

CHARACTERIZATION AND OPTIMIZATION OF  
BIOACTIVE COMPOUNDS EXTRACTED FROM GUM  
ARABIC VIA ULTRASONIC ASSISTED TECHNIQUE

BY

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## ABSTRACT

Gum Arabic is a natural antioxidant, which comes from Acacia complex group of gums (ACGG). It is rich in dietary fibres and polyphenolics compounds that can support healthy living due to its antioxidant activity (AA). Despite its rich AA and medical benefits, the research on the ACGG antioxidant extraction has not received the attention it deserves from researchers and governments. Therefore, the aim of this study is to create an optimized extraction conditions that will enhance antioxidant activity and effectiveness. To achieve this aim, the first experiment was conducted to extract the ACGG active compounds using methanolic crude extraction (MCE) method, and to determine the physiochemical properties of the extracts, e.g. flavonoids, phenolic compounds, moisture content, pH, metal profile and amino acid profile. Meanwhile, the second experiment was carried out to optimize the methods and extraction conditions to improve the AA using DOE and PCA. The third experiment was conducted to study the antioxidant materials regarding crude extract and its active sub-fractions using the optimized conditions, and the last experiment was carried out to investigate the anti-inflammatory of the ACGG methanolic crude extracts and active fractions (MF and AF) on Albino rats (*in-vivo*) and the antiproliferative activity against breast adenocarcinoma (MCF-7), colon adenocarcinoma (HCT-116), and prostate cancer (PC3) cell lines using *in-vitro* assay. Results from the extraction indicated a significant number of flavonoid compounds and phenolic compounds (amongst others) in both ACGG ( $6540 \pm 3.46 \mu\text{g}/100\text{g}$ ) and PBMT from ( $2560 \pm 3.49 \mu\text{g}/100\text{g}$ ) to ( $2710 \pm 4.04 \mu\text{g}/100\text{g}$ ), using HPLC analysis. The individual flavonoid detected was quercetin. For the phenolic acids, the active compound were identified in both the ACGG and the Prebio-T were caffeic acids and p-coumaric, p-hydroxybenzoic, and ferulic acid with caffeic acid being the most predominant phenolic compounds in the ACGG (lateritic soil sample:  $401770 \pm 3.52 \mu\text{g}/100\text{g}$ ; clay soil samples:  $77580 \pm 5.20 \mu\text{g}/100\text{g}$ ). The optimization result showed that the maximum AA and yield of extract (predicted by Design Expert software 7.00) were 11.10% and 15.56% for *Acacia seyal* gum (ASG) and Prebio-T (PTC), respectively, using the ultrasonic extraction; and methanol at experimental temperature conditions of  $43^\circ\text{C}$ , power of 40 kHz, for 3 hrs. Furthermore, the GC-MS/MS results of the MCE, MF and AF of both ASG and PTC confirm the presence of a total of 57 bioactive compounds (BCs). Compared to the amounts of the same BCs were almost doubled in PTC methanol crude extract (MCE). The *In-vivo* results (i.e. acute inflammatory test) under control conditions in the laboratory at 300 mg/kg dosage of both MCE of ASG and PTC developed a mean and maximum percentage inhibition of 23.63% and 23.54% respectively, during the 24 hours observation using *In-vitro* methodology. The MCE of PTC resulted in strong cytotoxic activity (CA) against MCF7 cell lines with an  $\text{IC}_{50}$  value of  $8.792 \mu\text{g}/\text{ml}$ . Compared to ASG, against MCF7, PC3, and HTC116 cell lines, showed  $\text{IC}_{50}$  values of 9.56, 11.53 and  $13.36 \mu\text{g}/\text{ml}$ , respectively. Furthermore, both MF and AF of PTC were found to possess the most efficient CA against PC3 cell lines that were stronger than the MF and AF of ASG with  $\text{IC}_{50}$  values of  $9.56 \mu\text{g}/\text{mL}$  and  $9.63 \mu\text{g}/\text{mL}$ , respectively. Finally, the antioxidant and antiproliferative properties of the bioactive compounds in GA have shown some evidence of effectiveness as traditional medicine as a preventive measure against the growth of cancer cell as well as acute inflammation.

## خلاصة البحث

الصمغ العربي هو إفراز طبيعي غني بالألياف الغذائية ومركبات البوليفينول، وعلى الرغم من فوائده الطبية والغذائية، إلا أنه لم يحظى بالاهتمام الكافي من قبل الباحثين. شملت الدراسة ست عينات صمغ خام من أشجار الهشاب والطلح والكاموت بالإضافة إلى عينتين تجاريتين تنحدران من أشجار الهشاب والطلح تسمى Prebio-M وب Prebio-T. تم تقدير مضادات الأكسدة في مستخلصاتها مستخدمين الميثانول النقي كفضل محلول تم اختياره باستخدام تصميم التجارب وبناءً على قوة المواد النشطة فيها بحيث يتم اختبار فعالية المستخلص والمشتقات النشطة على بعض الخلايا السرطانية حيث شملت سرطان الثدي MCF-7، القولون HCT-116، والبروستاتا PC3. اهتمت التجربة الأولى باستخراج المركبات النشطة بيولوجيا من الصمغ مع تحديد الخصائص الفيزيوكيميائية للخام. أما التجربة الثانية أجريت لغرض معرفة الظروف المثلى لاستخلاص مضادات الأكسدة "المواد الفعالة" من مجموعة الصمغ العربي المستهدفة في هذه الدراسة. ثالثاً تم استخلاص وتقدير مضادات الأكسدة من مستخلص الميثانول الخام ومشتقاته النشطة، والتي تمثلت في مجزء المانول والأستون على التوالي. أجريت التجربة الأخيرة لاختبار فعالية مستخلص الميثانول الخام بالإضافة إلى مشتقاته النشطة كمضاد للإلتهاب على فئران التجارب، بالإضافة إلى فعاليتها الحيوية ضد كل من السرطانات المذكورة في أعلاه. أوضحت النتائج أن كلا المجموعتين من الصمغ الطبيعي والتجاري تحتويان على مركبات الفلافونويد والمركبات الفينولية، حيث أعطى الصمغ العربي الطبيعي  $6540 \pm 3.46$  ميكروغرام/100 جرام، بينما الصمغ التجاري PBMT اعطى ما بين  $2560 \pm 3.49$  إلى  $2710 \pm 4.04$  ميكروغرام/100 جرام، على التوالي باستخدام كروماتوغرافية ذات الكفاءة العالية. علماً بأن الفلافونويد الوحيد الذي تم تقديره هو الكيروستين. بالنسبة للأحماض الفينولية، كانت المركبات النشطة المقدره في كل من مجموعة الصمغ الطبيعي و Prebio-T هي حمض الكافيين وبيتا كيوميرك، بيتا هيدروكسي بنزويك، وحمض الفيرويك علماً بأن حمض الكافيين هو أكثر المركبات الفينولية السائدة في الصمغ الطبيعي (عينة تربة القردود:  $401770 \pm 3.52$  ميكروغرام/100 جرام؛ بينما عينات التربة الطينية:  $77580 \pm 5.2$  ميكروغرام/100 جم). أظهرت نتائج الاستخلاص تحت ظروف المثالية للتجربة أن الحد الأقصى لقيمة مضادات الأكسدة مقارنة بنسبة انتاجية الاستخلاص كان 11.10% و 15.56% لصمغ الطلح بالإضافة إلى Prebio-T من العينات التجارية، على التوالي. ظروف الاستخلاص المثلى كانت باستخدام جهاز الموجات فوق الصوتية؛ والميثانول النقي في درجة الحرارة

المثلى 43 درجة مئوية، بقوة 40 كيلو هرتز، لمدة 3 ساعات. تم التأكد من المواد الفعالة في مستخلص الميثانول الخام ومشاقاته باستخدام تقنية كروماتغرافيه الغاز وطيف الكتله، حيث أعطى مستخلص الميثانول الخام ومشتقاته النشطة لكل من مجزء الميثانول والأستون من عينتى الطلح الطبيعى والتجارى وجود حوالى 57 مركباً حيويّاً. ايضاً اشارت النتائج المتحصل عليها من فئران التجارب المصابه بالحساسية الحاده الناجمة عن استخدام الكارجنان، بانالجرعة الفعالة كانت 300 ملغم/ كيلوغرام عند استخدام مستخلص الميثانول الخام لكل من عينتى صمغ الطلح الطبيعى والتجارى، حيث متوسط نسبة التثبيط القصى بلغت 23.63% و 23.54% على التوالي، خلال 24 ساعة من بداية التجربه. فيما يخص اختبار فعالية المستخلصات واثرها على الخلايا المسرطنة، وجد أن مستخلص الميثانول الخام الناتج من عينة الطلح التجارية كان الأفضل عند استخدامة للحد من نمو خلايا سرطان الثدي حيث كانت الكمية الكافية لقتل أو تثبيط 50% من خلايا سرطان الثدي، سرطان غدة البروستاتا وسرطان القولون 8.792، 9.56 و 9.63 ميكروجرام/مللتر على التوالي. مقارنة بالطلح الطبيعى، حيث كانت الكمية الكافية لقتل أو تثبيط 50% من خلايا سرطان الثدي، سرطان غدة البروستاتا وسرطان القولون حوالى 9.56 و 11.53 و 13.36 ميكروجرام/مللتر، على التوالي.

## **APPROVAL PAGE**

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## DECLARATION

I hereby declare that this thesis is the result of my own 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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Signature .....

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*For every moment I cherish the most*

*This humble thesis is especially dedicated to.*

*The love of my life.....my wife.*

*~ Rasha D.M.A. Ahmed*

*Both 'Princess' of mine...my lovely sisters.*

*Both of my adorable parents*

*My mother... Hawa Ahmed who passed away at the start of this study.*

*My father... Adam Hassan*

*also*

*My beloved siblings...*

*moreover, Gum Arabic farmers who spent their life under stress conditions for taping  
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## LIST OF SYMBOLES

$\mu$	Micro Unit
$O_2^{\bullet-}$	Superoxide anion
$OH^{\bullet}$	hydroxyl radical
$O_2^1$	Singlet oxygen
$^{\circ}C$	Degree Celsius
$\mu L$	Microgram per litter
ABTS $\cdot^+$	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ACGG	Acacia Complex Group of Gums
AF	Acetone Fraction
AGPs	Arabinogalactan-Protein
ANOVA	Analysis of Variance
ASG	<i>Acacia senegal</i> Gum
ASY	<i>Acacia seyal</i> Gum
BHA	Beta Hydroxy Acid
BHT	Butylated Hydroxytoluene
CAA	Cell Attachment Assay
CAA	Cell Attachment Assay
CCD	Central Composite Design
CCFAC	Codex Committee on Food Additives and Contaminants
CHF	Chloroform Fraction
CIPO	carrageenan-induced paw oedema
CUPRAC	Cupric iron Reducing Antioxidant Capacity
CVA	Cell Viability Assay
DMSO	Dimethyl Sulfoxide
DPPH	$\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl
FAO	Food and Agriculture Organization
FCI	Folin-Ciocalteu Index
FDA	Food and Drug Administration
FTIR	Fourier-Transform Infrared
GA	Gum Arabic
GC-MS/MS	Liquid Chromatography-Mass Spectroscopy
$H_2O_2$	Hydrogen Peroxide
HAT	Hydrogen Atom Transfer
HCT-116	Human Colorectal Adenocarcinoma Cell Lines
HPLC	High-Performance Liquid Chromatography
HPO	Hind Paw Oedema
HXF	Hexane Fraction
IACUC	Institutional Animal Care and Use
IC <sub>50</sub>	The concentration of compound that yields 50 % fewer cells compared to control
ICP-MS AES	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
JECFA	Joint Expert Committee for Food Additives
KHz	Kilohertz

M	Molarity (g/L).
MCE	Methanol Crude Extract
MCF-7	Human Breast Carcinoma Cell Lines
MF	Methanol Fraction
mg/g DW	Miligram per Gram Dry Weight
mgTE/100g DW	Milligram of Trolox Equivalent pre-Hundred Gram Dry Weight
mL	Millilitre
mM	Millimolar
NA	Natural Antioxidants
OFAT	One Factor At a Time
OH	Hydroxyl
ORAC	Oxygen Radical Absorbance Capacity
PBS	Phosphate-Buffered Saline
PC3	Human Prostate Cancer (PC3) Cell Lines
PCA	Principal Component Analysis
PMC	Prebio-M Commercial Sample Derived from <i>A. senegal</i> Gum
PMPT	Prebio -M and Prebio -T are Commercial Samples Belong to <i>A. senegal</i> and <i>A. seyal</i> Gum, Respectively.
PTC	Prebio-T Commercial Sample Derived from <i>A. seyal</i> Gum
RFAP	Ferric Reducing Antioxidant Power
ROS	Reactive Oxygen Radical Species
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
RT	Retention Time
SA	Synthetic Antioxidant
SDF	Soluble Dietary Fibres
SET	Single Electron Transfer
TBQH	Tert-butylhydroquinone
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TPTZ	2, 4, 6-tris (2- pyridyl)-s-triazine
UAE	Ultrasonic-Assisted Extraction
WHO	World Health Organization
$\alpha$	Alpha
$\beta$	Beta

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF STUDY

The mediators of intracellular signalling cascades are known as reactive oxygen species (ROS), which, come through cellular metabolism and are known to have both favourable and detrimental effects on living systems (Zhou, Shao, & Spitz, 2014). Comprehensive production of the ROS may, however, lead to oxidative stress (OS), and failure of the cell function (CFs) eventually leads to apoptosis or necrosis. A balance between oxidant and intracellular antioxidant systems is therefore vital for the CFs, regulation, and adaptation to diverse growth conditions (Nordberg & Arnér, 2005). However, the ROS benefits include good defence mechanism in the interactions between the cells and their surrounding environments, as well as responding to infections. Besides, non-enzymatic oxidants along with an imbalance effect between enzymatic activities and the ROS would result to high-level traces of the ROS in the biological systems.

Natural antioxidants (NA) are compounds capable of stabilizing and deactivating free radicals before any attack on the cells within the biological systems, that can help in eliminating the ROS through the use of either enzymatic or non-enzymatic means. Enzymes such as glutathione peroxidase, catalase, and superoxide dismutase are considered as the enzymatic antioxidants. On the other hand, enzymes such as the alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), flavonoids, phenolic acids, carotenoids, and other antioxidants are categorised as non-enzymatic antioxidants as

reported by Beddou et al., (2015). The use of dietary or enzymatic antioxidants may help to preserve optimal cellular functions (FAO/WHO, 1998). This is significant as the constituents of a cell are generally protected from oxidative damage by the antioxidants. Hence, they significantly lower the risk of degenerative diseases.

One of the vital antioxidants in fruits, tea, and vegetables is the polyphenol, which is an organic chemical micronutrient that plays a crucial role in lessening the risks of chronic diseases. Basically, fruits and beverages are the primary food sources of polyphenols for human beings. Consequently, this has increased the research interests of the antioxidant's unique attributes of the polyphenols.

Lately, utilizers of the renewable source were competing with those who were producing synthetic antioxidants in the ways that are cheaper and cost-effective for extracting such antioxidants (Carocho & Ferreira, 2013). The significance of this competition is based on the products' ability to prevent diseases which, are associated with oxidative stress, including neurodegenerative, cancer, and cardiovascular diseases (R. Sharma, 2014). Consequently, the concern of the cost-effectiveness brings about the consideration of an alternative approach that would be more financially desirable and of benefits.

Gum arabic (GA) is characterized as a natural antioxidant, and it can be obtained from three main types of Acacia gum trees. This characteristic makes it capable of having positive effects on pharmaceuticals and food processing industries. It is significant for various food applications since it serves as a natural resource, which is renewable and its natural ingredients are preferred over synthetic sources (Celli & Brooks, 2016). The demand for the GA increases annually, since it is considered a safe physiological substance, and effective drugs (Banerjee & Chen, 2010). Dietary fibres of the GA have proven in contemporary studies to be effective and play a significant

part in lipid metabolism (B. H. Ali & Al Moundhri, 2006; Trommer & Neubert, 2005). Various studies from around the world presented positive benefits of the GA's treatment results of such degenerative diseases including cardiovascular, gastrointestinal, and kidney failure (Glover, Ushida, Phillips, & Riley, 2009; M. Matsumoto, Poci, Rossetti, DePinho, & Accili, 2007), and the GA therefore, promises many benefits in medical, food, and pharmaceuticals.

As a raw material, the GA's export value recorded an average earning amounted to USD 40 million annually over the last twenty years (Couteaudier, 2007; Ibrahim, 2015). In their study of plants and food, Zhong and Shahidi (2015) classified the underlying antioxidants as heterogeneous molecules. Antioxidants are considered as compounds capable of preventing important molecules from being damaged. They do so by safely interacting with free radicals and causing the involved chain reaction to cease. Asimi et al., (2013) presented a description of the antioxidant's mechanisms that include; species that scavenge and start peroxidation, chelating metal ions that render them incapable of generating reactive, species or causing decomposing peroxides, and quenching super oxide hindering initiation of peroxides, causing a breakdown of the auto-oxidative chain reaction.

The effectiveness of the antioxidants of these compounds are determined by two underlying characteristics. They include the physical location of the plants, and the food as well as their chemical attributes, such as the components is considered the context of emulsion interfaces, aqueous phase, or closeness to membrane phospholipids (Brewer, 2011). Thus, antioxidant compounds are urgently needed to be determined.

A wide range of biological trails can be traced within these antioxidants such as anti-inflammatory, anti-carcinogenic, anti-atherosclerotic effects, and reduction of the prevalence of coronary disease, which contribute to the preservation of gut health by