

Preliminary Phytochemical Screening and GC-MS Analysis of Ethanol Extract of *Physalis Minima* L. (Solanaceae)

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Abstract—*Physalis minima* L. or locally known as pokok letup-letup is one of the interesting species in the genus *Physalis* due to the reports of its medicinal properties. This valuable plant can be found at warm temperate and subtropical regions throughout the world. This valuable plant is traditionally used as diuretic, purgative, analgesic, vermifuge, etc. Ethanolic extraction of leaf, root and fruit were screened by GS-MS to identify the phytochemical compounds. The obtained result showed that the plant have important compounds such as phytol, vitamin E, oleic acid and n-Hexadeconoic acid. Presence of antimicrobial and antioxidant in the three parts of the plant (leaf, root and fruit) confirms the application of *Physalis minima* for various ailments by practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Index Terms—*Physalis minima*, pokok letup-letup, phytochemical, GS-MS, medicinal plant, antioxidant

I. INTRODUCTION

Physalis played as one of the largest genera in Solanaceae family with of 80 to 100 species can be found around the world. *Physalis minima* L. are one of the popular species which can be found throughout India, Baluchistan, Afghanistan, Tropical Africa, Singapore, Australia and Malaysia [1]. This herb is commonly known as Cape gooseberry, bladder cherry, pygmy ground cherry and pokok letup-letup. *Physalis minima* is annual or- short lived perennial plants with less hairy as compared to few other species. The berry like fruit is almost round in shape and entirely hidden in calyx [2]. The fruiting calyx is the distinct characters of *Physalis*

and differentiates it from other genera in the Solanaceae family. This interesting plant grown very well in most of soil types but do well on sandy to gravelly loam under full of sun exposure [3]. *Physalis minima* tolerant to drought seasons and can grow up to 1.5 meter tall. It has broad leaves and grows rapidly on disturbed soil which makes it difficult to control. Whole body of the plant, from root to the shoot has been reported to be safe as traditional medicine except for calyx [4]. The decoction of the whole plant is consumed as a remedy by the Malay community in Malaysia for cancer and the leaves are used as a poultice for ulcer [4]. The fruit contained high amount of vitamin C (24.45 mg/100 ml of juices) and is considered to be diuretic, purgative and used to relieve pain and cure spleen disorder [5].

Phytochemical compounds are secondary metabolites produced by plant to act as protector against several of microorganisms, insects and higher herbivorous predators' infections [6]. Pharmaceutical industry nowadays is depending on medicinal plants to supply the raw materials for extraction of medicinally important compounds. The phytochemical compounds of plants have been used as therapeutic agent, new synthetic compound for drug formulations and as taxonomic markers for discovery of new compounds. Base compositions of more complex semisynthetic chemical compounds are also derived from phytochemical compounds of plants [7]. Unfortunately, statistics showed that demand on medicinal plants is increasing and exploring of new sources is encouraged. The literature review showed that the leaves, roots and fruits of *Physalis minima* have been used traditionally in the treatment of various ailments [1]. The extractions of *Physalis minima* plants particularly from India and China reported to have numerous of antioxidants and anticancer

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activities [2], [8]. With this information, an attempt was made to study the different phytochemical compounds that presence in leaf, root and fruit of *Physalis minima* L. from Malaysia through GC-MS analysis.

II. MATERIALS AND METHODS

A. Plant Sample Preparation

Fresh and healthy of leaves, roots and fruits of *Physalis minima* were collected from plants grown at Field 2, Universiti Putra Malaysia. It were dried at room temperature $\pm 30^\circ\text{C}$ for a period of 14 days and made into powder using homogizer.

B. Alcoholic Extraction

Ten grams of powdered plant materials (leaves, roots and fruits) of *Physalis minima* was soaked in 30 ml of ethanol overnight. It was filtered through Whatmann filter paper No. 41 along with 2 gm of Sodium Sulphate which has been wetted with absolute alcohol. The filtrates were then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1 ml. The extract contained both polar and non-polar phytochemical components.

C. Isolation and Analysis of Volatile Compounds

The volatile compounds were analyzed by gas chromatograph mass spectrometer (GC/MS) on an Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector with BP20 (WAX) capillary column (30 m \times 0.25 mm, 0.25 μm thickness, SGE). The ion source was set at 230°C and the ionization voltage at 70 eV. The temperature was programmed from 50°C - 250°C at rate of 5°C min^{-1} .

D. Identification of Peaks

The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0, and the results were combined in a single peak table.

III. RESULT AND DISCUSSION

Based on the GC-MS analyses, the ethanolic extractions of leaves, roots and fruits of *Physalis minima* contained numerous numbers of phytochemical compounds. The chemical components that had been identified, the percentage of peak area of each constituent and their running time of leaves, root and fruit of the plant were summarized in the Tables I, Table II and Table III and the GC-MS chromatogram were presented in Fig. 1, Fig. 2 and Fig. 3.

The major compounds that had been identified from the exthanolic extract of leaves were Acetamide, 2,2,2-trifluoro-N-methyl- (7.2%), 2-Cyclopenten-1-one, 2-methyl- (3.83%), Phytol (17.88%), n-Hexadecanoic acid (29.81%), Octadecanoic acid (5.04%), Oleic acid (3.55%), 9,12-Octadecadienoic acid (Z,Z)- (12.47%), Hexaethylene glycol monododecyl ether (5.6%) and

9,12,15-Octadecatrienoic acid, (Z,Z,Z) (14.63%). The major compounds identified from ethanolic extracts of leaf were presented in Table I and the GC-MS chromatogram of the same was presented in Fig. 1.

TABLE I. PHYTOCOMPONENTS IDENTIFIED IN ETHANOLIC EXTRACTION OF THE LEAVES OF *PHYSALIS MINIMA* BY GC-MS PEAK REPORT TIC.

Peak	R. Time	Compound identified	(%)
1	13.14	Acetamide, 2,2,2-trifluoro-N-methyl-	7.20
2	30.28	2-Cyclopenten-1-one, 2-methyl-	3.83
3	36.20	Phytol	17.88
4	40.50	n-Hexadecanoic acid	29.81
5	43.38	Octadecanoic acid	5.04
6	43.76	Octadec-9-enoic acid	3.55
7	44.44	9,12-Octadecadienoic acid (Z,Z)-	12.47
8	45.05	Hexaethylene glycol monododecyl ether	5.60
9	45.36	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	14.63

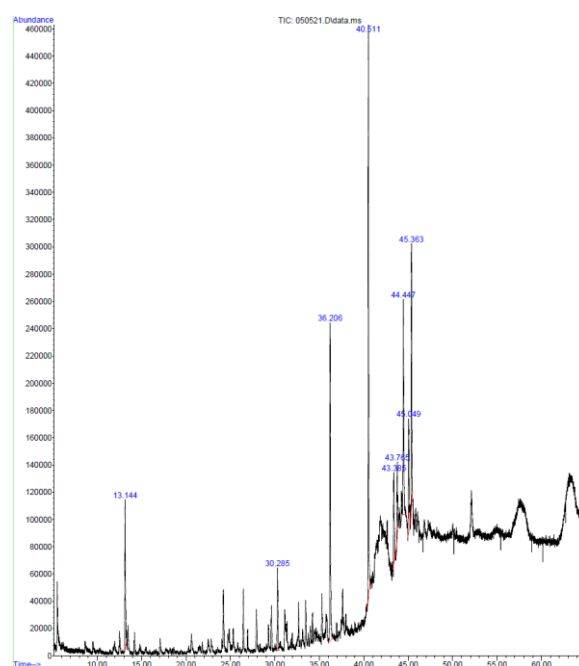


Figure 1. GC-MS chromatogram of the leaves of *Physalis minima*

The major phytochemical compounds that had been identified from ethanolic extract of roots were n-Hexadecanoic acid (4.06%), 2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol (1.43%), O-Methyl-DL-serine, N-dimethylaminomethylene-,ethyl ester (63.20%) and Ethyl dl-(1-naphthyl)glycolate (4.94%). All chemical compounds that had been identified from the extractions of root were summarized in Table II and the GC-MS chromatogram of the same was presented in Fig. 2.

The major compounds identified from ethanolic extract of fruits were Acetamide, 2,2,2-trifluoro-N-methyl- (3.90%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (8.40%), 5-Hydroxymethylfurfural (14.07%), (Z)-3-Phenyl-2-propenoic acid (3.33%), n-Hexadecanoic acid (28.98%), Octadecanoic acid (3.35%), Octadec-9-enoic acid (9.15%), 9,12-Octadecadienoic acid (Z,Z)- (16.09%),

2-Isopropoxyethyl propionate (9.28%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.47%). All the major compounds that had been identified from ethanolic extracts of fruit were summarized in Table III and the GC-MS chromatogram of the same was presented in Fig. 3.

TABLE II. PHYTOCOMPONENTS IDENTIFIED IN ETHANOLIC EXTRACTION OF THE ROOTS OF *PHYSALIS MINIMA* BY GC-MS PEAK REPORT TIC

Peak	R. Time	Compound identified	%
1	40.50	n-Hexadecanoic acid	4.06
2	45.17	2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethanol	1.43
3	59.30	O-Methyl-DL-serine, N-dimethylaminomethylene-, ethyl ester	63.20
4	59.45	O-Methyl-DL-serine, N-dimethylaminomethylene-, ethyl ester	4.49
5	59.47	Ethyl dl-(1-naphthyl)glycolate	4.94
6	59.51	O-Methyl-DL-serine, N-dimethylaminomethylene-, ethyl ester	24.74

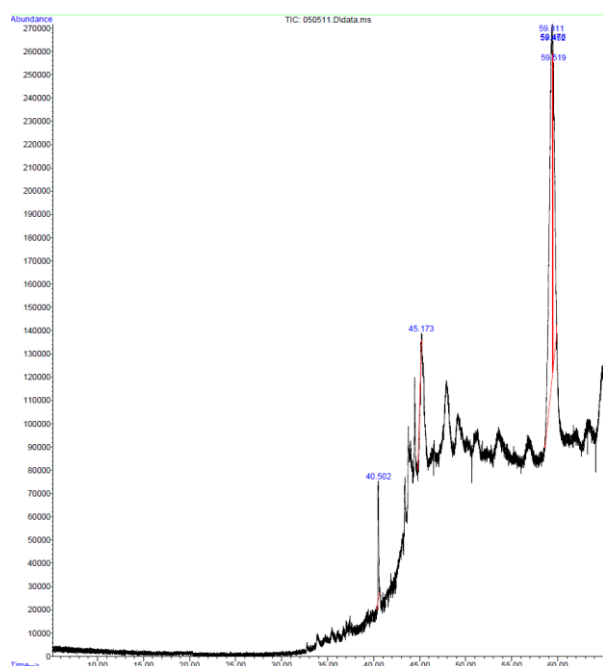


Figure 2. GC-MS chromatogram of the root of *Physalis minima*

n-Hexadecanoic acid ($C_{16}H_{32}O_2$) was the major compound that had been found in the leaf, root and fruit ethanolic extract with percentage of quality more than 90. This phytochemical compound was reported to have larvicidal activity, antibacterial, antifungal, antioxidant activity, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor and hemolytic activity [9]-[11].

Octadecanoic acid ($CH_3(CH_2)_{16}CO_2H$), Octadec-9-enoic acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$), 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- ($C_{18}H_{30}O_2$) were the fatty acids that can be found in leaf and fruit extracts. These fatty acids were reported to have various uses such as antimicrobial [12], hypercholesterolemic [13], dermatitogenic [14], anti-inflammatory and anti-tumor activity [15], [16].

Phytol ($C_{20}H_{40}O$) with percentage of quality more than 91 was found in ethanolic extract of leaf. This compound is precursor for the manufactured of synthetic form of vitamin E [17] and vitamin K1 [18]. Vitamin E is one of fat soluble compound that functioning most as antioxidant in human body system [19] and related with hypoglycaemic activity [20].

4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- ($C_6H_8O_4$) and (Z)-3-Phenyl-2-propenoic acid ($C_9H_8O_2$) were two flavonoids compounds that had been identified in the ethanolic extract of fruit. Flavonoid compound, 5-Methoxy-6, 7-methylenedioxyflavone was isolated from *Physalis minima* together with the known compound, 5,6,7-trimethoxyflavone [21].

TABLE III. PHYTOCOMPONENTS IDENTIFIED IN ETHANOLIC EXTRACTION OF THE FRUITS OF *PHYSALIS MINIMA* BY GC-MS PEAK REPORT TIC.

Peak	R. Time	Compound identified	%
1	13.16	Acetamide, 2,2,2-trifluoro-N-methyl-	3.90
2	30.26	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.40
3	34.22	5-Hydroxymethylfurfural	14.07
4	39.55	(Z)-3-Phenyl-2-propenoic acid	3.33
5	40.50	n-Hexadecanoic acid	28.98
6	43.38	Octadecanoic acid	3.35
7	43.75	Octadec-9-enoic acid	9.15
8	44.44	9,12-Octadecadienoic acid (Z,Z)-	16.09
9	45.07	2-Isopropoxyethyl propionate	9.28
10	45.35	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	3.47

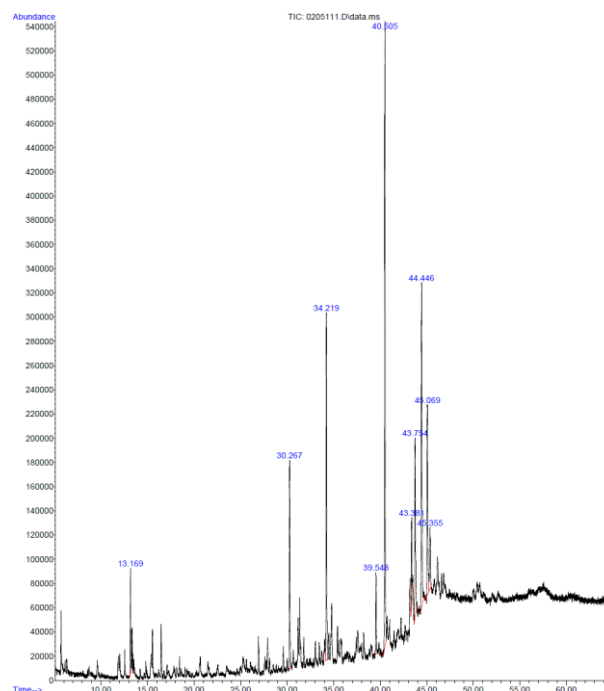


Figure 3. Figure 3: GC-MS chromatogram of the fruit of *Physalis minima*

IV. CONCLUSION

The phytochemical compounds screened from the leaves, roots and fruits of *Physalis minima* indicated the

presence of various antimicrobial and antioxidant compounds that are very important for health and can be implemented in pharmaceutical industry in Malaysia. The important compounds such as phytol, vitamin E, oleic acid and n-Hexadeconoic acid were reported to have therapeutic uses and the obtained results could form a good basis of selection of *Physalis minima* for further investigation in the potential discovery of new valuable bioactive compounds.

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REFERENCES

- [1] L. Chotani and H. U. Vaghasiya, "A phyto-pharmacological overview on *physalis minima* Linn," *Indian Journal of Natural Products and Resources*, vol. 3, pp. 477-482, December 2012.
- [2] M. Nathiya and D. Dorcus, "Preliminary phytochemical and anti-bacterial studies on *physalis minima* linn," *International Journal of Sciences*, vol. 1, pp. 24-30, January 2012.
- [3] G. J. Azlan, M. Marziah, M. Radzali, and R. Johari, "Establishment of *physalis minima* hairy roots culture for the production of physalins," *Plant Cell, Tissues and Organ Culture*, vol. 69, pp. 271-278, June 2002.
- [4] M. Zakaria and M. A. Mohamad, *Tradisional Medicinal Plant*, Selangor Malaysia: Fajar Bakti, 1994.
- [5] C. Parmar and M. K. Kaushal, *Physali minima In Wild Fruits*. 1st ed, Kalyani Publisher, New Delhi, India, pp. 62-65, 1982.
- [6] M. P. Prasad, M. Soumya and Brinda, "Phytochemical screening and in-vitro evaluation of anti-oxidant and anti-bacterial properties of medicinal plants," *CIB Tech Journal of Biotechnology*, vol. 3, pp. 18-25, March 2014.
- [7] O. Akerele, "WHO guidelines for the assessment of herbal medicine," *Fitoterapia*, vol. 62, pp. 99-110, 1992.
- [8] L. Ma, X. W. Gan, Q. P. He, H. Y. Bai, M. Arfan, F. C. Lou, and L. H. Hu, "Cytotoxic withaphysalins from *physalis minima*," *Helvetica Chimica Acta*, vol. 90, pp. 1406-1419, July 2007.
- [9] A. Manilal, S. Sujith, G. K. Seghal, J. Selvin, and C. Shakir, "Cytotoxic potentials of red alga, *laurencia brandenii* collected from the Indian Coast," *Global J Pharmacol*, vol. 3, pp. 90-94, 2009.
- [10] D. E. Okwu and B. U. Ighodara, "GC-MS evaluation of the bioactive compounds and antibacterial activity of the oil fraction from the stem barks of *dacryodes edulis* g. don (lam)," *Int. J. Drug Dev and Res*, vol 1, pp. 117-125, 2009.
- [11] P. PraveenKumar, S. Kumaravel, and C. Lalitha, "Screening of" 2010
- [12] A. F. Novak, C. Gladys, G. C. Clark, and H. P. Dupuy, "Antimicrobial activity of some ricinoleic acid, oleic acid derivatives," *Journal of the American Oil Chemist's Society*, vol. 38, pp. 321-324, 1961.
- [13] F. Natali, L. Siculella, S. Serafina, and G. V. Gnoni, "Oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells," *Journal of Lipid Research*, vol 48, pp. 1966-1975, 2007.

- [14] H. L. Newmark, "Squalene, olive oil, and cancer risk: A review and hypothesis," *Cancer epidemiology, Bio markers and Prevention*, vol. 6, pp. 1101-1103, 1997.
- [15] Y. Kimura, "Carp oil or oleic acid, but not linoleic acid or linolenic acid, inhibits tumor growth and metastasis in Lewis lung carcinoma-bearing mice," *J. Nutr.*, vol. 132, pp. 2069-2075, 2002.
- [16] Z. Yunfeng, W. Dong, G. Siyuan, Z. Xuewu, W. Mingfu, and C. Feng, "Chemical components and antioxidant activity of the volatile oil from *cassia tora* L. Seed prepared by supercritical fluid extraction," *Journal of Food Lipids*, vol. 14, pp. 411-423, 2007.
- [17] T. Netscher, "Synthesis of vitamin E," *Vitamins & Hormones*, vol. 76, pp. 155-202, 2007.
- [18] A. M. Daines, "The synthesis of naturally occurring vitamin K and vitamin K analogues," *Current Organic Chemistry*, vol. 7, pp. 1625-1634, 2003.
- [19] E. F. Bell, "History of vitamin E in infant nutrition," *American Journal of Clinical Nutrition* vol. 46, pp 183-186, 1987.
- [20] Sivan, Eyal, Homko, J. Carol, Reece, E. Albert, *et al.*, "Dietary vitamin E prophylaxis and diabetic embryopathy: Morphologic and biochemical analysis," *American Journals Obstetrics and Gynecology*, vol. 175, pp. 793-1086, 1996.
- [21] Ng Ang Sera, "Flavonoids from *physalis minima*," *Phytochemistry*, vol. 27, pp. 3708-3709, 1988



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