



Research article

Effect GC-MS Analysis and Antimicrobial Activity of Sudanese Cucumis melo L. (Cucurbitaceae) Fixed Oil

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Received – 20 November 2016, Revised - 25 December 2016, Accepted – 30 December 2016 (DD-MM-YYYY)

Refer This Article

Abdel Karim M, Asma H, Kamal M S, 2016 Effect GC-MS Analysis and Antimicrobial Activity of Sudanese Cucumis melo L. (Cucurbitaceae) Fixed Oil. Journal of Medical, Pharmaceutical, and Allied Sciences, V 5 - I 6, Pages -425 – 427. Doi: <https://doi.org/10.55522/jmpas.V5I6.0112>.

ABSTRACT

The present study was designed to investigate the chemical constituents of Sudanese Cucumis melo seed oil and to evaluate its potential antimicrobial activity. 28 components were detected by GC-MS analysis. Major constituents are: methyl 10-trans,12-cis-octadecadienoate (49.28%), 9-octadecenoic acid methyl ester(19.40%), hexadecanoic acid methyl ester(15.92%), methyl stearate(11.22%).

The antimicrobial activity of the oil was evaluated via cup plate agar diffusion bioassay against six standard human pathogens (Gram positive: Staphylococcus aureus and Bacillus subtilis; Gram negative : Escherichia coli and Pseudomonasa aeruginosa and the fungi Candida albicans and Aspergillus niger) . The oil showed different antimicrobial responses against test organisms. It gave significant activity against the fungus: Candida albicans and partial activity against Staphylococcus aureus.It seems that the oil is a lead for further optimization.

Keywords: Cucumis melo, Fixed oil, Cucumis melo, Fixed oil, activity.

INTRODUCTION

The Cucurbitaceae family comprises hundreds of wild and cultivated varieties. This family includes : gourds, melons , cucumbers, pumpkins and squashes. Cucurbitaceae is distributed in tropical and subtropical regions.

Plants of this family produce fruits with edible pericarp which could be important source of minerals, fiber, β- carotene (provitamin A) and vitamin C: Seeds are diuretic, beneficial for enlargement of prostate gland, chronic eczema.Fruits are tonic and laxative.The economic value of melons is quite substantial.

Cucumis melo L. (melon) genotypes differ largely in morphological and biochemical traits and intraspecific classification of such variability has been difficult.Still most taxonomists rely on the work of Noudin. Ninety-one accessions of *Cucumis melo* were investigated for oil content and oil characteristics. Oil content ranged from 12.5- 39.1%; iodine value 106.0-124%. GC analysis revealed the presence of linoleic , oleic , palmitic and stearic acids.

Cucumis melo extract was evaluated for antioxidant, anti-inflammatory and analgesic properties. The extract inhibited the production of superoxide anion in a dose – dependant manner. It also showed anti- inflammatory and analgesic properties. In another study, the therapeutic potential of traditionally used seeds was evaluated for antioxidant, analgesic and anti-inflammatory activities. Results showed potent anti-inflammatory, antioxidant and analgesic activity.

Yanty *et.al.* evaluated the physicochemical characteristics of *Cucumis melo* seed oil. The oil was predominated with linoleic acid (69.0%) followed by oleic acid (16.8%) and palmitic acid (8.4%)^[1, 2].

MATERIALS AND METHODS

Plant material

The fruits of *Cucumis melo* were purchased from the local market – Omdurman, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length; 0.25mm diameter; 0.25 μm, thickness) was used for GC- MS analysis.

Test organisms

Cucumis melo oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table 1: Test organisms

Microorganism	Type
<i>Bacillus subtilis</i>	G+ve
<i>Staphylococcus aureus</i>	G+ve
<i>Pseudomonas aeruginosa</i>	G-ve
<i>Escherichia coli</i>	G-ve
<i>Aspergillus niger</i>	fungus
<i>Candida albicans</i>	fungus

Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. (2ml) of supersaturated sodium chloride were added, then (2ml) of n- hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5µl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis

Cucumis melo fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25 µm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3 [3].

Table 2: Oven temperature program

Rate	Temperature (C)	Hold time (min .-1)
-	60.0	0.00
10.0 0	300.0	0.00

Table 3: Chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C

Preparation of bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline to produce a suspension containing about 10⁸-10⁹ colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism 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 in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions

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

Testing for antimicrobial activity

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antimicrobial activity. (2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates [4, 5].

RESULTS

GC-MS analysis of *Cucumis melo* fixed oil

GC-MS analysis of *Cucumis melo* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 28 components. The typical total ion chromatograms (TIC) [6-7].

Octadecenoic acid methyl ester (19.40%)

EI mass spectrum of 9- octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.530 in total ion chromatogram, corresponds to M⁺[C₁₉H₃₆O₂]⁺, while the peak at m/z 266 accounts for loss of a methoxyl function.

Oleic acid (9-octadecenoic acid) is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil.

Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is also used as soldening flux in stained glass work. Oleic acid is employed as emollient.

Hexadecanoic acid methyl ester (15.92%)

Mass spectrum of hexadecanoic acid methyl ester is depicted. The peak at m/z 270, which appeared at R.T. 15.804 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 is attributed to loss of a methoxyl function.

Hexadecanoic acid (palmitic acid) is a saturated fatty acid. It is wide-spread in plants and humans. This acid is produced first during the synthesis of fatty acids and is considered as precursor of long-chain fatty acids. Palmitic acid is a major lipid component of human breast milk^[14,15].The acid finds applications in soaps and cosmetics industries. It is also used in food industry.

Methyl stearate (11.22%)

Mass spectrum of methyl stearate. The peak at m/z 298, which appeared at R.T. 17.717 corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267 corresponds to loss of a methoxy function.

DISCUSSION

The normal chick embryo has shown devastating changes after the administration of Sodium Valproate. The drug administration resulted in a dose dependent massive reduction in the cells of all the layers of cerebrum as compared to the number from control. Sodium Valproate induced cytotoxicity manifested by dose dependent disturbance of cell-cycle resulted in an overall depression of proliferation activity clearly associated with the occurrence of malformations and embryonic death. The histological study of normal chick embryo brain- cerebellum tissue was compared with the drug administered chick embryo brain-cerebellum tissue at same age, which showed a gross loss in cellularity.

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