# Investigation on Antioxidants Compounds Composition Contains in *Leucaena Leucocephala* (*Petai Belalang*)

IOP Conf. Series: Materials Science and Engineering **551** (2019) 012016 doi:10.1088/1757-899X/551/1/012016

# Investigation on Antioxidants Compounds Composition Contains in *Leucaena Leucocephala* (*Petai Belalang*)

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**Abstract.** The main objective of the current work was to investigate the unknown compound composition contains in *Leucaena Leucocephala (Petai Belalang)* to prove that there were antioxidants properties presents in the leaves and seeds of the plant. High-performance liquid chromatography (HPLC) and Fourier-transform infrared spectroscopy (FTIR) were used to characterize the samples. It was found that for both leaves and seeds sample of *Leucaena Leucocephala,* the spectrum had shown the main existence of alcohol (O-H bond. As the 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 the leaves and seeds of *Leucaena Leucocephala*. Further analysis using HPLC, both seed and leaf sample shows for the presence of the same compounds, identified as *apigenin, caffeic acid, formic acid, isorhamnetin, keampferol, luteolin* and *quercetin,* but by comparing the peak, can be concluded that the compounds in seed are higher than the compounds in leaf.

## 1. Introduction

Antioxidant is one of the most widely used groups of elements for maintaining the physiological functions of human body such as the liver, kidney, digestive system, and prevention of cardiovascular diseases and cancer [1]. Natural antioxidants such as polyphenols and carotenoids are usually derived from plants. Most plants contain compounds that possess antioxidant activity which occur in all parts of the plant, for examples wood, bark, stems, leaves, fruit, roots, flowers, pollen and seeds [2].

The advantages of Leucaena Leucocephala such as it can be found easily in large amount and available throughout the year. Leucaena Leucocephala is a tropical tree which has been categorized into the group of tree legumes. Since long time ago, many tree legumes have been recognized for their capacity to enhance the productivity and sustainability of tropical agricultural systems, both for developed and less developed countries of the world. Studies show that tree legumes can provide fuel wood, nutrient-rich, erosion control and land stabilization. Other than that, it also was taken as food and as fencing materials for farmers [3].

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint".

For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds [4].

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products, HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants [5]. The present of bioactive compounds in plants was discovered to act as protection against bacterial, fungal and pesticides pathogens.

The common application of antioxidant from plant sources is usually being focused only on cosmetic and medicine industry. Indeed, at present, general research in looking for others used of antioxidant from plant's sources is still in its infancy.

In this work, the main types of equipment used for characterized and identified the antioxidants are fourier-transform infrared spectroscopy (FTIR) and the high performance liquid chromatography (HPLC), both of which are technically classified as efficient method for separation techniques related to plants materials [6,7].

## 2. Methodology

### *2.1 Sample preparation*

Samples of mature seeds and leaves were collected from different locations around Perlis by neglecting the effect of different location and morphological variation of the plants. The seeds were separating from its pod and was washed using water before undergoes for drying process. While for the leaf, it were separate from its twig before undergoes drying process at 45°C for 72 hours (3 days). Special precaution with proper actions and accurate awareness should be strictly considered to assure that potential bio active compound are not lost, distorted or destroyed during the preparation of the extract from plant samples.

### *2.2 Fourier-transform infrared spectroscopy (FTIR)*

The preparation of samples for FTIR analysis was done by collecting the fresh matured seeds and leaves from plant before undergoes drying proceed in the oven at 45°C for 72hours (3 days). The dried samples were blended using laboratory blender and was sieved (63µm) to get the uniform powder.

By using microspatula, about 1/8" of the solid sample and about 0.25-0.50 teaspoons of Potassium Bromide (KBr) were taken carefully and thoroughly mixed in a mortar while grinding with the pestle. The mixture powder was placed just enough amount to cover bottom in pellet die and pressing the die at the pressure of 5000-10000 psi. The pressed sample was carefully removed from die and placed in the FTIR sample holder [6].

FTIR analysis was carried out using a PerkinElmer FT-IR/FIR spectrometer frontier. The peaks obtained were analysed by using a standard IR spectra table.

### *2.3 High performance liquid chromatography (HPLC)*

Reverse-phase high performance liquid chromatography (RP-HPLC) analysis of samples was performed using PerkinElmer A-10 solvent and sample module, Altus with a PDA detector, and chromatographic separations were performed on a LiChrosorb Rp-18 column (5µm). The composition of solvents and the gradient elution conditions used were as described by Uzelac et al. (2005) and Butsat et al. (2009) with some modifications [7][8].

The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23% to 90% solvent B; from 44 to 45 min, linear gradient from 90% to 80% solvent B; from 45 to 55min, isocratic at 80% solvent B; from 55 to 60min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs.

Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of standard compounds and were detected using an external standard method [9].

## 3. Result And Discussion

*3.1* FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information.

In this part of analysis, both leaves and seeds sample from *Leucaena leucocephala* tree was undergoing for FTIR analysis. The result was shown in figure 1.

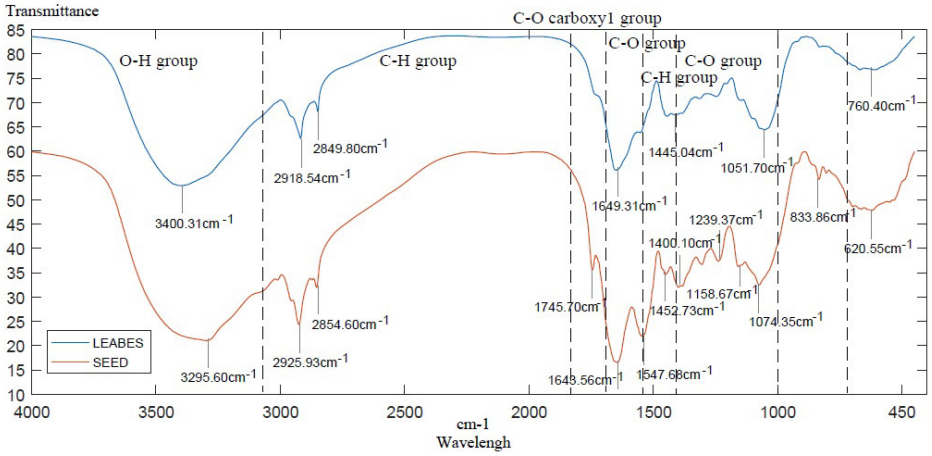


**Figure 1.** FTIR analysis.

**Table 1.** FTIR peaks value for extract of *L.Leucaena* extract**.**

| Functional Group | Wavelength range ($cm^{-1}$) |
|---|---|
| O-H | 3600-3200 |
| C-H | 3100-3000 |
| C-H | 3000-2850 |
| C-O | 1820-1670 |
| C=O carbonyl | 1700-1819 |
| C=C | 1620-1669 |
| C-C | 1600-1400 |
| C-H | 1480-1350 |
| C-O | 1300-1000 |
| 1,2-disubstituted | 1000-900 |
| 1,3-disubstituted | 1100-600 |

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Figure 1 shown for the IR spectrum appeared for leaves and seeds of *L.Leucaena* when analysed using FTIR. Table 1 recorded the range wavelength to distinct functional groups of organic compounds that present in leaves and seeds of *L.Leucaena*.

Peak shapes are sometimes very useful in recognizing what kind of bond is present. The rounded shape of most O-H stretching modes occurs because of hydrogen bonding between different hydroxyl groups [10].

Because protons are shared to varying extent with neighbouring oxygen, the covalent O-H bonds in a sample of alcohol all vibrate at slightly different frequencies and show up at slightly different positions in the IR spectrum. Instead of occur as single sharp peak, a whole lot of them usually all smeared out into one broad blob. Since C-H bonds (single bond) are actually a week bond, the peak appear differently for an ether, and an O-H peak is very easy to distinguish in the IR spectrum [11].

For the leaves sample, the peak observed were 3400.31, 2918.54, 2849.80, 1649.31, 1445.04, 1051.70, and 670.50 $cm^{-1}$. While for seeds sample, the peak observed were 3295.36, 2925.93, 2854.60, 1745.70, 1643.56, 1547.68, 1452.73, 1400.10, 1305.04, 1158.67, 1074.35, 833.86 and 620.55 $cm^{-1}$. The peaks proven for the existence of organic compounds (O-H bond), alkane (C-H bond), aldehyde (C-H), alkene (C-H bond), alkene (C=C bond), Cyclopentanone (C=O bond), Amine (C-N bond), vinyl ether (C-O bond), and tertiary alcohol (C-O bond) which all represent of hydrocarbon group.

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 the leaves and seeds of *Leucaena leucocephala*. The various functional groups observed in both leaves and seeds of *L.Leucaena* probably indicate the presence of carbohydrates ((CH2O)n), glycogen ($C_{24}H_{42}O_{21}$), starch ((C6H10O5)n), lipids (–COOH)), glycogen ($C_{24}H_{42}O_{21}$) and cellulose ((C6H10O5)n) [11].

*3.2 HPLC Analysis*

This part of research aims to prove and identified the presence of antioxidant compounds in samples with reversed phase High Performance Liquid Chromatography (HPLC). There are seven (7) type of compounds that was used as standard. The chosen for the standard was done by referred to previous study by Tradit et al where the same compounds are assuming to contains in tested sample [12]. The peak for standards was represented in figure 3 and table 1.

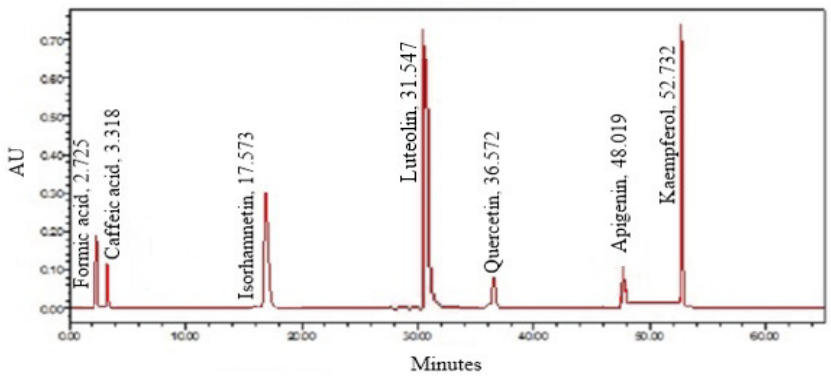


**Figure 2.** Run standard for HPLC analysis

**Table 2.** Peak versus retention time (RT).

| Compound | Retention time (RT) |
|---|---|
| Apigenin | 48.019 |
| Caffeic acid | 3.318 |
| Formic acid | 2.725 |
| Isorhamnetin | 17.573 |
| Kaempferol | 52.732 |
| Luteolin | 31.547 |
| Quercetin | 36.572 |

The samples, which is seed and leaf extract was run by referred to the previous standard. The peak which appeared on time same as retention time of the standard representation for the specific compounds that is contained in the sample. For both seed and leaf sample, there were proven for the presence of antioxidant (bioactive compounds). The results are shown in figure 3 and figure 4. Evidence for the presents of bioactive compounds (antioxidant) was provided by the result of HPLC analysis.

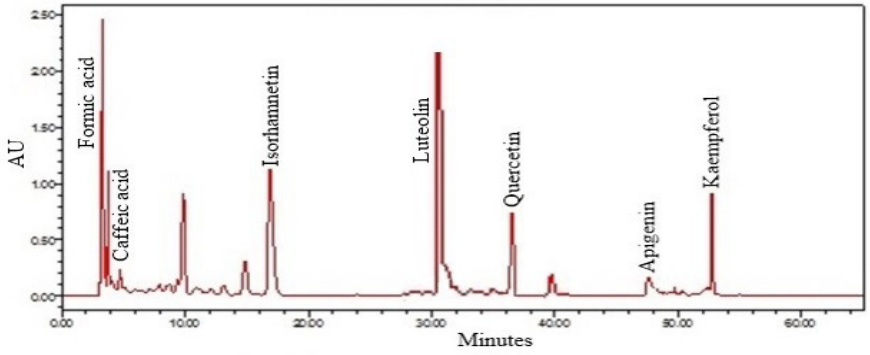


**Figure 3.** HPLC analysis of seed sample

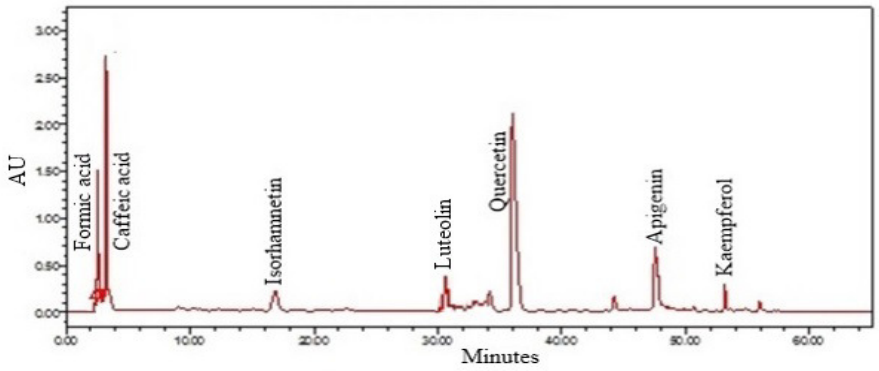


**Figure 4.** HPLC analysis of leaf sample.

The compounds were identified by comparing with standards of each identified compound using the retention time, the absorbance spectrum profile and also by running the samples after the addition of pure standards.

Despite both seed and leaf sample shows for the present of same compounds, identified as *apigenin*, *caffeic acid*, *formic acid*, *isorhamnetin*, *keampferol*, *luteolin* and *quercetin*, but by comparing the peak, can be concluded that the compounds in seed are higher than the compounds in

leaf. The results obtained agreed with the other work carried out by Al-Anbaril and Hassan, 2015 where was mentioned that the phenols in seeds were higher more than leaves [11].

The result has proved for the present of antioxidants in seeds and leaves of *Leucaena Leucocephala* that will be proposed as bio additives in high density polyethylene (HDPE). This compounds are expected to perform as UV protection for HDPE when exposed to the UV light as the general function of antioxidants is significantly to delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate [13].

## 4. Conclusions

The result obtained from this study has laid an important platform from which to investigate the antioxidant compound composition in *Leucaena leucocephala.* As the results has revealed in the present of antioxidant in samples, identified as *apigenin, caffeic acid, formic acid, isorhamnetin, keampferol, luteolin* and *quercetin*, it will become a platform to propose the plant for further used.

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