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RESEARCH ARTICLE

BIOLOGICALLY ACTIVE SECONDARY METABOLITES FROM *KALANCHOE TOMENTOSA*

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ABSTRACT

Phytochemical study of the ethanolic extract of *Kalanchoe tomentosa* (Crassulaceae) resulted in the isolation of 14 compounds identified as; α-amyrin acetate (**1**), friedelin (**2**), glutinol (**3**), 1-dotriacontanol (**4**), phytol (**5**), Stigmasta-7,25-dien-3β-ol (**6**), β-sitosterol (**7**), Isorhamnetin (**8**), 2,3-dihydroxypropyl tetradecanoate (**9**), Eriodictyol (**10**), Gallic acid (**11**), quercetin (**12**), kampferol-3-O-Rutinoside (**13**) and isovitexin (**14**). These compounds were isolated for the first time from this species. The structure elucidation of isolated metabolites was carried out using spectroscopic data (1D and 2D NMR). Antioxidant, cytotoxic and antimicrobial activities of different plant extracts were also studied and significant results were obtained.

Key words:Kalanchoetomentosa; Crassulaceae; cytotoxicity and antimicrobial activities.

INTRODUCTION:

The family Crassulaceae of 34 genera with 1,410 species distributed worldwide, usually in arid and/or rocky habitats, with centers of diversity in Mexico and South Africa. *Kalanchoe* is a large genus of succulent and a colorful plant popular in mild climates and a house plant standby[1].

Apart from its ornamental value, *Kalanchoe* is also very well known as a medicinal plant in the folk medicine. In recent years, an increased interest in the phytochemistry of the genus *Kalanchoe* which reported to contain interesting biologically active constituents such as bufadienolides, flavonoids, triterpenes and sterols that constitute the major secondary metabolites and showing interesting spectrum of activities[2]. In spite of the importance of the genus *Kalanchoe,* least number of species undergoes to phytochemical studies or biological activities. In recent years there has been renewed interest in natural medicines that are obtained from plants and microbial sources. The different crude extracts (methylene chloride, ethyl acetate and *n*-butanol) exhibited good radical scavenging properties towards DPPH radical. The *n*-butanol extract showed remarkable cytotoxic activities against two human tumor cell lines: Human colon carcinoma (HCT-116) and Human breast cancer (MCF-7) cell lines. The different crude extracts also showed selective antimicrobial activities against two Gram-positive bacteria, seven Gram-negative bacteria and two fungi.

MATERIAL AND METHODS:

***Experimental*:**

General experimental procedures: UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotte. NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer at 400 (^{1}H) and 100 MHz (^{13}C) in DMSO-d_6 or $CDCl_3$-*d* solution and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135^{o}). The EIMS spectra were measured using EI/MS 502 mass spectrometer having a direct inlet system and operating at 70eV. The ESIMS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI). Column chromatographic separation was performed on silica gel 60 (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia). TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2mm, Merck). Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min.

***Plant material*:**

Kalanchoe tomentosa aerial parts was collected from Orman Botanical Garden, Giza, Egypt and many cacti farms in Cairo, Kalubia and 6th October governorates and kindly identified by Agr. Eng. Treez and staff members of Orman Botanical Garden Herbarium.

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***Extraction and isolation*:**

The fresh plant material (20 kg) was subjected to washing with methylene chloride (2 x 4 L). The methylene chloride extract **(KA)** was concentrated under *vacuum* at 40°C to dryness (10 g). Directly after removing of the Cuticular waxes, the fresh plant material were subjected to exhaustive extraction with 70 % boiling ethyl alcohol (4 x 20 L). The ethanolic extract was concentrated by evaporation under *vacuum* at 40 ºC to yield (140 g) and the concentrated extract was suspended in distilled water (500 ml), then fractionated with methylene chloride **(KB)** to yield (15 g) , ethyl acetate **(KC)** to yield (16 g) , *n*-butanol **(KD)** to yield (25 g) and remaining aqueous **(KE)** extract (81 g). Further purification and separation of each fraction are conveniently achieved by chromatographic methods. Each fraction was subjected to series of silica gel column chromatography and gel filtration (sephadex LH-20) to afford different compounds.

Determination of antioxidant activity:

The free radical scavenging activity of the crude extracts of *K. tomentosa* was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay[3], at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University. The results were recorded in Table 1.

Determination of cytotoxic activity:

Cytotoxic activity of different plant extracts were measured against Human colon carcinoma (HCT-116) and Human breast cancer (MCF-7) cell lines[4] and the results are presented in Table 2. The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured two to three times a week, **Table 1**.

Determination of antimicrobial activity:

The *in vitro* antimicrobial activity was performed by agar cup plate diffusion method[5] at Microbiology and Immunology department, Faculty of Pharmacy, Al-Azhar University. The antibacterial activity[6-8]was carried out against two Gram-positive strains; *Bacillus cereus* and *Staphylococci aureus* (ATCC 6538), seven Gram-negative strains; *Escherichia coli* (ATCC 8739), *Salmonella typhi*, *Klebsiella pneumonia*, *Psedumonasa eruginosa* (ATCC 27853), *Proteus mirabilis*, *Acinetobacter baumanii* and *Shigella flexneri* and *in vitro* antifungal activity was carried out against two fungal strains; *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). The microbial strains were obtained from the culture collection of Department of Microbiology and Immunology (Faculty of Pharmacy, Al-Azhar University). The results were recorded in **Table 2**.

RESULTS AND DISCUSSION:

Dried aerial parts of *K. tomentosa* were extracted with ethanol and then fractionated with petroleum ether, ethyl acetate and *n*-butanol. From these extracts and by using combined chromatographic separations, 14 known compounds were isolated (Fig. 1).Their structures were elucidated using physicochemical and spectroscopic methods. The isolated metabolites were identified as;α-amyrin acetate[9] (**1**), friedelin[10] (**2**), glutinol[11] (**3**), 1-dotriacontanol[12] (**4**), phytol[13] (**5**), Stigmasta-7,25-dien-3β-ol[14] (**6**), *β*-sitosterol[15] (**7**), Isorhamnetin[16] (**8**), 2,3-dihydroxypropyl tetradecanoate[17] (**9**), Eriodictyol[18] (**10**), Gallic acid[19] (**11**), quercetin[20] (**12**), kampferol-3-*O*-Rutinoside[21] (**13**) and isovitexin[22] (**14**).

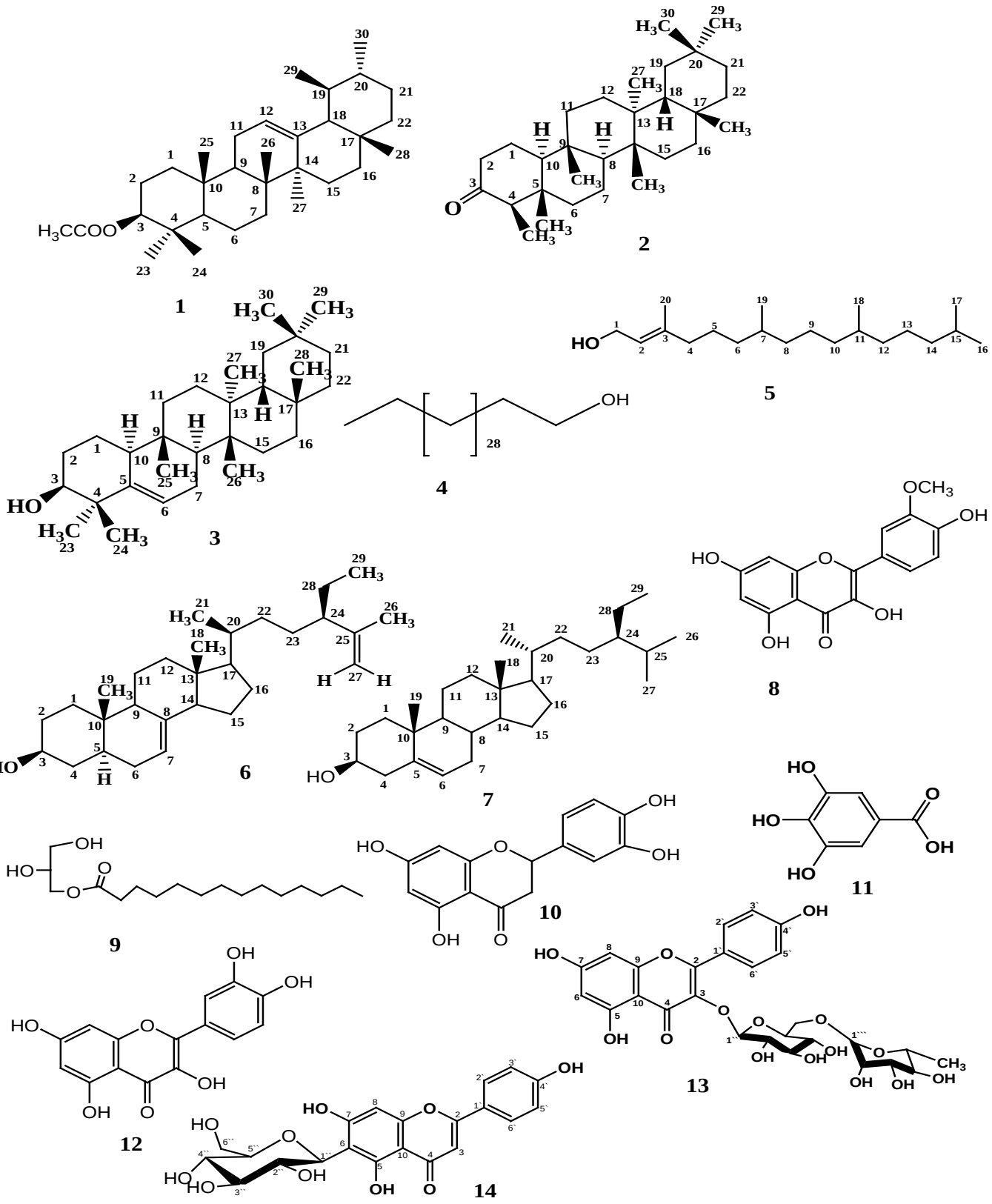

Figure 1: Compounds 1-14.

The order of potency of the different crude extracts as DPPH radical scavenging is ethyl acetate fraction (IC_{50} = 35.4 µg/ml) followed by methylene chloride fraction (IC_{50} = 71.3µg/ml) then *n*-butanol fraction (IC_{50} = 99.3µg/ml), Table (1).The *n*-butanol fraction exhibited the highest cytotoxic activity against the tested cell lines with values of IC_{50} from 2.4-3.7µg/ml, whereas the methylene chloride fraction showed a moderate cytotoxic activity

against the tested cell lines with values of IC_{50} from 16.3-20µg/ml. On the other hand, the ethyl acetate fraction showed cytotoxic activity against the tested cell lines with values of IC_{50} from 48.2µg/ml table 1.

Table 1: Scavenging effect on DPPH radicals and cytotoxic activity of crude extracts

Extracts	Antioxidant activity/(IC_{50} µg/ml)[1]	Cytotoxic activity (IC_{50} µg/ml)[2]	
		HCT-116	MCF-7
Methylene chloride	71.3	16.3	20
Ethyl acetate	35.4	48.2	>50
n-butanol	99.3	2.4	3.7
Ascorbic acid	11.2	-	-
Vinblastine	-	2.38 µg	4.6 µg

[1]IC_{50} denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.
[2]IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number.

The different crude extracts (methylene chloride, ethyl acetate and *n*-butanol) showed variable antimicrobial activity against most of the specific organisms tested (Table 2).

Table (2): Results of antimicrobial activity of different extracts of *Ficustrigonata*leaves

Sample / Tested microorganisms	KA	KB	KC	KD	KE	ST
FUNGI						***Amphotericin B***
Aspergillusfumigatus **(RCMB 02564)**	**16.2±0.25**	**13.6±0.58**	**NA**	**NA**	**14.1±0.63**	**23.7±0.10**
Candida albicans **(RCMB 05035)**	**14.7±0.37**	**11.4±0.44**	**NA**	**NA**	**12.6±0.44**	**21.9±0.12**
Geotricumcandidum **(RCMB 05096)**	**17.2±0.44**	**15.4±0.44**	**NA**	**NA**	**15.9±0.37**	**26.4±0.20**
Trichophytonmentagrophytes **(RCMB 09025)**	**15.9±0.25**	**13.6±0.37**	**NA**	**NA**	**14.3±0.44**	**25.4±0.16**
Gram Positive Bacteria:						***Ampicillin***
Staphylococcus aureus **(RCMB 010027)**	***18.6±0.44***	***14.2±0.25***	***11.2±0.25***	**NA**	***15.6±0.14***	***28.9±0.14***
Staphylococcus epidermidis **(RCMB 010024)**	***20.7±0.37***	***15.3±0.37***	***12.3±0.44***	**NA**	***16.7±0.18***	***25.4±0.18***
Staphylococcus pyogenes **(RCMB 010015)**	***21.6±0.37***	***15.9±0.25***	***14.3±0.19***	**NA**	***16.4±0.34***	***26.4±0.34***
Gram negative Bacteria:						***Gentamycin***
Proteous vulgaris **(RCMB 010085)**	***15.6±0.44***	***12.8±0.44***	***10.6±0.44***	**NA**	***13.8±0.44***	***23.4±0.3***
Klebsiella pneumonia **(RCMB 0010093)**	***16.0±0.44***	***13.6±0.58***	***12.8±0.58***	**NA**	***18.1±0.44***	***26.3±0.15***
Shigellaflexneri **(RCMB 0100542)**	***NA***	***NA***	***NA***	**NA**	***NA***	***24.8±0.24***
Pseudomonas aeruginosa **(RCMB 010043)**	***NA***	***NA***	***NA***	**NA**	***NA***	***17.3±0.12***
Escherichia coli **(RCMB 010056)**	***12.7±0.58***	***11.3±0.37***	***11.1±0.58***	**NA**	***11.2±0.37***	***25.3±0.18***

***NA: NO activity, data are expressed in the form of mean± SD.**

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