DETERMINATION OF QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL PROPERTIES OF *Salix tetrasperma* Roxb METHANOLIC LEAVES EXTRACT

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LIST OF SYMBOL

°C	Degree Celsius
%	Percentage
g	Gram
mL	Millilitre
m	Metre
ppm	Part per million

LIST OF ABBREVATIONS

$FeCl_3$	Ferum Chloride
KOH	Pottassium Hydroxide
HCL	Hydrochloric Acid
NH_4OH	Ammonium Hydroxide
Na_2CO_3	Sodium Carbonate
AOAC	Association of Analytical Communities

ABSTRAK

Salix tetrasperma adalah pokok "willow" atau dikenali sebagai dedalu yang biasanya dijumpai di kawasan berair dan paya di Malaysia. Pokok ini telah digunakan untuk tujuan perubatan bagi mengubati hepatitis, tahan sakit, sakit-sakit, demam, batuk, gigitan serangga, sengatan kala jengking, bengkak, ketuat, antirheumatik, anti bakteria, sedatif, anti diabetik dan anti-inflammatori. Tujuan kajian ini adalah untuk mengesan dan menetukan peratusan fitokimia yang terdapat dalam *Salix tetrasperma* melalui ekstrak methanol. Terdapat empat sebatian fitokimia yang penting dalam *Salix tetrasperma* iaitu alkaloid, tannin, saponin dan flavonoid. Hasil kajian mendapati kumpulan semulajadi yang dikesan melalui analisis kualitatif ialah tannin, saponin dan flavonoid manakala alkaloid tidak dapat dikesan. Manakala, jumlah kumpulan semulajadi melalui analisis kuantitatif dalam flavonoid, saponin, tannin dan alkaloid adalah 3.86%, 37.99%, 6.15% dan 0.48% mengikut turutan. Tannin mempunyai peratusan yang paling tinggi menunjukkan tannin ialah fitokimia utama dalam *Salix tetrasperma* manakala alkaloid mempunyai peratusan yang rendah berkemungkinan semasa ujian kualitatif perubahan warna tidak daptat dilihat kerana terlalu sedikit atau terdapat kesalahan semasa menjalankan ujian. Kumpulan semulajadi tersebut merupakan perkara yang penting dalam menentukan mekanisma yang terlibat bagi kesan farmakologi. Oleh itu, kepentingan *Salix tetrasperma* ini dalam perubatan tradisional boleh dikaitkan seperti anti kanser, anti diabetik, anti bakteria, tahan sakit dan sebagainya melalui kehadiran kumpulan semulajadi tersebut.

ABSTRACT

Salix tetrasperma is a willow tree or also known as dedalu commonly found in swampy and moist area in Malaysia. It was used for many medicinal purposes as alternative medicine such as for hepatits, analgesic, aches, fevers, whooping cough, bug bites, scorpion sting, sore, warts, antirheumatic sedative, antibacterial, antidiabetic and anti-inflammatory. The aim of this study was to screen and determine the amount of total phytochemical groups in *Salix tetrasperma* to produce methanol extraction. There are four important natural groups in plant namely alkaloids, tannins, saponins and flavonoids. The result of this study shows that from the qualitative analysis the phytochemical group presences are tannins, flavonoids and saponins while alkaloid was absence. While in quantitative analysis shows that the total amount of natural groups of flavonoid, saponins, tannins and alkaloid were 3.86%, 37.99%, 6.15% and 0.48% respectively. The amount of tannin is the highest among the phytochemical shows that the main phytochemical of *Salix tetrasperma* is tannin while the alkaloid can be measured in quantitative analysis may due to error during the test or the changes of color in qualitative is not seen because the amount is low. The natural groups detected in the test are important to determine the mechanism of pharmacological effect. So, the significance of the *Salix tetrasperma* in traditional medicine such as to treat cancer, diabetic, bacterial infection, painkiller, and so on can be related with the presence of these natural groups.

CHAPTER 1 INTRODUCTION

1.1 Identification of Problem

Basic health care is important for people in most developing countries and some of them facilities provided is lacking especially in the rural area. According to World Health Organisation (WHO) more than half of world's population does not have access to enough health care service which is may due to poor people does not have access or unaffordable for them to get heath care service (Batugal *et al.,* 2004). So, to overcome this problem they seek alternative remedies which are medicinal plants that offer huge opportunities. Medicinal plants can solve their access to medicine and it is also affordable medicine to poor people as well as some of them can generate income. Many traditional herbs and plant parts have been shown to have medicinal value especially in rural areas and these plants can be used to cure or alleviate several human diseases (Nina *et al.,* 2012). The use of plants for medicinal products have been practices since past century and in Malaysia is rich with plant biodiversity which open the opportunities to find new drug discovery by investigating the bioactive agents of plants that already used for traditional or alternative medicine. *Salix tetrasperma* is a semi parasitic plant which feed on the host tree has known medicinal uses and this plant considers as an unwanted plant to economically important medicinal plant Therefore, in this project an experiment will be done to evaluate the phytochemical properties of *Salix tetrasperma* to know the bioactive agents.

1.2 *Salix tetrasperma* Roxb

Salix tetrasperma Roxburgh commonly called as Indian Willow or dedalu. It is a medium sized of wet and swampy places and it shedding the leaves at the end of monsoon season. *Salix tetrasperma* belong to family Salicaceae. It deciduous and have size from small to medium sized tree. It is growing on wet and swampy areas while the bark is greyish brown or blackish. It has lax-flowered male and female catkins and glaborous with many seeded capsule. This plant has many medicinal values and produces a good quality timber and tannin is useful in wood and tannin industries. This plant has soft wooded type of stem with broad shaped plant and the leaf shape is lanceolate (Figure 1.1). The leaf arrangement is alternate with green coloured leaf. The plant can reach up to 20 m tall ant the actual height can reach maximum of 24 m. This plant is fast growing and most likely on Forest Plant or Hill Side Plant. The fruit on summer season is classified into Capsule, Dehiscent Fruit, Dry Fruit and Simple Fruit and the fruit habit is solitary plus with many seeds. On spring the flower type is dioeciously and has cluster flower characteristics. It can be found usually on hill slopes, moist place and water courses mostly in India, mainland Asia and Malaysia.

1.3. Scientific Classification of *Salix tetrasperma*

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliate

Order : Salicales

Family : Salicaceae

Genus : *Salix*

Species : *tetrasperma*

1.4. Study Objective

The objective of this study is to :

1. To know the yield of the extracted *Salix tetrasperma* per gram of powder used.

2 To extract the leaves of *Salix tetrasperma* using methanol extraction.

3 To examine the qualitative phytochemical properties of *Salix tetrasperma.*

4 To determine the quantitative phytochemical properties of *Salix tetrasperma.*

Figure 1.1 : *Salix tetrasperma* on a plant

CHAPTER 2 LITERITURE REVIEW

2.1 Phytochemical in Medicinal Herb

The usage of plant for medicinal purpose has been practiced by human is as old as the history of mankind and with the increase population of people plus with many disease have been encountered everyday become more common and it has cause for increase mortality (Saiful *et al.,* 2011). The number of plant species that have been reported to be used by human in different culture and practice around the world for medicinal purpose are approximately 35,000 species (Lewington, 1993) but the number could be higher as knowledge on the various uses of plants will always be discovered and passes orally from one generation to another plus some of them are not documented (Jantan, 2004). In Malay Peninsula, there is not less than 1300 plant have been used in traditional medicine as recorded by (Burkill, 1966).

The active compound substances called phytochemicals is responsible for the medicinal value of these plants that can cause physiological action on human body (Manjamalai *et al.*, 2010). Phytochemical screening of plants acts as the initial steps for selection of plant in further phytochemical studies and this tests are simple, cheap, sensitive, selective and rapid chemical test to discover the presence of chemical compounds. In addition, the discovery of new drug is beginning with the identification of bioactive compound in a plant (Jantan, 2004). Alkaloids, tannins, flavonoids and phenolic compounds are the most important bioactive constituent of plants (Edeoga *et al.*, 2005).

From previous study on the phytochemical of this plant there are various types of sapogenins such as quinovic acid, salicortin, saligenin, phenolic glycosides and pyrocatechol was isolated from the barks and leaves (Maliya and Singh, 1997). The

plant also has been reported to have tannins, triterpines, viz. β-amyrin, lupeol, chalcinasterol, steroids, viz. β-sitosterol and stigmasterol (Saiful *et al.,* 2011). The acetone and ethanol bark extract of *Salix petiolaris* Sm. Was shown to contain salicin, picelin, vimalin, salicyloylsalicin, salireposide, grandidentatin, populin, tremulacin and tremuloidin, salicyloysalicin-2-O-benzoate, (+)-catechin and beta sitosterool (Steele *et al.,* 1972). *Salix babylonica* and *Salix alba* also have been reported contain flavonols rhamnazin 3-glucoside, flavonol apigenin 7-glucoside, luteiolin 4-glucoside, tannins and cyanidin (Frank Santamour ,1992).

2.2 Medicinal uses of *Salix tetrasperma*

There are many medicinal uses of *Salix tetrasperma* for example dried leaves of the plant have cardiotonic and neurotonic activity (Bhakuni *et al.*, 1971). It is also have been mentioned in ancient test form Sumer, Egypt and Assyria the usage of leaves and bark of the willow tree as ointment for aches and fevers (Dhawan *et al.,* 1977). Furthermore, treatment of whooping cough in children also uses the decoction of bark and leaf of the plant as well as the paste of both and leaf applied externally to treat bug bites, scorpion sting, sore and warts. Other than that, the decoction of dried root of *Salix tetrasperma* is used to treat hepatitis by taking it orally. (Mondal *et al.,* 2010). Dysmenorrhea treatment in female also used the sap of the stem of *Salix tetrasperma* via oral and hot water extract of the entire plant has been applied to vaginal cavity to induce abortion in pregnant females. The local sore in rectum also use *Salix tetrasperma* plant for treatment by administered it rectally (Chhetree *et al.,* 2010). In traditional medicine, *Salix tertrasperma* has been used as antirheumatic sedative and analgesic (Mohamed *et al.*, 2010).

CHAPTER 3 MATERIAL AND METHOD

3.1. Instruments and Apparatus

The instrument and apparatus used in this research were Rotavac Heidolph Laborate 4000 series for evaporation of *Salix tetrasperma* after soxhlet extraction and spectrophotometer Thermofisher Genesys 20.

3.2. *Salix tetrasperma* Collections

The collection of *Salix tetrasperma* was collected from Pasir Hor, Kelantan. The leaves of the plant was washed and dried in oven at 55°C for 2 to 4 days. Then, the dried leaves of *Salix tetrasperma* were blended into fine powder form with blender. The powdered form of leaves was weighed and kept in a container.

3.3. Extraction of *Salix tetrasperma*

The extraction of *Salix tetrasperma* was done by using soxhlet extractor in maceration method. 50 g of plant sample was weighed and put into a thimble and then, 350 mL of methanol was filled into 500 mL round boiling flask. The temperature was set between 40°C to 60°C. The process was run until the colour in the chamber become clear and transparent. The resulting extract was concentrated using rotary evaporator Rotavac Heidolph Laborate 4000 series. The residual extract in the thimble is place into fume hood to evaporate at room temperature overnight or until methanol was completely dried. The semi solid paste like extract was weighed and kept into container (Ayoola *et al.*, 2008, Nikhal *et al.*, 2010).

3.4. Qualitative Phytochemical analysis

3.4.1. Detection of flavonoids

50 mg of the plant extract was dissolved in 30 mL of 80% ethanol and filtrated. The filtrate was mixed with 1 mL of 1% potassium hydroxide (KOH) solution. The discolouration of yellow color or the presence of dark yellow colour was considered as a positive result for flavonoid compound (Ayman *et al.*, 2012).

3.4.2. Detection of alkaloids

50 mg of methanol extract of plant was mixed with 8.0 mL of 1% Hydrochloric acid (HCL). The solution was heated in the water bath and filtered using Whatman filter paper. The filtrate was added with several drops of Dragendroff reagent. Formation of orange precipitate indicates for the presence of alkaloids (Rafia *et al.*, 2010)

3.4.3. Detection of tannins

50 mg of methanol plant extract was dissolved in 20 mL of distilled water and filtered using Whatman filter paper. 2 mL of 1% Ferum (III) chloride ($FeCl_3$) was added into the filtered plant extract. The development of yellow brown precipitate indicates the present of tannins (Jigna and Sumitra, 2007)

3.4.4. Detection of saponin

50 mg of methanol plant extract was added with 5 mL of distilled water in test tube. The solution was vigorously shaken and stable persistent froth is examined. The frothing as mixed with 3 drops of olive oil and shaken vigorously. The development of emulsion indicates the presence of saponin (Manjamalai *et al.*, 2010).

3.5. Quantitative Phytochemical Analysis

3.5.1 Determination of total alkaloids

The determination of total alkaloids was done by using alkaline precipitation gravimetric method. 5 g of plant extract was dispersed in 10% acetic acid solution in ethanol to form a ratio 1:10 (10%). The mixture was left to stand for 4 hours at 28°C. Then, the solution was filtered with Whatman no. 42 grade filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with addition of concentrated aqueous Ammonium hydroxide (NH_4OH) by drop wise until the alkaloid was precipitated. The alkaloid precipitate was collected in a weighed filter paper and washed with 1% ammonia solution. The filter paper was dried in the oven at 80°C. The total alkaloid content was calculated and it was expressed as a percentage of the weight of sample analyzed (Harborne, 1973)

3.5.2. Determination of total flavonoids

The determination of flavonoids was done by weighing 5 g of the plant extract which was boiled in 50 mL of 2M HCL solution for 30 minutes under reflux. Then, it was allowed to cool and filtered through Whatman No. 42 filter paper. The volume of solution with extract after the boil was measured and it was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitate was collected by filtration using weighed filter paper and the resulting weight differences indicate the weight of flavonoid in the plant sample (Harborne, 1973).

3.5.3. Determination of total tannins

0.2 g of plant extract was measured into 50 mL beaker and 20 mL of 50% methanol was added which then covered with paraffin film. It was placed in a water bath at 77°C – 80°C for 1 hour and to prevent lumping it was stirred with glass rod. The extract was quantitatively filtered using double layered Whatman No. 1 grade filter paper into a 100 mL volumetric flask by using 50% methanol to rinse. The volume in the volumetric flask was made up to mark with distilled water and thoroughly mixed. 1 mL of from sample extract solution was pipette into 50 mL volumetric flask and added along with 20 mL distilled water, 2.5 Folin-Derris reagent and 10 mL of 17% Na_2CO_3 and mixed properly. The mixture was made up to mark with distilled water, mixed well and left to stand for 20 minutes when formation bluish-green coloration can be seen. Standard Tannic Acid solution from range 0 to 10 ppm was prepared and treated similarly as 1 mL of plant sample. The absorbances of Tannic Acid Standard solution as well as the sample were read after colour development in the spectrometer Thermofisher Genesys 20 at a wavelength of 760 nm. The percentage of tannin in the plant extract was calculated (Swain, 1979)

3.5.4. Determination of total saponin

The determination of saponin was based on the method of Association of Analytical Communities (AOAC). The extraction of saponin was done using two different solvent. The first solvent was acetone which used to extract crude lipid from the plant extract while the second solvent was methanol which used for the extraction of saponin proper. 2.0 g of sample was added into a thimble and put into Soxhlet extractor and a reflux condenser fitted on top. The first extraction using the acetone was done in a 250 mL round bottomed flask for 3 hours and after that the apparatus was dismantled.

Another 150 mL capacity round bottomed flask added with 100 mL of methanol was fitted to the extractor and extraction was carried for 3 hours. The weight of flask was taken before and after the second extraction in order to make the change in weight. At the end of second extraction, the methanol was recovered by distillation and the flask was oven dried to remove any remaining solvent in the flask. The flask was allowed to cool and the weight of flask was taken. Thus:

$$Sp = \frac{A-B}{SM} \times 100$$

Where A = mass of flask and extract, B = mass of empty flask and SM = sample mass (Nwinuka *et al.*, 2005).

CHAPTER 4 RESULT

4.1. Percentage of Extraction Product

The percentage of extraction product for methanol was 25% where the return weight is 12.5 g for every 50 g of plant sample used. The extracted *Salix terasperma* sample acquire from soxhlet methanol extraction method give out about one quater of its original weight that can be consider this method can provide many extracted sample.

4.2. Qualitative Phytochemical Analysis

The result for qualitative phytochemical analysis of *Salix tetrasperma* was shown in table 4.2. The result shows that the presence of secondary metabolite for tannin, saponin and flavonoid in *Salix tetrasperma* by using methanol extraction method.

Table 4.1: The Qualitative Phytochemical Analysis Result

Test	**Extract**
	Methanol
Alkaloids	-
Tannins	+
Saponins	+
Flavonoids	+

ST - *Salix tetrasperma*

(-) = absence of natural group

(+) = present of natural group

4.3. Quantitative Phytochemical Analysis

4.3.1. Alkaloid

The weight of filter paper before and after the precipitation of alkaloid taken was 0.838 g and 0.862 g respectively. So, from 5 g of sample used for the test, the weight differences give out the percentage of alkaloid in the plant extract for methanol extraction is 0.48% per 5 gram of sample extract used in the test. There were low amount of alkaloid presence in the plant extract shows the significant of the qualitative phytochemical result where there no alkaloid presence for the screening test. Figure 4.2 shows the overall percentage of total phytochemical in extracted *Salix tetrasperma.*

4.3.2. Flavonoids

The weight of filter paper before and after the precipitation of flavonoid taken was 0.608 g and 0.801 g respectively. So, from 5 g of sample used for the test, the weight differences give out the percentage of alkaloid in the plant extract for methanol extraction is 3.86% per 5 gram of sample extract used in the test. The percentage shows that there was presence of flavonoids in the methanol extraction of the plant which is significant with the qualitative phytochemical analysis where the flavonoid was positive in the plant extract. Figure 4.2 shows the overall percentage of total phytochemical in extracted *Salix tetrasperma.*

4.3.3. Tannins

The straight line of the standard was draw by preparing 5 solutions of tannic acid with concentration within the interval of 0.002% and 0.01% ranging from 2 to 10 ppm or solution number. The solutions with concentration between 0.001% and 0.01% at interval of 0.002 have been obtained and the absorbance has been determined using

distilled water as blank as well as the absorbance of sample which was presented in the table 4.1. Figure 4.1 show the straight line graph obtained between the absorbance and concentration of tannic acid to get the value of the slope for the calculation of tannic acid percentage in the plant extract. The calculation of percentage tannic acid based on the graph where the concentration of tannic acid in the sample is 0.0615 so the percentage of tannic acid was 6.15% per gram of extract used for the test. There were high amount of tannin in the plant extract based on the percentage of tannic acid so this result was significant with the qualitative analysis with the presence of tannic acid in the plant extract. Figure 4.2 shows the overall percentage of total phytochemical in extracted *Salix tetrasperma.*

Table 4.2: Values of absorbance for standard straight line, used for the determination of tannic acid.

Solution number / sample	Concentration of tannic acid	Absorbance
0	0.000	0.000
2	0.002	0.017
4	0.004	0.036
6	0.006	0.063
8	0.008	0.080
10	0.010	0.105
Sample	0.062	0.628

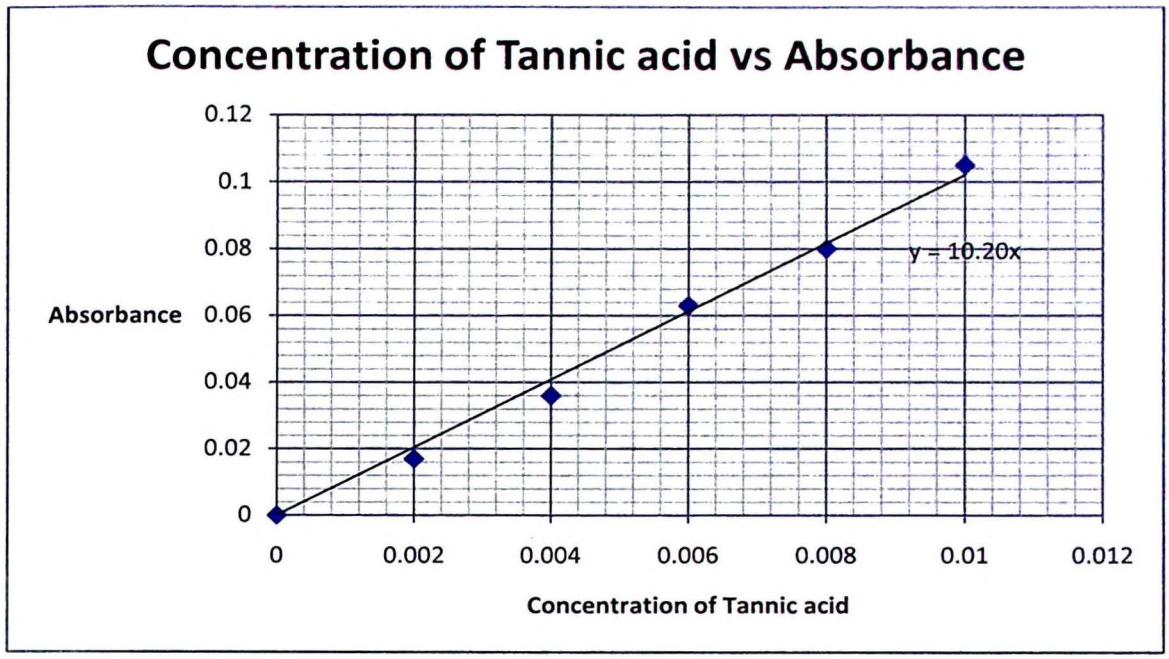

Figure 4.1 : Graph between concentration of tannic acid versus absorbance

4.3.4. Saponin

The mass of flask and extract after the extraction process and mass of empty flask taken was 76.5412 g and 77.4133 g respectively. From 2 g of sample used for the test, the weight differences give out the percentage of saponin in the plant extract for methanol extraction is 37.99% per 2 gram of sample extract used in the test. There were high percentages of saponin in the plant extract which indicate there were presence of saponin in the methanol extraction of the plant and this was significant with the qualitative phytochemical analysis where the flavonoid was positive. Figure 4.2 shows the overall percentage of total phytochemical in extracted *Salix tetrasperma.*

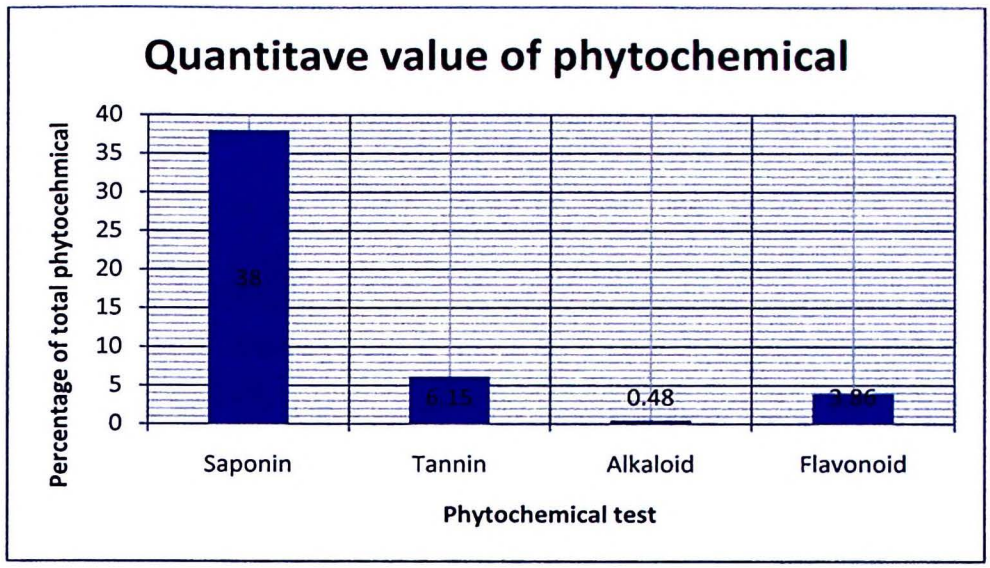

Figure 4.2 Quantitative value of percentage phytochemical in *Salix tetrasperma* methanol extract

CHAPTER 5 DISCUSSION

Phytochemical screening is important to investigate the bioactive agents in a plant which is important for medicinal purposes. The plant has required agents which act as primary product for synthesis of some useful drugs or remedy. The presence of phenolic compound in methanol extract of *Salix tetrasperma* suggests that the plant might have anti microbial agent (Okwu and Josiah, 2006). This is supported by a study where the leaves extract is effective in inhibition of Gram positive bacterial strain and *Shigella flexneri* and the root of the plant extract was effective in inhibition of *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli* while the bark of *Salix tetrasperma* is effective against *Shigella flexneri, E. coli* and *Bacillus cereus* (Saiful *et al.,* 2011). There are different mechanisms of antimicrobial activity by phytochemical for example tannin act by iron deprivation, hydrogen bounding or non specific interactions with important proteins for example enzymes (Damintoti *et al.,* 2005). This study also shows that from the quantitative and qualitative phytochemical analysis the main chemical substance contain high percentage in *Salix tetrasperma* leaves are flavonoids, tannins as well as saponins thus support medicinal use of this plant to treat certain illness.

Free radical is responsible for the cause of certain disorder to human such as arthritis, respiratory disease, and cancer and so on. Herbal medicine contain phytochemical that posses antioxidant that act as free radical scavengers which have health promoting properties (Padma *et al.,* 2013). One of the phytochemical that have these properties is flavonoids which is strong water soluble antioxidant and free radical scavengers that prevent cell damage and have potent anticancer activity (Okwu, 2004). The phenolic compound such as tannins, flavonoids and so on has been reported to have

chemo preventive role in cancer through their effect on signal transduction in cell proliferation and angiogenesis (Sutharsingh *et al.,* 2011). Other than that, saponin also can inhibit cancer where immune system modulating effect of saponin increase anti-tumor activity in the body plus the reduction of cancer also due to the stimulation of bile acid secretion in intestinal tract as well as antioxidant activity (John *et al,* 2004). Flavonoids also shows to have antimutagenic and antiproliferative effect in various cancer line and presence of tannin, terpenoids, steroids and other bioactive compound also promote cytotoxicity activities of *Salix tetrasperma* (Saiful *et al.,* 2011, (Mieczysław *et al.,* 2009, Padma *et al.,* 2013).

Furthermore, this plant also provides anti-inflammatory activity where flavonoids act as the anti-oxidant (Okwu, 2004). The anti-inflammatory activity of methanol *Salix tetrasperma* leaves extract may be cause by the presence of flavonoids (Mohamed *et al.,* 2010). The mechanism of action for the anti-inflammatory effect is due to inhibition of pathway of arachidonic acid metabolism where flavonoid turn out to have a significant role in anti-inflammatory activity as in a study where the comparison with Indomethacin and Hyrocortisone and the result proved flavonoid anti-inflammatory activity (Delta, 1986). The presence of flavonoid, tannin and saponin that have anti inflammatory activity proved the usage of this plant traditionally to treat hepatitis, sore, wart and so on. The major phenolic glycoside which is Salicin present in extracts of *Salix tetrasperma* expected to be the pharmacological active principle because of its chemical structure similar to aspirin (Mohamed *et al.,* 2010).

The other pharmacological effect of flavonoids are analgesic, anti-spasmodic and anti-allergic (Ayman *et al.,* 2012). The *Salix tetrasperma* extract also have hypoglycaemic activity as reported where extract show significant reduction in blood glucose levels in normal and alloxan induced diabetic rat at tested dose levels thus

support the use of this plant on anti-diabetic in traditional medicine (Chhetree *et al.*, 2010).

Salix tetrasperma extract also have high amount of tannin which have stringent properties that fasten the healing of wound and inflamed mucous membrane (Okwu and Josiah, 2006). This plant also contain high amount of saponin from the methanol extract where this phytochemical have the ability to stop bleeding to be used for wound treatment and ulcers as saponin help in coagulation of red blood cell. The other characteristics of saponin are can form foams in aqueous solution, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). Saponins also have the ability to reduce elevated cholesterol levels where it will form complexes with cholesterol and bile acids that prevent it absorbed through the small intestine. Cholesterol and bile complexes will excreted through the stool that lower cholesterol levels in blood and liver (John *et al.*, 2004). Stimulation of immune system also can be triggered by saponins as been reported where saponin is used as adjuvant in vaccines plus oral intakes of saponins has been used to treat retroviral infection (John *et al.*, 2004). Antibody production is stimulated by saponins which inhibit viruses and induced the response by lymphocyte to fight infection (John *et al.*, 2004). The present of alkaloid in quantitative analysis of *Salix tetrasperma* may due to error during the procedure and the amount of alkaloid is very low that during the qualitative analysis the changes in color cannot be seen.

CHAPTER 6 CONCLUSION

In this study, the screening and quantitative analysis of four phytochemical compounds which are alkaloid, saponin, tannin and flavonoid has been done on methanol extract of *Salix tetrasperma*. There are three natural group are presence for qualitative analysis in methanol plant extract which are flavonoid, tannin and saponin with the amount of the substances in the extract are 3.86%, 37.99% and 6.15% respectively but alkaloid is measured in quantitative analysis with 0.48%. The presence of the compound proves the usage and effectiveness of this plant in traditional medicine such as bug bites, scorpion sting, aches, fever, hepatitis, antidiabetic, anti-inflammatory, analgesic and so on.

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