World Applied Sciences Journal 17 (Towards the Traceability of Halal and Thoyyiban Application): 67-70, 2012
ISSN 1818-4952

GC-MS Analysis of Various Extracts from Leaf of *Plantago major* Used as Traditional Medicine

[1]J. Jamilah, [1]A.A. Sharifa and [2] N.R.S.A. Sharifah

[1]Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia,
Kampus Pandan Indah, Tingkat 13, Menara B, Persiaran MPAJ,
Jalan Pandan Utama, 55100 Kuala Lumpur, Malaysia
[2]Faculty of Science and Technology, Universiti Sains Islam Malaysia,
Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia

Abstract: *Plantago major* L. leaves have been used as a wound healing remedy for centuries in the treatment of a number of diseases. The objective of this study is to analyse the chemical composition in the leaf extract of *P. major*. The chemical composition of various extract (petroleum ether, methanol, ethyl acetate, n-butanol and aqueous) from leaf of *Plantago major* have been examined by Triple Quadrupole GC-MS. Results have showed the main constituents in petroleum ether extract were phytol 13.22%, benzofuranone 10.48%, penthyne-diol 10.26% and benzene propanoic acid 10.18%; methanol extract were group of diglycerol 30.31% and glycol 18.91%; ethyl acetate extract were glycerine 30.70%, benzene 21.81% and dibuthyl phthalate 16.22%; n-butanol were phtalic acid 24.62%, benzene propanoic acid 16.83% and group of phenol 10.20%; and aqueous extract were phenol 27.47%, diathiapentene 14.53%, napthalenone 14.13% and glycerine 12.02%. Chemical composition identified in all five extracts has showed that all of them have phenol's group in their extract while having different variation of organic acid groups, flavonoids and terpenoids. These data would be constructive for future ethno-pharmacological studies in *P.major*.

Key words: Plantago major · Soxhlet extraction · GC-MS · Chemical composition · Halal traditional medicine

INTRODUCTION

Halal traditional medicine is one of the alternative ways for Muslim consumers beside conventional medicine. This is includes *Plantago major* L. (*Plantago major* ssp. Major L.) a perennial plant that belongs to the Plantaginaceae family. Many people called it as weed, an old medicinal plant that has been used for centuries [1] for wound healing remedy and in the treatment of a number of diseases which include diseases related to the skin, respiratory organs, digestive organs, reproduction, the circulation, against cancer, pain relief and against infections.

P. major contains biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, terpenoids, alkaloids and some organic acids that involved in the wound healing activity, anti-inflammatory, analgesic, antioxidant, weak antibiotic, immune modulating and antiulcerogenic, antileukemic and antihypertensive activity effects [2-7].

The remarkable medicinal properties of *P. major* are due to the high content of phenols, flavonoids and tannin especially in its leaves. Quite recently, ethanolic extract of *P. major* leaves possessed the greatest effect on tumor cell growth (Dead 74%) followed by hot water extract of *P. major* leaves (Dead 54.6%) which gave astonishing finding to the its beneficial list [8].

As of to date, to our best knowledge, study on chemical composition of *P. Major* leaves by Gas Chromatography-Mass Spectrometry (GC-MS) is very limited. Thus, objective of this study is to determine the chemical composition in various extracts from *P. major* leaves by using GC-MS analysis.

Corresponding Author: J. Jamilah, Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia (USIM), Kampus Pandan Indah, Tingkat 13, Menara B, Persiaran MPAJ, Jalan Pandan Utama, 55100 Kuala Lumpur, Malaysia. Tel: +603-4289 2473, H/P: +6012-2010241, E-mail: jamilah@usim.edu.my.

MATERIALS AND METHODS

Sample Preparation: The whole plant of *Plantago major* was collected from Cameron Highlands and identified at Forestry Research Institute of Malaysia (FRIM). Leaf part was separated and dried at room temperature for one week. The dry leaves were then grinded in WARING blender and stored in tight container at room temperature.

Extraction of *Plantago major* Leaves: 250 g of dry leaves were submitted to soxhlet extraction with petroleum ether as solvent for 16 hours followed by methanol with the same duration time. Methanol extract were then proceed for separation with ethyl acetate, n-butanol and aqueous phase using liquid-liquid extraction method in separatory funnel. Each extract were then evaporate by rotary evaporator and dried in room temperature for one week. 40mg of each extract were then weighted and diluted in 10 ml 50 % DMSO. Prior to GC-MS analysis, each extract solution were filtered through sterile 0.22µm WHATMAN filter and kept in amber vial at 4°C. All chemicals used were analytical reagent (AR) grade.

Chemical Compound Analysis: Chemical analysis was analyzed by Triple Quadruple Gas Chromatography - Mass Spectrometry (Agilent 7000A). Analysis was performed in MS1 Scan Mode and DB-5MS fused-silica column (30m X 250µm i.d. film thickness 0.25µm, Agilent) was used. Oven temperature was set at 50°C for 2 min and then programmed at 50°C to 230°C at a rate of 4°C/min and hold at 230°C for 2 min resulting in the complete elution of all peaks analyzed. Injector and detector temperatures were 350°C. Carrier gas was helium. Mass spectra were taken at 70eV. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial NIST libraries using Mass Hunter Software (Agilent).

RESULTS AND DISCUSSION

Chromatographic analysis of five different extracts (petroleum ether, methanol, ethyl acetate, n-butanol and aqueous) obtained by soxhlet extraction enabled the identification of 51 compounds which are listed in Table 1 in order based on their retention times and peak area percentage.

The main constituents in petroleum ether extract were phytol 13.22%, benzofuranone 10.48%, penthyne-diol 10.26% and benzene propanoic acid 10.18%; methanol extract were group of diglycerol 30.31% and glycol 18.91%; ethyl acetate extract were glycerine 30.70%,

Table 1: Chemical composition of various extracts of *Plantago major* leaves

RT	Compound	Petroleum Ether	Methanol	Ethyl Acetate	n-Butanol	Aqueous
10.527	Glycerin			30.70		12.02
10.994	Pentanoic Acid	1.44				
11.327	Group of Diglycerol		30.31			
11.583	Glycol		18.91			
11.756	Group of Diglycerol		5.26			
13.445	Group of Phenol	0.81			2.31	
14.035	Adenosine					8.87
14.340	Glucosamine	0.78				
14.738	Trans-Dueos	2.45				
15.523	Cinnamic Acid	0.68				
15.864	Octanoic Acid	4.88				
16.012	Methane	3.35			3.15	
16.305	Diathiapentene		8.43			14.53
16.369	Butane			5.02		
16.473	Catchin				1.67	
16.598	Group of Dodecane	1.40				
16.682	Pyrocatecho		2.19			
16.745	Catechin					9.22
16.920	Thiophene	0.84				
17.044	Group of Dodecane	0.65				
17.299	Benzofuran		3.30		4.47	
17.789	Pyrrole	0.98			1.74	
19.437	Silicic Acid	1.25				

Table 1: Continue

RT	Compound	Petroleum Ether	Methanol	Ethyl Acetate	n-Butanol	Aqueous
19.602	Isosorbide			4.05	5.03	
19.618	Dianthydro mannitol	1.80				
20.031	Tridecane	2.31				
20.354	Ethanone				2.63	7.40
20.365	Group of Phenol		3.99		1.33	
21.592	Napthalenone		3.01			14.13
22.421	DL-proline		5.21	7.58		
23.051	Acrylic Acid	1.30				
23.059	Vanilin				1.90	
23.315	Tetradecane	3.33				
24.374	Benzene			21.81		27.47
26.568	Group of Phenol	2.11	7.11	3.86	10.20	6.37
27.161	Benzofuranone	10.48			1.52	
28.310	Fumaric Acid	2.03				
29.961	Megastigmatrienone			5.04		
30.432	Cyclohexanoine	4.24				
31.690	Hydroxy-B ionone	3.88				
33.844	Penthyne-diol	10.26	7.59		6.00	
35.898	Pentadecanone	5.65			4.12	
37.832	Elosenoic Acid	3.20			1.73	
38.025	Benzene propanoic Acid	10.18	3.00	5.72	16.83	
38.657	Dibutyl phtalate			16.22		
38.815	Hexadeanoic Acid	1.72	1.67		2.34	
39.771	Propiolic Acid	1.07				
42.107	Linolenin				2.54	
42.314	Phytol	13.32			5.86	
46.350	Pthalic Acid				24.62	
46.546	Heptacosane	3.63				

benzene 21.81% and dibuthyl phthalate 16.22%; n-butanol were phtalic acid 24.62%, benzene propanoic acid 16.83% and group of phenol 10.20%; and aqueous extract were phenol 27.47%, diathiapentene 14.53%, napthalenone 14.13% and glycerine 12.02%.

All those five extracts had given different chemical composition due to different polarity of the extraction solvent. Compared with previous study, the following organic acids; fumaric acid, syringic acid, vanillic acid, p-hydroxy benzoic acid, ferulic acid, p-coumaric acid, gentisic acid, traces of salicylic acid, benzoic acid and cinnamic acid were isolated in methanol extract [9]. Most of these of component are organic acid, flavonoid, terpenoid and all these five extracts have the same phenol's group.

Zubair *et al*. [10] have also discovered major phenols in leaves, flower stalks and seeds of *P. major* plants by using High Performance Liquid Chromatography, HPLC. This has showed that result from GC-MS analysis was found appropriate to be use to in analysing secondary metabolite composition from *P. major* leaves.

CONCLUSION

As conclusion, chemical composition identified in all five extracts has showed that all of them have phenol's group in their extract while having different variation of organic acid groups, flavonoids and terpenoids. These data would be constructive for future ethno-pharmacological studies in *P. major*.

ACKNOWLEDGEMENT

This research was fully supported by Research Grant (PPP/PSK-06-12409) from Universiti Sains Islam Malaysia (USIM), Bandar Baru Nilai, Negeri Sembilan, Malaysia.

REFERENCES

1. Roca-Garcia, H., 1972. Weeds: a link with the past. Arnoldia, 30: 23-24.
2. Beara, I.N., M.M. Lesjak, E.D. Jovin, K.J. Balog, G.T. Anackov, D.Z. Orcic and N.M. Mimica-Dukic, 2009. Plantain (Plantago L.) species as novel sources of flavonoid antioxidants. J. Agric. Food Chem., 57(19): 9268-9273.
3. Beara, I.N., D.Z. Orcic, M.M. Lesjak, N.M. Mimica-Dukic, B.A. Pekovic and M.R. Popovic, 2010. Liquid chromatography/tandem mass spectrometry study of anti-inflammatory activity of plantain (Plantago L.) species. J. Pharm. Biomed. Anal., 52(5): 701-706.

4. Chiang, L.C., W. Chiang, M.Y. Chang and C.C. Lin, 2003. In vitro cytotoxic: antiviral and immunomodulatory effects of *Plantago major* and *Plantago asiatica*. Am. J. Chinese Med., 31(2): 225-234.
5. McCutcheon, A.R., T.E. Roberts and E. Gibbons, 1995. Antiviral screening of British Colombian medicinal plants. J. Ethnopharmacol., 49(2): 101-110.
6. Nyunt, T.M., K.K. Lwin, T.T. Aye, M.A. Than, K. Chit, T. Kyaw, O.M.T. Hlaing, M. Wun and N.N. Win, 2007. Antihypertensive effect of *Plantago major* Linn. whole plant (Ahkyawpaung-tahtaung) on mild to moderate hypertensive patients. Myanmar Health Sci. Res. J., 19: 97-102.
7. Samuelsen, A.B., 2000. The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. Journal of Ethnopharmacol., 71(1-2): 1-21.
8. Mohamed, I.K., M.A. Osama, M.A.E. Samiha and E.M.M. Zahrat, 2011. Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. International Journal of Biodiversity and Conservation, 3(3): 83-91.
9. Pailer, V.M. and E. Haschke-Hofmeister, 1969. Inhaltstoffe aus *Plantago major*. Planta Medica. 17(2): 139-145.
10. Zubair, M., H. Nyboma, C. Lindholmb and K. Rumpunena, 2011. Major polyphenols in aerial organs of greater plantain (*Plantago major* L.) and effects of drying temperature on polyphenol contents in the leaves. Scientia Horticulturae, 128 (4): 523-529.