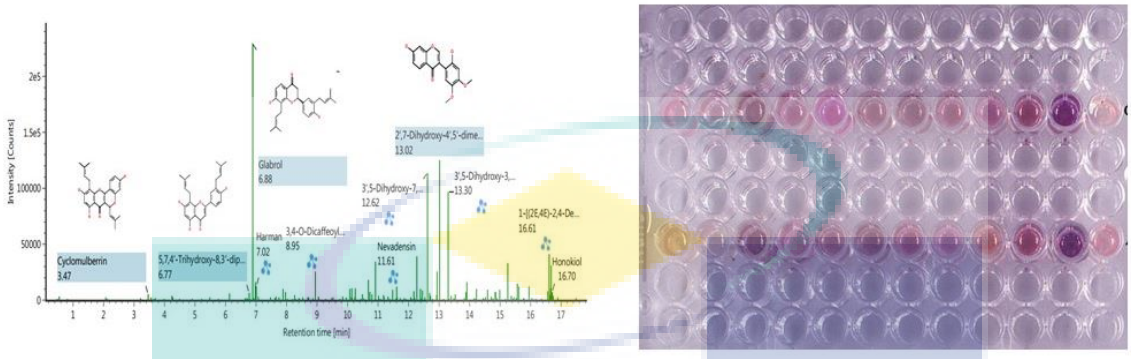


ISOLATION AND CHARACTERIZATION OF ALKALOIDS FROM PASSIFLORA FEOTIDA FOR POTENTIAL OF ANTIDEPRESSANT COMPOUNDS

Hazrulrizawati Abd Hamid
Mohd Hairul Ab. Rahim
Aizi Nor Mazila Binti Ramli
Mashitah Mohd Yusoff
Ahmad Faizal bin Abdull Razis

Faculty of Industrial Sciences & Technology
Universiti Malaysia Pahang
Hazrulrizawati@ump.edu.my
Natural and Cultural Heritage (Biodiversity)

ABSTRACT (120 words)

The potential antimicrobial activity and acetylcholinesterase enzyme inhibition assay of water and methanol *P. feotida* extracts from fruits, leaves and stems were evaluated. Extracts from methanol fruits of *P. feotida* extract has found to be able to inhibit and resist the growth of pathogenic bacterial strains either Gram positive bacteria (*Bacillus subtilis* and *Enterococcus faecalis*) or Gram negative bacteria (*Escherichia Coli* and *Pseudomonas aerogenosa*). The identification of chemical constituents in *P. feotida* was done by UPLC-QTOF/MS and the results showed that this plant extract possessed a

great number flavonoid compounds. With the presence of these compounds, it has contributed to the antimicrobial activity of *P. feotida*. Results of the present biological activity investigation further points to the potential of this plant species as a good source of new AChE inhibitors.

1. INTRODUCTION

Nowadays, human around the world have been extensively worried regarding to their health. Air pollutions, water pollutions, and unhealthy food consumption are the causes of many types of diseases including cancer, diabetic, hypertension, cataracts, immune system decline, and Alzheimer. Due to this, new drug discovery and the development in supplement have a very high potential and demand to consumers throughout the world. According to the World Health Organization (WHO), around 80% of world population in developing countries use plants as a source for the treatment of various diseases and ailments such as cancer treatment, anti-inflammatory, malaria, and other chronic diseases such as cardiovascular, heart diseases and hypertension (Yahaya, Dash, Abdullah, & Mathews, 2015).

Plants have been the source of many traditional medicines throughout the world for centuries. Due to this, our ancestors obtained knowledge of these species after serving distinguish between eating and those who had any effect on the body, so from this they began to differentiate and select; thus, we have inherited an extensive knowledge of the use of plants to several diseases, including the nervous system. (Grosso, 2016).There are one of the richest sources which contain bioactive compounds. Some researched has been reported that, people from the Indian subcontinent believe that plants are useful to be one

of main remedy in traditional medicines and give beneficial therapeutically effects. The investigations of bioactive natural products were mainly concerned with discovering of bioactive constituents. A research on isolation and characterization of pharmacologically active compounds from plants continue until today.

Thus, current research is focussed on natural products as they are rich source of potent new drug leads. *Passiflora incarnate* origin from European countries is one of a high value antidepressant plant. In Malaysia one of endangered species in the same genus, *Passiflora feotida* (pokok letup/ulat bulu menjalar) also had been utilized for the treatment of depression related symptoms such as hysteria and insomnia but no scientific proof for that therapeutic effect. Due to the lack of scientific information, many Malaysians are not aware about the medicinal importance of P. *feotida*. Thus in this study we are attempting to isolate and elucidate the structure of bioactive compounds and further study their antidepressant property.

The compounds will be purified by chromatographic procedure (column chromatography and preparative HPLC) and the structure will be elucidated by spectroscopic techniques (FTIR, NMR, MS). Isolated compounds will be subjected to biological activities assay. The project is expected to isolate new compounds with indole alkaloids moiety and able to show antidepressant property. Finding from this project will be useful for development of new natural antidepressant compounds and in the future it will be beneficial for drugs development.

2. RESEARCH METHODOLOGY

2.1 Plant materials

The whole plant of P.*feotida* was collected in September 2014 from herbal farm in Alor Setar, Kedah. Dr. Shamsul Kamis, authenticated the plant Botanist at Institute of Bioscience, University Putra Malaysia.. The whole plant include leaves (2kg), stems (2kg) and the fruits (500g) were dried in the oven (50 ˚C) and grounded into small pieces (*ca*. 0.5-1.0 cm) thickness.

2.2 Extraction of Plant

The overall research begins with extraction of sample according to our previous work (Yusoff et al., 2014). Powdered, dried P. *feotida* leaves, stems and fruits were first extracted via percolation with hexane followed by methanol and then filtered to get fraction A and fraction B. The extracts were sonicated for 30 minutes for three cycles and then filtered to give a brown extract. The filtrates evaporated using rotary evaporator at temperature. Leaves extract was chosen to be eluted in column chromatography for isolation process.

2.3 Isolation of compounds

Leaves extract was chromatographed over a column of silica gel, slurry packed in chloroform. Elution was initiated with chloroform and progressed through the solvent series of 10%, 15%, 30%, 50% and 70% of methanol in chloroform, 100% methanol. The

fractions were collected from CC which further purified by column chromatography and preparative HPLC.

2.4 Identification of Chemical Constituents

The extracts obtained were analysed for its bioactive compounds by using UPLC-QTOF/MS. UPLC data were produced using the Waters ACQUITY UPLC I-Class systems (Waters, Milford, USA) equipped with a binary pump, an autosampler, a degasser, and a diode-array detector (DAD). The system was controlled with Waters UNIFI Vion software. The chromatographic column UPLC HSS T3 C18 (2.1 mm×100 mm, 1.8 µm) was used and eluted with a linear gradient of A (0.1% formic acid in deionized water) and B (ACN) at a flow rate of 0.5 mL/min for 16 minutes. The injection volume was 3 µL.

MS^E data were recorded using the Xevo G2-S QTOF/MS (Waters, Milford, USA) equipped with an electrospray ion source, ion mobility system and quadrupole-time-of-flight (Q-TOF) mass spectrometer. The Q-TOF instrument was operated in positive and negative ion mode for MS experiments with the TOF data had been collected between m/z 100 and 1000 Da. The condition was desolvation gas at 1000 L/h at a temperature of 500 ◦C, cone gas at 30 L/h and source temperature at 120 ◦C, capillary voltages at 2.5kV (ESI^+) and 1.8kV (ESI^-) respectively. Leucine-enkephalin was utilized as the lock mass with m/z 556.2771 for positive mode and negative mode m/z 554.2615. The MS data were collected and search against the integrated Traditional Medicine Library. The structures of matched components are verified byMassFragment ^{TM} using corresponding

fragment ions and detailed information of the identified component are displayed automatically in UNIFI software

2.5 Antimicrobial Activity Assay

The five microorganisms were used to determine the antimicrobial activities of the six extracts of *P. feotida*. Two gram-positive bacteria (*B. subtilis* and *E. faecalis*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) will be taken into consideration. The bacterial strains were sub-cultured to almost similar absorbance (~0.100 A) under wavelength 600 nm. Gentamicin and Fluconazole was used as the positive control and solvents were used as the negative control. For the bacterial test, 12 petri dishes were coated with respective bacterial strains (100 µL) for each dish. Each paper disc was coated with the three different extracts and stuck on the bacterial agar. There were three plates containing pathogenic bacterial strains and added with Gentamicin, Fluconazole as the positive control and solvents as the negative control respectively. The agar petri dishes containing the paper disc extract were incubated at 37^0C for about 24 hours for the complete diffusion. In order to determine the antimicrobial activity of the plant extract, the diameter of zone of inhibition was measured in millimetre. The diameter of the inhibition zone measured has to be compared with the positive and negative control. For the same concentrations tested under different bacterial strains, it will be repeated for three times and obtain the average value (Baur et al., 1996)

The antimicrobial activities can be evaluated by the diameter of the paper discs. The extract can be considered to be highly susceptible towards bacteria when the

diameter of inhibition zone exceeds 15 mm. When the diameter is greater than 11.00 mm, it is considered as intermediate susceptible to bacterial growth. It was said that the extract is resistant to bacterial growth when the diameter of the inhibition zone was less than 8.00 mm. However, when there was uniform lawn growth of the bacteria around the disk which means that the extract has no inhibitory properties against the bacteria growth. When there is no zone of inhibition around the paper disc, the susceptibility of the bacteria test is considered negative.

2.6 Acetylcholinesterase Activity by Microplate Assay

Acetylcholinesterase activity was evaluated using a 96-well microplate reader (Rhee et al., 2001) based on Ellman's method (Ellman et al., 1961). In this method the enzyme hydrolyzes the substrate acetylthiocholine resulting in the production of thiocholine which reacts with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 405 nm. Three buffers were prepared for the assay, Buffer A (50mM Tris-HCl, pH 8), Buffer B (50mM, pH 8, containing 0.1% bovine serum albumin) and Buffer C (50mM Tris-HCl, pH 8, containing 0.1M NaCl and 0.02M $MgCl_2.6H_2O$).

In the 96-well plates, 25 µL of 15 mM acetylthiocholine iodide (ATCI) in water, 125 µL of 3 mM DTNB in buffer C, 50 µL of buffer B, 25 µL of isolated compounds dissolved in MeOH at concentrations ranging from 62.5-1000 µg/mL, were added. Then 25 µL of 0.22 UmL^{-1} of the enzyme acetylcholinesterase were added and the absorbance was read at 405 nm. Physostigmine served as the positive control. All assays were carried

out in duplicate in 96-well microplate reader (Tecan Infinite 200 Pro). The percentage inhibition was calculated by following the formula:

$$\% \text{ Inhibition} = [(E - S) / S] \times 100$$

where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound. The IC_{50} values were calculated by plotting graph of percentage inhibition against extract concentration. The IC_{50} values obtained in unit µg/mL had been converted into µM.

3. LITERATURE REVIEW

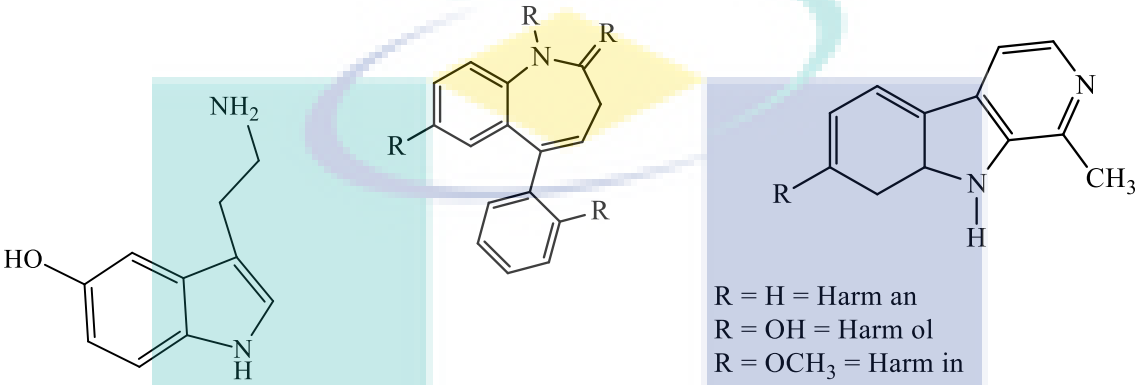
*Passiflora incarnat*a and other species such as P. *alata Curtis*, P. *Coerulea L*. and P. *edulis Si*ms are widely used as a sedative in traditional medicine in most European countries and in America (Fajemiroye et al., 2016). The drugs that are commonly used for anxiety and depression treatment is benzodiazepines. The structure of benzodiazepines drugs consists of a benzene ring attached with seven membered heterocyclic compound with two nitrogen atoms while indole alkaloids isolated from P. *feotid*a namely Harmane (1) , harmol (2) , harmine (3) , harmalol (4) and but harmaline (5) consist of a benzne ring attached with five membered heterocyclic compounds with one nitrogen atom (Figure 1). Some studies indicate that P. *feotid*a has a pharmacological profile similar to benzodiazepines and acts through gamma-aminobutyric acid (GABA) receptors (Jawna-Zboińska et al., 2016). P. *feotid*a from Galapagos also contained tetraphyllin A (6) and B (7), deidaclin (8) and volkenin (9), and P. *feotid*a from Reunion

tetraphyllin B (7), volkenin (9) as well as linamarin (10). Extraction and fractionation of the aerial parts of both plants revealed tetraphyllin B sulphate (11) as the major cyanogemic constituent in both collections.

A further study conducted by (Aslam, Ahmad, & Mamat, 2014) show the major phytochemical constituents like hydrocyanic acid, groups of flavonoid, such as, 2"-Xyloslyvitexin (12), 3,5-dihydroxy-4,7-dimethoxy flavanone (13), 4,7-O-dimethyl-naringenin (14) and 7,4'-Dimethoxyapigenin (15). Passifloricins: passifloricin A (16), passifloricin B (17) and passifloricin C (18). Three polyketides α-pyrones,named passifloricins, were isolated from Passiflora foetida resin; their structures and relative configurations were assigned through 2-D NMR spectroscopic analyse. P. foetida supposed to be an enormous source of chrysoeriol (19), apigenin (20), isovitexin (21), vitexin, 2-xylovitexin (22), 2-xylosylso(23) vitexin (24), lutelin7-β-d-glucoside (25) and kaempferol (26).

The use of *Passiflora* species as medicinal plants has been well documented. The leaves, flowers, roots, and fruits of wild and commercial species are used in folk medicine for the treatment of insomnia, anxiety, helminthic infestations and stress (Pacheco et al., 2016). *Passiflora feotida L.* is South American in origin and found in riverbeds, dry forest floors and growing near hamlets. The ethno botanical views of P. *feotida*, suggest that decoction of leaves and fruits is used for the treatment of asthma and the root decoction used for hysteria and the leaf paste is applied on the head for giddiness and headache (Shuayprom et al., 2016). The major phytoconstituents of P. *feotida* are alkaloids, phenols, glycosides, flavonoids (figure 2) and cyanogenis compounds

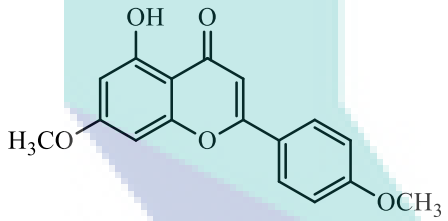
(Fajemiroye et al.,2016). Research publications on phytochemicals of P. *feotida* are mostly origin from India and only two publications about morphological of *Passiflora* species were conducted from Malaysia by Ramaiya et al.(2014) and Veeramohan et al.(2015).



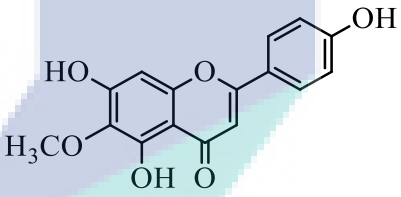
Serotonin

Benzodiazepine

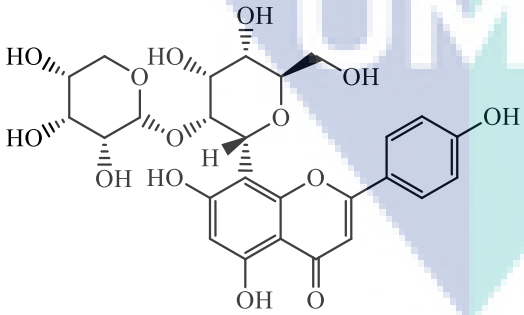
Figure 1



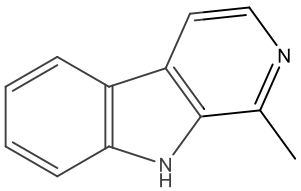
4,7-O-dimethyl-naringenin flavanone



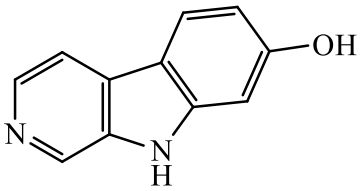
3,5-dihydroxy-4,7-dimethoxy flavanone



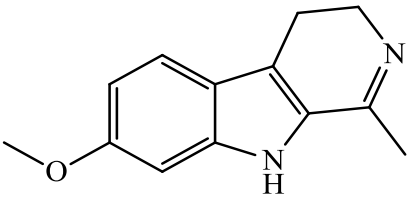
2"-Xyloslyvitexin



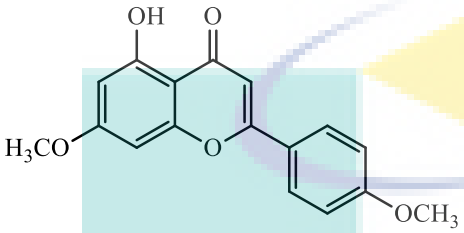
Hermane



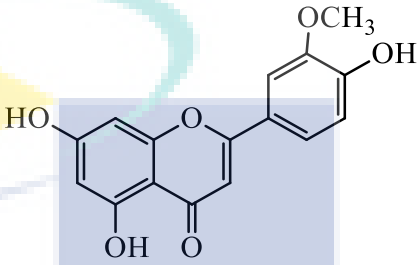
Harmol



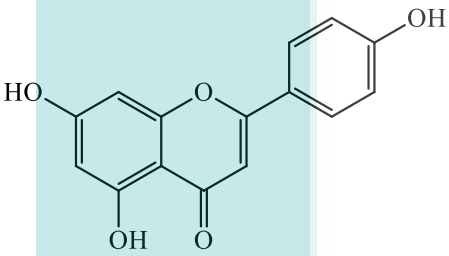
But hermaline



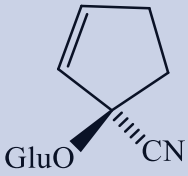
4,7-O-dimethyl-naringenin



Chrysoeriol

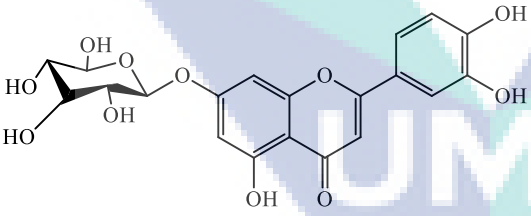


Epigenin

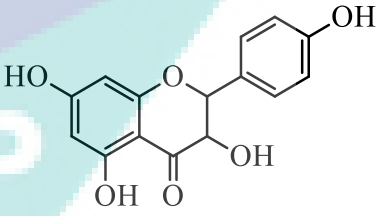


Glu = *β-D*-glucopyranosyl

Deidaclin



Luteolin-7-β-D-glycoside



Kaempferol

4. FINDINGS

4.1 Isolation of compounds

Leave extract of *P. feotida* (200.31g) was subjected to pass over a silica gel column (70-230 mesh), slurry packed in hexane. Elution was initiated with ethyl acetate

and progressed through the solvent series of 10% and 20% of ethyl acetate in hexane, 100% chloroform and 10%, 20%, 30%, 35% and 40% of chloroform in methanol and 100 mL fractions were collected. Each fraction was monitored on TLC silica gel plate with visualisation of ultraviolet light. Fractions showing similar TLC profiles were assembled to give nine combined fraction. Subfraction F were continuous further column chromatography to give yield F4 and F6. Then both of them are mixed and continued column chromatography give F41. Now, the subfraction F41 still on going to give pure compound.

4.2 Identification of chemical compounds

4.2.1 Methanol Extracts

Figure 1a and Figure 1b report base peak ion (BPI) chromatogram and the confirmed major compounds plotted on (+) ESI-MS of fruit methanollic extract. Glabrol show the most abundant compound followed by 2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone, 5,7,4′-Trihydroxy-8,3′-diprenylflavone and Cyclomulberrin at a flow rate of 0.1 mL/min for 10 min at 20 °C. the other compound also detected in fruit methanollic extract such as 3,4-O-Dicaffeoylquinic acid, Harman, 3′,5-Dihydroxy-7,4'-dimethoxy flavone, Nevadensin, 1-[(2E,4E)-2,4-Dodeca-dienoyl] pyrrolidine, 3',5-Dihydroxy-3,4',7-trimethoxy flavone and Honokiol. The abundant ions were detected as $[M - H]^+$ at *m/z* 392.202 for glabrol , $[M - NA]^+$ at *m/z* 314.0786 for 2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone, while 5,7,4′-Trihydroxy-8,3′-diprenylflavone was $[M - NA]^+$ at *m/z* 406.1809 and for Cyclomulberrin was $[M - H]^+$ at *m/z* 420.1603. Other ions also were detected as $[M - NA]^+$ at *m/z* 516.1264 for 3,4-O-Dicaffeoylquinic acid , $[M - H]^+$

at *m/z* 182.0839 for Harman , $[M - NA]^+$ at *m/z* 314.0786 for 3′,5-Dihydroxy-7,4'-dimethoxy flavone, $[M - NA]^+$ at *m/z* 344.0892 for Nevadensin, $[M - NA]^+$ at *m/z* 249.211 for 1-[(2E,4E)-2,4-Dodeca-dienoyl] pyrrolidine, $[M - NA]^+$ at *m/z* 314.0786 for 3',5-Dihydroxy-3,4',7-trimethoxy flavone and $[M - NA]^+$ at *m/z* 266.1282 for Honokiol, respectively.

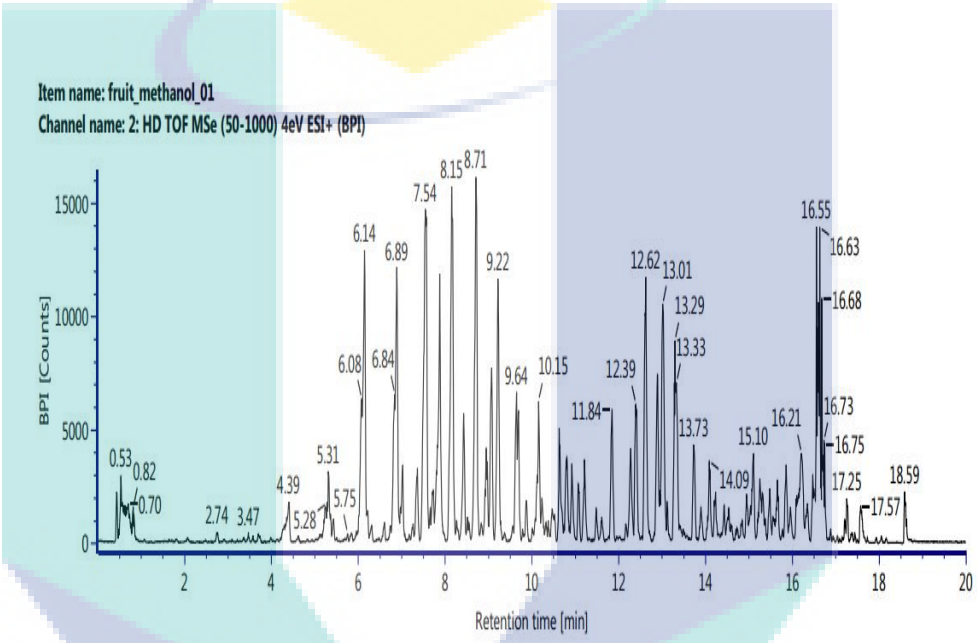


Figure 1a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* fruit methanolic extract

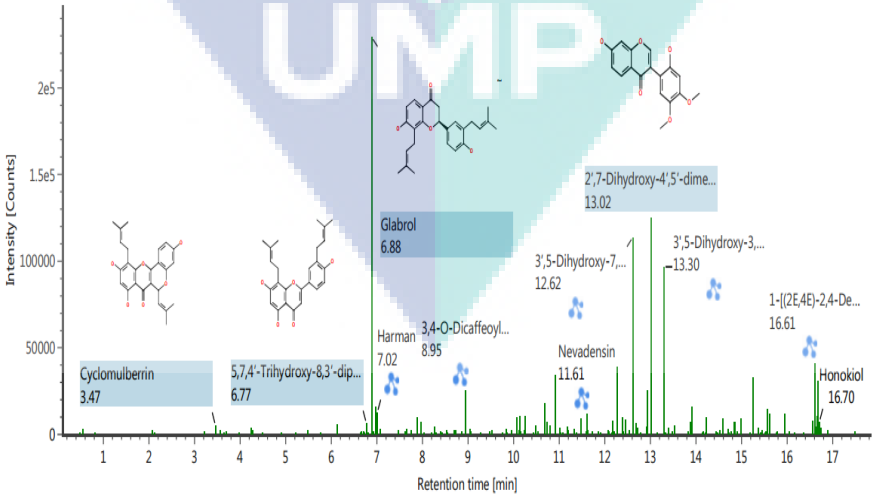


Figure 1b Confirmed major compounds plotted on (+) ESI-MS of methanolic extract

Figure 2a shows base peak ion (BPI) chromatogram in leaves methanollic extract. Based on the plotted chromatogram, the most abundant compound was found were glabrol, Isopenniclavine, (3R, 4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman and followed by 6,7-Dihydroxy-2-(2-phenylethyl) chromone. Picrasidine S, Meliadanoside A and Ergosine were also traced through base peak ion (BPI) chromatogram. Figure 2b describes data of confirmed compounds on (+) ESI-MS of leaves methanolic extract. the $[M - H]^+$ at *m/z* 392.2023 ion of glabrol was collected to be the most abundant ion followed by $[M - NA]^+$ at *m/z* 270.1369 Of Isopenniclavine, $[M - H]^+$ at *m/z* 346.1387 For (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman and for 6,7-Dihydroxy-2-(2-phenylethyl) chromone $[M - H]^+$ at *m/z* 310.1201. Other ions were reported by $[M - NA]^+$ at *m/z* 508.2133 for Picrasidine S, $[M - H]^+$ at *m/z* 376.1345 of Meliadanoside A and for Ergosine $[M - H]^+$ at *m/z* 547.284.

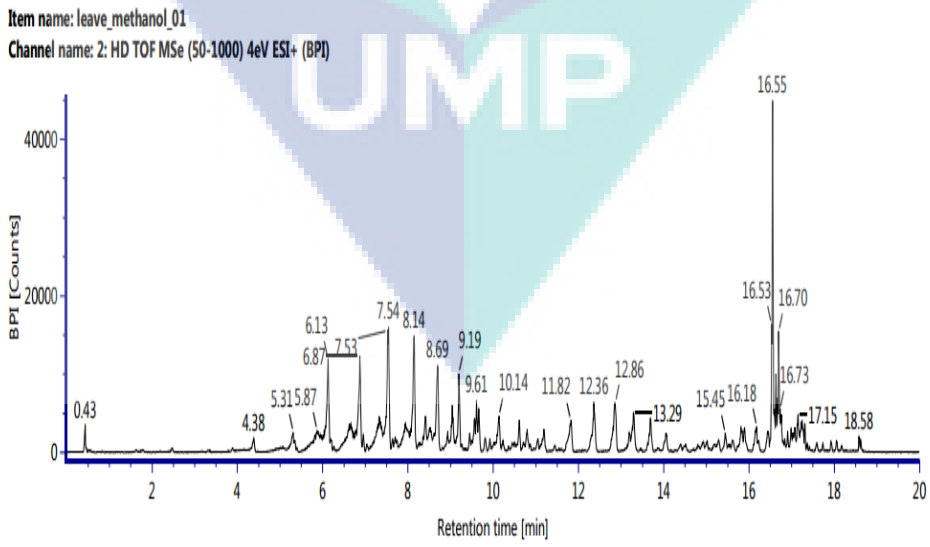


Figure 2a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* leave methanolic extract

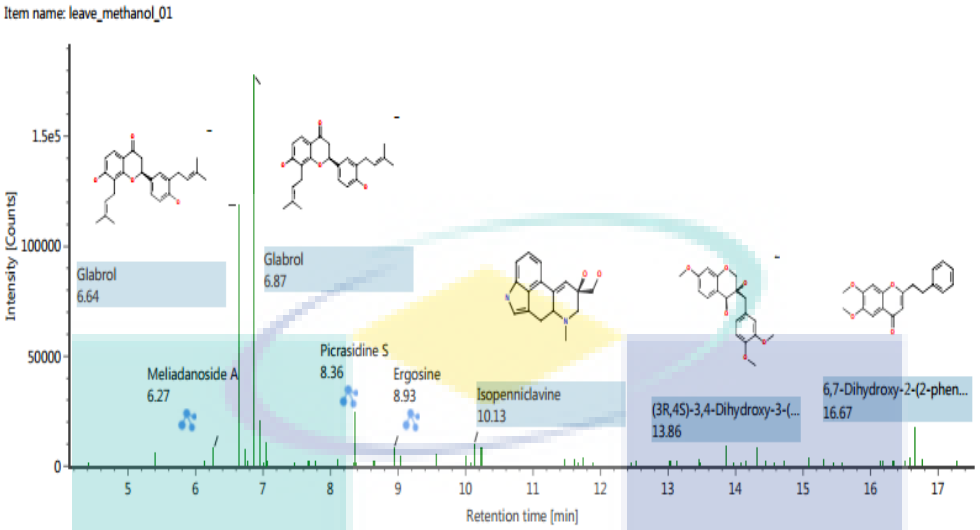


Figure 2b Confirmed major compounds plotted on (+) ESI-MS of methanolic extract

Based on the peak ion (BPI) chromatogram by UPLCQTOF/MS Figure 3a the most abundant in stem methanollic extract were compounds glabrol, β-Carboline, 7-Hydroxy-5,3',4'-trimethoxy flavone and 1,7-Dimethoxy-2,3-methylenedioxyxanthone. Compounds detected in stem methanollic extract of *P. feotida* included Picrasidine F, Santin, Lupinifolin followed by licoricone. While figure 3b performed data of confirmed compounds on (+) ESI-MS of *P. feotida* stem methanol extract. According to the data, compound glabrol at $[M - H]^+$ at *m/z* 392.202 was selected as the most abundant ion followed β-Carboline $[M - NA]^+$ at *m/z* 168.0683, $[M - NA]^+$ at *m/z* 328.0965 of 7-Hydroxy-5,3',4'-trimethoxy flavone and $[M - H]^+$ at *m/z* 300.0631 of 1,7-Dimethoxy-2,3-methylenedioxyxanthone.

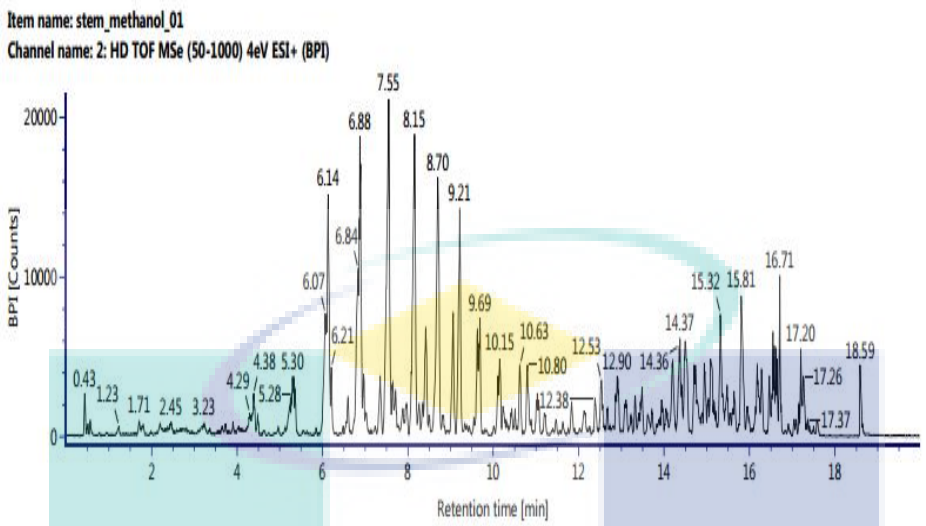


Figure 3a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* stem methanolic extract

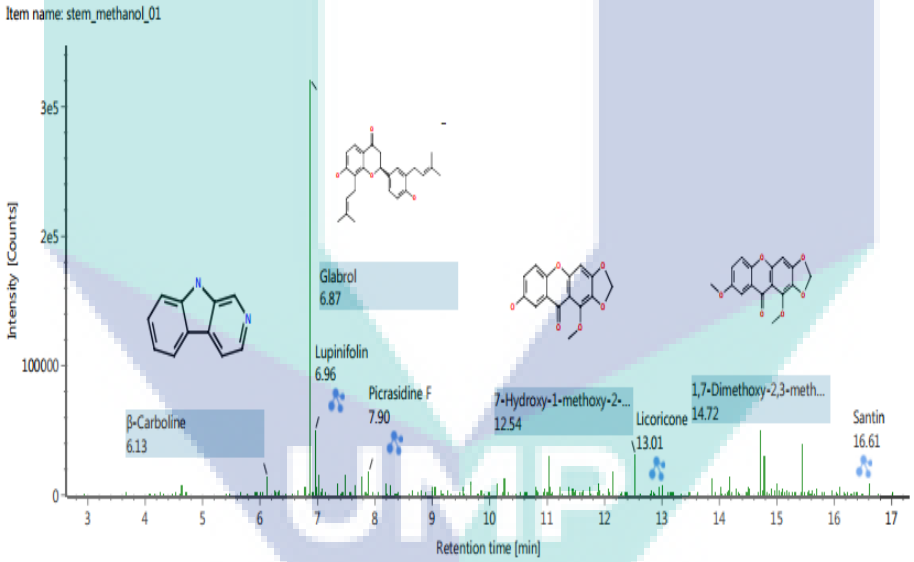


Figure 3b Confirmed major compounds plotted on (+) ESI-MS of methanolic extract

For conclusion, based on the data collected (), the UPLC-QToF/MS base peak ion (BPI) chromatogram of *P. feotida* MeOH extract and based on the confirmed component plotted, major compounds that can be found in MeOH extract was glabrol. All the parts of *P. feotid*a (leaves, stem, and fruit) methanol extract shows glabrol is the abundant compound in this extract and the confirmation was performed by the confirmed compounds on (+)ESI of MeOH extract from *P. feotida* with $[M - H]^+$ at *m/z*

392.202 ion. Compound glabrol is one of the major flavanone compound classes that can be found in medicinal herbs plants. Flavonoids are found as free, prenylated and glycosylated compounds in nature. One of researched reported that, Glabrol having two prenyl moieties, is the main flavanone in the hairy root [Asada, 2000].

4.2.2 Water Extract

Figure 4a describes UPLC-QToF/MS positive BPI chromatogram of *P. foetida* fruit water extract. compounds glabrol was performed one of the most abundant compound followed 1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one And (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman at higher concentration . Others compounds also were noted form the chromatogram such as 3β, 6 exo-Dihydroxy nortropane , Picrasidine F, Sanggenon J and Kushenol H. These compounds was confirmed by data of confirmed compounds on (+)ESI-MS of *P. feotida* fruit water extract (Figure 4b). The most abundant ions eluted with $[M - H]^+$ at *m/z* 392.202 for glabrol , 1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one $[M - H]^+$ at *m/z* 326.1124 and $[M - H]^+$ at *m/z* 346.1393of (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman. The others ion also was traced for 3β,6 exo-Dihydroxy nortropane $[M - H]^+$ at *m/z* 143.0945 , Picrasidine F with $[M - NA]^+$ at *m/z*478.2025 , $[M - H]^+$ at *m/z* 488.2234 of Sanggenon J and for Kushenol H $[M - H]^+$ at *m/z* 472.2079

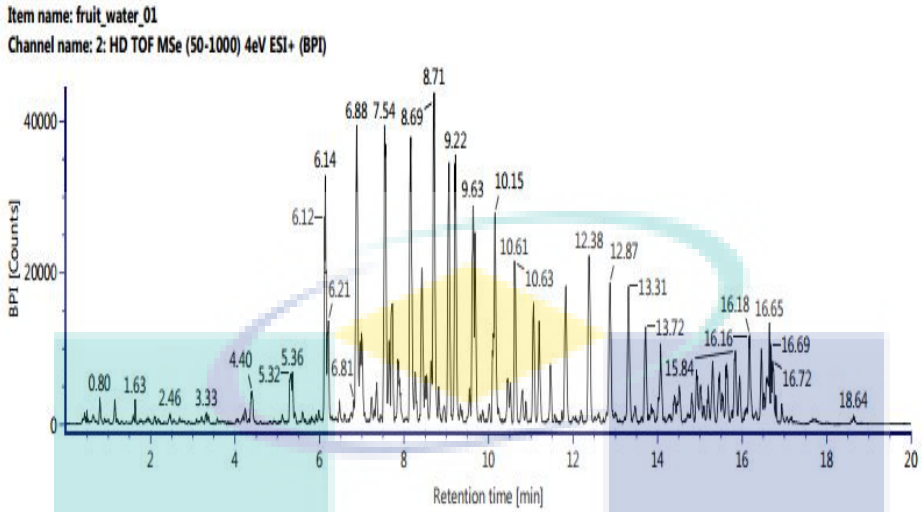


Figure 4a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* fruit water extract

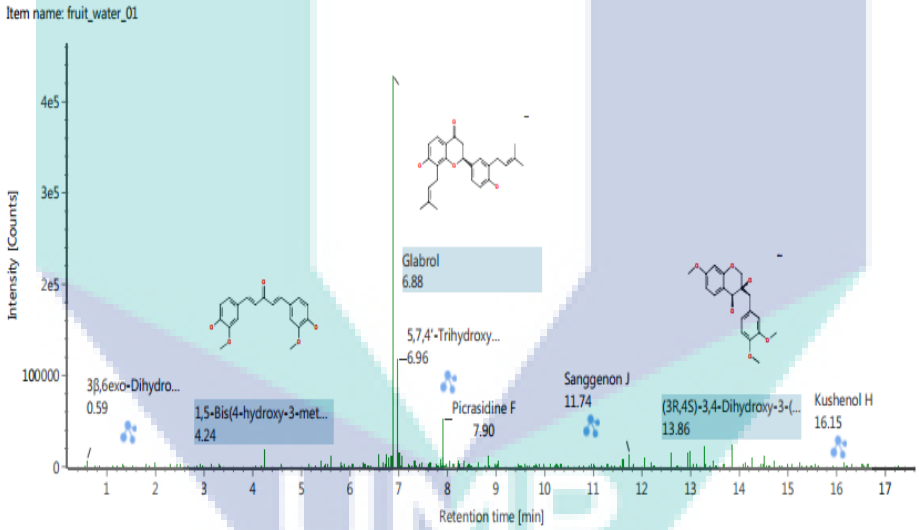


Figure 4b Confirmed major compounds plotted on (+) ESI-MS of water extract

Figure 5a shows base peak ion (BPI) chromatogram in leaves water extract. Based on the plotted chromatogram, the major compound was found were glabrol, 2α,3β-Dihydroxy nortropan, 5,7,4′-Trihydroxy-8,3′-diprenylflavone and (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman. Sanggenon J, Kushenol H and Ergosine were also eluted through base peak ion (BPI) chromatogram.,Figure 5b describes data of confirmed compounds on (+) ESI-MS of *P. feotida* leaves water extract.

the most abundant ions $[M - H]^+$ at *m/z* 392.2023 ion of glabrol was collected followed by 2α,3β-Dihydroxy nortropan $[M - NA]^+$ at *m/z* 143.0944 , 5,7,4′-Trihydroxy-8,3′-diprenylflavone $[M - H]^+$ at *m/z* 406.1812 and (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman $[M - H]^+$ at *m/z* 346.1389, Other ions were reported by $[M - NA]^+$ at *m/z* 488.2229 for Sanggenon J, $[M - H]^+$ at *m/z* 472.207 of Kushenol H and for Ergosine $[M - H]^+$ at *m/z* 561.2997

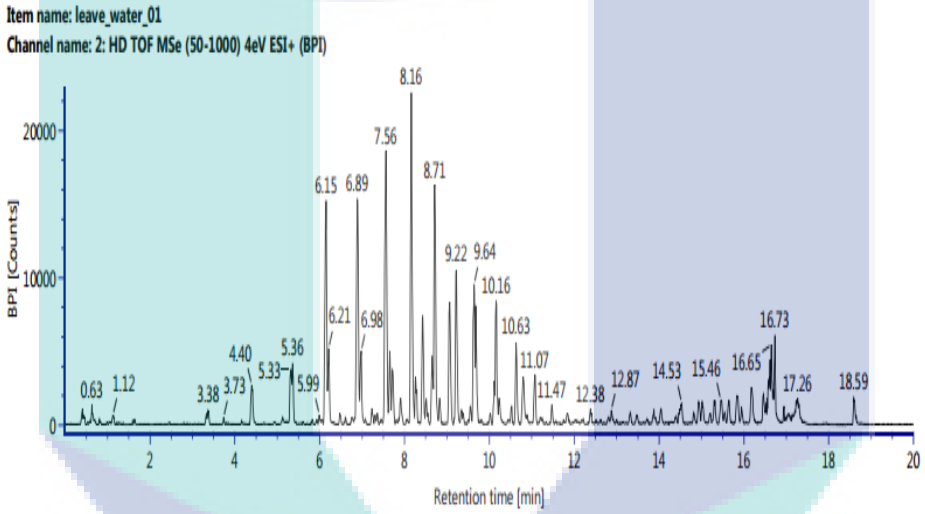


Figure 5a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* leave water extract

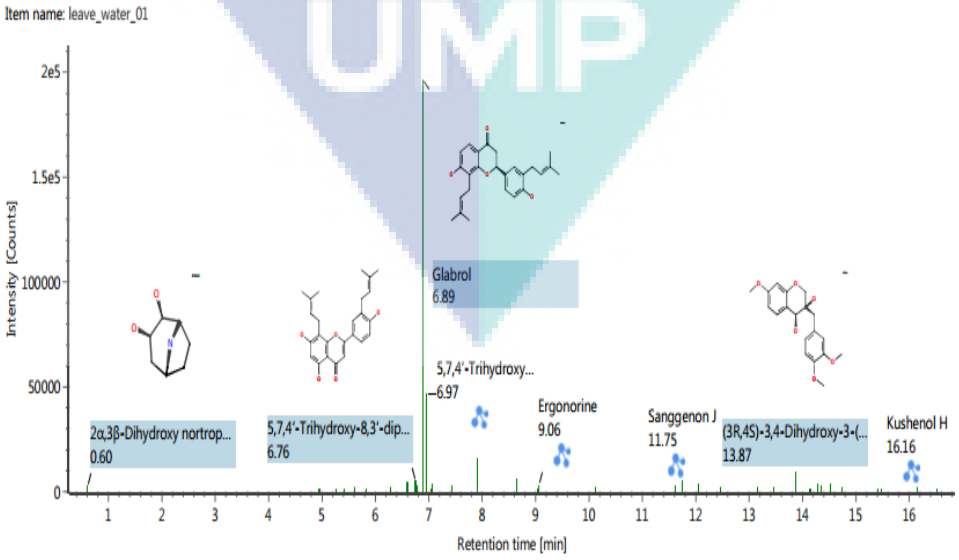


Figure 5b Confirmed major compounds plotted on (+) ESI-MS of water extract

.Figure 6a shows UPLC-QToF/MS positive BPI chromatogram of *P. foetida* stem water extract. Glabrol show the most abundant compound followed by 5,7,4′-Trihydroxy-8,3′-diprenylflavone and (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman. The other compounds also detected in stem water extract such as Forsythoside D, Picrasidine F, Sanggenon J and Kushenol H. According on confirmed component plotted on (+) ESI-MS of water extract in Figure 6b, a major compound detected was $[M - H]^+$ at *m/z* 392.202 for glabrol followed by 5,7,4′-Trihydroxy-8,3′-diprenylflavone $[M - H]^+$ at *m/z* 406.1812 and (3R,4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman $[M - NA]^+$ at *m/z* 346.1389. Othes ions was also collected $[M - H]^+$ at *m/z* 478.1663, $[M - NA]^+$ at *m/z* 478.203, for Forsythoside D and Picrasidine F, repectively followed Sanggenon J and Kushenol H, $[M - NA]^+$ at *m/z* 488.2233, $[M - H]^+$ at *m/z* 472.2079 respectively.

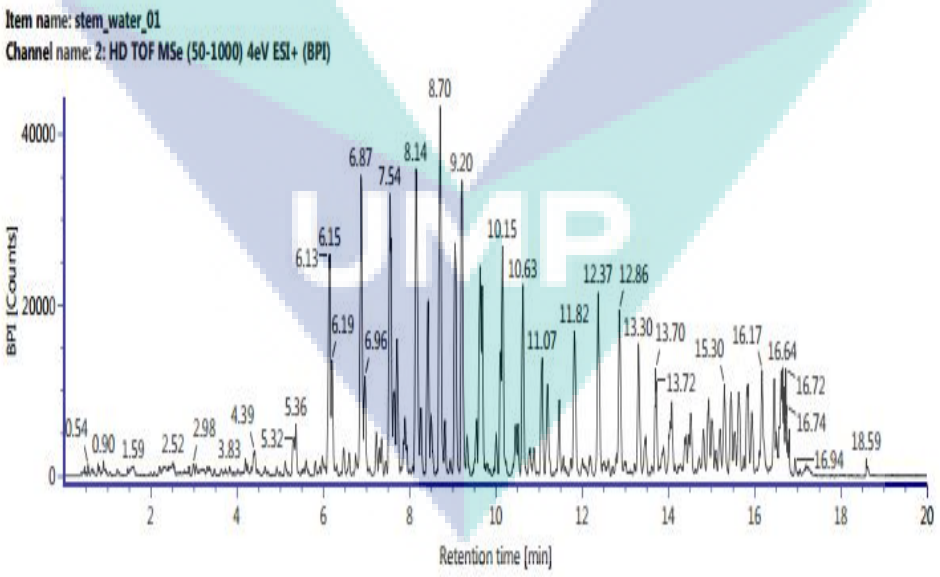


Figure 6a UPLC-QToF/MS positive BPI chromatogram of *P. foetida* stem water extract

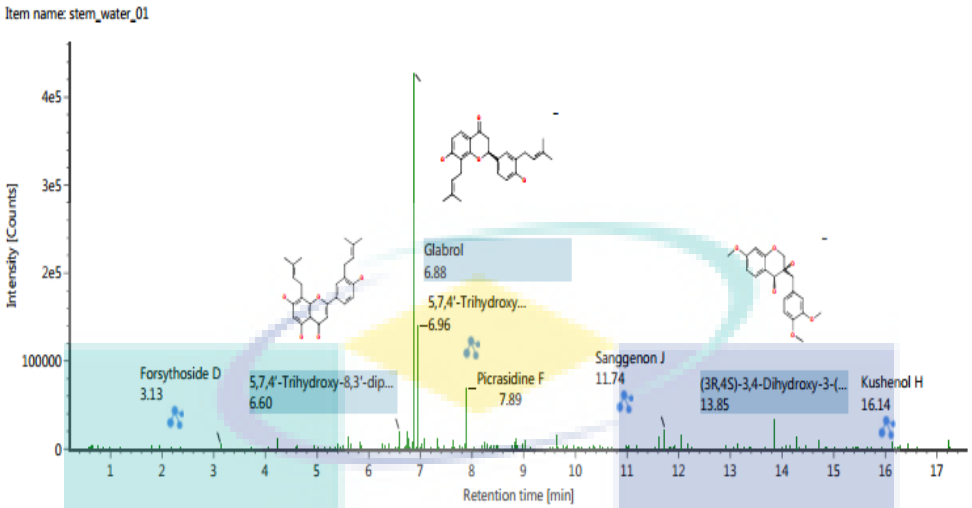


Figure 6b Confirmed major compounds plotted on (+) ESI-MS of water extract

As conclusion for of 3 parts of *P.feotida* which is leaves, stem and fruit using water as extraction solvent. Based on UPLC-QToF/MS positive BPI chromatogram of *P. foetida* and Confirmed major compounds plotted on (+) ESI-MS of water extract (figure 2.1.1 until figure 2.3.2) performed very good result. Two compounds are detected the most abundant from all water extracts which is glabrol and (3R, 4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman which gives $[M - H]^+$ at *m/z* 392.202, $[M - NA]^+$ at *m/z* 346.1389 ion, respectively.

For summarization, 3 parts of *P.feotida* which is leaves, stem and fruit has been extracted with water as a solvent. All the extracts were analyse using UPLC-QToF/MS for phytochemical screening. Both extracts (methanol and water) detected same major compound which are glabrol and (3R, 4S)-3,4-Dihydroxy-3-(3′,4′-dimethoxybenzyl)-7-methoxy-chroman $[M - H]^+$ at *m/z* 392.202, $[M - NA]^+$ at *m/z* 346.1389 ion, respectively. Both compounds can be categorizes as flavanone compound and hydroxyl compound which are very polar compound. So, Isolation and characterization of

P.feotida are still in progress and these two compounds will be used as a marker compound for the next isolation process.

4.3 Antimicrobial Activity

The antimicrobial test was done on *B. subtilis* (gram positive bacteria) and the results showed (Table 4.1) certain zone of inhibition. When methanol fruits extract was tested on *B. subtilis* having Gentamicin as positive control, the zone of inhibition showed was 11.67 ± 0.10 mm. However, for the methanol extract of leaves and stems showed, the zone of inhibition was 5.05 ± 0.03 mm and 5.11 ± 0.01 mm respectively. This results showed that for both t extracts are very susceptible by the bacteria. The other gram-positive bacteria used was *E. faecalis* and there were also some zone of inhibition on the bacterial strain. From the results, it has shown that the methanol extract from fruits *P. feotida* can inhibit the *E.faecalis* bacterial strain up to 9.00 ± 0.15 mm. Methanol extract of leaves and stems has not shown ability in inhibiting the bacterial growth with only 5.10 ± 0.01 mm and 5.02 ± 0.02 mm of zone of inhibition respectively.

For the gram negative bacteria, *E. coli* and *P. aerogenosa*, they showed certain zone of inhibition. As shown in the table, the methanol fruits extract showed 10.33 ± 0.92 mm of inhibition zone on the *E. coli* bacterial strain which is also considered as moderately inhibited the bacteria growth. The leaves and stems methanol extracts f have both shown 5.33 ± 0.01 mm and 5.32 ± 0.20 mm, zone of inhibition. For *P. aerogenosa,* the zone of inhibition by methanol fruits extract was 9.00 ± 0.04 mm. Then, the zone of inhibition by water fruit extract was shown to be 7.34 ± 0.21 mm while the methanol and

water extracts for both leaves and stem fraction of extract showed 5.00-5.67 mm of zone of inhibition on the *P. aerogenosa* bacterial strain.

According to the antimicrobial tests on the plant extract, it has been found that the methanol fruits extract has shown the highest antimicrobial activity compared to the other extracts. This was due to the presence of antimicrobial chemical compounds within this sample extract.

Table 4.1 Antimicrobial activity of fruits, leaves and stems extracts of *P. feotida*

Pathogenic Bacterial Strains	Zone of Inhibition (mm)						
	Positive control	Fruits		Leaves		Stems	
	Gemtamicin	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
E. faecalis	20.00 ± 0.20	9.00 ± 0.15	7.50 ± 0.10	5.10 ± 0.01	5.15 ± 0.05	5.02 ± 0.02	5.13 ± 0.03
B. subtilis	20.00 ± 0.43	11.67 ± 0.10	6.5.0 ± 0.05	5.05 ± 0.03	5.25 ± 0.10	5.11 ± 0.01	5.12 ± 0.01
E. coli	20.00 ± 0.50	10.33 ± 0.92	5.33 ± 0.01	5.33 ± 0.01	5.13 ± 0.04	5.32 ± 0.20	5.42 ± 0.20
P. aerogenosa	19.67 ± 0.15	9.00 ± 0.04	7.34 ± 0.21	5.67 ± 0.02	5.31 ± 0.15	5.15 ± 0.05	5.51 ± 0.25

4.4 Acetylcholinesterase inhibitory activity

During the microplate assay, hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine at a wavelength of 406 nm.

Table 4.2 Acetylcholinesterase inhibitory activity by fruits, leaves and stems extract of *P. feotida* and the IC_{50} values

Extract	Solvent	IC_{50} (µM)
Fruits	MeOH	415.26 ± 2.70
	H_2O	564.60 ± 2.08
Leaves	MeOH	417.64 ± 5.34
	H_2O	NA
Stems	MeOH	276.07 ± 1.84
	H_2O	616.44 ± 5.88
Positive control	Physostigmine	31.40 ± 0.54

The IC_{50} values are the mean ± standard deviations of two independent experiments. The inhibitory effects are represented as compounds concentration (µM) giving 50% inhibition on AChE activity (IC_{50}). Physostigmine was used as a positive control. Based on the results, there was no extract found to be a competitive inhibitor as the IC_{50} value close to the IC_{50} value of physostigmine which was the positive control. One extract that showed moderately AChE inhibitory activity in microplate assay was stems, fruits and leaves methanol extract. Other compounds with IC_{50} more than 500 µM were considered as weak AChE inhibitors. The efficiency of inhibition of AChe was found to decrease in the order of water fruits extract followed by water stems extract.

5. CONCLUSION

Plants are an important source for the development of new therapeutic and antioxidant agents. This study indicates both antioxidant and acetylcholinesterase inhibitory activity of the traditional important plant *P. feotida* . The antioxidant effect of *P. feotida* and acetylcholinetrese potential may due to the contents of flavonoids compounds. The recent results help in providing a base for the further investigation of *P. feotida* and potential identification of novel bioactive compounds with therapeutic properties.

ACHIEVEMENT

i) Name of articles/ manuscripts/ books published

This project produced two journal papers

Mutazah, R., Hamid, H. A., Ramli, A. N. M., Aluwi, M. F. F. M., Yusoff, M. M. J. F., & Toxicology, C. (2019). In vitro cytotoxicity of *Clinacanthus nutans* fractions on breast cancer cells and molecular docking study of sulphur containing compounds against caspase-3. 110869.

Zamri, N., & Hamid, H. A. J. P. F. f. H. N. (2019). Comparative Study of Onion (Allium cepa) and Leek (Allium ampeloprasum): Identification of Organosulphur Compounds by UPLC-QTOF/MS and Anticancer Effect on MCF-7 Cells. 74(4), 525-530.

ii) Title of Paper presentations (international/ local)

In vitro cytotoxicity of Clinacanthus nutans fractions on breast cancer cells and molecular docking study of sulphur containing compounds against caspase-3, First International Conference On Natural Toxicology And Pharmacology (1-ICNTP), Jinan University, Guangzhou, China, 7-11 August 2019, Guangzhao, China

iii) Human Capital Development

The following student works for this project

Izzah Hayati Binti Yahya (PSI18001)
Isolation and Characterization of Passiflora feotida for Potential of Antidepressant Compounds.
Ongoing

Siti Radhiah Binti Rahman (SA15025)
Determination of alkaloids and acetylcholineterase inhibitory activity of *Passiflora feotida*
Graduated 2019

iv) Awards/ Others

Awards Title	*Date/Year*	*Organizer*	*Level*
Silver medal dalam Creation, Innovation Technology & Research Exposition (CITREX) 2018 -Clitoria Ternatea-Starch Film as Ph Indicator	12-13 Feb 2018	UMP	Universiti

REFERENCES

1. Fajemiroye, J.O., Silva, D.M., Oliveira, D.R., and Costa, E.A. 2016. Treatment of anxiety and depression: medicinal plants in retrospect. Fundamental & clinical pharmacology.
2. Hamid, H., Yusoff, M., Liu, M., Karim, M., 2015. α-Glucosidase and α-amylase inhibitory constituentsof Tinospora crispa: Isolation and chemical profile confirmation by ultra-high performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. Journal of Functional Foods 16, 74-80.
3. Herraiz, T., Chaparro, C., 2016. Human monoamine oxidase enzyme inhibition by coffee and β-carbolines norharman and Harman isolated from coffee. Life sciences 78, 795-802.
4. Jawna-Zboińska, K., Blecharz-Klin, K., Joniec-Maciejak, I., Wawer, A., Pyrzanowska, J., Piechal, A., Mirowska-Guzel, D., Widy- Tyszkiewicz, E., 2016. Passiflora incarnata L. Improves Spatial Memory, Reduces Stress, and Affects Neurotransmission in Rats. Phytotherapy Research.
5. McCarthy, A., Wafford, K., Shanks, E., Ligocki, M., Edgar, D.M., Dijk, D.-J., 2016. REM sleep homeostasis in the absence of REM sleep: Effects of antidepressants. Neuropharmacology 108, 415-425.
6. Shuayprom, A., Sanguansermsri, D., Sanguansermsri, P., Fraser, I.H., Wongkattiya, N., 2016. Quantitative determination of vitexin in Passiflora foetida Linn. leaves using HPTLC. Asian Pacific Journal of Tropical Biomedicine 6, 216-220.
7. Ota, K.T., Liu, R.-J., Voleti, B., Maldonado-Aviles, J.G., Duric, V., Iwata, M., Dutheil, S., Duman,C., Boikess, S., Lewis, D.A., 2014. REDD1 is essential for

stress-induced synaptic loss and depressive behavior. Nature medicine 20, 531-535.

8. Pacheco, G., Simão, M.J., Vianna, M.G., Garcia, R.O., Vieira, M.L.C., Mansur, E., 2016. In vitro conservation of Passiflora—A review. Scientia Horticulturae 211, 305-311.
9. Ramaiya, S.D., Bujang, J.S., Zakaria, M.H., 2014. Genetic diversity in Passiflora species assessed by morphological and ITS sequence analysis. The Scientific World Journal 2014.
10. Veeramohan, R., Haron, N.W., 2015. Macromorphological and micromorphological studies of four selected passiflora species in peninsular malaysia. Pak. J. Bot 47, 485-492.
11. Vijeepallam, K., Pandy, V., Kunasegaran, T., Murugan, D.D., and Naidu, M. 2016. Mitragyna speciosa leaf extract exhibits antipsychotic-like effect with the potential to alleviate positive and negative symptoms of psychosis in mice. Frontiers in Pharmacology 7.
12. Yusoff, M., Hamid, H., Houghton, P., 2014. Anticholinesterase inhibitory activity of quaternary alkaloids from Tinospora crispa. Molecules 19, 1201-1211.

APPENDIXES

Plant Foods for Human Nutrition (2019) 74:525–530
https://doi.org/10.1007/s11130-019-00770-6

ORIGINAL PAPER

Comparative Study of Onion (*Allium cepa*) and Leek (*Allium ampeloprasum*): Identification of Organosulphur Compounds by UPLC-QTOF/MS and Anticancer Effect on MCF-7 Cells

Normaiza Zamri[1] · Hazrulrizawati Abd Hamid[1]

Published online: 6 November 2019

Abstract

Onion (*Allium cepa)* and leek (*Allium ampeloprasum* var. *porrum*) are common herbs and vegetables found in our daily life. It belongs to the genus *Allium*, which is usually known for their high antioxidant and anticancer properties. Medical researchers highly recommend the exploitation of herbs and plants as alternative ways in the treatment of cancer. This research was designed to study the anticancer effects of onion and leek extracts on MCF-7 human breast cancer cell. Crude extracts of ethanol, methanol, and water of onion and leek were obtained by maceration. MCF-7 cells were cultured in complete media at 37 °C and subjected to different treatments that involved varying concentrations (10, 50, and 100 μg/mL) of onion and leek extracts for 24, 48, and 72 h of incubation. The percentage of cell viability and the concentration of extracts on MCF-7 cells were determined using MTT assay. The water leek extract proved to be the most effective extract at 50 μg/mL, whereby it showed a significant inhibition ability due to the presence of entadamide A-β-D-glucopyranoside as identified by ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UPLC-QTOF/MS). Further studies about the mechanism of both extracts in causing cell death and the determination of the presence of other bioactive compounds in the extracts are needed.

Keywords Onion (*Allium cepa*) · Leek (*Allium ampeloprasum*) · Organosulphur compounds · UPLC-QTOF/MS, anticancer · MCF-7 cells

Introduction

Chemoprevention is a cancer preventive strategy to inhibit, delay, or reverse carcinogenesis. Altering dietary habits by reducing consumption of processed foods while increasing the intake of vegetables may decrease cancer risks. There is an increasing public health demand to identify those dietary patterns, bioactive foods, and components that may decrease cancer risks. Approximately 30–40% of cancers are preventable by appropriate food and nutrition intake, physical activity, and maintenance of healthy body weight [1]. One particular group of foods that has raised considerable interest in their putative cancer-preventive properties is the genus Allium.

Allium is a large genus of onion or garlic-scented bulbous herbs of the Amaryllidaceae family. Allium vegetables, such as garlic (*Allium sativum*), onion (*Allium cepa*), and leek (*Allium ampeloprasum* var. *porrum*) are widely consumed for their characteristic flavours (as spices) and their health-promoting effects [2]. Garlic is one of the most extensively studied functional species among Alliums, and it has been considered a medicinal food for centuries, being used as a traditional remedy for common disorders. Garlic consumption is associated with decreased risk of some types of cancer, cardiovascular diseases, and neurodegenerative disease [3]. Besides, various other biological activities have been reported for garlic, including antimicrobial, antioxidant, and anti-inflammatory properties [4]. Onion has also been utilised as a medicinal agent according to sources dating from ancient times. This broad spectrum of health-benefits is mainly attributed to the presence of organosulphur compounds, a distinct characteristic of garlic and other Allium species. It must be

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11130-019-00770-6) contains supplementary material, which is available to authorized users.

✉ Hazrulrizawati Abd Hamid
hazrulrizawati@ump.edu.my

[1] Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang Kuantan, Pahang, Malaysia

Food and Chemical Toxicology 135 (2020) 110869

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

In vitro cytotoxicity of *Clinacanthus nutans* fractions on breast cancer cells and molecular docking study of sulphur containing compounds against caspase-3

Roziasyahira Mutazah, Hazrulrizawati Abd Hamid*, Aizi Nor Mazila Ramli, Mohd Fadhlizil Fasihi Mohd Aluwi, Mashitah M. Yusoff

Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Kuantan, Pahang, Malaysia

ARTICLE INFO

Keywords:
Clinacanthus nutans
Cytotoxicity
MDA-MB-231
MCF-7
Sulphur containing compounds
Molecular docking
Caspase-3

ABSTRACT

Clinacanthus nutans has attracted Malaysian public interest due to its high medicinal value in the prevention of cancer. Currently, the specific compound or compounds giving rise to the anticancer potential of *C. nutans* has not been investigated thoroughly. The extraction was carried out by MeOH at room temperature using the powdered bark of *C. nutans*, while chromatography was carried out on a silica gel RP-18 column using the crude methanolic extract. Six fractions collected from column chromatography were evaluated by MTT assay against two breast cancer cell lines: MDA-MB-231 and MCF-7. Amongst the fractions, A12 and A17 were shown to exhibit the highest activity. Two sulphur-containing compounds, viz., entadamide C (1) and clinamide D (2), were isolated from these fractions. Molecular docking simulation studies revealed that entadamide C and clinamide D could bind favourably to the caspase-3 binding site with the binding energy of −4.28 kcal/mol and −4.84 kcal/mol, respectively. This study provides empirical evidence for the presence of sulphur-containing compounds in the leaves of *C. nutans* that displayed anticancer effects which explains its ethnomedicinal application against breast cancer. The docking simulation study showed that both compounds could serve as important templates for future drug design and development.

1. Introduction

Cancer is presently considered a substantial public health issue in the world. One of the primary cancers inflicting women worldwide is breast cancer. The treatment of cancer is a significant challenge. Cancer is classified and treated solely according to the organs of origin or simplistic histomorphologic features (Zugazagoitia et al., 2016). The clinical modalities presently employed in the treatment of cancer such as surgical removal, radiotherapy, and specialised chemotherapy or hormone therapy are systemic anti proliferative proxies that distort cell division (Huang et al., 2017). These drugs do not limit themselves to cancerous cells, and their therapeutic efficiency is restricted as they can cause damage to healthy cells and tissues as well. Consequently, there is an essential need for new natural anticancer compounds in chemotherapeutics. In the past few years, many biological properties of several potential plants and herbs have been studied by various researchers.

However, to date, the potential chemotherapeutic properties of many compounds present in vegetables, fruits and traditional herbs that are ingested by humans are not well understood. The incidence of chemoprevention, a condition when apoptosis is induced in pre-cancerous and cancerous cells caused by these agents, instigate the interest of researchers to study their properties. The advancement in the development of novel chemopreventive agents might be possible with better and concise knowledge of the molecular mechanisms that cause these effects. Sulphur-containing compounds (OSC) such as garlic constituents (GCs) and isothiocyanates (ITCs) are some of the naturally occurring dietary chemopreventive agents that have shown anticancer effects (Wu et al., 2005). *Clinacanthus nutans* possesses sulphur-containing compounds that exhibit anticancer effects.

The potential use of *C. nutans* as an anticancer agent has been reported by Yong et al. (2013), whereby the chloroform extract of the plant was capable of inhibiting cell proliferation of seven tested cancer cell lines, namely, the HepG2, IMR32, NCL-H23, SNU-1, HeLa, LS-174 T, K562, and Raji cells. Although several compounds such as C-glycosyl flavones, sulphur-containing glycosides, cerebrosides, monoacyl-monogalactosylglycerol, and chlorophyll derivatives (phaeophytins) have been discovered in the leaves of *C. nutans* (Alam et al., 2016),

* Corresponding author.
E-mail address: hazrulrizawati@ump.edu.my (H.A. Hamid).

https://doi.org/10.1016/j.fct.2019.110869
Received 6 March 2019; Received in revised form 17 September 2019; Accepted 2 October 2019
Available online 15 October 2019
0278-6915/

S1: *In Vitro* Cytotoxicity of *Clinacanthus Nutans* Fractions on Breast Cancer Cells and Molecular Docking Study of Sulphur Containing Compounds Against Caspase-3

Hazrulrizawati Abd Hamid*

Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang Kuantan, Pahang, Malaysia

Clinacanthus nutans has attracted Malaysian public interest due to its high medicinal values in the prevention of cancer. Currently, the specific compound demonstrating the anticancer potential of C. nutans has not been investigated thoroughly. The purpose of this study is to explore the in vitro cytotoxic activity of semi-purified fractions of C. nutans. The extraction was done by MeOH while chromatography was done on a silica gel RP-18 column. Six fractions collected from column chromatography were evaluated by the MTT assay against two breast cancer cell lines: MDA-MB-231 and MCF-7. Amongst the fractions, A12 and A17 were shown to exhibit the most activity, and the compounds were mostly isolated from these fractions. Two sulphur-containing compounds, entadamide C and clinamide D were isolated[1]. Molecular docking simulation studies revealed that entadamide C and clinamide D could bind favourably to the caspase-3 binding site with the binding energy of -4.28 kcal/mol and -4.84 kcal/mol, respectively. This study provided empirical evidence for the presence of sulphur-containing compounds in the leaves of C. nutans that displayed anticancer effects and its usage as an ethnomedicine against breast cancer. The docking simulation study showed that both compounds could serve as important hit compounds for future chemical modification.

Keywords: Clinacanthus nutans; cytotoxicity; sulphur containing compounds; molecular docking; caspase-3

Acknowledgements:
The authors are grateful to the Ministry of Higher Education Malaysia (MOHE) for the financial support provided under the Fundamental Research Grant Scheme, FRGS/1/2017/WAB13/UMP/02/2 (RDU170139).

References:

[1] Tu, S.-F., Liu, R., Cheng, Y.-B., Hsu, Y.-M., Du, Y.-C., El-Shazly, M., Wu, Y.-C., Chang, F.-R.J.M., Chemical constituents and bioactivities of Clinacanthus nutans aerial parts. Molecules. 2014. 19 (12), pp 20382-20390.

Universiti Malaysia PAHANG
Jabatan Penyelidikan & Inovasi
Research & Innovation Office

Canselerí Tun Abdul Razak
Universiti Malaysia Pahang
26600 Pekan
Pahang Darul Makmur
Tel : +609 424 5000
Fax : +609 424 5055

Our Ref.: UMP.05/26.12/RDU170139/973
23 November 2018

IZZAH HAYATI BINTI YAHYA
Pelajar
FAKULTI SAINS & TEKNOLOGI INDUSTRI

Saudara/i,

PERLANTIKAN SEBAGAI PEMBANTU PENYELIDIK (SKIM PEMBANTU PENYELIDIK SISWAZAH) PROJEK: VOT RDU170139
- IZZAH HAYATI BINTI YAHYA (PSI18001)

Sukacita dimaklumkan bahawa permohonan saudara/i sebagai Pembantu Penyelidik (Skim Pembantu Penyelidik Siswazah) telah diluluskan seperti maklumat di bawah:

Lantikan	: Pembantu Penyelidik
Taraf	: Skim Pembantu Penyelidik Siswazah
Vot Projek	: RDU170139
Tarikh Kuatkuasa	: 01/10/2018 - 31/08/2019

2. Perlantikan ini adalah tertakluk kepada peraturan Skim Pembantu Penyelidik yang telah dipersetujui oleh UMP mengikut kadar bulanan yang telah dikuatkuasakan seperti berikut:

Elaun/Upah	: RM 1000/bulan

3. Tawaran jawatan ini adalah tertakluk kepada syarat-syarat seperti berikut:

i. Tempoh perlantikan saudara/i adalah bergantung kepada status projek penyelidikan atau tarikh perlantikan bergantung kepada tarikh terdahulu.
ii. Sebagai Pembantu Penyelidik (Skim Pembantu Penyelidik Siswazah), saudara/i bukanlah seorang kakitangan dalam perkhidmatan tetap UMP dan dengan sedemikian saudara/i tidak berhak mendapat keistimewaan yang biasa diberikan kepada kakitangan dalam perkhidmatan tetap UMP.
iii. Saudara/i dikehendaki menjalankan tugas di mana-mana tempat pada bila-bila masa seperti yang diarahkan oleh Ketua Projek.
iv. Ketua Projek tidak terikat untuk memberi saudara/i jawatan tetap di dalam perkhidmatan universiti setelah tamat tempoh perlantikan ini.
v. Saudara/i hendaklah mengambil perhatian bahawa segala maklumat rasmi yang diperolehi semasa bertugas di universiti ini tidak boleh dibocorkan atau disampaikan kepada sesiapa juga yang tidak berkenaan.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Yang Benar,

ERNIE NURAZLIN BINTI LIZAM
Jabatan Penyelidikan & Inovasi
Universiti Malaysia Pahang

c.c **Profesor Madya Dr. Hazrulrizawati Binti Abd Hamid**
Ketua Projek RDU170139
FSTI
Dekan,
Fakulti Sains & Teknologi Industri
Bursary, Bursary Department
(u.p: Pegawai Kewangan Unit Tabung Penyelidikan)

Universiti Malaysia PAHANG
Jabatan Penyelidikan & Inovasi
Research & Innovation Office

Canseleri Tun Abdul Razak
Universiti Malaysia Pahang
26600 Pekan
Pahang Darul Makmur
Tel : +609 424 5000
Fax : +609 424 5055

Our Ref.: UMP.05/26.12/RDU170139/1758
19 September 2019

IZZAH HAYATI BINTI YAHYA
Pelajar
FAKULTI SAINS DAN TEKNOLOGI INDUSTRI

Saudara/i,

PERLANTIKAN SEBAGAI PEMBANTU PENYELIDIK (SKIM PEMBANTU PENYELIDIK SISWAZAH) PROJEK: VOT RDU170139
- IZZAH HAYATI BINTI YAHYA (PSI18001)

Sukacita dimaklumkan bahawa permohonan saudara/i sebagai Pembantu Penyelidik (Skim Pembantu Penyelidik Siswazah) telah diluluskan seperti maklumat di bawah:

Lantikan	: Pembantu Penyelidik
Taraf	: Skim Pembantu Penyelidik Siswazah
Vot Projek	: RDU170139
Tarikh Kuatkuasa	: 01/09/2019 - 29/02/2020

2. Perlantikan ini adalah tertakluk kepada peraturan Skim Pembantu Penyelidik yang telah dipersetujui oleh UMP mengikut kadar bulanan yang telah dikuatkuasakan seperti berikut:

Elaun/Upah	: RM 2000/bulan

3. Tawaran jawatan ini adalah tertakluk kepada syarat-syarat seperti berikut:

i. Tempoh perlantikan saudara/i adalah bergantung kepada status projek penyelidikan atau tarikh perlantikan bergantung kepada tarikh terdahulu.
ii. Sebagai Pembantu Penyelidik (Skim Pembantu Penyelidik Siswazah), saudara/i bukanlah seorang kakitangan dalam perkhidmatan tetap UMP dan dengan sedemikian saudara/i tidak berhak mendapat keistimewaan yang biasa diberikan kepada kakitangan dalam perkhidmatan tetap UMP.
iii. Saudara/i dikehendaki menjalankan tugas di mana-mana tempat pada bila-bila masa seperti yang diarahkan oleh Ketua Projek.
iv. Ketua Projek tidak terikat untuk memberi saudara/i jawatan tetap di dalam perkhidmatan universiti setelah tamat tempoh perlantikan ini.
v. Saudara/i hendaklah mengambil perhatian bahawa segala maklumat rasmi yang diperolehi semasa bertugas di universiti ini tidak boleh dibocorkan atau disampaikan kepada sesiapa juga yang tidak berkenaan.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Yang Benar,

IDAYU BINTI MAT ALI
Pegawai Sains Kanan
Jabatan Penyelidikan & Inovasi
Universiti Malaysia Pahang

c.c **Profesor Madya Dr. Hazrulrizawati Binti Abd Hamid**
Ketua Projek RDU170139
FSTI
Dekan,
Fakulti Sains Dan Teknologi Industri
Bursary, Bursary Department

Universiti Malaysia PAHANG

CREATION, INNOVATION, TECHNOLOGY & RESEARCH EXPOSITION (CITREx)

Certificate of Award

SILVER MEDAL

This Certificate of Award is presented to

DR. HAZRULRIZAWATI BINTI ABD HAMID, DR. NORMAIZA BINTI ZAMRI, DR. FARAH HANANI BINTI ZULKIFLI, DR. AIZI NOR MAZILA BINTI RAMLI

For the invention entitled

CLITORIA TERNATEA-STARCH FILM AS PH INDICATOR

CREATION, INNOVATION, TECHNOLOGY & RESEARCH EXPOSITION 2019
12th – 13th February 2019, Universiti Malaysia Pahang

PROFESSOR DATO' DR. MASHITAH MOHD. YUSOFF
ACTING DEPUTY VICE CHANCELLOR (RESEARCH & INNOVATION)
UNIVERSITI MALAYSIA PAHANG