

# Analysis of antioxidant activity, total phenolic and flavonoid contents of ethanol extract of *Litsea cubeba* Lour. Bark

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**Abstract.** Free radicals also play a role in the pathology of various degenerative diseases such as cancer, rheumatism, coronary heart disease, cataracts, and others. Free radicals can come from within the body (endogenous) and outside the body (exogenous). *Litsea cubeba* (Lour.) is a Lauraceae family plant which have contents volatile oils which used as antimicrobial, anticancer on breast cancer, pesticide, antidepressants, antiinflammation, antioxidant, and neuro pharmacology. The extract was prepared using water with the soxhletation method. The antioxidant activity was determined with the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-Azinobis [3-ethylbenzothiazoline-6)-sulfonic acid] -diammonium salt (ABTS) and Ferric Reducing Antioxidant Power (FRAP) methods. Total flavonoid and total phenolic content were determined with colorimetric methods. Antioxidant activity measured as IC<sub>50</sub> was 23.37 ± 0.42 µg/mL; 111.21 ± 0.42 and 109.01 ± 0.28 respectively. The extract was found to contain high levels of total phenolic (282.93 ± 0.33 mg GAE/g) and total flavonoid 7.49 ± 0.51 mg QE/g). The results reveal that ethanol extract of *Litsea cubeba* Lour. Bark has antioxidant potential. The further analysis is to isolation antioxidant compound.

## 1 Introduction

The antioxidant has the capacity to protect the cell from free radical harm cause of its activity in donates one or more electrons. Free radicals are reactive entities with various number of unpaired electrons that try to obtain electrons from other molecules or release the unpaired electron. Antioxidants are molecule that can slow or stop the process of oxidation in general. The majority of plant chemicals with secondary metabolites that are highly rich biogenic resources for the identification of new drugs. Natural products and derivatives account for more than half of all drugs in clinical trials throughout the world, according to a recent research.

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Free radicals are also having important role in pathophysiology of diseases including cancer, rheumatism, coronary heart disease, cataracts, and others. Free radicals can arise from both inside and outside the body (endogenous and exogenous) [1-5].

*Litsea cubeba* (Lour.) is from Lauraceae group that contains various of metabolites that are utilized as antiinflammation, antimicrobials, pesticides, anticancer, neuropharmacology, antioxidants and antidepressants. It was discovered active on HeLa cells that cause apoptosis by triggering caspase 3/7 activation [6,7]. Antibacterial activity of isoquinoline alkaloids found in the *Litsea* genus [8]. The heartwoods had significant levels of flavonoid and phenolic compounds that serve as antioxidants and slow the growth breast and pancreatic cancer by arresting the cell cycle. The alkaloid extract have the capacity to decrease expression of the phosphatidylinositol-3-kinase, and AKT-serine threonine (1 and 2) genes. With the ABTS and DPPH techniques, alkaloid compounds from heartwood show an antioxidant effect [9-15]. The goal of this research was to determine antioxidant activity, total content of phenol and flavonoid of ethanol extract of *Litsea cubeba* Lour. barks using a Soxhlet extraction technique.

## 2 Materials and method

### 2.1 Material

*Litsea cubeba* Lour. Fresh barks were collected from Parsoburan district, Toba Samosir regency, North Sumatera province, Indonesia. ABTS (2,2'-Azinobis[3-ethylbenzothiazoline-6]-sulfonic acid]-diammonium salt), DPPH (1,1-diphenyl-2-picrylhydrazyl), aluminium chloride (Merck), gallic acid (Sigma), ethanol (Merck), quercetin (Sigma), iron (III) chloride (Merck), aquadest, Folin Ciocalteu (Sigma), methanol (Merck), potassium persulfate (Merck).

### 2.2 Method

#### 2.2.1 Preparation of extraction

*Litsea cubeba* Lour. barks were pulverized after being dried at 45°C. Simplicia powder of *Litsea cubeba* Lour. barks (30 g) was weighed and placed in a Soxhlet extractor thimble. In a conical flask, 600 mL of ethanol was measured. With an extraction duration of 4 hours, a heating mantle was utilized to heat the solvent. When the extraction time was completed, the extraction solution was allowed to cool to room temperature. Then, using a rotary evaporator and a water bath, it was filtered through a cone of Whatman paper and concentrated to dryness [16].

#### 2.2.2 DPPH radical scavenging activity test

Blois (1958) technique of (DPPH•) was applied to assess scavenging activity for free radicals. The procedure was followed as previously describe with quercetin as positive control [3,17].

### 2.2.3 ABTS radical scavenging activity test

The ABTS radical cation decolorization assay was used to determine the scavenging activity for free radicals of plant materials. The procedure was followed as previously describe with quercetin as positive control [3].

### 2.2.4 Ferric reducing antioxidant power

5 mg of extract in ethanol was pipetted 1 mL and 1 mL 0.2 M phosphate buffer (pH 6.6), and 1 mL(1%)  $K_3Fe(CN)_6$  were added, and the procedure was followed as previously describe [3].

### 2.2.5 Determination of total phenol concentration

The total phenol concentration (TPC) of the sample was determined using a Folin ciocalteu technique with gallic acid as a standard [15,16,18].

### 2.2.6 Determination of total flavonoid concentration

As previously reported, using quercetin as a reference, the total flavonoids in the extract were quantified spectrophotometrically. [3,19,20].

## 3 Results and discussion

### 3.1 Antioxidant activity, total phenol, and total flavonoid

The color change of DPPH and ABTS has a correlate with the radical reduction of DPPH and ABTS by antioxidants and can be used to measure an antioxidant molecule's free radical scavenging capability. FRAP method is based on a reduction reaction in an acidic environment to the yellow  $Fe^{3+}$  complex (potassium hexacyanoferrate) to the bluish-green  $Fe^{2+}$  complex compound due to electron donors from antioxidant compounds. The method of determining the quantity of phenolic substance in samples is known as total phenol activity. Plant phenolic compounds have redox characteristics, which allow them to serve as antioxidants [19]. Table 1 shows the inhibitory concentration for 50% ( $IC_{50}$ ), and Table 2 shows total phenolic and flavonoid content.

**Table 1.**  $IC_{50}$  value with DPPH, ABTS and FRAP methods

No	Sample	$IC_{50}$ Value ( $\mu g/mL$ )		
		DPPH	ABTS	FRAP
1	<i>Litsea cubeba</i> Lour. barks	23.37 $\pm$ 0.42	111.21 $\pm$ 0.42	109.01 $\pm$ 0.28

**Table 2.** Total phenolic and flavonoid content

No	Sample	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
1	<i>Litsea cubeba</i> Lour. barks	282.93 $\pm$ 0.33	7.49 $\pm$ 0.51

Radical scavenging actions are critical for preventing free radicals to cause different diseases, including cancer. The IC<sub>50</sub> for *Litsea cubeba* Lour. barks ethanol extract with DPPH, ABTS and FRAP assay were  $23.37 \pm 0.42$ ;  $111.21 \pm 0.42$  and  $109.01 \pm 0.28$  µg/mL respectively. The ethanol extract of *Litsea cubeba* Lour. bark had a high phenolic concentration of  $282.93 \pm 0.33$  mg GAE/g. Phenolic chemicals are well-known as powerful antioxidants, with their hydroxyl groups playing a key role in their capacity to scavenge free radicals. The total flavonoid content of the ethanol extract of *Litsea cubeba* Lour. barks was  $7.49 \pm 0.51$  mg QE/g. Antioxidants activity is influenced the amount of phenolic and flavonoids present; phenol and flavonoids components contribute linearly to antioxidant activity [21,22].

## 4 Conclusion

The results shown that *Litsea cubeba* Lour. barks ethanol extract which extraction with soxhletation method have very strong antioxidant activity and high level of phenolic and flavonoid content.

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