EXTRACTION OF THORNS OF CEIBA PETANDRA FOR PHYTOCHEMICALS STUDIES

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ABSTRACT

Thorns of Ceiba petandra (kekabu plant) is widely used in complementary medicine and has been recommended for the treatment of many diseases such as bronchitis, diarrhoea and skin disease. The thorn of its extract via soxhlet extraction was investigated for optimum operating condition of extraction process and major phytochemical constituent presence (flavanoids, alkaloids and tannins). Methanol and ethanol were used as a solvents in the extraction process with vary the solid to solvent ratio (w: v); 1:10, 1:15 and 1:20. The phytochemical constituents were investigated by using standard procedure and also Gas Chromatography Mass Spectrometry (GC-MS) analysis is only for the optimum condition. The phytochemical analysis revealed that the presence of alkaloid in the entire sample while tannins and flavanoids at solid to solvent ratio in a range of 1:10 to 1:15 for both type of solvents. Based on Gas Chromatography Mass Spectrometry (GC-MS) analysis have prove that the extracted oil by using methanol contain high amount of active compound compare to sample extract using ethanol. This is due to the polarity of the solvents. Results showed that the methanol extract was found to be generally more effective than ethanol extract with solid to solvent ratio is 1:15.

ABSTRAK

Duri Ceiba petandra (tumbuhan kekabu) digunakan secara meluas dalam perubatan sampingan dan telah disyorkan untuk rawatan pelbagai penyakit seperti bronkitis, cirit-birit dan penyakit kulit. Duri ekstrak menerusi pengekstrakan soxhlet telah disiasat untuk mengenalpasti keadaan optimum dalam proses pengekstrakan dan kehadiran unsur fitokimia (flavanoids, alkaloid dan tannin). Methanol dan ethanol digunakan sebagai pelarut dalam proses pengekstrakan dengan berbeza-pepejal nisbah pelarut (w: v); 1:10, 1:15 dan 1:20. Juzuk fitokimia telah disiasat dengan menggunakan prosedur standard dan juga Gas Chromatography Mass spektrometri (GC-MS) analisis adalah hanya untuk keadaan optimum. Analisis fitokimia mendedahkan bahawa kehadiran alkaloid di seluruh sampel manakala tanin dan flavanoids hanya pada pelarut nisbah dalam pelbagai 1:10-1:15 untuk kedua-dua jenis pelarut. Berdasarkan Gas Chromatography Mass spektrometri (GC-MS) analisis telah membuktikan bahawa minyak yang diekstrak dengan menggunakan methanol mengandungi jumlah yang tinggi kompaun aktif berbanding dengan sampel ekstrak menggunakan ethanol. Ini adalah kerana kekutuban pelarut. Hasil kajian menunjukkan bahawa ekstrak metanol yang didapati umumnya lebih berkesan daripada ekstrak etanol dengan kukuh untuk pelarut adalah nisbah 1:15.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Medicinal plants are of great importance to the health of individuals and communities in general. They are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemical are naturally occurring in the medicinal plants, leaves, thorns, vegetables and roots that have defence mechanism and protect from various diseases. Phytochemical are primary and secondary compounds. Primary constituents include the common sugars, amino acids and proteins. While, secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) (Wadood et al. 2013). Reactive of free radical from the secondary constituents can be used in several activities, such as anti-fungal activities, antimicrobial activities and anti-diarrhoeal activity.

Ceiba petandra is one of the medicinal plants used for researches in extraction of oil for phytochemical studies. Ceiba Petandra which is locally known as kapok or kekabu is a native in America and West Africa. Ceiba Petandra belongs to the Malvaceae family (Rajeswari et al. 2013).Ceiba petandra have been recommended for the treatment of bronchitis, diabetes, diarrhoea, skin diseases, painful aye diseases and chronic fever (Elumalai et al. 2012). All the parts of this plant (leaves, stems, roots and even whole parts) have numerous therapeutic activities for the treatment of variety diseases.(Activity et al. 2009) reported that the extraction of stem bark oil can be used as an anti-microbial because the phytochemical contents exist. While, (Odinma 2013)

presented the work on extraction of stem bark oil consists flavanoids, saponins and alkaloids. These phytochemical can be used in anti-antibacterial.

Among the part of medicinal plants used for researches in extraction of oil for phytochemical studies, thorns of ceiba petandra is a new part as a raw material for extraction process have been explored and it is found that thorn of ceiba petandra has potential for the extraction process (Dangi et al. 2014).Many studies had been done on extraction of oil from ceiba petandra for phytochemical studies by using soxhlet apparatus (Anosike et al. 2012) and (Odinma 2013).

Figure 1-1: Thorns of ceiba petandra

Therefore, this project comes out with a well-established technique to extract thorns of ceiba petandra by using soxhlet apparatus at optimum conditions. It offers higher yield and selectivity within hours. None of the reported literature on extraction of ceiba petandra using thorns as a plant sample for phytochemical analysis. Therefore, the present projects attempts to explore the potential of utilizing indigenously available thorns of ceiba petandra oil in the phytochemical study.

1.2 PROBLEM STATEMENT

Several problem statements of this research are:

i. In Malaysia, the application of the ceiba petandra as a medicine is not commercially used.
ii. Little research had been done on thorns of ceiba petandra.

1.3 OBJECTIVES

This study embarks on the following objectives:

i. To study the optimum operating condition for process of extraction of ceiba petandra.
ii. To extract phytochemical exist in the ceiba petandra plant.

1.4 SCOPE OF STUDY

This project focus on the application of soxhlet apparatus assisted technique to extract oil from thorn of ceiba petandra. Optimization of the two parameters affecting extraction process including type of solvent and weight ratio between raw material and solvent used will be studied. Comparison will be made between results of experiments using different type of solvent which are methanol and ethanol. Study also will be made to see the suitable ratio between raw material and solvent used; 1:10, 1:15 and 1:20 to achieve high yield of the extracted oil. The extracted oil product produced from optimized condition with highest yield will be analyzed phytochemical content exist in the thorn of ceiba petandra plant. In this project, three type of phytochemical content will be analyzed by using several tests based on Table 1-1. The gas chromatography mass chromatography analysis also will be done for the confirmation of the result obtained.

Table 1-1: Analysis of the phytochemical content in thorn of ceiba petandra

Phytochemical	Test
Flavanoids	Zinc hydro chloric acid
Tannins	Gelatin test
Alkaloids	Mayer

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION OF CEIBA PETANDRA

Ceiba petandra (Bombacaleae) commonly called kapok tree or silk cotton (English). The plant has a spreading crown, huge trunk and large palm shaped leaves (Rameshwar et al. 2014) .The mature tree bears hundreds pods, up to 15 m long filled with fibrous seeds. Various morphological parts of the plant have been reported to be useful as effective remedies against diabetes, hypertension, headache, dizziness, constipation, mental diseases and fever. In West Africa, it is generally used in the treatment of diarrheal (Elumalai et al. 2012).That why, it is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia (Rameshwar et al. 2014).

Ceiba petndra is grows under a wide variety of conditions, but thrives better below 500 m elevation and with at least 1,000 mm annual rain, particular important during the vegetative growth period. Flowering and fruiting occurs during the dry season, but fruiting fails at night temperatures below 20°C. The ceiba petandra is consists of stem and branches with conical spines. It have branches horizontal, leaves compound palmate, ripe fruits packed with whitish floss kapok.

2.2 APPLICATION OF CEIBA PETANDRA

Ceiba petandra also can be divided into several parts which are roots, stem barks, thorns and also leave. For each part have their advantage and applications especially in medical. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care.

According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhoea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health.Table 2-1 below shows the summary of the usage of ceiba petandra plant.

Table 2-1: The applications of ceiba petandra plant
(Alagawadi, 2011) and (S. Ibrahim, 2012)

Parts	Medicines usage	Other usage
Leaves	Anti-fungal, Anti-ulcer , Anti-venom, fevers and headaches.	-Used as an alternative to down as filling in mattresses, pillows, and stuffed toys such as teddy bears, and for insulation.
Stem barks	Anti-diarrheal, fevers and headaches, wounds.	-It was previously much used in life jackets and similar devices until synthetic materials largely replaced the fibre.
Roots	Anti-ulcer	
Seeds	Antioxidant, Anti-inflammatory	-The seeds produce oil used locally in soap and that can be used as fertilizer.

2.3 PHYSICAL AND CHEMICAL PROPERTIES OF ESSENTIAL OIL

Essential oils are the odours and volatile products of various plant and animal species. An essential oil is a liquid that is generally distilled from the part of the plant (stem barks, leaves, roots, seeds and thorns). Most of the essential oils are clear.(Silitonga, Mahlia, and Ong n.d.) Ceiba petandra is a non-edible oils that have a lot of potential and does not compete with food crop for limited gowning regions (Dauda, Sallau, and Chindo 2013). The oil of the plants has a potential as a biodiesel because of its properties. However, in this study, the research is focus on the phytochemical contents in the oil of the plants. Table below show the physiochemical properties of the ceiba petandra oil.

Table 2-2: Physiochemical properties of the ceiba petandra oil

(Dauda et al. 2013) and (Vedharaj et al. 2013)

Properties	Value/Characteristics
Density (kg/m^3)	905.2
Viscosity (mm^2/s)	34.455
Flash point (^oC)	183
Cloud point (^oC)	170
Physical state	Slightly viscous
Colour	Brightly yellow
Odour	Slightly offensive

2.4 PHYTOCHEMICAL STUDIES

Phytochemical is a non-nutritive chemicals that have protective or disease preventative properties. They are non-essential nutrients, meaning that they are not required by the human body sustaining life. It is well known that plant produce these chemicals to protect them but recent research demonstrate that they can also protect human against disease. They are more than thousand known phytochemicals. Some of the well known phytochemicals are:

i. Flavanoids
ii. Saponins
iii. Glycosides
iv. Alkaloids
v. Tannins
vi. Phenolics
vii. Steroids
viii. Terpenoids

Many researchers have found that the phytochemicals content in the ceiba petandra plant have potential to prevent many diseases as mention in the applications of the ceiba

petandra plants. 8 types of phychemical content have found in the stem barks of the plants by (Doughari I, 2009) (Nwachukwu, 2008), (Ezigbo V.O., 2013) and (Sule, 2009). They are saponins, glycosides, tannins, phenolics, alkaloids, flavanoids, steroids and terpenoids. While, (G.Madhukumar, 2014) and (S. Ibrahim, 2012) analysed flavanoids are the only phytochemicals that exist when the extraction of the leaves were done. The extraction of the roots of ceiba petandra plant consists of saponins, tannins, alkaloids, flavanoids and steroids (Nwachukwu, 2008). (Alagawadi, 2011) has done the extraction of the ceiba petandra plant by using seeds as a raw materials. Based on the finding, tannins, phenolics, alkaloids and steroids are exists in the sample used. From the analysis above, all the parts of ceiba petandra plants have potential to protect and prevent human from disease.

By comparing the contents of the phytochemicals exist in the part of the ceiba petandra plant; there are three major phytochemicals that exist which are flavanoids, alkaloids and tannins. The comparison were made within others part of the plant because generally many researchers used stem barks, roots and leaves as their extraction material**.** Table below shows the comparison of the phytochemical content that exists in the several parts of the plants.

Table 2-3: Qualitative of phytochemical studies on ceiba petandra trees

Part of ceiba petandra tree	Phytochemicals								References
	Saponins	**Glycosides**	**Tannins**	**Phenolics**	**Alkaloids**	**Flavanoids**	**Steroids**	**Terpenoids**	
Stem barks	+	+	+	+	+	+	+	+	(Doughari I, 2009), (Nwachukwu, 2008), (Ezigbo V.O., 2013), (Sule, 2009)
Leaves	-	-	-	-	-	+	-	-	(G.Madhukumar, 2014) (S. Ibrahim, 2012), (Alagawadi, 2011)
Seeds	-	-	+	+	+	-	+	-	(Nwachukwu, 2008)
Roots	+	-	+	-	+	+	+	-	

2.4.1 Alkaloids

Alkaloids are the largest group of secondary chemical constituents made largely of ammonia compound comprising basically of nitrogen bases synthesized from amino acids building blocks. The compounds have basics properties and are alkaline in reaction, turning red litmus paper blue. Majority of alkaloids exist in crystaline solid such as atropine and some as liquids containing carbon, hydrogen and nitrogen. Most alkaloids are readily soluble in alcohol though they are soluble in water, their salts are usually soluble. The solutions of alkaloid are intensely bitter. Other important alkaloids of plant origin include the addictive stimulants caffeine, nicotine, morphine, codeine and sanguinary based on Figure (Lo 2002) and (Grover and Patni 2013).

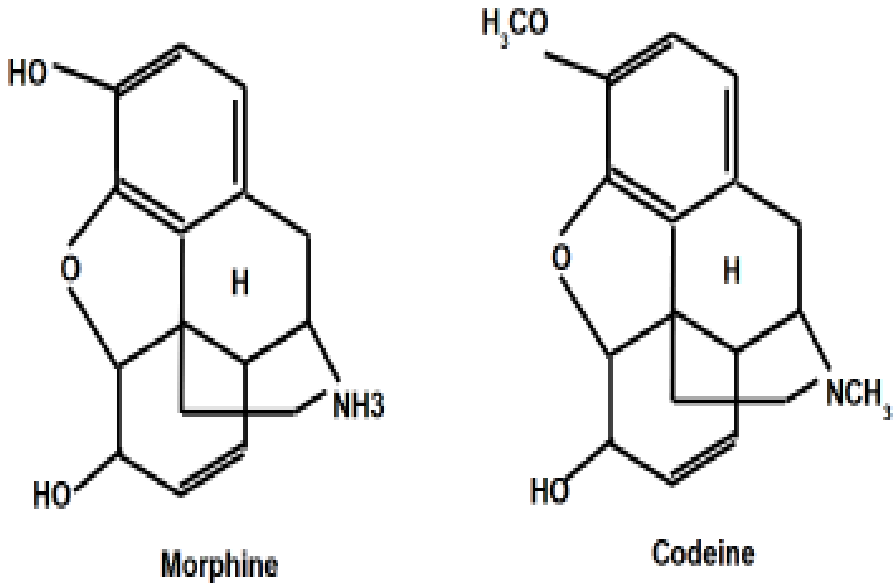

Figure 2-1: Basic structures of some pharmacologically important plant derived alkaloids

Alkaloids are generally classified by their common molecular precursors, based on the biological. From a structure point of view, alkaloids are divided according to shape andrue alkaloid structure. There are three main types of alkaloids; true alkaloids, protoalkaloids and pseudoalkaloids. True alkaloids and protoalkaloids are derived from amino acids wheresas pseudoalkaloids are not derived from these compounds.

The structures of the alkaloids give good antimicrobial activity against microorganism. An antimicrobial is an agent that kills microorganism or inhibits their

growth. Antimicrobial activity can be grouped according to the microorganism they act primarily against. Among the compounds isolated from ceiba petandra plants, its alkaloids appeared to be of great interest in pharmacological studies. These alkaloids belong to the family of indoloquinolines (Karou, Dicko, et al. 2005). Many researches have been done on this family compounds and the results showed that they are new leads in the establishment of drugs against many diseases. More recently, found that polyphenols extract of the plant had a weak antioxidants activity, on the other hand the extract was very active on pathogenic bacteria and this activity may be influenced by the polymerisation size of the phenolics compounds (Karou, Savadogo, et al. 2005). Some screening methods for the detection of alkaloids are summarized in Table .

Table 2-4: Methods for detection of alkaloids

Reagent/test	Composition of the reagent	Result
Mayer's reagent	Potassiomercuric iodide solution.	Cream precipitate
Wagner's reagent	Iodine in potassium iodide.	Reddish- brown precipitate
Tannic acid	Tannic acid	Precipitate
Hager's reagent	A saturated solution of picric acid.	Yellow precipitate

2.4.2 Flavanoids

Flavanoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in the structure and numerous reports support their use as antioxidants or free radical scavengers. The compounds are derived from parent compounds known as flavans. Over 4000 flavanoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavanoids present in nearly 70% of plants. Other groups of flavanoids include flavanols, flavans, anthocysnidins and dihydroflavons based on Figure .

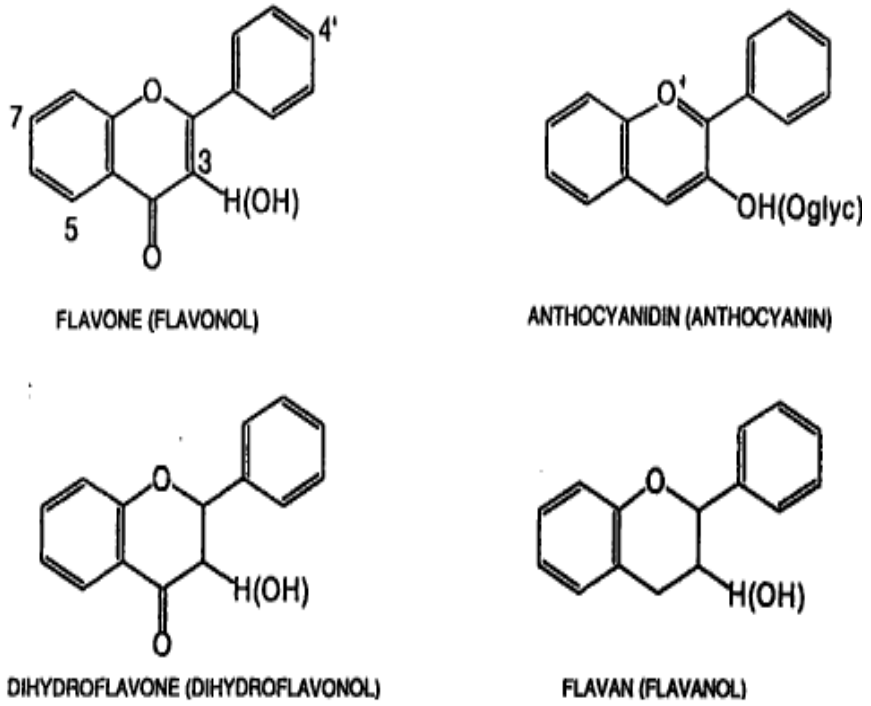

Figure 2-2: Basic structures of some pharmacologically important plant derived flavanoids

Increasingly, flavanoids are becoming the subject of medical research. They have been reported to process many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition and also anti microbial activity (Cushnie and Lamb 2005). Because of the function flavanoids that are widely distributed in edible plants and have previously been used in a traditional medicine, it has been likely to have minimal toxicity (Hendra et al. 2011).

2.4.3 Tannins

Tannins are phenolics compound of height molecular weight. They are soluble and water and alcohol and are found in the root, bark, stem and outer layer of the plant tissues. They are acidic in reaction and the acidic reactions are attributed to the presence of phenolics or carboxylic group. Tannins are used as antiseptics and this activity is due to the presence of the phenolics group. Tannin-rich medicinal plants are used as healing agents in the number of diseases. They form complex with protein, carbohydrates, gelatine and alkaloids. Tannins are divided into two hydrolysable tannins and

condensed tannins. Theaflavin and gallic acid are example of the some pharmacologically important plant derived from the tannins based on Figure .

There are a lot of activities when a plant presence a tannins. (Reddy et al. 2007) analysis tannins can be used as antioxidant, anti- malarial and antimicrobial. Tannins are known to provide the typical tanning effect which is important for the treatment of inflamed or ulcerative tissues (Parekh and Chanda 2007). Tannins are phytochemicals exert antimicrobial activity through different mechanism; hydrogen bonding or specific interactions with vital proteins such as enzymes (Agricultural et al. 2008).

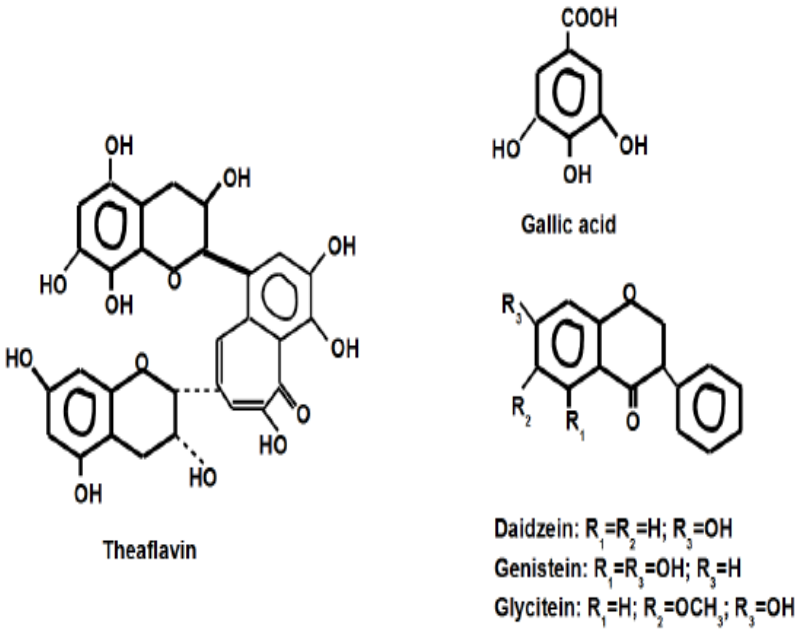

Figure 2-3: Basic structures of some pharmacologically important plant derived tannins

2.5 EXTRACTION METHOD/PROCEDURE

Extraction in term of pharmaceutically is the separation of the medicinally active portions of the plants or animals tissues from the inactive/inert components by using selective solvent through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity. Generally, the researchers used soxhlet apparatus for

extraction of ceiba petandra process. So, in this project, soxhlet extraction method was used to extract the thorns of ceiba petandra.

2.5.1 Soxhlet extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. (Prashant Tiwari, 2011).In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus from Figure .The extracting solvent in flask A is heated, and its vapours condense in condenser D. The condensed extracting drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

Figure 2-4: Soxhlet apparatus

Temperature and time are the most important parameter in the extraction process using soxhlet apparatus. Temperature for the process are commonly used at the boiling point of the solvent used in the. While, the best time for the process is analysed at the higher yield of the extraction product. Based on the (G.Madhukumar, 2014), 58 °C within 6 hours is used in the extraction process by using ethanol as solvent. From the (Nwachukwu, 2008) analysis, the high yield achieved within 12 hours at 60 °C by using methanol and ethanol as a solvents. The summary of the comparison for the soxhlet apparatus process is in Table **.**

Table 2-5: Comparison of the condition for the soxhlet apparatus

References	**Method**	**Conditions**		
		Solvent	**Time (hours)**	**Temperature (°C)**
(G.Madhukumar, 2014)	Soxhlet extraction	Ethanol	6	58
(Nwachukwu, 2008)		Hot water, methanol, ethanol	12	60
(Prashant Tiwari, 2011)		Petroleum ether	6	60

2.6 FACTOR AFFECTING EXTRACTION PROCESS

The purpose of standardized extraction procedures for medicinal plant parts is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as men strum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavanoids and lignin (Prashant Tiwari, 2011).

The basic parameters influencing the quality of an extract are:

i. Plant part used as starting material (raw material used)
ii. Solvent used for extraction
iii. Solid to solvent ratio

2.6.1 Plant material

Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many researchers had reported about the plant extract preparation from the fresh plant tissues. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers due to water content within different plant tissues. Dried or wet plant parts are grinded in the blender to fine particles. The highest the surface area of the sample will increase the rate of extraction reaction. For the ceiba petandra plant, dried part of plant will be used. The part of plant (thorns of ceiba petandra) will be dried in the oven at 30 ^{o}C within 2 hours.

2.6.2 Choice of solvents

Properties of good solvents in plant extraction include low toxicity, ease of evaporation at low heat and inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of extracted, rate of extraction, toxicity of solvent in the bioassay process and the potential health hazard of the extractants. Since the end product will contain trace of residual solvent, the solvent should be non- toxic and should not interfere with the bioassay. The choice of solvents will also depend on the targeted compounds to be extracted and also solubility of the solutes in particular solvent.

Therefore, it is difficult to predict the solubility of the pytochemical compund on the solvent. An alternative way of considering solubility is to use the concept of polarity. (Andri Cahyo Kumoro, 2009) defined polarity as the relative ability of the molecule to engage in strong interactions with other polar molecules. Polarity therefore represent the ability of a molecule to enter ito interactions of all kinds and relative

polarity is the sum of all possible interactions. By comparing the polarity of methanol and ethanol, methanol is more polar than ethanol. (Farooq Anwar, 2012) reported that the methanol extract gave higher yield than ethanol extract, indicating the most polar solvent used caused further extraction of polar components.Based on (Andri Cahyo Kumoro, 2009), high extract yield were obtained from extraction employing polar organic solvents containing hydroxyl group and have similar solubility properties. Therefore,its indicates that the polarity of the solvent influenced the solubility of the solute in solvent, thus influenced the presence of the constituents compound in plant extract. Figure showed the ranking of the polarity of solvents.

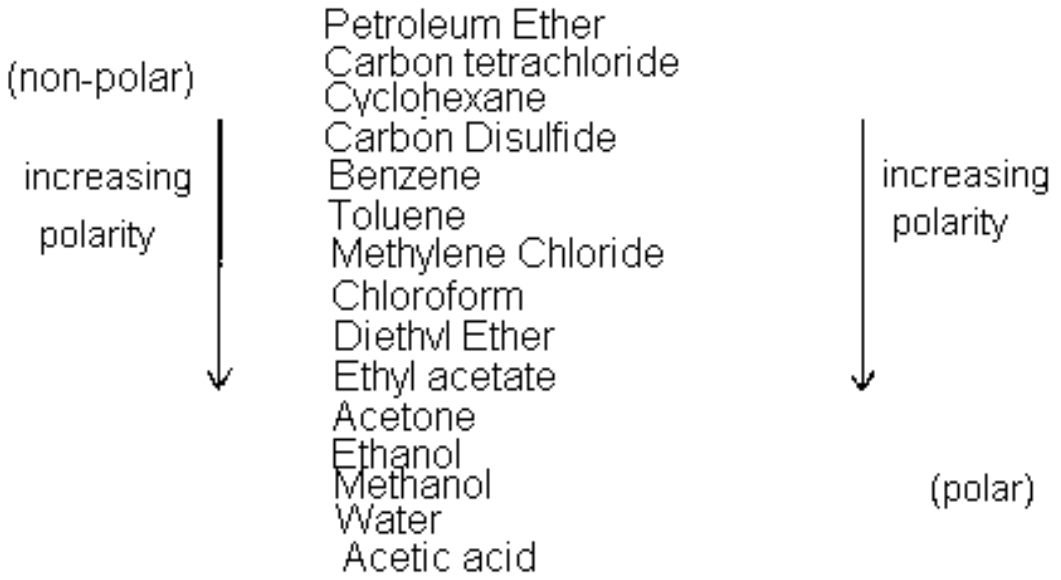

Figure 2-5 Polarity of solvents

The various solvents that are used in the extraction procedure are; water, alcohol and ether.For the ceiba petandra plant, many researchers used alcohol as a solvent for the extraction process. (Nwachukwu, 2008) Reported the alcohols extract of ceiba petandra give higher yields than respective water extracts. The alcohol extract gave 7.98 % higher that water extract that gave 5.74 % only. By comparing methanol and ethanol as a solvents, ethanol gives higher yield compare to ethanol. Based on the result obtain from (Anosike et al. 2012), the yield of the ethanol extracts is higher than methanol extracts with 0.34 % and 0.30 %.The further research is study by comparing the percentage of the yield of oil extract from ceiba petandra based on below Tabele 2-6.