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Isolation and characterization of a constituent from the leaves of *Kalanchoe crenata* and its evaluation for antioxidant activity

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ABSTRACT

Kalanchoe crenata (Adrews) Haw. is an ornamental plant belonging to the family Crassulaceae is probably native to Africa and drier parts of south-east Asia. Commonly known as "never die" or "Dog's liver", this plant is widely used in traditional medicine in the treatment of inflammation, earache, headache, asthma, palpitation, abdominal pain, convulsion and general debility [1]. The leaves of K.crenata possess analgesic, anticonvulsant & spasmolytic properties [2, 3, 4].The present research includes isolation and characterization of chemical constituent and in vitro evaluation of antioxidant potentials by assay method of isolated constituent from leaves of Kalanchoe crenata. Chemical constituent (hentriacontan-1-ol) was isolated and the structures of this compound have been established by spectroscopic methods (UV, ^{1}H-NMR, IR, MS). This constituent was isolated from this plant for the first time. This chemical constituent showed significant antioxidant activity in dose dependent manner assay method. The result obtained in the present study indicates that leaves of Kalanchoe crenata could be a potential source of natural antioxidant. This also justified the traditional use of herb in preventing disease induced by oxidative stress.

Keywords: *Kalanchoe crenata*, Crassulaceae, Antioxidants, DPPH Scavenging activity

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INTRODUCTION

Kalanchoe crenata (Adrews) Haw. is an ornamental plant belonging to the family Crassulaceae. Commonly known as "never die" or "Dog's liver", this plant is widely used in traditional medicine in the treatment of inflammation, earache, headache, asthma, palpitation, abdominal pain, convulsion and general debility [1]. The plant has several health promoting benefits like spasmolytic, anti inflammatory, analgesic, anticonvulsant, cardiovascular, antimicrobial, antioxidant and activities [2, 3, 4]. In early studies, it was found that petroleum ether extracts of *K. crenata's* leaves contain triterpenoids, steroids, saponins and fatty acids [5, 6].

In continuation of investigation of bioactive metabolites from *Kalanchoe crenata*, the present work deals with the isolation, structure elucidation and identification of chemical constituent from the petroleum ether extract of leaf part of the plant and further the isolated constituent was evaluated for its antioxidant potential.

MATERIALS AND METHODS

General

Identification and characterization of isolated component was done by chemical tests i.e. test for alcohol & also by UV, IR, NMR & Mass spectroscopy. UV spectral analysis was done by using Shimazdu UV/VIS spectrophotometer and λmax was determined by dissolving isolated component in ethanol. For IR Spectral Analysis Perkin Elmer

Spectrum 400 FT-IR/FT-FIR Spectrometer was used and isolated component was examined by preparing KBr pelletes and from stock solution spectra was recorded. NMR Spectral Analysis was done by Bruker Avance II 400 NMR, Isolated compound was dissolved in chloroform and the ^{1}H Nuclear magnetic spectrum was recorded and for Mass Spectral Analysis, isolated compound was dissolved in chloroform and the mass spectrum was recorded using (ESI-MS) Bruker Daltonics Esquire 300 mass spectrometer. TLC was performed with silica gel GF254. All solvents were analytical reagent grade.

Compound was isolated from petroleum ether extract of *K. crenata* by increasing polarity of solvent system (n-hexane: ethyl acetate) using silica gel. The ^{1}H-NMR and other spectral data for this compound revealed that compound has been identified as long chain alcohol from their physical constants and spectral data.

Plant Collection & Preparation

K. crenata whole plants were collected from Landran campus Mohali (Punjab) in month of July were subjected to first morphological identification and followed by authentication by Taxonomist Dr. H.B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. After confirmed authenticity leaves were manually separated. The plant material were washed with water to remove soil, mud, debris and other adhering materials and dried thoroughly in air under shade at room temperature. Coarse powdered of drug was prepared, passed through sieve no. 40 and stored in air tight containers.

Extraction

About (500g) of powdered leaves of *Kalanchoe crenata* was extracted with petroleum ether using Soxhlet apparatus. The extract was concentrated in rotary evaporator under controlled temperature (40^0-50^0C) and dried in desiccator yields 15g. The petroleum ether extract was further saponified using 1M alcoholic potassium hydroxide and then subsequently picked up in anhydrous ether. This fraction contained a lesser number of components than the unsaponified extract. The ether fraction was reduced to a minimum volume.

Isolation

4g of the extract after saponification was subjected to column chromatography on silica gel (60-120 mesh, Merck) and was eluted with solvents in increasing order of polarity using n-hexane, n-hexane: chloroform, pure chloroform, chloroform: ethyl acetate and methanol.

170 fractions each of volume 40 ml were collected. The solvent of each of the fraction was completely removed by evaporation at 50^0C under a steam. The residue was reconstituted with 0.5 ml chloroform. After reconstitution, TLC of these fractions was carried out to find out which fractions contain similar compounds. Fractions with components having same Rf value were combined together and labeled. Fractions 26-42 were combined and labeled as A ((Hexane, Hexane: Ethyl acetate) But none of these fractions obtained were exclusively pure. So, further purification was carried out. Compound was purified using re-column chromatography and Preparative Thin Layer Chromatography (PTLC) over silics gel G.

Pharmacological Evaluation

Antioxidant Activity

DPPH Radical Scavenging Capacity

The DPPH radical-scavenging activity or the Hydrogen donating capacity was determined in the presence of stable DPPH radical. DPPH (0.002%) was dissolved in pure methanol. Freshly prepared stock solution was used. The DPPH solution (1 ml) was added to 1 ml of sample extracts of different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorbance of the resulting solution was monitored at 517 nm using ascorbic acid as standard. The degree of discoloration indicates the scavenging efficacy of the extracts. Methanol (1ml) with DPPH solution (0.002%, 1ml) was used as blank. The equation used to measure free radical scavenging activity is:

$$\%DPPH\ ScavengingActivity = \frac{Ao - A1}{Ao} \times 100$$

Where 'control' was the absorbance of DPPH, 'Test' was the absorbance in the presence of the sample of compound and standard. The experiment is performed in triplicate and average absorbance is noted for each concentration. Ascorbic acid is used as a positive control. Results are expressed as mean inhibitory concentration (IC_{50}). A lower value of IC_{50} indicates a higher free radical scavenging activity [7-8].

RESULTS AND DISCUSSION

Although large number of isolates were separated from successive column chromatography but quantitatively only one compound was isolated, purified and characterized from petroleum ether extract of leaves part of *Kalanchoe crenata* by column chromatography. Isolated component was further purified by PTLC and re-column chromatography method. Isolated compound was identified and confirmed by melting point, TLC and UV, ^{1}HNMR, IR, MS spectra of the compound. The compound was characterized on the basis of spectroscopic analysis and compared with reported data in literature.

Characterization of Compound:

Compound was isolated from petroleum ether extract of *K. crenata* as white amorphous powder. The melting point was 65^0-67^0C [8, 9].

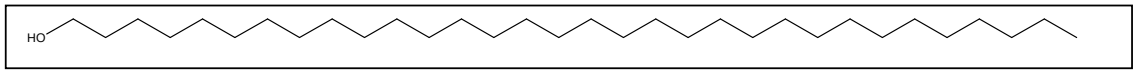
The mass spectral data of the compound gave a molecular formula $C_{31}H_{64}O$ [m/z 453].

IR spectra showed absorption peaks at 3306.57 cm^{-1} indicate the presence of alcohol group 2868.94 & 2917.04 cm^{-1} indicate the presence of C-H stretching and band at 730.33, 719.27 cm^{-1} was due to long aliphatic nature of molecule.

^{1}H- NMR spectra showed presence of triplet at δ_H 0.88 (J=6.6Hz) for the three end protons while the methylene group α to the hydroxyl group or carbinolic proton (CH_2OH) as a triplet at δ_H 3.64 (J=6.6Hz). The rest of methylene protons merged into a broad singlet at δ_H 1.25. The methylene protons β- to carbinolic group merged with OH proton resonated at δ_H 1.54.

Mass spectrum of the compound exhibited base peak at m/z 453 and other characteristic fragments peaks were observed at m/z 453.4, 435.4, 360.4, 338.4, 321.3, 210.2, 149, and 133.

Hence the melting point and spectral analysis such as NMR, IR and MS spectrum of isolated compound C obtained from petroleum ether extract of *K. crenata* confirmed that the compound to be in good agreement with hentriacontan-1-ol in the literature.



Structure of Hentriacontan-1-ol ($C_{31}H_{64}O$; Mol.Wt. 453)

***In vitro* evaluation of Antioxidant and Free Radical Scavenging Activity**

DPPH Scavenging Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. DPPH is the stable nitrogen centered free radical the color of which changes from violet to yellow upon reduction by the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and radical scavengers [5]. Radical scavenging activity of all the isolated compounds was found to be increase with increase in concentration. The IC_{50} of isolated compound and Ascorbic acid (standard) was found to be 107.57 & 15.32 µg/ml respectively. Antioxidant potential by DPPH method was found.

Table 1: DPPH Radical Scavenging Activity of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Conc. µg/ml	% Scavenging activity Mean± SD (n=3) Isolated compound & Ascorbic acid	
20	9.7± 0.23	46.8±0.03
40	19.4± 0.14	53.52±0.02
60	26.3± 0.12	62.63±0.01
80	33.3± 0.22	69.74±0.03
100	39.6± 0.18	98.79±0.03
IC_{50}	107.57	15.32

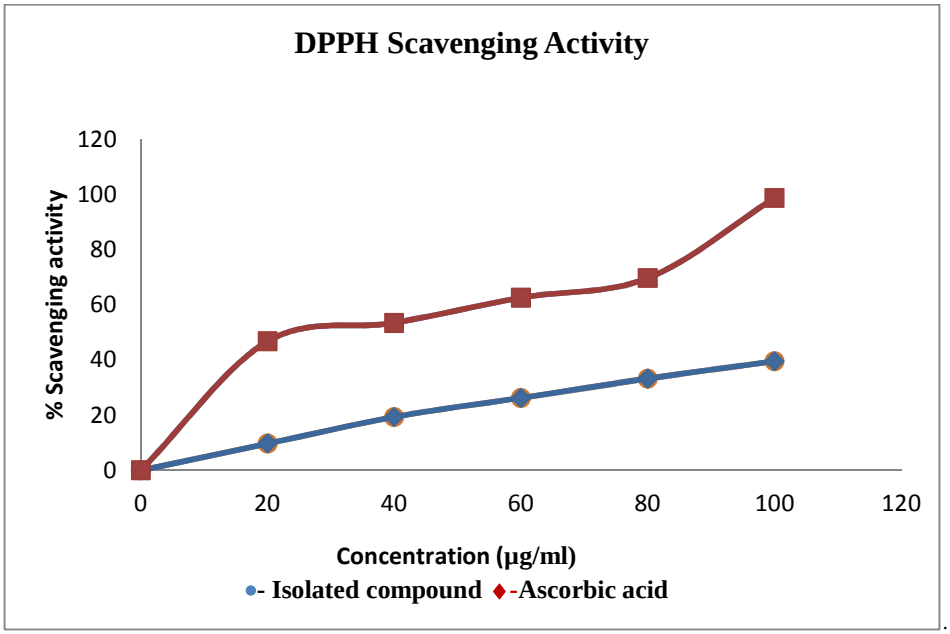


Figure 1: Antioxidant Capacity by DPPH Radical Scavenging Method of Compound Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

DISCUSSION

The plants was studied for Isolation of Phytoconstituent and and *In-vitro* evaluation of antioxidant activity by DPPH Scavenging Activity method. It was found that isolated component was alcoholic in nature. It was first preliminary tested by identification tests than confirmed by various spectroscopic methods ie (UV ^{1}H-NMR IR & MS).*In-vitro* studies showed that the isolated component also sowed antioxidant profile.

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