Australian Journal of Basic and Applied Sciences, 11(3) Special 2017, Pages: 91-97

AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

GC-MS analysis of bioactive constituents of Hibiscus flower

Hesham H. A. Rassem, Abdurahman H. Nour, Rosli M. Yunus

Faculty of chemical and Natural Resources Engineering, Universiti Malaysia Pahang (UMP), Pahang, Malaysia.

Address For Correspondence:
Hesham H. A. Rassem, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang (UMP), Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.
E-mail:hesham_rassem@yahoo.com.

ARTICLE INFO

Article history:
Received 18 September 2016
Accepted 21 January 2017
Available online 26 January 2017

Keywords:
Microwave-assisted Hydrodistillation (MAHD); GC-MS; Phytochemical constituents; Ethanol extract; Ethanimidic acid.

ABSTRACT

GC-MS the best technique to identified the compounds of essential oils by comparison of mass spectra data obtained from the sample with that taken from pure commercially available standards injected under the same conditions. **Objective:** To characterize the chemical constituents of Hibiscus Flower using GC-MS**.** The shade dried flower powder was extracted with methanol by using Microwave-assisted Hydrodistillation (MAHD). The Agilent 5975C Series GC/MS and a DB-WAX fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was programmed to 60 ^{0}C for 10 min, and then increased at 20^{0}C/min to 230^{0}C and held at 250^{0}C for 10 min. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The GC-MS analysis provided different peaks determining the presence of fifteen compounds. These compounds have biological activity namely Ethanimidic acid, ethyl ester (31.43%), Propanal, 2,3-dihydroxy (12.58%), 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl (10.69%), Ethylenediamine (6.71%), o-Methylisourea hydrogen sulfate (4.06%), Ethene, ethoxy- (3.63%), Methyl palmitate (2.99%), 7-Formylbicyclo[4.1.0]heptanes (2.80%), 2-Butanamine, (S)- (2.72%), 1,3,5-Triazine-2,4,6-triamine (2.48%), N-Formyl-β-alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), 1,2-Ethanediamine, Butanedial (1.65%), N-methyl-1-Propanol, 2-methyl-(1.57%) and Methanecarbothiolic acid (1.08%)**.** From the results, it can be concluded that Hibiscus flower extract shows the presence of 15 phytocompounds. The presence of various bioactive compounds justifies the use of the whole flower for various ailments by traditional practitioners.

INTRODUCTION

Medicinal plants have occupied an important position in the socio-cultural in Malaysia and another country. Plants considered one of the main sources of biologically active compounds. compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries (Bobbarala *et al*., 2011). Plant-based natural constituents can be derived from any part of the plant like flowers, leaves, bark, roots, fruits, seeds (Gordon, 2001). Screening active compounds from plants has lead to the invention of new medicinal drugs which have different protection and treatment roles against various diseases, including cancer (Sheeja and Kuttan, 2007) and Alzheimer's disease (Mukherjee *et al*., 2007) and also has a biological activity like antibacterial, antioxidants and antifungal. The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardization of herbal and its formulations. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, ethers etc (Muthulakshmi *et al*., 2012).

Hibiscus Rosa-Sinensis a well-known member of the family Malvaceae, it grows as an evergreen herbaceous plant. Hibiscus rosa-Sinensis is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft)

To Cite This Article: Hesham H. A. Rassem, Abdurahman H. Nour, Rosli M. Yunus., GC-MS analysis of bioactive constituents of Hibiscus flower. ***Aust. J. Basic & Appl. Sci.,*** *11(3): 91-97, 2017*

tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. Various parts of this plant, like leaves, flowers, and roots, have been known to possess medicinal properties like an aphrodisiac, menorrhagia, oral contraceptive, laxative. Hibiscus flower was reported to contain fats, acids, flavonoids, carbohydrates, proteins and minerals (Mahadevan & Kamboj, 2009). Several studies describing the antihypertensive, hepatoprotective, anticancer, antidiabetic, cytotoxicity, antibacterial, antinociceptive, anti-inflammatory and antioxidant activities of the flowers among others have been published (Kristen *et al*., 2014).

Microwave-assisted Hydrodistillation (MAHD) is one of the current techniques that are used to extract bioactive components and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are: the reduction of extraction time, less volume of solvents, selectivity, volumetric heating and controllable heating process. Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere (Lucchesi *et al*., 2004; Ferhat *et al*., 2006) and consuming only a fraction of the energy used in conventional extraction methods (Farhat *et al*., 2009). The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions.

With this background, the present study was aimed to identify the phytoconstituents in Hibiscus flower by using GC-MS analysis.

Experimental:

Collection and preparation sample:

The fresh Hibiscus flowers were collected from a location in Gambang Campus, Universiti Malaysia Pahang, Malaysia in February 2016. The flowers were washed thoroughly in running up tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. Drying the flowers by the oven in 70 °C for 1h.

Extraction Method:

The powdered sample of Hibiscus flowers(35g) was extracted with methanol (280 ml, 2 h) at a temperature around 60 °C by using Microwave-assisted Hydrodistillation (MADH). The microwave oven was operated at 300 W power for a period of 120 minutes. The essential oil present in the flask is evaporated. Steam and essential oil vapors are passed through a condenser. The condensate, which has a mixture of water and the essential oil is collected and put into the separating funnel with solvent dichloromethane to be separated.

GC-MS analysis:

The Agilent 5975C Series GC/MS used in the analysis employed a fused silica column packed with DB-WAX (100 % dimethyl polysiloxane, 30 m × 0.25 mm ID × 2.5 μm). Oven temperature was programmed to 60 °C for 10 min, and then increased at 20 °C/min to 230 °C and held at 250 °C for 10 min. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s.

Identification of components:

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractionations of the methanolic extract of Hibiscus flowers. These compounds were identified by mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on National Institute Standard and Technology Database. Table 2 Listed the major photocomponents and its biological activities obtained through GC-MS study of Hibiscus flowers. The results revealed that the presence of Ethanimidic acid, ethyl ester (31.43%), Propanal 2,3-dihydroxy (12.58%), Propanamide N-ethyl- (10.69%), Ethylenediamine (6.71%), O-Methylisourea hydrogen sulfate (4.06%), Ethene ethoxy- (3.63%), Hexadecanoic acid, methyl ester (2.99%), 7-Formylbicyclo(4.1.0) heptanes (2.80%), 2-Butanamine (S)- (2.72%), 1,3,5-Triazine-2,4,6-triamine (2.48%), N-Formyl-β-alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), Butanedial (1.65%), 1-Propanol 2-methyl- (1.57%) and Methanecarbothiolic acid (1.08%). The spectrum profile of GC-MS confirmed the presence of fifteen major components with the retention time 2.29, 3.03, 3.21, 7.85, 9.38, 9.42, 9.52, 9.57, 9.64, 9.77, 13.06, 13.08, 39.67, 44.54 and 44.63 respectively (Figure 1). Figures 2-8 shows mass spectrum and structures of Propanal, 2,3-dihydroxy, Propanamide, N-ethyl, Ethylenediamine, Hexadecanoic acid, methyl ester, 1,3,5-Triazine-2,4,6-triamine, 1-Propanol, 2-methyl and Methanecarbothiolic acid. Thus this type of GC-MS analysis is the step to understanding the nature of active principles in this medicinal flowers and this type of study will be helpful for further studies. The pharmacological of Hibiscus flowers and their variety and detailed phytochemistry may add new knowledge to the information in the traditionalism medical.

Table 1: Components detected in the plant of methanol extract of *Hibiscus flowers MW: Molecular Weight, RT: Retention Time*

No	Compounds	Molecular Formula	MW	RT	Area%
1	Ethanimidic acid, ethyl ester	C_4H_9NO	87.12	3.03	31.43
2	Propanal, 2,3-dihydroxy-	$C_3H_6O_3$	90.08	2.29	12.58
3	Propanamide, N-ethyl-	$C_5H_{11}NO$	101.15	9.77	10.69
4	Ethylenediamine	$C_2H_8N_2$	60.10	3.21	6.71
5	o-Methylisourea hydrogen sulfate	$C_2H_8N_2O_5S$	172.16	9.52	4.06
6	Ethene, ethoxy-	C_4H_8O	72.10	9.38	3.63
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	39.67	2.99
8	7-Formylbicyclo[4.1.0]heptanes	$C_8H_{12}O$	124.18	44.54	2.80
9	2-Butanamine, (S)-	$C_4H_{11}N$	73.13	9.57	2.72
10	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12	7.85	2.48
11	N-Formyl-β-alanine	$C_4H_7NO_3$	117.10	13.06	2.36
12	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224.38	44.63	1.70
13	Butanedial	$C_4H_6O_2$	86.09	9.42	1.65
14	1-Propanol, 2-methyl-	$C_4H_{10}O$	74.12	9.64	1.57
15	Methanecarbothiolic acid	C_2H_4OS	76.12	13.08	1.08

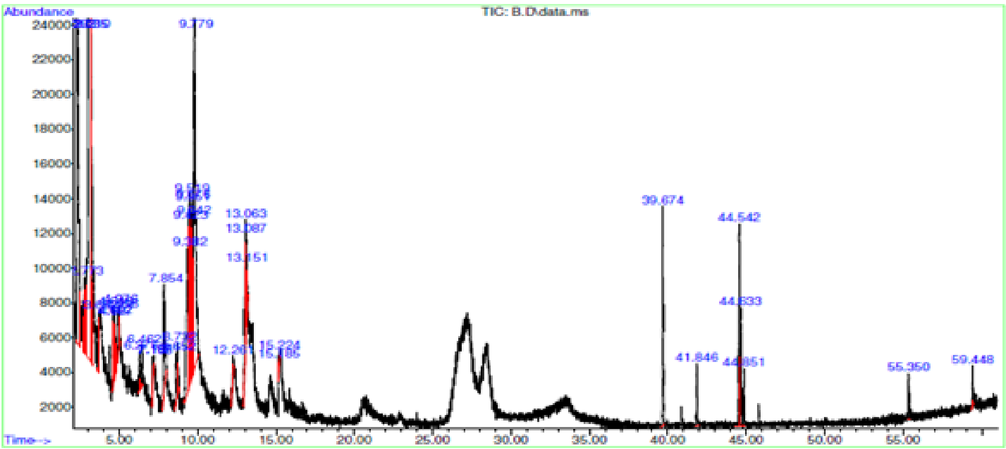

Fig. 1: GC-MS Chromatogram of Methanolic extract of the leaves of Hibiscus flower

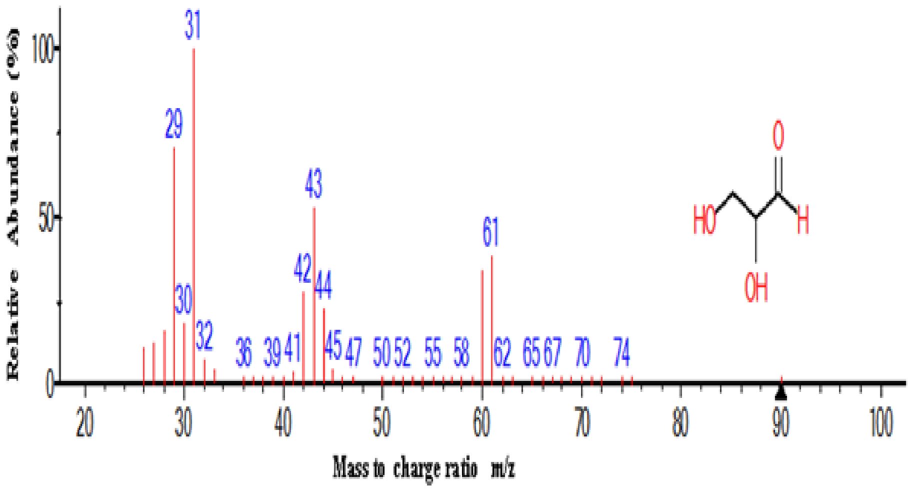

Fig. 2: Mass spectrum of Propanal, 2,3-dihydroxy. (RT: 2.29)

Australian Journal of Basic and Applied Sciences, 11(3) Special 2017, Pages: 91-97

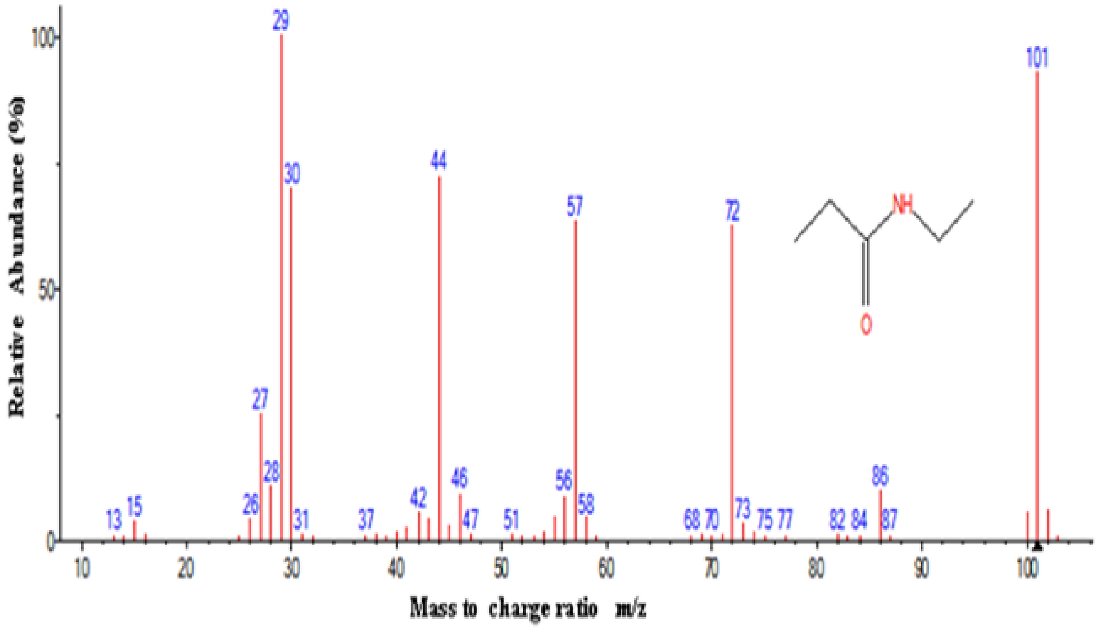

Fig. 3: Mass spectrum of Propanamide, N-ethyl. (RT: 9.77)

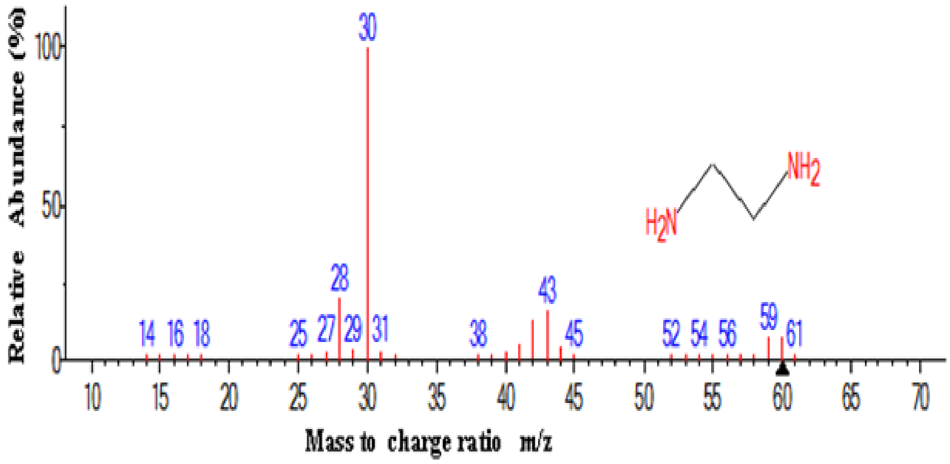

Fig. 4: Mass spectrum of Ethylenediamine. (RT: 3.21)

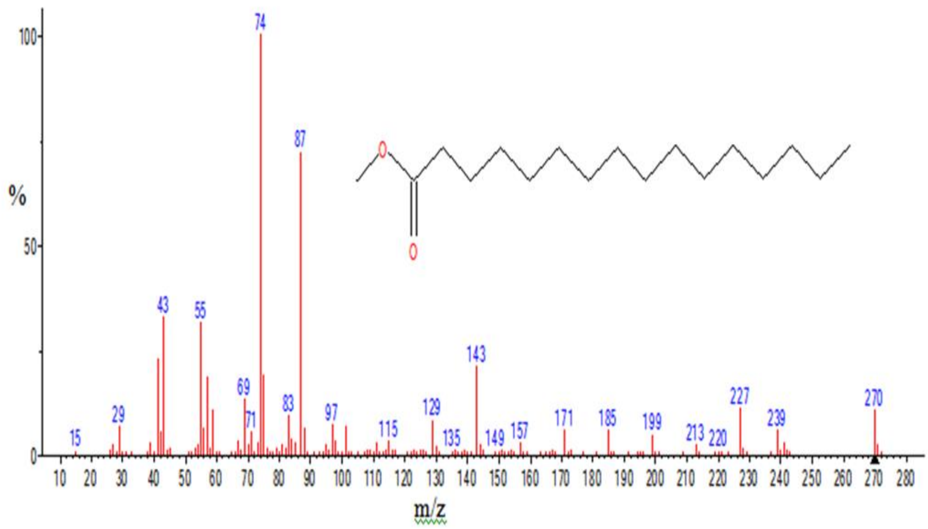

Fig. 5: Mass spectrum of Hexadecanoic acid, methyl ester. (RT: 39.67)

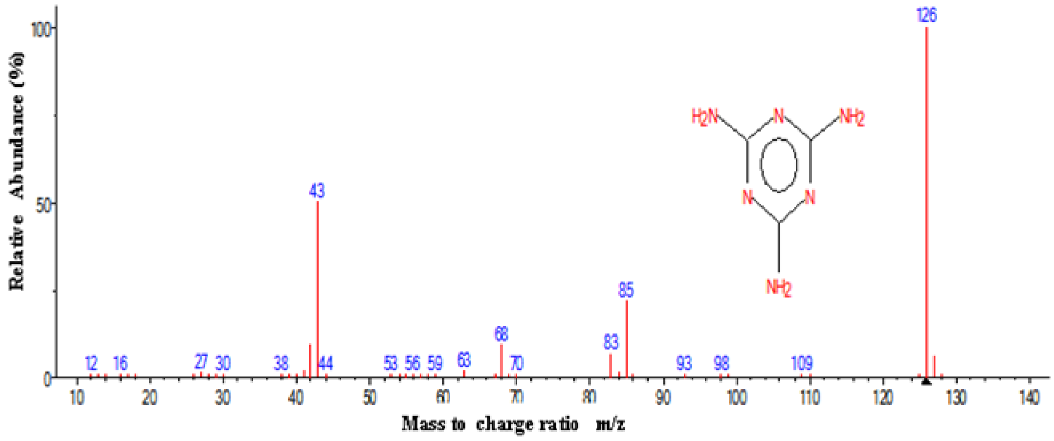

Fig. 6: Mass spectrum of 1,3,5-Triazine-2,4,6-triamine. (RT: 7.85)

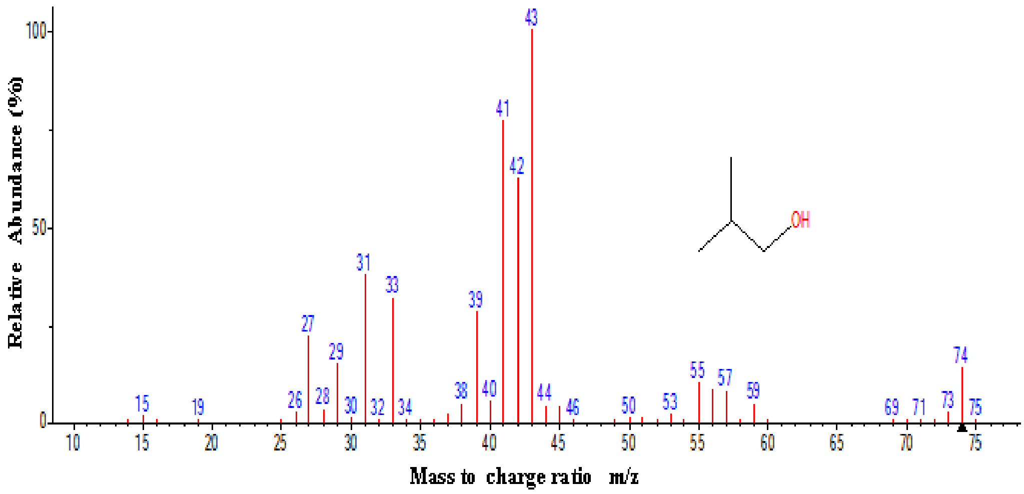

Fig. 7: Mass spectrum of 1-Propanol, 2-methyl. (RT: 9.64)

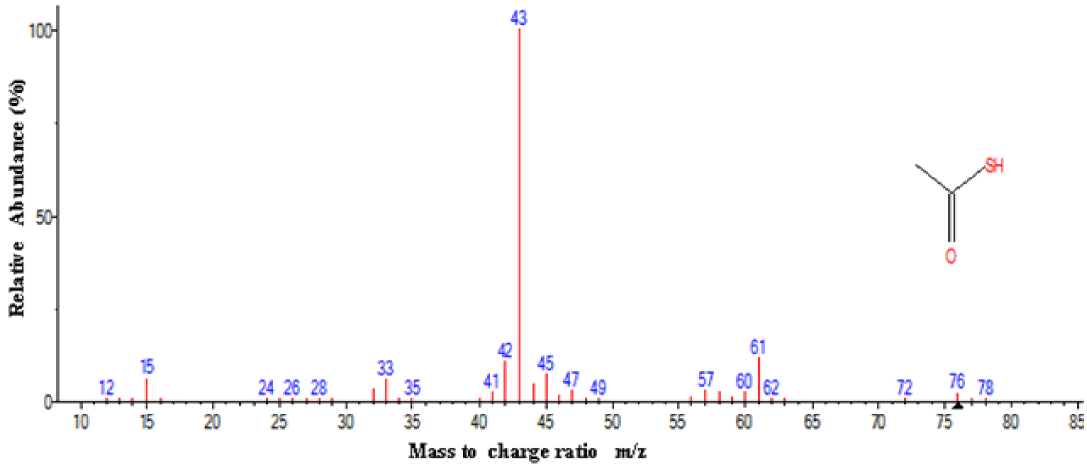

Fig. 8: Mass spectrum of Methanecarbothiolic acid. (RT: 13.08)

Table 2: Activity of Phyto-Components identified in the methanol extracts of Hibiscus flowers.

No	Compounds	Molecular Formula	**Activity
1	Ethanimidic acid, ethyl ester	C_4H_9NO	Antioxidant, Cancer preventive.
2	Propanal, 2,3-dihydroxy-	$C_3H_6O_3$	Antioxidant, Hypochloesterolemic, Antimicrobial.
3	Propanamide, N-ethyl-	$C_5H_{11}NO$	Antioxidant, Pesticide, Cancer preventive.
4	Ethylenediamine	$C_2H_8N_2$	Antioxidant, Antimicrobial.
5	O-Methylisourea hydrogen sulfate	$C_2H_8N_2O_5S$	Antioxidant, Antimicrobial.
6	Ethene, ethoxy-	C_4H_8O	Antioxidant, Cancer preventive.
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Antioxidant.
8	7-Formylbicyclo[4.1.0]heptanes	$C_8H_{12}O$	Antioxidant, Anemiagenic,
9	2-Butanamine, (S)-	$C_4H_{11}N$	Antioxidant, Cancer preventive Antimicrobial.
10	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	Antioxidant, Cancer preventive.
11	N-Formyl-β-alanine	$C_4H_7NO_3$	Cancer preventive, Anemiagenic, Dermatitigenic.
12	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	Antioxidant, Antimicrobial.
13	Butanedial	$C_4H_6O_2$	Antioxidant,
14	1-Propanol, 2-methyl-	$C_4H_{10}O$	Antioxidant, Antimicrobial.
15	Methanecarbothiolic acid	C_2H_4OS	Antimicrobial.

***Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database*

Conclusion:

In this study, the GC-MS analysis of the methanolic extract of Hibiscus flowers showed the presence of fifteen compounds. In terms of percentage amounts Ethanimidic acid, ethyl ester (31.43%), Propanal, 2,3-dihydroxy (12.58%), Propanamide, N-ethyl- (10.69%), Ethylenediamine (6.71%), O-Methylisourea hydrogen sulfate (4.06%), Ethene, ethoxy- (3.63%), Hexadecanoic acid, methyl ester (2.99%), 7-Formylbicyclo(4.1.0) heptanes (2.80%), 2-Butanamine, (S)- (2.72%), 1,3,5-Triazine-2,4,6-triamine (2.48%), N-Formyl-β-alanine (2.36%), (Z)6,(Z)9-Pentadecadien-1-ol (1.70%), Butanedial (1.65%), 1-Propanol, 2-methyl- (1.57%) and Methanecarbothiolic acid (1.08%) were predominant in the extract.

These compounds were shown to have antioxidant, cancer preventive, pesticide, Hypocholesterolemic, Dermatitigenic, and Anemiagenic. Antioxidant and antimicrobial are shown by Propanal, 2,3-dihydroxy, Ethylenediamine, o-Methylisourea hydrogen sulfate, 2-Butanamine, (S), (Z) 6,(Z) 9-Pentadecadien-1-ol and 1-Propanol, 2-methyl-. There is growing awareness in correlating the phytochemical compounds and their biological activities. Hibiscus flowers are used in medicine in Malaysia. We in this report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will lead to further detailed studies.

REFERENCES

Bobbarala, V., P.V. Bramhachari, J. Ravichand, Y.H.K. Reddy, D. Kotresha, K.V. Chaitanya, 2011. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of Aegle marmelois (L.) Correa extracts. J Pharm Res., 4(1): 252-255.

Farhat, A., C. Ginies, M. Romdhane and F. Chemat, 2009. “Eco-friendly and cleaner process for isolation of essential oil using microwave energy: Experimental and theoretical study”, Journal of Chromatography A, 1216(26): 5077-5085.

Ferhat, M., B. Meklati, J. Smadja and F. Chemat, 2006. “An improved microwave Clevenger apparatus for distillation of essential oils from orange peel”, Journal of Chromatography A, 1112(1-2): 121-126.

Gordon, D.M., 2001. Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. Microbiology, 147: 1079-1085.

Sheeja, K., G. Kuttan, 2007. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by Andrographis paniculate extract and andrographolide. Immunopharmacol Immunotoxicol., 29: 81-93.

Mukherjee, P.K., V. Kumar, P.J. Houghton, 2007. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. Phytother Res., 21: 1142-1145.

Muthulakshmi, A., R. Joshibhi Margret, V.R. Mohan, 2012. GC-MS analysis of bioactive components of Feronia elephantum Correa (Rutaceae). App. pharmac.sci., 2: 69-74.

Mahadevan, N.S., P. Kamboj, 2009. Hibiscus sabdariffa Linn. An overview. Natural Product Radiance, 8(1): 77-83.

Kristen, L.H., P.B. Kellie, Z. Svetlana, P.D. Michael, J.R.C.N. Stewart, 2014. Antimicrobial activity of Hibiscus sabdariffa aqueous extracts against Escherichia coli O157: H7 and Staphylococcus aureus in a microbiological medium and milk of various fat concentrations. Journal of Food Protection, 77(2): 262-268.

Lucchesi, M.E., F. Chemat and J. Smadja, 2004. Original solvent free microwave extraction of essential oils from spices. Flavor and Fragrance Journal, 19: 134-138.

http://www1.biologie.uni-hamburg.de/b-online/ibc99/dr-duke/index.htm