Journal of Science and Mathematics Letters, Vol 10, Issue 1, 2022 (91-96)
ISSN 2462-2052, eISSN 2600-8718

RESEARCH PAPER

Chemical Constituents from *Piper magnibaccum* C.DC.

Nur Athirah Hashim*, Farediah Ahmad

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,
81310 Skudai, Johor Bahru, Johor, MALAYSIA

*Corresponding author: nurathirah136@gmail.com

Received: 15 April 2022; **Accepted:** 20 June 2022; **Published:** 28 June 2022

To cite this article (APA): Hashim, N. A., & Ahmad, F. (2022). Chemical Constituents from Piper magnibaccum C.DC. *Journal of Science and Mathematics Letters*, *10*(1), 91-96. https://doi.org/10.37134/jsml.vol10.1.9.2022

To link to this article: https://doi.org/10.37134/jsml.vol10.1.9.2022

Abstract

The chemistry of *Piper* species has been widely investigated and phytochemical investigations conducted in all parts of the world have led to the isolation of a number of physiologically active compounds. Thus, this study was carried out to investigate the phytochemicals from *Piper magnibaccum* from Malaysia. Fractionation and purification of the leaves led to the isolation and identification of linoleic acid (**1**), 24*S*-ethylcholesta-5,22,25-trien-3β-ol (**2**), oleic acid (**3**), β-sitosterol (**4**), cepharadione A (**5**), piperumbellactam A (**6**), and stigmast-3,6-dione (**7**). The structures of these compounds were obtained by analysis of their spectroscopic data, as well as the comparison with that of reported data.

Keywords: *Piper magnibaccum*, Piperaceae, phytochemical, isolation

INTRODUCTION

Isolation and characterisation of pharmacologically active compounds from medicinal plants are continuously demanded today. In recent years, interest in traditional medicine has increased greatly among researchers and the general public (Fakim, 2006). The considerable interest in replacing synthetic drugs with natural sources from parts of plants has led to intensified exploration and research for various purposes to cure illness. Thousands of plants have been used traditionally to treat various diseases; thus, natural remedies have become popular, especially in the part of lower risk of adverse reactions (Natarajan et al., 2006). Several traditional plants in Malaysia are well known to possess medicinal values and largely consumable as an 'ulam', which is chewed alone or with other plants or food materials. The plant's leaves, fruits, seeds, tuber and roots are enriched with nutrients (Zaifuddin et al., 2014). Several traditional plants in Malaysia are consumed as 'ulam' and used as ingredients for traditional medicine. Plants from the genus *Piper* such as *Piper sarmentosum*, *P. betle* and *P. nigrum* are also categorised among the important medicinal plants used in various systems of medicine in Malaysia (Jamal, 2006).

The Piperaceae family is assigned in the order of Piperales and widely distributed in the tropics and subtropics regions. The family has about five genera and over 1950 species (Mabberley,

1997). *Manekia*, *Verhuellia*, *Zippelia*, *Piper* and *Peperomia* are the genera in Piperaceae plant taxonomy. *Piper* and *Peperomia* contributed the most number of species in this family with the latter used as ornamental plants. Commonly many species of Piper were used as spices, folk medicines and pest control agents (Arnason, 2005).

Piper, as the largest genus in the family of this pantropical group, are estimated to contain 2000 species dispersed widely in American and Asian tropic including India, Indonesian and Malaysian tropical rainforest (Jaramillo and Paul, 2001). Most species of *Piper* appeared to be restricted to altitudes ranging from 0 to 2500 m, and very few occurred above 3000 m which grow in wet and shaded places. It consists of a wide variety of species of high economic value, as they are used as food aromas, perfumes, fish venom, and insecticides, as well as in the treatment of gynaecological and gastrointestinal disturbances, depression, anxiety, pain, and inflammations, as well as bacterial and fungal infections. Moreover, *Piper* species are used to treat diseases, including fever, jaundice, rheumatism, and neuralgia, in various countries' folk medicine. Chemical studies have shown that Piper has many classes of compounds, such as amides, alkaloids, flavonoids, lignans, neolignans, aristolactams, terpenes, steroids, and phenylpropanoids (Salleh et al., 2011; 2012a, 2012b, 2014a, 2014b, 2014c, 2015a, 2015b). Due to the endless traditional uses of *Piper* species, the search for chemical compositions and active constituents from different *Piper* species has been intensified in recent years as a source of natural products with potential bioactivity properties (Peres et al., 2009).

Therefore, the objective of this study was to isolate, purify and elucidate phytochemicals from *P. magnibaccum*. Thus, a comparison of the phytochemical profiles of the current findings with previous reports can also be compared. These results must be investigated for developing pharmaceutical and herbal formulation documentation.

MATERIALS AND METHODS

Plant Material

The leaves of *P. magnibaccum* (SK2330/14) were collected in January 2014 from Hutan Simpan Fraser, Fraser Hill, Perak and identified by Shamsul Khamis from the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). The voucher specimens were deposited at the Herbarium of IBS. UPM.

General Experimental Procedures

Solvents systems used in the chromatographic method were *n*-hexane, chloroform ($CHCl_3$), dichloromethane (DCM), and methanol (MeOH). Soxhlet extraction technique was applied to extract the phytochemicals from the dried sample. Vacuum liquid chromatography (VLC) was performed on Merck silica gel 60 (230-400 mesh), while column chromatography (CC) on Merck silica gel 60 (70-230 mesh) was the stationary phase. Thin-layer chromatography (TLC) analysis was performed on Merck precoated silica (SiO_2) gel F_{254} plates with 0.2 mm thickness to detect and monitor compounds in the samples. The spots were visualised under UV light at 254 and 365 nm, and spraying reagent vanillin-sulphuric acid in MeOH was followed by heating. For spectroscopic analysis, the ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 Spectrophotometer. Chemical shifts were reported in ppm and $CDCl_3$ as the solvent. Residual solvent was used as an internal standard. The IR spectra were recorded on Perkin Elmer ATR and 1600 spectrophotometer series as KBr disc or thin film of NaCl discs. Mass spectral data were obtained from the Mass Spectrometry Service, National University of Singapore (NUS).

Extraction and Isolation

The leaves of *P. magnibaccum* (560 g) were sequentially subjected to cold extraction with n-Hex, EtOAc and MeOH which yielded the crude extracts of n-Hex (PMGLH: 5.34 g, 0.95 %), EtOAc (PMGLE: 12.89 g. 2.30 %) and MeOH (PMGLM: 8.10 g, 1.45 %), respectively. Fractionation of PMGLH extract by using VLC afforded 19 fractions, F1-19. The purification of the selected combination of fractions by CC method afforded four compounds from the PMGLH characterised as linoleic acid (**1**), 24-ethylcholesta-5,22,25-trien-3β-ol (**2**), oleic acid (**3**) and β-sitosterol (**4**). The fractionation of PMGLE crude extract yielded 29 fractions, F1-29. Fraction 5-10 were submitted to column chromatography to afford cepharadione A (**5**) and piperumbellactam A (**6**). Column chromatography of the methanol extract of *P. magnibaccum*, PMGLM yielded stigmast-3,6-dione (**7**).

RESULTS AND DISCUSSION

Investigation of the chemical constituents of the leaves part of *P. magnibaccum* has led to the isolation of seven compounds. These metabolites were identified by analysing their spectroscopic data and comparing it with the literature data. Previously, Emrizal et al., (2008) reported the anti-inflammatory activity of a crude extract of *P. magnibaccum* and of compounds isolated from it was established using the TPA-induced mouse ear edema model and an in vitro quantitative lipoxygenase inhibition assay. They successfully isolated five compounds which are β-sitosterol, *N*-isobutyl-(2*E*,4*E*)-tetradecadienamide, linoleic acid, 13-(4', 5'-dimethoxytridecanoyl)piperidine, and piperine. In addition, the light petroleum extract of the plant exhibited significant anti-inflammatory activity in both models. Meanwhile, *N*-isobutyl-(2*E*,4*E*)-tetradecadienamide showed significant activity, with an IE% value of 70.2 in the TPA-induced mouse ear edema model and 87.2 in the in vitro quantitative lipoxygenase inhibition assay. Many of these compounds were isolated previously from various *Piper* species. The secondary metabolism in most Piperaceae species appears to be restricted to the production of only a few classes of compounds. For instance, in the case of *P. regnellii*, only benzofuran lignans are biosynthesized and accumulate, together with other phenylpropanoids. For *P. solmsianum* several benzofuran lignans have been reported, while in *P. wightii* and *P. clarkia* a species accumulation of tetrahydrofuran lignans was investigated.

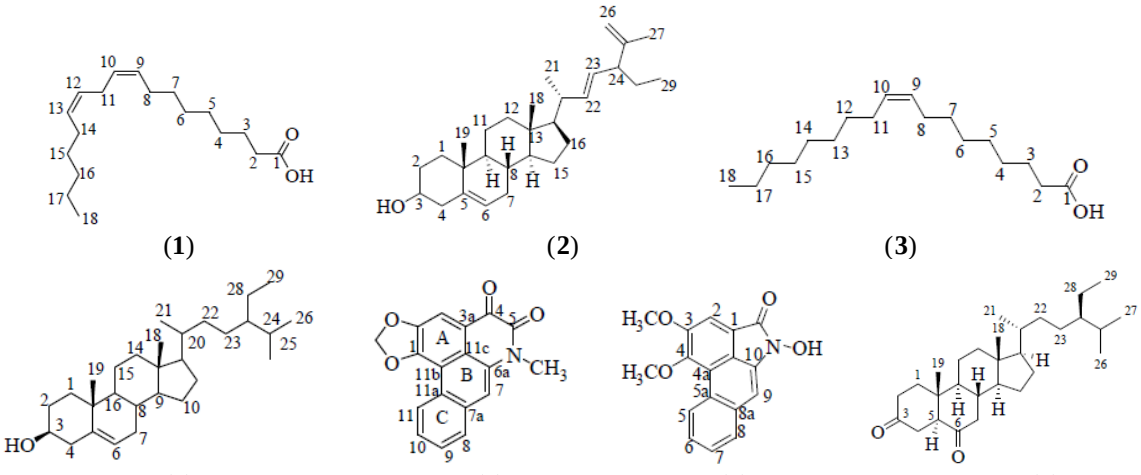

Figure 1. Chemical structures of isolated compounds

Isolation of Linoleic acid (1)

Fraction PMGLH F 1-9 (2.01 g) was purified by CC packed with silica gel (70 g), column size: 2.5 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ to yield linoleic acid (**1**) (68.2 mg) as a yellow oil. R_f 0.69 (*n*-Hex: $CHCl_3$; 4:1); IR (ATR) υ_{max} cm^{-1}: 3451, 2921, 1735; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 0.93 (3H, t, *J*= 2.8 Hz, 18-CH_3), 1.30-1.35 (14H, m, 7×CH_2), 1.61 (2H, m, H-3), 2.06 (4H, m, H-8, H-14), 2.20 (2H, t, *J* = 7.6 Hz, H-2), 2.79 (2H, t, *J* = 6.8 Hz, H-11), 5.35 (4H, m, H-9/H-10/H-12/H-13); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 13.0 (C-18), 22.2 (C-17), 22.3 (C-3), 25.1 (C-8), 25.9 (C-14), 26.7 (C-11), 28.9-29.4 (C-15/(C-13), 180.2 (C=O); EIMS *m/z* (% rel. int.) 280 [M^+, $C_{18}H_{32}O_2$] (21), 147 (68), 95 (54), 81 (75), 67 (100).

Isolation of 24*S*-ethylcholesta-5,22,25-trien-3β-ol (2)

Fraction PMLH F 10-12 (2.1 g) was purified by CC packed with silica gel (70 g), column size: 2.5 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ to yield 24*S*-ethylcholesta-5,22,25-trien-3β-ol (**2**) (32.4 mg) as a white solid; R_f 0.67 (*n*-Hex: $CHCl_3$; 4:1); m.p. 140-146°C; IR (KBr) υ_{max} cm^{-1}: 3407, 2958, 2853, 1459; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 0.72 (3H, m, 18-CH_3), 0.85 (3H, t, *J* = 7.6 Hz, 29-CH_3), 0.94 (1H, m, H-9), 1.03 (3H, s, 19-CH_3), 1.04 (3H, s, H-21), 1.08 (1H, m, H-14), 1.09 (1H, m, H-1b), 1.15 (2H, m, H-12), 1.19 (1H, m, H-17), 1.20 (1H, m, H-16b), 1.27 (2H, m, H-28), 1.46 (1H, m, H-20), 1.49 (1H, m, H-11a), 1.52 (1H, m, H-15a), 1.55 (1H, m, H-16a), 1.56 (1H, m, H-1a), 1.59 (2H, m, H-2), 1.67 (3H, s, 27-CH_3), 1.85 (1H, m, H-8), 1.99 (2H, m, H-7), 2.44 (1H, q, *J* = 7.6 Hz, H-24), 3.55 (1H, m, H-3), 4.72 (2H, br d, *J* = 2.0 Hz, H-26), 5.21 (1H, dd, *J* = 7.6, 13.2 Hz, H-23), 5.24 (1H, dd, *J* = 7.6, 13.2 Hz, H-22), 5.37 (1H, t, *J* = 5.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 12.0 (C-18), 20.8 (19-CH_3), 12.1 (29-CH_3), 19.4 (C-21), 20.2 (C-27), 20.8 (C-19), 21.1 (C-11), 24.3 (C-15), 25.7 (C-16), 25.8 (C-28), 31.9 (C-2), 31.7 (C-7), 31.9 (C-8), 36.5 (C-10), 37.3 (C-1), 39.7 (C-12), 40.2 (C-20), 42.3 (C-13), 42.3 (C-4), 50.2 (C-9), 52.0 (C-24), 56.0 (C-17), 56.9 (C-14), 71.8 (C-3), 109.5 (C-26), 121.7 (C-6), 130.1 (C-23), 137.2 (C-22), 140.8 (C-5), 148.6 (C-25); EIMS *m/z* (% rel. int.) 410 [M^+, $C_{29}H_{46}O$] (40), 392 (18), 273 (40), 271 (100), 255 (60).

Isolation of Oleic acid (3)

Fraction PMLH F 2-12 (2.54 g) was purified by CC packed with silica gel (70 g), column size: 3.0 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ to yield oleic acid (**3**) (51.3 mg) as yellowish oil; R_f 0.54 (*n*-Hex: $CHCl_3$; 4:1); IR (ATR) υ_{max} cm^{-1}: 3006, 2922, 2857, 1707, 1462, 1283; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 0.91 (3H, t, *J* = 2.8 Hz, 18-CH_3), 1.34-1.29 (20H, s, 10×CH_2), 1.64 (2H, q, *J* = 7.2 Hz, H-3), 2.04 (1H, d, *J* = 5.6 Hz, H-8), 2.36 (2H, t, *J* = 7.2 Hz, H-2), 5.36 (2H, dt, *J* = 12.0, 5.6 Hz, H-9/H-10), 11.39 (1H, br.s, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 14.0 (C-18), 22.7 (C-17), 24.6 (C-3), 27.1 (C-8), 27.2 (C-11), 29.0 (C-6), 29.1 (C-5), 29.2 (C-4), 29.3 (C-13, C-15), 29.5 (C-14), 29.7 (C-7), 29.8 (C-12), 31.9 (C-16), 34.1 (C-2), 129.7 (C-10), 129.9 (C-9), 180.5 (C=O); EIMS *m/z* (% rel. int.) 284 [M^+, $C_{18}H_{34}O_2$] (51), 256 (100), 229 (23), 213 (37), 185 (44), 148 (80), 129 (84).

Isolation of β-Sitosterol (4)

Fraction PMLE F 1-3 (1.54 g) was purified by recrystallisation with *n*-Hex/$CHCl_3$ to yield β-sitosterol (**4**) (11.8 mg) as white crystalline needles; R_f 0.58 (*n*-Hex: $CHCl_3$; 4:1); m.p. 130-135ºC; IR (KBr) υ_{max} cm^{-1}: 3435, 2966, 1461; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 0.71 (3H, s, H-18), 0.76 (3H, d, *J* = 6.4 Hz, H-27), 0.82 (3H, d, *J* = 6.4 Hz, H-26), 0.87 (3H, t, *J* = 3.8 Hz, H-29), 0.93 (3H, t, *J* = 3.8 Hz, H-21), 1.03 (3H, s, H-19), 1.05-2.31 (m, -CH_2-) 3.53 (1H, m, H-3), 5.37 (1H, d, *J* = 5.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 11.8 (C-29), 11.9 (C-18), 18.7 (C-21), 19.0 (C-27),

19.3 (C-19), 19.8 (C-26), 21.0 (C-11), 23.0 (C-28), 24.3 (C-15), 26.0 (C-23), 28.2 (C-7), 29.1 (C-25), 31.6 (C-2), 31.9 (C-8, C-16), 33.9 (C-22), 36.1 (C-20), 36.5 (C-10), 37.2 (C-1), 39.7 (C-12), 42.3 (C-4), 42.3 (C-13), 45.8 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5); EIMS *m/z* (rel. int.) 414 [M^+, $C_{29}H_{50}O$] (100).

Isolation of Cepharadione A (5)

Fraction PMGLE F 1-8 (2.56 g) was purified by CC packed with silica gel (90 g), column size: 3.5 cm × 30.0 cm and eluted with $CHCl_3$/EtOAc to yield cepharadione A (**5**) (2.1 mg) as orange needles; R_f 0.32 (*n*-Hex: $CHCl_3$; 2:3); m.p. 350-354°C; IR (ATR) υ_{max} cm^{-1}: 2925, 1710, 1652, 1020; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 3.87 (3H, s, N-CH_3), 6.48 (2H, s, OCH_2O), 7.53 (1H, s, H-7), 7.69 (2H, m, H-9/H-10), 7.92 (1H, m, H-8), 8.15 (1H, s, H-3), 9.01 (1H, m, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 30.5 (*N*-CH_3), 103.0 (OCH_2O), 109.0 (C-3), 114.2 (C-7), 115.2 (C-11b), 123.2 (C-3a), 125.5 (C-11a), 126.7 (C-10), 128.3 (C-8), 128.6 (C-11), 131.6 (C-7a), 132.4 (C-6a), 147.8 (C-2), 151.4 (C-1), 156.5 (C-5, C=O), 174.6 (C-4, C=O); EIMS *m/z* (% rel. int.) 305 [M^+, $C_{18}H_{11}NO_3$] (69), 277 (100), 248 (100), 232 (5), 163 (17).

Isolation of Piperumbellactam A (6)

Fraction PMGLM (8.10 g) was purified by CC packed with silica gel (90 g), column size: 3.5 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ and recolumn again (F180-184) by CC packed with silica gel (90 g), column size: 2.5 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ to yield piperumbellactam A (**6**) (1.5 mg) as a yellow needle; R_f 0.29 (*n*-Hex: $CHCl_3$; 2:3); m.p. 208-210°C; IR (ATR) υ_{max} cm^{-1}: 3156, 3017, 2951, 1710, 1652; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 4.11 (3H, s, 4-OCH_3), 4.15 (3H, s, 3-OCH_3), 7.11 (1H, s, H-9), 7.60 (2H, m, H-6/H-7), 7.83 (1H, dd, *J* = 6.4, 2.4 Hz, H-8), 7.85 (1H, s, H-2), 8.06 (1H, br.s, *N*-OH), 9.27 (1H, dd, *J* = 6.4, 2.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 57.0 (4-OCH_3), 60.4 (3-OCH_3), 105.6 (C-9), 110.0 (C-2), 121.1 (C-1), 124.4 (C-11), 127.6 (C-6), 127.0 (C-5), 127.6 (C-6), 129.0 (C-8), 134.0 (C-8a), 126.0 (C-5a), 141.3 (C-4a), 151.6 (C-4), 154.6 (C-3), 169.1 (C=O); EIMS *m/z* (% rel. int.): 295 [M^+, $C_{17}H_{13}NO_4$] (69), 279 (25), 250 (40), 237 (100), 223 (17), 194 (18), 165 (18), 152 (12).

Isolation of Stigmast-3,6-diene (7)

Fraction PMGLH F 14-15 (0.9 g) was purified by CC packed with silica gel (30 g), column size: 2.5 cm × 30.0 cm and eluted with *n*-Hex/$CHCl_3$ to yield stigmast-3,6-diene (**7**) (13.1 mg) as white needles; R_f 0.66 (*n*-Hex: $CHCl_3$; 4:1); m.p. 194-196°C; IR (KBr) υ_{max} cm^{-1}: 2924, 2864, 1708; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 0.71 (3H, s, 18-CH_3), 0.81 (3H, d, *J* = 6.8 Hz, H-27), 0.85 (3H, d, *J* = 6.8 Hz, H-26), 0.86 (3H, t, *J* = 6.8 Hz, H-29), 0.98 (3H, d, *J* = 6.8 Hz, H-21), 1.04 (3H, s, 19-CH_3), 1.15 (2H, m, H-23), 1.32 (1H, H-22 (H-b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 11.9 (C-29), 12.0 (C-18), 12.6 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 21.7 (C-11), 23.1 (C-28), 24.0 (C-15), 26.1 (C-23), 28.0 (C-16), 29.2 (C-25), 33.9 (C-22), 36.1 (C-20), 37.0 (C-4), 37.4 (C-2), 38.0 (C-1), 38.1 (C-8), 39.4 (C-12), 41.3 (C-10), 43.0 (C-13), 46.6 (C-7), 45.8 (C-24), 53.5 (C-9), 56.0 (C-17), 56.6 (C-14), 57.5 (C-5), 209.1 (C-6), 211.2 (C-3); GCMS (Ultra-1) t_R 57.61 min MS *m/z* (% rel. int.) 428 [M^+, $C_{29}H_{46}O$] (100), 399 (18), 287 (32), 245 (62).

CONCLUSION

Fractionation and purification of the crude extracts using the various chromatographic technique have resulted in the isolation of seven compounds of several classes of phytochemicals. Those classes of phytochemicals were identified spectroscopically as aporphine alkaloids, triterpenes, and

fatty acids. However, more detailed experiments are needed to explore the underlying biological activity in order to verify their mechanisms of action. Next, to validate the bioactivity, clinical trials should be carried out to ensure the safe use of the compounds as therapeutic agents.

Acknowledgement
The authors would like to thank the Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia for research facilities.

REFERENCES

Arnason, J.T., Steven, R.S. & Ian, M.S. (2005). Natural products from plants as insecticides. *Phytochemistry and Pharmacognosy*, *5*, 37-46.

Emrizal, Ahmad, F., Sirat, H.M., Jamaludin, F., Mustapha, N.M., Ali, R.M. & Arbain D. (20080. Anti-inflammatory activity of *Piper magnibaccum* (Piperaceae). *Natural Product Communications*, *3*(10), 1719-1721.

Fakim, A.G. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, *27*, 1-93.

Jamal, J.A. (2006). Malay Traditional Medicine: an overview of scientific and technological progress. *Technology Monitor*, *5*, 37-49.

Jaramillo, M.A. & Paul S.M. (2001). Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *American Journal of Botany*, *88*, 706-716.

Mabberley, D.J. (1997). The Plant-book. A Portable Dictionary of the Higher Plants (13th Edition). New York, USA: Cambridge University Press.

Natarajan, K.S., Narasimhan, M., Shanmugasunduraram, K.R. & Shanmugusundaram, E.R.B. (2006). Antioxidant activity of a salt-spice-herbal mixture against free radical induction. *Journal of Ethnopharmacology*, *105*, 76-83.

Péres, V.F.1., Moura, D.J., Sperotto, A.R., Damasceno, F.C., Caramão, E.B., Zini, C.A. & Saffi, J. (2009). Chemical composition and cytotoxic, mutagenic and genotoxic activities of the essential oil from *Piper gaudichaudianum* Kunth leaves. *Food Chemical and Toxicology*, *47*, 2389-95.

Salleh, W.M.N.H.W., Ahmad, F., Yen, K.H., & Sirat, H.M. (2011). Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Piper caninum* Blume. *International Journal of Molecular Sciences*, *12*, 7720-7731.

Salleh, W.M.N.H.W., Ahmad, F., Yen, K.H. & Sirat, H.M. (2012a). Chemical compositions, antioxidant and antimicrobial activity of the essential oils of *Piper officinarum* (Piperaceae). *Natural Product Communications*, *7*(12), 1659-1662.

Salleh, W.M.N.H.W., Ahmad, F., Sirat, H.M. & Yen, K.H. (2012b). Chemical compositions and antibacterial activity of the leaf and stem oils of *Piper porphyrophyllum* (Lindl.) NE Br. *EXCLI Journal*, *11*, 399-406.

Salleh, W.M.N.H.W., Ahmad, F. & Khong, H.Y. (2014a). Chemical composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. essential oils, and their antioxidant, antimicrobial and tyrosinase inhibition activities. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 13, 488-497.

Salleh, W.M.N.H.W., Ahmad, F., & Yen, K.H. (2014b). Chemical compositions and antimicrobial activity of the essential oils of *Piper abbreviatum*, *P. erecticaule* and *P. lanatum* (Piperaceae). *Natural Product Communications*, *9*(12), 1795-1798.

Salleh, W.M.N.H.W., Hashim, N.A., Ahmad, F. & Yen, K.H. (2014c). Anticholinesterase and antityrosinase activities of ten *Piper* species from Malaysia. *Advance Pharmaceutical Bulletin*, *4*(2), 527-531.

Salleh, W.M.N.H.W., Kamil, F., Ahmad, F. & Sirat, H.M. (2015a). Antioxidant and anti-inflammatory activities of essential oil and extracts *of Piper miniatum*. *Natural Product Communications*, *10*(11), 2005-2008.

Salleh, W.M.N.H.W., Ahmad, F. & Yen, K.H. (2015b). Chemical constituents from *Piper caninum* and antibacterial activity. *Journal of Applied Pharmaceutical Science*, *5*, 20-25.

Zaifuddin, F.A.M., Hassan, N.M. & Othman, R. (2014). Quantification of pro-vitamin A activities and content in 22 selected 'Ulam' species or Malaysian traditional vegetables. International *Journal of Pharmacology and Pharmaceuticals*, *6*, 9-12.