UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURE

UMS
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LIST OF PHOTO

UMS
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LIST OF SYMBOL AND ABBREVIATION

ACN	Acetonitrile (solvent)
α	Separation factor
Å	Armstrong (length unit)
HPLC	High performance liquid chromatography
k'	Capacity factor
MeOH	Methanol
ml/minute	ml per minute (flow rate unit)
mV	Milli-Volt (detection unit for HPLC)
pH	Potential of hydrogen
PTFE	Polytetraflouroethylene
UV	Ultraviolet
UV-VIS	Ultraviolet and visible light
THF	Tetrahydrofuran
v/v	volume per volume (concentration unit)

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CHAPTER 1

INTRODUCTION

1.1 History of Medicinal Plant

Medicinal plant, or herb, is a plant with one or more parts of it contain active compounds that can be used for therapeutic purposes or can be the initiator for drugs synthesizing. These kinds of plants are usually used as traditional medicine by rural folks started from the ancient time (Ahmad and Raji, 1993).

The starting point of medicinal plants utilization was remained unknown. Nevertheless, it was used to treat many serious or minor illnesses or used as preservative for food. There are a few countries, especially those countries that rich of plants, that strongly believe in the use of medicinal plants in their daily routine even until now. These countries include China, Egypt, India, some Islamic countries and many more.

Malaysia is a country that rich of medicinal plants. Among 7000 angiosperm species, about 15% of it was reported as plants that have its medicinal value. This is because most of the plants that being recognized within out planet were found in tropical area. Thus, it would not be surprise if we know the fact of more than 70%

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resident in the world, especially in the third world countries, are still depend on herbs as their major medicine (Ahmad and Raji, 1993).

Aloe vera is one of the popular medicinal plants among the world. It is widely used in India, Malaysia, America, Egypt, Mexico, Taiwan and many other countries (Ross, 1999). Aloes today are mostly cultivated for decoration in Southern California and many other dry, frost-free places (Taylor, 1965).

1.2 Usage of *Aloe vera*

Aloe vera was well known as a useful medicinal plant for decades. Traditionally, it has different usage and ways of used at different places. For example, fresh leaf juice is used as a contraceptive before and after coitus in Egypt; while in Taiwan, decoction of dried leaves is taken orally to treat hepatitis. In Malaysia, hot water extract of leaf juice is taken orally as cholagogue and emmenagogue (Ross, 1999). However, internal consumption of aloe juice is not recommended especially in the condition of pregnancy, breast-feeding or menstruating (Fetrow and Avila, 2000).

Recently, *Aloe vera* becomes the subject of vigorous scientifically based investigations and begins to take place in the pharmacopoeia of modern medical practices. Gel preparations of *Aloe vera* have been reported to be effective against radiation burns, skin ulcer and peptic ulcer (Fetrow and Avila, 2000).

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1.3 Objective

The objectives of this study are as below:

i. to obtain extract from different parts of *Aloe* leaves.

ii. to do elution programme optimization of the HPLC profiles.

iii. to compare the HPLC profiles of leaf gel and leaf skin.

1.4 Scope

A plant of *Aloe* genus was chosen for the experiment and this experiment was only include leaves part. The aloes in this experiment are taken from a Syarikat Hortic Nursery Plantation at Jalan Tuaran, Kota Kinabalu, Sabah. Each leaf was separated into two parts for extraction, partition and HPLC profiles analysis. The extract was partitioned between water and chloroform for partitioning and subsequently all collected specimen was subjected to HPLC by different settings of elution programme. Finally, the HPLC profiles comparison and data analysis was made (Zonta, *et al.*, 1995).

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CHAPTER 2

LITERATURE REVIEW

2.1 Phytochemistry

Phytochemistry, or plant chemistry is a study on plant in between natural products organic chemistry and plant biochemistry. This topic deals with variety of organic substances and its chemical structure, their biosynthesis and their natural distribution. By understanding phytochemistry, we can, not only exploit the exist techniques but continuing develop new techniques to solve outstanding problems whenever as they appear (Harborne, 1984).

Nowadays, the research on phytochemistry has become more and more important, especially the research of phytochemicals determination in medicinal plants since many peoples are more concern on health. The importance of phytochemistry on medicinal plants is that the separated compound can be used as a therapeutic agent, for example morphine, an analgesic that much more stronger than aspirin; digitoxin, a cardiac stimulant; atropine and others (Ahmad and Raji, 1993; Bruice, 2001). Besides, the compounds from plants can be used as an initial material for useful drugs synthesis. One of these examples is hormone adrenal cortex, which is synthesized from sapogenin steroid that acquired from plants. The compounds from plants can

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also be the pharmacology active compound models in drugs synthesis. This is because some useful compound in plants cannot be used as the limited amount or there are side effects with it because of some functional groups bonded. By these models, we can generate the useful one by ourselves (Ahmad and Raji, 1993).

2.2 Phytochemicals in Medicinal Plants

Plant chemicals can roughly be classified as primary and secondary constituents depending on their role in plant metabolism. The primary constituents are those involving plant metabolism such as sugar, amino acid and chlorophyll; while the secondary constituents are alkaloids, terpenoids, phenolic compounds and other substances that vary from plant to plant. These constituents usually do not play essential role in plant metabolism but have a role in protecting the plant from environmental pressure or controlling plant growth (Harborne, 1999). Since there is no standard classification available, we can just roughly group some widely distributed compounds as major plant chemicals.

2.2.1 Sterol

Among sterol, cholesterol is one of the wide spread component, which found in all animal tissues. Other sterols include ergosterol and sitosterol. Sterols are important in the process of steroid-hormone synthesizing (Ahmad and Raji, 1993). The general structure of sterol is as in Figure 2.1.

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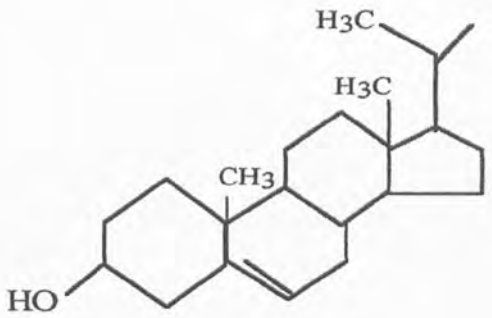

Figure 2.1 General structure of sterol

2.2.2 Saponin

Saponin can be separated into two groups, which are steroid saponin and triterpanoid saponin. Saponin have a few special properties to be recognized such as it can form a quite thick layer of bubbles and have the ability to hemolysis red blood cells. Saponin will produces sapogenin and sugar as by-product when it is being hydrolyzed. The examples of saponins are diosgenin, hekogenin and cholegenin. Saponin is an important compound as it can be changed into cortisone, estrogen and others. The best example is *Dioscrorea sp.* that provides diosgenin, which is an important source to produce steroid hormone (Ahmad and Raji, 1993). The general structure of saponin is as in Figure 2.2.

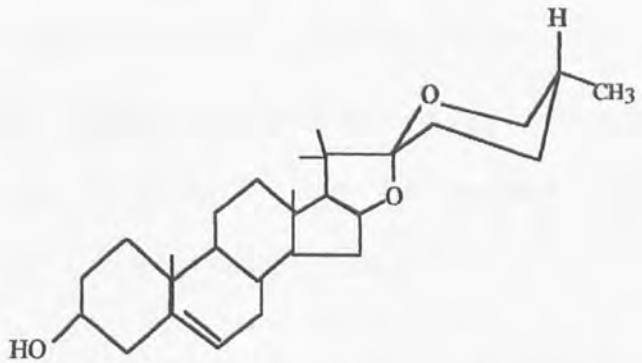

Figure 2.2 General structure of saponin

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2.2.3 Tannin

Tannin is a big phenolic compound with high molecule weight. It is an important agent for cytotoxin and anti-neoplastic (Ahmad and Raji, 1993). Traditionally, tannins are used as an agent to convert animal hides to leather. This is because of the tannin's ability to interact with and precipitate proteins, include animal's protein (Hagerman, 2002). There are generally two types of tannin: condensed tannin (catechol tannin) and pyrogallol tannin (Ahmad and Raji, 1993).

a. Catechol tannin

This type of tannin cannot be hydrolyzed, it is usually formed by a few groups of flavonoids for example flavon-3-ol and flavon-3,4-diol. This tannin only has phenolic groups, which condensed between each other to form huge molecule. (Ahmad and Raji, 1993; Hagerman, 2002).

b. Pyrogallol tannin

Pyrogallol tannin can be hydrolyzed by heated with diluted acid to form phenolic compound. This kind of tannin is generally an ester formed by gallic acid and glucose such as galotannin. However, it may also contain phenolic acid as in heksahydroxydiphenic (Ahmad and Raji, 1993).

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2.2.4 Flavonoid

Flavonoids are an ever-present group of big polyphenolic substances, which are present in most plants, concentrating in fruit, fruit skin or peel, bark, and flowers (Miller, 1996). Flavonoids are available in different colour but most of it are yellow. Differences between the structures of flavonoids are caused by different stage of oxidation on pyran ring, the appearance of various hydroxyl groups and its amount at both sides of the benzene ring (Ahmad and Raji, 1993). The general structure of flavonoid is as in Figure 2.3.

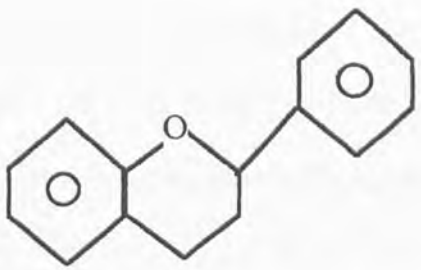

Figure 2.3 General structure of flavonoid

a. Antosianin

Antosianin is normally available in flowers as it gives red colour, blue colour or the mixture of both of it. The different colours of antosianin compounds are influence by its pH value that caused by the location and amount of the hydroxyl groups. Antosianin can dissolve in water and posses maximum absorbance between 475-560nm (Ahmad and Raji, 1993).

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b. Flavonol

Flavonol appear as a co-pigment with antosianin in flowers and leaves. Besides free compound, flavonol also can appear as glycosides for example rutine. Flavonol is colourless and has the maximum absorbance around 350-390nm (Ahmad and Raji, 1993).

c. Flavon

Flavon has close relationship with flavonol. Their only difference is the unavailable of hydroxyl group at the third position of pyran ring. The maximum absorbance for flavon is lower than flavonol, which is about 330-350nm. The most common flavon are apigenin compound and luteoline compound (Ahmad and Raji, 1993).

d. Isoflavon

Isoflavon is a colourless constituent with the maximum absorbance at 255-265nm. It is usually found in plant from Leguminosea family. Isoflavon is quite important as it has evident pharmacology effect. This is because its structure is quite similar with steroid. Hence, it has similar properties with steroid or the ability to inactivate steroid. Examples of isoflavon include genistein and diethylstilbestrol (Ahmad and Raji, 1993).

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2.2.5 Alkaloid

Alkaloids are basic compound with nitrogen, generally in the form of heterocyclic. It is produces by certain plants and animals and gives or posses very clear physiology activities on human and animals. Alkaloids may contain one or more nitrogen atom especially in cyclic form. However, alkaloids are not stable in high temperature or sunlight exposure. It will decompose at about 70°C. Most of the alkaloids show pharmaceutical activities even in low concentration. Classification for alkaloids is quite difficult, as it involves many different kinds of bonding and structure. Table 2.1 shows the explanation for some of the alkaloids (Harborne, 1999).

Table 2.1 Description of alkaloids in plants

Alkaloid Compound	Description
Amaryllidaceae	275 structures, restricted to daffodil family
Betalain	Yellow or purple pigments of the Centropermae
Diterpenoid	Mainly in the Ranunculaceae; often poisonous
Indole	Many structures; in Apocynaceae, Loganiaceae
Isoquinoline	Largest group of alkaloids, widely distribute
Lycopodium	150 structures, restricted to club mosses
Monoterpene	Related and co-occurring with iridoids
Peptide	130 structures, especially in the Rhamnaceae
Quinoline	Common in Rutaceae
Steroidal	In Apocynaceae, Buxaceae, Liliaceae and Solanaceae
Tropane	Mainly found in Solanaceae

Source: Harbone, 1999

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