EXTRACTION OF BIO ACTIVE COMPOUND FROM ACACIA SEYAL GUM AND *IN VITRO* EVALUATION OF ANTITUMOR ACTIVITY AGAINST LEUKEMIA CELL LINES

BY

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ABSTRACT

The current chemical treatment for leukemia is not effective and often has negative health consequences. Alternative herbal medicine is rising in popularity because it is more compatible with the human body and has fewer side effects. While alternative herbal therapies have been shown to reduce symptoms in traditional medicine, many of them have not been scientifically validated. As a result, it is critical to keep looking for ways to improve its efficiency against cancer cells. Due to their minimal or close to zero toxicity to normal tissues, herbal therapies contain several phytochemicals that have safe anticancer activity comparable to standard cancer treatments. *Acacia seyal* gum (AS), known as arabic gum, is a well-known traditional medicinal therapy with various restorative characteristics. In this study, the yield of AS extract was optimised using Response Surface Methodology (RSM) followed by chemical characterization of a bioactive compound for the last yield, and then the therapeutic potential of AS crude extracts against leukemia cancer cells was investigated in *vitro*. RSM optimized the AS extraction using Ultrasound-Assisted Extraction (UAE), considering the effect of three parameters on the yield of AS extracts, namely time, temperature, and solid-liquid ratio, and applying the optimum conditions suggested by previous studies. Raman spectroscopy (RS), Fourier transform infrared (FTIR) spectroscopy, and GC-TOFMS analyses were used to characterize AS crude hydroethanolic extract bioactive components. The anti-leukemic activity of AS crude extracts was investigated *in vitro* against tumoral Jurkat, T-cell ALL, and K562 leukemia cancer cell lines, as well as non-tumoral WIL2NS cells. The optimum extraction conditions resulted in a yield of 75.89 % after 45 min of extraction at a temperature of 40°C and a solid/liquid ratio of 1:25g/mL. Many phytoconstituents, such as polyphenols, tannins, saponins, coumarins, flavonoids, and terpenoids, were discovered through phytochemical screening for the optimized extract. Anticancer compounds were found in crude AS after GC-TOFMS analysis, such as Lycopene, Oleanolic acid, and carotene. The cytotoxicity assays of AS and Taxol revealed that both treatments inhibited the growth of K562 and Jurkat T cancer cells and exhibited the lowest IC_{50} for K562 and Jurkat T cancer cells (IC_{50}=10g/ml and IC_{50}=5.11g/ml respectively), while it showed an insignificant inhibition effect on WIL2NS cells (IC_{50}=80 g/ml). The AS crude ethanol extract showed a low IC_{50} value. In conclusion, these findings show that AS could be a good candidate to be developed into a new natural anticancer agent and to tap the potential benefits of using AS as a novel treatment approach for leukemia.

خلاصة البحث

اليوم، العلاج الكيميائي للسرطان ليس فعالًا بدرجة كافية وعادة ما يكون له آثار جانبية ضارة على صحة الإنسان بينما يستمر الطب البديل بالنمو بشكل جيد لأنه متوافق مع جسم الإنسان مع آثار جانبية أقل. تُستخدم الأدوية العشبية البديلة كبديل للعلاج الطبي في الطب التقليدي حتى أنها أثبتت فعاليتها في التخفيف من الأعراض رغم أنه لم تثبت بشكل علمي بعد. لذلك، كان من المهم الاستمرار بالبحث لاثبات فعاليتها على الخلايا السرطانية.تحتوي هذه العلاجات العشبية على العديد من المواد الكيميائية النباتية التي تمتلك تأثيرا مضادا للسرطان وامناحين يتم مقارنته بعلاجات السرطان الشائعة بسبب قله سميته والتي تكاد تكون صفرا على الخلايا الطبيعية. صمغ الأكاسيا سيال (AS) المعروف بالصمغ العربي والذي يعتبر من أشهر العلاجات الطبية التقليدية لما له من خصائص علاجية مختلفة. في هذا الاقتراح، سيتم البحث في القدرة العلاجية لمستخلصات ASG ضد خلايا سرطان الدم في المختبر وهذا بعد تحسين طريقة استخلاصAS من خلال منهجية الاستجابة السطحية (RSM) والتي يتبعها القيام بالفحص الكيميائي للمستخلص. تم تحسين كمية AS المستخلصة بواسطة الموجات فوق الصوتية(UAE) عبر منهجية الاستجابة السطحية RSM مع مراعاة تأثير العوامل الثلاثة، وهي الوقت ودرجة الحرارة ونسبة المادة الصلبة إلى السائل على عملية الاستخلاص، مع اتباع الظروف المثلى التي توصلت اليها الدراسات السابقة لزيادة كمية وكفاءة مستخلص AS.كما سيتم فحص المركبات النشطة بيولوجيًا للمستخلص المائي الإيثانولي الخام لل ASG باستخدام مطياف رامان (RS)، والتحليل الطيفي للأشعة تحت الحمراء(FTIR) وتحليل GC-TOFMS.سيتم اختبار فعالية مستخلص AS الخام كمضاد للسرطان الدم على خلايا T- Jurkat (cell ALL)وK562 التابعة لخلايا سرطان الدم وخلايا WIL2NS الغير سرطانية. أعطت ظروف الاستخلاص المثلى مستخلص ASبنسبة تصل الى 75.89٪ عند استخلاصه في وقت 45 دقيقة ودرجة حرارة 40 درجة مئوية ونسبة صلبة/سائلة 1:25 جم/مل. في حين كشف الفحص الكيميائي النباتي لمستخلص ASG المحسن عن وجود العديد من المواد الكيميائية النباتية مثل البوليفينول والصابونين والكومارين والفلافونويد والتربينويد. وقد كشف تحليل GC-TOFMS عن وجود مركبات مضادة للسرطان مثل الليكوبين وحمض أولينوليك وبيتا كاروتين. علاوة على ذلك أظهر فحص السمية لخلايا سرطان الدم لـ AS وTaxol أن كلا العلاجين يثبطان نمو الخلية السرطانية K562 وJurkat T وأظهرت أقل IC50 تجاه خلية السرطان K562 وJurkat T حيث IC_{50} = 10 ميكروغرام/مل و5.11 = IC50 ميكروغرام/مل على التوالي في حين تأثير تثبيط غير ملحوظ تجاه خلايا WIL2NS الغير سرطانية، IC = 80 ميكروغرام/مل. في الختام، توضح هذه النتائج أن AS قد يكون مرشحًا جيدًا لمزيد من البحوث العلمية لتطوير مركب طبيعي فعال لعلاج سرطان الدم، وكذلك يدعم بحوثا اخرى تضيف مزايا جديدة لاستخدام AS كعلاج جديد لسرطان الدم.

APPROVAL PAGE

I **certify** that I have supervised and read this thesis and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science (Biotechnology Engineering).

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DECLARATION

I hereby declare that this thesis is the result of my investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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All glory is due to Allah, the Almighty, whose Grace and Mercies have been with me throughout my life.

In the Name of Allah, the Most Gracious, Most Merciful and Salam upon His Messenger, Prophet Mohammed (SAW).

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LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
±	Plus-Minus
<	Less than
g	Gram
mg/L	Milligram per Milliliter
µg/mL	Microgram per Millilitre
R^2	Coefficient of Determination

LIST OF ABBREVIATION

AG	Arabic Gum
ALL	Acute Lymphocytic Leukemia
AML	Acute Myelogenous Leukemia
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
A-549	Human Lung Carcinoma Cells
AS	*Acacia seyale*
BMT	Bone Marrow Transplantation
CML	Chronic Myelogenous Leukemia
CLL	Chronic Lymphocytic Leukemia
CV	Coefficient of Variation
(CV)	Cell Viability
DF	Dilution Factor
DMSO	Dimethyl Sulfoxide
DOE	Design of Experiments
et al	Et alia – and Others
etc	and other types
EtOH	Ethanol
FCCCD	Face-Centered Central Composite Design
FTIR	Fourier Transform Infrared
GCMS	Gas Chromatography-Mass Spectrometry
GC-TOF-MS	Gas Chromatography Time-Of-Flight Mass Spectrometry
K562	Chronic Myelocytic Leukemia
kHz	Kilohertz
HepG2	Human Hepatoma Cell Line
HCT-116	Human Colon Cancer Cell Line
HL-60	Human Leukemia Cell Line
IFN-a	Interferon Alpha
IC_{50}	Half Maximal Inhibitory Concentration
Jurkat	Acute Lymphoblastic Leukaemia
LOF	Lack of Fit
MAE	Microwave-Assisted Extraction
MCF-7	MCF-7 Human Breast Adenocarcinoma Cell Line
MTT	MTT 4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2- (4-sulfophenyl)-2H-tetrazolium)
OFAT	One-Factor-at-a-Time
PBS	Phosphate Buffered Saline
PEE	Pressurized Fluid Extraction
PC-3	Human Prostate Cancer
RSM	Response Surface methodology
RT	Retention Time
rpm	Rotation Per Minute
SD	Standard Deviation
SE	Standard Error

UAE	Ultrasound-Assisted Extraction
WST	1;2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2H-tetrazolium, monosodium salt)
WIL2 NS	Human B-lymphocyte-Derived Cell
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-Tetrazolium-5-Carboxanilide

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Leukemia is a cancerous tissue-forming disease that triggers the excessive production of immature blood cells entering the bloodstream (Pal et al., 2016). The proliferation of leukemic cells occurs primarily in the bone marrow and some in the lymphoid tissue (Naumburg & Jonzon, 2002).

Leukemia has been recognised as a disease since 1845 when a report on a patient who died of the disease revealed an abnormally large number of blood cells. According to the World Health Organization, death from cancer is expected to grow 104% worldwide by 2021 (Das & Hospital, 2020). Currently, leukemia is among the most prevalent types of cancer in various parts of the world. In the US, leukemia may cause mortality of an estimated 58,100 people in 2018, is found commonly in adolescents and young adults below 20 years of age (Steven et al., 2018). In Malaysia, for both males and females, the incidence rates of lymphoid leukemia were 2.8 and 1.7 per 100,000 populations, respectively, while the incidence rates of myeloid leukemia for both males and females were 3.0 and 2.7 per 100.000 populations, respectively (Saedi et al., 2014).

Leukemia patients are treated with a combination of treatments, chemotherapy (as the initial treatment), antibiotics, blood transfusion, radiation therapy, and bone marrow transplantation. While these treatments have improved the survival rate of patients with leukemia, some of them are difficult to implement. Thus, there is a need to look for alternative remedies to cure leukemia. Therefore, the use of such medical plants has increased gradually for medicinal purposes. As a result, these natural plant compounds should be investigated to learn more about their qualities, protection, and

efficacy. It would result in reduced use of most common therapies for cancer such as radiation therapy, surgery, and chemotherapy. Some cancer cells show resistance to chemotherapy treatment, thus, prompting a need to discover new cytotoxic drugs that function through distinctive mechanisms.

In developing countries, using therapeutic herbs as cures for leukemia is gaining popularity. The National Cancer Institute collected approximately 35,000 plant samples from 20 countries and is currently evaluating approximately 114,000 extracts for anticancer activity (Prakash et al., 2013).

Plants are well-known as a significant source of biologically active materials. Numerous studies showed that constituents in plants demonstrated various biological and pharmacological activities. For thousands of years, plants were utilized for the treatment of multiple diseases. Numerous types of herbs and fruits have chemicals that behave as anticancer through diverse modes of action, such as inhibitors of carcinogen synthesis, blockers of carcinogen interaction, and suppressors of tumour advancement.

Acacia seyal (AS)tree belongs to the *Mimosaceae* family of the genus *acacia*. It is usually distributed in tropical Africa, mainly found in both western and eastern Africa, and also on the Arabian Peninsula (Awad et al., 2018).

AS is well-known for its therapeutic properties and scientific novelty originating from the *Acacia* genus. It contains various active chemical components such as tannins, flavonoids, polysaccharides, alkaloids, terpenes, fatty acids, anthocyanins, and saponins.AS is well-known for its therapeutic properties and scientific curiosity stemming from the *Acacia* genus, which contains various families of active chemical components (Rather et al., 2015).

AS gum powder mainly used as a natural gum is made of hardened sap from *acacia* trees as shown in Figure 1.1.

Figure 1.1 The *Acacia seyal* gum powder

The gum of AS tree is used for a variety of therapeutic applications, including as a therapeutic component in medications used to treat throat and stomach inflammation, as well as a film-forming substance in peel-off skin masks, colds, haemorrhage, jaundice, headache, burns, and chronic renal failure (Andrew et al., 2012). Various chemical compounds were isolated and characterized from AS tree such as flavonoids, particularly flavanols' quercetin which showed cytotoxic activity *in vitro* (Murakami et al., 2008). Therefore, this study investigates the therapeutic potential of the inhibitory effect of crude extract of AS against leukemia cell lines that may be a promising alternative for new, safe, biodegradable, and renewable sources of anticancer drugs with a high therapeutic index.

1.2 PROBLEM STATEMENT AND SIGNIFICANCE OF THE STUDY

Leukemia is among the most common causes of death globally and the leading cancer death in children. It is not a solid tumor like other cancer that can be surgically removed. For this reason, therapy is more complex (M. Mohammadian et al., 2018).

Many treatments for leukaemia such as radiotherapy, chemotherapy, blood transfusion, bone marrow transplantation or a combination are frequently accompanied by severe side effects. However, more people are suffering from the side effects of chemotherapy every day, which include hair loss, immune system weakness, loss of appetite, hormonal fluctuation, anxiety, depression, and death. Some people die not because of cancer but because they are unable to survive with the side effects.

Furthermore, these drugs are expensive for patients. Moreover, these treatments are limited in bioavailability, toxic to normal cells, non-specific, fast clearance and cannot prevent cancer metastasis. Therefore, the search for new alternative anticancer drugs from plants remains one of the most challenging research areas.

Herbal medicine more effective for a long term, chronic illness because it is well tolerated and contains so many effective compounds that work together that have less severe side effects than modern methods of cancer treatment. AS possesses various pharmacological effects, including anti-cancer (Revathi et al., 2017), anti-inflammatory (Kamal et al., 2018), antioxidant (Daoub et al., 2018), anti-diabetic (Hegazy et al., 2013), and antimicrobial activity (Alawi et al., 2018), attached to its phytoconstituents. Most AS phytochemical compounds are extracted through conventional extraction methods, with many disadvantages, including a large amount of extractant waste, time-consuming and potential toxicity emissions during extractions.

Modern extraction method is commonly used because it saves time and increases the extract yield's quality and keeps the extract's biological properties.

It is strongly believed that the discovery of anticancer properties from AS could lead to the development of new anticancer drugs that possess both chemo preventive and chemotherapeutic properties, which are safer and more effective without weakening the patients' health.

1.3 RESEARCH HYPOTHESIS

A natural medicinal plant can consume an alternative treatment that may not have any risk on individuals because of its potential pharmaceutical importance. Nowadays, many research and studies focus on the extraction, separation, purification, and phytochemical screening of medicinal plants and evaluate their therapeutic advantages to be used as an alternative treatment.

Today, traditional knowledge and practices have contributed to modern medicine. Recent records reported that medicinal herbs are used by 80% of people living in rural areas as the principal health care systems (Sakarkar & Deshmukh, 2011). Different parts of AS tree have been reported and used traditionally to treat many diseases (Rosnah et al., 2015). AS extracts have many pharmacological properties due to their many useful phytochemicals, and many research explained their cytotoxicity against different cancer cell lines for example breast cancer (MCF-7) (Elnour et al., 2020). Therefore, from this study results, the crude extract of AS may be suitable to be used as a chemoprotective medicine and a potential new anticancer agent, especially against leukemia.

Ultrasound-assisted extraction (UAE) is considered as a "clean technology" due to its excellent advantages for extracting bioactive compounds from medicinal plants (Dzah et al., 2020). The reasons for using UAE are mainly because of reduction in extraction and processing time and energy. Optimizing the condition of UAE can be

more useful and lead to an optimum yield of extract. UAE will be approved to be an environment-friendly extraction technique for AS gum and other medicinal herbs.

Hence, the UAE method could be a valuable and suitable method to get more bioactive anticancer compounds from AS that could be safer for human consumption for leukaemia cancer treatment.

1.4 RESEARCH QUESTIONS

1. What is the optimum UAE condition to extract high quality and quantity of bioactive compounds from AS?
2. What are the bioactive compounds in AS gum ethanol extract?
3. What is the effective concentration of the AS extract on WIL2NS, K562, and Jurkat T human leukaemia cell lines that cause 50% inhibition of cell growth and proliferation?
4. What is the relation between the identified compound and the anticancer biological activity against leukemia cancer cell lines?

1.5 RESEARCH OBJECTIVES

Based on the current treatment pattern using natural sources, this study aims to explore a new procedure of extraction for AS to improve the yield by defining the most effective extraction condition. Screening of AS extract bioactive compound will be carried out to clarify the reason for its cytotoxicity effect to be offered as a new treatment for one of the most prevalent cancers in childhood leukaemia. The main objective is to discover the cytotoxicity of the crude AS extract on Jurkat (T-cell ALL), K562, and WIL2-NS cell lines. The study aimed to achieve the following objectives:

1. To extract AS using UAE as a method of extraction and identify the optimum condition of extraction to get a high yield of AS.
2. To characterize AS bioactive compounds through Raman spectroscopy, FTIR spectroscopy, and GC-TOFMS chemometric methods.
3. To investigate the effects of AS crude extract on WIL2NS, K562 and Jurkat -T human leukaemia cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay.

1.6 RESEARCH METHODOLOGY

The research began with the extraction and determination of the optimised processing conditions of UAE to get a high yield based on a Face-Centred Central Composited Design (FCCCD) under response surface methodology (RSM) following the method of Fan et al. (2020). Next, the qualitative identification of the most bioactive compounds available in AS using Raman spectroscopy, FTIR spectroscopy, and GC-TOF-MS analysis. Finally, the crude extract was tested *in vitro* for antiproliferative effects against WIL2NS, K562, and Jurkat-T human leukaemia cell lines using the MTT assay, with Taxol as a positive control. Figure 1.2 summarizes the methodologies used in this study.

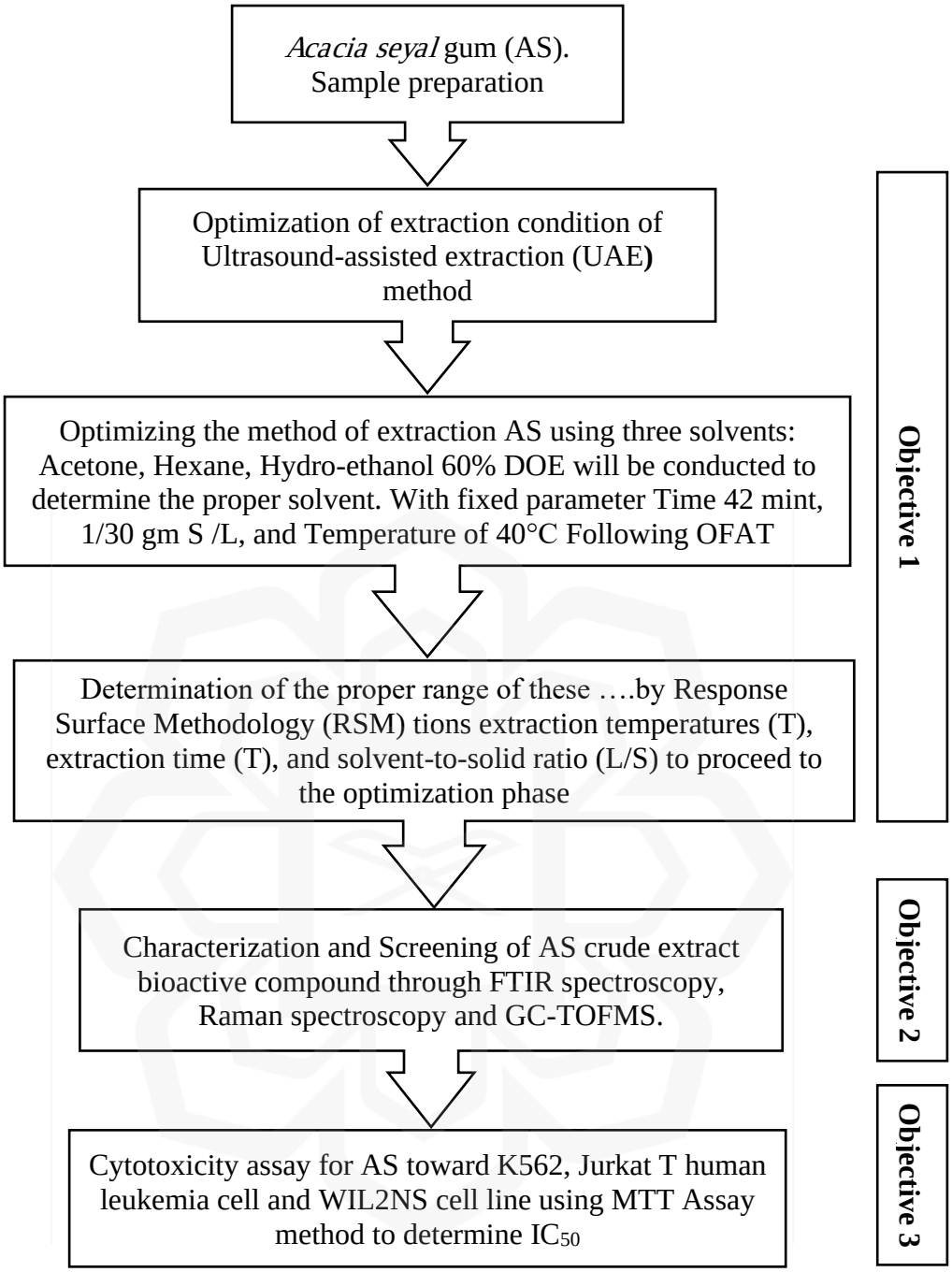

Figure 1.2 The flowchart that describes the general methodology applied in this research

1.7 SCOPES OF STUDY

1. The scope of this research started with extracting and optimising the extraction conditions of the UAE to maximise the yield. Firstly, OFAT optimization was carried out to identify the best solvent for extraction by comparing the AS yield (%) of three different organic solvents.