

## Aqueous Extraction of Hydroxychavicol from *Piper Betle L.* Leaves

K.Y. Pin<sup>\* 1</sup>, T.G. Chuah<sup>2</sup>, A. Abdull Rashih<sup>1</sup>, M.A. Rasadah<sup>1</sup>, C.L. Law<sup>3</sup>, & T.S.Y. Choong<sup>2</sup>

<sup>1</sup>Medicinal Plants Program, Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.

<sup>2</sup>Department of Chemical and Environmental Engineering,

Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>3</sup>Faculty of Engineering and Computer Science, University of Nottingham, 43500 Semenyih, Selangor, Malaysia.

### Abstract

*Piper betle L.* is locally named as *Sirih* and Betel in English. It is a native medicinal plant of central and eastern parts of Peninsular Malaysia. The betel plant is an attractive and aromatic creeper with alternate, heart shaped, smooth, shining, and long stalked leaves with pointed apex. Betel leaf is well known for its use as masticatory, which consists of fresh betel leaf, betel nut and slaked lime paste. The phenolic compound, Hydroxychavicol, found in the aqueous extract of betel leaf is reported to exhibit useful bioactivities - anticarcinogenic and antimutagenic. Batch experiments of betel leaves extraction were conducted using distilled water as solvent and under different operating parameters including temperature, duration and ratio of solvent to sample. After freeze-drying, the yield of each experiment was determined and analyzed using High Performance Liquid Chromatography (HPLC) to investigate the effects of operating parameters on the major compound, Hydroxychavicol.

*Keywords:* *Piper betle L.* – Extraction – Hydroxychavicol – Yield – HPLC.

### 1.0 Introduction

*Piper betle L.* belongs to the genus *Piper* of Piperacea family. It is called *Sirih* locally and Betel in English. The betel plant is an attractive and aromatic creeper. It has an alternate, heart shaped, smooth, shining, and long stalked leaves with pointed apex. This plant is originated from the central and eastern part of Peninsular Malaysia (Anon., 2002). Today betel spread throughout Asia's tropical region and east Africa. Betel is widely cultivated by thousands of betel growers in many parts of India.

Traditionally, betel leaf is used as masticatory or better known as betel quid, which consists of fresh betel leaf, betel nut, slaked lime paste. The betel quid chewing with or without tobacco acts as natural tonic and breath refresher. This is a popular practice among 200-600 million people in India, Taiwan and Southeast Asian countries (Ko at el., 1992). This habit is found to be responsible of the incidence of oral cancer among the chewers (Kwan at el., 1976; Sanghvi L.D., 1981; Thomas at el., 1985). However, the betel leaf plays a protective role against the carcinogenic effect. Amonkar at el. (1985) found that the two phenolic compounds, Hydroxychavicol (HC) and Eugenol, isolated from betel leaf extract exhibited antimutagenicity and HC was more potent than eugenol. Synthesized HC suppressed the mutagenic effects of two tobacco-specific carcinogens in the in-vitro and in-vivo systems (Amonkar at el., 1989). The betel leaves extract also exhibited anticarcinogenic effect against tobacco-induced cancer using long term studies in Swiss male mice (Padma at el., 1989).

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\* Corresponding author: E-mail: pin@frim.gov.my

Aqueous extraction is a method to obtain the essence of the herbs. By taking the extract, the users are able to consume the active ingredients directly instead of taking together the fibers and cellulose that is insoluble and indigestible. The operating parameters including temperature, duration, amount of solvent, type of solvent, and particle size of the solid (plant parts) would influence the effectiveness of the extraction process (Dibert et al., 1989; Wongkittipong et al., 2004; Sim et al., 2004).

In this study, batch experiments of betel leaves extraction were conducted using distilled water as solvent and under different operating parameters including temperature, duration and ratio of solvent to sample. The yield of process was determined after freeze-drying. The aqueous extract was analyzed using High Performance Liquid Chromatography (HPLC) to investigate the effects of operating parameters on the major compound, Hydroxychavicol.

## 2.0 Materials and Methods

### 2.1 Samples

*Piper betle L.* leaves were collected from Santan, Perlis. The leaves were washed with clean water before drying in oven at a temperature of 40°C until the moisture content was below 14%. This is to prevent fungi infection on the samples. The dried leaves were grinded and stored in airtight container.

### 2.2 Aqueous Extraction

#### Experiment E1: Extraction for studying the effect of duration

The extraction was conducted using a 60-litre-jacketed vessel with agitator. The betel leaves was extracted at the temperatures of 27°C and 60°C for 2 hours. 100ml of extract was sampled every 30 minutes. The samples were filtered to remove the residue particles. 50ml of filtrate was freeze-dried using Freeze Dryer (Model 35 XL Genesis, Virtis) to remove the solvent and 50ml used for HPLC analysis. The calculation of yield was based on weight of the freeze-dried extract, given by

$$Yield = \frac{\left[ \frac{W_d}{V_e} \right] \times V_t}{W_s} \times 100\% \quad (1)$$

Where

$W_d$  = Weight of dried extract (g)

$W_s$  = Weight of sample (g)

$V_e$  = Volume of aqueous extract used for freeze-drying (ml)

$V_t$  = Volume of aqueous extract obtained after filtration (ml)

Assume that the solvent is recovered totally from filtration,

$\therefore V_t$  = Volume of solvent used.

#### Experiment E2: Extraction for studying the effects of temperature and ratio of solvent to sample

The lab scale extraction was carried out by using laboratory Flask Heater. The heater consists of six heating places with temperature control for each position. The solvent used in the extraction was distilled water. The operating parameters selected were:

Temperatures selected, T (°C): 27 (Room temperature), 40, 60, 80, and 90.

Duration, t (hrs) : 2

Solvent to sample ratio, R (ml/g) : 10, 20, and 30

For each run, the sample was extracted in a 250 ml round-bottom flask. After the extraction, the sample was filtered to obtain the aqueous extract. The aqueous extracts were then freeze-dried to remove the solvent. The freeze-drying process was carried out using Freeze Dryer (Model 35 XL Genesis, Virtis). The yield of each run was determined using equation (1).

### 2.2.1 High Performance Liquid Chromatography (HPLC) Analysis

The freeze-dried extracts from E1 were analyzed using HPCL to determine its chemical profiles and concentration of Hydroxychavicol (HC). The calibration curve was developed using pure crystal of HC provided by Forest Research Institute Malaysia (FRIM). The HPLC analysis was carried out using Waters 600E System Controller coupled with Waters 996 Photodiode Array Detector. A Phenomenex Luna C18 100A column (250mm x 4.6mm, 5 $\mu$ m particle size) was used as stationary phase. The mobile phase was in gradient mode by changing the content of 0.1% Orthophosphoric Acid, H<sub>3</sub>PO<sub>4</sub> (solvent A) and 100% Acetonitrile, CH<sub>3</sub>CN (solvent B). The changes of mobile phase content was shown in Table 1. The flow rate of the mobile phase was 1ml/min and the detection wavelength was 200.0 nm.

Table 1 The mobile phase changes in HPLC analysis.

Time	Solvent A (%)	Solvent B (%)
0	35	65
1	45	55
10	45	55
15	35	65

## 3.0 Results And Discussion

### 3.1 Extraction of Hydroxychavicol (HC)

The presence of HC in the aqueous extract was determined using the HPLC analysis. As shown in Fig. 1, the important parameters, including retention time and wavelength with maximum absorbance ( $\lambda_{max}$ ), of the HC peak in the pure crystal and aqueous extract were the same. The concentration of HC in the aqueous extract was determined using the calibration curve. From the HPLC analysis, HC is the major chemical component found in the aqueous extract of betel leaves.

The HC concentration increased rapidly in first 30 minutes and reached the equilibrium state in the extraction at 60°C. However, continuous increase of HC concentration was observed in the extraction at 40°C. The rate of extraction reduced gradually after the first 30 minutes. This might due to the decline of the concentration gradient between the particle and solvent as the saturation took place in the solvent.

The final concentration of HC increased with the temperature. This is because the rise of temperature resulted in the increase of solubility and diffusivity of HC. Diffusivity or diffusion coefficient is given by Einstein equation as a function of the absolute temperature and the dynamic viscosity coefficient (Loncin et al., 1979). Referring to Fig. 2, the HC was stable at 60°C as no reduction was observed after the equilibrium.

### *3.2 Yield of the Aqueous Extraction*

A similar trend was observed in the change of yield (see Fig.3) as compared to the change of HC concentration (see Fig. 2). The similarity is mainly because HC is the major component in the aqueous extract with which the yield is closely related. The yield increased with time and temperature and the process reached its state of equilibrium reached at about 30 minutes. There was an increase of 3% in the final yield for the extraction carried out at 60°C. The results implied that the temperature was the limiting factor in the experiment E1. Although a higher yield could be achieved if a higher temperature used, the maximum tolerable temperature of HC should be identified to avoid its degradation during the extraction process.

Referring to the results from experiment E2, the rise of ratio of solvent to sample ratio resulted in the increase of the yield (see Fig. 4). This shows that the total amount of solute that transfer into the solvent increases when more solvent used. However, the amount of solvent used is compromised by the cost of removing it. This is because more solvent means more energy is required during the drying process. Besides that, the yield responded consistently with the temperature as compared to experiment E1.

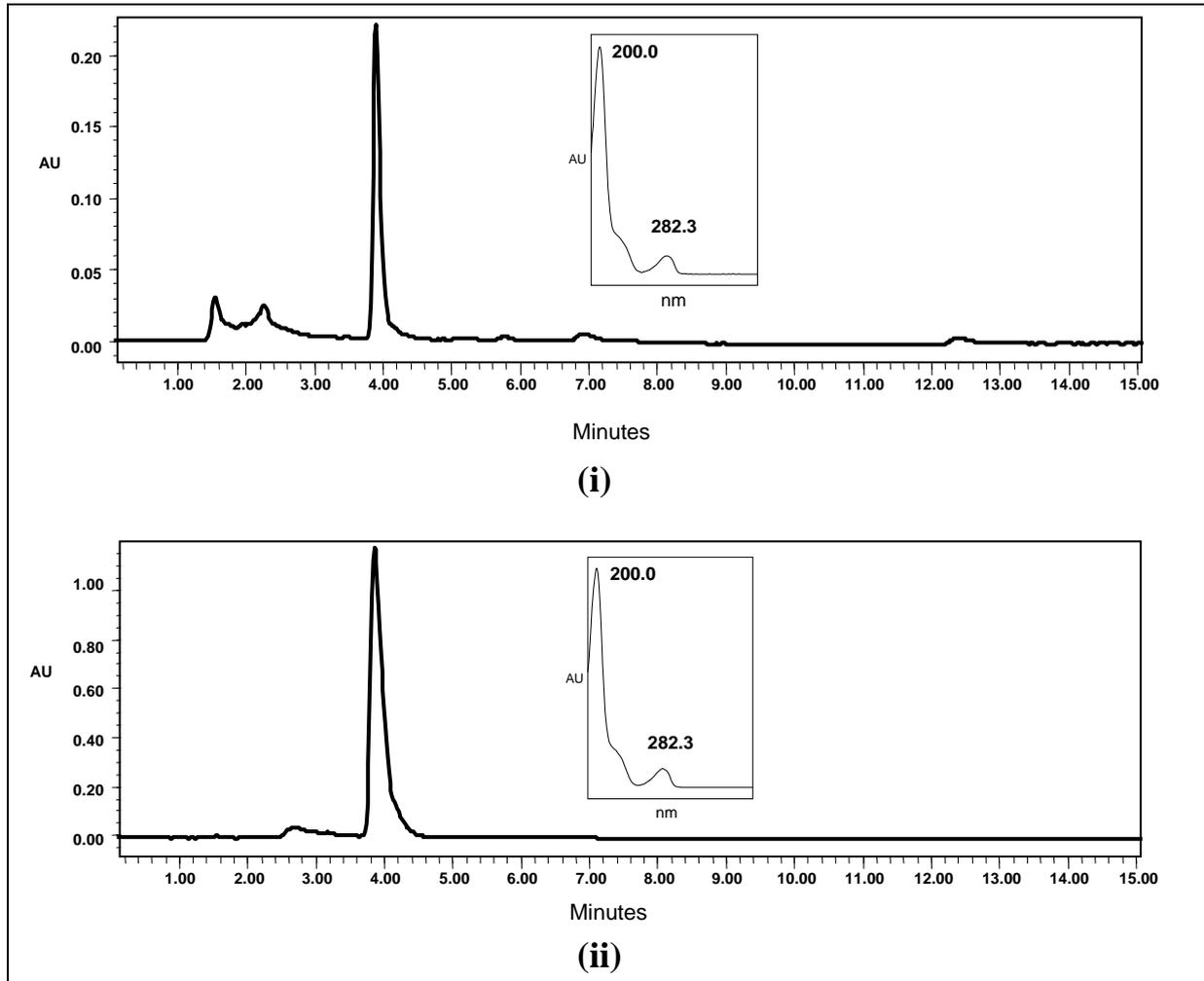


Figure 1. Chromatograms of i) Aqueous extract and ii.) Pure HC Crystal.

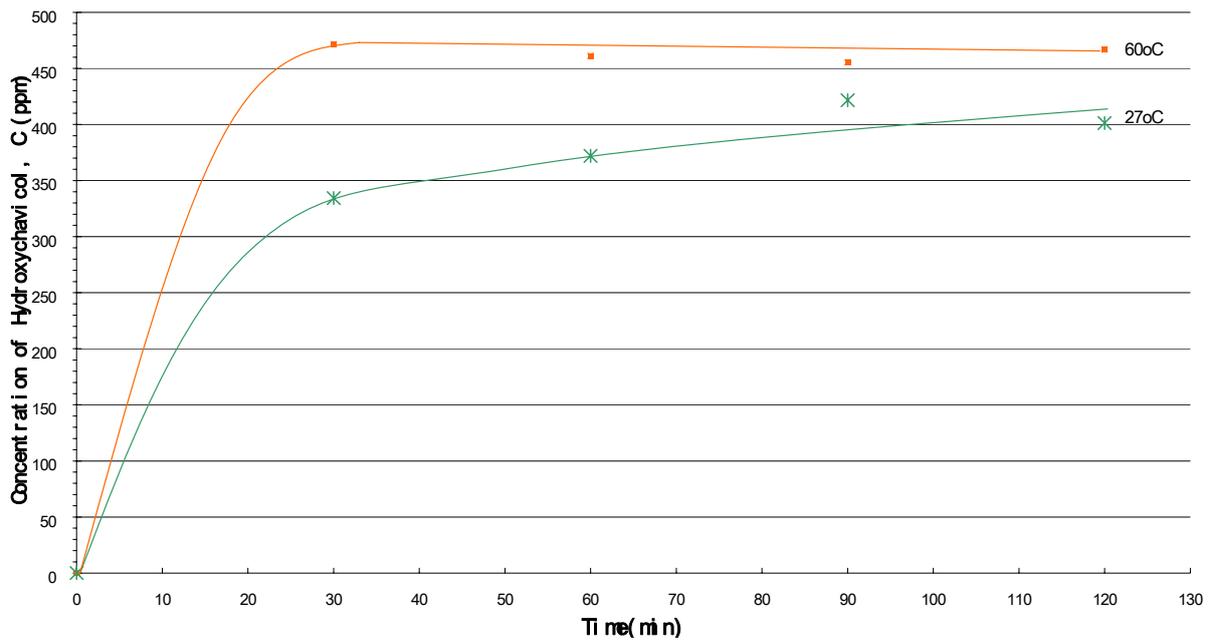


Figure 2. The change of concentration of HC for 2-hours extraction at 27°C and 60°C.

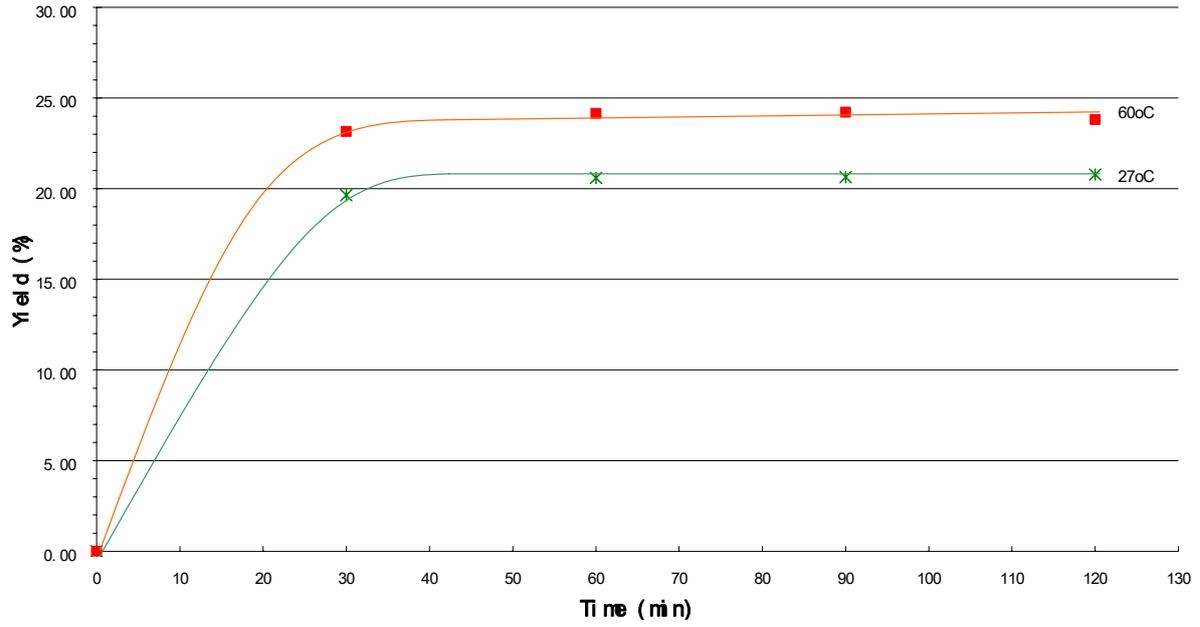


Figure 3. The change of yield for 2-hours extraction at 27°C and 60°C.

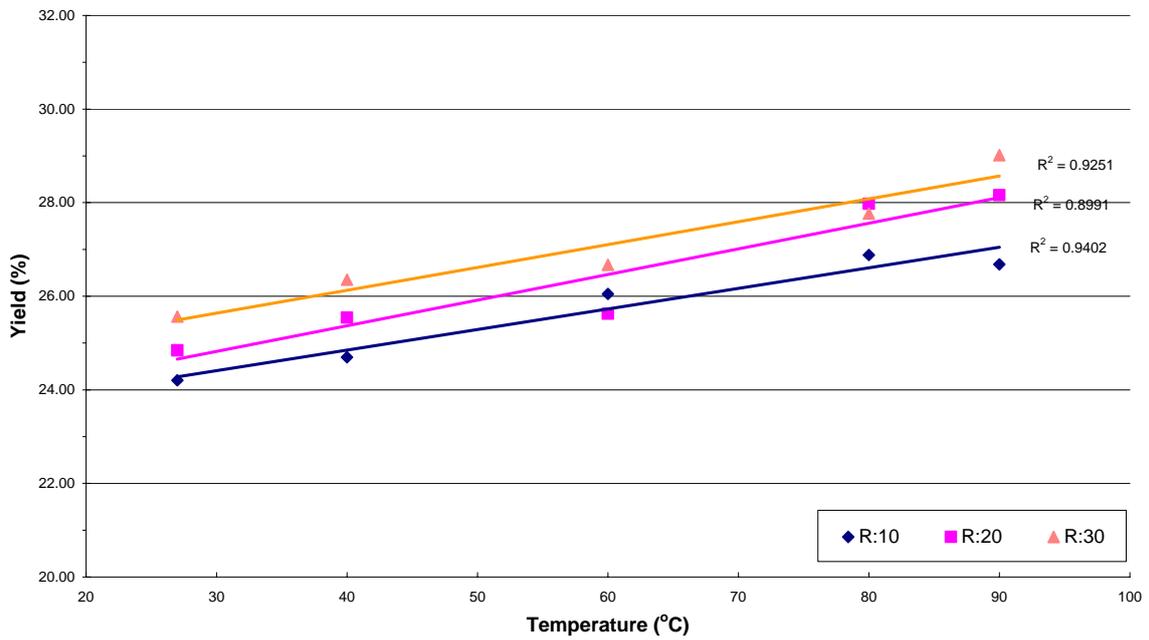


Figure 4. The yield of extraction under different T and R for t = 2 hrs.

#### 4.0 Conclusion

Results of this work indicates that Hydroxychavicol, a beneficial phytochemical, can be extracted from *Piper betle* L. leaves using the aqueous extraction. In fact, it is the major component of the betel leaves extract. The yield of aqueous extraction increases with the temperature and ratio of solvent to sample. The kinetic data of the process reveals that the equilibrium state was reached about 30 minutes. Thus, the duration of the extraction should be kept within the same time to avoid waste of energy.

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#### References

- [1] Amonkar, A. J., Nagabhushan, M., D'Souza, A. V., & Bhide, S. V. 1986. Hydroxychavicol: A new phenolic antimutigen from betel leaf. *Food and Chemical Toxicology*, Vol. 24, No. 12, pp. 1321-1324.
- [2] Amonkar, A. J., Padma, P. R. & Bhide, S. V. 1989. Protective effect of hydroxychavicol, a phenolic component of betel leaf, against the tobacco-specific carcinogens. *Mutation Research*, 210, pp. 249-253.
- [3] Anon. 2002. *Compendium of Medicinal Plants used in Malaysia 2002*, Herbal Medicine Research Centre, Institute for Medical Research, Kuala Lumpur. 225-226.
- [4] Dibert, K., Cros, E. & Andrieu, J. 1989. Solvent extraction of oil and chlorogenic acid from green coffee. Part II: Kinetic Data. *Journal of Chemical Engineering*, Vol. 10, 199-214.
- [5] Ko, Y. C., Chiang, T.A., Chang, S. J., & Hsieh, S. F. 1992. Prevalence of betel quid chewing habit in Taiwan and related sociodemographic factors. *J. Oral Pathol. Med.* **21**:261-264.
- [6] Kwan, H.W. 1976. A statistical study on oral carcinomas in Taiwan with emphasis on the relationship with betel nut chewing: a preliminary report. *J. Formos. Med. Assoc.* **75**:497-505.
- [7] Padma, P.R., Lalitha V.S., Amonkar A. J., & Bhide S.V. 1989. Anticarcinogenic effect of betel leaf extract against tobacco carcinogens. *Cancer Letters*, Vol. 45, Issue 3, 195-202.
- [8] Sanghvi, L. D. 1981. Cancer epidemiology: Indian scene. *J. Cancer Res. Clin. Oncol.* **99**, 1.
- [9] Sim, C.C., Kumaresan, S., & Sarmidi, M.R. 2004. Mass transfer coefficients of *Eurycoma longifolia* batch extraction process. In: *Proceedings of 18<sup>th</sup> Symposium of Malaysia Chemical Engineers 13-14 December 2004*, Vol. 1, 362-367.
- [10] Thomas, S., Kearsley, J. 1993. Betel quid and oral cancer: a review. *Oral Oncol.*, **9**:13-19.
- [11] Wongkittipong, R., Prat, L., Damronglerd, S., Gourdon, C. 2004. Solid-liquid extraction of andrographolide from plants – experimental study, kinetic reaction and model. *Separation and Purification Technology*, Vol. 40, 147-154.
- [12] Loncin, M., & Merson, R. L. 1979. *Food engineering. Principles and selected applications*, pg.494. New York: Academic Press Inc.