SCREENING FOR PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *Annona muricata* L. LEAF

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ABSTRACT

Annona muricata L. *(A. muricata* L.) or traditionally known as soursop is well known for its deliciously sweet sour taste of its fruits. The other parts of the tree also have been used in remedies in traditional medicinal history including the bark, leaves, and root, fruit, and fruit seeds. Previous research on *A. muricata* L. has focused on the bark of the tree and root for pharmaceutical purposes by testing it on laboratory animals. Little attention has been paid to the research on the leaves which actually possessed valuable phytochemical constituents that have the potential as antimicrobial properties in treating diseases that caused by some bacterial strains. The main objective of this study is to do phytochemical and antimicrobial activity screenings of the *A. muricata* L. leaves extract. The analysis result obtained had been compared to previous study done in other countries. The leaves of *A. muricata* L. collected randomly from rural area in Jerantut, Pahang. The process of extraction, screenings and analysis were done in Laboratory of Sekolah Pertanian Lestari, UMS Kampus Sandakan. The solvents used for the extraction of plants are aqueous, chloroform and ethanol. The *in vitro* antibacterial activity was performed by disc diffusion method and tested on bacteria strains which were three Gram negative; *Escherichia coli*, *Salmonella enteritidis, Citrobacter freundii* along with one Gram positive bacteria which is *Staphylococcus aureus* . In this study, it was found that aqueous leaves extract gave the highest percentage of yield extraction. In phytochemical screening, the leaves extract showed the presence of glycoside, tannin, saponin and flavonoid. For antimicrobial activity screening, aqueous leaves extract showed the highest zone of inhibition on tested bacterial strains .The phytochemical and antimicrobial activity screenings suggest that *A. muricata* has antimicrobial properties.

Keyword: phythochemical screening, antimicrobial, bacteria strains, solvents

ABSTRAK

Annona muricata L. (*A. muricata* L.) atau tradisinya dikenali sebagai Durian Belanda terkenal dengan rasanya yang manis-manis masam. Bahagian-bahagian lain pokok ini termasuk kulit, daun, akar, buah-buahan, dan biji buah-buahan juga telah digunakan sebagai ramuan dalam sejarah perubatan tradisional. Kajian terdahulu terhadap *A. muricata* L. telah memfokuskan pada kulit pokok dan akar untuk tujuan farmaseutikal dengan melakukan ujian ke atas haiwan makmal. Sedikit perhatian telah diberi kepada kajian terhadap daun yang sebenarnya memiliki juzuk fitokimia berharga yang mempunyai potensi sebagai bahan antimikrob dalam merawat penyakit-penyakit yang disebabkan oleh beberapa jenis bakteria. Objektif utama kajian ini adalah untuk melakukan penyaringan fitokimia dan aktiviti antimikrob ekstrak daun *A. muricata*. Hasil analisis yang diperolehi telah dibandingkan dengan kajian lepas yang dilakukan di negara-negara lain. Daun *A. muricata* L. dikutip secara rawak dari kawasan luar bandar di Jerantut, Pahang. Proses pengekstrakan, penyaringan dan analisis telah dilakukan di Makmal Sekolah Pertanian Lestari, UMS Kampus Sandakan. Bahan pelarut yang digunakan untuk pengekstrakan tumbuhan ialah akues, kloroform dan etanol. Aktiviti antibakteria *in vitro* yang dilakukan oleh kaedah penyebaran cakera dan diuji pada strain bakteria yang tiga daripadanya ialah Gram negatif; *Escherichia coli, Salmonella enteritidis, Citrobacter freundii* bersama-sama dengan satu bakteria Gram positif iaitu *Staphylococcus aureus*. Dalam kajian ini, ia telah mendapati bahawa ekstrak akueus daun memberikan peratusan tertinggi pengeluaran hasil. Dalam penyaringan fitokimia, ekstrak daun menunjukkan kehadiran glikosida, tanin, saponin, dan flavonoid. Untuk saringan aktiviti antimikrob, ekstrak akueus daun menunjukkan zon tertinggi perencatan pada strain bakteria yang diuji. Pemeriksaan aktiviti fitokimia dan antimikrob mencadangkan bahawa A. muricata mempunyai bahan antimikrob.

Kata kunci: saringan fitokimia, antimikrob, strain bakteria, pelarut

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
±	Plus-minus
cm	Centimetre
ft	Feet
FeCl3	Iron (III) chloride
ml	Millilitre
mg	Milligram
µl	Microlitre

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia can be called as a blessed country which has fertile soil, good climatic condition, and rich in biodiversity. Malaysia also owned the oldest rainforest, which have a lot of natural good plants, especially medicinal plants. Malaysia has been classified as one of mega diversity of the world. Altogether, these twelve countries comprise at least 60% of world's known species (Norhajar *et al.*, 2010).

Malaysia has an abundance of flowering plants as well as non flowering plants, which is said that quarter of it has medicinal values. In Malaysia rainforest, some 8,100 plants species were found, and about 10% are reported to have medicinal value (Syamkumar *et al.*, 2003; Zaidah *et al.*, 2006). However, only few had been fully investigated for their potential (Zaidah *et al.*, 2006).

According to the World Health Organization (WHO), medicinal plants could be a good source in foundation of new drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000). Medicinal plants can be define as various plants that being used in herbalism practices, thought to have medicinal properties. Basically from the plants, the roots, leaves, stem,

bark, or seeds of some medicinal plants are known for their medicinal value. Their effectiveness and popularity depend not only on new research findings but also the usage experience and ethnic beliefs of the multi-ethnic society.

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicine are still included as part of the habitual treatment of various maladies (Doughari *et al.*, 2008). However, scientific research needed in order to give better understanding about phytoconstituents compound that have may have different potential value in treating difference and various diseases.

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Pathak *et al.*, 2010). As a result, a number of medicinal plants used in indigenous medicine have been tested and found to possess bactericidal properties (Vieira *et al.*, 2001).

In the past five decades, medicinal plants research in Malaysia has been carried out mainly by researches from government-funded universities and research institutes with little involvement of industries and multinationals. The earliest report on medicinal plant research in Malaysia was on the phytochemical screening of 205 plants in Sabah (Arthur, 1954), followed by few years later by screening of 200 species in Peninsular Malaysia for presence of alkaloids (Douglas and Kiang, 1957). These two publications marked the beginnings of medicinal plant research in Malaysia (Ibrahim, 2004).

Phytochemical screening is very important methods of identifying bioactive compound that useful in creating new drugs. These simple, cheap, sensitive, selective and rapid chemical tests to determine the presence of certain groups of compounds is an initial step to select plants for further phytochemical studies (Ibrahim, 2004).

Along with the increasing public interests on medicinal plants, recently there are lots of researches done on various potential medicinal plants. One of them is *Annona muricata* L. (*A. muricata* L.) or commonly known as soursop. However, previous research on *A. muricata* L. has focused on the bark of the tree and roots for pharmaceutical purposes (Kimbonguila *et al.*, 2010) and little attention has been paid to the leaves, in which usually used in traditional medicine remedies. To date, there are only few research publications about phytochemical screening of *A. muricata* L. leaves and their antimicrobial activity against Gram-positive and Gram-negative bacteria.

1.2 Justification of the study

This study was conducted in order to carry on screening of the potential phyotochemical constituents of the *A. muricata* L. *leaves* for its biological activities against bacteria. It is well-known that *A. muricata* L. fruits are good to keep a good health. The leaves are also used traditionally to keep away pests such as cockroach in the houses due its strong aroma. Nevertheless, there are lacks of scientific research conducted especially in Malaysia to reveal the potential of *A. muricata* L. leaves on antimicrobial purposes since the research mainly focus on pulp, seeds and bark of the plant. A very little research also done with regards to ascertain the extraction yield based on solvent polarity (Mohd *et al.*, 2012). Thus, the influence of solvents with different polarities on extraction yield was investigated. Thus, hopefully this study will help to add more findings on *A. muricata* L. leaves properties and potential, so it can be further developed for more advanced purposes in medicinal aspect.

1.3 Objective of the study

To determine the effects of different solvents extraction by carry on preliminary screening of *A. muricata L.* leaves extract for its antimicrobial activities against selected bacterial strains that cause common illness such as skin infection, diarrhea and others.

CHAPTER 2

LITERATURE REVIEW

2.1 Botany of Plant Studied

2.1.1 Family of *Annonaceae*

Annonaceae, the custard apple, or annona family is the largest family of magnolia order (Magnoliales). Some authorities sated that it contain 129 genera and 2,220 species, and many of species are valuable for its large pulpy fruits, some useful for their timber also as ornamentals. The family consists of trees, shrubs and woody climbers found mainly in the tropic, although few species extended into temperate regions. Among the characters that give *Annonaceae* their unmistakable appearance is the fibrous and aromatic bark, wood with fine tangential bands of parenchyma, alternate, distichous leaves, a trimerous perianth, and ruminate endosperm.

The *Annonaceae* economic importance is derived from the considerable range of non-timber products obtainable from its species including kernels, edible fruits and medicines while the woods of some species are valued for fuel wood, furniture and in pharmaceutical research (Focho *et al.*, 2010). Recently some Annonaceae became important in pharmaceutic research because of the antifungal, bacteriostatic, and especially cytostatic capability of some chemical constituents of the leaves and bark.

2.1.2 Genus of *Annona*

Annona is a genus of flowering plants in the *Annonaceae*. It is the second largest genus in the family after Guatteria containing approximately 110 species of mostly neotropical and afrotropical trees and shrubs. *Annona* species typically grown for it domestic or commercial use, mostly for the edible and nutritious fruits.

Many of the species are used in traditional medicines for the treatment of a variety of diseases. The fruits of Annona are haematinic, cooling, sedative, stimulant, expectorant, maturant, and tonic. They are useful in anaemia, burning sensation. The seeds are abortifacient and insecticidal (Mona *et al.*, 2012).Several annonacaeous species have been found to contain acetogenins, a class of natural compounds with a wide variety of biological activities.

2.1.3 *Annona muricata* L. species

A. muricata L. or infamously known as soursop is one of Malaysian exotic fruits from family *Annonaceae.* In Malay language, it is called *durian belanda.* The tree is a low-branching and bushy but slender plant. It can reach a height of 25 to 30 ft. It is a typical tropical tree with heart shaped edible fruits with the flesh is white and juicy. The leaves are lanceolate with glossy and dark green in color. This species are widely distributed in most of tropical countries (De Feo, 1992; Sulaiman *et al.*, 2012).

A. muricata L. has a long, rich history of use in herbal medicine as well as a lengthy recorded indigenous use. All parts of the soursop plant are used in natural medicine in the tropics, including the bark, leaves, roots, fruit, and fruit seeds. Different properties and uses are attributed to the different parts of the tree.

Generally, the fruit and fruit juice are taken for worms and parasites, to cool fevers, to increase mother's milk after childbirth, and as an astringent for diarrhea and dysentery. The crushed seeds are used against internal and external parasites, head lice, and worms. The barks, leaves, and roots are considered sedative, antispasmodic, hypotensive, and nervine, and a tea is made for various disorders toward those effects (Stephen and Ezkiel, 2006).

2.2 Chemical Constituents of *Annona* sp.

2.2.1 Nutritional value and chemicals of *Annona* species

A. muricata L. has quite high nutritional value. The white juicy pulp of the fruit is high in carbohydrates and sugars and fair amount of vitamin C, vitamin B1, vitamin B2, potassium and dietary fibre. However, it is poor in vitamin A.

Recent studies have supported many of *A. muricata*'s traditional medicinal uses and also showed that various parts of the tree contain acetogenins, which have been showen to be responsible for its myriad array of its medicinal attributes.

Annonaceous acetogenins are only found in the Annonaceae family (to which *Annona muricata L.* belongs). These chemicals in general have been documented with antitumorous, antiparasitic, insecticidal, and antimicrobial activities.

2.2.2 Previous study on phytochemical screening of *Annona* sp.

The evaluation requirement of the toxicity profile of *A. muricata* leaves extract was prompted by the increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicine. Herbal medicines have been receiving greater attention as alternatives to orthodox therapy, leading to their increase in demand (Crook, 2006).

Recently, there are lots of studies conducted on Annonaceae sp. on their leaves, bark, seeds, and fruits for their phytochemical constituents. In one of studies, a preliminary phytochemical analysis revealed the presence of secondary metabolites like tannins, steroid, cardiac glycosides, etc. were present in trace amounts in the leaves of *A. muricata* (Pathak *et al.*, 2010).

Other phytochemical analysis of the n-butanolic leaf extract of *A.muricata* revealed the presence of flavonoids, terpenoids, tannins, cardiac glycosides and reducing sugars. Whereas, the extract showed the absence of saponins, steroids, phlobatannins, oil and anthraquinones tested (Kumar *et al.*, 2009).

The phytochemical screening of the *A. muricata* different plant parts also showed the presence of flavonoids, terpenoids, reducing sugar, anthraquinone, tannins and cardiac glycosides. Phytoconstituents in the leaves of *A. muricata* L. contain an alkaloidal principle named 6-Hydroxyundulatine and other alkaloids (Vimala *et al.*, 2012).

In other study of *Annona squamosa*, the results of phytochemical screening of ethanolic extract, chloroform and water fractions of the plant revealed the presence of alkaloids, flavonoids, reducing sugars, saponins, steroids,

tannins and glycosides. These metabolites have been reported to possess antimicrobial activity (Yusha'u *et al.,* 2011). In particular the flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003; Yusha'u *et al.,* 2011).

In each studies there are different findings on the phytochemical constituents obtained, thus it is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. (Pathak *et al.*, 2010).

Phytochemical analysis helps detect the chemical constituents of plants extract in search of bioactive agents as basis for drug synthesis (*Ogbonnia et. al.*, 2009). The presence of saponins, condensed tannins and glycosides as the major constituents and trace amounts of flavonoids contribute immensely to the bioactivity of *A. muricata* and also to its usage in treating various diseases. These have included antioxidant activity (Adewole *et. al.*, 2009) as well as hepatoprotective effect and antibacterial agent by (Chukwuka *et. al*, 2011).

2.3 Biological Activities Properties of *Annona* sp.

2.3.1 Previous study on antimicrobial screening of *Annona* sp.

Along with the phytochemical screening of *Annona* sp., their antimicrobial properties also had been evaluated through screening process. In one of study, it was revealed that the aqueous extracts of *A. muricata* L showed an antibacterial effect against *S. aureus* and *V. cholerae*, but the antibacterial activity by the ethanol extracts of this plant was not demonstrated (Vieira *et al.*, 2010).

A study also had been conducted in which *A. muricata* extract was screened against Herpes simplex virus-1 (HSV-1) and clinical isolate (obtained from the human keratitis lesion) in order to check whether they inhibit the cytopathic effect of HSV-1 on vero cells which is the indicative of anti-HSV-1 potential. The minimum inhibitory concentration of ethanolic extract of *A. muricata* was found to be 1 mg/ml which shows that the *A. muricata* could be used as the potential antiherptic drugs (Isela *et. al.*, 2008).

In one studies of *Annona squamosa* antimicrobial properties, it was observed that water fraction were active against *S. pneumoniae* and α-haemolytic *streptococci* but inactive against the other test isolates while chloroform fraction was active against *S. aureus* and *S. pneumoniae* respectively but inactive against all other test isolates. In contrast, ethanolic extract was inactive against the other test isolates at the same disc concentration of 50µg (Yusha'u *et al.,* 2011).

2.4 Screening for Major Phytochemical Constituents

2.4.1 Alkaloid

Alkaloid occurs in plant as salts. They are found in seeds, barks, leaves, roots and other parts of plant. The extraction of the alkaloids is based on their basic character and solubility pattern. The bassist of alkaloid depends upon number of nitrogen items in the molecule, structure of the molecule and presence of other functional group.

The alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structure types, biosynthetic pathways, and pharmacological activities. Alkaloids have many other pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effects (quinidine, ajmaline, sparteine), antimalaria activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine) (Margaret and Micheal, 1998).

2.4.2 Flavonoid

Flavonoids are polyphenolic compounds that are ubiquitously present in practically all dietary plants, like fruits and vegetables. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, vasodilatory actions (Alan and Miller, 1996), radical scavenger and antileukemic. In the present study, the total phenol content & total flavonoidal content were determined and this in terms helps in gauging the antioxidant potential of the tuberous plant not only helping for establishing the phytochemical standardization but also in authentication of this drug.

2.4.3 Saponin

Saponins are secondary plant metabolites that occur in a wide range of plant species (Hostettmann and Marston, 1995). They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. The natural role of saponins in plants is thought to be protection against attack by pathogens and pets (Price *et al.* 1987; Morrissey and Osbourn, 1999).

2.4.4. Glycoside

Glycoside are condensation products of sugar and aglycon. These are soluble in water as well as alcohol. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications.

2.4.5 Tannin

Tannins are present in cell sap soluble in water and alcohol. They are classified into two major categories, the hydrolysable and condensed tannins (Koukoura and Nastis). They are distributed is different parts of plants depending upon the source of the however, tannins usually found in the bark but often in fruits and leaves. It is used as herbs since it possessed complex chemicals which act as an astringent, drawing the tissues together and contracting them and hence was useful for treating surfaces such as the inflamed mucous membranes characteristic of coughs and colds and bathing wounds.

2.5 Bacterial Strains Used - Gram positive and Gram negative Bacteria

2.5.1 *Staphylococcus aureus (S. aureus)*

S. aureus or also known as "golden staph" and Oro staphira, it is a facultative anaerobic Gram-positive coccal bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passagesis. It has been known for as long as we have

had medical literature, that it is a pathogen that capable to causing human infection. *S. aureus* secrets exotoxins called superantigens, which stimulate a large proportion of T cell that induce allergic reactions (for example, the release of inflammatory mediators such as leukotrienes and histamine) (Mustafa *et al.*, 1996).

S. aureus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis.

Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. In fact, when this microorganism enters the blood, it represents one of the most lethal human pathogens also because it is often characterized by multidrug resistance. It can survive for hours to weeks, or even months, on dry environmental surfaces, depending on strain.

2.5.2 *Escherichia coli (E. coli)*

E. coli is a Gram-negative, is a rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for people making product recalls. *E. coli* is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation. It is the leading pathogen causing urinary tract infections (Wagenlehner *et al*, 2008). It is also among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans. *E. coli* is also the most common cause of food and water-borne human diarrhea worldwide and in developing countries, causing many deaths in children under the age of five years (Turner *et al.*, 2006).

2.5.3 *Salmonella enteritidis (S. enteritidis)*

S. enteritidis are presented separately from other sero-types of Salmonella because these bacteria are often specifically cited in zoonosis control legislation and also because there are differences in the epidemiology as compared to other salmonellae, which are the predominant sero-types associated with human disease in most

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