



## GC-MS analysis of bioactive compounds in the methanolic extract of *Kalanchoe pinnata* fresh leaves

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### ABSTRACT

Secondary metabolites and different phytochemicals are present in a great deal in the plant *Kalanchoe pinnata* belonging to family Crassulaceae. However, few reported the scientific data regarding GC-MS analysis of methanolic extract of *Kalanchoe pinnata* fresh leaves till date during literature review. The study was aimed to verify the phytochemical in the methanolic extract of *Kalanchoe pinnata* fresh leaves. Methanolic extraction of fresh leaves *Kalanchoe pinnata* was performed and GC-MS analysis data was provided by IIT, Powai, Mumbai. Phytochemical investigation was carried out to identify the possible components from *Kalanchoe pinnata* fresh leaves by GC-MS. It has revealed only major three peaks of GC-MS spectrum that phytol, squalene and 5-oxotetrahydrofuran-2,3-dicarboxylic acid dimethyl ester. Other two phytocomponents remained unidentified and still reported no activity. Squalene is generally reported for anticancer, antioxidant and additional activities. From the results, it is firstly reported that phytol and squalene present as major bioactive compounds in methanolic extract of *Kalanchoe pinnata* fresh leaves.

**Keywords:** GC-MS analysis, phytochemicals, *Kalanchoe pinnata*

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### INTRODUCTION

*Kalanchoe pinnata* plant is rich source of phytochemicals with interesting biological activities such as antioxidant [1], anticancer [2] activities. The scientific data regarding GC-MS analysis of methanolic extract of *Kalanchoe pinnata* fresh leaves has few reported till date during literature review. Therefore, in our present study, it was undertaken into consideration to characterize GC-MS chromatogram for evaluation of bioactive phytochemicals from methanolic extract of the plant.

### EXPERIMENTAL SECTION

#### Plant material

Plant material was collected from Karad, Satara District, Maharashtra, India. It was authenticated from the Department of Botany, Yashwantrao Chavan College of Sciences, Karad.

#### Preparation of extract

Fresh leaves of *Kalanchoe pinnata* was collected, washed under running tap water and were chopped into small pieces by hand and put into a conical flask. 100ml of methanol was added to the conical flask and was macerated for 7 days in order to maximize the extraction. After 7 days it was filtered through Whatmann filter paper, prepared as

methanolic stock solution and stored in a suitable container. 1.5ml of methanolic extract was pipetted into Eppendorf tube and sent to IIT, Powai, Mumbai for GC-MS analysis of different compounds.

#### GC-MS analysis [3]

GC-MS analysis of methanolic extract of *K. pinnata* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 μl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °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 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

#### Identification of phytochemical constituents

GC-MS mass-spectrum was interpreted by using the database of National Institute Standard and Technology (NIST). Mass spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. Three major peaks of GC-MS spectrum were ascertained while two phytochemical components were remained unidentified.

### RESULTS

GC-MS chromatogram analysis of the methanolic extract of *Kalanchoe pinnata* [Fig. 1] showed five peaks which indicating the presence of five major phytochemical constituents. In accordance to reference mass spectra of the constituents available in the NIST library, only major three peaks of GC-MS spectrum revealed that phytol, squalene, and 5-oxotetrahydrofuran-2,3-dicarboxylic acid dimethyl ester were identified. Other two phytocomponents remained unidentified [Table 1]. Out of the five compounds, the most current compounds were squalene; a triterpene compound and phytol have been reported for various activities. Three compounds were not still reported to have any pharmacological activity in general.

**Table 1: Phytochemical constituents of methanolic extract of *Kalanchoe pinnata* fresh leaves**

Peak Number	Retention Time	Name of compound	Molecular formula	Molecular Weight
1	11.48	5-Oxotetrahydrofuran-2,3-dicarboxylic acid dimethyl ester	C <sub>8</sub> H <sub>10</sub> O <sub>6</sub>	641
2	21.27	Phytol	C <sub>20</sub> H <sub>40</sub> O	841
3	29.08	Squalene	C <sub>30</sub> H <sub>50</sub>	410
4	36.69	Not identified	Unknown	
5	38.16	Not identified	Unknown	

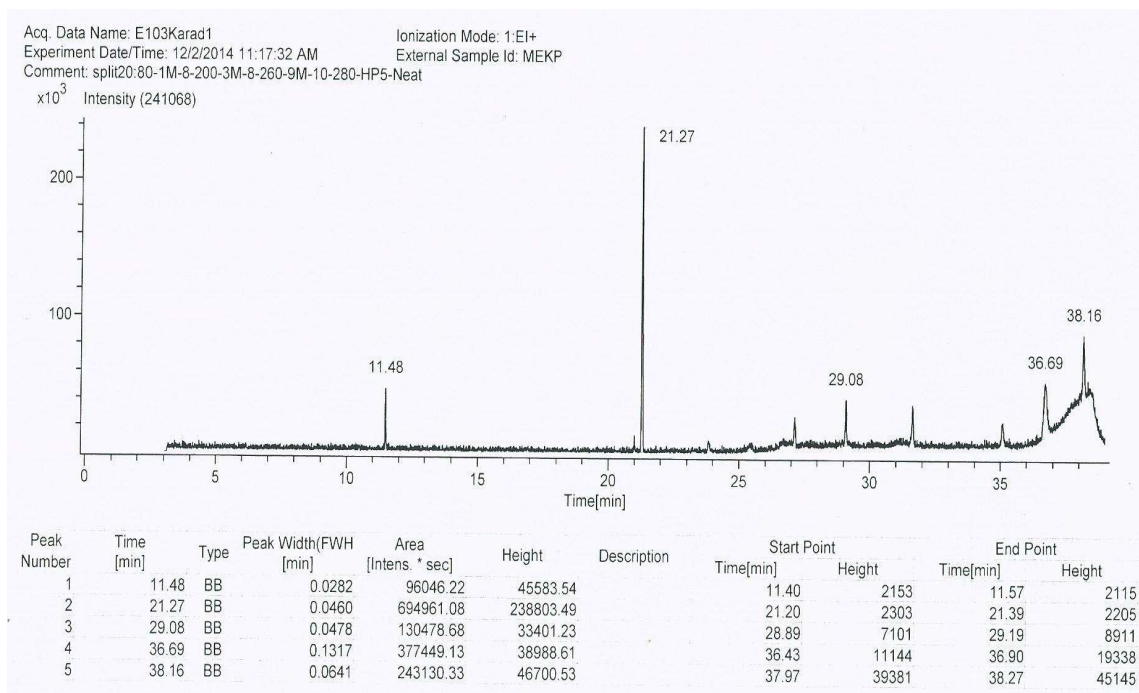


Fig.1: Gas chromatogram of of methanolic extract of *Kalanchoe pinnata* fresh leaves

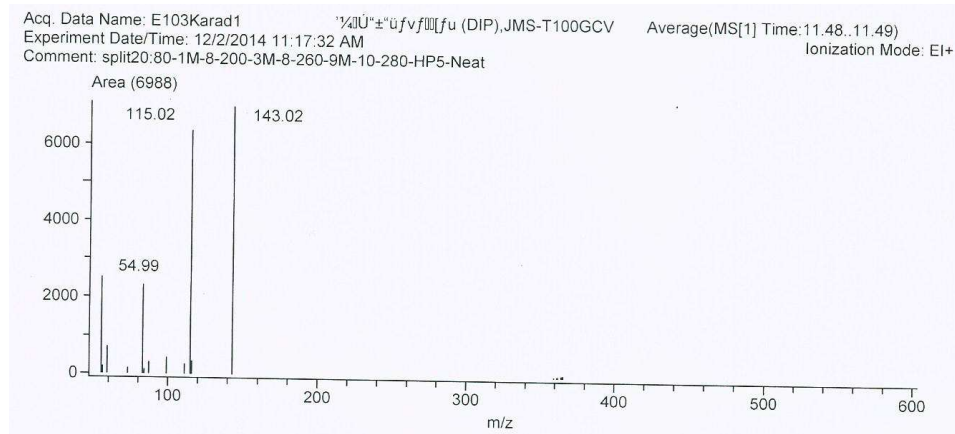


Fig. 2 a: Mass spectrum of major part of first fragment of methanolic extract of *Kalanchoe pinnata* fresh leaves

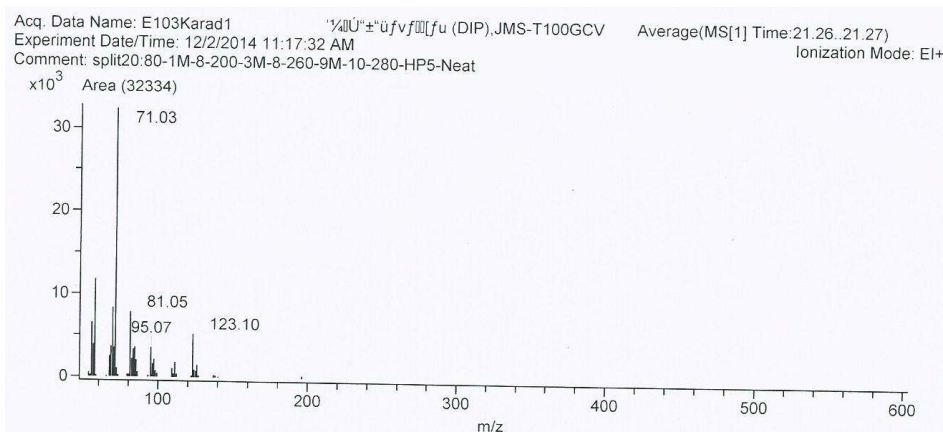


Fig.2b: Mass spectrum of major part of second fragment of methanolic extract of *Kalanchoe pinnata* fresh leaves

### DISCUSSION

Squalene was also reported to have anticancer [4], anti-oxidant [5] and other pharmacological activities [6]. Squalene (triterpene) is a phenolic compound generally found in latex and resins of some plants and physiological function of these compounds are generally believed to be a chemical in defense against certain pathogens causing human and animal diseases [7].

### CONCLUSION

From the results, it is firstly reported that phytol and squalene present as major bioactive compounds in methanolic extract of *Kalanchoe pinnata* fresh leaves. Isolation of phytochemical components present in the methanolic extract of *Kalanchoe pinnatum* and its characterization should be further investigated.

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