QUALITATIVE PHYTOCHEMICAL SCREENING AND GC-MS PROFILING OF *Azadirachta excelsa* LEAF EXTRACT

NURUL 'IZZATI SHAFIE[1*], NURDIANA SAMSULRIZAL[2], NURFARHANA AHMAD SOPIAN[1], MOHAMED ALI RAJION[1], GOH YONG MENG[1], MOHD MOKRISH MD. AJAT[1] and HAFANDI AHMAD[1]

[1]*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPMSerdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Biology, Faculty of Applied Sciences, UniversitiTeknologi MARA (UiTM), Shah Alam, 40450 SelangorDarul Ehsan, Malaysia*
E-mail: nurul_izzati90@ymail.com

ABSTRACT

Azadirachta excelsa is traditionally used by the locals to treat diabetes mellitus but the active compounds of the leaves have not been identified yet. Hence, the aim of the study was to identify the components of *Azadirachta excelsa* leaf extract by qualitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS). Five grams of leaf extract were extracted using various solvents for phytochemical screening, while another five grams of ethanolic leaf extract was subjected for GC-MS analysis where the mass spectra of the compounds detected were matched with the National Institute of Standards and Technology (NIST) library. The results showed the presence of flavonoids, condensed tannin, triterpenes and steroids in phytochemical screening tests, while GC-MS analysis revealed the presence of seven major compounds in the ethanolic leaf extract. The major component of *A.excelsa* ethanolic leaf extract was 9, 12, 15-octadecatrienoic acid (42.34%), followed by pentadecanoic acid, 14-methyl-, methyl ester (28.99%), phytol (10.63%), 9, 12, 15-octadecatrien-1-ol (5.37%), octadecanoic acid, methyl ester (4.36%), 9, 12-octadecadienoic acid, methyl ester (4.24%) and hexadecanoic acid, ethyl ester (4.06%). Therefore, the findings of this study form a basis for the characterization of the compounds and allow researchers to investigate the potential of this plant in treating diabetes mellitus.

Key words: *Azadirachta excelsa*, phytochemical screening, GC-MS analysis

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia, resulting from the defect of insulin secretion, action of insulin or both (Patel *et al*., 2012). According to the World Health Organization, about 346 million people were estimated to be affected by this disease. To date there is no satisfactory effective therapy or drugs available to completely cure diabetes mellitus. The synthetic drugs used often causing unwanted side effects such as hypoglycemia, drug resistance and weight gain (Tahrani *et al*., 2010). This has led the researchers to find other alternative ways for treating diabetes mellitus by investigating various potential medicinal plants with less or no side effects. Based on the ethnobotanical information, about 800 plants possessing antidiabetic potential have been found (Warjeet Singh, 2011; Venkatesh *et al*., 2010; Patel *et al*., 2011).

Most of the plants contain secondary metabolites accounted for their antidiabetic activity such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids and so forth (Malviya *et al*., 2010). *Azadirachta excelsa* has been traditionally used by the locals to treat diabetes mellitus where the young shoots and leaves are eaten raw as vegetables. However, this plant has not received any scientific scrutiny and validation regarding its antidiabetic activity. Keeping this in view, the present study has been conducted to investigate the phytochemical constituents present in *Azadirachta excelsa* leaves and its ethanolic extract by qualitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Chemicals and raw materials
All the chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St Louis,

---

* To whom correspondence should be addressed.

Missouri). *Azadirachta excelsa* leaves were collected from Forest Research Institute Malaysia (FRIM) in Kepong, Selangor, Malaysia.

Preparation of leaf powder and extract

Fresh leaves were collected and shade dried for one to two weeks. After drying, the leaves were ground into fine powders using a mechanical grinder. About five grams of the powdered leaves were used for the qualitative phytochemical screening tests by soaking in chloroform and methanol solvents, while for GC-MS analysis the powdered leaf extract was soaked in 70% ethanol for two days at room temperature. Leaf extract was then filtered and concentrated by using a rotary evaporator at 40°C. The dark semi-solid material was stored at 4°C until further analysis using GC-MS.

Phytochemical screening tests

Test for alkaloids

Dried leaves were macerated in chloroform followed by addition of ammoniacal chloroform. The mixture was then treated with 10% sulphuric acid and tested with Mayer's reagent. Formation of white precipitates indicated the presence of alkaloids.

Test for saponins

Methanolic leaf extract was mixed with distilled water in a test tube. The formation of stable froth for at least 15 minutes indicated the presence of saponins.

Test for flavonoids

Dried leaves were extracted with chloroform and dissolved in ether and shaken in 10% ammonia solution. Formation of yellow colour in ammonia layer indicated the presence of flavonoids.Test for tannins and polyphenolic compounds.

Ethanolic leaf extract was mixed with 1% ferric chloride solution. Formation of blue-black colour indicated the presence of hydrolysable tannins, while brownish-green colour indicated the presence of condensed tannins.

Test for triterpenes/steroids

Chloroform leaf extract was tested using Liebermann-Buchard reagent. Formation of reddishcolour indicated the presence of triterpenes and greenish colour for steroids.

GC-MS analysis

Ethanolic extract of *Azadirachta excelsa* was analyzed by using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a fused silica capillary column (30 x 0.25 mm i.d. x 0.25 μm film thickness) coupled with a Perkin Elmer

Clarus 600C MS. An electron ionization system with ionization energy of 70 eV was used to detect the present compounds in the extract. The carrier gas used was helium at a flow rate of 1 ml/min. Mass transfer line and injector temperatures were set at 220°C and 300°C respectively. Oven temperature was started from 50°C to 150°C at 3°C/min, and held for 10 min and eventually increased to 300°C at 10°C/min. Particle-free diluted extract (1 μl) was injected into injector with a split mode. The ratio of the split mode was 1:120. The percentage composition of theextract compounds was expressed as a percentage by peak area. The identification of the peaks was matched with the National Institute of Standards and Technology (NIST) Library and also by direct comparisons with published data.

RESULTS AND DISCUSSION

Phytochemical constituents of *Azadirachta excelsa* leaf extract

Table 1 shows the presence of phytochemical constituents from *Azadirachta excelsa* leaves. Flavonoids were found to be strongly presence in the leaf extract, while tannins, triterpenes and steroids were found to be moderately presence. However, there were no alkaloids nor saponins found to be presence in the leaf extract. Flavonoids are important secondary metabolites under polyphenols groupthat are widely distributed in plants. There are plenty of reports that support their used as antioxidants or free radical scavengers (Kar, 2007). A very close example is *Azadirachta indica*, one of the tree species sharing the same genus with *Azadirachta excelsa*. The aqueous extract of *Azadirachta indica* leaves contained flavonoid whichwas quercetin that helped in reducing glycemia in streptozotocindiabetes animal model (Chakraborthy *et al.*, 1989).

Tannins are phenolic compounds that might also contribute to the antidiabetic effect of the plant such as in *Ficus deltoidea* where insulin mimetic and

Table 1. Phytochemical constituent's of *Azadirachta excelsa* leaf extract

Phytochemical constituent	Absence	Presence
Alkaloids	–	
Saponins	–	
Flavonoids		3+
Tannins		2+
Triterpenes and steroids		2+

Key: - (Absent); + (Present, low); 2+ (Present, moderate); 3+ (Present, strong)

sensitizing activities were observed (Zunoliza *et al*., 2009). The presence of triterpenes and steroids in this plant was also consistent with other researchers' findings. For instance, azadirachtin and marragin, a triterpenoid of the class of limonoids were found in the seed kernels and leaves (Mordue and Blackwell, 1993). Triterpenes might as well contribute to the antidiabetic effect of medicinal plant. For example, nimbin and nimbidin the triterpenes from *Azadirachta indica* seed oil managed to delay the rise of glucose level in an oral glucose tolerance test in rabbits (Pillai and Santhakumari, 1981).

GC-MS analysis

Figure 1 as shown below presented GC-MS chromatogram of *Azadirachta excelsa* ethanolic leaf extract while Table 2 revealed the presence of seven compounds in the GC-MS analysis. The name, retention time, total composition, class and the bioactivity of the compounds were listed in Table 2. Interestingly, the major components found in the ethanolic extract were polyunsaturated fatty acids (51.95%) while another 37.41% were saturated fatty acids and about 10.63% was made up of phytol, an acyclic diterpene alcohol which is a part of chlorophyll and also a precursor of vitamin E and K1 (Sermakkani and Thangapandian, 2012).

Diabetes mellitus is reported to elevate the synthesis of saturated fatty acids in the tissues and reduce the polyunsaturated fatty acid levels as polyunsaturated fatty acids were more prone to the reactive oxygen species (ROS) attack (Saravanan and Ponmurugan, 2012). It has been proven that a dietary consisting of polyunsaturated fatty acids has significantly lowered the prevalence of steatosis in patients with type II diabetes mellitus (Petit *et al*., 2012). As this ethanolic plant extract also contained higher polyunsaturated fatty acid levels, it could be a promising treatment for hyperlipidemia and tissue steatosis in type II diabetes mellitus.

Meanwhile, phytol was also found to be presence in a small amount from ethanolic extract. Phytol administration to the diabetic insulin-resistant rats has been reported to successfully repress insulin and fructosamineto normal levels, decreased glucose, HOMA-index and TNF-α levels and normalized serum lipid profile (Elmazar *et al*., 2013). Thus, this present study suggests that ethanolic extract of *Azadirachta excelsa* possesses a great potential to treat diabetes mellitus based on the identified compounds that might work as a synergy and hence, enable this extract to exert an antidiabetic effect.

CONCLUSIONS

The present study showed the phytochemical constituents present in *Azadirachta excelsa* leaf extract. To our knowledge, this study is the first to report the chemical composition of ethanolic extract of *Azadirachta excelsa* by GC-MS analysis. Further study − needs to perform in order to identify individual relevant compounds from ethanolic extract for better understanding of their mechanism of action as a plant with antidiabetic and antioxidant effects. In addition, toxicology and *in vivo* studies should also be performed before deducing that this plant is actually safe for consumption and diabetes mellitus treatment.
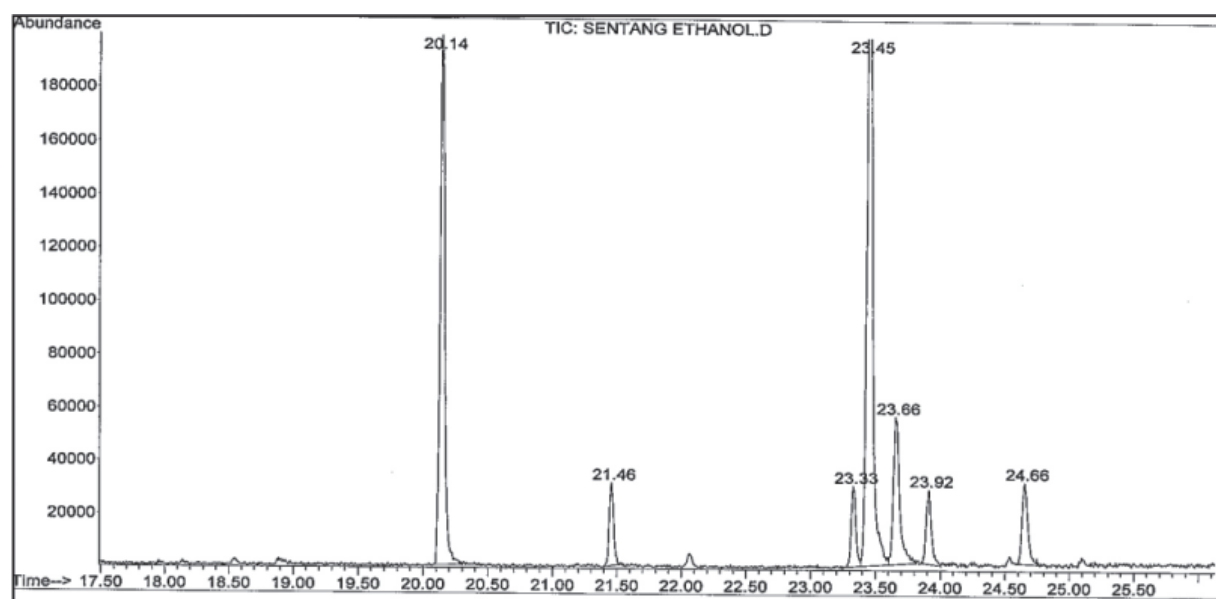

Fig. 1. GC-MS chromatogram of the ethanolic leaf extract of *Azadirachta excelsa.*

Table 2. Compounds identified in the ethanolic extract of *Azadirachta excelsa* by GC-MS analysis

Compound name	RT	Total composition (%)	Class	Bioactivity
Pentadecanoic acid, 14-methyl-, methyl ester	20.14	28.99	Isopalmitic acid; Saturated fatty acid	Antifungal, antimicrobial (Bashir *et al.*, 2012)
Hexadecanoic acid, ethyl ester	21.46	4.06	Palmitic acid; saturated fatty acid	Antioxidant, flavor, hypo-cholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-Alpha reductase inhibitor (Gopalakrishnan & Vadivel, 2011; Rajeswari *et al.*, 2012; Sermakkani & Thangapandian, 2012)
9, 12-Octadecadienoic acid, methyl ester	23.33	4.24	Linoleic acid; polyunsaturated fatty acid	–
9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	23.45	42.34	Linoleic acid; polyunsaturated fatty acid	Antiinflammatory (Jones, 2002; Lalitharani *et al.*, 2009; Sermakkani & Thangapandian, 2012) hypocholesterolemic, cancer preventive (Praveen *et al.*, 2010; Sermakkani & Thangapandian, 2012); hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anticoronary, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic Sermakkani & Thangapandian, 2012); antimicrobial, antioxidant (Praveen *et al.*, 2010)
Phytol	23.66	10.63	Acyclic diterpene alcohol	Antiarthritic (Ogunlesi *et al.*, 2009); antimicrobial (Arunkumar & Muthuselvam, 2009; Ogunlesi *et al.*, 2009); antidiabetic, insulin sensitizer (Elmazar *et al.*, 2013); antibacterial (Inoue *et al.*, 2005); anticancer, diuretic, anti-inflammatory (Rajeswari *et al.*, 2012; Thanga Krishna Kumari *et al.*, 2012); antifungal, antimalaria (Hema *et al.*, 2011; Okeje *et al.*, 2009; Syeda *et al.*, 2011)
Octadecanoic acid, methyl ester	23.91	4.36	Stearic acid; saturated fatty acid	Antifungal, antimicrobial, antibacterial (Gehan *et al.*, 2009)
9, 12, 15-Octadecatrien-1-ol, (Z, Z, Z)-	24.66	5.37	Omega-3 fatty acid; polyunsaturated fatty acid	–

REFERENCES

Arunkumar, S. and Muthuselvam, M. 2009. Analysis of phytochemical constituents and anti-microbial activities *of Aloe vera* L. against clinical pathogens. *World Journal of Agricultural Sciences.* **5(5):** 572-576.

Bashir, A., Ibrar, K., Shumaila, B. and Sadiq, A. 2012. Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal, and antibacterial activities of the essential oils of *Acacia modesta*. *Journal of Medicinal Plants Research.* **6(31):** 4653-4659.

Chakraborthy, T., Uerotta, L. and Poddar, G. 1989. Evaluation of *Azadirachta indica* leaf extract for hypoglycemic activity in rats. *Phytotherapy Research.* **3:** 30-32.

Elmazar, M.M., El-Abhar, H.S., Schaalan, M.F. and Farag, N.A. 2013. Phytol/phytanicacid and Insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. *PLoS One.* **8(1):** 1-10.

Gehan, M.A., Hanan, A.E., Hassan, A.H.I. and Okbah, M.A. 2009. Marine natural products and their potential applications as antiinfective agents. *World Science Journal.* **7(7):** 872-880.

Gopalakrishnan, S. and Vadivel, E. 2011. GC-MS analysis of some bioactive constituents of *Mussaendafrondosa* Linn. *International Journal of Pharma & BioSciences.* **2(1):** 313-320.

Hema, R., Kumaravel, S. and Alagusundaram. 2011. GC/MS determination of bioactive components of *Murrayakoenigii*. *Journal of American Sciences.* **7(1):** 80-83.

Inoue, Y., Hada, T.A., Shiraishi, K., Hirore, Hamashima, H. and Kobayashi, S. 2005. Biphasic effects of geranylgeraniol, terpenone and phytol on the growth of *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy.* **49(5):** 1770- 1774.

Jones, P.J. 2002. Clinical nutrition: 7 functional foods – more than just nutrition. *Canadian Medical Association Journal.* **166(12):** 1555-1563.

Kar, A. 2007. In Pharmacognosy and Pharmaco-biotechnology New Age International Limited Publishers, New Delhi. (Revised-Expanded Second Edition). 332-600 pp.

Lalitharani, S., Mohan, V.R., Regini, G.S. and Kalidass, C. 2009. GC-MS analysis of ethanolic extract of *Pothosscandens* L. leaf. *Journal of Herbal Medicine Toxicology.* 159-160.

Malviya, N., Jain, S. and Malviya, S. 2010. Anti-diabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica.* **67(2):** 113-118.

Mordue (Luntz), A.J. and Blackwell, A. 1993. Azadirachtin: an update. *Journal of Insect Physiology.* **39(11):** 903-924.

Ogunlesi, M., Okiei, W., Ofor, E. and Osibote, A.E. 2009. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma. *African Journal of Biotechnology.* **8:** 7042-7050.

Okeje, W., Ogunlesi, M., Ofor, E. and Osibote, E.A.S. 2009. Analysis of essential oil constituents in hydro-distillates of *Calotropisprocera* (Ait.). *R.Br. Research Journal of Phytochemistry.* **3:** 44-53.

Patel, D.K., Kumar, R., Prasad, S.K., Sairam, K. and Hemalatha, S. 2011. Antidiabetic and *in vivo* antioxidant potential of *Hybanthusennea-spermus* (Linn) F. Muell in streptozotoicn-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine.* **1:** 316-322.

Patel, D.K., Kumar, R., Laloo, D. and Hemalatha, S. 2012. Diabetes mellitus: an overview on its pharmacological aspects andreported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine.* 411-420.

Petit, J.M., Guiu, B., Duvillard, L., Jooste, V., Brindisi, M.C., Athias, A., Bouillet, B., Habchi, M., Cottet, V., Gambert, P., Hilton, P., Cercueil, J.P. and Verges, B. 2012. Increased erythrocytes n-3 and n-6 polyunsaturated fatty acids is significantly associated with a lower prevalence of steatosis in patients with type 2 diabetes. *Clinical Nutrition.* **31:** 520-525.

Pillai, N.R. and Santhakumari, G. 1981. Hypoglycemic activity of *Melia azadirachta* Linn. (Neem). *Indian Journal of Medicinal Research.* **74:** 931-933.

Praveen, P.K., Kumaravel, S. and Lalitha, C. 2010. Screening of antioxidant activity, total phenolicsand GC-MS study of *Vitexnegundo*. *African Journal of Biochemistry Research.* **4(7):** 191-195.

Rajeswari, G., Murugan, M. and Mohan, V.R. 2012. GC-MS analysis of bioactive components of *Hugoniamystax* L. (Linaceae). *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* **3(4):** 301-308.

Saravanan, G. and Ponmurugan, P. 2012. Ameliorative potential of S-allyicysteine: effect on lipid profile and changes in tissue fatty acid composition in experimental diabetes. *Experimental Toxicology and Pathology*. **64:** 639-644.

Sermakkani, M. and Thangapandian, V. 2012. GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*. **5(2):** 90-94.

Syeda, F.A., Habib-Ur-Rehman, Choudahry, M.I. and Atta-Ur-Rahman. 2011. Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bioassays of crude extract of *Iris germanica*. *International Journal of Genetics and Molecular Biology.* **3(7):** 95-100.

Tahrani, A.A., Piya, M.K., Kennedy, A. and Barnett, A.H. 2010. Glycaemic control in type 2 diabetes: targets and new therapies. *Pharmacology & Therpeutics*. **125:** 328-361.

Thanga Krishna Kumari, S., Muthukumasary, S. and Mohan, V.R. 2012. GC-MS analysis of ethanolic extract of *Sarcostemmasecamone* (L) Bennet (Asclepiadaceae). *Scientific Research Report*. **2(3):** 187-191.

Venkatesh, S., Mahdava Reddy, B., Dayanand Reddy, G., Mullangi, R. and Laskhman, M. 2010. Antihyperglycemic and hypolipidemic effects of *Helicteresisora* roots in alloxan-induced diabetic rats: a possible mechanism of action. *Journal of Nature Medicine*. **64:** 295-304.

Warjeet Singh, L. 2011. Traditional medicinal plants of Manipur as anti-diabetics. *Journal of Medicinal Plants Research*. **5:** 677-687.

World Health Organization. Keyfacts. In Diabetes. Retrieved March 20, from http://www.who.int/mediacen tre/factsheets/fs312/en/

Zunoliza, A., Khalid, S., Zhari, I., Rasadah, M.A., Mazura, P., Fadzureena, J. and Rohana, S. 2009. Evaluation of extracts of leaf of three *Ficusdeltoidea* varieties for antioxidant activities and secondary metabolites. *Pharmacognosy Research.* **1(4):** 216-223.