

# GC-MS Analysis Of Isolated Ethyl Acetate Extract Of Citrus Sinuses Peel

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**Abstract:** Orange (*Citrus sinuses*) is well known for its nutritional and medicinal properties through the world. With multiple active molecular components, citrus peels and derived extracts have demonstrated potent efficacious properties against various cancers due its abundant flavonoids content. The orange peel was shadow dried for 7-10 days and the residue was extracted by using Ethyl acetate overnight (16 hours) at RT in a magnetic stirrer at 2000 RPM. The extract was evaporated to dryness and reconstituted in 1ml of Ethyl Acetate. 1ml of the concentrated Ethyl Acetate extract was loaded onto the silica gel column (300mm x 10mm). Elution was done using 25ml of ethyl acetate and 5ml fractions were collected. All these fractions were pooled and analyzed using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library for identification of probable compounds and detailed insight about the phytochemical profile present in the purified sample which could be exploited for the development of plant based drugs. Results showed that the purified Ethyl Acetate Extract from Orange peel contains Camphor and Eucalyptol.

**Keywords:** Citrus Sinuses, Silica gel Column, Ethyl Acetate, GC-MS, Camphor, Eucalyptol.

## I. INTRODUCTION

Recently, the development of natural-product-based and therapeutically sound anti-cancer agents have gained popularity in the fields of functional and medical foods, which may exhibit advantages of minimal toxicity and multiple active molecular components. Citrus peel or its extract has been reported to have potent pharmacological activities and health benefits because of abundant flavonoids present in citrus fruits, particularly in the peels [1]. The biological properties of flavonoids include antioxidant, anti-inflammatory, anti tumoral, antiviral and antibacterial, as well as a direct cytoprotective effect on coronary and vascular systems, the pancreas, Breast and the liver [2, 3, 4]. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [5, 6, 7]. Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with

the detection feature of mass spectrometry to identify different substances within a test sample [8].

## II. MATERIALS AND METHOD

Citrus sinuses were purchased from super market, Silica gel column (300mm x 10mm), Thermo Scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000

### PREPARATION OF EXTRACT

The orange peel was shadow dried for 7-10 days. 100g of powdered orange peel was weighed and extracted with 500ml of nhexane for 4 hours at RT in a magnetic stirrer maintained at 2000rpm. The extract was filtered using 0.4µM filter and the residue was air dried at RT. The residue was extracted by using 500ml of Ethyl acetate overnight (16hours) at RT in a magnetic stirrer at 2000 RPM. The extract was evaporated to dryness and reconstituted in 1ml of Ethyl Acetate [9, 10]. This

1ml of the concentrated Ethyl Acetate extract was loaded onto the silica gel column (300mm x 10mm). The column was washed with 25 ml of hexane and 5ml fractions were collected [11]. Elution was done using 25ml of ethyl acetate and 5ml fractions were collected [12]. Then the 5ml fractions were pooled and checked for GC-MS Analysis.

### SAMPLE PREPARATION

Concentrated extract (10 $\mu$ L) were reconstituted in 990  $\mu$ L Ethyl acetate. Ethyl acetate extracts (1  $\mu$ L) were injected for GC-MS analysis.

### GC-MS ANALYSIS

GC-MS analysis of the Purified Ethyl Acetate extract of Citrus sinuses peel of was performed

On Thermo Scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000

The conditions were as follows:

Method type:	: Acquisition - General
MS transfer line temperature :	300 °C
Ion source temperature :	230 °C
Ionization mode :	EI
Temperature Program :	Initial 40° C hold for 2 mins Ramp at 5° C to 240° C Ramp at 20° C to 300°C hold for 2 mins
Flow Rate :	1 ml/min
Carrier Gas :	Helium
Column Used :	Agilent DB 5MS (30 meter X 0.25 mm)

### III. DATA INTERPRETATION AND IDENTIFICATION OF COMPONENTS

The sample subjected to GCMS and the total separated peaks are shown in Figure 1 and 2. Extracted ion chromatograms were obtained from all the major peaks (Figure 3-4). The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the sample. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

The mass of compounds and fragments matching with NIST database and chemical structures of major compounds matched with NIST Library is shown in Figure 5-6.

### IV. RESULTS AND DISCUSSION

The results shown that the purified Ethyl Acetate fraction contains compounds called Eucalyptol and Camphor. The health benefits of camphor includes a stimulant, antispasmodic, antiseptic, decongestant, anesthetic, sedative,

and nervous pacifier. It also acts as an ant neuralgic, anti-inflammatory, disinfectant, and insecticide. Eucalyptol is an ingredient in many brands of mouthwash and cough suppressant, as well as an inactive ingredient in body powder. Eucalyptol is used as an insecticide and repellent. It is also having anti-Allergic and Anti Inflammatory properties.

### V. CONCLUSION

GC-MS is an advanced technique and it has broad range of applications that caters to academic research, quality control as well as industrial applications. From these results, it could be concluded that Citrus sinuses peel contains various bio-active compounds and opens a new area of investigation of individual components. Evaluation of pharmacological activity is under progress. This versatile analytical technique could be explored for better prospects in future.

### CHROMATOGRAMS

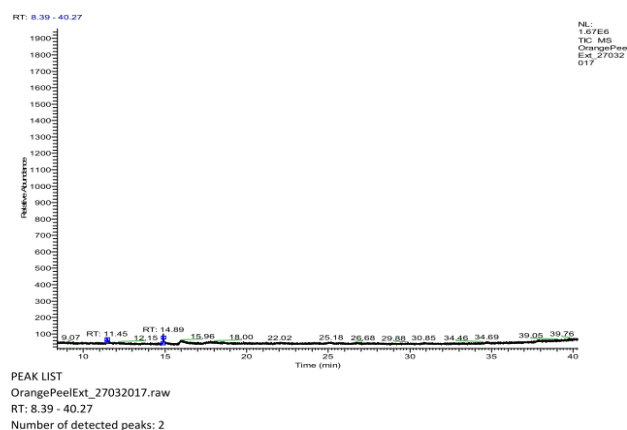


Figure 1: GCMS Total Ion Chromatogram

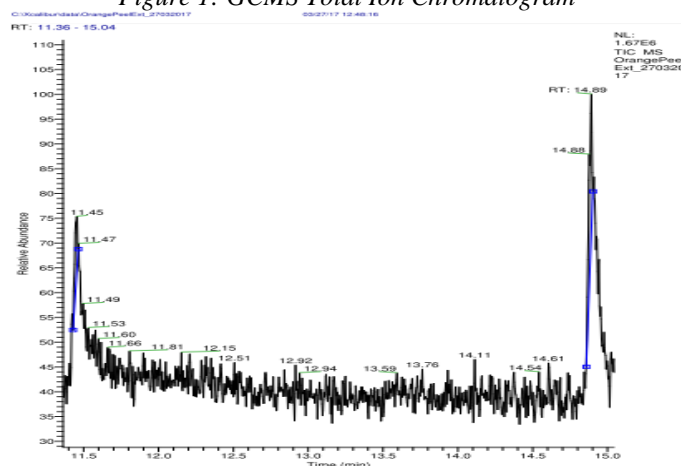


Figure 2: GCMS Total Ion Chromatogram

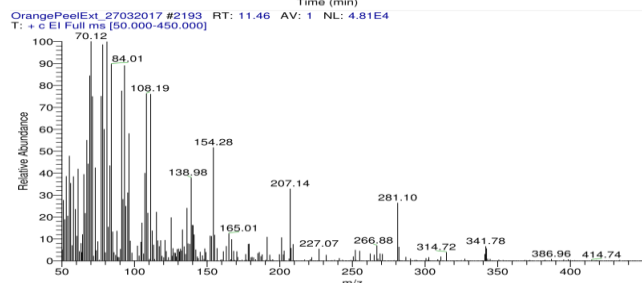


Figure 3: Extracted ion chromatogram of 11.46 min

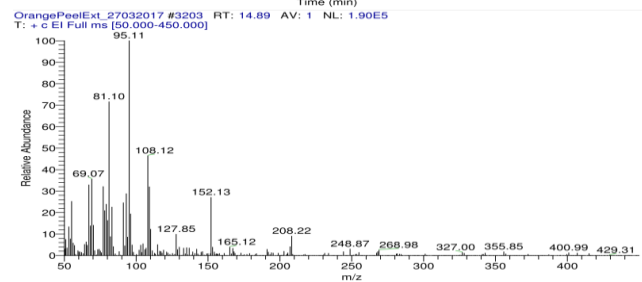
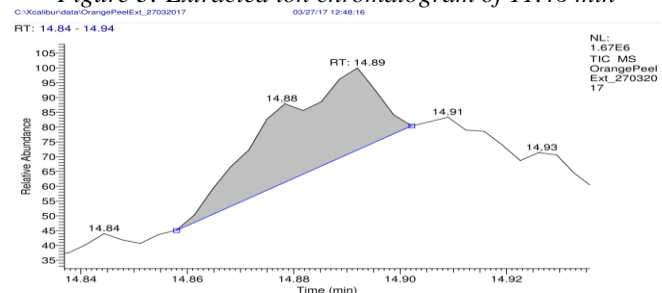


Figure 4: Extracted ion chromatogram of 14.89 min

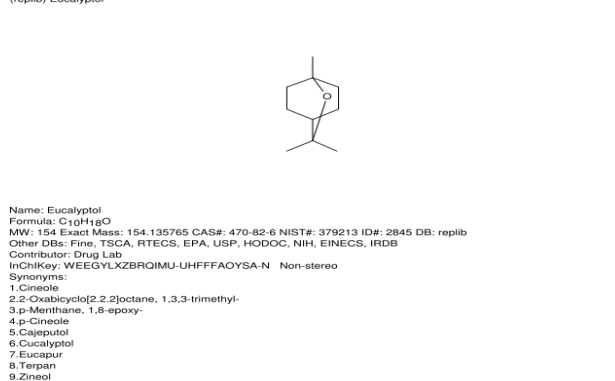
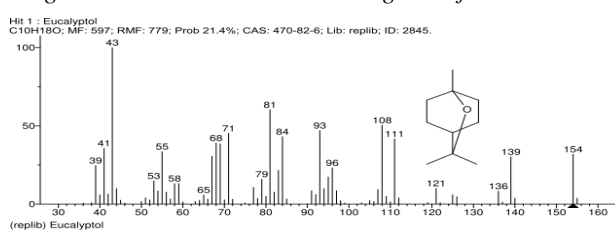


Figure 5: NIST library Hits for 11.45 min peak

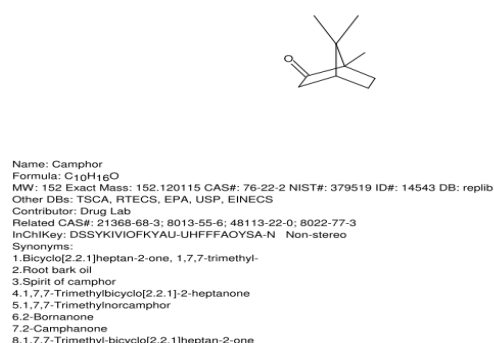
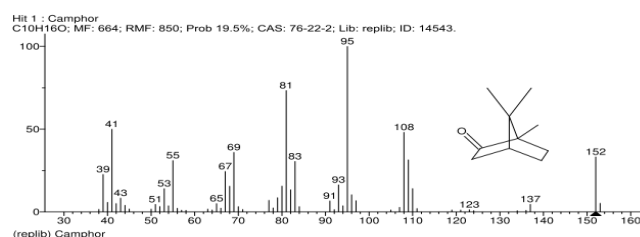


Figure 6: NIST library Hits for 14.89 min peak

## REFERENCES

- [1] Michiko Suzawa, Limin Guo, Min-Hsiung Pan, Chi-Tang Ho, Shiming Li. (2014). In vivo anti-carcinogenic property of a formulated citrus peel extract, Functional Foods in Health and Disease; 4(3):120- 129.
- [2] Parle Milind and Chaturvedi Devi, Orange Range of Benefits, ISSN 2230-8407, Vol-3, pp 56-79.
- [3] W.W.Widmer and A.M.Montanari, Citrus waste stream as a source for phytochemicals. (1994), Vol.107, pp 284-288.
- [4] ZunliKe, Yupan, Xiaodan Xu, Chao Nie, Zhiqin Zhou, CitrusFlavonoids and Human Cancer, Vol. 3, No.5, pp 341-351.
- [5] Robertson DG. (2005). Metabonomics in toxicology: A review. Toxicol. Sci; 85:809-22.
- [6] Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. (2004). Metabolite profiling: From diagnostics to Systems biology. Nat Rev Mol Cell Biol; 5:763-9.
- [7] Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. (2005). Metabolic footprinting and systems biology: The medium is the message. Nat Rev Microbiol; 3:557-65.
- [8] Sahil K, Prashant B, Akanksha M, Premjeet S, Devashish R (2011) GC-MS: Applications. International Journal Pharma & Biological Archives 2:1544-1560.
- [9] Yoshiaki MIYAKE, Kanefumi YAMAMOTOI and ToshikoOsAWA, Isolation of Eriocitrin (Eriodictyol7-rutinoside) BURM. f.) and Its Antioxidative Activity from Lemon Fruit (Citrus on BRUM.f) and its Anti Oxidative Activity, 3(1), pp84-89.
- [10] Bushra Sultana, Farooq Anwar and Muhammad Ashraf, Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts, 14, pp 2167-2180.
- [11] Yoichi Nogata, Hideaki Ohta, Toshinao Ishii and Keizo Sekiya. (2007). Isolation of eriocitrin (eriodictyol 7-

Orutinoside) as an arachidonate lipoxygenase inhibitor from Lumie fruit (Citrus lumia) and its distribution in Citrus species, 87, pp 82–89.

[12]Corradocaristi, ErsiliaBellocco, Vincenza Panzer, Giovanni Toscano, Rosa Vadala and Ugo Leuzzi, Flavonoids Detection by HPLC-DAD- MS-MS in Lemon juices from Sicilian Cultivars, 51, 12, pp 3528-3534.

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