

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 optimized 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 IC₅₀ for K562 and Jurkat T cancer cells (IC₅₀=10g/ml and IC₅₀=5.11g/ml respectively), while it showed an insignificant inhibition effect on WIL2NS cells (IC₅₀=80 g/ml). The AS crude ethanol extract showed a low IC₅₀ 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 فعالاً 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 تأثيراً مضاداً للسرطان وامنحين يتم مقارنته بعلاجات السرطان الشائعة بسبب قلة سميته والتي تكاد تكون صفراً 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 استخلاص AS 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 إليها الدراسات السابقة لزيادة كمية وكفاءة مستخلص AS. كما سيتم فحص المركبات النشطة بيولوجياً 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 AS بنسبة 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 ل AS وTaxol أن كلا العلاجين يشيطان نمو الخلية السرطانية K562 و Jurkat T وأظهرت أقل IC50 تجاه خلية السرطان K562 و Jurkat T حيث IC50 = 10 ميكروغرام/مل و IC50 = 5.11 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 مرشحاً جيداً لمزيد من البحوث العلمية لتطوير مركب طبيعي فعال لعلاج سرطان الدم، وكذلك يدعم بحوثاً أخرى تضيف مزايا جديدة لاستخدام AS كعلاج جديد لسرطان الدم.

APPROVAL PAGE

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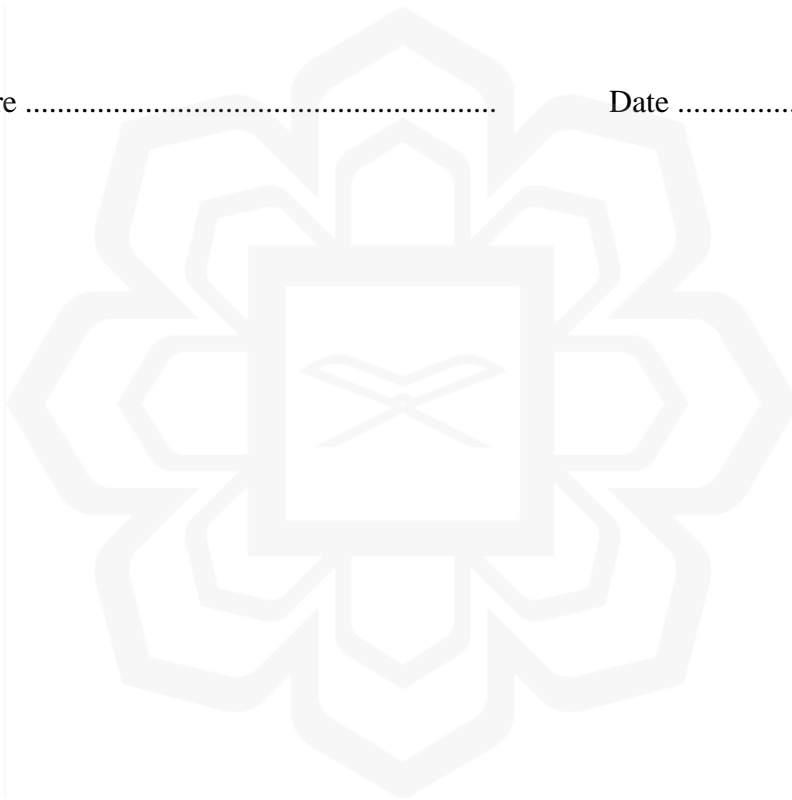
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All glory is due to Allah, the Almighty, whose Grace and Mercies have been with me throughout my life.

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TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval Page.....	iv
Declaration.....	v
Copyright Page.....	vi
Acknowledgements.....	vii
Table of Contents	viii
List of Tables	xi
List of Figures	xii
List of Symbols	xiv
List of Abbreviations	xv
CHAPTER ONE: INTRODUCTION	1
1.1 Background of The Study	1
1.2 Problem Statement and Significance of The Study	4
1.3 Research Hypothesis	5
1.4 Research Questions	6
1.5 Research Objectives	6
1.6 Research Methodology	7
1.7 Scopes of The Study	8
1.8 Thesis Organization.....	9
CHAPTER TWO: LITERATURE REVIEW.....	11
2.1 Introduction	11
2.2 <i>Acacia seyal</i> Gum	12
2.2.1 Botanical Description and Distribution	12
2.2.2 Ethnomedicinal Use.....	13
2.2.3 Chemical Constituents of <i>Acacia seyal</i> Gum (AS)	14
2.2.4 Pharmacological Property of <i>Acacia seyal</i> Gum (AS).....	19
2.2.4.1 <i>Antibacterial and Antimicrobial Properties</i>	19
2.2.4.2 <i>Antioxidant Properties</i>	20
2.2.4.3 <i>Antidiabetic Properties</i>	21
2.2.4.4 <i>Anti-inflammatory Properties</i>	21
2.2.4.5 <i>Anticancer Properties</i>	22
2.3 Leukemia Disease.....	23
2.3.1 Common Types of Leukemia	25
2.3.1.1 <i>Acute Myelogenous Leukemia (AML)</i>	26
2.3.1.2 <i>Chronic Myelogenous Leukemia (CML)</i>	26
2.3.1.2.1 <i>K562 Cell line</i>	27
2.3.1.3 <i>Acute Lymphocytic Leukemia (ALL)</i>	27
2.3.1.3.1 <i>Jurkat (T-cell ALL) Cell Line</i>	28
2.3.1.4 <i>Chronic Lymphocytic Leukemia (CLL)</i>	28
2.3.2 Treatment of Leukemia	29
2.3.2.1 <i>Chemotherapy</i>	30
2.3.2.2 <i>Radiation Therapy</i>	30

2.3.2.3 Stem Cell Transplant.....	30
2.3.2.4 Alternative Leukemia Treatment from the Medicinal Plant...	31
2.4 Extraction of Bioactive Compounds	34
2.4.1 Ultrasonication Assisted Extraction (UAE)	35
2.4.2 Solvent for Extraction	37
2.5 Characterization and Analysis of Extract Bioactive Compound	37
2.5.1 Fourier Transform Infrared (FTIR) Spectroscopy.....	38
2.5.2 Raman Spectroscopy	39
2.5.3 Gas Chromatography Time-Of-Flight Mass Spectrometry Analysis.	39
2.5.4 Response Surface Methodology (RSM).....	40
2.6 In-Vitro Mammalian Cell Culture Assay	43
2.6.1 Cytotoxicity Assay Method.....	43
2.6.1.1 Antiproliferative Assay (MTT Assay).....	44
2.7 Research Gap	45
2.8 Summary.....	47
CHAPTER THREE: MATERIALS AND METHODS	48
3.1 Introduction	48
3.2 Materials and Equipment.....	49
3.2.1 Raw Material	49
3.2.2 Cell Lines	50
3.2.3 Chemicals and Reagents.....	50
3.2.4 Consumable Items	50
3.2.5 Laboratory Apparatus and Equipment	51
3.2.6 Computer Application Programs and Software.....	51
3.3 Methodology.....	52
3.3.1 Sample Preparation.....	52
3.3.2 <i>Acacia seyal</i> Gum Extraction	52
3.3.3 Ultrasound-Assisted Extraction (UAE) Optimization.....	54
3.3.4 Statistical Analysis and Statistical Optimization Experiments	54
3.3.4.1 One-Factor-at-a-Time (OFAT) Experimental Design	55
3.3.4.2 Response Surface Methodology (RSM) Experimental	
Design	55
3.3.4.3 Validation of the Experimental Model	57
3.3.5 Characterization and Analysis of Extract Bioactive Compound.....	57
3.3.5.1 Raman spectroscopy	57
3.3.6 Fourier Transform Infrared Spectroscopy (FTIR).....	58
3.3.6.1 Gas Chromatography Time-Of-Flight Mass Spectrometry	
(GC-TOF-MS) Analysis	59
3.4 Preparation of Solutions and Reagents	60
3.4.1 Preparation of Cell Culture Medium	61
3.4.2 Preparation of Complete Media	61
3.4.3 Preparation of Phosphate Buffered Saline (PBS).....	62
3.5 Evaluation of Cytotoxic Effects	62
3.5.1 Cell Culture	62
3.5.2 Thawing Frozen Cells.....	62
3.5.3 Cell Revival	63
3.5.4 Subculture of Suspension Cell Lines.....	63
3.5.5 Cell Counting	64

3.5.6 Cryopreservation of Cells.....	64
3.5.7 Stock Concentration Preparation	65
3.5.8 Cell Viability (MTT Assay)	65
3.6 Estimation of the Inhibitory Concentration At 50% (IC ₅₀)	66
3.7 Cell Morphology Observation	66
3.8 Statistical Analysis	67
3.9 Summary.....	67
CHAPTER FOUR: RESULTS AND DISCUSSION	68
4.1 Introduction	68
4.2 Extraction of <i>Acacia seyal</i> Gum (AS)	68
4.3 Optimisation of Solvent by OFAT	69
4.4 Optimisation of <i>Acacia seyal</i> Gum by RSM.	71
4.4.1 Analysis of Variance	72
4.4.2 Analysis of Variance (ANOVA) of the Extraction Yield	73
4.4.3 Test for Significance of the Regression.....	75
4.4.3.1 <i>The Interaction Response Effects</i>	75
4.5 Validation of the Optimized Parameter	78
4.6 Qualitative Phytoconstituent Identification <i>Acacia seyal</i> Gum.....	79
4.6.1 Raman Spectroscopy	79
4.6.2 Fourier Transform Infrared (FTIR)Spectroscopy	83
4.6.3 Chemical Profile of Ethanol AS Extract Using GC-TOFMS.....	86
4.7 DISCUSSION.....	90
4.8 Antiproliferative Activities.....	92
4.8.1 Evaluation of the Antiproliferative Activity of Crude <i>Acacia seyal</i> Gum (AS).....	92
4.8.1.1 <i>Cytotoxicity Assay Against WIL2NS Cells</i>	93
4.8.1.1.1 <i>Morphological changes</i>	93
4.8.1.1.2 <i>Cytotoxicity Assay Against JURKAT Cells</i>	94
4.8.1.1.2.1 <i>Morphological changes</i>	95
4.8.1.1.3 <i>Cytotoxicity Assay Against K526 Cells</i>	96
4.8.1.1.3.1 <i>Morphological changes</i>	97
4.9 DISCUSSION.....	98
CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS	101
5.1 Conclusion	101
5.2 Recommendations	103
REFERENCE	105

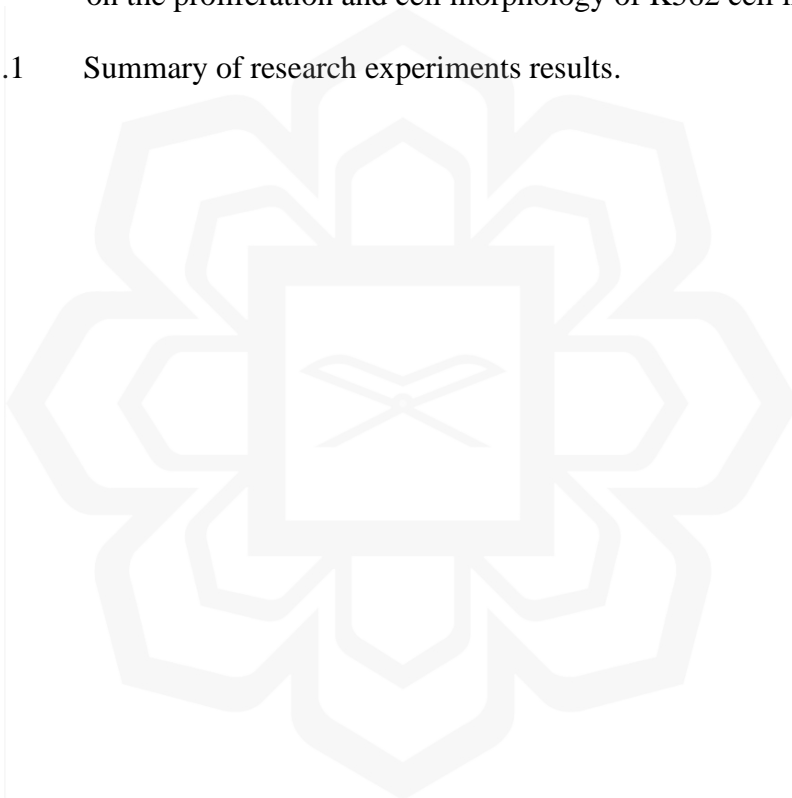
LIST OF TABLES

Table 2.1	Structures of the Identified Compounds From <i>Acacia seyal</i> Gum (AS)	17
Table 2.2	List for Plant Attributed to Potential Antileukemic Effect	33
Table 2.3	List for Plant-derived antileukemia agents	33
Table 2.4	Comparison of various extraction methods for natural products	34
Table 2.5	Summary of the Research Published on Different UAE Plant Extraction Optimized by RSM	42
Table 3.1	Experimental Design and Levels of Independent Process Variables	56
Table 3.2	Experimental Design for Optimization of Extraction Parameters using RSM	56
Table 4.1	FCCCD Experimental Design and Result of the Responses using RSM	72
Table 4.2	ANOVA for Yield (%) Fitted Quadratic Model of Extraction Conditions	74
Table 4.3	The Analysis of the Model Fitting	75
Table 4.4	<i>Acacia seyal</i> Gum Chemical Compound Wavenumber Assigned using Raman Spectroscopy	82
Table 4.5	Characteristic Absorption Band Analysis Results of AS Extract Using FTIR Spectrophotometer	85
Table 4.6	List of Compounds of <i>Acacia seyal</i> Gum Ethanolic Extracts Observed in GC-TOFMS with their Biological Activity	87

LIST OF FIGURES

Figure 1.1	The <i>Acacia seyal</i> gum powder	3
Figure 1.2	The flowchart describes the general methodology applied in this research	8
Figure 2.1	The shapes of arabis gum (pre-harvest) and commercial material (post-harvest)	13
Figure 2.2	Structure of arabic gum	16
Figure 2.3	Medicinal properties of <i>Acacia seyal</i> gum(AS)	21
Figure 2.4	Anatomy of the bone (adopted from the National Cancer Institute Web page)	24
Figure 2.5	Schematic diagram of cell cytotoxicity assays	44
Figure 3.1	Flow diagram of the major experiments used in the research	49
Figure 3.2	Water bath sonicator	51
Figure 3.3	Flow diagram of <i>Acacia seyal</i> gum Ultrasound-Assisted Extraction	53
Figure 4.1	Effect of different solvents on <i>Acacia seyal</i> gum (AS) extraction yield	70
Figure 4.2	3D plots representing the effects (A) extraction time and temperature, (B) time and solid–liquid ratio, and (C) temperature and solid–liquid ratio on the yield. Where A, extraction time, B. temperature, C, solid-to-solvent ratio.	77
Figure 4.3	Typical Raman spectrum of <i>Acacia seyal</i> gum powder sample	80
Figure 4.4	F TIR spectra in the region of 400-4000 cm^{-1} for <i>Acacia seyal</i> gum powder sample	83
Figure 4.5	The cell viability percentage of WIL2NS cells lines vs different concentrations of Taxol treatment and ethanol extract of <i>Acacia seyal</i> gum (AS).	94
Figure 4.6	Effect of A; 60% ethanol extract of <i>Acacia seyal</i> gum (AS), and B; Taxol on the proliferation and cell morphology of WIL2NS cells lines	95

Figure 4.7	The cell viability percentage of Jurkat cell Lines vs different concentrations of Taxol treatment and ethanol extract of Acacia seyal gum (AS).	95
Figure 4.8	Effect of A; 60% ethanol extract of Acacia seyal gum (AS), and B; Taxol on the proliferation and cell morphology of Jurkat cells.	96
Figure 4.9	The cell viability percentage of K652 cell lines vs different concentrations of Taxol treatment and ethanol ext of Acacia seyal gum (AS).	97
Figure 4.10	Effect of A; 60% ethanol extract of Acacia seyal gum (AS), and B; Taxol on the proliferation and cell morphology of K562 cell lines.	98
Figure 5.1	Summary of research experiments results.	102



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	<i>Acacia seyal</i>
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- α	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 Compositated 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.

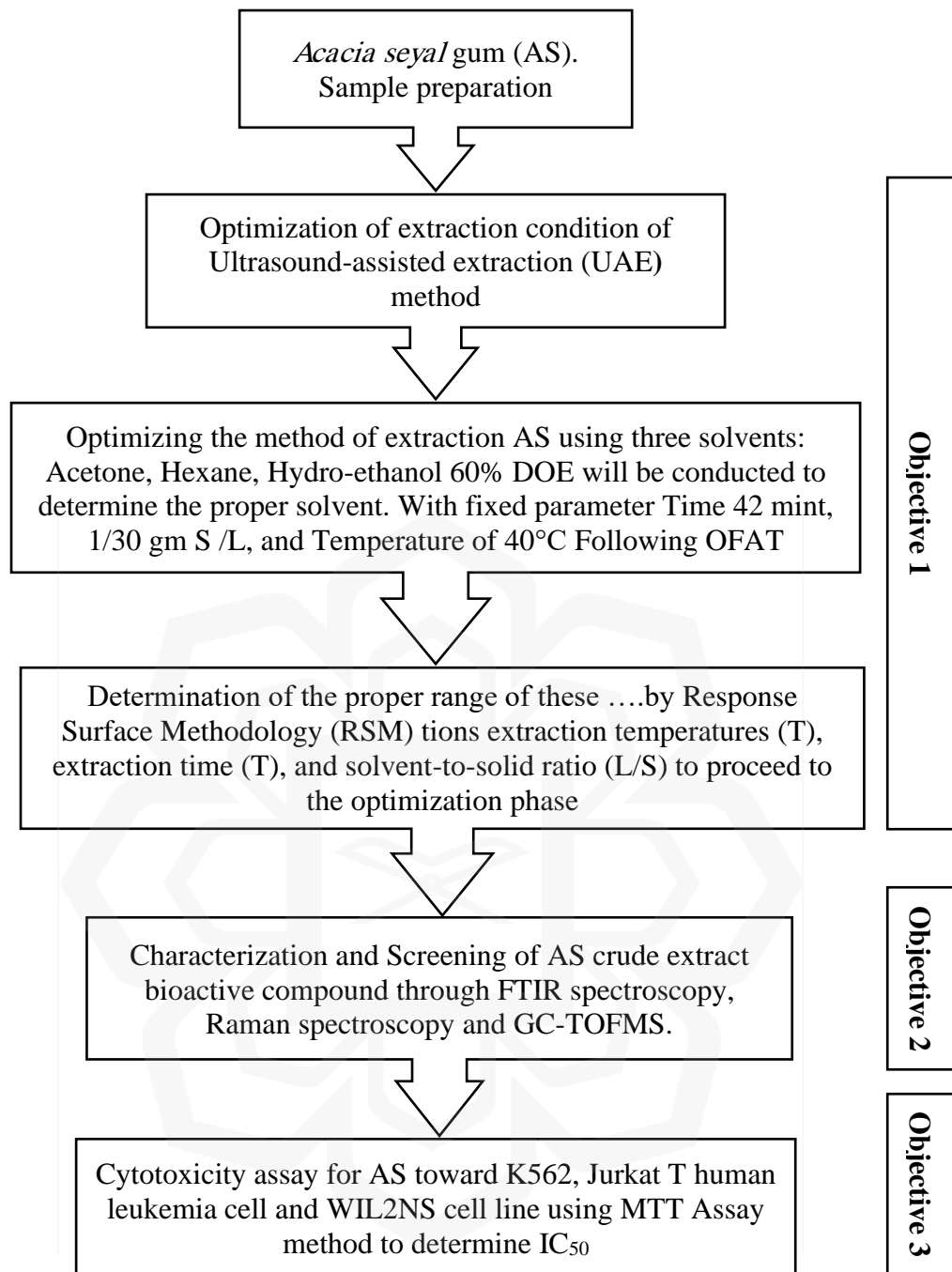


Figure 1.2 The flowchart that describes the general methodology applied in this research

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.