Antioxidant Activity and Chemical Compositions of Fresh and Dry *Litsea glutinosa* Leaves Analysis by GC-MS

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Abstract:

*Litsea glutinosa* is a plant that can be easily found in mixed deciduous forest and can be used for many purposes such as cosmetics, herbal shampoo or herbal treatment. This study aimed to determine antioxidant activity and chemical components in fresh and dry *Litsea glutinosa* leaves. Essential oils obtained by steamed distillation of fresh and dry leaves were analysed by gas chromatography-mass spectrometry (GC-MS). Crude extracts obtained by evaporating the boiled water of steam distillation part to dryness were determined for antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Crude extracts from fresh leaves contained the higher antioxidant activity than that of dry leaves. The GC-MS results of essential oil from fresh leaves were found phytol (29.31%), caryophyllene oxide (14.25%), and isopropyl myristate (6.08%). In dry leaves essential oil phytol (26.42%), caryophyllene oxide (12.39%), and isopropyl myristate (5.75%) were found as major components.

1. Introduction

*Litsea glutinosa* (Lour.) C.B. Robinson (Lauraceae), also known as “Mee Hmen” is a perennial plant, height of 5-15 meters, distributed throughout Southeastern Asia. In Thailand, it is abundant in the northern and northeastern parts of the country.\(^1\)\(^2\)

Leaves are mucilaginous and considered for antispasmodic, emollient, and poultice.\(^3\) It is believed that mucilage from the leaves helps clean the hair and promotes hair growth. Hence some herbal shampoo more often found to contained this herbs.\(^4\)

The leaves are also used in diarrhea and dysentery as well as in wounds and bruises.\(^5\)

Fresh *L. glutinosa* was used for remedy traumatic injury, fracture, furuncle and hemorrhage from trauma in the folk.\(^6\) The leaf extract has been evaluated for cardiovascular and anti-inflammatory activities.\(^7\) The plant is very important from economical, medicinal and conservation point of view.

Moreover, essential oil from leaves of *L. glutinosa* harvested from Guangxi Zhuang Autonomous region of China\(^1\) has been reported for their chemical compositions by GC-MS. The essential oil were consisted of β-caryophyllene, β-ocimene, α-pinene, phytol, β-pinene, bicyclogermacrene, caryophyllene oxide and α-caryophyllene.

This study aimed to investigate the free radical scavenging of DPPH of fresh and dry *L. glutinosa* leaves and analysis chemical compositions by GC-MS of essential oils of fresh and dry *L. glutinosa* leaves.

2. Materials and Methods

2.1 Herbal plants

*L. glutinosa* leaves were collected from Sansai District, Chiang Mai Province, Thailand 50290, during October-November, 2018.

2.2 Plant extraction

*L. glutinosa* leaves were cleaned by water, oven-dried at 60°C for 3 days, chopped into pieces and kept at room temperature in darkness until ready for extraction. For fresh leaves can be prepared the same process without drying. The fresh and dry leaves were steamed distillation for 3 hours, then the essential oils were obtained and evaporated the boiled water part to
dryness and the crude extracts were obtained. Crude extracts were furthered analysed for DPPH scavenging activity and the essential oils were analysed by GC-MS for their chemical compositions.

2.3 Free radical scavenging activity
A free radical scavenging activity was determined by DPPH assay using the method of Anaberta with some minor modifications. Crude extracts were dissolved in methanol at 50 ppm and performed two-fold dilution to 25, 12.5, 6.25, 3.125, and 1.563 ppm, respectively. DPPH reagent of 180 µL was added into the extract solution of 20 µL and mixed well for 20 minutes, measurements were taken at 517 nm by a microplate reader (model spectrostar nano, BMG Labtech, USA Manufacturer). The DPPH inhibition percentage was calculated by the following equation.

\[
\%\text{inhibition} = \frac{[A_{\text{control}} - A_{\text{sample}}]}{A_{\text{control}}} \times 100
\]

When \(A_{\text{control}}\) and \(A_{\text{sample}}\) are the absorbance of the control and the sample, respectively. The sample concentration of 50% inhibition (IC_{50}) was calculated by plotting the percentage of inhibition against concentrations of the samples. IC_{50} of samples were compared to IC_{50} of gallic acid, because gallic acid is a well-known natural antioxidant that is basically a secondary polyphenolic metabolite.

2.4 Chemical composition by GC-MS
The essential oils of \(L.\text{glutinosa}\) was subjected to GC-MS, an Agilent system model 6980 consisting of a mass selective detector (electron ionization, EI, 70eV, scan range 40-400m/z, and solvent delay 2.00min). The GC column was HP-5MS, capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas used was helium at the constant flow rate mode of 1 mL/min.

The temperature of the oven was programmed from 60°C-246°C at temperature ramp of 3°C/min. The temperature of the injector was set at 220°C. The essential oils of \(L.\text{glutinosa}\) leave were prepared at 10% in hexane and 1 µL of solution was injected using a splitless injection mode.

Identification of oil components was achieved based on their retention indices and by the National Institute of Standards and Technology (NIST98) MS search.

3. Result & Discussion
3.1 DPPH free radical scavenging activity
The DPPH free radical scavenging activity of fresh leave crude extract was higher than dry leave extract and IC_{50} of fresh leave extract was lower than dry leave extract one. Fresh crude leave extract exhibited higher antioxidant activity than gallic acid, which gallic acid as a standard substance used for comparison. The results of the investigation are shown in Fig. 1.

![Fig. 1 The DPPH free radical scavenging activity of crude extracts.](image)

3.2 Chemical composition by GC-MS
The yield of fresh and dry \(L.\text{glutinosa}\) leave essential oils were 0.07 and 0.05%, respectively. Analysis of the chemical composition of crude extracts, none of peaks in the chromatogram was found and identified. The fresh leave essential oil was found to contain 22 compounds (Fig. 2A)

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constituting 89.32% of the total essential oils. The main compounds are phytol (29.31%), caryophyllene oxide (14.25%), isopropyl myristate (6.08%), germacrene D (3.46%), isophorone (3.19%), and caryophyllene (2.54%). For essential oil of dry L. glutinosa leaves were found to contain 20 compounds (Fig. 2B) constituting 82.98% of the total essential oils. The main compounds are phytol (26.42%), caryophyllene oxide (12.39%), isopropyl myristate (5.75%), germacrene D (3.37%), isophorone (2.46%), and caryophyllene (1.90%).

![Fig. 2 Total ion chromatogram of L. glutinosa essential oils; fresh leaves (A) and dry leaves (B).](image)

In the essential oils of fresh and dry leaves of L. glutinosa, most of components found were terpenoids as shown in Fig. 3. Among the 22 components in the essential oils of L. glutinosa fresh leaves, were found alcohol (4.27%), ketone (5.51%), ether (2.68%), hydrocarbon (8.88%), terpene (49.56%), alkane (9.87%), alkene (2.47%) and ester (6.08%). The 20 components in the essential oils of L. glutinosa dry leaves, including alcohols (3.67%), ketone (6.09%), ether (1.45%), hydrocarbon (8.83%), terpene (44.08%), alkane (9.93%), alkene (3.18%), and ester (5.75%). Less of compounds were found than the fresh one, it might be because of some compounds were evaporated or converted to other compounds during drying process.

![Fig. 3 Chemical compounds in essential oils of fresh and dry L. glutinosa leaves.](image)

### 4. Conclusion

The DPPH free radical scavenging activity from crude extracts of L. glutinosa fresh leaves gave the lowest IC$_{50}$ value which exhibited the higher antioxidant activity than gallic acid. It might be a new promising source of antioxidant. Essential oils from fresh and dry leaves were contained similar components, but more compounds and higher concentration of components were identified in the fresh one. However, both dry and fresh leaves of L. glutinosa could be utilized but the better is the fresh one.

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