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# Extraction and Qualitative Analysis of *Piper Betle* Leaves for Antimicrobial Activities

**Lee Weng Foo, Eraricar Salleh and Siti Nur Hana Mamat**
Bioprocess Engineering Department, Faculty of Chemical Engineering,Universiti Teknologi Malaysia,81310 Skudai, Johor,Malaysia.

## ABSTRACT

The *Piper betle* is a plant with medicinal properties. In Malaysia, the*Piper betle* leaves is used in many commercial product and its function can act as flavouring, folk medical, aromatherapy and Ayurveda, dental and oral product due to its contain many bioactive compound. The main objectives of this study are to extract bioactive compound from dried*Piper betle* leaves to determine major bioactive compound from the extracts using qualitative analysis. The extraction of dried*Piper betle* leaves was prepared using Soxhlet apparatus and the solvent used for extraction was deionised water and ethanol (70%). Gas chromatography mass spectrometry (GC/MS) was used to analyse the bioactive compound in the leaves extract. Phytochemical screening of the extract showed the presence of alkaloids, phenolic compounds, alcoholic compound, organic acids and other solvent soluble compound. The yield of aqueous extraction (1.57g) was slightly higher than ethanol (70%) extraction (1.23g). Based on the GCMS data, a percentage for the aqueous extraction for antimicrobial function is 96.01% and for the ethanol (70%) extraction is 83.68%. The presence of various bioactive compounds from dried *Piper betle* leaves justified the use of various treatments by traditional practitioners in Malaysia. Dried *Piper betle* leaves extract is a complex with many bioactive components which can show significant antimicrobial activities.The GCMS information will be useful to formulate the new commercial product in market.

## 1. INTRODUCTION

*Piper betle* plant is a tropical plant and is a native plant from central and eastern part of peninsular Malaysia (Pin *et al*., 2010, Periyanayagam*et al*., 2012). This plant has a very unique love shaped leave (Vandana and Shalini, 2014). In Malaysia, this love shaped leaf is call 'DaunSirih' by local.*Piper betle* plant easy grows in Malaysia and can be easily obtained from local traditional market.*Piper betle* plant is useful for many diseases (Deshpande and Kadam, 2013).The leaves areused by the local and believed useful for skin diseases, halitosis, cuts and injuries. In many Asian countries, fresh *Piper betle* leaf was chewed together with areca nut (pinang) and lime (kapur) (Norton, 1998) and as a medicinal herb (Arambewela*et al*., 2005). The fresh *Piper betle* leaves is good for body and healthy because its contain vitamins, minerals, protein, essential oil, fibre, carbohydrate and fat (Guha, 2006).The aroma of *Piper betle* leaf is due to the present of essential oils inside the leaf and the younger leaves reported to yield more essential oil (Bhaleraoet *al*., 2013).Hydroxychavicol (HC) and Eugenol (EU) is part of the bioactive compound in *Piper betle*essential oils (Pin *et al*., 2010).The *Piper betle*leaves from different regionscan vary in smell and taste (Mubeen*et al*., 2014).Taking into consideration the importance commercial value and medicinal value of the *Piper betle* leaves,Soxhlet technique was used for the extraction of the bioactive compound of the dry *Piper betle* leaves and Gas chromatography mass spectrometry (GC/MS) was employed to separate and detect the bioactive compound of the dry leaves extract. GCMS can separate more than 50 individual components in one sample and is consider one of the best technique for identify the bioactive compound of long chain hydrocarbon,alcohols, acids esters,alkaloids, steroids, amino acids and nitro compounds (Muthulakshmi*et al*.,2012).

## 2. MATERIALS AND METHODS

### 2.1 Collections and preparation of plant materials

Fresh *Piper betle* leaves were obtained from the local market at Taman University, Skudai, Johor, Malaysia. The *Piper betle*leaves were washed with tap water in lab to remove earthly matters. After washing, the leaves were left in the fume hood to remove the excess water. The fresh leaves were cut into small pieces and flatly

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spread in small plastic trays. The small pieces leaves in the small plastic trays were dried in the oven-drier set at 40ºC. After 72 hour of drying, the leaves were powdered by using a blender for 3 minutes. The powder were put inside the sealed plastic bag and kept in refrigerator in 4ºC for further use.

### 2.2 Soxhlet Method Extraction Process

#### Aqueous extraction

10 grams of dried powder were extracted by using Soxhlet equipment with 150 mL deionized water. Ratio 1:15 of the dried powder and solvents were select for this Soxhlet extraction process. The extraction process was run for 24 hour and temperature was set at 150ºC. Extract was dried in oven for 3 days in 40ºC until the solvent is evaporated and only left dried extract. The dried extract was weighed and recorded.

#### Ethanol (70%) extraction

10 grams of dried powder were extracted by using Soxhlet equipment with 150 mL of ethanol (70%). The solvents used were of analytical grade. Ratio 1:15 of the dried powder and solvents were select for this Soxhlet extraction process. The extraction process was run for 24 hour and set at 80ºC for ethanol extraction. Extract were dried in oven for 3 days in 40ºC until the solvent is evaporated and only left dried extract. The dried extract was weighed and recorded.

### 2.3 Identification of components

GC-MS analysis of the extract was performed using aAgilent 6890N/5973I with mass selective detector. Sample was injected into silica capillary column (30m X 0.25mm I.D. X 0.25µm film thickness). The initial oven temperature was programmed from 70ºC; hold 2.0 min to 305ºC at 20ºC/min and hold for 1 min. Helium gas (99.999%) was use as carrier gas at constant flow rate 1.2ml/min. The injector temperature set at 250ºC and the ion source temperature was set at 230ºC. Total GC running time was 14 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total area. The mass spectrums of the unknown component was comparing with the spectrum of the known components stored in the National institute of Standard and Technology (NIST) Ver.2.0 computer library. The database of NIST has more than 62,000 patterns.

## 3. RESULTS& DISCUSSION

### Soxhlet Method Extraction

Table 3.1: Yield of extract in gram

| | Yield (g) |
|---|---|
| Aqueous | 1.57 |
| Ethanol (70%) | 1.23 |

Table 3.1 and Figure 3.1 show the yield of aqueous extract and ethanol (70%) extract. 10 grams of dried *Piper betle* leaves can produce of 1.57 grams of extract in aqueous extraction and 1.23 grams of extract in ethanol(70%) extraction. The yield of aqueous extraction better than ethanol (70%) extraction because of polarity water is greater than ethanol and the polar compounds are easier to be extract compared with non-polar compounds. Water and ethanol contain hydroxyl group and can form hydrogen bonding with the bioactive compounds. Aqueous extraction is more effective than ethanol extraction in antimicrobial compound because water has higher polarity and shorter chain than ethanol (Pin *et al*., 2010). This can know from the water capability in extract the polar compound like aromatic carboxylic acids. The carboxylic acids are considered to be highly polar organic functional group.

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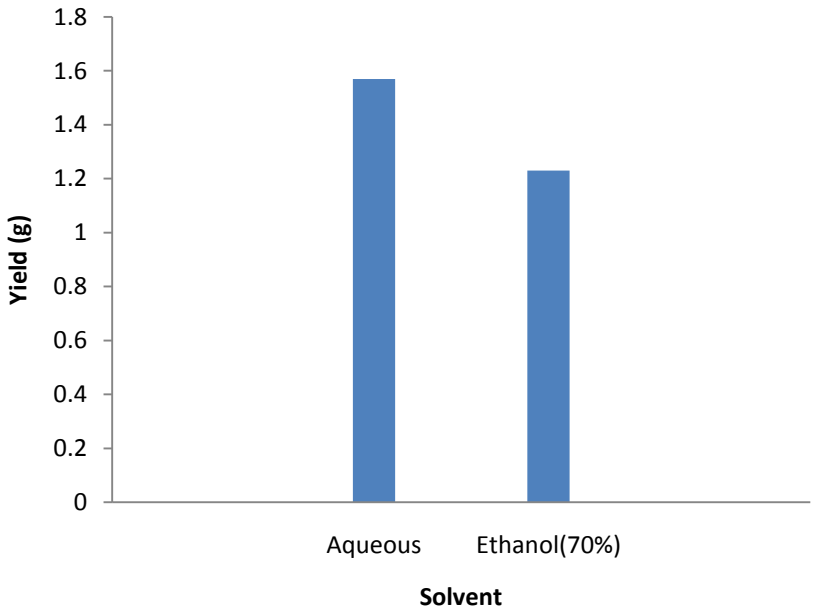


Figure 3.1: Yield of extraction

**GCMS Analysis**

Table 3.2: GCMS data of importance bioactive compound with their function in aqueous extraction

| | RT (min) | Peak area (%) | Name | Compound Nature | Function |
|---|---|---|---|---|---|
| 1 | 3.02 | 2.45 | Isopropyl isothiocyanate | Ester | Antimicrobial, Flavouring agent, Antifungal |
| 2 | 3.78 | 2.24 | 3-Heptanone, 6-methyl- | ketones | Flavouring agent, Fragrance agent |
| 3 | 5.73 | 0.48 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | Flavonoid compound | Antimicrobial, Anti-inflammatory, Antioxidant agent |
| 4 | 6.50 | 0.19 | 1,2,3-Propanetriol, monoacetate | Fatty acid and their ester | Antimicrobial and Antifungal properties at low pH |
| 5 | 7.25 | 0.18 | Triacetin | Ester | Antifungal, Flavouring agent, Humectants, Plasticizer, and as a solvent |
| 6 | 7.64 | 0.84 | Pyrene, 4,5,9,10-tetrahydro- | Pyrene and its derivatives | Dye and dye precursors |
| 7 | 7.70 | 0.34 | Pyrimidin-4(3H)-one, 2-amino-6-(3-fluorophenyl)- | Heterocyclic aromatic organic compounds | DNA repair for cancer in research |
| 8 | 7.86 | 0.75 | 1-Penten-3-ol | Alcoholic compound | Antimicrobial ,Antifungal |
| 9 | 8.15 | 91.51 | Benzoic acid, 2,5-dimethyl- | Aromatic carboxylic acid/Benzoic acid compound | Antimicrobial, Food preservation |
| 10 | 8.51 | 0.11 | Benzoic acid, 3,5-dimethyl- | Aromatic carboxylic | Antimicrobial, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | acid/Benzoic acid compound | Food preservation |
| 11 | 8.53 | 0.19 | Benzoic acid, 2,3-dimethyl- | Aromatic carboxylic acid/Benzoic acid compound | Antimicrobial ,Food preservation |
| 12 | 8.79 | 0.21 | trans-3-(2-Nitrovinyl)pyridine | Pyridine derivatives /Alkaloid | - |
| 13 | 10.01 | 0.10 | 2-Methoxy-4-vinylphenol (synonyms: p-Vinylguaiacol) | Phenolic compound | Antimicrobial, Antioxidant, Anti-inflammatory, Analgesic |
| 14 | 13.55 | 0.20 | 2,5-Dimethoxy-4-ethoxybenzonitrile | Benzoic acid amides | - |
| 15 | 13.63 | 0.20 | Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl | Alkaloid/ Aromatic compound | Antimicrobial |
| 16 | 13.96 | 0.03 | 2-Ethylacridine | Alkaloid | Antimicrobial |

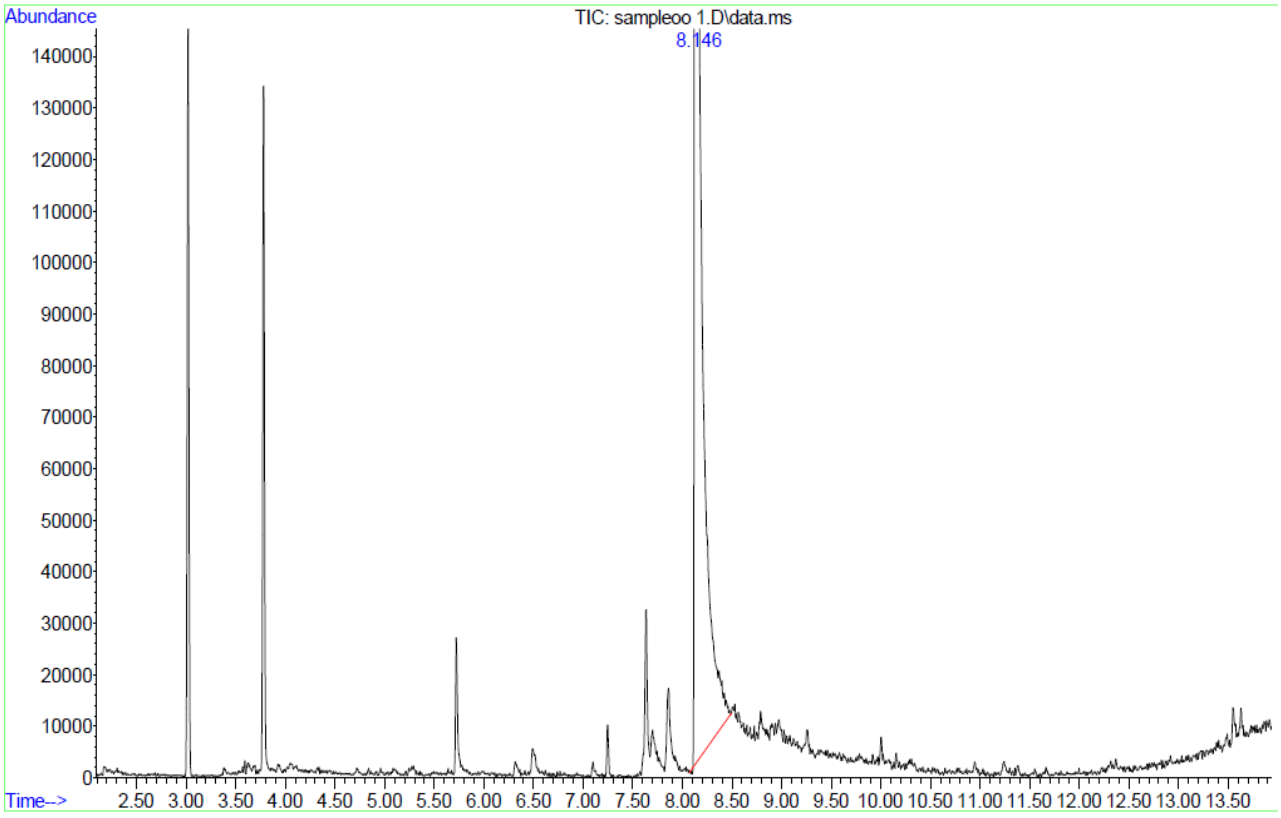


Figure 3.2: GCMS Chromatogram of aqueous extract of dried *Piper betle* leaves

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Table 3.3: GCMS data of importance bioactive compound with their function in ethanol (70%) extraction

| | RT (min) | Peak area (%) | Name | Compound Nature | Function |
|---|---|---|---|---|---|
| 1 | 3.03 | 0.46 | Isopropyl isothiocyanate | Ester | Antimicrobial, Flavouring agent, Antifungal |
| 2 | 5.726 | 0.07 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | Flavonoid compound | Antimicrobial, Anti-inflammatory, Antioxidant agent |
| 3 | 6.30 | 0.19 | Benzofuran, 2,3-dihydro- | Coumaran | Antimicrobial, Anti-inflammatory |
| 4 | 6.71 | 0.03 | Hydroquinone | Phenolic compound | Antimicrobial, Antioxidant , Antimalarial, FLavor |
| 5 | 6.97 | 0.03 | Indole | Alkaloid | Antimicrobial, Anticariogenic, Antiseptic, Cancer-Preventive, Perfumery |
| 6 | 7.09 | 0.18 | 2-Methoxy-4-vinylphenol | Phenolic compound | Antioxidant,Antimicrobial, Anti-inflammatory |
| 7 | 7.28 | 0.05 | Triacetin | Ester | Antifungal |
| 8 | 7.50 | 4.88 | Phenol,2-methoxy-4-(1-propenyl)-(isoeugenol) | Phenolic compound | Antimicrobial |
| 9 | 7.60 | 0.03 | Quinolin-5(6H)-one, 7,8-dihydro-2-hydroxy-4,7,7-trimethyl- | Quinolines compound/Alkaloid | Anticancer, Antimicrobial, Anticonvulsant, Anti-inflammatory and cardiovascular activity |
| 10 | 7.85 | 0.10 | Trans-Cinnamic acid | Phenolic compound | Antitumor activity, Antioxidant |
| 11 | 8.18 | 76.22 | Benzoic acid, 2,5-dimethyl- | Aromatic carboxylic acid/Benzoic acid compound | Antimicrobial, Food preservation |
| 12 | 8.41 | 0.40 | Benzoic acid, 2,3-dimethyl- | Aromatic carboxylic acid/Benzoic acid compound | Antimicrobial, Food preservation |
| 13 | 8.44 | 0.38 | Benzoic acid, 3,5-dimethyl- | Aromatic carboxylic acid/Benzoic acid compound | Antimicrobial, Food preservation |
| 14 | 8.50 | 0.41 | Eugenol | Phenolic compound | Antimicrobial, Antimutagenic, Antiseptic, Antiviral, Insecticide, Ulcerogenic, Fungicide, |

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| | | | | | |
|---|---|---|---|---|---|
| | | | | | Cancer-Preventive |
| 15 | 9.28 | 0.19 | Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- | Alkenes/Terpenes | Antimicrobial |
| 16 | 9.65 | 0.03 | phytol | Diterpene | Antimicrobial, Anticancer, Anti-inflammatory, Anti-diuretic and Anti-diabetic |
| 17 | 9.67 | 0.05 | 4-Chromanol | Alcohol | Antioxidant |
| 18 | 10.84 | 0.33 | n-Hexadecanoic acid | Fatty acids | Anti-inflammatory, Antioxidant |
| 19 | 11.83 | 0.11 | 9,12-Octadecanoic acid (Z,Z)- | Fatty acids | Hepotoprotective |
| 20 | 11.85 | 0.12 | 6-Octadecenoic acid | Steric acids | Cancer preventive |
| 21 | 12.55 | 0.04 | Oleic acid | Fatty acids | Antioxidant, cancer preventive |
| 22 | 13.12 | 0.18 | Undecanal,2-methyl- | Aldehyde compound | Antimicrobial |

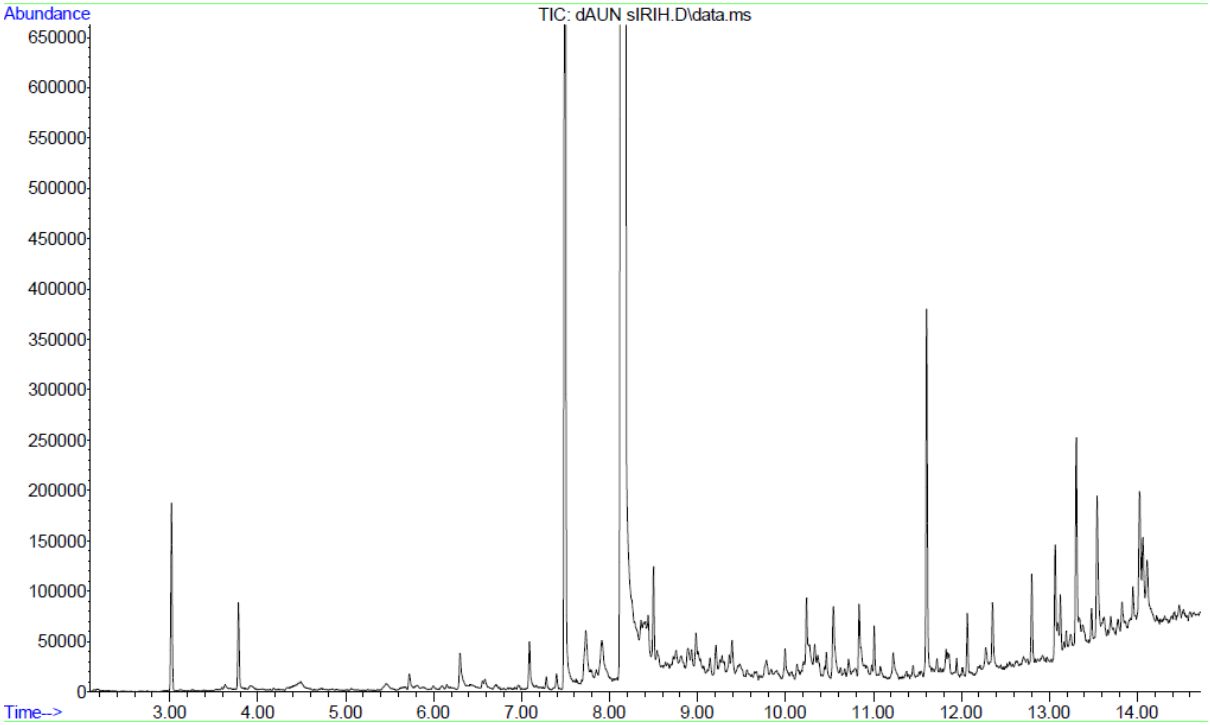


Figure 3.3: GCMS Chromatogram of ethanol (70%) extract of dried *Piper betle* leaves

The bioactive compound present in the aqueous extract and ethanol (70%) extract of dried leaves of *Piper betle* were identified by GCMS (Figure 3.2 and Figure 3.3). GCMS data of importance bioactive compound with their function in aqueous extraction and ethanol (70%) extraction were shown in table 3.2 and 3.3.Mostly antimicrobial compound in bothextractions were extract out in the GCMS retention time range 1-10 minute.

GCMS analysis showed the presence of Benzoic acid, 2,5-dimethyl- as a main compound in both extraction. The aqueous extraction showed 91.51% and ethanol (70%) extraction showed 76.22% of Benzoic acid, 2,5-dimethyl-.The presence of various bioactive compounds from both plants justified the use of various treatments by local traditional practitioners. Phytochemical screening of extracts in GCMS showed the presence of various bioactive compound for example alkaloids, fatty acids, phenolic compounds, alcoholic compounds, flavonoids compounds, terpenes compounds, coumaran compounds and organic acids.The dried *Piper betle* leaves extract is a complex with many bioactive components (AraniDatta*et al*., 2011) which show significant biological activities (Fawad*et al*. 2012). The phenolic compounds, terpenes, alcohols compounds, aldehydes compounds, ketone, acid compounds and flavonoid compound have been known with antimicrobial properties since long time ago (Tiwari*et al*., 2009). The aqueous extraction of *Piper betle* plant mainly showed antimicrobial properties. Other than antimicrobial, Piper betle extract in aqueous extraction also showed antifungal, antioxidant and anti-inflammatory properties. The ethanol (70%) extraction of dried *Piper betle* leaves showed antimicrobial activity, anti-inflammatory activity, antifungal, antitumor properties or cancer-preventive, anti-diabetic, hepotoprotective and etc.

Ethanol and water mixtures are commonly used for the extraction of phenolic compound from plant materials (Alothman*et al*., 2009). This is because water had its limited ability to extract oil-based components such as eugenol (Phenolic compound).This is proved that some bioactive compound were only soluble in organic solvent were not present and detected in aqueous extract in the GCMS result. However, based on the GCMS result, the both method of extraction (water and ethanol (70%)) were significantly showed inhibition to bacterial.

## CONCLUSION

In conclusion, dried *Piper betle* leaves contain various bioactive compound. Based on this preliminary study, the aqueous extraction and ethanol (70%) extraction of dried*Piper betle* leaves can consider as a promising tool for antibacterial. Aqueous extraction is less cost and green process if compare to using ethanol as solvent because water is non-toxic and eco-friendly. Further investigations in detail are needed on antimicrobial efficacy and toxicology of both extract to formulate the new commercial product in market.

**NOMENCLATURES** GCMS: Gas Chromatography Mass Spectrometry

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