Asian Journal of Chemical Sciences

2(2): 1-6, 2017; Article no.AJOCS.33182

Phytochemical Composition and Evaluation of Antimicrobial Activity of *Blepharis linariifolia* (Pers.) Seeds

Alsiddig Osama[1*], Sufyan Awadelkarim[1], Noha Ali[1], Safa Khalid[1], Shima Mohammed[1] and Nosiba Hashim[1]

[1]Department of Chemistry, Faculty of Science and Technology, Omdurman Islamic University, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AO and SA designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors NA, SK, SM and NH managed the analyses of the study. Author AO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2017/33182
Editor(s):
(1) Zygadlo Julio Alberto, Professor of Chemistry, National University of Cordoba, Argentina.
Reviewers:
(1) C.D.Dayanand, Sri Devaraj Urs academy of Higher Education and research, India.
(2) Fernanda Maria Marins Ocampos, Federal University of Paraná, Brazil.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/19075

Original Research Article

Received 1st April 2017
Accepted 16th April 2017
Published 16th May 2017

ABSTRACT

Blepharis linariifolia (Pers.) originates from Sudan and grows widely in Africa. The current study has been designed to evaluate the bioactive compounds and antimicrobial activity of the n-hexane extract of *Blepharis linariifolia* seeds. The extract was analyzed by GC-MS (Model GCMS-QP2010 Ultra, Shimadzu Co., Japan) which revealed the presence of 2-Pentanol, 2-methyl;3-Pentanol, 3-methyl; 2-Hexanone; Hexanal; Benzaldehyde, 4-fluoro; 4-Methyl-4-(tetrahydropyran-2-yl)oxypentane-2,3-dione; 4-methyl-4-[3',4',5',6'-tetrahydro-2'-H-pyranyl-2'-oxy]-2,3-pentanedione; Hexane, 1,1'-oxybis; 1-Pentanol, 2,2-dimethyl; Butanoic acid, 2-ethyl-2-methyl; 2-Heptene, 5-ethyl-2,4-dimethyl; Undeca-4,8-dione; Acetic acid, 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl ester; 1-(3,3-dimethyl-bicyclo[2.2.1]hept-2-yl)pentan-2-one 3-cyano-2-oxa-1-ethoxyadamanane; Ethyl 3-methyl-2-oxobutyrate; Heneicosane 4,8,12,16-Tetramethylheptadecan-4-olide; 1,2-Benzenedicarboxylic acid, diisooctyl ester; Hexatriacontane and Dotriacontane. All compounds were identified from the spectral libraries of National Institute of Standard and Technology and WILEY.

**Corresponding author: E-mail: alsiddigosama@gmail.com;*

FTIR analysis revealed the presence various functional groups related to different type of organic substances including: Organic halogen, Ester, nitro compound, aromatics, amines, Aldehyde, Alkenes, Phenol, Alcohol and silicon compounds. The in vitro antimicrobial assays showed there is non-significant activity of this extract against five microorganism tested for.

Keywords: Blepharis linariifolia; oil; GCMS; FTIR; antimicrobial.

1. INTRODUCTION

Natural products are a well-known source of new valuable compound for medicinal or industry proposes [1]. Amongst the various natural sources, plants are predominant source of bioactive possess, including anticancer, antifungal and antimicrobial properties. The use of plant compounds as prototypes of new drugs has a historical and economic importance [2-3].

The analysis of plant extracts is extremely valuable to discover the chemical composition and to give better understanding of plant biological activities that may possess. Analysis of small amounts of chemicals has become easier and more and accurate after the discovery and development of chromatographic and spectroscopic techniques such as GC, HPLC, MS, NMR, etc. GC-MS are among the most powerful techniques used for both isolation and detection of samples which can be analyzed sufficiently even in trace amounts, less than 1 ng/ml [4].

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined [5].

B. linariifolia (family: Acanthaceae) distributed in Africa especially the areas from Mauritania to Sudan, through Arabia to Northwestern India. *B. linariifolia* is a low-growing, wiry herb with prickly bracts and blue flowers. Seeds have analgesic activity and are also used in veterinary medicine [6]. However, genus Blepharis have not investigated much [7].

2. MATERIALS AND METHODS

2.1 Extraction

B. linariifolia seed samples were collected from River Nile state, Sudan. The fresh seeds were dried in shades for 7 days and then powdered then used for extraction. method was used as per the method described by Osama and Awdelkarim, [8].

2.2 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

The above processed sample was used for FTIR analysis using KBr disk methodology. 1 mg of sample was encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The powdered sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm $^{-1}$ with a resolution of 4 cm $^{-1}$. Each analysis was repeated ten times for the spectrum confirmation.

2.3 GC-MS Analysis

GC-MS analysis was carried out by using the instrument (Model GCMS-QP2010 Ultra, Shimadzu Co., Japan) equipped with a capillary column Rtx-5 (0.25 μm film × 0.25 mm i.d. × 30 m length). The instrument was operated in electron impact mode at ionization voltage (70 eV), injector temperature (250°C), and detector temperature (280°C). The carrier gas used was helium (99.9% purity) at a flow rate of 1.2 mL/min and about 1 μL of the sample was injected. The oven temperature was initially programmed at 35°C (3 min) to 240°C at 5°C/min and from 240-280°C at 3°C/min withhold time up to 4 minutes. The identification of compounds from the spectral data was based on the available mass spectral records (NIST and WILEY libraries).

2.4 Antimicrobial Evaluation

2.4.1 Preparation of bacterial suspensions

One ml aliquots of pure standard bacteria of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 cfu/ml.

The suspension was stored in a refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes on drop of the appropriate dilutions were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.4.2 Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

2.4.3 In vitro testing of extracts for anti-microbial activity

A) Testing for Antibacterial Activity

The cup-plate agar diffusion method was adopted according to published method of Eltayeb et al. [9] One ml of the standardized bacterial stock suspension 108 –109 C.F.U./ml were thoroughly mixed with 100ml of sterile molten nutrient agar which was maintained at 45°C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars were left to dry and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml seed extract sample using automatic Microliter-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extracts against each of the test organisms. Simultaneously addition of extracts was carried out as controls. After incubation, the diameters of the resultants and growth inhibition zones were measured, averaged and the mean values were tabulated.

B) Testing for Antifungal Activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used, which is considered to be more selective for fungal. The inoculated medium was incubated at 25ºC for three days for *Aspergillus niger*.

3. RESULTS AND DISCUSSION

The n-hexane dissolved about 2.7% of total sample mass. Hexane is a nonpolar solvent capable to isolate the nonpolar compounds especially fatty acids, terpenes and steroids. This low yield percentage indicates the low amount of oil in this plant. The nature of sample is not the only factor that affect its content of group of compounds. The environmental factors, the part of plant used, methodology of extraction, duration of extraction, solvent used for extraction, etc. all these are factors can effect on percentage yield [10].

In the current study the hexane extract was analyzed using spectrometric techniques (FTIR and GCMS) to determine the functional groups and chemical constituents. The FTIR is the most powerful technique to determine the functional groups according to the response of compounds to the radiation with adjusted wave length. These groups are most likely responsible for the chemical and biological activities of this extract. The functional groups are the active parts of compounds they affect to its reactivity to certain kind of compounds or receptors. The study of the structure and its activities is the base of valuable part of medicinal chemistry which named as structure activity relationship [11]. The results showed the presence of different groups, shown in Table (1).

The functional groups detected by FTIR was mainly belong to aliphatic hydrocarbons, the highest absorption was found in 2958.6 (symmetric C–H stretching) which indicates the high amount of hydrocarbons alkenes. The hydrocarbons are well known for their non-polar properties therefore they can be isolated by hexane, the solvent used for extraction.

However, the alkenes are not active compounds compared to phenols or carboxylic acids. Low absorption was noticed for OH and C=O groups which are known for their antimicrobial activity.

Gas chromatography- mass spectrometry is one of the most updated techniques to isolate and detected the volatile chemical substances. In the present study the GCMS analysis detect different kind of compounds with variable molecular weight. The chromatogram showed the presence of 22 compounds, which they were identified by MS. The MS showed that the molecular weight of these compounds which vary from 100 to 506 amu. These compounds are mainly aliphatic hydrocarbons, shown in Table (2).

The *B. linariifolia* seed extract was evaluated for its antimicrobial activity and was found to be inactive to five type of bacteria and fungal. Table (3). Compounds or plant extracts are considerd to be active if the inhbition zone excees 15 mm [12].

Table 1. Functional groups and its wave number

Cm^{-1}	Bond	Functional Group
721.33	C–X stretching (X = F, Cl, Br or I)	organic halogen
1149.50	Aliphatic C–O stretching	Ester
1377.08	Aliphatic NO_2 symmetric stretching	nitro compound
1460.01	C–C stretching	(in–ring) aromatics
1537.16	N–O asymmetric stretching	nitro compounds
1581.52	N–H bend	1° amines
1668.31	C=O stretching	carbonyls (general)
1822.61	Overtone and combination bands	Other
2352.99	Combination C–H stretching	Common near-infrared bands of organic compounds
2731.02	C–H stretching	Aldehyde
2866.02	symmetric C–H stretching	Alkenes
2925.81	asymmetric C–H stretching	Alkenes
2958.60	symmetric C–H stretching	Alkenes
3544.92	O–H stretching	Phenol
3589.28	O–H stretching	Alcohol
3627.85	O–H stretch	free hydroxyl alcohols, phenols
3652.93	O–H stretch,	free hydroxyl alcohols, phenols
3670.28	O–H stretching	free hydroxyl alcohols, phenols
3712.72	O–H stretching	free hydroxyl alcohols, phenols
3780.22	Si–OH stretching	silicon compounds
3876.65	O–H stretching	free hydroxyl alcohols, phenols
3926.80	O–H stretching	free hydroxyl alcohols, phenols

Table 2. GCMS analysis of *B. linariifolia*

Peak no.	R. Time	Area %	Compounded name	Molecular Formula	Mass
1	4.655	0.44	2-Pentanol, 2-methyl	$C_6H_{14}O$	102
2	5.157	0.22	3-Pentanol, 3-methyl	$C_6\ H_{14}\ O$	102
3	6.199	0.46	2-Hexanone	:$C_6H_{12}O$	100
4	6.474	0.72	Hexanal	$C_6H_{12}O$	100
5	11.685	0.57	Benzaldehyde, 4-fluoro	C_7H_5FO	124
6	19.309	0.50	4-Methyl-4-(tetrahydropyran-2-yl)oxypentane-2,3-dione	$C_{11}H_{18}O_4$	214
7	19.643	0.16	4-methyl-4-[3',4',5',6'-tetrahydro-2'-H-pyranyl-2'-oxy]-2,3-pentanedione	$C_{11}H_{18}O_4$	214
8	19.988	0.34	Hexane, 1,1'-oxybis	$C_{12}H_{26}O$	186
9	20.064	0.19	1-Pentanol, 2,2-dimethyl	$C_7H_{16}O$	116
10	20.273	0.52	Butanoic acid, 2-ethyl-2-methyl	$C_7H_{14}O_2$	130

Peak no.	R. Time	Area %	Compounded name	Molecular Formula	Mass
11	21.346	2.18	2-Heptene, 5-ethyl-2,4-dimethyl	$C_{11}H_{22}$	154
12	23.226	1.94	Undeca-4,8-dione	$C_{11}H_{20}O_2$	184
13	29.413	11.60	Acetic acid, 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl ester	$C_{13}H_{22}O_4$	242
14	31.695	3.10	1-(3,3-dimethyl-bicyclo[2.2.1]hept-2-yl)pentan-2-one	$C_{14}H_{24}O$	208
15	32.078	13.09	3-cyano-2-oxa-1-ethoxyadamanane	$C_{12}H_{17}NO_2$	207
16	34.041	15.49	Ethyl 3-methyl-2-oxobutyrate	$C_7H_{12}O_3$	144
17	43.429	1.56	Heneicosane	$C_{21}H_{44}$	296
18	44.396	0.58	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324
19	46.706	2.13	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390
20	47.756	2.87	Hexatriacontane	$C_{36}H_{74}$	506
21	50.337	8.18	Hexatriacontane	$C_{36}H_{74}$	506
22	54.058	16.03	Dotriacontane	$C_{32}H_{66}$	450

Table 3. Antimicrobial evaluation of *B. linariifolia* against five standard organisms

Type of microbe	Gram possitive		Gram negative		Fungai
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Aspergillus niger***
MDIZ (mm)	11	11.5	12	14	zero

MDIZ = mean diameter of growth inhibition zone

4. CONCLUSION

Study concludes that*, B. linariifolia* seed extract contains different types of chemical compounds amongst major components were alkenes. bioactive compounds have minimal bactericidal and fungicidal activity. Further studies for both chemical and biological proposes are recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhao S, Liu JY, Chen SY, Shi LL, Liu YJ, Ma C. Antioxidant potential of polyphenols and tannins from burs of *Castanea mollissima* blume. Molecules. 2011;16: 8590-8600.
   DOI: 10.3390/molecules16108590
2. Mann J, Davidson RS, Hobbs JB, Banthorpe DV, Harborne JB. Natural Products. Their Chemistry and Biological Significance, Addison Wesley Longman, Harlow, UK; 1994.
3. Trease GE, Evans MD. A text book of Pharmacognosy, 13th Edn. Baillier, Tindal and Caussel, London. 1989;144-148.
4. Prajna SS, Priya KS, Swarnalatha S, Bhojaraju P, Kanthal LK, Satyavathi K. GC–MS analysis and *In-Vitro* cytotoxic activity of methanolic extract of *Antigonon Leptopus* flowers. Int J Pharm Sci Res. 2015;6(7):3083-87.
   DOI: 10.13040/IJPSR.0975-8232.6
5. Hollas JM. Modern spectroscopy. Fourth edition. John Wiley & Sons. England; 2004.
6. Vijayalakshmi S, Kripa KG. Theraoutic uses of plants genus Blepharis- a systematic review. Int J Pharm Bio Sci. 2016;7(4):236–243.
7. Osama A, Awdelkarim S, Ezeldin M. Spectrophotometric determination of total phenol and flavonoid contents of aerial parts of *Blepharis edulis* (Pers.). Chem Sci Int J. 2017;18(2):1-7.
   DOI: 10.9734/CSIJ/2017/25916
8. Osama A, Awdelkarim S. Phytochemical screening of *Ficus sycomorus* L. bark and Cleome gynandra L. aerial parts. J. Pharmacog. and Phytochem. 2015;4(4): 24-27.
9. Eltayeb OM, Jobara MI, Mohamed MA, Masaad AM, Elshikh AA, Elrofaei NA,

Ahmed AO, FadulAllA EF, Mohamed AK. *In vitro* evaluation of antimicrobial, antioxidant activities and phytochemical screening of *Quercus infectoria* (galls) used in traditional medicine. Curr Res Microbiol Biotechnol. 2015;3(2):595-599.

10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A review. Internationale Pharmaceutica Sciencia. 2011;1:98-106.
11. Thomas G. Medicinal chemistry. 2nd edition. John Wiley & Sons Ltd. England; 2007.
12. Voukeng IK, Beng VP, Kuete V. Antibacterial activity of six medicinal Cameroonian plants against Gram-positive and Gram-negative multidrug resistant phenotypes. BMC Complement Altern Med. 2016;16:388. DOI 10.1186/s12906-016-1371-y

© 2017 Osama et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/19075