Study of Antioxidants contains in *Leucaena Leucocephala* (Petai Belalang) and Potential Application as Natural Based UV Protection in High Density Polyethylene (HDPE)

by

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(In The Name of Allah, The Most Graceful, The most Merciful)

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LIST OF ABBREVIATIONS

AB-DTPA	Ammonium bicarbonate diethylenetriaminepentaacetic acid
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
$AlCl_3$	Aluminium chloride
DPPH	(1,1-diphenyl-2-picrylhydrazyl)
FTIR	Fourier-transform infrared spectroscopy
g	Gram
HDPE	High density polyethylene
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
L	Liter
ml	Mililiter
mm	milimeter
NA	Nutrient agar
NH_4HCO_3	Ammonium bicarbonate
Nm	Nanometer
UV	Ultra violet
µg	microgram
°C	Degree Celsius

LIST OF SYMBOLS

C	For sample without bio additives (control)
L	Leaf
S	Seed

Kajian Antioksidan di dalam Leucaena Leucocephala (Petai Belalang) dan potensi kegunaan sebagai Perlindungan UV berasaskan Semulajadi dalam High Density Polyethylene (HDPE)

ABSTRAK

Leucaena Leucocephala (Lam.) De Wit (Petai Belalang) adalah tumbuhan sederhana yang tergolong dalam kumpulan baka tropika, yang secara tradisinya digunakan sebagai makanan untuk manusia dan haiwan, dan digunakan dalam perubatan dan kosmetik. Objektif utama adalah untuk mengkaji pelarut yang berbeza untuk mengekstrak komposisi sebatian bio aktif dalam biji dan daun *L. leucocephala*, untuk mengenal pasti dan mencirikan jenis komposisi sebatian bio aktif dalam biji dan daun *L. leucocephala* dan juga untuk menilai potensi antioksidan daripada ekstrak *L. leucocephala* di.dalam polietilena ketumpatan tinggi (HDPE). Bagi aktiviti antioksidan daripada daun dengan menggunakan kaedah diphenylpicrylhydrazyl (DPPH) radikal bebas, kepekatan yang lebih tinggi dicatatkan melalui kaedah pengekstrakan dengan menggunakan metanol (sampel kering) iaitu 8247.0 mg/L, kepekatan jumlah kandungan fenolik yang lebih tinggi pula dicatatkan melalui kaedah pengekstrakan dengan menggunakan air ternyahion (sampel kering) iaitu 4276.0 mg/L, bagi jumlah kandungan flavonoid, kandungan yang tertinggi direkodkan melalui kaedah pengekstrakan menggunakan metanol (sampel kering) iaitu 4439.0 mg/L, dan untuk analisis fosforus pula kandungan yang tertinggi dicatatkan melalui kaedah pengekstrakan menggunakan metanol (sampel kering) iaitu 71.057 mg/L. Aktiviti antioksidan untuk biji lebih tinggi apabila melalui pengekstrakan menggunakan metanol (sampel kering) iaitu 593.09 mg/L, jumlah kandungan fenolik yang lebih tinggi dengan kaedah pengekstrakan menggunakan air ternyahion (sampel kering) iaitu 5587.50 mg/L, jumlah kandungan flavonoid lebih tinggi melalui pengekstrakan menggunakan metanol (sampel kering) iaitu 445.05 mg/L, dan analisis fosforus lebih tinggi melalui kaedah pengekstrakan menggunakan metanol (sampel kering) iaitu 73.817 mg/L. Analisis spektroskopi inframerah (FTIR) dan analisis Kromatografi Cair Prestasi Tinggi (HPLC) dilakukan untuk mengesan dan mengenal pasti sebatian bio aktif dalam sampel. Oleh kerana ikatan O-H adalah unsur utama kepada antioxidant dan sebatian fenolik, analisis FTIR menunjukkan kebarangkalian yang tinggi kepada kewujudan antioxidant dan sebatian fenolik dalam *L. leucocephala*. Analisis HPLC untuk biji dan daun menunjukkan kehadiran asid kafein, apigenin, quercetin, asid formik, isorhamnetin, dan luteolin yang mewakili sebatian bagi kumpulan antioksidan. Pelarut yang terbaik telah dipilih untuk aplikasi selanjutnya sebagai bahan tambah dalam HDPE. Produk akhir akan menjalani ujian tegangan, resolusi tinggi mikroskopik optik, analisis degradasi warna dan ujian antimikroorganisma untuk mengukur kesan penambahan bahan tambah. Dengan bahan tambah bio telah memberi peningkatan kepada ketahanan produk selepas terdedah selama 1000 jam terhadap sinaran UV apabila peratus penurunan ketahanan menunjukkan perubahan yang lebih sekata berbanding produk tanpa bahan bio tambahan. Antioksidan juga bertindak balas sebagai antimikroorganisma positif. Secara keseluruhannya, keputusan yang diperolehi menunjukkan bahawa sebatian aktif bio yang diekstrak daripada *Leucaena Leucocephala* mempunyai potensi tinggi untuk digunakan sebagai perlindungan UV berasaskan semulajadi dalam plastik.

Study of Antioxidants contains in Leucaena Leucocephala (Petai Belalang) and Potential Application as Natural Based UV Protection in High Density Polyethylene (HDPE)

ABSTRACT

Leucaena Leucocephala (Lam.) de Wit (*Petai Belalang*) is a medium plant which belong to the group of tropical breed, traditionally used as food for both human and animal, and being applied in medical and cosmetic. The main objective of this work was to investigate the different solvent for extracting bioactive compound composition in seeds and leaves of *L. leucocephala* (*Petai Belalang*), to identify and characterize the type of bioactive compound composition in both seed and leaves of *L. leucocephala* (*Petai Belalang*) and last but not least to evaluate the potential of antioxidant activity of *L. leucocephala* (*Petai Belalang*) extract in high density polyethylene (HDPE). For antioxidant activity of leaves by using free radical diphenylpicrylhydrazyl (DPPH) method, higher concentration was recorded by extraction using methanol (dried sample) which is 8247.0 mg/L, for total phenolic content higher concentration was recorded by extraction using deionized water (dried sample) which is 4276.0 mg/L, for total flavonoid content by using colorimetric assay higher concentration was recorded by extraction using methanol (dried sample) which is 4439.0 mg/L, and for phosphorus analysis higher concentration was recorded by extraction using methanol (dried sample) which is 71.057 mg/L. While, Antioxidant activities for seeds are higher under extraction using methanol (dried sample) which is 593.09 mg/L, total phenolic content are higher under extraction using deionized water (dried sample) which is 5587.50 mg/L, total flavonoid content are higher under extraction using methanol (dried sample) which is 445.05 mg/L, and phosphorus analysis are higher under extraction using methanol (dried sample) which is 73.817 mg/L. The Fourier-transform infrared spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC) analysis was done in order to detect and identified the bioactive compound in sample. As O-H bond is the main element of antioxidant and phenolic compound, the FTIR analysis shows for the high possibilities for the present of antioxidant and phenolic compound in *L. leucocephala*. HPLC analysis for both seeds and leaves revealed the presence of *caffeic acid, apigenin, quercetin, formic acid, isorhamnetin,* and *luteolin* which is all the compounds are belong to the group of antioxidant. The strongest extraction solvent which extracted higher concentration of antioxidant was chosen for further application as bio additives in high density polyethylene (HDPE). The final product will undergo tensile test, higher resolution optical microscopic, colour fastness and degradation analysis and antimicrobial assay to measure the effect of added bio additives. Sample with bio additives resulted for improvement in its strength after 1000 hours UV exposure as the percent of degradation shows for smooth pattern compared to sample without bio additives. The antioxidants also react as positive antimicrobial when undergoes antimicrobial assay. Overall, the presented results show that bio active compounds extracted from *Leucaena Leucocephala* has a high potential to be used as natural based UV protection in plastic.

CHAPTER 1 : INTRODUCTION

1.1 Background

Generally, pure plastic based product are not strong to exist alone and usually the additives are added to upgrade performance. The common types of additives are inorganic fillers that reinforce the material, plasticizers to render the material pliable, thermal and ultraviolet stabilizers, flame retardants and colourings (Meeker, Sathyanarayana, and Swan, 2009).

The most commonly examples of additives used in plastic product are phthalate plasticizers, bisphenol A (BPA), brominated flame retardants and anti-microbial agents. The BPA and phthalates are widely found in medical devices, food packaging, perfumes, cosmetics, toys, flooring materials, computers and CDs. Phthalates can leach out of products because they are not chemically bound to the plastic matrix, and they have attracted particular attention because of their high production volumes and wide usage (Thompson, Moore, Saal, & Swan, 2010).

Cherif Lahimer et al (2013) in their study on characterization of plastic packaging additives: food contact, stability and toxicity has stated that approximately eighty percent of plastics in the world are thermoplastics. Polystyrene, polyethylene, polyvinyl chloride are the most

common thermoplastic used in food packaging industry. Additives are introduced in polymers in order to improve the physical (mechanical, thermal) and chemical properties of the packaging. Others than that, plasticizers antioxidants, anti-UV are also been used. However, it has been reported that these adjuvants may contain compounds with adverse effects on human health and environment (Cherif Lahimer, Ayed, Horriche, & Belgaied, 2013).

Based on the analyses performed on several additives, researchers has made conclusion for most of the additives used in plastic industries contain compounds harmful to human health and environment as it is not always pure products. They are usually mixed with resins and waxes (rosin), oils (paraffin), which do not safe when contact to the food. These substances are commonly appeared when the additives are fat or liquid form (Cherif Lahimer et al., 2013).

In antioxidants analysis, the present of antioxidants in commercial plastics material is not stable and highly sensitive to hydrolysis and heat. There are either other additives to stabilize the polymer (synergistic effect), or intermediates for the adjuvants synthesis. Some of these molecules are not very toxic but most of them are not mentioned in the positive list. As the health issues is important for serious concerns about the use of harmful additives. In the case of plasticizers for example, researchers has proposed sebacate or citrate molecules as the replacer for phthalates (Cherif Lahimer et al., 2013).

Polymer additives have had a dramatic improved on packaging materials. Improvements in polymer performance have opened the door for new and innovative ways of production.

Numerous additives provide processing and manufacturing performance improvements, others allow materials to have an assortment of enhanced properties (Fox, 2008).

An additive is a material that is added to a polymer melt to enhance process ability, performance, or appearance. The ability to modify polymers to has allowed the packaging industry to produce better and stronger materials. Polymeric materials are used in packaging products such as films, moulded containers, and cushioning. Packaging materials benefit greatly from inclusion of additives to polymers to enhance process ability and performance (Fox, 2008).

Increased and accelerated global economic activities over the past century have led to interlinked problems that require urgent attention. The current patterns of production and consumption have raised serious concerns. In this context, greater emphasis has been put on the concept of sustainable economic systems that rely on technologies based on and supporting renewable sources of energy and materials. Average UK households produce around 3.2 million tonnes of packaging waste annually whereas 150 million tonnes of packaging waste is generated annually by industries in the UK. Hence, the development of biologically derived biodegradable polymers is one important element of the new economic development (Philip, Keshavarz, & Roy, 2007).

1.2 Problem Statement

Generally, *Leucaena leucocephala* is a plant which grow randomly, easily spread and can be found almost everywhere in Malaysia. Focusing in Perlis, this plant was found grows wildly without any specific usage of it and simply was abandoned. Only few who used this plant to

feed livestock while many sees it as a non-function plant which just grows creating a bush. Even though this plant was destroyed, it is able to regrow easily over again and rather than it been wasted, this research comes out with the idea to evaluate the benefits of *Leucaena leucocephala.*

Plants in the tropical zone including Malaysia contain a high concentration of phenolic compounds formed as secondary metabolites in plants. In a different way, this plants have been used as medicines because it contain active phytochemicals including phenolic compounds. For centuries, it was applied as antibiotics, act as structural materials to give plants stability and provide protection against ultraviolet (UV) light (Maisuthisakul, 2012).

In almost all commercial plastics, there are always present others added monomeric materials to improve their processing and end-use performance such as reinforcing fibres, fillers, and coupling agents; plasticizers; colorants; stabilizers (halogen stabilizers, antioxidants, ultraviolet absorbers, and biological preservatives); processing aids (lubricants, others, and flow controls); flame retardants; peroxides; and antistats.

Since chemical additives in plastic are not covalently linked to the host polymer, they can leach into the environment. Although they are generally non-persistent, contamination in the environment is still significant due to widespread use; low- and high-molecular weight chemical additives are commonly found in household dust, soil, and indoor and outdoor air, and as detectable residues in foods. Chemical additives exposures may occur through ingestion, inhalation, dermal absorption, and parenteral administration (Beach, Weeks, Stern, & Anastas,

2013). This lead to the concern regarding the effect of the added materials into human health and environment.

As an alternative for new solution about the issue, this research come out with the idea of using wasted *Leucaena Leucocephala* as a natural additives in plastic industry. The outcome product is biodegradable, better quality which safe to human and environment.

1.3 Scope of Research

The very initial part in this research is by focusing on the different extraction solvents of targeted samples. Two different type of samples are collected from different parts of *Leucaena leucocephala*'s tree which are matured seed and matured leaves. Applying the same samples preparation, fours (4) different types of extraction solvent was used in this study.

Both the matured seed and the matured leaves were extracted with four (4) different types of extraction solvent which is methanol, glacial acetic acid, deionized water, and distilled water. The samples will undergoes further analysis to evaluate which types of solvent give the higher concentration of targeted bioactive compounds. The analysis begin with separation techniques by using (Fourier-transform infrared spectroscopy) FTIR and High Performance Liquid Chromatography (HPLC) to certify the present of organic bonding in the samples and to identify the compounds presents in the samples. Next, Phytochemical screening assays, including antioxidant activity (DPPH free radical scavenging activity), antioxidant activity (ABTS free radical scavenging activity), total phenol contents analysis using folin-ciocalteu method, total flavonoid contents analysis using colorimetric assay and total phosphorus contents analysis using molybdenum blue method will conducted to check for the concentration of bioactive compounds in the samples. The best extraction solvent which gives the higher concentration of targeted bioactive compounds will be choosing for the next step.

The bio active compounds extracted from *Leucaena leucocephala* will be used as natural additives in High density polyethylene (HDPE). The final product will undergoes UV exposure until 1000 hours before being tested for its color fastness and degradation, surface morphology, and tensile analysis.

1.4 Objective

1. To study different solvent for extracting bioactive compound composition in seeds and leaves of *Leucaena leucocephala* (Petai Belalang).
2. To identify and characterize the type of bioactive compound composition in both seed and leaves of *Leucaena leucocephala* (Petai Belalang).

3. To evaluate the antioxidant function of *Leucaena leucocephala* (Petai Belalang) extract in high density polyethylene (HDPE) through tensile analysis.