

## Study on the Chemical Constituents of *Piper betle* L. in Relation to their Possible Insect Attractant Property

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**ABSTRACT** Chemical constituents of *Piper betle* extract were studied to identify the active component that could be responsible for any attractant property on the Oriental Corn Borer, *Ostrinia salentialis*. Extraction process was conducted on locally collected *Piper betle* leaves using petroleum ether and methanol. Essential oil was also extracted using hydrodistillation and analysed by GC-MS. Two major components isolated from the petroleum ether extract were identified as chavibetol [2-methoxy-5-(2-propenyl)phenol] and  $\beta$ -sitosterol while one major component isolated from methanol extract was identified as allylpyrocatechol [4-(2-propenyl)-1,2-benzenediol]. All the three components were identified based on their GC-MS,  $^{13}\text{C}$  and  $^1\text{H}$  NMR data and also by comparison with literature. Field evaluation using traps containing petroleum ether extract, methanol extract and essential oil of *Piper betle* were conducted in a cornfield and adult moths of *Ostrinia salentialis* were not detected.

**ABSTRAK** Komponen kimia ekstrak daun *Piper betle* telah dikaji untuk menentukan komponen aktif yang mungkin mempunyai sifat penarik terhadap serangga pengorek jagung *Ostrinia salentialis*. Proses pengekstrakan daun *Piper betle* telah dijalankan menggunakan petroleum eter dan metanol. Minyak pati daun *Piper betle* juga telah diekstrak melalui kaedah penyulingan hidro dan dianalisa dengan GC-MS. Dua komponen utama telah diasingkan daripada ekstrak petroleum eter dan dikenalpasti sebagai kavibetol [2-metoksi-5-(2-propenil)fenol] dan  $\beta$ -sitosterol. Komponen utama daripada ekstrak metanol dikenalpasti sebagai allilpirokatekol [4-(2-propenil)-1,2-benzenadiol]. Kesemua komponen dikenalpasti melalui data KG-SJ,  $^{13}\text{C}$  dan  $^1\text{H}$  RMN dan juga perbandingan dengan literatur. Kajian diladang menggunakan beberapa perangkap yang mengandungi ekstrak petroleum eter, metanol dan minyak pati daun *Piper betle* telah dijalankan di ladang jagung dan didapati kupu-kupu dewasa *Ostrinia salentialis* tidak dikesan.

(*Piper betle*, *Ostrinia salentialis*, essential oil, allylpyrocatechol [4-(2-propenyl)-1,2-benzenediol], NMR)

### INTRODUCTION

*Piper betle* locally known as sireh is a native plant of central and eastern Malaysia and became a cultivated plant spreading throughout tropical Asia and Malaysia [1]. The chemical constituents of *P. betle* leaves had been previously investigated in relation to some of its biological activities. Evans *et al.* [2] had isolated antifungal and antinematocidal components from the chloroform extract of *P. betle* leaves, identified as propenyl phenols namely chavicol, chavibetol, allylpyrocatechol, chavibetol acetate and allylpyrocatechol. The fungicidal property of the essential oil of *P. betle* (Indian betel leaves) as reported by Dubey and Tripathi was due to the presence of eugenol [3]. Wang *et al.* [4] had isolated antioxidants from the leaf extract of

*P. betle* in Taiwan of which hydroxychavicol was the major component and they also showed *P. betle* leaves contained high amount of ascorbic acid and carotene. Zeng *et al.* [5] had isolated four neolignans from the vines of *P. betle* identified as piperbetol, methylpiperbetol, piperol A and piperol B. These neolignans were found to be active as a platelet activating factor (PAF) antagonist.

Nor Azah *et al.* [6] identified the presence of chavibetol (69%), eugenyl acetate (8.3%), chavicol (6.0%),  $\gamma$ -muroloen (5.2%) and methyleugenol (0.6%) in the GC-MS analysis of the essential oil of *P. betle* leaves of our local species. They also found the methanol extract of *P. betle* to exhibit antioxidative, anti-inflammatory and antimicrobial activity.

We had previously conducted a preliminary research work to investigate the insecticidal effect of the *P. betle* extract in the control of the oriental corn borer, *Ostrinia salentialis* Snell. From our study, the maize plant sprayed with *P. betle* extract, although not significant, seemed to show higher damage by *Ostrinia* compared to control [7, 8]. Based on these observations, we suspect that the *P. betle* extract might have some attractant property toward the corn borer instead. This led us to carry out another experiment to investigate whether the extract of *P. betle* leaves could act as an attractant toward *Ostrinia salentialis*. This paper will discuss on the isolation of the major components present in various extracts of *P. betle* and also highlight on some preliminary observation on their attractant property toward the oriental corn borer.

## MATERIALS AND METHODS

### Extraction Method

Leaves of *Piper betle* L were collected from Padang Lati area, in Perlis. A total of 3 kg of the fresh *P. betle* leaves were air dried for about five days, and then ground to fine powder for extraction. The fine powder was first soaked in petroleum ether for one month, filtered and dried under reduced pressure. The extraction was repeated once and the combined dried extract obtained was 21.4 grams. The remaining residue was then further extracted twice using methanol, filtered and the solvent was removed using rotatory evaporator. The total extract obtained was 18 grams. Extraction of the essential oil of *Piper betle* leaves was also carried out using Dean and Stark like apparatus. A total of 276.35 g of the fresh leaves (cut into small pieces) were subjected to a series of hydrodistillation extraction. In each serie 30-40 g of leaf samples and 300 ml of water was hydrodistilled for about 2 hours and the oil obtained was trapped in 3.0 mL hexane.

### Isolation and Purification of Crude Extract

Both petroleum ether and methanol extract were analysed by TLC and isolated by column chromatography on silica gel. Five grams of the petroleum ether extract was subjected to column chromatography eluted using hexane with increasing ratios of ethyl acetate. The elution was continued using a more polar solvent system, ethyl acetate : methanol mixture. Fifty fractions were collected and were monitored by TLC and recombined based on the Rf values. Fractions

18-20 contained the major components and fraction 43-45 was an amorphous solid. Fractions 18-20 was further purified using repetitive preparative TLC developed with hexane : ethyl acetate (8.5:1.5) to afford an orange oil (0.137 g). While fraction 43-45 was purified using preparative TLC developed with petroleum ether : ethyl acetate (8:2) to give an amorphous pale white solid (8 mg). For the methanol extract (5 g), the eluent used was dichloromethane with increasing ratios of methanol of which thirty-nine fractions were collected. Fraction 11-14 of the methanol extract was further purified using preparative TLC (hexane : ethyl acetate; 7:3) which was repeated several times to yield a pale light green gummy solid (0.101 g).

The essential oil was analyzed by using an Agilent GC-MSD 19015-433 series: 70 eV. The capillary column (30.0m x 250 $\mu$ m x 0.25 $\mu$ m) was HP-5MS (0.25mm x 30m x 0.25 $\mu$ m) which is an intermediate polar column. The carrier gas used was helium (pressure: 8.78psi; flow: 1.0m/min; Average velocity: 37cm/sec). The temperature program for the column was as follows: Initial temperature : 70  $^{\circ}$ C (2 min. hold) then 50–230  $^{\circ}$ C with temperature program 20  $^{\circ}$ C/min and the injection temperature was 250  $^{\circ}$ C.

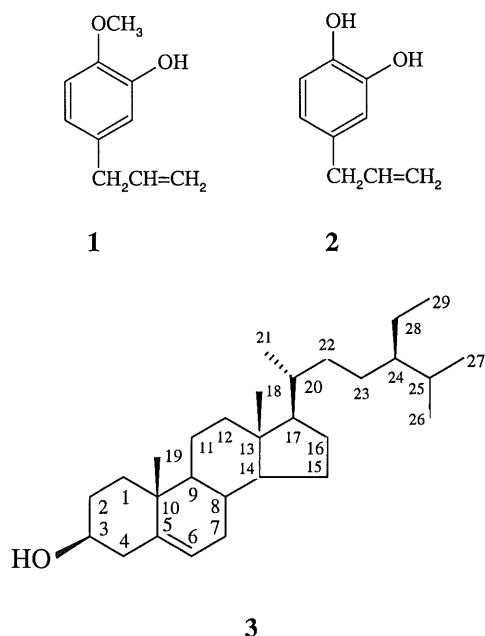
### Field Trapping of the Pest

A quick field evaluation was conducted to investigate the attractant property of *P. betle* extract towards the Oriental Corn Borer, *Ostrinia salentialis* in the August planting season of corn at the UiTM farm. In this trial, the petroleum ether extract, the methanol extract and the essential oil of *P. betle* were tested for their potential attractant property. In the evaluation, 40 simple traps made from PVC tubes measuring 7.5 cm in diameter and 20 cm in length were placed randomly in the cornfield. Each PVC tube was fixed at both ends with 2 pieces of filter papers (125 mm diameter) that have been folded into cones. Each of the cone was cut at the center so that it has a smaller opening (35 mm) which is pointing inward in the tube. This was done to prevent the moth from escaping once they are trapped inside. Inside each tube, another piece of filter paper (125 mm diameter) which has been sprayed with a sticker (Neopeace®) was carefully placed. A cotton bud, which has been dipped with the extract, was then placed onto the filter paper inside the tube. The experiment was set in a completely randomized design with 4

treatments (petroleum ether extract, methanol extract, essential oil and control) and 10 replicates. The traps were left for 1 week and checked daily for any moths.

### RESULTS AND DISCUSSION

Field experiment, which involved trapping of *Ostrinia salentialis* in the field was conducted using three samples, which are the petroleum ether, methanol extract and the essential oil of *P. betle*. Meanwhile in our laboratory, we had also carried out isolation work on the petroleum ether and the methanol extract of *P. betle* leaves. Two major components isolated from petroleum ether extract were identified as chavibetol [2-methoxy-5-(2-propenyl) phenol] **1** and  $\beta$ -sitosterol **3**. The methanol extract yielded one major component, which was identified as allylpyrocatechol [4-(2-propenyl) 1,2-benzenediol] **2**. All the three components were identified based on their GC-MS,  $^{13}\text{C}$  and  $^1\text{H}$  NMR data and also by comparison with the literature [2, 9, 10].



GC-MS analysis of chavibetol gave the molecular ion peak at  $m/z$  164, which corresponded to the molecular formula of  $\text{C}_{10}\text{H}_{12}\text{O}_2$ . The  $^{13}\text{C}$  NMR showed the presence of 10 carbons, and the  $^1\text{H}$  NMR showed the presence of a methoxyl group at  $\delta$  3.91. One of the aromatic hydrogens resonated at  $\delta$  6.71 and the two more at  $\delta$  6.82. The two terminal alkene protons resonated at  $\delta$  5.13 and  $\delta$  5.09 and one more at  $\delta$  5.98.

The  $^1\text{H}$  NMR of compound **2**, allylpyrocatechol [4-(2-propenyl) 1,2-benzenediol] was similar to that of **1** except that it lacked the methoxyl peak at about  $\delta$  3.9. The EIMS spectrum showed the molecular ion peak at  $m/z$  150 thus suggesting a molecular formula of  $\text{C}_9\text{H}_{10}\text{O}_2$ . The  $^{13}\text{C}$  NMR showed the presence of nine peaks. This compound is very sensitive as can be seen from the way it slowly changed to darker colour after we obtained it in pure form. This sensitive behavior could be due to the presence of two hydroxyl groups in its structure. Table 1 showed the comparison of  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra of chavibetol **1** and allylpyrocatechol **2**.

Compound **3** was isolated as a white solid. The GC-MS analysis gave a molecular peak at  $m/z$  414 corresponding to the molecular formula of  $\text{C}_{29}\text{H}_{50}\text{O}$ . The GC-MS analysis suggested this compound to be  $\gamma$ -sitosterol (S1: 90) or  $\beta$ -sitosterol (S1: 82). The  $^{13}\text{C}$  NMR data of this compound showed quite a good correlation with those reported for  $\beta$ -sitosterol and the assignment of the carbon chemical shifts in the table was based on the reported values for  $\beta$ -sitosterol [9].  $\beta$ -sitosterol was reported to be present in several species of *Piper* such as *P. acutislegium*, *P. aduncum*, *P. attenuatum*, *P. brachystachyum*, *P. khasiana*, *P. pedicellosum*, *P. peepuloides*, *P. thomsoni* and also *P. betle* [10].

We have also carried out the GC-MS analysis of the oil extracted from the leaves of *P. betle* using hydrodistillation. Five major components were identified. The component with the highest relative abundance was chavibetol (41.4%) which was identified based on the comparison of its retention time with that of the isolated component. The other four components were 2-methoxy-4-(1-propenyl)phenol (22.9%), 4-allyl-1,2-diacetoxybenzene (9.8%), 4-(2-propenyl)phenol (4.86%) and 4-(2-propenyl)phenolacetate (4.86%) as suggested by the GC-MS library data.

Eugenol, which is isomeric to chavibetol and was reported to be the major component of the *Piper betle* of Indian variety, was almost absent from our sample. The TIC chromatogram gave a very small peak (0.54%), which has the same retention time as the authentic eugenol. Though methyleugenol was detected in our sample, its relative abundance in the oil is low (1.3%). The essential oil, the petroleum ether extract and the methanol extracts were subjected to a field

experiment which was designed to evaluate whether they can serve as attractants towards *Ostrinia*. Several attractants consisting of volatile compounds such as esters, alcohol, aldehydes and ketones had been successfully used in the management of pest in agriculture. Some of these compounds are present in plants, while the others could be synthesised from the essential oils of the plants [11]. Amongst the known insect attractants (pheromones) include methyleugenol which was an attractant to the oriental fruitfly, *Daccus dorsalis* Hendel [11] and eugenol, an attractant to beetles, *Diobrotica cristata* [12-14]. Our preliminary field trial experiment on the possible attractant properties of the *Piper betle* extracts however showed a negative result. After one week of observations, none of the traps including control has any catch of the moth. This indicated that the *Piper betle* does not have any attractant property towards *Ostrinia salentialis*. Although the results in the previous study [8] showed some increased infestation in the plants treated with *Piper betle*, this could have be so due to chance, as indicated in the statistical analysis.

#### CONCLUSION

The chemical constituents of the extracts of *Piper betle* (sireh melayu) leaves were identified as chavibetol, **1**, allylpyrocatechol **2** and  $\beta$ -sitosterol **3**. The essential oil consists of prophenylphenols and their acetates of which chavibetol is the major component. These findings are identical to the chemical constituents reported in other studies of *Piper betle* leaves [2, 6, 10] except that in the essential oil of *Piper betle* of Indian variety (Indian betel leaves), eugenol present as the major component. The preliminary result obtained in the trapping of pest of maize, the corn borer, *Ostrinia salentialis* in the field indicated that the oil, pet. ether and the methanol extract of *Piper betle* did not show any attractant properties.

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