Gas chromatography–mass spectrometry analysis of *Pulicaria crispa* (whole plant) petroleum ether extracts

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Abstract

Pulicaria crispa is used in traditional Sudanese medicine for a number of indications. The petroleum ether extract of the whole the plant was exhibited significant cytotoxicity, antibacterial and antioxidant activities. The antibacterial activity of the plant extract against four pathogenic bacteria was exhibited activity with MIZD (20.5 - 17). The antioxidant activity of this extract was determined using DPPH method, and the result was showed (85±0.06). Also the invitro cytotoxicity using brine shrimp lethality method was used and the result was exhibited (37.9). GC- MS was used to study the bioactive compounds present in petroleum ether extract of *Pulicaria crispa.* The GC- MS analysis showed the presence of 14 compounds.

KeyWords: *Pulicaria crispa* ; Antimicrobial; Antioxidant; Cytotoxicity; GC- MS; Tetratriacontane; Heneicosane; Heptacosane; *Asteraceae*

{**Citation:** Yasmin Hassan Elshiekh, Mona A M abdelmageed. Gas chromatography–mass spectrometry analysis of *Pulicaria crispa* (whole plant) petroleum ether extracts. American Journal of Research Communication, 2015, 3(3): 58-67} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Pulicaria crispa (*Asteraceae)* is a deciduous herb flowering plant. Geographically it is distributed in northern and central Sudan(1). The antimicrobial activity of the plant was reported against standard bacterial strains(2-7). Also the moderate to high cytotxicity of the whole plant

extract was reported (2, 3, 8-11). Many member of this chemically rich family have long been used in folk medicines(4-6, 10-12). The qulalitative chemical analysis of the whole plant was reflected by the presence of wide range of secondary metabolites extended from less lipophilc to hydrophilic including amphotric compounds (11-26).

Methodology

Plant material

Pulicaria crisp (whole plant) was obtained from (Elobaied) North Kordofan, The plant was identified and authenticated by the Medicinal and Aromatic plants Research Institute (2012). The whole Plant was cleaned, freed from dust and foreign material, and then dried under the shade and finally crushed to powder form.

Extraction and identification

The coarsely powdered material (75 g) was successively extracted using. Soxhelt with petroleum ether for 48hr. the extract was separated by filtration and evaporated to dryness on rotary evaporator under reduced pressure, which afforded to 0.75g.

Phytochemical Screening

Phytochemical screening of the secondary metabolites was determined as the method described by(23) with some modification.

Analysis of petroleum ether extract

GC was carried out on Perkin Elmer Auto System XL fitted with a PE-5 (5% phenyl, 95% dimethyl polysiloxane), capillary column (50 mm x 0.32 mm); film thickness 0.20 µm; carrier gas H2 . Oven temperature 100º C for 2 min and then programmed from 100-280º C at 3º C/min. injector and detector temperature 220º C and 300º C respectively.

GC/ GCMS Fragmentation

GC-MS analysis was carried out on a Perkin Elmer Turbo -mass coupled with GC-Auto- XL, MS at 70 eV; column and temperature programmed same as above using carrier gas Helium. Inlet pressure 10 psi. The constituents were identified by comparing their retention indices with

those of authentic samples or identified in ether extract of known compounds. The mass spectra were compared with those stored in spectrometer database and built in libraries (19-21)

Antibacterial activity

The antibacterial activity of the extract was tested against four standard pathogenic microorganisms: two gram positive bacteria *Staphylococcus aureus and Bacillus subtilis;* and two gram negative bacteria *Escherichia coli and Pseudomonas aerogenosa* using agar disc diffusion method (7, 8)

Antioxidant activity

It was determining using DPPH method. The antioxidant activity of the oil sample was assessed by quantifying the scavenging ability to stable free radical 2, 2′- diphenyl-1-picrylhydrazyl (9). The sample from 5.5 µg/ml

was mixed with 1 ml of 90 µM DPPH solution followed by addition of 95% MeOH up to final volume of 4 ml. The absorbance of the resulting solution and the blank was recorded after 1 h at room temperature. Synthetic antioxidant, BHT was used as a positive control. The disappearance of DPPH was read spectrophotometrically at 515 nm. Inhibition of free radical by DPPH in percent[1 (%)] was calculated in following way:

I (%) = 100 × (Ablank - Asample / Ablank)

Where A_{blank} is the absorbance of the control reaction mixture excluding the test sample, and A_{sample} is the absorbance of the test sample. IC50 values, which represented the concentration of petroleum ether extract that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Invivo Cytotoxicity of the ether extract

Brine shrimps (*Artemia salina*) was used to determine the invivo cytotoxicity of *Pulicaria crispa,* the eggs were filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to ph 8.5 using 1N naoh) under constant aeration for 48 h. After hatching, active nauplii were collected from brighter portion of the hatching chamber and used for the assay. 20mg of crude extract dissolved then 500 µl, 50 µl and 5 µl of stock solutions was transferred to vials corresponding to 1000, 100 and 10 µg/ml ,respectively. The solvent was evaporated overnight. After two days of hatching, 10 Nauplii /larvae were placed into each vial and the volume was adjusted with sea water to 5ml per vial, and incubated at 27 °C for 24 hours under illumination.

Then, the number of survivors were counted and recorded. Cyclophosphamide an anticancer drug was used as a positive control in the bioassay. LD50 values were obtained from the best-fit line plotted concentration verses percentage lethality. The data were processed using a Finney computer Programme and LD_{50} values were obtained at 95 % confidence intervals.[2 , 9].

Results

Phytochemical Screening of Plant Extracts

The phytochemical Screening of *Pulicaria crispa* (whole plant) petroleum ether extract was revealed the presence of Alkaloids , Coumarins, Triterpenoids and Fatty acid (Table 1).

Table 1: The Chemical Constituents of *Pulicaria crispa* Whole plant petroleum ether extract

Phytochemical	Results
Alkaloids	+
Coumarins	+
Triterpenoids	+
Fatty acids	+

Key: (+): present , (-): absent.

The GC- MS analyses indicated the qualitative content in the petroleum ether extract of the *Pulicaria crispa* (whole plant). The extract is a complex mixture of many constituents, 14 compounds have been identified (table 2). The major components identified were Heneicosane , Tetratriacontane , Heptacosane , Benzene, 1-ethyl-2,3,4,5,6-pentafluoro-,7b-Phenyl-2a,7b-

dihydro-3H-cyclobuta[a]indene , Hexane, 1-(hexyloxy)-3-methyl- , Hop-22(29)-en-3.beta.-ol , 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-,[4R-(4.alpha.,4a.alpha.,6.beta.)]- , Decane, 1-iodo- , Stannane, diethenyldimethyl- , 3-Ethyl -3-Methyl heptane , Tetradecane , 2-Propenamide (Figure 1).

Table 2 : GC-MS Analysis of the Petrolium Ether Extract of *Pulicaria crispa*

S. No.	Name of the Compounds	RT	Peak Area %	Qual	Activity Referencs
1	Benzene, 1-ethyl-2,3,4,5,6-pentafluoro-	9.7415	18.9707	64	Antiasthmatics , Prostate disorder Kidneys disorder Antipsoriatics
2	2-Propenamide	11.5374	0.2875	5	Antispasmodics , Urolithiasis , Antiasthmatics , Anti tussive agent .
3	Hexane, 1-(hexyloxy)-3-methyl-	13.3828	1.1871	43	No activity reported
4	Heptacosane	14.3468	1.8301	72	antimicrobial
5	Heneicosane	15.5476	4.4537	90	Antiasthmatics urine acidifiers Antimicrobial
6	3-Ethyl-3-methylheptane	16.2803	0.4251	23	No activity reported
7	Tetratriacontane	17.1617	12.588	90	No activity reported
8	Decane, 1-iodo-	18.1918	1.1129	37	No activity reported
9	Tetratriacontane	19.4588	5.4907	86	No activity reported
10	Tetradecane	22.8191	0.7229	9	Antimicrobial Diuretic
11	7b-Phenyl-2a,7b-dihydro-3H-cyclobuta[a]indene	24.6204	5.514	58	No activity reported
12	Stannane, diethyldimethyl-	25.7552	2.2508	35	No activity reported
13	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-, [4R-(4.alpha.,4a.alpha.,6.beta.)]-	25.7717	2.6009	42	No activity reported
14	Hop-22(29)-en-3.beta.-ol	28.6307	42.5655	43	No activity reported

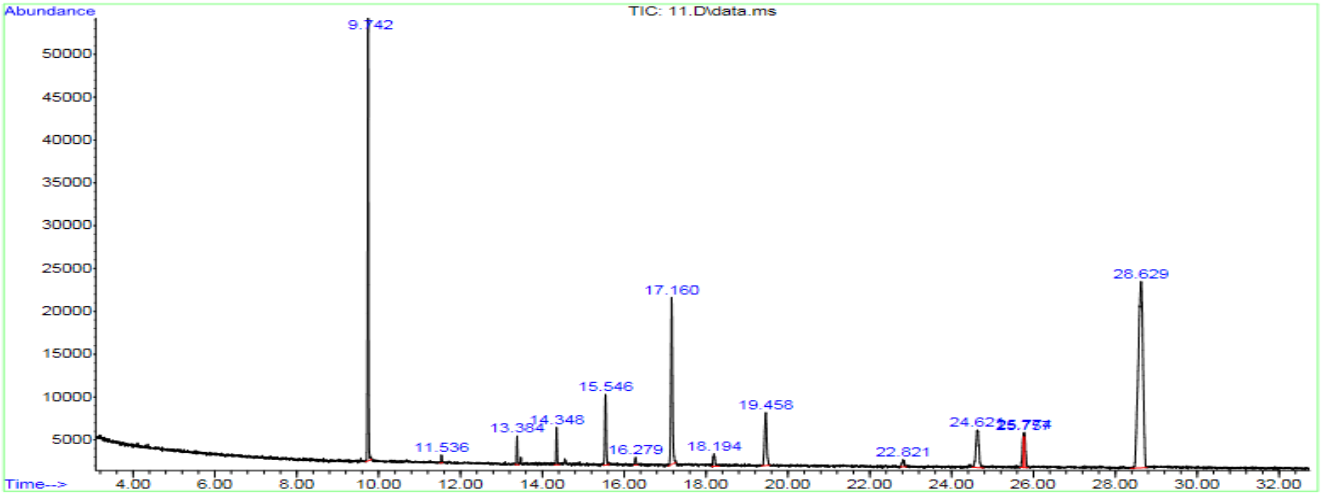

Figure 1 : GC-MS Chromatogram of petroleum ether extract of *Pulicaria crispa.*

The biological activity of the plant ether extract as table (3), were exhibited anti bacterial activity against *Staphylococcus aureus* , *Bacillus subtilis* , *Escherichia coli* , *Pseudomonas aeruginosa* ranging from (20.5 – 17) and high antioxidant activity with DPPH (85±0.06) while the invitro cytotoxicity was showed IC_{50} 37.9 .

Table 3: the biological activity of the Petroleum ether extract of *Pulicaria crispa*

Biological Bioassay					
Bacterial strains				Antioxidant	Brine Shrimp lethality (IC50)
Antibacterial activity (MIZD mm)					
S. a.	*B. s.*	*E. c.*	*Ps. a.*	85±0.06	37.9
17	20.5	19	21		

Discussion

From this study the presence of Flavonoids , Terpinoids, Alkaloid was reported and this explained the multi uses of plant in traditional medicine . the compounds hat determined by the GC-MS most are explained the antimicrobial activity of this plant extract.

On the other hand ; the presence of Heneicosane , Heptacosane , Benzene, 1-ethyl-2,3,4,5,6-pentafluoro-, Tetradecane , 2-Propenamide in the Petrolium ether extract of *Pulicaria crispa* explained the uses of the plant in folk medicine for the treatment of colds, coughs, colic, excessive sweating and as carminative .

Conclusion

The objective of this work was to find the chemical constituents of the plant ***Pulicaria crispa*** and reflect the relation between the secondary metabolites of the ether extract of the plant and the different biological activity. The result was exhibited a potential antibacterial activity explain the traditional uses of the plant as wound healer. On the other hand the potential antioxidant activity with the invitro cytotoxicity of the plant was explained by the present of the flavonoids and the alkaloids. In the general this is the first report of isolation and bioassay of the ether extract of this plant.

Acknowledgements

The authors are grateful to Department of Pharmacognosy , Omdurman Islamic University.

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