Jurnal
Teknologi

Full Paper

EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM HIBISCUS FLOWER VIA MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD)

Article history
Received
X x 2017
Received in revised form
X x 2017
Accepted
X x 2017

Hesham H. A. Rassem[a]*, Abdurahman H. Nour[a], Rosli M. Yunus[a]

[a]Faculty of chemical and Natural Resources Engineering, Universiti Malaysia Pahang (UMP), Malaysia

*Corresponding author
hesham_rassem@yahoo.com

Graphical abstract

Abstract

In this study, the influence of several experimental parameters on the Microwave-assisted Hydrodistillation (MAHD) extraction of Hibiscus flower was investigated: extraction time, solvent polarity, sample amount, solvent volume and sample particle size. It was concluded that the most influential variables were extraction time and solvent polarity. The optimized procedure employed 35 g of ground flowers, 280 mL of methanol and 120 min of extraction. The extracts were fractionated using preparative silica columns (30 m * 0.25 mm i.d., film thickness 0.25 mm). 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 and the resulting fractions were analyzed by Gas Chromatography-mass Spectroscopy (GC-MS). Some saturated hydrocarbons and fatty acid methyl esters were identified in theHIbiscus flowers extracts. Thirty-seven compounds were identified, representing 99.9.% of the oil. The main components were Ethanimidic acid, ethyl ester (31.43%), Propanal, 2,3-dihydroxy (12.58%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl (10.69%).

Keywords: Essential oil, Hibiscus flower, Microwave-assisted Hydrodistillation (MAHD), Gas Chromatography-mass Spectroscopy (GC-MS)

1.0 INTRODUCTION

Essential oils are factory the volatile oils with aromatic components that are made up of chemical compounds. For example esters, alcohols, aldehydes, ketones, hydrocarbons, and phenols, are some of the major components of essential oil [1]. Oils of plant species are available commercially in the market [2]. Numerous studies have demonstrated the efficiency of Essential Oils in low doses in the activity biological [3][4].

Hibiscus Rosa-Sinensis a well-known member of the family Malvaceae, Hibiscus rosa-Sinensis 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) 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.

Microwave-assisted Hydrodistillation (MAHD) is one of the current techniques that are used to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are the reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process. Application of Microwave-assisted Hydrodistillation (MAHD) has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO2 into the atmosphere [5] and consuming only a fraction of the energy used in conventional extraction methods such as steam distillation, SD [6].

[7] reported some recently published studies have successfully utilized a microwave oven for the extraction of active components from plants. The efficiency of Microwave-assisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample [8]. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step [9]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique.

The use of Microwave-assisted hydrodistillation (MAHD) in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions. The principle of heating using Microwave-assisted hydrodistillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously [10].

Hibiscus flower was reported to contain fats, acids, flavonoids, carbohydrates, proteins and minerals [11]. Several studies describing the antihypertensive, hepatoprotective, anticancer, antidiabetic, cytotoxicity, antibacterial, antinociceptive, anti-inflammatory and antioxidant activities of the flowers among others have been published [12].

2.0 METHODOLOGY

2.1 Materials and Sample preparation

Mature and fresh flowers of Hibiscus flower were collected from a location in Gambang Campus, Universiti Malaysia Pahang, Malaysia in February 2016. After the collection, the flowers were washed with water to remove some impurities. Dry the flowers by the oven in 70 °C for 1h. Grind the flowers to powder. In extraction process, the rate of extraction is increased when the area of contact between the solvent and solid is high. So, the higher surface area of flowers, an essential oil can be extracted from the flowers. The ratio of a flower to water is 1:8 for the period of three to seven days in order to break down the parenchymatous and oil glands [13]. For this experiment, one hour was chosen in order to maximize the soaking effect. A number of flowers used are 35 gm powder of Hibiscus flowers and Methanol equal to 280 mL.

2.2 Microwave-assisted Hydrodistillation (MADH)

Microwave oven (Milestone MWS Ethos E Solvent Extraction System: 2.5KW;230 V-60Hz; 2450 MHz) (Figure 1) was modified for MAHD operation. 35-gram flowers samples were placed in a 1L flask containing 280 ml of Methanol. The flask was set up within the microwave oven cavity and a condenser was used on the top (outside the oven) to collect the extracted essential oils. The microwave oven was operated at 300 W power levels 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 separate.

Figure 1 Microwave-assisted Hydrodistillation (MADH)

2.3 Extraction of essential oil

The air-dried and pulverized flowers of Hibiscus flowers (35 g) were subjected to hydrodistillation in an all glass Clevenger apparatus in Microwave-assisted hydrodistillation (MAHD) for 1 h according to the established procedure. The yield of the essential oil 1.417% (v/w), calculated on a dry weight basis.

2.4 Analysis of the oil sample

2.4.1 Analysis with Gas Chromatography-Mass Spectrometry (GC-MS)

Analyses were performed using an Agilent 5975C Series GC/MSD 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 ⁰C for 10 min, and then increased at 20 0C/min to 230 ⁰C and held at 250 ⁰C for 10 min. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s (Figure 2).

Both samples for MAHD extracts were diluted in Dichloromethane to the concentration of 3% by adding the 1 μL of pure essential oil of flowers with 10 μL of Dichloromethane. Then the dilutions samples were injected into the GC with the same split ratio. The compounds were identified by comparison of mass spectra data obtained from the sample with that taken from pure commercially available standards injected under the same conditions (Table1).

Figure 2 Gas Chromatography-Mass Spectrometry (GC-MS)

2.4.2 Analysis with Fourier transform infrared spectroscopy (FTIR)

Analyses were performed using a Thermo Scientific Nicolet iS5 FT-IR Spectrometer. The FT-IR spectrum has been recorded in the region between 4000 and 400 cm−1.To calibration, the FT-IR instrument used the blank. After this take the small amount of essential oil Hibiscus flower in FT-IR. The FTIR data of the compounds in essential oil for Hibiscus flower are given in Table 2 and shown (Figures 3).

Table 2 FT-IR spectral analysis functional theory calculations studies

NO.	Vibration assignment (v) (cm-1)	Absorption band
1	3276.50	O-H
2	1714.83	C-C stretching
3	1633.29	C=O stretching
4	1454.48	C=C stretching
5	1377.41	Ring stretching

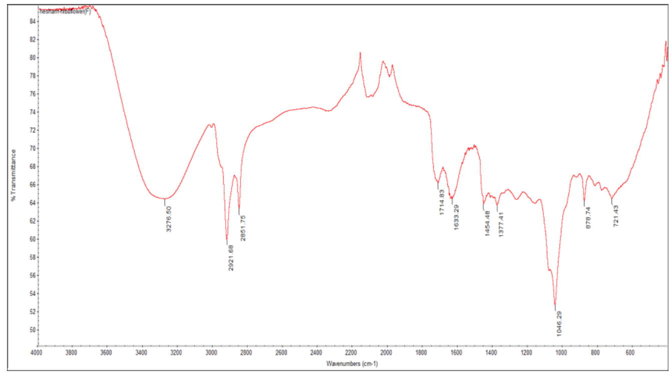

Figure 3 FT-IR spectrum of essential oil for Hibiscus flower

3.0 RESULTS AND DISCUSSION

The essential oil of Hibiscus flower contained 37 compounds representing 99.99 % of the total oil content. The constituents identified in the essential oil for Hibiscus flower as well as the experimental and literature retention can see in Table 1. The compounds were arranged in order of rinse on DB-WAX fused silica column. The major compounds identified in the essential oil for Hibiscus flowers were the ester compounds and oxygenated compounds represented by Ethanimidic acid, ethyl ester (31.43%), Propanal, 2,3-dihydroxy (12.58%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl (10.69%). The minor constituents include 1,3-Dihydroxyacetone dimer (6.71%), o-Methylisourea hydrogen sulfate (4.6%) and Ethoxyethane (3.63%) (Figure 4). And also the results by used FT-IR Spectrometer showed that the O-H functional group in compounds was assigned at 3276.50 cm-1. The azomethine stretching at vibration for the compounds are observed at 1633.29 cm-1 [14]. Moreover, a strong bond at the frequency range of 1454.48 cm-1 can be assigned to the C=C stretching [15].

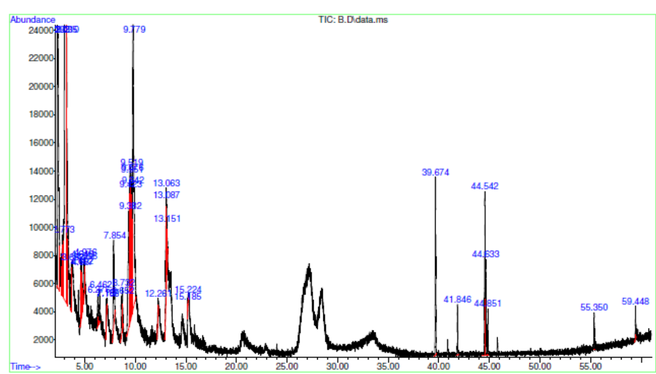

Figure 4 GC/MS chromatogram of the volatile constituents of Hibiscus Flowers

Table 1 Chemical compositions for essential oil of Hibiscus flowers obtained by (MADH)

Pk	Compounds	RT	Area%
1	Propanal, 2,3-dihydroxy-	2.295	12.58
2	1-Isopropyldiaziridine	2.771	0.75
3	Ethanimidic acid, ethyl ester	3.034	31.43
4	Ethylenediamine	3.210	6.71
5	Carbamic acid, ethylnitroso-, ethyl ester	3.681	0.82
6	Acetaldehyde	4.601	0.62
7	2-Propen-1-ol	4.643	0.98
8	3,4-Furandiol, tetrahydro-, trans-3,4-Furandiol,	4.686	0.91
9	Succinamic acid	4.975	0.66
10	3-Piperidinol	4.997	0.28
11	1,5-Pentanediol	6.275	0.48
12	1-Octyne	6.462	0.45
13	Ethanone, 1-(3-methyloxiranyl)-	7.157	0.18
14	Propanedioic acid, oxo-, bis(2-methylpropyl) ester	7.189	0.08
15	1,3,5-Triazine-2,4,6-triamine	7.853	2.48
16	Topotecan	8.650	0.80
17	Oxirane, trimethyl-	8.730	0.78
18	Ethene, ethoxy-	9.382	3.63
19	Butanedial	9.425	1.65
20	o-Methylisourea hydrogen sulfate	9.521	4.06
21	Guanidine, methyl-	9.554	0.73
22	2-Butanamine, (S)-	9.575	2.72
23	1,2-Ethanediamine, N-methyl-1-Propanol, 2-methyl-	9.644	1.57
24	2-Tetrazene, 1,1-diethyl-4,4-dimethyl-	9.778	10.69
25	Isothiazole	12.260	0.17
26	N-Formyl-β-alanine	13.062	2.36
27	Methanecarbothiolic acid	13.089	1.08
28	Citramalate	13.153	0.36
29	2,3-Dioxabicyclo[2.2.1]heptanes	15.186	0.07
30	6-Acetyl- β -d-mannose	15.223	0.08
31	Methyl palmitate	39.672	2.99
32	Decanoic acid, ethyl ester	41.843	0.77
33	7-Formylbicyclo[4.1.0]heptanes	44.544	2.80
34	(Z)6,(Z)9-Pentadecadien-1-ol	44.635	1.70
35	Propanamide, 2-methyl-	44.849	0.58
36	1-(2-Adamantylidene)semicarbazide	55.349	0.68
37	Dodecahydropyrido[1,2-b]isoquinolin-6-one	59.446	0.35

4. Conclusions

The chemical constituents of essential oil obtained from Hibiscus Flowers from Malaysia indicated that both qualitative and quantitative variation exists between the present results. This may be attributable to factors such as environmental conditions and the nature of flowers and also may be due to the geographical variations for places growth the flowers. Thirty-seven compounds were identified, representing 99.9 % of the oil of Hibiscus flower by Microwave-assisted Hydrodistillation (MAHD).

Acknowledgement

The authors would like to thank Malaysia Ministry of Education and Universiti Malaysia Pahang for providing assistance for this study under the research (PGRS grant, 160363). Also, the authors would like to extend special thanks to Hodeidah University, Yemen to help me in the study.

References

[1] Younis A., Riaz A., Khan MA., Khan AA., and M.A. Pervez. 2008. Extraction and identification of chemical constituents of the essential oil of Rosa species. Acta Hort. 766: 485-492.

[2] Form check V., Kubeczka KH., 1982. Essential oil by capillary gas chromatography and carbon 13 NMR spectroscopy. John Wiley and Sons: Chichester, U.K. 51: 341-348.

[3] Oussalah M., Caillet S., Saucier L., Lacroix M. 2006. Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. Meat Sci. 73: 236–244.

[4] Oussalah M., Caillet S., Saucier L., Lacroix M., 2007. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. Food Control. 18: 414–420.

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

[6] Farhat A., Ginies C., Romdhane M., Chemat F. 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.

[7] Golmakani M. T., Razaei K. 2008. Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from Thymus vulgaris L. Food Chemistry. 109: 925-930.

[8] Brachet A., Christen P., Veuthey JL. 2002. Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. Phytochemical Analysis. 13: 162-169.

[9] Mandal V., Mohan Y., Hemalatha S. 2007. Microwave-assisted extraction-An innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews. 1(1): 7-18.

[10] Letellier M., Budzinski H., Charrier L., Capes S., Dorthe A. M. 1999. Optimization by factorial design of focused microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. J. Anal. Chem. 364: 228-37.

[11] Mahadevan NS., Kamboj P. 2009. Hibiscus sabdariffa Linn. An overview. Natural Product Radiance. 8(1): 77-83.

[12] Kristen LH., Kellie PB., Svetlana Z., Michael PD., Stewart JRCN. 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.

[13] Nor Azah M. A., Chang Y.S., Mailina J., Abu Said A. AbdMajid J., Saidatul Husni S., Nor Hasnida H., Nik Yasmin Y. 2008. Comparison of chemical profiles of selected gaharu oils from peninsular Malaysia. The Malaysia Journal of Analytical Sciences. 12(2): 338-340.

[14] Stamatoiu O., Bubnov A., Rcomnicu I., Iovu M. 2008. Synthesis and spectral characterization of new amido-rther Schiff bases. Journal of Molecular Structure. 886: 187-196.

[15] Yeap G. Y., Ha S. T., Ishizawa N., Suda K., Boey P. L., Mahmood W. A. K. 2003. Synthesis, crystal structure and spectroscopic study of para-substituted 2-hydroxy-3-methoxybenzalideneanilines. Journal of Molecular Structure. 658: 87-99.