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Original Research Article

Chemical Composition and Antimicrobial Evaluation of *Blepharis linariifolia* Seeds

Abstract

The current study has been designed to evaluate the bioactive compounds and antimicrobial activity of the n-hexane extract of *Blepharislinariifolia*seeds. The oil was analyzed by GC-MS (Model GCMS-QP2010 Ultra, Shimadzu Co., Japan) which revealed the presence of 22 diversified compounds. All compounds were identified from NIST and WILEY spectral libraries. FTIR analysis revealed the presence of different functional groups. The in vitro antimicrobial assays showed there is no significant activity of this extract against five microorganism tested for.

Keywords:*Blepharislinariifolia;* 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 the most important source of bioactive constituents including anticancer, antifungal and antimicrobial drugs. The use of plant compounds as prototypes of new drugs has a historical and economic importance[2-3].

The chemical 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 ofchemicals has become easier and more and accurate after the discover and development of chromatographic and spectroscopictechniques 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 [illegible]g[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].

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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, are also used in veterinary medicine [6]. however, genus Blepharis have not investigated much[7].

2. Materials and methods

2.1. Extraction

The fresh samples were dried in shades for 7 days, powdered then used for extraction. Cold maceration methodology was used and it was carried out according to published method of Osama and Awdelkarim, 2015 [8].

2.2. Fourier Transform Infrared Spectrophotometer (FTIR) analysis

Dried powder of 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-MSAnalysis

GC-MS analysis was carried out by using the GCMS 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 (3 and 4 min respectively. 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 a 24 hours broth culture of the test organism 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 c.f.u/ml. The suspension was stored in a refrigerator at 4° C till used.The average number of

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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 were stored in the refrigerator until used.

2.4.3. In vitro testing of extracts for antimicrobial activity

A) Testing for Antibacterial Activity

The cup-plate agar diffusion method was adopted according to published method of Eltayeb et al., 2015 [9] with some minor modification to assess the antibacterial activity of the prepared extract.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 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes.The agars was 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 sample of each extracts using automatic Microlitre-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC 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

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92 The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar
93 was used. The inoculated medium was incubated at 25 ºC for three days for Aspergillus niger.

94 **3. Results and discussion**

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

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

110

111 **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

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2731.02	C–H stretching	Aldahyde
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

112

113 The functional groups detected by FTIR was mainly belong to aliphatic hydrocarbons, the
114 highest absorption was found in 2958.6 (symmetric C–H stretching) which indicates the high
115 amount of hydrocarbons alkenes. The hydrocarbons are well known for their non-polar
116 properties therefore they can be isolated by hexane, the solvent used for extraction. However, the
117 alkenes are not active compounds compared to phenols or carboxylic acids. Low absorption was
118 noticed for OH and C=O groups which are known for their antimicrobial activity.

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

125 **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	C6H14O	102
2	5.157	0.22	3-Pentanol, 3-methyl	C6 H14 O	102
3	6.199	0.46	2-Hexanone	:C6H12O	100
4	6.474	0.72	Hexanal	C6H12O	100

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5	11.685	0.57	Benzaldehyde, 4-fluoro	C7H5FO	124
6	19.309	0.50	4-Methyl-4-(tetrahydropyran-2-yl)oxypentane-2,3-dione	C11H18O4	214
7	19.643	0.16	4-methyl-4-[3',4',5',6'-tetrahydro-2'-H-pyranyl-2'-oxy]-2,3-pentanedione	C11H18O4	214
8	19.988	0.34	Hexane, 1,1'-oxybis	C12H26O	186
9	20.064	0.19	1-Pentanol, 2,2-dimethyl-	C7H16O	116
10	20.273	0.52	Butanoic acid, 2-ethyl-2-methyl	C7H14O2	130
11	21.346	2.18	2-Heptene, 5-ethyl-2,4-dimethyl	C11H22	154
12	23.226	1.94	Undeca-4,8-dione	C11H20O2	184
13	29.413	11.60	Acetic acid, 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl ester	C13H22O4	242
14	31.695	3.10	1-(3,3-dimethyl-bicyclo[2.2.1]hept-2-yl)pentan-2-one	C14H24O	208
15	32.078	13.09	3-cyano-2-oxa-1-ethoxyadamanane	C12H17NO2	207
16	34.041	15.49	Ethyl 3-methyl-2-oxobutyrate	C7H12O3	144
17	43.429	1.56	Heneicosane	C21H44	296
18	44.396	0.58	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324
19	46.706	2.13	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390
20	47.756	2.87	Hexatriacontane	C36H74	506
21	50.337	8.18	Hexatriacontane	C36H74	506
22	54.058	16.03	Dotriacontane	C32H66	450

The *B. linariifolia* seed oil 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 (MDIZ) exceeded 15 mm[12].

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

Type of microbe	Gram +ve		Gram -ve		Fungal
	Bacillus subtilis	*Staphylococcus aureus*	Escherichia coli	*Pseudomonas aeruginosa*	*Aspergillusniger*
MDIZ (mm)	11	11.5	12	14	zero

MDIZ = mean diameter of growth inhibition zone

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4. Conclusion

In conclusion the *B. linariifolia*oil was analyzed with FTIR and GCMS also was evaluated for their antimicrobial activity against four type of bacteria and a fungal. Different functional groups were detected. The main type of compounds was found to be alkenes. The GCMS revealed the presence of variable compounds with diverse structures. Low antimicrobial activity was noticed. Further studies for both chemical and biological proposes are recommended.

References

[1] Zhao S, Liu JY, Chen SY, Shi LL, Liu YJ and Ma C. Antioxidant Potential of Polyphenols and Tannins from Burs of *Castaneamollissima* 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 and Satyavathi K. GC–MS Analysis and In-Vitro Cytotoxic Activity of Methanolic Extract of *AntigononLeptopus* Flowers. Int J Pharm Sci Res. 2015; 6 (7): 3083-87.doi: 10.13040/IJPSR.0975-8232.6 (7). 3083-87.

[5] Hollas JM. Modern spectroscopy. Fourth edition. John Wiley & Sons. England. 2004.

[6] Vijayalakshmi S and 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 and Ezeldin M. Spectrophotometric Determination of Total Phenol and Flavonoid Contents of Aerial Parts of *Blepharisedulis* (Pers.). ChemSciInt J. 2017; 18 (2): 1-7.DOI: 10.9734/CSIJ/2017/25916.

[8] Osama A and Awdelkarim S. Phytochemical screening of *Ficussycomorus* 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 *Quercusinfectoria*(galls) used in Traditional Medicine. Curr Res MicrobiolBiotechnol. 2015, 3(2): 595-599.

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162 [10] Tiwari p, Kumar B, Kaur M, Kaur G and Kaur H (2011). Phytochemical screening and
163 Extraction: A Review. InternationalePharmaceuticaSciencia. 1, 98-106.
164 [11] Thomas G. Medicinal Chemistry. 2nd edition. John Wiley & Sons Ltd. England. 2007.
165 [12] Voukeng IK, Beng VP and Kuete V. Antibacterial activity of six medicinal Cameroonian
166 plants against Gram-positive and Gram-negative multidrug resistant phenotypes. BMC
167 Complement Altern Med. 2016; 16:388. DOI 10.1186/s12906-016-1371-y.