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Analysis of anti –inflammatory active fractions of *Tribulus terrestris* by high resolution GC-MS

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Abstract
High resolution GC-MS Analysis of anti –inflammatory active fractions of *Tribulus terrestris* have been studied. The phytoconstituents of the anti –inflammatory active fractions of *Tribulus terrestris* were investigated using Thermo Scientific TM DFS high resolution GC-MS Thermo fisher scientific Inc. The GC-MS analysis revealed the existence of 25 compounds, some of them with either known anti-inflammatory activity or shows another activities strongly related to the inflammation process.

Keywords: High resolution GC-MS, *Tribulus terrestris,* anti-inflammatory fractions.

1. Introduction
Plants are potent biochemical factories as being a component of phytomedicine. Since time immemorial, man is able to obtain from them a wondrous assortment of industrial chemicals. Plant-based natural constituents can be derived from different parts of the plant like bark, leaves, flowers, roots, fruits, seeds, etc., that is, any part of the plant may contain active components sometimes with variable concentrations [1]. Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanism or action is known [2]. Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions [3]. The public is becoming increasingly aware of problems with the over prescription and misuse of traditional anti-inflammatory drugs. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace [4].
Traditionally, people have been using powerful anti-inflammatory plants for thousands of years as part of their diet and pharmaceutical arsenal, which have been found to have anti-inflammatory properties [5].
Sudan with its unique variable climatic conditions possesses a huge wealth of flora, cultivated or wild. These found their way to folk medicine and are used widely and effectively for the treatment of various human and animal aliments, especially by natives in rural areas. Many of these medicinal plants were extracted and used successfully in treatment of various diseases that are considered as inflammation in nature e.g.: asthma, arthritis, rheumatism, fever, edema, infections, and related inflammatory diseases, but in most cases their effectiveness has never been evaluated nor received any comprehensive scientific evaluation. Little information is documented with reference to their other pharmacological effects. *Tribulus terrestris L* (Zygophyllaceae), is a taprooted herbaceous perennial plant that grows as a summer annual in colder climates and it is known for its use in the traditional medicine of many countries for the treatment of cardiac diseases, edema, eye trouble, skin itch and impotency [6]. In Sudanese traditional medicine the Infusion of the aerial parts are used as demulcent and renal nephritis [7].
Gas chromatography-mass spectroscopy (GC-MS) is one of the so-called hyphenated analytical techniques; it is used extensively in the medical and pharmacological fields. GC/MS was applied very early as it allowed determining a large number of metabolites, the main application area of plant metabolites are phytochemistry [8]. The identification of markers by primary metabolites using GC/MS analysis was advantageous because of its reproducibility and the use of a constructed database [9].

2. Materials and Methods

2.1. Plant material

T. terrestris (aerial parts) was collected from Kordofan and Nuba Mountain (Western Sudan). The plant was authenticated at the Medicinal and Aromatic Plant Research Institute, Sudan and voucher specimens deposited in the Herbarium.

2.2. Preparation of extract

The powdered plant material (300 g) was extracted by the cold maceration method with sufficient quantity of chloroform and 80% methanol at room temperature for 48 hr. The process of extraction was repeated twice to complete extraction. The extracts were filtered, concentrated under reduced pressure which afforded 28 g methanol and 25 g chloroform concentrated extracts. Concentrated extracts were subjected to anti-inflammatory activity by carrageenan induced rat paw edema. Methanolic extract showed good anti-inflammatory activity, aliquots of which were then re-dissolved in 100 mL of distilled water and fractionated with chloroform, ethyl acetate and *n*-butanol respectively and the process repeated with three different portions. Different fractions were collected and concentrated and subjected for anti-inflammatory activity. Chloroform fractions of *T. terrestris* showed good anti-inflammatory activity, then the chloroform extract was subjected to further purification by normal phase silica gel column chromatography which eluted with petroleum ether-chloroform and chloroform- ethyl acetate mixtures of increasing polarity to give different fractions (1, 2, 5, 7, 9 and 14).

2.3. GC-MS Analysis of anti–inflammatory active fractions

mass-spectrum GC-MS was conducted using Thermo Scientific TM DFS high resolution GC-MS Thermo fisher scientific Inc and different phytoconstituents were identified using NIST and WILLY mass spectrum database where the spectrum of the unknown components was compared with known components stored in the library.

3. Results and Discussion

The study on anti–inflammatory active fractions of *Tribulus terrestris* (1, 2, 5, 7, 9 and 14) revealed the presence of compounds corresponding to 3, 5, 4, 2, 5, 1 and 5 peaks when 10 µl applied, respectively at different R_f values (Table-1).The major peaks at the chromatograms of the fractions showed compounds with known anti-inflammatory activity and other with activities strongly related to inflammation process; e.g. the mono terpene lactone (−)-loliolide having immunosuppressive activity [10], benzenedicarboxylic acid and octadecadienoic acid derivatives having antitumor activities [11] [12] and the bis phenols having remarkable anti-inflammatory activity [13]. Consequently, this GC-MS fingerprinting may serve as reference to check the ability of other batches of chloroform extract of these plants for their inflammation suppressive activity.

Table 1: GC-MS profile of the Fractions (F1, F2, F4, F7, F9, F11 and F14)

Fractions	Peak	Compound name	Rt	Area	Height Area	Intensity %	Area %
F1	1	2-(Hydroxymethyl) Benzoic acid	11.8	48297	943487	47.9	11.0
	2	E-14-hexadecenal	22.3	39772	719422	39,4	9.1
	3	1,2-benzenedicarboxylic acid	33.2	100879	1616814	100.0	23.0
F2	1	2-(Hydroxymethyl) Benzoic acid	11.7	55498	1177803	12.3	3.9
	2	1-hexadecene (cas) centene	22.3	66650	1135354	14.7	4.7
	3	Phenol,4,4'-(1-methylethylidene)bis	26.4	452818	6078851	100.0	32.1
	4	1,2-benzenedicarboxylic acid	33.2	174160	2301669	38.5	12.3
	5	9,12-octadecadienoic acid(z,z)	44.0	48306	382887	10.7	3.4
F5	1	2-(Hydroxymethyl) Benzoic acid	11.7	81863	1679919	6.9	1.9
	2	(-)-loliolide	19,2	226976	3759695	19.2	5.3
	3	Hexadecanoic acid, methyl est	21.1	1182436	11971308	100.0	27.4
	4	Octadecanoic acid,2-hydroxy-1	32.9	307954	2947980	26.0	7.1
F7	1	E-15-heptadecenal	22.3	103425	1866708	28.0	7.7
	2	1,2-benzenedicarboxylic acid	33.2	368805	5967818	100.0	27.3
F9	1	2-(Hydroxymethyl) Benzoic acid	11.8	72429	1373975	56.7	11.9
	2	Bis-(3,5,5-trimethylhexyl)ether	19.3	39654	390292	31.0	6.5
	3	E-14-hexadecenal	22.3	62478	1097271	48.9	10.3
F9	4	1-heptadecene (cas)hexahyd	26.8	27233	445956	21.3	4.5
	5	1,2-benzenedicarboxylic acid	33,2	127734	2019260	100.0	21.0
F11	1	(-)-loliolide	19.3	3265012	52665824	100.0	58.1
F14	1	2-(Hydroxymethyl) Benzoic acid	11.8	68225	1294852	46.3	10.7
	2	(+)-isololiolide	18.9	18186	309187	12.3	2.9
	3	1-heptadecene (cas) hexahyd	22.3	44565	786365	30.2	7.0
	4	Eicosyl acetate	26.9	16672	263115	11.3	2.6
	5	1,2-benzenedicarboxylic acid	33.2	147469	2355720	100.0	23.1

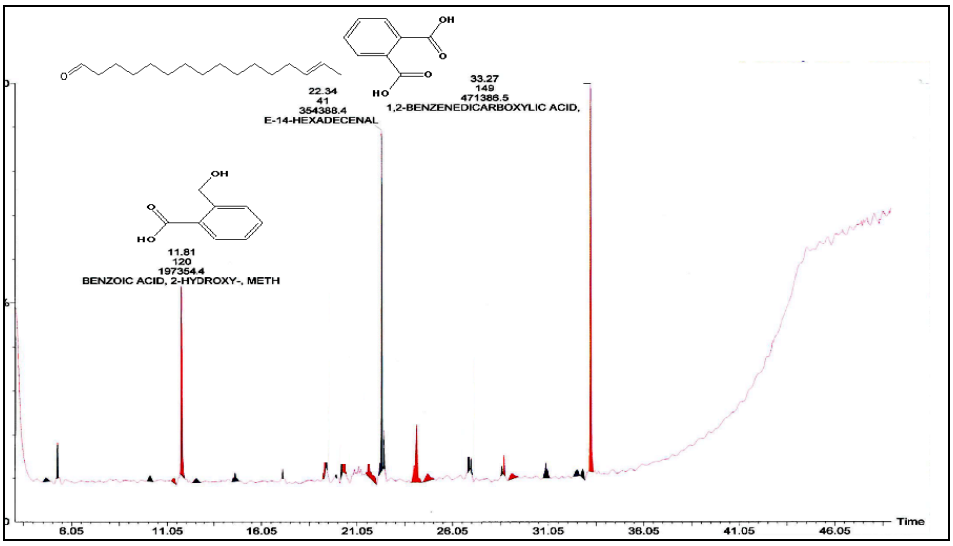

Fig 1: GC-MS of F1

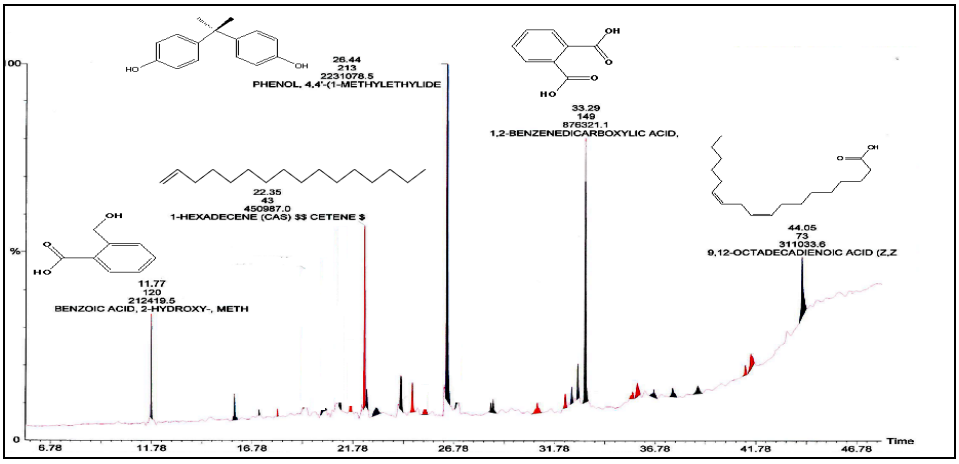

Fig 2: GC-MS of F2

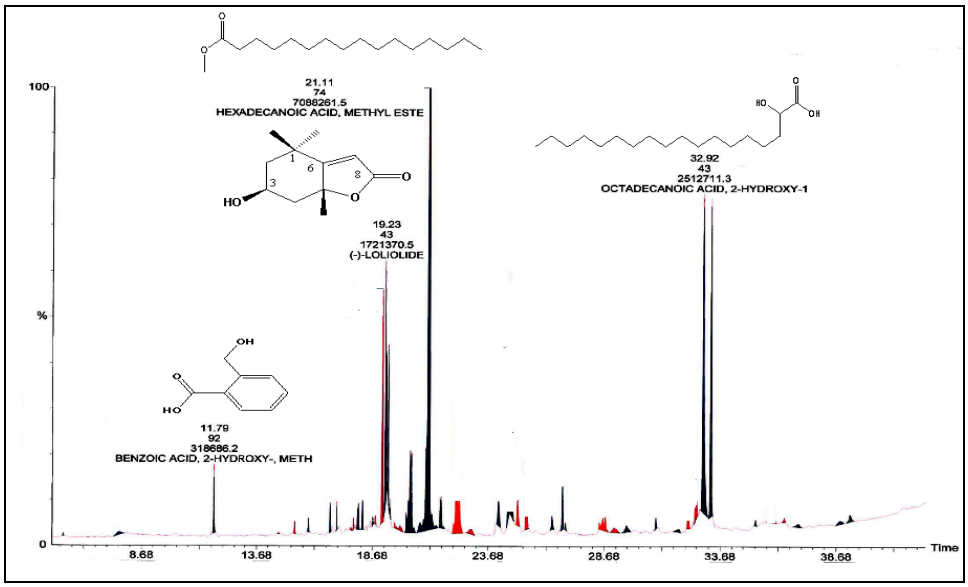

Fig 3: GC-MS of F5

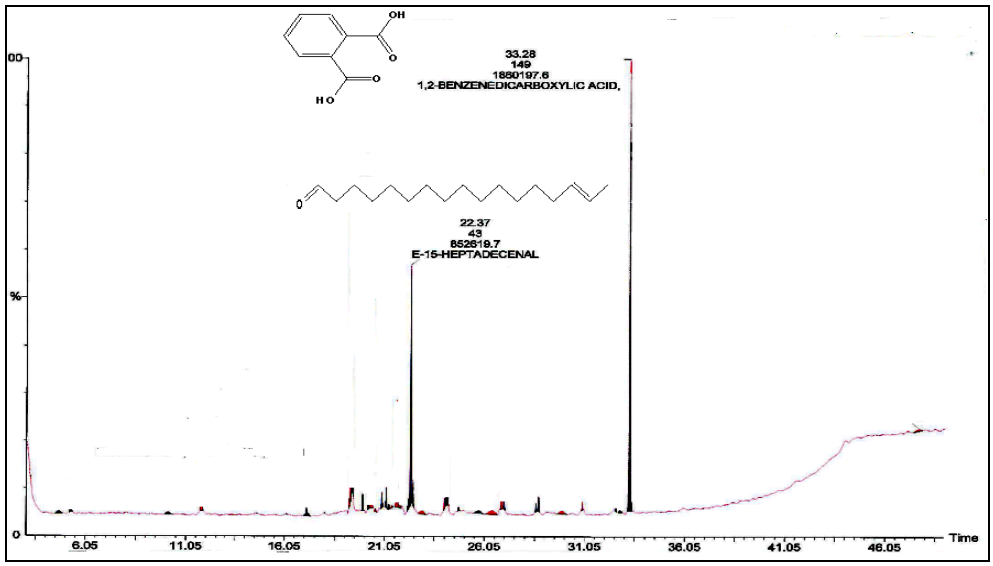

Fig 4: GC-MS of F7

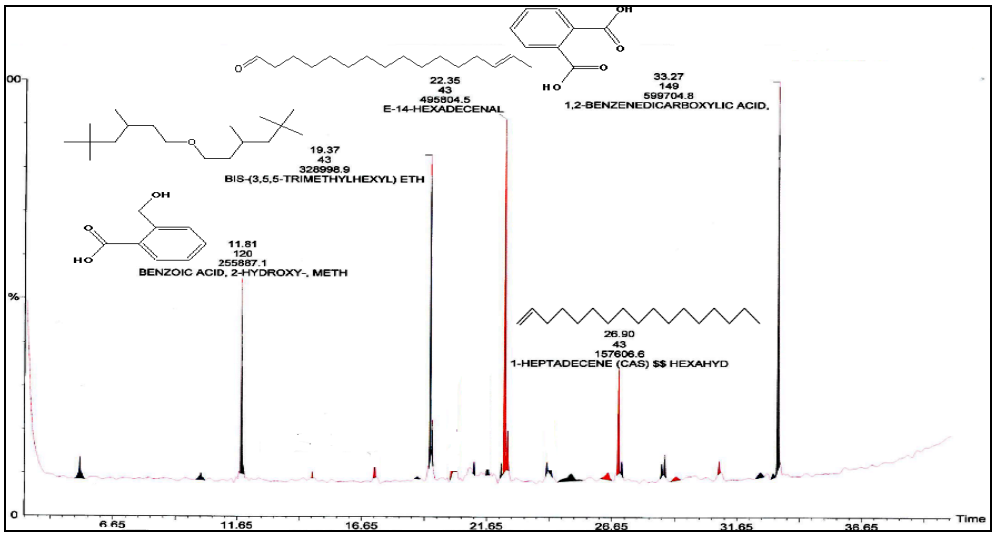

Fig 5: GC-MS of F9

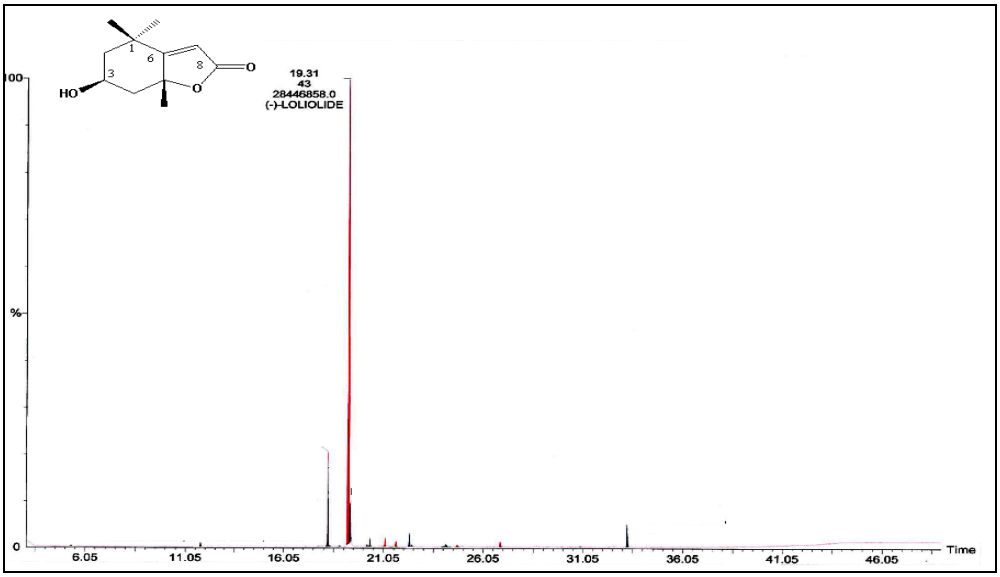

Fig 6: GC-MS of F11

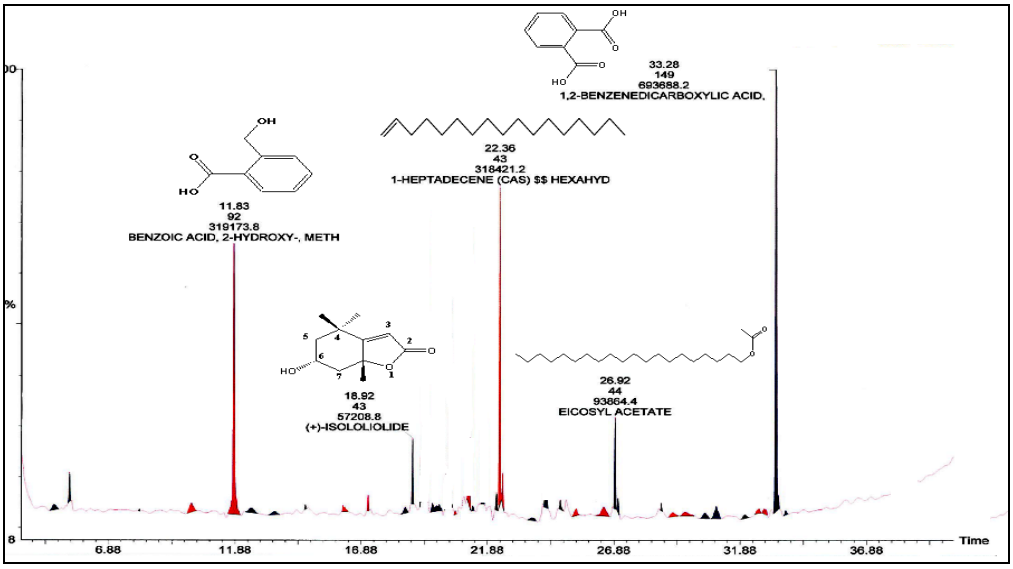

Fig 7: GC-MS of F14

4. Conclusion

This study represents an advanced methodology of the qualitative analysis of complex matrix, such as herbal medicine by using high resolution GC/MS techniques where the identification of compounds was ascribed. Chemical content is the driving force for the medicinal value of the plant. This promotes the use of such new techniques as GC/MS to assure not only identity, but it may also help in assessing of quality against adulterant and act as a biochemical marker for those medicinally important plants in the pharmaceutical industry and the systematic studies of plants.

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