Bioactive Compounds In Buasbuas (*Premna pubescens*. Blume) Shoots With Proteomic Approaches Using GC-MS

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ABSTRACT: Buasbuas (*Premna pubescens*, Blume) is one the most widely used plant in the Melayu traditional food and medicine. Several pharmacological activities including antioxidant effects and phytochemical investigations have been previously reported for the various parts of plant tissues. In the present study, the bioactive compound active compound of the leaf tissues were investigated. Bioactve compound from leaf was monitored using GC-MS investigation. A Gas chromatography-mass spectrometry (GC-MS) system, combined with an in vivo assay for detecting active compounds, was developed in order to identify and inventory the major bio active compound chemicals present in Buasbuas (Premna pubescens, Blume). Through the use of comprehensive spectroscopy studies, the isolated bio active principle was identified. Bio active coumpounds, which was able account for most of the reported biological activity on *P. pubescens*.

KEYWORDS: Premna pubescens; Lamiaceae; bio actve compounds; shoot leaf; GC-MS

1. INTRODUCTION

The genus Premna (Lamiaceae) is widely distributed in tropical and subtropical regions of Africa, Asia, Australia and the Pacific islands (Leeratiwong et al, 2016). Buasbuas (*Premna pubescens* Blume) is a tree that occur in Sumatera and Malaysia. Various parts of the plant especially the leaf are extensively used for thousands of years in Melayu traditional medicine formulations such as Bubur Pedas (Restuati et al, 2016). Among the various medicinal uses reported for the roots are for treating diabetes, chyluria, gonorrhoea, inflammation, swelling, bronchitis, dyspepsia, headach, liver disorder, piles, constipation and fever (Restuati et al, 2014).

Some pharmacological studies have revealed that the plant possess anti-coagulant (Harley et al, 2004), anti-inflammatory (Marbun and Restuati, 2015), anti-arthritic (Simanjuntak, 2012), antinociceptive (Hidayat, 2015), hypoglycemic (Hanim, 2016), gastrprotective (Jothi et al., 2010), antimicrobial properties (Restuati et al, 2015) and cardioactivity (Hardiyanti, 2016). Wahyuni (2014) have recently studied the antioxidant activity of the leaves of *P. pubescens* along with its active constituents. It was reported that 4″-hdroxy-E-globularinin, 10-O-trans-p-coumaroylcatalpol, premnosidic acid, 10-O-trans-p-coumaroyl-6-O-α-l-rhamnopyranosyl catalpol and a new dimeric lignin were responsible for the antioxidant effect of the stem bark (Yadav et al., 2011). Similarly the leaves and roots of *P. pubescens* have also been demonstrated to possess antioxidant effect (Restuati et al., 2015). To date a number of phytochemical studies on the leaves stem bark and root bark of the plant are reported but the leaf that plays major role in the proteomic and metabolomic has not yet been studied (Diningrat et al, 2014 and Diningrat et al, 2015). We herewith report the identification of the bioactive compounds principles of the leaf tissues using proteomic approach (Roessner et al, 2000).

2. MATERIALS AND METHODS

2.1. Samples and Extraction of Bioactive Compounds

Unpasteurized Buasbuas young leaf were derived from the same tree source. The leaf juice was processed with a juice extractor. The leaf juice samples were stored at -20 °C until analyzed. The manual SPME device equipped with a 50/30 µm DVB/CAR/PDMS fiber (Supelco, Bellfonte,PA, USA) was used for extraction volatile compounds of leaf juice. The fiber was conditioned in the GC injector port at 270 °C for 1 h prior to use. The Buasbuas leaves juice (5 mL) were placed into a 20 mL vial containing a micro stirring bar, respectively. The sample was equilibrated at 40 °C±1 °C for 15 min and headspace extracted by DVB/CAR/PDMS fiber for 40 min at the same temperature under stirring (500 rpm). After extraction, the fiber was inserted into the injection port of the GC to desorb the analytes for 5 min. Each analytical sample was measured in triplicate. The results are reported as the mean values of relative peak area percent ± SD (standard deviation) (Roessner et al, 2000).

2.2. Chemicals

Authentic standards were obtained as follows: α-pinene were purchased from Aldrich Chemical Co. (Germany) n-alkanes (C8-C20), ethyl butyrate, β-myrcene, linalool, decanal, were obtained from FluKa Chemical Co. (Germany). Hexanal, trans-2-

hexenal, benzaldehyde, octanal, limonene, nonanal, α-terpineol, neral, geranial, neryl acetate, geranyl acetate, citronellyl acetate and valencene were gifts from the GLD-Boton Essential Company (Roessner et al, 2000).

2.3. GC-MS analysis

GC-MS was carried out using a HP 5975B quadropole mass selective detector (Agilent Technologies, USA). The Mass spectral ionization temperature was set at 230 °C. The mass spectrometer was operated in the electron impact ionization mode at a voltage of 70 eV. Mass spectra were taken over the m/z range 30–400. The flow rate of the helium carrier gas on HP-5 column (30 m×0.25 mmI.D, 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA) was 1 mL/min. The analysis performed in the splitless mode and injector temperature was 250 °C. The column was held at 40 °C for 3 min, and then increased from 40 °C to 160 °C at 3 °C/min, held at 160 °C for 2 min, and finally increased to 220 °C at a rate of 8 °C/min, then held for 3 min.

Volatile components were identified by comparing their mass spectra with the mass spectra from MS libraries (NIST 05, WILEY 7.0). When available, MS identifications were confirmed by comparing GC retention times of the analytes with those from pure standards. The identification was confirmed using retention indices (RI), and the value was compared with those reported in the literature. Linear retention indices (RI) of the compounds were calculated using a series of n-alkanes (C8–C20, Sigma-Aldrich, Germany) injected in the same conditions. When standard chemicals were not available, tentative identification was carried out by matching the mass spectra (Roessner et al, 2000).

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3. RESULT AND DISCUSSION

The bioactive compounds of the crude ethanolic extracts of *P. pubescens* leaf tissues was assessed by using the GC-MS assay as described previously (Diningrat et al, 2014). The typical concentration shown in Figure 1 was in agreement with previous studies that revealed the leaves of P.serratifolia (Diningrat et al, 2015). When the leaf tissues were separately assessed for their bio active compunds, we observed a profile (Figure 1) that suggests that the leaf must be considered for establishing the scientific evidence of the reputed medicinal uses of *P. pubescens* leaf. Since the chemistry of the leaf has been studied in recent years, emphasis was given in the present study on the leaf tissues that is obtained in similar yield as the root bark.

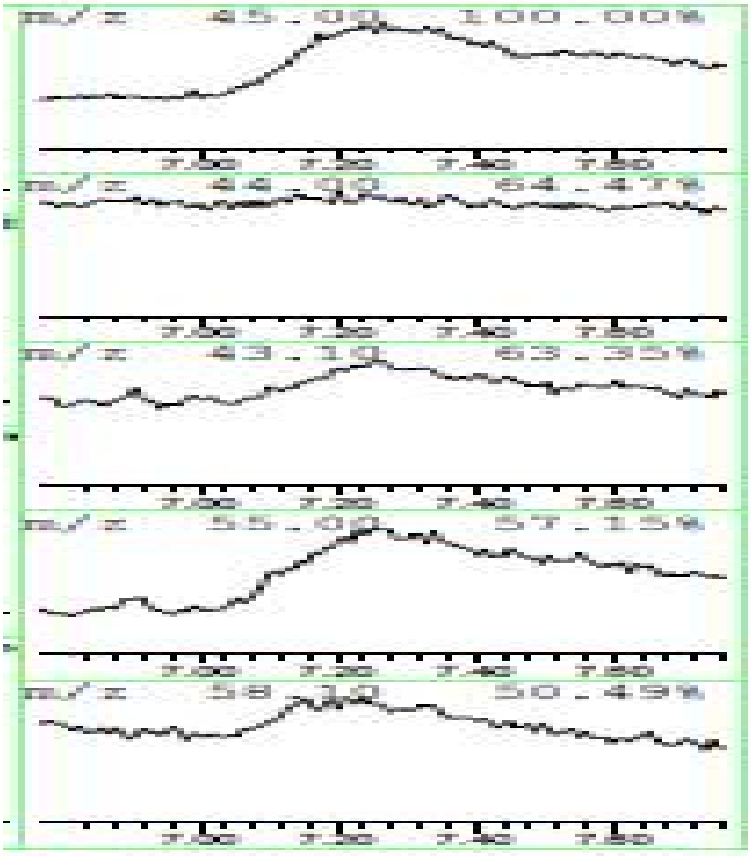

Figure 1. GC-MS profile of the crude leaf extract of *P. pubescens*

GC-MS analysis of the bio acitve compounds leaf tissue extract revealed one prominent peak (retention time of 23.9 min) within the 50 min analysis time (Figure 1). It appears from this figure that the extract was made of one major constituent in an incredibly pure form instead of being complex mixture as expected in crude extracts. GC-MS analysis, however, revealed that the extract is a crude mixture suggesting that there are lots of compounds that were not picked up by the UV detection system. Further purification was done by a fast Combiflash ISCO chromatography system that allows the processing of large scale separations. Repetitive purification using this system afforded the bio active principle that is shown as a major chromatogram peak (Figure 1). Proteomic study of the isolated bio active compound revealed a better activity profile than the crude extracts (Figure 1). The IC50 value of the compound was also 18.3 ± 3.7 µg/ml and was about 4-times more potent the crude extracts. On the basis these spectroscopic data, the proteomic principle isolated from the leaf tissues was identified. The compound was previously isolated from the leaves of Premna corymbosa that is considered to be the same with *P. pubescens*.

4. CONCLUSION

In conclusion, the shoot leaves of *P. pubescens* are extensively used in the Melayu traditional system of medicine for treating various disease conditions. As with the other plant parts, the leaves have been shown to display bio active compounds but the proteomic principles have so far not identified. Our proteomic analysis using GC-MS assay as a guide has resulted in the identification of bio active compounds principle of the leaf tissues. This compound could account for most of the bio active reported for *P. pubescens* leaf.

5. REFERENCES

[1] Diningrat, D. S., Widiyanto, S. M., Pancoro, A., Shim, D., Panchangam, B., Zembower, N., & Carlson, J. E. (2014). Transcriptomes of Teak (Tectona grandis, Lf) in Vegetative to Generative Transition Stage Development. International Journal of Innovation and Applied Studies, 9(3), 1416.

[2] Diningrat, D. S., Widiyanto, S. M., Pancoro, A., Shim, D., Panchangam, B., Zembower, N., & Carlson, J. E. (2015). Transcriptome of Teak (Tectona grandis, Lf) in Vegetative to Generative Stages Development. Journal of Plant Sciences, 10(1), 1.

[3] Hanim, N. (2016), Pengaruh Ekstrak Etanol Daun Buasbuas (Premna pubescens Blume) Terhadap Penurunan Kadar Gula Darah Dan Gambaran Histologi Pankreas Tikus Putih Jantan (Rattus norvegicus) Yang Diindiksi Aloksan., skripsi, FMIPA, UNIMED, Medan

[4] Hardiyanti, Y. (2016), Efek Toksik Formaldehid Terhadap Organ Reproduksi Tikus Betina (Rattus norvegicus. L) Yang Diberikan Ekstrak Etanol Daun Buasbuas (Premna pubescens Blume)., skripsi, FMIPA, UNIMED, Medan.

[5] Harley, R. M., Atkins, S., Budantsev, A. L., Cantino, P. D., Conn, B. J., Grayer, R., Harley, M. M., de Kok, R. P. J., Krestovskaja, T., Morales, R., Paton, A. J., Ryding, O. & Upson, T. (2004). Labiatae. In: K.Kubitzki & J. W. Kadereit (eds), The Families and Genera of Vascular Plants vol. VII:167 – 275. Springer- Verlag, Berlin.

[6] Hidayat, U. (2015), Pengaruh Ekstrak Daun Buasbuas (Premna pubescens Blume) Terhadap Pertumbuhan Bakteri Bacillus cereus Secara In Vitro., skripsi, FMIPA, UNIMED, Medan.

[7] Leeratiwong, C., Chantaranothai, P dan Paton A. (2016), Taxonomic Notes On The Genus Premna L. (Lamiaceae) In Thailand, Thai Forest Bull., Bot 44(2): 122-124.

[8] Marbun, E.M.A Dan Martina, R (2015), Pengaruh Ektrak Etanol Daun Buas-Buas (Premna pubescens Blume) Sebagai Antiinflamasi Pada Edema Kaki Tikus Putih (Rattus novergicus), Jurnal Biosains 1(3): 2443-1230.

[9] Restuati, M., Ilyas, Syafruddin., Hutahaean, Salomo. dan Sipahutar, Herbert. (2014), Study of the extract activities of Buas buas leaves (Premna pubescens) as immunostimulant on rats (Rattus novegicus), American Journal of BioScience . 2(6): 244-250.

[10] Restuati, M., U. Hidayat, A. S. S. Pulungan, N.Pratiwi. Dan D. S.Diningrat. (2016), Antibacterial Activity Of Buasbuas (Premna pubescens Blume) Leaf Extracts Against Bacillus cereus And Escherichia coli, Journal of Plant Sciences 1816-4951.

[11] Roessner, U., Wagner, C., Kopka J Trethwey R.N And Wilmitzer, L, (2000), Simultaneous Analysis Of Metabolites In Potato Tuber By Gas Chromathography-Mass Spectrometry, the plant journal. 23(1) 131-142.

[12] Simanjuntak, K. (2012), Pengaruh Pemberian Ekstrak Daun Buasbuas (Premna pubescens Blume) Terhadap Imunitas Humoral Tikus Putih (Rattus norvegicus. L)., skripsi, FMIPA, Unimed, Medan.

[13] Wahyuni, S., Mukarlina1., dan Yanti, A.H (2014) Aktivitas Antifungi Ekstrak Metanol Daun Buas-Buas (Premna Serratifolia) Terhadap Jamur Diplodia Sp. Pada Jeruk Siam (Citrus Nobilis Var. Microcarpa), Protobiont 3 (2) : 274 – 279.