



Chemical Composition and Antimicrobial Activity of Essential Oil of *Belpharis linariifolia*

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Abstract: The study was aimed to investigate essential oil chemical composition and antimicrobial activities of essential oils extracted from seeds of *Belpharis linariifolia*. The oil was extracted according to the method described by Harborne (1984). and analyzed by Fourier Transform Infrared Spectrophotometer (FTIR) and Gas Chromatography Mass Spectrometry (GC/MS) techniques to determine the chemical composition of the volatile fraction and identify their chemo-types. The essential oil of *Belpharis linariifolia* seeds were tested against four standard bacterial species: two Gram-positive bacteria viz, *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the paper disc diffusion method. Twenty two components were identified in the essential oil of *Belpharis linariifolia* representing 82.87% of the total components, the major compounds were Acetic acid (11.60%), 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl (11.60%), 3-Cyano-2-Oxa-1-Ethoxyadamanane (13.09%), Ethyl 3-methyl-2-oxobutyrate (15.49%), Hexatriacontane (8.18%) and Dotriacontane (16.03%). Antimicrobial activity of essential oil of *Belpharis linariifolia* dissolved in methanol (1:10), showed low activity against the Gram-negative bacteria (*P. aeruginosa* & *E. coli*) (14 & 11 mm). It also showed against Gram positive bacteria (*S. aureus* & *B. subtilis*) (11.5 & 14 mm) and against (*C. albicans*) (zero mm). This study conducted for essential oil of *Belpharis linariifolia* seeds presence of variable compounds with diverse structures and low antimicrobial activity.

Keywords: *in-vitro*, FT-IR, Gas Chromatography–Mass Spectrometry (GC-MS), Antimicrobial Activity, Essential Oils, *Belpharis linariifolia* (Seeds)

1. Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in-recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases

[2]. The drug resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases [3]. The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern [4] [5]. The value of natural products in the treatment of ailments is well-known. Amongst the various natural sources, plants are an important source of bioactive constituents. More than 1000 plant species are known for their anticancer potential. The use of plant compounds as prototypes of new drugs has a

historical and economic importance. Some plants extracts were defined as effective in treating cancer, they action was attributed to additional or synergistic effect of compounds present in the extract. In consequence, the cytostatic effect of the extract observed in tumor cells seems to be more effective than the effect of isolated and biologically active compounds [6].

Belpharis linariifolia belonging to the family: Acanthaceae is distributed in Africa - the drier areas from Mauritania to Sudan, through Arabia to Northwestern India and it is a plant for the drier areas of the tropics and subtropics. *Blepharis linariifolia* is a low-growing, wiry herb with prickly bracts, flowers blue, eaten by cattle, goats, sheep, camels and donkeys. The plant is gathered from the wild for local use as food, medicine for ailments such as tuberculosis, chest pain and wounds. A tisane of the whole plant is used in the treatment of syphilis [7]. Decoction is used as a wash and powder of the dried plant parts are applied in powdered form for local applications, skin burns and urogenital infections. Seeds and leaves have analgesic activity, are also used in veterinary medicine as lactogenic and also in retained placenta. The antioxidant activity and the total phenolic and flavonoid contents of the plant *Belpharis linariifolia* have been studied and the results suggested that the plant might be considered as a good source of antioxidant, thus supporting its use in cardiovascular and anti-inflammatory diseases [8]. Some plants are known as medicinal because they contain active substances that cause certain reaction, from relating to the cure of disease on the human organism [10]. The study was aimed to investigate the chemical composition and antimicrobial activity of essential oil of *Belpharis linariifolia* (seeds).

2. Materials and Methods

2.1. Plant Materials

Belpharis linariifolia seeds were collected from alpine north Kordfan and identified and ethnocatated by plant taxonomist at ministry of agriculture in Khartoum, Sudan. Seeds were air-dried, under the shade, pulverized and stored prior to extraction. Shade with good ventilation and then ground finely in a mill and kept in the herbarium until oil extract preparation Figure (1).



Figure 1. *Belpharis linariifolia* (Seeds).

2.2. Extraction of Oil

Seeds samples were taken from hem of fresh plant powdered and then used for extraction. Extraction was carried out according to the method described by Harborne (1984). The shade-dried samples were soaked in 95% hexane at a ratio of (1:10) at room temperature for 3 days, filtered and left to dry at room temperature. This process was repeated till the solvent returned to colorless. The weight of the solid residues was recorded and taken as yield of crude extracts. Yield percentage was calculated as follows:

$$\text{Yield \%} = (\text{weigh of extract/weigh of sample}) \times 100$$

2.3. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Principle: 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.

Assay: Fixed oil 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 liquid sample of plant specimen 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.

2.4. GC-MS Analysis

GC-MS technique was used in this study to identify the phytochemicals present in the most active fractions. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 μL of sample was injected in GC-MS equipped with a split injector. The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min. The analytical column connected to the system was an Rtx-5 capillary column (length-30 m \times 0.25 mm i.d., 0.25 μm film thickness). The column head pressure was adjusted to 93.9 kPa. Column temperature programmed from 110°C (7 min) to 200°C at 10°C/min and from 200-280°C at 5°C/min with hold time 0 and 9 min respectively. A solvent delay of 4.50 min was selected. The injector temperature was set at 250°C. The GC-MS interface was maintained at 280°C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

2.5. Test Microorganisms

The oil solution of *Belpharis linariifolia* was tested against four standard bacteria species: two Gram-positive bacteria viz., *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and one standard fungal strains viz, *Candida albicans* (ATCC 7596) using the Disc diffusion method. The standard bacterial and fungal strains used in the study were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 18 h and then used for the antimicrobial test.

Testing of Antibacterial and Antifungi Susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial and fungi activity of plant oil and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines [9]. Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 μ l of a solution of plant oil. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2.6. Statistical Analysis

All data were presented as means \pm S.D. Statistical analysis for all the assays results were done using Microsoft Excel program (2010).

3. Results and Discussion

Belpharis linariifolia oil has low yield percentage with

hexane solvent as shown in Table (1).

Table 1. Yield percentage of *Belpharis linariifolia* oil.

Name of plant	Part used	Weight of sample (g)	Volume of oil	Yield %
<i>Belpharis linariifolia</i>	Seeds	200	5.4	2.7

The n-hexane was used to extract the plant samples and was able to dissolve about 2.7%. Hexane is a very non polar solvent capable to isolate the non-polar compounds especially fatty acids (oil). This low yield percentage indicates the low amount of oil in this plant. However the oil can be isolated with other solvent e.g. petroleum ether or chloroform, but their properties are similar to hexane so no expectation for significant difference if we used other solvent.

3.1. FTIR Analysis

The FTIR spectrum was used to identify the functional groups of the chemical components present in the tested fractions based on the peak value in the region of infrared radiation. Essential oil of *Belpharis linariifolia* FTIR spectrum confirmed the presence of alkynes, alkenes, alkanes, carboxylic acids, esters, ethers, carbonyls, carboxylic acids, H-bonded alcohols and phenols and other compounds as shown in Table (2) and Figure(2).

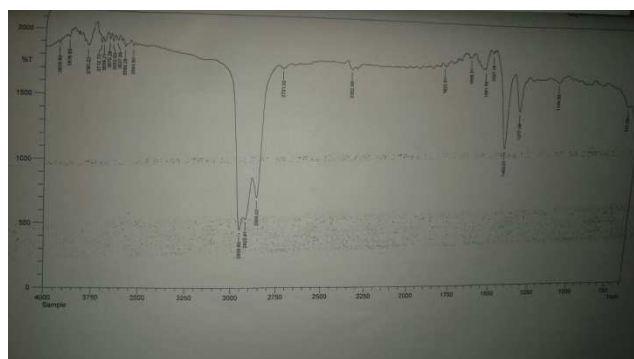


Figure 2. FT-IR spectra of *Belpharis linariifolia* oil.

Table 2. FT-IR spectral analysis of *Belpharis linariifolia* oil.

Frequency 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	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

Frequency CM ⁻¹	Bond	functional group
3652.93	O–H stretch,	free hydroxyl alcohols, phenols
3670.28	O–H stretching	free hydroxyl alcohols, phenols
3699.21	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

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 compare with phenols or carboxylic acids. Low absorption was noticed for OH and C=O groups which are known for their antimicrobial activity.

3.2. GC-MS Analysis

The results pertaining to GC-MS analysis lead to the identification of number of compounds. These compounds were identified through mass spectrometry attached with GC. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of these compounds. Interpretation of mass spectrum GC–MS was conducted using the database of National Institute of Standards and Technology (NIST). The name, molecular

weight and structure of the components of the test materials were ascertained, the components identified by the GC-MS are illustrated in Tables (3) and Figure (3).

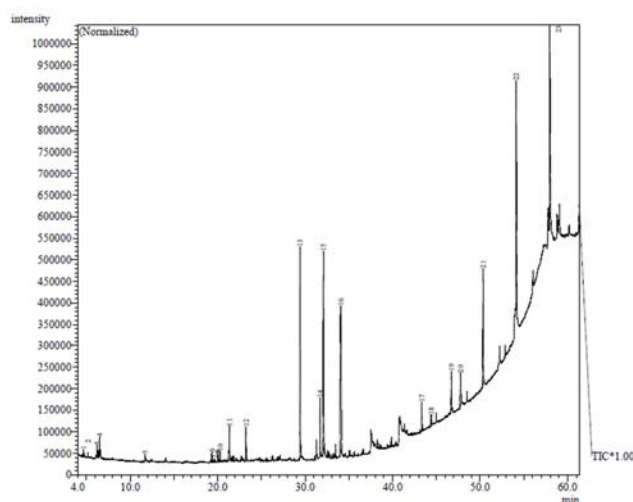


Figure 3. GC-MS chromatogram of *Belpharis linariifolia* oil.

Table 3. GC-MS spectral analysis of *Belpharis linariifolia* oil.

Peak no.	R. Time	Compoundname	Molecular Formula	Area %	Mass
1	4.655	2-Pentanol, 2-methyl	C ₆ H ₁₄ O	0.44	102
2	5.157	3-Pentanol, 3-methyl	C ₆ H ₁₄ O	0.22	102
3	6.199	2-Hexanone	C ₆ H ₁₂ O	0.46	100
4	6.474	Hexanal	C ₆ H ₁₂ O	0.72	100
5	11.685	Benzaldehyde, 4-fluoro	C ₇ H ₅ FO	0.57	124
6	19.309	4-Methyl-4-(tetrahydropyran-2-yl)oxypentane-2,3-dione	C ₁₁ H ₁₈ O ₄	0.50	214
7	19.643	4-methyl-4-[3',4',5',6'-tetrahydro-2'-H-pyran-2'-oxy]-2,3-pentanedione	C ₁₁ H ₁₈ O ₄	0.16	214
8	19.988	Hexane, 1,1'-oxybis	C ₁₂ H ₂₆ O	0.34	186
9	20.064	1-Pentanol, 2,2-dimethyl-	C ₇ H ₁₆ O	0.19	116
10	20.273	Butanoic acid, 2-ethyl-2-methyl	C ₇ H ₁₄ O ₂	0.52	130
11	21.346	2-Heptene, 5-ethyl-2,4-dimethyl	C ₁₁ H ₂₂	2.18	154
12	23.226	Undeca-4,8-dione	C ₁₁ H ₂₀ O ₂	1.94	184
13	29.413	Acetic acid, 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl ester	C ₁₃ H ₂₂ O ₄	11.60	242
14	31.695	1-(3,3-dimethyl-bicyclo[2.2.1]hept-2-yl)pentan-2-one	C ₁₄ H ₂₄ O	3.10	208
15	32.078	3-Cyano-2-Oxa-1-Ethoxyadamanane	C ₁₂ H ₁₇ NO ₂	13.09	207
16	34.041	Ethyl 3-methyl-2-oxobutyrate	C ₇ H ₁₂ O ₃	15.49	144
17	43.429	Heneicosane	C ₂₁ H ₄₄	1.56	296
18	44.396	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	0.58	324
19	46.706	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	2.13	390
20	47.756	Hexatriacontane	C ₃₆ H ₇₄	2.87	506
21	50.337	Hexatriacontane	C ₃₆ H ₇₄	8.18	506
22	54.058	Dotriacontane	C ₃₂ H ₆₆	16.03	450
Total content fraction of determinates compounds				82.87%	

Results obtained from gas chromatography mass detector showed the presence of high number of active constituents in all tested fractions. This could give a clue to a wide medicinal activity they may possess. Twenty two components were identified in the essential oil of *Belpharislinariifolia*

represented 82.87% of the total components, the major compounds were Acetic acid (11.60%), 4-acetyl-2-isopropyl-5,5-dimethyltetrahydrofuran-2-yl (11.60%), 3-Cyano-2-Oxa-1-Ethoxyadamanane(13.09%), Ethyl 3-methyl-2-oxobutyrate(15.49%), Hexatriacontane (8.18%),

Dotriacontane (16.03%).

3.3. Antimicrobial Activity

The oil of *Belpharis linariifolia* seeds were tested against four standard bacterial species: two Gram-positive bacteria viz, *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), two Gram-negative bacterial strains *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and fungal strains viz, *Candida albicans* (ATCC 7596) using the disc diffusion method. but it showed slight activity against *Bacillus subtilis* with inhibition zone 14 mm. Table (4). Compounds or plant extracts are considered to be active if the inhibition zone (IZ) exceeded 15 mm. however in other literature the compound can be active also if the IZ was > 13. This low activity may be due the presence of low concentration of active constituents. The FTIR showed low amount of OH and C=O groups which are interact with microbes and cause their death.

Table 4. The antimicrobial activity oil of *Belpharis linariifolia* and reference antibiotics against the standard bacteria and fungi.

Standard microorganisms	Concentration (mg/ml)	Gentamicin 30 µg/ml
	Zone of Inhibition in (100 mm)	
<i>Bacillus subtilis</i>	14	20
<i>Escherichia coli</i>	11	29
<i>Staphylococcus aureus</i>	11.5	30
<i>Pseudomonas aeruginosa</i>	14	25
Tested fungi used		Nystatin
<i>Candida albicans</i>	-	15



Figure 4. Antimicrobial activity of *Belpharis linariifolia* of seeds oil. Zone of inhibition of oil against standard bacteria and fungi.

4. Conclusion

In conclusion the *B. linariifolia* was extracted with hexane. The oil was analyzed with FT-IR and GC-MS and also evaluated for their antimicrobial activity against four types 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.

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