

**IDENTIFICATION OF CHEMICAL COMPOUNDS FROM THE FRUITS OF
CUCUMIS TRIGONUS ROXB. BY GC-MS ANALYSIS**

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Abstract

Cucumis trigonus Roxb. (Fam. Cucurbitaceae) commonly known as “Thummittikai” in Tamil, and “Vishala” in Sanskrit is reported to possess a number of medicinal values. In Indian Traditional Systems of Medicine the fruit pulp of the plant is used as expectorant, liver tonic, stomachic and purgative. The present study has been carried out for the identification of the phytochemicals present in the fruits of *Cucumis trigonus* using GC-MS analysis. The GC-MS analysis of the ethanolic extract of *Cucumis trigonus* showed the presence of fourteen phytochemical constituents. The major chemical constituents are: demeclocycline, glycodeoxycholic acid, 3 α ,7 α ,12 α -trihydroxycoprostanic acid, chlortetracycline, azafrin methyl ester, giganteumgenin N, phorbol 12,13-dihexanoate, astaxanthin, tetrahydrospirilloxanthin. These different phytochemical constituents have been found to possess a wide range of activities. Hence *Cucumis trigonus* may serve as candidate for the discovery of new drugs in the treatment of liver disorder.

Keywords: *Cucumis trigonus*, GC-MS, phytochemical constituents, bile acids, liver disorder

1. Introduction

Cucumis trigonus known as ‘Bitter gourd’ in English belonging to the Cucurbitaceae family is indigenous to India, Ceylon, Malaya, North Australia, Afghanistan and Persia¹⁻². It is used for various ailments in Indian Traditional Systems of Medicine. The fruits of *cucumis trigonus* are reported to be useful in treating leprosy, fever, jaundice, diabetes, cough, bronchitis, anaemia, constipation and other abdominal disorders³⁻⁴. Alcoholic extract of *cucumis trigonus* fruit is shown to possess various activities such as anabolic activity⁵, analgesic, anti-inflammatory and diuretic activity⁶. Recently it’s proteolytic and serine protease activity has been reported⁷⁻⁸. However there is no report on the chemical investigation of this potent medicinal plant. In the present investigation the GC-MS analysis of the ethanolic extract of the fruits of *Cucumis trigonus* has been carried out.

2. Material and Methods

2.1 Plant materials: *Cucumis trigonus* was collected in the month of March from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai- 600 045, Tamil Nadu.

A voucher specimen (MSU/PHAR/HER–140) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli -627 012, Tamil Nadu.

2.2 Extraction of plant material: The fruits were cut into pieces, shade-dried at room temperature and powdered. The dried fruit powder (500 gm) was successively extracted using petroleum ether (40°- 60° C), benzene, chloroform, ethanol and water by using a Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. The ethanolic extract of the fruits has been used for the GC-MS analysis.

2.3 GC-MS Analysis

2.3.1 Preparation of extract: 2 μ l of the ethanolic extract of the fruits of *Cucumis trigonus* was employed for GC-MS analysis⁹.

2.3.2 Instruments and chromatographic conditions: GC-MS analysis of the extracts was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 mm x 0.25 mm ID x 1 μ m film, composed of 100 % Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 μ l was employed (split ratio of 10:1); injector temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

2.3.3 Identification of photochemical constituents: Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the unknown component was compared with that of the known components stored in the NIST library. The name, molecular weight and structure of the phytochemical constituents of the ethanolic extract were ascertained¹⁰⁻¹¹.

3. Results and Discussion

GC-MS chromatograms of the ethanolic extract of *Cucumis trigonus* showed fourteen peaks indicating the presence of fourteen compounds and are presented in Fig.1. When the mass spectra of these fourteen peaks were compared with those of the compiled data for known compounds, nine major phytochemical constituents were identified and are presented in Table.1. with their retention time (RT), molecular formula, molecular weight and peak area (%). The mass spectra of the nine major constituents are presented in Fig.2 to Fig. 10. Antibiotic compounds such as demeclocycline (31.37 %), and chlortetracycline (9.80 %), and bile acids such as glycodeoxycholic acid (13.73 %), and 3 α ,7 α ,12 α -trihydroxycoprostanic acid (9.80 %), are recorded predominantly. Three carotenoid compounds, viz. azafrin methyl ester (7.84 %), tetrahydrospirilloxanthin (7.84 %), and astaxanthin (5.88 %). and a steroidal compound, giganteumgenin N (11.76 %), are also present considerable amounts, phorbol 12,13-dihexanoate (1.96 %) is present in least quantity. The various phytochemical constituents which contribute to the medicinal activity of *Cucumis trigonus* are presented in Table. 2.

4. Conclusion

The nine phytochemical constituents are responsible for the various pharmacological actions. The ethanolic extract of the fruits of *Cucumis trigonus* is reported to possess anabolic activity⁵, analgesic, anti-inflammatory and diuretic activity⁶. The proteolytic and serine protease activity has also been reported^{7,8}. GC-MS analysis of the ethanolic extract showed the presence of bile acids such as glycodeoxycholic acid, and 3 α ,7 α ,12 α -trihydroxycoprostanic acid, which may be responsible for the liver disorder curing effect. The traditional use of *Cucumis trigonus* fruits for liver disorder by medical practitioners has been scientifically validated. In conclusion *Cucumis trigonus* contains biologically active compounds that may serve as candidate for the discovery of new drugs in the treatment of liver disorder.

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Table.1. Phytocomponents identified in the ethanolic extract of the fruits of *Cucumis trigonus* by GC-MS analysis.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	5.54	Demeclocycline	C ₂₁ H ₂₁ ClN ₂ O ₈	464	31.37
2.	7.62	Glycodeoxycholic acid	C ₂₆ H ₄₃ NO ₅	449	13.73
3.	8.16	3 α ,7 α ,12 α -Trihydroxycoprostanic acid	C ₂₇ H ₄₆ O ₅	450	9.80
4.	13.10	Chlortetracycline	C ₂₂ H ₂₃ ClN ₂ O ₈	478	9.80
5.	17.86	Azafrin methyl ester	C ₂₈ H ₄₀ O ₄	440	7.84
6.	24.54	Giganteumgenin N	C ₃₀ H ₅₀ O ₆	506	11.76
7.	25.57	Phorbol 12,13-dihexanoate	C ₃₂ H ₄₈ O ₈	560	1.96
8.	32.43	Astaxanthin	C ₄₀ H ₅₂ O ₄	596	5.88
9.	34.42	Tetrahydrospirilloxanthin	C ₄₂ H ₆₄ O ₂	600	7.84

Table.2. Activity of phytochemicals identified in the ethanolic extract of the fruits of *Cucumis trigonus* by GC-MS analysis.

S. No	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1.	5.54	Demeclocycline	$C_{21}H_{21}ClN_2O_8$	464	31.37	Antibiotic compound	Antimicrobial.
2.	7.62	Glycodeoxycholic acid	$C_{26}H_{43}NO_5$	449	13.73	Bile acid	Detergent to solubilise fat.
3.	8.16	3 α ,7 α ,12 α - Trihydroxycoprostanic acid	$C_{27}H_{46}O_5$	450	9.80	Bile acid	Used in digestive activity.
4.	13.10	Chlortetracycline	$C_{22}H_{23}ClN_2O_8$	478	9.80	Antibiotic compound	Antimicrobial.
5.	17.86	Azafrin methyl ester	$C_{28}H_{40}O_4$	440	7.84	Carotenoid ester compound	Antioxidant, Anti-inflammatory, Antiarthritic Lowering hypertension, Anticancer, Drug for eye problems, Natural color pigment.
6.	24.54	Giganteumgenin N	$C_{30}H_{50}O_6$	506	11.76	Steroid compound	Antimicrobial, Anti-inflammatory, Antiarthritic, Anti asthma, Diuretic.
7.	25.57	Phorbol 12,13-dihexanoate	$C_{32}H_{48}O_8$	560	1.96	Phorbol ester	Antimicrobial, Anti-inflammatory.
8.	32.43	Astaxanthin	$C_{40}H_{52}O_4$	596	5.88	Carotenoid compound	Antioxidant, Anti-inflammatory, Antiarthritic, Lowering hypertension, Anticancer, Drug for eye problems Natural color pigment
9.	34.42	Tetrahydrospirilloxanthin	$C_{42}H_{64}O_2$	600	7.84	Carotenoid compound	Antioxidant, Anti-inflammatory, Antiarthritic, Lowering hypertension, Anticancer, Drug for eye problems Natural color pigment

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

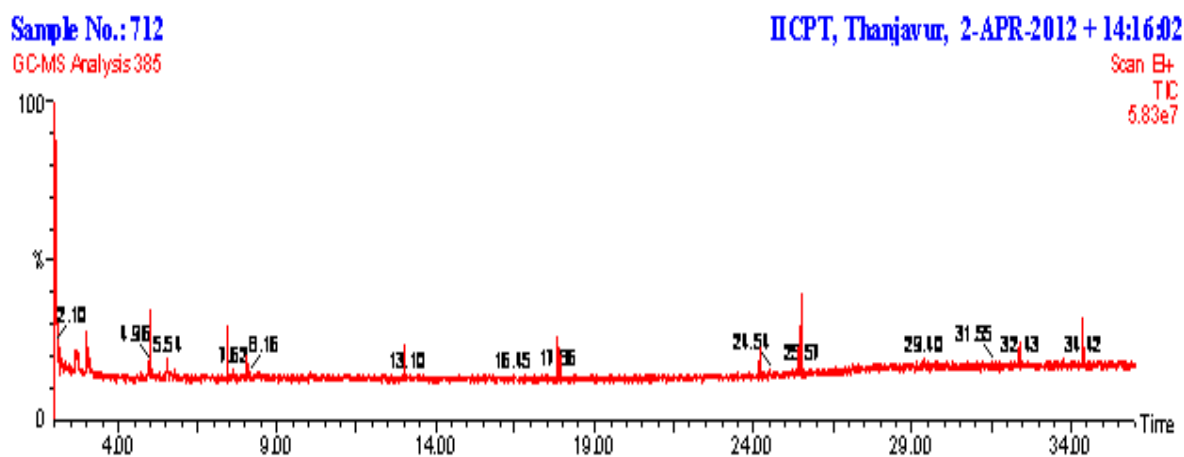
Fig.1. GC-MS Chromatogram of ethanolic extract of the fruits of *Cucumis trigonus*

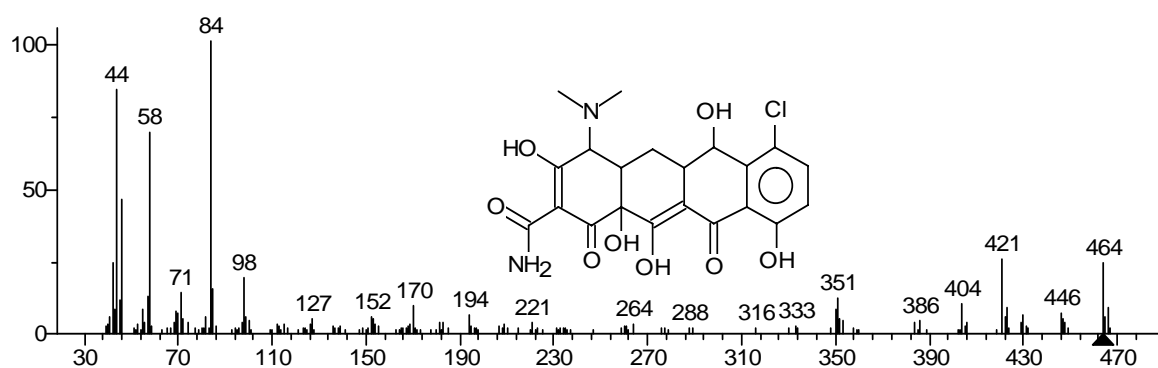
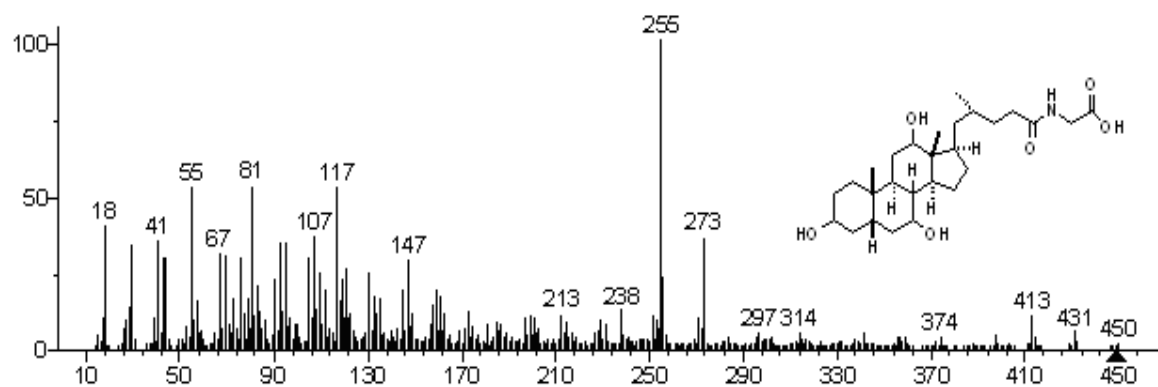
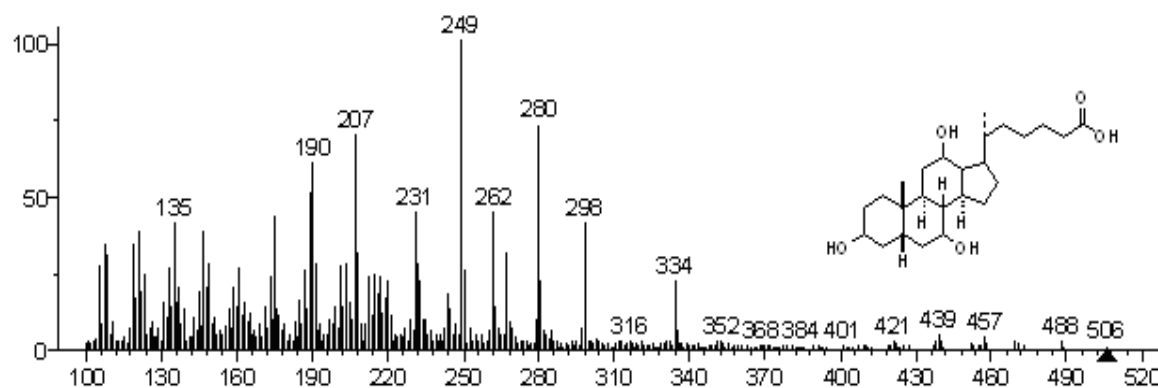
Fig.2. Mass spectrum of Demeclocycline (RT: 5.54)**Fig.3. Mass spectrum of Glycodeoxycholic acid (RT: 7.62)****Fig.4. Mass spectrum of 3 α ,7 α ,12 α -Trihydroxycoprostanic acid (RT: 8.16)**

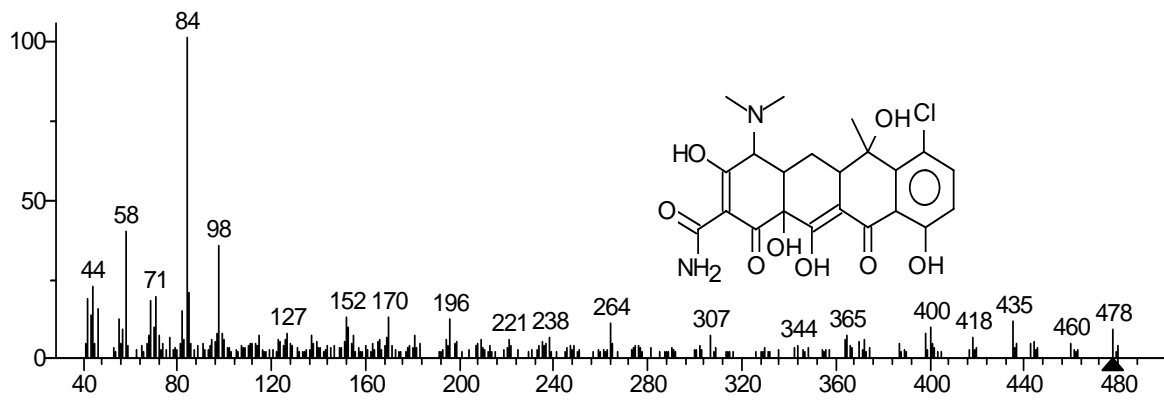
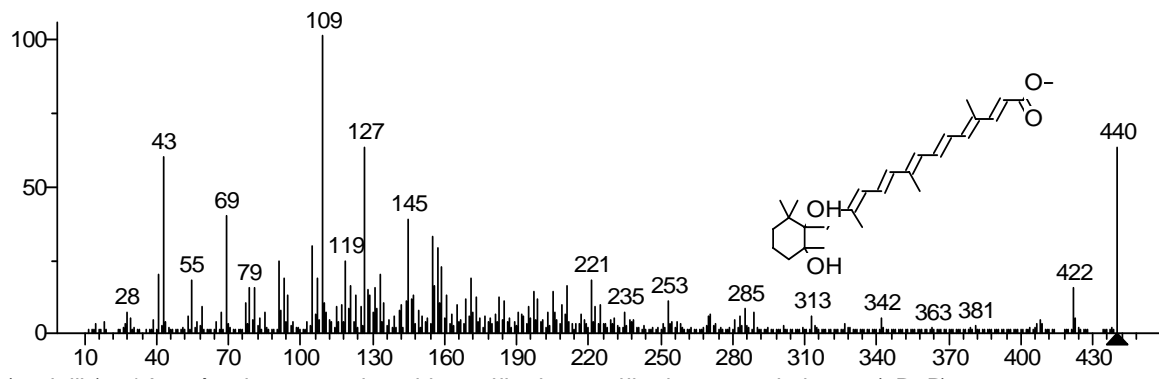
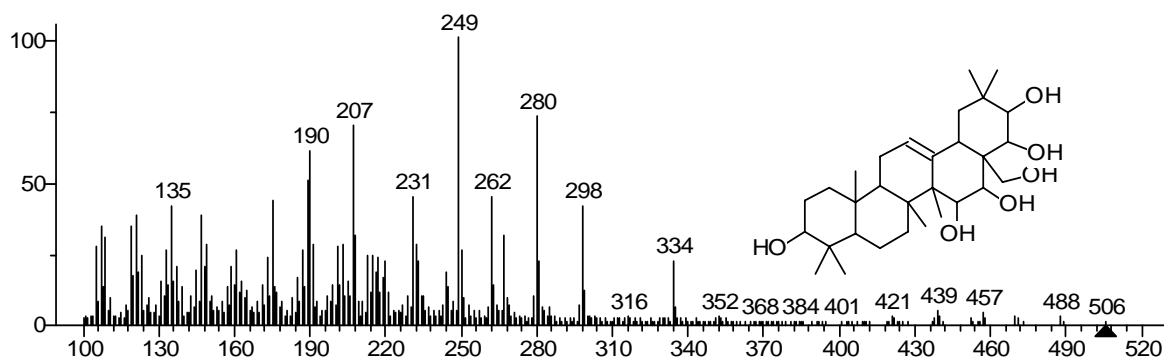
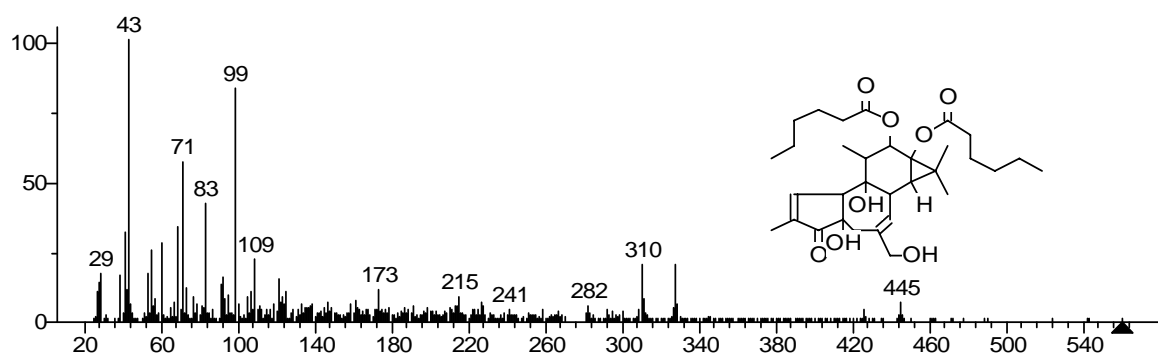
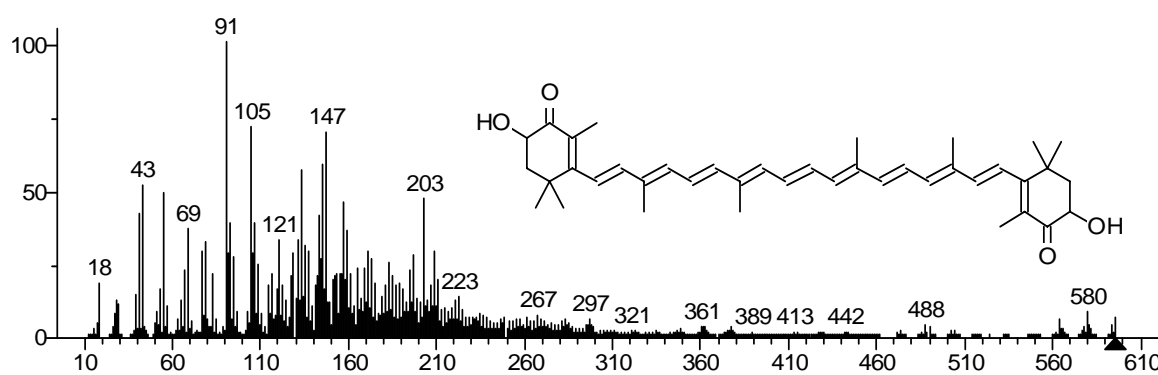
Fig.5. Mass spectrum of Chlortetracycline (RT: 13.10)**Fig.6. Mass spectrum of Azafrin methyl ester (RT: 17.86)****Fig.7. Mass spectrum of Giganteumgenin N (RT: 24.54)**

Fig.8. Mass spectrum of Phorbol 12,13-dihexanoate (RT: 25.57)**Fig.9. Mass spectrum of Astaxanthin (RT: 32.43)****Fig.10. Mass spectrum of Tetrahydrospirilloxanthin (RT: 34.42)**