

ISOLATION AND CHARACTERIZATION OF CONSTITUENTS FROM THE LEAVES OF *KALANCHOE CRENATA* AND THEIR EVALUATION FOR ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

Aarti Rathour, Anjoo Kamboj*, Mandeep Kaur, Ajay Kumar Saluja

Chandigarh College of Pharmacy, Landran (Mohali) 140307, INDIA

Article Received on
02 January 2013
Revised on 28 January 2013,
Accepted on 19 February
2014

***Correspondence for
Author**

Dr. Anjoo Kamboj
Chandigarh College of
Pharmacy, Landran
(Mohali) 140307, INDIA.

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. The present research includes isolation and characterization of chemical constituents and *in vitro* evaluation of antioxidant potentials by three different assay methods of isolated constituents from leaves of *Kalanchoe crenata*. Three constituents (α -amyrin, Stearyl alcohol, and β -sitosterol-d-glycoside)

were isolated and the structures of these compounds have been established by spectroscopic methods (UV, $^1\text{H-NMR}$, IR, MS). All these constituents were isolated from this plant for the first time. These chemical constituents showed significant antioxidant and antimicrobial activity in dose dependent manner by different assay methods. The result obtained in the present study indicates that leaves of *Kalanchoe crenata* could be a potential source of natural antioxidant and antimicrobial. This also justified the traditional use of herb in preventing disease induced by oxidative stress and microorganism.

Keywords: *Kalanchoe crenata*, DPPH scavenging activity, Gram positive, Gram negative.

INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant [1-2]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality [3]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial and antioxidant drugs [4-5].

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 [6]. The plant has several health promoting benefits like spasmolytic, anti-inflammatory, analgesic, anticonvulsant, cardiovascular, antimicrobial, antioxidant and antinephropathic activities. In early studies, it was found that petroleum ether extracts of *K. crenata*’s leaves contain triterpenoids, steroids, saponins and fatty acids.

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

MATERIAL AND METHODS

General

IR spectra were recorded (KBr discs) on FT-IR spectrometer, validation (ν_{\max} in cm^{-1}). Bruker ADVANCE II 400 NMR spectrophotometer was used to record ^1H -NMR chemical shifts (δ) are reported as downfield displacements from TMS used as internal standard and coupling constants are reported in Hz. WATERS Q-T OF MICROMASS (LC-MS) was used to record mass spectrum of compounds. SHIMAZDU UV -1800 spectrophotometer was used for UV analysis. Electronic balance (CY 200) of Denver instruments was used for weighing of compounds. TLC was performed with silica gel GF₂₅₄. All solvents were analytical reagent grade.

Isolated phytoconstituents were tested by performing chemical test such as Libermann-buchard's reagent, Salkowski test, Spot test, Litmus paper test etc and determining the physical parameters like appearance, solubility and melting point by comparing with literature.

Plant Material

Fresh and fully grown plants were collected Aman plant nurseries, Morinda, Ropar (Pb.) in the month of September and October. The sample was authenticated by comparing the morphological characters as described in the literature. The authentication was further confirmed by Dr. H.B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. A herbarium sample of this plant was preserved in the department, Chandigarh College of Pharmacy, Landran (Mohali) for future reference CCP/HB/AR/05.

Collection and Preparation of Plant Materials

Kalanchoe crenata were collected in bulk quantity after confirmed authenticity from Aman plant nurseries, Morinda, Ropar (Punjab) in months of September and October 2012. The plant parts such as leaves, stems, and fruits were manually separated. The plant material was washed thoroughly 2-3 times with water to remove soil, mud, debris and other adhering materials and dried in air under shade at room temperature. Coarse powder of each drug was prepared and stored in air tight container in a dry place at room temperature.

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 (40⁰-50⁰C) and dried in dessicator yields 14g. 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

6g of the extract after saponification was subjected to chromatography on silica gel (60-120 mesh, Merck) and was eluted with solvents in increasing order of polarity using hexane, hexane: chloroform, pure chloroform, chloroform: ethyl acetate and methanol.

350 fractions each of volume 50 ml were collected. The solvent of each of the fraction was completely removed by evaporation at 50°C 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 R_f value were combined together and labeled. Fractions 1-8 were combined and labeled as A (Hexane), fractions 39-50 were combined and labeled as B (Hexane: Ethyl acetate) (90:10), fractions 120-150 were combined and labeled as C (Hexane: Ethyl acetate) (20:80).

But none of these fractions obtained were exclusively pure. So, further purifications of these compounds were carried out. Compound B was purified by recrystallization and compound A and C were 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:

$$\%DPPH \text{ Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

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 (IC₅₀). A lower value of IC₅₀ indicates a higher free radical scavenging activity [7-8].

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was determined according to the method of Beara *et al.* 2 ml of compound solution (5-100 µg/ml), 1.0 ml of *ortho*-phenanthroline (7.5 mmol L⁻¹), 5.0 ml of phosphate buffer (0.2 M, pH 6.6), 1.0 ml of ferrous sulfate (7.5 mmol L⁻¹) and 1.0 ml of H₂O₂ (0.1 %) were mixed and diluted to 25 ml with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm using ascorbic acid as standard [9].

The equation used to measure free radical scavenging activity is

$$\% \text{ Scavenging Activity} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Where 'control' was the absorbance value of the system with all solution including H₂O₂ and '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 (IC₅₀).

A lower value of IC₅₀ indicates a higher free radical scavenging activity.

Phosphomolybdenum Method

The total antioxidant capacity of sample was evaluated by phosphomolybdenum complex formation method. 0.3ml sample (5-100 µg/ml) of each sample was mixed with 3ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in test tubes. The blank solution contained 3 ml of reagent solution. The test tubes were capped and incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm using a spectrometer against blank. Ascorbic acid was used as standard [10]. The experiment is performed in triplicate and average absorbance is noted for each concentration.

Antimicrobial Activity

Microorganisms, Media & Standard drugs

Three bacterial strains (one Gram positive i.e. *Staphylococcus aureus* MTCC Code – 737 and two Gram negative i.e. *Escherichia coli* MTCC Code – 68 & *Pseudomonas aeruginosa* MTCC Code – 1688) and two fungal strains (*Candida albicans* MTCC Code – 3017 &

Kluyveromyces marxianus MTCC Code – 9769) were used for evaluating antimicrobial activity. These strains were obtained from Microbiology Department, Institute of Microbial Technology (IMTECH) Chandigarh sec-39A. The microorganisms, which were maintained by sub-culturing, were used at regular intervals in nutrient agar medium and Sabouraud media. Ciprofloxacin was used as standard drug for antibacterial activity and Fluconazole for antifungal activity.

Experimental Procedure (Agar Diffusion Method)

Nutrient agar was prepared, sterilized and cooled to 45°C with gentle shaking to bring about uniform cooling. It was then poured into the sterilized petri dishes. The poured material was allowed to set and plates were incubated for 24 hours to check for sterility. Plates which showed any kind of contamination were discarded. Thereafter, 100 µl culture of microorganism was spread uniformly over the solidified agar with the help of spreader. Cups were made by punching into the agar surface with sterile cork borer and scooping out the punched part of the agar. 0.1ml of test compounds was added into the cups with the help of micropipette.

Two-fold diluted solutions of the compounds and reference drugs were used (2000, 1600, 800, 400, 100, 6.5 µg/ml). The drug solution was allowed to diffuse for sometime into the medium for 30 minutes. The plates were incubated at 30-35 °C for 24-48 hours. The incubation chamber was kept sufficiently humid. The minimal inhibitory concentration (MIC) values were determined at the end of the incubation period. DMF was used as control.

Statistical Analysis

All data were expressed as the mean \pm SE and where applicable, the data were analyzed statically by student's t-test using and the level of significance was from $P < 0.05$.

RESULT AND DISCUSSION

Although large number of isolates were separated from successive column chromatography but quantitatively only three compounds (A, B and C) were isolated, purified and characterized from petroleum ether extract of leaves part of *Kalanchoe crenata* by column chromatography. Isolated components were further purified by PTLC and recrystallization method. Isolated compounds were identified and confirmed by melting point, TLC and UV, ¹HNMR, IR, MS spectra of the compounds. The compounds were characterized on the basis of spectroscopic analysis and compared with reported data in literature.

α -amyrin (Compound A)

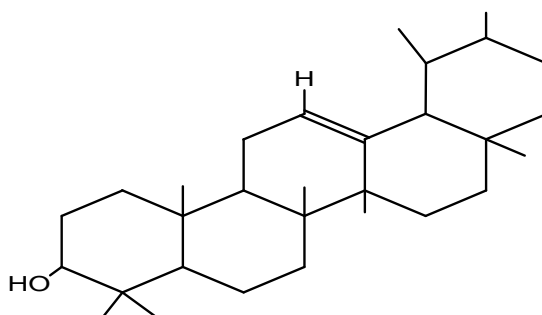
The compound A isolated from petroleum ether extract of leaves part of *Kalanchoe crenata* appeared as colorless crystals with R_f 0.48 in Hexane: Chloroform (9:1). It gave positive Salkowski test. From the positive test it was assumed to be a triterpenoids. The melting point of compound A (183°C - 185°C) was in good agreement with melting point of α -amyrin (185°C). The λ_{max} of the compound A was observed at 292nm in DMF.

IR spectra showed absorption peaks at 3367.67cm^{-1} indicate the presence of alcohol group, at 2943.52cm^{-1} indicate the presence of C-H stretching vibration, at 1042.67cm^{-1} due to C-O stretching.

^1H - NMR spectra revealed a triplet at δ 5.15 for a proton which indicate the presence of $=\text{C-H}$ proton, a multiplet at δ 3.15 for a proton due to the presence of hydroxyl group ($-\text{CH-OH}$) and other hydrogen signals are reported between δ 2.3-0.88 which are in agreement with the corresponding data described for α - amyrin in literature.

Mass spectrum of compound A exhibit M^+ base peak at m/z 426 and other characteristic fragment peaks were observed at m/z 411, 408, 393, 302, 274, 246, 219, 218 (100%), 207, 202, 191, 89.

Hence melting point and spectral data from ^1H NMR, IR and MS spectrum of isolated compound A obtained from petroleum ether extract of *K. crenata* confirmed that the compound to be in good agreement with α - amyrin [11-12].



Structure of α - amyrin ($\text{C}_{30}\text{H}_{50}\text{O}$; Mol. Wt. 426)

Stearyl alcohol (Compound B)

The compound B isolated from petroleum ether extract of leaves part of *Kalanchoe crenata* appeared as colorless crystals with R_f 0.85 in Hexane: Chloroform (9:1). The melting point of compound B (50 - 55°C) was in good agreement with melting point of stearyl alcohol (55°C).

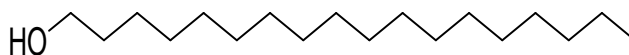
λ_{max} of the compound B was observed at 290nm in DMF. The molecular formula was established as $\text{C}_{18}\text{H}_{38}\text{O}$ [m/z 270] from mass spectrum. The fragmentation pattern was found in line with that of long chain alcohol i.e. stearyl alcohol.

IR spectra showed absorption peaks at 3327.37 cm^{-1} indicate the presence of alcohol group, at $2849.10\text{--}2919.5\text{ cm}^{-1}$ indicate the presence of C-H stretching and at 724.53 cm^{-1} due to long aliphatic chain for $(\text{CH}_2)_n$ group.

^1H - NMR spectra showed presence of triplet at δ_{H} 0.88 for the three end protons while the methylene group α to the hydroxyl group or carbinolic proton (CH_2OH) as a triplet at δ 3.64. The rest of methylene protons merged into a broad multiplet at δ 1.25. The methylene protons β - to carbinolic group appeared as multiplet at δ 1.54.

Mass spectrum of compound B exhibit M^+ base peak at m/z 270 and other characteristic fragment peaks were observed at m/z 256, 200, 126, 119, 102, 71.

Hence melting point and spectral analysis such as $^1\text{HNMR}$, IR and MS spectrum of isolated compound B obtained from petroleum ether extract of *K. crenata* confirmed that the compound to be in good agreement with stearyl alcohol.



Structure of Stearyl alcohol ($\text{C}_{18}\text{H}_{38}\text{O}$; Mol. Wt. 270)

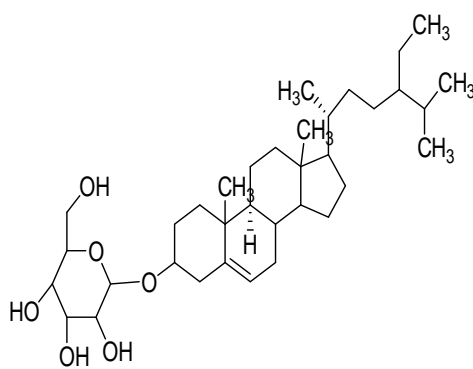
β - Sitosterol-d-glycoside (Compound C)

The compound C isolated from petroleum ether extract of leaves part of *Kalanchoe crenata* appeared as yellow semisolid compound with R_f 0.60 in Chloroform: Methanol (9:1). It gave positive test for Libermann Burchard's test. From the positive test it was assumed to be a steroid. The melting point of compound C ($283^{\circ}\text{--}286^{\circ}\text{C}$) was in good agreement with melting point of β -sitosterol-D-glycoside (284°C). The λ_{max} of the compound C was observed at 206nm in DMF. IR spectra showed broad absorption peaks at 3428.52 cm^{-1} indicate the presence of alcohol group, at 1727.47 cm^{-1} indicate the presence of C=C Stretching, at 2958.38 and 2928.35 cm^{-1} indicate the presence of C- H Stretching due to aliphatic group and at 1286.47 cm^{-1} was due to C-O group.

^1H - NMR spectra revealed a multiplet for double bond proton (C-H) and (1') proton in sugar moiety at δ 5.15 – 5.11, a multiplet for protons at 2'-6' positions in sugar moiety appeared at δ 4.32-3.49 and multiplet at δ 1.07-0.84 due to methyl protons which are in agreement with corresponding data described for β -sitosterol-D-glycoside in literature.

Mass spectrum of compound exhibit base M^+ peak at m/z 577 and other characteristic fragment peaks were observed at m/z 414, 329, 315, 301.2, 245.1, 219, 149.0, 87.

Hence melting point and spectral analysis such as ^1H 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 β -Sitosterol-d-glycoside [13].



β - Sitosterol-d-glycoside

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 b either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and radical scavengers [14].

Radical scavenging activity of all the isolated compounds was found to be increase with increase in concentration. The IC_{50} of compound A, compound B, compound C and Ascorbic acid (standard) was found to be 77.64, 137.57, 185.65, 11.41 $\mu\text{g/ml}$ respectively.

Antioxidant potential by DPPH method was found to maximum with compound A followed by compound B and compound C (**Table 1and Figure 1**).

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)			
	A	B	C	Ascorbic acid
5	09.10±0.05	10.13±0.01	00.99±0.02	44.5±0.01
10	10.02±0.01	12.46±0.03	01.90±0.05	48.6±0.03
15	13.56±0.02	13.56±0.04	03.10±0.03	52.74±0.02
20	16.03±0.03	14.98±0.02	04.68±0.01	57.92±0.01
25	20.52±0.03	16.32±0.01	06.10±0.02	62.63±0.03
100	38.52±0.04	25.68±0.01	18.97±0.03	98.79±0.03
IC ₅₀	77.64	137.51	185.65	11.41

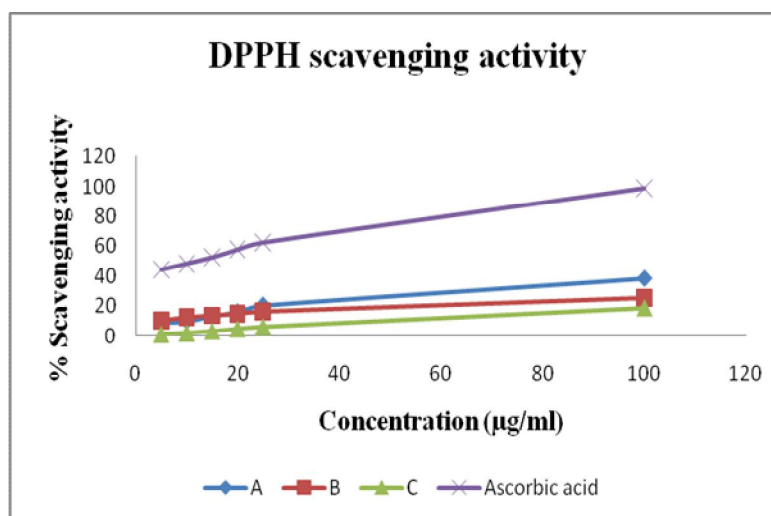


Figure 1 Antioxidant Capacity by DPPH Radical Scavenging Method of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Hydroxyl Scavenging Activity

The compounds were capable of scavenging hydrogen peroxide in a concentration dependent manner. Scavenging capacity increases with increase in concentration. Hydrogen peroxide itself is not very reactive; it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H₂O₂ is very important throughout food systems [15].

The isolated compounds were capable of scavenging hydrogen peroxide in a concentration dependent manner. Scavenging capacity of all compounds was found to be increase with increase in concentration. The IC₅₀ of compound A, compound B, compound C, Ascorbic acid (standard) was found to be 75.21, 77.95, 194.40, 56.93 μg/ml respectively. Scavenging capacity by hydroxyl method was found to maximum by the compound A followed by compound B and compound C. (Table: 2 and Figure: 2)

Table 2 Hydroxyl Radical Scavenging Activity of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Conc. μg/ml	% Scavenging activity Mean±SD (n=3)			
	A	B	C	Ascorbic acid
5	8.92±0.02	2.12±0.01	1.05±0.03	12.05±0.01
10	12.74±0.03	8.30±0.02	1.29±0.01	19.21±0.03
15	16.56±0.01	11.96±0.04	2.20±0.02	20.56±0.02
20	19.32±0.02	13.22±0.01	2.65±0.04	25.98±0.01
25	19.96±0.04	15.46±0.03	4.35±0.02	26.09±0.03
100	49.65±0.01	22.23±0.02	6.28±0.01	59.24±0.02
IC ₅₀	75.21	77.95	194.40	56.93

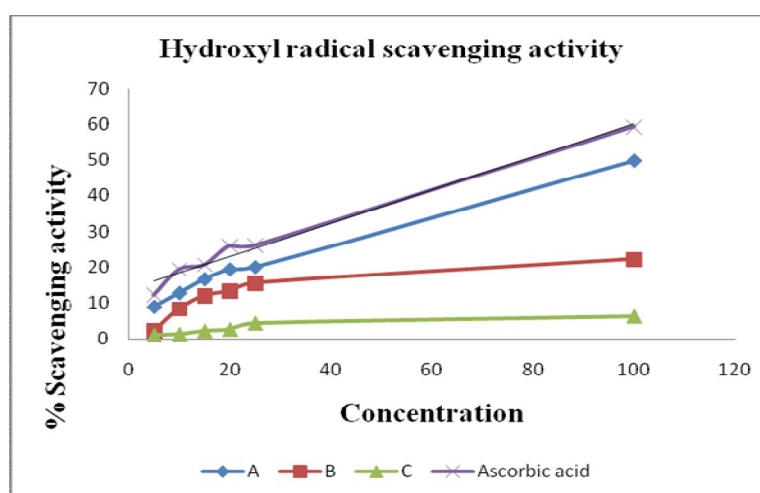


Figure 2 Antioxidant Capacity by Hydroxyl Radical Scavenging Method of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Table 3 Comparison of IC₅₀ (μg/ml) values of Antioxidant Activity by DPPH and Hydroxyl Ion Scavenging Method of Isolated Compounds from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Compound	DPPH Scavenging Activity (IC ₅₀ μg/ml) Mean±SD (n=3)	Hydroxyl ion Scavenging Activity (IC ₅₀ μg/ml) Mean±SD (n=3)
A	77.64±1.13	75.21±1.02
B	137.51±3.20	77.95±1.14
C	185.65±3.15	194.40±2.17
Ascorbic acid	11.40±1.50	56.93±2.10

Comparing the antioxidant results, it revealed that total antioxidant potential increases in a concentration dependent manner. It further revealed that the total antioxidant capacity was higher with the Compound A followed by Compound B & Compound C respectively (Table 3 and Figure 3).

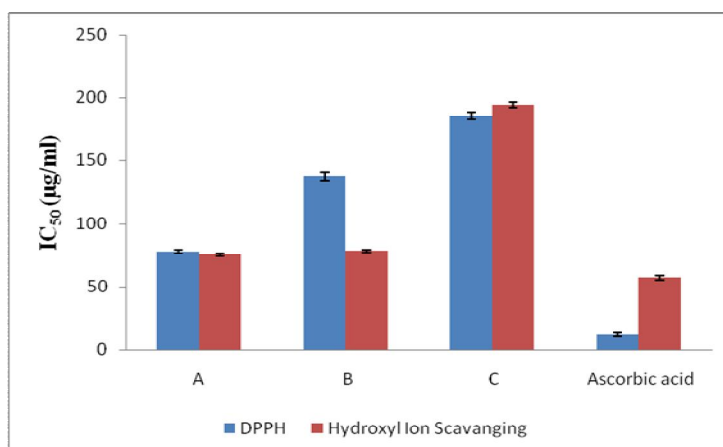


Figure 3 Comparison of IC₅₀ (µg/ml) values of Antioxidant Activity of Isolated Compounds from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Phosphomolybdenum Method

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since total antioxidant activity is expressed as the number of equivalents of ascorbic acid [16].

Result indicate that the total antioxidant capacity increases in a concentration dependent manner and further revealed that the total antioxidant capacity was higher with the compound A followed by compound C and compound B respectively (Table 4 and Figure 4)

Table 4 Antioxidant activity by phosphomolybdenum method of Isolated compounds from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Conc. µg/ml	Absorbance Mean±SD (n=3)			
	A	B	C	Ascorbic acid
5	0.021±0.01	0.013±0.02	0.019±0.01	0.021±0.01
15	0.022±0.01	0.026±0.03	0.024±0.03	0.039±0.04
25	0.024±0.03	0.030±0.02	0.026±0.02	0.044±0.02
35	0.028±0.02	0.032±0.01	0.028±0.02	0.062±0.01
100	0.067±0.01	0.046±0.03	0.053±0.00	0.084±0.03

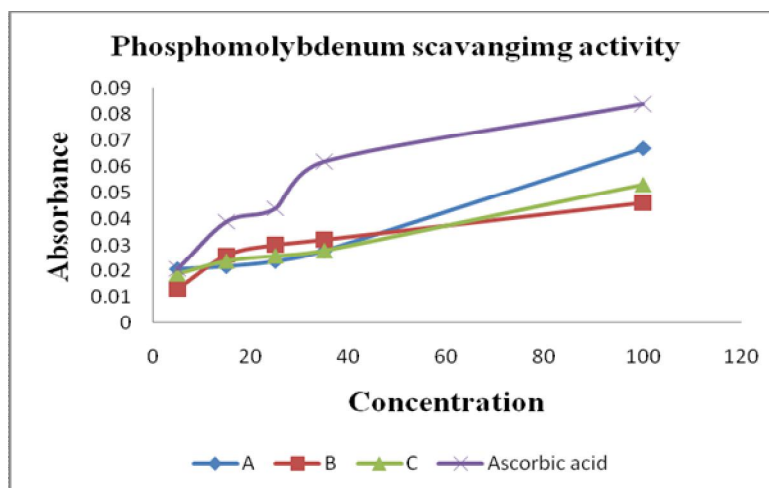


Figure 4 Antioxidant Capacity by Phosphomolybdenum Method of compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Evaluation of Antimicrobial activity

Antimicrobial activity of isolated compounds from petroleum ether extracts of leaves of *K. crenata* was also evaluated. Result indicates that all the isolated compounds showed good antimicrobial potential. From the result, it was observed that Compound A exhibit higher antibacterial potential followed by Compound C and Compound B. All the isolated compounds also exhibit antifungal potential. Result indicates that Compound A exhibit higher antifungal potential followed by Compound B and Compound C (Table 5 and Figure 5, 6).

Table 5 Comparison of Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$ of isolated compounds from Petroleum Ether Extract of Leaves of *Kalanchoe crenata*

Compound	Minimum inhibitory concentration (MIC) in $\mu\text{g/ml}$ Mean \pm SD (n=3)				
	EC	PA	SA	CA	KM
A	5.25 \pm 0.05	12.25 \pm 0.06	15.25 \pm 0.07	3.25 \pm 0.02	8.25 \pm 0.03
B	7.25 \pm 0.07	15.25 \pm 0.07	14.25 \pm 0.05	4.25 \pm 0.05	9.25 \pm 0.02
C	5.25 \pm 0.02	12.25 \pm 0.05	17.25 \pm 0.09	8.65 \pm 0.02	15.25 \pm 0.06
D	1.25 \pm 0.01	1.25 \pm 0.02	2.25 \pm 0.03	-	-
S	-	-	-	1.25 \pm 0.01	1.25 \pm 0.01
Control	-	-	-	-	-

Where, EC- *Escherichia coli*, PA- *Pseudomonas aeruginosa*, SA- *Staphylococcus aureus*, CA- *Candida albicans*, KM-*Kluyveromyces marxianus*, D- Ciprofloxacin, S-Fluconazole

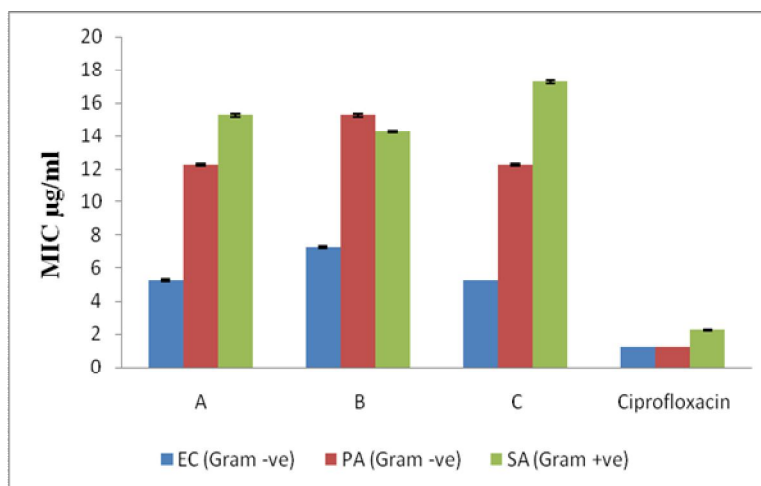


Figure 5 Comparison of Minimum Inhibitory Concentration (MIC) of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata* against bacterial strains (EC, PA and SA)

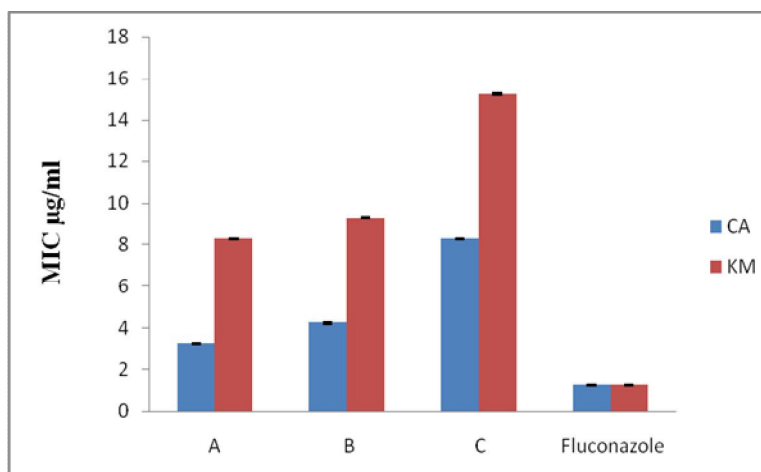


Figure 6 Comparison of Minimum Inhibitory Concentration (MIC) of Compounds Isolated from Petroleum Ether Extract of Leaves of *Kalanchoe crenata* against fungal strains (CA and KM)

CONCLUSION

Three compounds isolated from the petroleum ether extract of leaves of plant *Kalanchoe crenata* were identified as triterpenoid, long chain alcohol & steroidal glycoside respectively. By spectroscopic data compounds A, B & C were characterized as α - amyryn, stearyl alcohol & β - sitosterol-d-glycoside. From the results it was concluded that isolated compounds (α - Amyryn, Stearyl alcohol and β -sitosterol-d-glycoside) may have some role in exhibiting the antioxidant and antimicrobial activities of the plant. This also justified the traditional use of herb in preventing disease induced by oxidative stress and microorganism.

REFERENCES

1. Aibinu I E, Ohaegbulam V C, Adenipekun E A, Ogunsola FT, Odugbemi TO, Mee BJ. Extended-Spectrum Beta-Lactamase Enzymes in Clinical Isolates of *Enterobacter* species from Lagos, Nigeria. *Journal of Clinical Microbiology*, 2003;41(5):2197-2200.
2. Aibinu I, Adenipekun E, Odugbemi T. Emergence of Quinolone Resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. *Nigerian Journal of Health and Biomedical Science*, 2004; 3(2):73-78.
3. Williams R. Antimicrobial resistance a global threat. *Essential Drug Monitor*, 2000; 28-29:1.
4. Pretorius JC, Magama S, Zietsman PC. Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species *South African Journal of Botany*, 2003; 20: 188-192.
5. Moreillon P, Que YA, Glauser MP. *Staphylococcus aureus* (Including Staphylococcal Toxic shock). In 'Principles and Practice of Infectious diseases.' (Ed.) Mandell G.L, Bennett J.E, Dolin R. Published by Churchill livingstone Pennsylvania, 6th ed., 2005; 2: 2333- 2339.
6. Nguelefack T B, Sontia B, Dongmo A B, Dimo T, Kamanyi A, Veirling W. Spasmolytic effects of extracts from *Kalanchoe crenata* (Andrews) Haworth (Crassulaceae) leaves, *Pharmacologyonline*, 2006; 1: 30-39.
7. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, *Songklanakarin, J Sci Technol*, 2004; 26:211-219.
8. Mani RP, Pandey A, Goswami S, Tripathi P, Kumudhavalli V, Singh AP. Phytochemical Screening and In- vitro Evaluation of Antioxidant Activity and Antimicrobial activity of the Leaves of *Sesbania sesban* (L) Merr., *Free Radic Antioxid*, 2011; 1:412-414.
9. Peiyuan L, Huo L, Su W, Lu R, Deng C, Liu L, Deng Y, Guo N, Lu C, He C. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. *J. Serb. Chem. Soc.*, 2011;76 (5): 709–717.
10. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr J. Biotechnol.*, 2008; 32: 43-49.
11. Viviane GCA, Jacqueline AT, Lucienir PD, Dorila P, Policarpo ASJ, Rosana OA, Alvaro JR, Antônio FCA. Evaluation of the bactericidal and trypanocidal activities of triterpenes

- isolated from the leaves, stems, and flowers of *Lychnophora pinaster*. Brazilian Journal of Pharmacognosy, 2011; 21(4): 615-621.
12. Vazquez¹ LH, Palazon J, Navarro-Ocana A. The Pentacyclic Triterpenes, α , β -amyryns: A Review of Sources and Biological Activities. Phytochemicals- A Global Perspective of Their Role in Nutrition and Health: 487-502.
 13. Arunachalam G, Bag P, Chattopadhyay D. Phytochemical and phytotherapeutic evaluation of *Mallotus peltatus* (Geist.) Muell. Arg. var *acuminatus* & *Alstonia macrophylla* wall ex A. DC: Two ethnomedicine of Andaman Islands, India. Journal of Pharmacognosy and Phytotherapy, 2009; 1 (1):01-13.
 14. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Inter. J. Pharmacognosy. 1997; 35: 313-317.
 15. Tirzitis G, Bartosz G. Determination of antiradical and antioxidant activity: Basic principles and new insights. 2010; 57(1): 139–142.
 16. Abbasi M A, Saleem H, Rehman A, Riaz T, Ajaib M. Determination of Antioxidant Activity and Phytoconstituent Screening of *Euphorbia heterophylla* Linn., British Journal of Pharmaceutical Research, 2013; 3(2):202-216.