

# Betel (*Piper betle* L.) leaf essential oil extraction using steam distillation

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**Abstract**— The highest essential oil content was obtained at distillation conditions as below raw material's moisture content of 81.72%, distillation time of 5 hours, NaCl concentration of 15%, the ratio of raw materials and solvent of 1:3 (w/v), and the material size of  $0.5\text{mm} < d < 1.5\text{mm}$ . The quality of essential oils was then refined by using different method such as crystallization, anhydrous  $\text{Na}_2\text{SO}_4$  filtration and double distilling. By gas chromatographic mass spectrometry (GC-MS), the main chemical components in betel essential oil are identified including eugenol, acetoeugenol, 4-allyl-1,2-diacetoxybenzene.

**Keywords**— betel leaf, essential oil, *Piper betle* L., steam distillation.

## I. INTRODUCTION

Betel vine (*Piper betle* L.) belongs to genus *Piper* of the family *Piperaceae*. Leaves of *Piper betle* have several bioactivities properties and are used in traditional medicinal systems to cure indigestion, stomach ache, diarrhea, flatulence and to heal wounds, scales, burns, swelling etc. The leaves are nutritive and contain anticarcinogens, showing future opportunities in anticancer drugs. The main biochemical component betel leaf is essential oil and it contributes flavor to the leaf. It is also reported that essential oil contributed to the medicinal property of betel vine [1]. The main ingredient in betel essential oil is phenols (Chavicol, Chavibetol, Eugenol, ..) along with some other phenolic compounds including beta-Caryophyllen, beta-Cadinen, 4-allyl-1,2-diacetoxybenzene (27.51%) [2]. These phenol derivatives presented in betel leaf essential oil have multi biological effects such as antibacterial, antifungal, and antioxidant effects, etc. Betel leaf essential oil exhibits inhibitory activity against 3 strains of microorganisms: Gram (+) *Bacillus subtilis*, *Aspergillus niger* and *Fusarium oxysporum* molds with values of 100, 200 and 200  $\mu\text{g/mL}$ , respectively [3]. In the previous research the essential oils of betel plants contain phenol group compounds such as safrol and eugenol which have insect repellent and insecticidal activity [4,5]. Compounds such as eugenol, limonene, terpinolene, citronellal, and camphor contained in volatile oils indicate rejection activity in mosquitoes and other biting insects, which can be used as an alternative mosquito repellent replace synthetic chemicals [6, 7].

In this study, extraction essential oil from betel leaves (*Piper betle* L.) by using steam distillation will be carried out. This objective of study is evaluate different conditions for *Piper betle* L. essential oils extraction. The antibacterial and antioxidant activity of *Piper betle* L. essential oil are also evaluated in this study.

## II. MATERIALS AND METHODS

### 2.1 Materials

Preparation of material samples: The material samples used for this study were collected in the region of Hoc Mon, Ho Chi Minh city in Viet Nam. The betel leaves were washed and dried at room temperature. A grinder (LC-1416B, Alaska, Vietnam) was used to change the size of materials.

### 2.2. Essential oil extraction

The betel leaf essential oil was extracted by the steam distillation method. An instrument has five main parts, including a 1000 mL volume flask containing solvent, which was distilled water in this case, another flask of 500 mL capacity was used to contain material of lemon peels. The system was heated by a VELP heating device (Model: ARE, Italy) placed under the solvent flask. A condensing equipment was placed on the top of 500 mL flask containing materials, in order to condense the steam to collect the essential oils.

### 2.3. Experimental design

The chemical compositions of betel leaves were firstly identified including: carbohydrates, Glycoside,

Flavonoid, Phenol, Tannins, Saponin và Terpenoid. The moisture content, total solid content and color of raw material were also analysed.

In this study, four factors influencing betel essential oil distillation were investigated. The effect of raw material's moisture content on essential oil content was evaluated through different drying time of sample, which varies from 15 to 75 minutes of drying under temperature of 50°C by using tray drying. Time of extraction varied at 60 minutes intervals from 60 to 360 minutes. Concentration of NaCl varied from 5 to 25%. A ratio of betel leaves and water varied from 1/2 to 1/4 (w/v). All the experiments were repeated three times and average values were expressed.

The essential oil content was used to compared the efficiencies of different experiments. The obtained essential oil was dehydrated using different technique and calculated the content using the following equation:

$$\text{Essential oil content (mL/g db)} = \frac{\text{Volume of betel essential oil obtained (mL)}}{\text{Amount of betel leaves originally used (g)}}$$

Chemical composition of essential oils was analysed using GC-MS method using THERMO SCIENTIFIC Trace GC Ultra – ISQ instrument. Antimicrobial activity of betel essential oil was evaluated through MIC value by using agar-well diffusion method. Antioxidant activity of betel essential oil was carried out using DPPH (1,1-diphenyl- 2-picrylhydrazyl) method.

### III. RESULTS AND DISCUSSION

#### Physicochemical characterisation of raw material

Table 1: Chemical composition identification in betel leaf

Composition	Present	Absent
Carbohydrates		X
Glycoside	X	
Flavonoid		X
Phenol	X	
Tannins		X
Saponin	X	
Terpenoids	X	

Glycosides, phenols, sapiens and terpenoids are present in betel leaf (Table 1). Glycosides and terpenoids are ingredients that make the acrid taste and pungent aroma of betel leaves. Phenol and sapiens are the components that make up the bioactive of betel leaves, especially in betel leaves contain many phenol derivatives, so betel oil has good anti-inflammatory, antifungal and antioxidant activity. Moisture content and total solid content of betel leaf are  $81.72 \pm 3.45$  %wb and  $2.27 \pm 0.18$  %, respectively. The colour of betel leaf is present in Table 2.

Table 2: Physicochemical of betel leaf

Parameter	Result
Moisture content (%)	$81.72 \pm 3.45$ % wb
Total solid content (%)	$2.27 \pm 0.18$ %
Light side	$L^* = 43.46$ $a^* = -15.93$ $b^* = 20.15$
Colour	
Dark side	$L^* = 32.89$ $a^* = -10.45$ $b^* = 11.33$

#### Effect of moisture content of raw material on essential oil content

Essential oils are volatile compounds and may be evaporated along with steam. When increasing drying time, moisture content of the material decreases caused a reduction of essential oil content. At the moisture content of 81.72% (corresponding to the fresh sample), the highest concentration of essential oil was obtained.

#### Effect of extraction time on essential oil content

Table 5 shows that the content of essential oils varies with the distillation time. At 6h of steam distillation, the content of the essential oil tends to decrease. At 5h of distillation time, the content of essential oil is highest. This result is consistent with the results of Nguyen Nho Dung, 2011. The distillation time of 5h is used to conduct the further experiment.

Table 5: Effect of extraction time on essential oil content

Extraction time (hour)	2	3	4	5	6
Essential oil content (ml/g db)	6.753	9.709	13.236	<b>15.416</b>	10.044

*Effect of NaCl concentration on essential oil content*

Table 6: Effect of NaCl on essential oil content

NaCl concentration (%)	0	5	8	10	12	15	20	25
Essential oil content (ml/g db)	7.274	9.603	12.309	12.758	15.513	<b>19.289</b>	14.923	12.031

The betel essential oil content varies with the concentration of NaCl added (Table 6). The essential oil content tends to decrease at the NaCl concentration of 20%. Using a high content of NaCl, the outer epidermis containing essential oils shrinks, preventing the diffusion of essential oils, results an decrease of essential oil content. At NaCl

concentration of 15%, the highest content of essential oil. This result is consistent with the research results of Nguyen Thien Chi, 2016. NaCl concentration of 15% is used to conduct the further experiment.

*Effect of raw material size and ratio of raw material/ solvent content on essential oil content*

Table 7: Effect of raw material size and ratio of raw material/ solvent on essential oil content

Ratio of raw material/ solvent (w/v)	1:2		1:3		1:4	
Raw material size	Grinded	Non grinded	<b>Grinded</b>	Non grinded	Grinded	Non grinded
Essential oil content (ml/g db)	11.725	10.193	<b>20.678</b>	13.147	14.953	11.142

Table 7 shows that the essential oil content varies with the size of the material and the ratio of material/solvent. A grinded sample has a higher extracted essential oil than a non-grounded material. The contact surface between the

grinded material and the solvent is larger which help the oil diffuse more easily. At the ratio of material/solvent at 1/ 3 (w/v), the highest content of essential oil is obtained.

Table 8: The suitable condition for betel essential oil extraction using steam distillation

Raw material (g)	Moisture content of raw material (%wb)	Distillation time (h)	Distillation temperature (°C)	NaCl concentration (%)	Raw material size	Ratio of material/solvent (w/v)
150	81.72%	5	100	15	Grinded sample	1/3

*Chemical properties of betel essential oil*

Table 9: Chemical composition of betel essential oil analysed by GC-MS

TT	Compostion	Hàm lượng
1	$\beta$ -Phellandrene	0.840
2	Terpinolene	0.384
3	Sabinen	0.458
4	Eucalyptol	0.630

5	$\gamma$ -Terpinene	0.779
6	Terpinen-4-ol	2.668
7	Terpineol	0.306
8	4-Allylphenyl acetate	0.825
<b>9</b>	<b>Eugenol</b>	<b>22.693</b>
10	$\beta$ -Elemen	0.650
11	Caryophyllene	0.657
12	Humulene	0.406
13	$\gamma$ -Muurokene	1.758
14	Germacrene D	1.343
15	$\alpha$ - Muurokene	1.086
16	$\gamma$ -Cadinene	0.623
<b>17</b>	<b>Aceteugenol</b>	<b>27.503</b>
18	1,10-Diepicubenol	0.286
<b>19</b>	<b>4-Allyl-1,2-diacetoxybenzene</b>	<b>27.391</b>
20	$\delta$ -Cadionol	0.698
21	$\alpha$ - Cadionol	8.015

The major components in betel essential oil is phenol compounds (> 77.59%) including aceteugenol (27,503%), 4-Allyl-1,2-diacetoxybenzene (27,391%), and Eugenol (22,693%). Other chemical components of betel essential oil are terpene compounds (terpinen-4-ol,  $\gamma$ -Muurokene, Germacrene D,  $\alpha$ -Muurokene,  $\alpha$ - Cadionol, ..). The phenol derivatives present in the essential oil have good biological effects such as anti-inflammatory, antibacterial and antioxidant.

#### IV. CONCLUSION

The suitable conditions for steam distillation of betel essential oil are as below the moisture content of raw materials of about 81.72% (fresh sample), distillation temperature of 100°C, distillation time of 5 hours, NaCl concentration of 15%, the ratio of material/solvent of 1/3 (w/v) and the size of raw material varied from 0.5mm to 1.5mm.

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