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Phytochemical, Biological and Botanical Studies of *Klanchoe blossfeldiana* Poelln

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Abbreviations:
Kalanchoe blossfeldiana: *K. blossfeldiana*, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, NP/PEG: Natural products-polyethylene glycol reagent, TLC: Thin-Layer Chromatography, VLC: Vacuum Liquid Chromatography, PC: Paper Chromatography

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Abstract
Kalanchoe blossfeldiana Poelln. (Crassulaceae) is a plant cultivated in Egypt. As many Kalenchoe species proved biological actions, the present study aimed to assess the Egyptian plant regarding its phytochemicals, possible biological actions and microscopical characters. GLC analysis of lipoidal matter was carried out using standard method and the alcohol extract of the plant was fractionated using different solvent of which ethyl acetate and butanol fractions were subjected to column chromatography. Isolated phenolics were identified using physico-chemical and spectral analyses. Antimicrobial study was carried out using agar diffusion method. The diuretic activity was determined using frusemide as reference drug. Antioxidant activity was assessed using DPPH assay. Palmitic acid (21.64 %) was the major fatty acid, while n-eicosane and n-octacosane, 26.68% and 30.84%, respectively were the major hydrocarbons. Four phenolic compounds were isolated and identified as methyl gallate , gallic acid, quercetin 3-O-β-galactopyranoside and kaempferitin Alcohol extract was tested for several activities as anti-inflammatory , hepatoprotective, cytotoxic, diuretic, antimicrobial .It showed potent diuretic, anti-bacterial and antioxidant activities. The studied microscopical features of the stems and leaves of the plant showed great agreement with the reported characters of family Crassulaceae.

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1. Introduction

1.1 History

Kalanchoe species (syn. Bryophyllum, family: Crassulaceae) are succulent perennial plants. There are over hundred species of *Kalanchoe*, native to tropical Africa, but have naturalized throughout the tropics (Costa et al., 2008). Different species viz; *Kalanchoe pinnata* Pers., *K. blossfeldiana* Poelln. and K. fedtschenkoi Raym.-Hamet & E.P. Perrier are flowering and growing in Egypt.

1.2 Genus Kalanchoe in Egypt

Some species *viz; Kalanchoe pinnata* Pers., *K. blossfeldiana* Poelln and *K. fedtschenkoi* Raym.-Hamet & E.P. Perrier are flowering and growing in Egypt.

1.2 Reports on K. blossfeldiana Pollen

Reports concerning the active constituents of *K. blossfeldiana* Poelln. have appeared since a pioneered study in 1963 (Neyland et al., 1963), then a recent study on the flavonoidal content (Nielsen et al., 2005).

1.3 Needs for this study

Nothing was reported dealing with the chemical constituents or macro and micromorphological, as well as, biological studies of the plant cultivated in Egypt as a member of genus Kalanchoe.

2. Objectives of Research

As some *Kalanchoe* species proved potent biological activities that was attributed to its phytochemicals, this study deals with one of *kalanchoe* species cultivated in Egypt that may be valuable in discovery of new herbal drugs. Evaluation of *K. blossfeldiana* Poelln includes:

1. Study of biological activities based on the reports of the genus to suggest a possible use of the drug in phytotherapy.

2. Phytochemical screening of the plant searching for its active phytochemicals and characterization of isolated compounds.

3. Establishment of pharmacognostic profile of the leaves and stem will assist in standardization and identification of samples from other related species.

3. Materials

3.1. Materials for Phytochemical Study
3.1.1 Plant material
Samples of the aerial parts of *K. blossfeldiana* Poelln. were collected in February to May (2006-2008) from the plant cultivated in El-Orman Botanical Garden Giza, Egypt. Identity of the plant material was kindly verified by Dr. Reem Hamdy, botanist specialist. Specimens were kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

3.1.2 Reference material
Reference material for fatty acids, hydrocarbons and sterols were obtained from E-Merck (Darmstadt, Germany).

3.1.3 Material, solvent system and spray reagents for chromatographic study
Precoated silica gel F_{254} for thin-layer chromatography (TLC) from Fluka (Sigma-Aldrichchemicals-Germany); Silica gel H 60 for vacuum liquid chromatography (VLC) was purchased from E-Merck (Darmstadt, Germany); Sephadex LH-20, Pharmacia Fine Chemicals AB Uppsala, Swedenand sheets of Whatmann No. 1 for paper chromatography (PC).

The following solvent systems were prepared from analytical grade chemicals:
S_1-Ethyl acetate-Methanol-Water-Formic acid (100:16.5:13.5:0.2 v/v, TLC)
S_2-n-Butanol-Acetic acid-Water (4:1:5 v/v upper layer PC)

The following spray reagents (Stahl, 1969) were used:

Reagent 1: Aluminium chloride for flavonoids. Reagent 2: Ferric chloride for phenolics. Reagent 3: Natural products-polyethylene glycol reagent (NP/PEG): for phenolics.

The Shift reagents (Mabry et al., 1996), and chemicals used during performance of the UV spectroscopic analysis of flavonoids were: sodium methoxide 2.5%, aluminium chloride solutions, hydrochloric acid, sodium acetate and boric acid anhydrous powders.

3.1.4 Apparatus for Phytochemical Study
Thermo Pye Unicam Series 304 Gas Chromatograph, UK, for GC analysis of saponifiable and unsaponifiable matter; Varian Mercury NMR-Spectrophotometer (Japan) 1H-NMR, 300 MHz, ^{13}C-NMR, 75 MHz spectra were recorded in DMSO, $CDCl_3$ and CD3OD using TMS as internal standard and chemical shift values expressed in δ ppm; Triple Quadropole (TQD) Mass Spectrophotometer, Waters, Milford, MA, USA, for ESI-MS; Mass Spectrometer, Varian Mat 711, Finnigan SS Q 7000 for recording mass spectra by EI mode; UV-Visible Spectrophotometer, Shimadzu UV-1650 PC was used forrecording UV spectra and measuring the absorbance in UV range; Ultraviolet lamp (λ_{max}= 254 and 365 nm, Shimadzu) a product for Hanovia lamps, for localization of spots on chromatograms.

3.2 Materiasl for Biological study
3.2.1 Experimental animals
Male albino mice (20-30 g) were used for determination of LD_{50} and analgesic activity. Adult male albino rats of Sprague Dawley strain (120-200 g) were utilized for assessment of different biological effects. All animals were obtained from the animal house (Cairo University), kept in laboratory house, at constant environmental conditions and allowed free access to food and water.

3.2.2 Carcinoma cell lines
Five human cell lines viz.,liver (HEPG2), breast (MCF-7), larynx (HEP-2), cervical (HELA) and colon (HCT116) human carcinoma cell lines were obtained frozen in liquid nitrogen (-180° C) from the American Type Culture Collection. The tumor cell lines were maintained in the Cancer Biology Department, National Cancer Institute (Kasr El Aini Street, Cairo, Egypt) by serial sub-culturing.

3.2.3 Microorganisms
a series of microorganisms (available in stockcultures at the Micro Analytical Center, faculty of Science, Cairo University) was used for susceptibility testing comprising: *Staphylococcus aureus* (ATCC 12600), *Bacillus subtilis* (ATCC 6051) as representative Gram-positive, *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 10145) as Gram-negative, *Aspergillus flavus* (ATCC) and *Candida albicans* (ATCC 26555) as the tested fungal strains.

3.2.4 Authentic reference materials
3.2.4.1 Carrageenan: used for induction of inflammation (Sigma, USA).
3.2.4.2 Acetyl salicylic acid: aspocid infantile tablets (75 mg) as anti-inflammatory from CID Phaemaceuticals, Giza, Egypt;
3.2.4.3 Diclofenac sodium
3.2.4.4 Voltaren tablets (25 mg)asanalgesic from Novartis Pharma S.A.E., Cairo, Egypt, under licence from Novartis Pharma A G., Basle, Switzerland.

3.2.4.5 Furosemide
Lasix tablets (40 mg) as diuretic Hoechst.
3.2.4.6 Silymarin: Silymarin (140 mg) as hepatoprotective Sedico Pharmaceutical Co., 6 October City, Egypt

3.2.4.7 2, 2-Diphenyl-1-picrylhydrazyl (DPPH reagent) used for in-vitro antioxidant activity, Sigma-Aldrich, Germany dissolved in methanol HPLC grade, Sigma-Aldrich, Germany in a concentration 0.004 %

3.2.4.8 Gallic acidas reference standard for in-vitro antioxidant activity,Sigma-Aldrich, Germany.

3.2.4.9 Transaminase kits (Bio-merieux Co., France) biochemical kits foraspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

3.3. Material for Botanical Study
Fresh samples of *Kalanchoe blossfeldiana* Poelln. were used, as well as, samples kept in alcohol 70 % containing 5 % glycerin. Photographs were taken using a Casio Digitalcamera.Anatomical investigations were performed on crosssections of the stem and leaf and on powder. The photographs were taken using a LeicaDFC500 digital camera.

4. Methods

4.1 Phytochemical Study
4.1.1 Preliminary Phytochemical Screening
Fresh samples of the aerial partsof the plant under investigationwere subjected to microsublimation (Claus and Tyler, 1967), steam distillation and chemical tests for different constituents viz.; carbohydrates and/or glycosides (Coutts and Snail, 1973), sterols and/or triterpenes (Wall et al., 1954, Libermann and Burchard, 1890), flavonoids (Geissman, 1962), tannins (Wall et al., 1954), anthraquinones (Balbaa and Hilal, 1981), cardinolides (Balbaa and Hilal, 1981), proteins and/or amino acids (Coutts and Snail, 1973), alkaloids and/or nitrogenous bases (Peach and Tracey, 1955) and saponins (Walform et al., 1940).

4.1.2 Extraction, Fractionation and Isolation
Samples of the fresh aerial parts (9 Kg) were communized into small pieces using a blender and macerated in hot ethanol 90% (for deactivation of enzymes). Then, extract with ethanol 90 % till exhaustion. The ethanolic extract was evaporated under reduced pressure to yield 134 g. An aliquot portion of the ethanolic extract (110 g) was suspended in 200 ml of distilled water and partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol till exhaustion. The solvents were then evaporated under reduced pressure. The residues were weighed and kept for phytochemical study. The solvent-free extractives were physico-chemically examined and screened by PC and/or TLC.

4.1.3 Investigation of Lipoidal Content
The solvent-free n-hexane extract (4.2 g) was saponified, the saponifiable and non-saponifiable fractions were separated (British Pharmacopoeia 1998).

4.1.3.1 Investigation of the saponifiable fraction
The saponifiable fraction, as well as, authentic reference fatty acids were methylated (Finar, 1985) and subjected to GLC analysis adopting the following conditions: column dimension (30m x 0.25mm I.D. x 0.25 µm film); stationary phase, TR-FAME, Technique, 70- 190° C, 8oC/min then isothermally at 190° C for 25 min; nitrogen flow-rate, 30 ml/min. Identification of compounds was carried out by comparison of the relative retention times of the peaks with those of the available authentics. Quantitative determination was based on relative peak area measurement.

4.1.3.2 Investigation of the non-saponifiable fraction

The non-saponifiable fraction as well as, the available reference samples were analysed by GLC adopting the following conditions: column dimension (30m x 0.25mm I.D. x 0.25 µm film); stationary phase, TR-5MS, Technique, 70-270° C, 10° C/min then isothermally at 270° C for 25 min; other operating conditions, as mentioned under the saponifiable fraction. Identification of sterols and/or triterpenes, as well as, hydrocarbons was carried out by comparison of the relative retention times of the peaks with those of the available authentics. Quantitative determination was based on relative peak area measurement.

Results of GLC analyses of fatty acid methyl esters and non-saponifiable fractions are recorded in tables 1 and 2.

4.1.4 Investigation of the Ethyl Acetate Extract:

The ethyl acetate extract of *K. blossfeldiana* Poelln. (13 g) was fractionated on a silica gel column. Elution was started with CH_2Cl_2 followed by increasing polarity through 5 % increments of CH_3OH until 100% CH_3OH was reached. Fractions (100 ml, each) were collected and monitored on precoated TLC silica gel F_{254}plates using S_1 as the solvent system. Spot visualization was carried out by examination under UV light (254 and 365 nm) before and after exposure to ammonia vapor, as well as by spraying with reagents 1, 2 and 3. Based on their chromatographic pattern, similar fractions were pooled together and combined to yield three main fractions. Fractions yielding mixtures of compounds were subjected to repeated refractionation on series of sephadex LH-20 columnsusing methanol-water (1:9) as eluent resulted in isolation of three pure phenolic compounds namely E_1-E_3.

4.1.5 Investigation of the n-Butanol Extract

The n-butanol extractive (22 g) was fractionated on a VLC column. Elution was started with CH_2Cl_2 followed by increasing polarity through 5 % increments of CH3OH until 100% CH_3OH was reached. Fractions (200 ml, each) were collected and monitored on precoated silica gel F_{254}plates using S_1 as the solvent system. Spot visualization was carried out as previously mentioned under ethyl acetate extract. Based on their chromatographic pattern, similar fractions were pooled together and combined to yield five main fractions. Fractions yielding mixtures of compounds were subjected to repeated refractionation on series of sephadex LH-20 columns (Pharmacia) using methanol-water (1:9) as eluent. This procedure afforded 4 pure compounds E_1-E_3 (previously isolated from the ethyl acetate fraction) and E_4.

4.1.6 Characterization

The structure elucidation was established on the basis of physico-chemical data. Physical, UV, MS and 1H-NMR spectral data of the isolated compounds are as follows:

CompoundE_1: White crystalline powder (33 mg), m.p. 201-203° C, soluble in methanol and water, R_f value: 0.93 (TLC-S_1), 0.78 (PC-S_2). Itappeared as violet spot in UV (254 nm), the color was intensified upon exposure to ammonia vapors and turned bluish black after spraying with spray reagent 2.The UV spectral data of compound E1in MeOH showed two bands at 220 and 278.E_I-MS (70eV) m/z, $[M^+]$ = 184. ^{1}H-NMR (300 MHz- D_2O) data: δ ppm 7.14 (2H, s, H-2, 6), 3.71 (3H, d, O-CH_3)

Compound E_2: White crystalline powder (140 mg), m.p. 248-250° C, soluble in methanol and water, R_f value: 0.87 (TLC-S_1), 0.48 (PC-S_2). Itappeared as violet spot in UV (254 nm), the color was intensified upon exposure to ammonia vapors and turned dark blue after spraying with spray reagent 2. The UV spectral data of compound E2 in MeOH showed two bands at 215, 270. ^{1}H-NMR (300 MHz- DMSO) data: δ ppm 7.005 (2H, s, H-2, 6).

Compound E_3: yellow needle crystals (30 mg), m.p. 225-227, soluble in methanol,Rf value: 0.51 (TLC-S_1), 0.48 (PC-S_2). It appeared brown under UV light (365 nm), upon exposure to ammonia vapors, spray reagent 1 and turned to orange upon spraying with spray reagent 3. UV spectral data, λ_{max} (nm), (MeOH): 257, 268sh, 302sh, 356; (+NaOMe): 270, 327, 409; (+$AlCl_3$): 275, 302sh, 334, 438 ; (+AlCl3/ HCl): 268, 300sh, 366, 404; (+NaOAc): 274, 324, 380 and (+NaOAc/H_3BO_3) 260, 300sh, 370. ESI-Ms: $[M-H]^+$: 465. ^{1}H-NMR (300 MHz, CD3OD) data: δ ppm7.82 (^{1}H, d, J=2.76 MHz, H-6′), 7.58 (1H, d, J=2.76 MHz, H-2′), 6.86 (1H, d, J=10.1 MHz, H-5′), 6.39 (1H, d, J=2.76 MHz, H-6), 6.19 (1H, d, J=1.8 MHz, H-8), 5.14 (1H, d, J=10.98 MHz, H-1″) and 3.28-3.62 (sugar protons).

Compound E_4: yellow needle crystals (10mg), m.p. 217-219° C, soluble in methanol.Rf value: 0.34 (TLC-S1), 0.42 (PC-S_2). It appeared brown under UV light (365 nm), turned dark yellow upon exposure to ammonia vapors and

yellow upon spraying with spray reagents 1 and 3. UV spectral data, λ_{max} (nm), (MeOH): 265, 315 sh, 352; (+NaOMe): 242, 271, 301sh, 350 sh, 389; (+$AlCl_3$): 255 sh, 274, 301sh, 354, 400 ; (+$AlCl_3$/ HCl): 273, 300 sh, 348 sh, 395 ; (+NaOAc): 265, 318 sh, 358, 406 sh; (+NaOAc/H_3BO_3) 266,319 sh, 352.ESI-Ms: [M-H]-: 577.^{1}H-NMR (300 MHz, CD3OD) data: δ ppm 7.79 (2H, d, J=8.4, H-2′/6′), 6.94 (2H, d, J=8.1, H-3′/5′), 6.73 (1H, d, J=2.5 MHz, H-8), 6.46 (1H, d, J=2.5 MHz, H-6), 5.55 (1H, brs, H-1″), 5.37 (1H, brs, H-1‴), 3.26-4.18 (m, remaining sugar protons) 1.25 (3H, d, CH_3-6″), 0.9 (3H, d, CH_3-6‴) and 0.94 (3H, d, CH3-6‴).

It is noteworthy to mention that compounds E_1-E_4 are reported here for the first time from *Kalanchoe blossfeldiana* Poelln. cultivated in Egypt. Although, compound E_2 was previously reported in the aerial parts of *kalanchoe pinnata* Pers growing abroad (Ogungbamila et al., 1997) and compound E_4 was previously reported from family Crassulaceae (Mulinacci et al., 1995).

4.2. Biological Study

4.2.1. Determination of Median Lethal Dose (LD_{50})

The LD_{50} of the alcoholic extract (10% in water) of the aerial parts of *K. blossfeldiana* Poelln. was estimated by oral treatment of male albino mice (20-25 g) adopting Karber's procedure (1931). The animals were divided into seven groups, each of six mice. Preliminary experiments were carried to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses (up to 4 g/Kg b.wt.) at equal logarithmic intervals were selected; each dose was applied to a group of animals by subcutaneous injection. The symptoms of toxicity and mortality were recorded within the first 24 hours after administration.

4.2.2 Effect of Prolonged Administration

The effect of prolonged oral administration of the alcoholic extract of the plant under investigationwas studied on liver enzymes (AST, ALT and ALP), total proteins, albumin, globulin, and total bilirubin serum levels, as well as, serum creatinine, urea and uric acid were determined to evaluate the effect on kidney function. Animals were divided into 2 groups (of 5 rats each, 150-200 g): the first group received saline solution serving as control, the second received alcoholic extract of *K. blossfeldiana* Poelln. in a dose of 200 mg/Kg b.wt. for 30 days. Blood samples were taken to study the effect of prolonged use on the liver and kidney serum enzymes level. The effect on kidney and liver functions was evaluated by colorimetric determination of related serum enzymatic activities. Results obtained are recorded in table 3.

4.2.3. Anti-inflammatory Activity

This activity was carried out adopting the carrageenan-induced rat paw oedema method (Winter et al., 1962). Fifteen albino male rats, weighing 130-150 g were divided into three groups of five animals each: the first groupreceived saline serving as control, the second group: rats received acetyl salicylic acid as reference drug in a dose of 100 mg/Kg b.wt. by intraperitoneal route and the third group: rats received alcoholic extract in an oral dose of 200 mg/Kg b.wt.

One hour later, the animals had a subplantar injection of 0.1 ml of 1% carrageenan solution in salineinto the right hind paw. Paw size was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a meter rule (Bambgose and Noamesi, 1981). Measurement was carried out immediately before and three hours after carrageenan administration.Percent inhibition of tested extracts and standard drug was calculated in comparison with saline control (100%).

The results of anti-inflammatory activity of the extracts on carrageenan induced paw oedema in rats are recorded in table 4.

4. 4.2.4. Analgesic Activity

This activity was evaluated adopting writhing method according to Collier et al., (1968). Fifteen mice weighing (25-30 g) were divided into three groups of five animals each: the first group received saline serving as control, the second group: rats received diclofenac sodium as reference drug in a dose of 50 mg/Kg b.wt. by intraperitoneal route and the third group: rats received alcoholic extract in an oral dose of 200 mg/Kg b.wt.

Writhing was induced thirty minutes later, by intraperitoneal injection of 0.1 ml of 0.6 % acetic acid. The number of abdominal contractions (writhes/min) was counted in all animals at one hour intervalfor four hours after administration. Moreover, percentage inhibition of writhing reflex was calculated by counting the number of writhes in the control mice and those pre-treated with the tested extracts according to the formula:

% inhibition = (control mean – treated mean) X 100 / control mean

Results of analgesic effect of both extracts represented by number of writhes and by % protection against writhing were recorded in tables 5 and 6, respectively.

4.2.5 Diuretic Activity

Diuretic activity was performed (Lipschitz et al., 1943) in healthy male albino rats (180-200 g). The animals were private of food from 3:00 p.m. from previous day and water was suppressed to them at 7:00 a.m. of the following day. At the beginning of the experiment, the urinary bladder was emptied by gentle compression of the abdomen. Animals were divided into three groups of five rats, each. The first group received saline serving as control, the second group: rats received frusemide as reference drug in a dose of 20 mg/Kg b.wt. by intraperitoneal route and the third group: rats received alcoholic extract in an oral dose of 200 mg/Kg b.wt.The doses were given by mean of introgastric cannula beginning at 8:00 a.m. Rats were kept for six hours in metabolic cages under fluid and food deprivation and the urine volume is measured at the end of this period.

Results of diuretic effect of the tested extract against frusemide on urine volume of rats are illustrated in table 7.

4.2.6. Hepatoprotective Activity

Alcoholicextract was administered orally in a daily manner for 30 days to adult male albino rats (130-140g). The animals were divided into three groups of ten animals each. Thefirst groupreceived saline serving as control, the second group: rats received silymarin as reference drug in a dose of 25 mg/Kg b.wt. by intraperitoneal route and the third group: rats received alcoholic extract in an oral dose of 200 mg/Kg b.wt.

After one month of drug administration,liver damage in rats was induced by intraperitoneal injection of 5 ml/Kg b.wt. of 25 % CCl_4 in liquid paraffin adopting the method of Klassan and Plaa, (1969). Seventy two hours after administration of CCl_4, blood samples were withdrawn to be used for the biochemical study.

Whole blood samples were obtained from the retro-orbital venous plexus through the eye canthus of anaesthetized rats by means of clean dry heparinized capillary tubes and separately transferred into clean dry centrifuge tubes. Blood samples were allowed to clot by standing at room temperature for 20 minutes.Serum was isolated by centrifugation and divided for analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman and Frankel 1957), alkaline phosphatase (ALP) (Kind and King 1954) enzymes, as well as total bilirubin (Walter and Gerarde 1970), total proteins (Henry et al., 1974), albumin (Doumas et al., 1971) and globulin (obtained by the difference between total proteins and albumin). Results obtained are recorded in table 8.

4.2.7 Antitumor Screening

Five human cancer cell lines: HEPG-2 (liver cancer cell line), MCF-7 (breast cancer cell line), HEP-2 (head and neck squamous cell line), HELA (cervical carcinoma cell line) and HCT-116 (colon carcinoma cell line)were maintained in the cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt, were used. The antitumor activity of the alcoholic extract was studied using the Sulpho-Rhodamine-B (SRB) assay adopting the method of Skehan et al., (1990). The IC_{50} (dose of the extract which reduces survival to 50 % by µg/ml) was calculated and the results are summarized in table 9.

4.2.8 Antioxidant Screening

The antioxidant activity was assessed using a modified quantitative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (Hosny et al., 2002). The DPPH was prepared at a concentration of 0.004 % in methanol (HPLC grade). The alcohol extract of the studied plant was dissolved in 50% methanol at a concentration of 0.1, 0.2, 0.4, 0.6 and 0.8 mg/ml.Sample of the tested solution, 200 µl, was added to 6 ml DPPH solution. Blank was carried out using 200 µl methanol and 6 ml DPPH solution. Solutions were incubated in dark for 30 minutes at room temperature. The absorbance of each sample was measured against methanol at 517 nm. Gallic acid was used as a standard control at a concentration of 0.005, 0.01, 0.02, 0.04, 0.06 mg/ml. The antioxidant activity data are presented in Table 10, in terms of IC_{50} ± SD, which is the concentration in mg/ml causing 50% inhibition of the free radical.

4.2.9 Antimicrobial Screening

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method (Bauer et al., 1966) against representatives of Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis)*, Gram-negative bacteria (*Escherichia*

coli, Pseudomonas aeruginosa), fungi (*Aspergillus flavus* and *Candida albicans*).The samples were dissolved in DMSO at a concentration of 20 mg/ml. Mueller-Hinton agar was used as culture media. A filter paper disc (8 mm in diameter) impregnated with 10µl of the tested sample is placed on agar. The plates were incubated while inverted, at 35-37° C for 24-48 hours in case of bacteria and at 25° C for 48 hours in case of fungi. DMSO (10 µl) was used as negative control; tetracycline and amphotericin B were used as positive controls for bacteria and fungi, respectively. Zone of inhibition were measured (in mm) diameters less than 5 mm were considered as having an inhibitory effect. Diameter of zone of inhibition (mm) and potency percentage of the tested samples are recorded in table 11.

All the data obtained were analyzed using students t-test where means of the treated groups were compared to that of the control for each variable (Snedecor and Cochran, 1982).

5. Results and Discussion

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the aerial parts of *K. blossfeldiana* Poelln. revealed the presence of carbohydrates and/or glycosides, flavonoids, tannins, sterols and/or triterpenes. Volatile constituents, crystalline sublimates, proteins and/or amino acids, saponins, alkaloidsand/or nitrogenous bases, anthraquinones, bufadienolides and cardenolides were not detected.

Table 1: Identified fatty acids of *K. blossfeldiana* Pollen.

RRT(min.)	Components	Relative Percentage
0.62	Capric acid C10	4.25
0.79	Lauric acid C12	6.67
0.94	Myristic acid C14	8.74
1.00	Palmitic acid C16	21.64
1.51	Stearic acid C18	3.29
1.60	Oleic acid C18:1	8.13
1.80	Linoleic acid C18:2	19.11
2.01	Linolenic acid C18:3	13.79
4.04	Arachidic acid C20	0.52
4.42	Arachidonic acid C20:4	2.42
Percentage of identified components		88.56
Percentage of saturated fatty acids		45.11
Percentage of unsaturated fatty acids		43.45

RRT: Relative retention time to palmitic acid (Rt .04)

GLC analysis of the non-saponifiable fraction (table 2) revealed that the total identified hydrocarbons and sterols represented 80.42% and 13.99 %, respectively. The major detected hydrocarbons were n-eicosane C20 and n-octacosaneC28 (26.68 % and 30.84 %, respectively). The identified sterols were stigmasterol and β-sitosterol (8.03% and 5.96 %, respectively).

Table 2: Identified non-saponifiable constituents of *K. blossfeldiana* Pollen.

RRT(min.)	Components	Relative Percentage
0.69	n-Decane (C10)	1.12
0.73	n-Dodecane (C12)	0.27
0.74	n-Tridecane (C13)	0.20
0.77	n-Tetradecane (C14)	1.69
0.81	n-Pentadecane (C15)	3.22
0.85	n-Hexadecane (C16)	0.93
0.92	n-Heptadecane (C17)	1.96
0.98	n-Octadecane (C18)	0.53
1.00	n- Eicosane(C20)	26.68
1.09	n-Heneicosane (C21)	0.95
1.26	n-Decosane (C22)	2.54
1.29	n- Tetracosane (C24)	0.44
1.34	n-Pentacosane (C25)	0.50
1.45	n- Hexacosane (C26)	1.21
1.60	n-Heptacosane (C27)	7.40
1.65	n-Octacosane (C28)	30.84
1.66	Stigmasterol (C29)	8.03
1.69	β-Sitosterol (C29)	5.96
Percentage of identified hydrocarbons		80.48
Percentage of identified sterols		13.99

RRT: Relative retention time to n-eicosane (Rt 27.01)

3.2 Lipoidal Content

GLC analysis of the saponifiable fraction (table 1) showed the presence of at least 19 components. Ten of which were identified under the experimental conditions and amounted to 88.54%. The major saturated fatty acid was palmitic acid (21.64 %).The predominant unsaturated fatty acids were linoleic acid and linolenic, amounted to 19.11 % and 13.79 %, respectively.

3.3 Constituents of Ethyl Acetate & butanol fractions (figure 1).

Compound E_1: It exhibited a molecular weight of 184 as indicated by EI-MS (70eV) m/z, [M^+] = 184, 153 (M^+-OCH_3), 123 (M+- CO_2Me),

corresponding to a molecular formula $C_8H_8O_5$. ^{1}H-NMR (300 MHz- D_2O) spectrum showed sharp singlet at δ-7.14 ppm assigned to two equivalent *meta* –coupled aromatic protons H-2, 6 with two protons integration which was characteristic to gallic acid moiety, it also showed sharp singlet at δ-3.71 ppm with three protons integral characteristic for the methoxy group. It was identified as methyl gallate. Identification was confirmed by comparing with published data (Gudej, 2003; Mahjan and Pai, 2010)

Compound E_2: The UV spectral data of compound E_2 showed one two bands at 215, 270 in MeOH. It exhibited a molecular weight of 170 indicated by EI-MS (70eV) m/z, [M+] = 170, 153 (M+-H_2O), 125 (M+-H-CO_2), corresponding to a molecular formula of $C_7H_6O_5$. ^{1}H-NMR (300 MHz-DMSO) spectrum displayed the characteristic signal of gallic acid (Wang et al., 2007) at δ 7.005 ppm with two protons integration assigned to H-2 and H-6. It was identified as gallic acid.

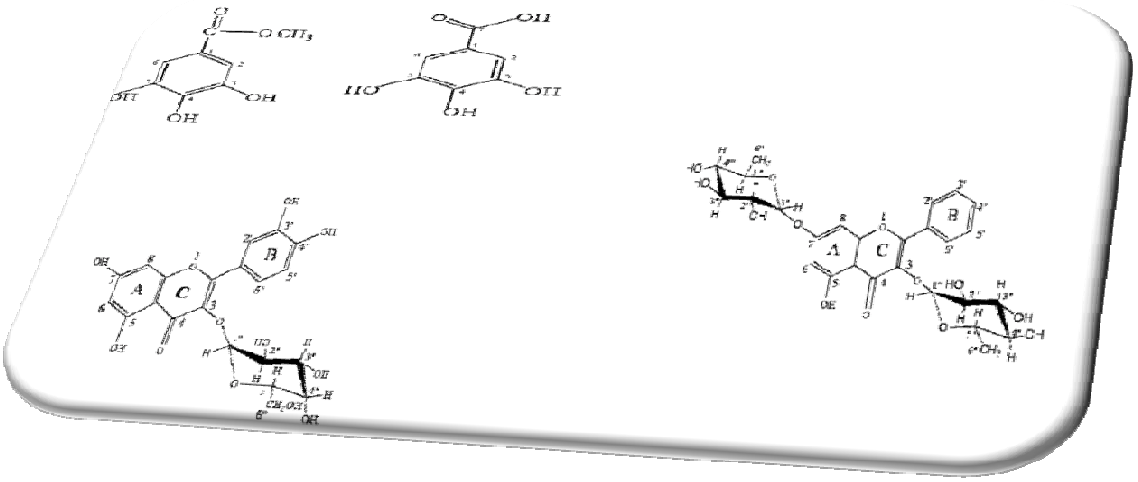
Compound E_3: UVspectral data in MeOH and in the presence of different shift reagents suggested compound E_3 to be a flavonol structure type (band I and II at 356 and 271 nm, respectively) (Mabry et al., 1970; Geissman, 1962; Markham, 1982 and Wagner et al., 1983). A bathochromic shift in band I (53 nm) with increased intensity upon addition of NaOMe revealed the presence of a 4′ hydroxyl group and absence of free 3-hydroxyl group (no decomposition after 5 min). The bathochromic shift in band II on addition of NaOAc (17 nm) indicated the presence of a free 7-OH. While a hypsochromic shift (34 nm) in band I in the $AlCl_3$/ HCl relative to $AlCl_3$, as well as, a hypsochromic shift (10 nm) in band I with NaOAc/H_3BO_3 relative to NaOAc confirm the presence of 3′, 4′ *ortho*-dihydroxyl group on ring B. Complete acid hydrolysis of compound E3 revealed the presence of quercetin and D-galactose (co-chromatography with authentic samples). The compound exhibited a molecular weight of 464 as shown by positive ESI-MS [M+H] + was detected at m/z 465. It corresponds to a molecular formula $C_{21}H_{20}O_{12}$.

^{1}H-NMR (300 MHz, CD_3OD), displayed a spectrum typical to that of 3-OH substituted quercetin. The ^{1}H-NMR spectrum showed two doublets signals at δ-6.19 and 6.39 ppm (J=2.76 MHz), indicated the presence of two *meta* protons at C-6 and C-8 of ring A, respectively. B ring protons were represented by two doublets at 6.86 (1H, d, J=10.1 MHz) and 7.57 (1H, d, J=2.76 MHz,) assigned for H-5′*ortho*-coupling and H-2′ meta-coupling with H-6′, respectively. While H-6′ was displayed as doublet signal at δ 7.82 ppm (1H, d, J = 2.76 MHz). The anomeric proton of sugar unit appeared at δ 5.14 ppm (1H, d, J = 10.98 MHz) and showed a β-configuration evidenced by its coupling constant. The remaining sugar protons appeared in the range δ 3.28-3.62 ppm. From the above physicb ochemical and spectral results compound E_3 (figure 7) was suspected to be quercetin 3-O-β-galactopyranoside. Identification was confirmed by comparing with published data (Shigematsu et al, 1982; Zalabani et al, 2004; Kim et al., 2011).

Compound E_4: UVspectral data of compound E4in MeOH and in the presence of different shift reagents are in accordance with 3, 7-disubstituted flavonol (Mabry et al., 1970; Geissman, 1962). Band I and II appeared at 352 and 265 nm, respectively. The bathochromic shift (37 nm) in band I with NaOMe relative to MeOH is diagnostic for the presence of free 4′-OH, the hypsochromic shift in band II with $AlCl_3$ (10 nm) that persisted on the addition of HCl indicated the presence of 5-OH. Also, the absence of bathochromic shift in band II with NaOAc confirms the absence or glycosylation of 7-OH.The compound exhibited a molecular weight of 578 as shown by negative ESI-MS, [M-H] - was detected at m/z 577. The ^{1}H-NMR (300 MHz, CD_3OD) spectrum showed the expected signals of kaempferol: the two meta-coupling protons resonated at δ 6.46 and 6.73 ppm assigned for the ring A H-6 and H-8, respectively represented by doublet, J=2.5 Hz, their downfield shift revealed the glycosylation at 7-hydroxy position. It showed also, two *ortho*-coupled doublets at δ 7.79 ppm assigned for H-2′, 6′, and the broad singlet at δ 6.94 ppm was assigned for H-3′, 5′. The anomeric protons at δ 5.55 and d 5.37 were attributed to two L-rhamnosyl moieties directly linked to the aromatic rings at the 7 and 3 positions, respectively. The rhamnosyl methyls appeared as doublets at δ 1.25 and 0.90 ppm. The remaining sugar protons were observed in the range δ 3.26-4.18. Based on the above discussion and spectral results compound E_4 (figure 7) could be identified as Kaempferol 3, 7- di -O-rhamnoside (Kampferitin). Identification was confirmed by comparing with published data (Iwashina et al., 1995; Mulinacci et al., 1995; El-Sayed et al., 1999). It is noteworthy to mention that compounds E1-E4 are reported here for the first time from Kalanchoe blossfeldiana Poelln. Cultivated in

Egypt. Compound E2 was previously isolated from the aerial parts of Kalanchoe pinnata Pers growing abroad (Ogungbamila et al., 1997) and compound E4 was previously reported from family Crassulaceae (Mulinacci et al., 1995).

Figure 1: Structure of the isolated phenolic compounds from *Kalanchoe blossfeldiana* Poelln



3.4 Biological Study

3.4.1 Determination of Median Lethal Dose (LD_{50})

Oral administration of the alcoholic extract of the aerial parts of *K. blossfeldiana* Poelln. in doses up to 4 g/Kg b.wt. failed to kill mice within 24 hours. We conclude that the tested extracts were not toxic.

3.4.2 Effect of prolonged administration

Prolonged administration for 30 days of alcoholic extract have no significant effect on the levels of liver enzymes (AST, ALP, ALT), total proteins, albumin, globulin, total bilirubin and kidney enzymes (creatinine, urea, uric acid).

Prolonged administration for 30 days of alcoholic extract have no significant effect on the levels of liver enzymes (AST, ALP, ALT), total proteins, albumin, globulin, total bilirubin and kidney enzymes (creatinine, urea, uric acid).

3.4.3 Anti-inflammatory Activity (table 4)

The alcoholic extracts of both plants showed a significant moderate anti-inflammatory activity at a tested dose (200 mg/Kg b.wt.) when compared to the acetyl salicylic acid (100 mg/Kg b.wt. by intraperitoneal route).

3.4.4 Analgesic Activity (table 5&6)

The alcoholic extract showed significant analgesic activity after one hour of writhing induction by i.p. injection of 0.6 % acetic acid at a tested dose (200 mg/Kg b.wt.) when compared to diclofenac sodium (50 mg/Kg b. wt.). The observed analgesic effect decreases by time to be ineffective after 4 hours of writhing induction.

Table 3: Effect of prolonged administration of ethanolic extract of *K. blossfeldiana* Poelln. on kidney& liver functions

Groups	Control	Ethanolic extract
AST (U/L)	130.2±6.82	138.2±7.83
ALT (U/L)	57.7±3.07	58.3±2.74
ALP (U/L)	85.22±3.60	87.50±4.15
Total proteins (mg/dl)	7.49±0.28	7.54±0.22
Albumin (mg/dl)	4.58±0.11	4.62±0.15
Globulin (mg/dl)	2.91±0.05	2.92±0.06
Total bilirubin (mg/dl)	0.55±0.02	0.54±0.02
Urea (mg/dl)	47.4±2.33	40.7±2.88
Creatinine (mg/dl)	0.76±0.04	0.68±0.02
Uric acid (mg/dl)	2.06±0.10	1.98±0.07

Table 4: Anti-inflammatory activity of the ethanolic extract of *K. blossfeldiana* Poelln

Groups	Paw size (mm) (mean ± S.E.M)	% of Reduction in paw size
Control	29.80±1.65	-
acetylsalicylic acid	15.72±1.72***	47.24
Ethanolic extract	23.50±1.82*	21.14

Significant at: * P ≤ 0.05 **P≤ 0.01 ***P≤ 0.001

3.4.5 Diuretic Activity

The alcoholic extract of *K. blossfeldiana* Poelln. showed a significant potent diuretic effect at tested dose (200 mg/Kg b.wt.) when compared to frusemide (in a dose of 20 mg/Kg b.wt.).

Table 5: Analgesic activity of *K. blossfeldiana* Poelln. Represented by reduction number of writhes

Groups	Number of writhes/minute				
	0	1 hour	2 hours	3 hours	4 hours
Control	42.5±2.6	42.3±2.7	43.2±3.0	44.7±3.2	45.5±3.1
Diclofenac sodium	42.2±2.7	10.2±2.4	9.8±2.4***	12.3±1.9***	17.8±2.4***
Ethanolic extract	41.7±3.0	24.3±2.9***	25.1±2.8***	33.9±2.8*	38.8±3.2

Significant at: * P ≤ 0.05 ***P≤ 0.001

Table 6: Analgesic activity represented by % protection against writhing of ethanolic extract of *K. blossfeldiana* Poelln.

Groups	% Protection against writhing				
	0	1 hour	2 hours	3 hours	4 hours
Control	-	-	-	-	-
Diclofenac sodium	-	75.82	76.77	70.85	57.81
Ethanolic extract	-	41.72	39.80	18.70	6.954

Table 7: Diuretic effect of ethanolic extract of *K. blossfeldiana* Poelln. Against frusemide

Groups	Volume of urine (ml)
Control	2.93 ± 0.18
Frusemide	4.25 ± 0.19***
Ethanolic extract	3.49 ± 0.19*

Significant at: * P ≤ 0.05 ***P≤ 0.001

3.4.6 Hepatoprotective

Activity Hepatoprotective activity of the alcoholic extract of *K. blossfeldiana* Poelln. was investigated in CCl_4 intoxicated rats (by intraperitoneal injection of 5 ml/Kg b.wt.).It showed significant decrease in AST, ALT, ALP and total bilirubin serum levels when compared to silymarin (in a dose of 25 mg/Kg b.wt.). This results support a moderate significant hepatoprotective activity

Table 8: Hepatoprotective effect of ethanolic extract of *K. blossfeldiana* Poelln

Groups	Control	Ethanolic extract	Silymarin	CCl_4
AST	88.30±4.71	214.80±8.23**	161.60±6.66**	255.30±9.1***
ALT	46.50±2.33	74.70±4.36**	65.30±4.35***	93.40±4.14***
ALP	88.60±4.52	158.00±6.54***	158.20±6.85***	186.50±7.34***
Total proteins (g/dl)	6.25±0.19	5.60±0.19*	5.82±0.17**	5.06±0.16***
Albumin (g/dl)	4.10±0.15	3.62±0.09*	3.82±0.11**	3.33±0.11***
Globulin (g/dl)	2.15±0.11	1.98±0.08*	2.00±0.05*	1.73±0.07**
Total bilirubin (mg/dl)	0.25±0.01	0.48±0.02***	0.42±0.02***	0.65±0.03***

Significant at: *P ≤ 0.05 **P ≤ 0.01 ***P ≤ 0.001

3.4.7 Cytotoxic Screening

The alcoholic extract of *K. blossfeldiana* Poelln. was inactive against all tested human carcinoma cell lines.

Table 9: IC_{50} (µg/ml) of the total ethanolic extract of *K. blossfeldiana* Poelln. on HEPG-2, MCF-7, HEP-2, HELA and HCT-116 carcinoma cell lines

Sample	HEPG-2	MCF-7	HEP-2	HELA	HCT-116
Ethanolic extract	21.0	30.0	28.8	22.0	25.8

3.4.8 Antioxidant Screening

The alcoholic extract had significant scavenging effects on the DPPH radical, so it possessed a potent antioxidant activity when compared to gallic acid.

Table 10: The antioxidant activity of ethanolic extract of *k. blossfeldiana* Poelln. against gallic acid in terms of IC_{50} ± SD

Sample	DPPH % Inhibition*
Ethanolic extract	0.32 ± 0.01
Gallic acid	0.03 ± 0.002

*Values are presented as mean ± SE of 3-test sample observations, P <0.05 for all values.

3.4.9 Antimicrobial Screening

The alcoholic extract showed significant antibacterial activity against the available Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), but no antifungal activity against *Aspergillus flavus* and *Candida albicans*.

Table11: Antimicrobial activity of *K. blossfeldiana* Poelln ethanolic extract

Samples	Diameter of zone of inhibition (mm) (% potency*)					
	G+ bacteria		G- bacteria		Fungi	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. flavus*	*C. albicans*
Tetracycline	19 100%	29 100%	30 100%	30 100%	-	-
Amphotericin B	-	-	-	-	17 100%	19 100%
Ethanolic extract	12 63.15%	12 41.38%	14 46.67%	13 43.33%	-	-

3.5 Botanical Study

3.5.1 Macromorphology (figure 2)

It is an ornamental, succulent, perennial herb, native to Madgascar. It reproduces by sprouting (when a leaf is removed from the plant and placed in a warm moist place, young plants will very soon appear in the notches around the margin of the leaf) or by cutting. It carries ovate, fleshy leaves and red, small, tubular flowers in terminal or axillary cymes. The plant starts blooming in October and continues till May.

3.5.1.1 The Stem

The stem is erect, cylindrical, suffruticose (herbaceous in the upper part and woody at the basal part). It measures from 25 to 60 cm in height and 1 cm in diameter at the basal part. The stem is smooth, green in color and turned to greenish red color when exposed to sunlight. The stem has a faint characteristic odor and an astringent taste.

3.5.1.2 The Leaf

The leaf is simple, opposite decussate, petiolate, exstipulate, fleshy, green in color and turned greenish-red when exposed to sunlight. The lamina is ovate,glabrous, with an obtuse apex and a symmetric base.Leafmargin is crenate and venation is pinnatereticulate. It measures from 1-4-6 cm in length, 1-2.5- 4 cm in width. The petiole is glabrous, cylindrical and measuring 0.3-1-2 cm in length and 0.2-0.5-1 cm in diameter. The leaf has a faint characteristic odor and an astringent taste.

3.5.2 Micromorphology

3.5.2.1 The Stem

3.5.2.1.1 The Herbaceous Part of the Stem (figure 3)

A transverse section in the herbaceous part of the stem is circular in outlineThe epidermal cells arepolygonal, axially elongated with straight beaded anticlinal walls, covered withthinsmooth cuticle;they measure 50-156-215 µ in L., and 21-38-78 µ in W and 27-44-56µ in H.Fewstomata of anisocytic type are present.Trichomes are absent.

The cortex consists of 12 to18 rows of rounded, thin-walled parenchyma cells. Some cortical cells contain simple, rarely compound, rounded starch granules with no hilum and no striations measuring from 3-12-19 µ in diameter. Small prisms of calcium oxalate (measuring from 6-10-17 µ in L. and 3-5-7 µ in W.) are found in the corners of cortical parenchyma.Tannin cells containing dark brown contents (gives dark blue color with $FeCl_3$ reagent) are scattered, especially in the first two layers. The endodermis is undifferentiated. The pericycleis parenchymatous showing scattered tannin cells similar to those of the cortex.

The vascular tissue consists of a continuous ring of phloem and xylem separated by 2 - 3 layers of thin-walled tangentially elongated cambial cells and traversed by uniseriate or biseriate lignified medullary rays. The phloem consists of thin-walled cellulosic elements of sieve tubes, companion and parenchyma cells. The phloem is devoid of bast fibres and shows scattered tannin cells. The xylem is formed of spiral and annular lignified xylem vessels measuring from 7-12-25µ inD. Tannin cells are scattered in the vascular tissue. Simple starch granules, similar to that of the cortex and small prisms of calcium oxalate are present in the pith.

3.5.2.1.2 The Woody Part of the Stem (figure 4)

The structure of the woody part of the stem is almost similar to that of the herbaceous one with the following differences:

- The cork: It is formed of 4-8, radially arranged rows of tangentially elongated, polygonal cells with thick brown, suberized, non-lignified walls. It measures 50-150-175 µ in L., 25-40-94µ in W and 10-30-40 µ in H.
- The vascular tissue is wider and the pith is narrower.

3.5.2.1.3 The Powdered Stem (figure 5)

The powdered stem of *K. blossfeldiana* Poelln. Is greenish brown in color, has a faint characteristic odor and an astringent taste. By

microscopical examination of the powdered stem, the following fragments were observed:

1. Fragments of polygonal axially elongated epidermal cells of the herbaceous part of thestem with straightbeaded anticlinal walls, covered with smooth cuticle, anisocytic stomata rarely found, and no trichomes.

2. Fragments of brown, polygonal and suberized non-lignified cork cells of the woody part of the stem.

3. Fragments of scattered thin-walled parenchyma cells with small prisms of calcium oxalate present in the corner of the cells and simple, rarely compound rounded starch granules.

4. Fragments of tannin cells containing dark brown content (give blue with $FeCl_3$).

5. Fragments of lignified xylem vessels with spiral and annular thickening.

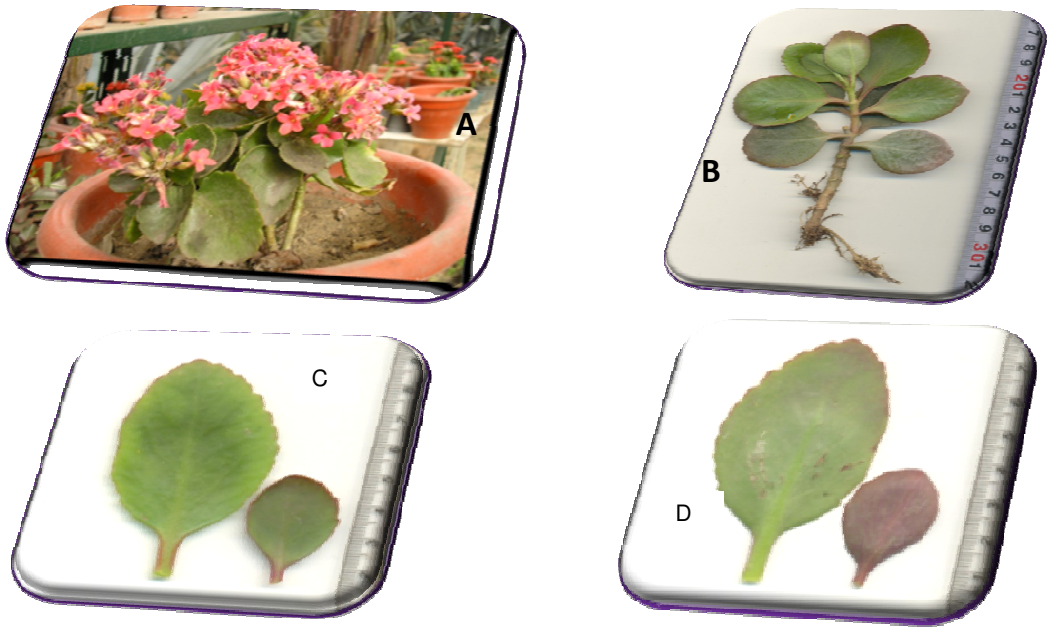
3.5.2.2 The Leaf

3.5.2.2.1 Leaf lamina (fig. 6) shows a planoconvex midrib being slightly prominent on the lower side and slightly depressed to the upper side. The epidermal cells of both surfaces are similar in shape and differ in size. They are polygonal isodiametric, papillosed thin-walled with straight anticlinal walls, covered by thin smooth cuticle. The upper epidermal cells measure 25-66-100 µ in L., 10-40-84 µ in W, 33-40-84 µ in H. and the lower epidermis measures 20-40-100 µ in L., 10-26-67 µ in W and 33-40-55 µ in H. Stomata of anisocytic type are present and more frequent on the lower surface. They are oval in shape and measure 23-32-35 µ in L. and 6-12-15 µ in W. Trichomes are absent.

The mesophyll is homogenous. Midrib shows small crescent shape collateral vascular bundle with parenchymatous pericycle. Simple starch granules (measuring 3-10-14 µ in D.) and small prisms of calcium oxalate are present, measuring from 6-10-17 µ in L. and 3-5-7 µ in W. The xylem is composed of lignified vessels, wood parenchyma and separated by uni- or biseriate medullary rays of thin-walled parenchyma. The vessels show spiral and annular lignified thickenings and measuring from 13-16-24 µ in D. They are mostly solitary, rarely in groups of 2 or 3.The phloem consists of soft tissue (phloem elements and phloem parenchyma) and is devoid of bast fibres.

3.5.2.2.2 A transverse section in the petiole (fig. 6 C) is oval in outline showing a slight depression to the upper side. It shows the same arrangement of tissues found in the midrib region of the leaf lamina.

Figure 1: Macromorphology of *Kalanchoe blossfeldina* Poelln.



(A) A photograph of the herb X 0.45
(B) A Photograph of a branch X 0.30
(C) Upper surface of the leaf X 0.55
(D) Lower surface of the leaf X 0.56

Figure 2: Micromorphology of the herbaceous part of the stem of *Kalanchoe blossfeldiana* Poelln.
(A)Transverse section in the herbaceouspart of the stem (X14.14)
(B) Transverse section of the herbaceouspart of the stem (X 73.33)
cam.: cambium, co: cortex, epi: epidermis, m.r.: medullary rays, per.: pericycle, ph: phloem, pi: pith, t.c.: tannin cells, w.p: wood parenchyma, x.: xylem, x.v.: xylem vessels

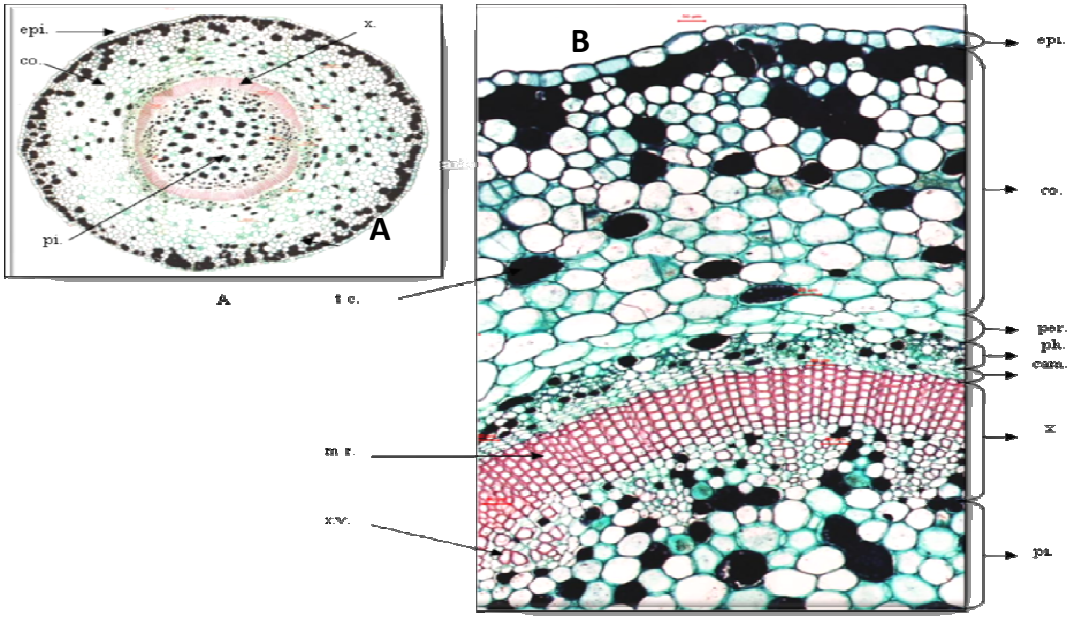


Figure 3: Micromorphology of the woody part of the stem of *Kalanchoe blossfeldiana* Poelln.
(A)Transverse section in the woodypart of the stem (X 24.32)
(B) Transverse section of the woodypart of the stem (X 65.33)
cam., cambium; co, cortex; ck., cork, epi: epidermis, m.r.: pericycle, ph, phloem, pi: pith, t.c: tannin cells, w.p: wood parenchyma, x: xylem, x.v: xylem vessels

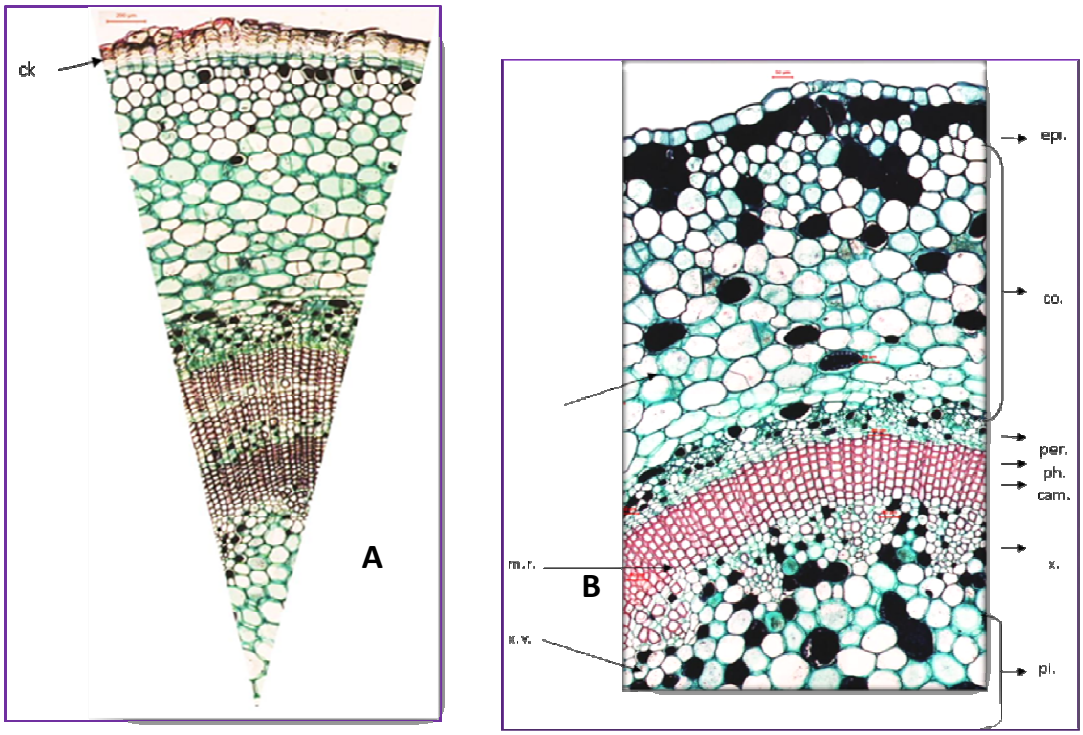
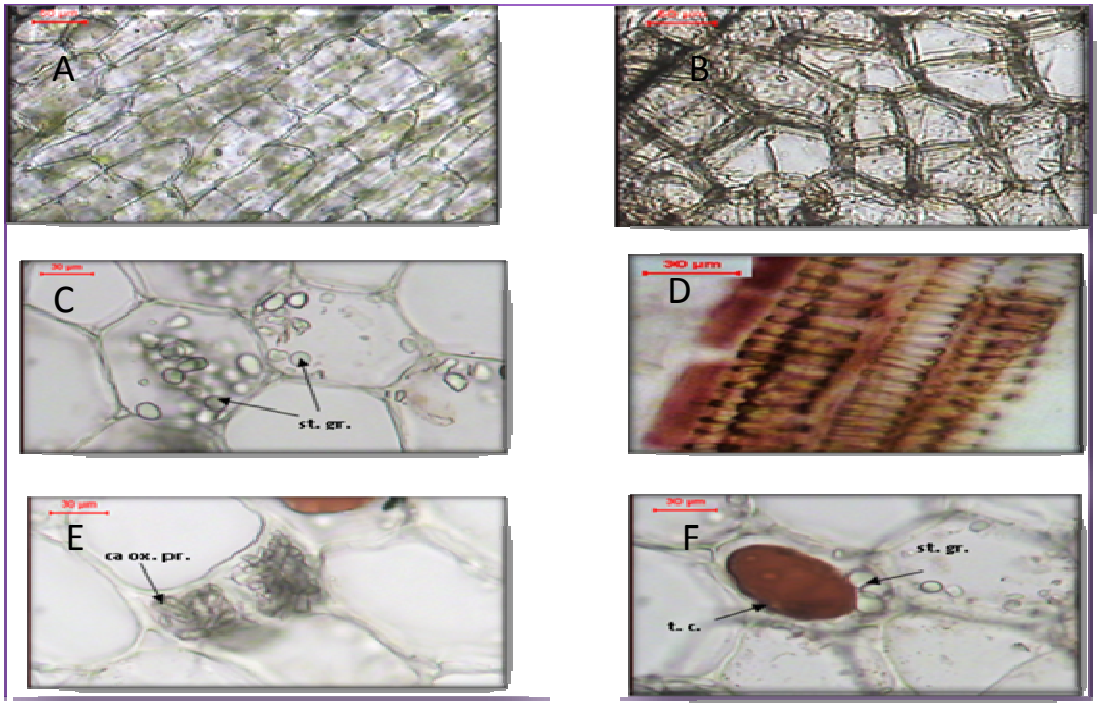
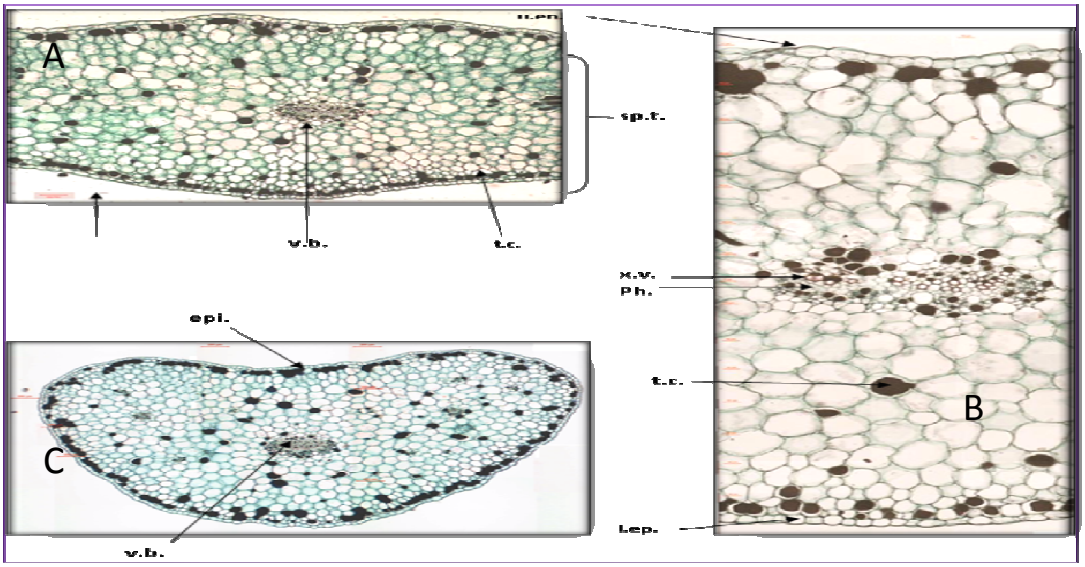


Figure 4: Micromorphology of the woody part of the stem of *Kalanchoe blossfeldiana* Poelln.



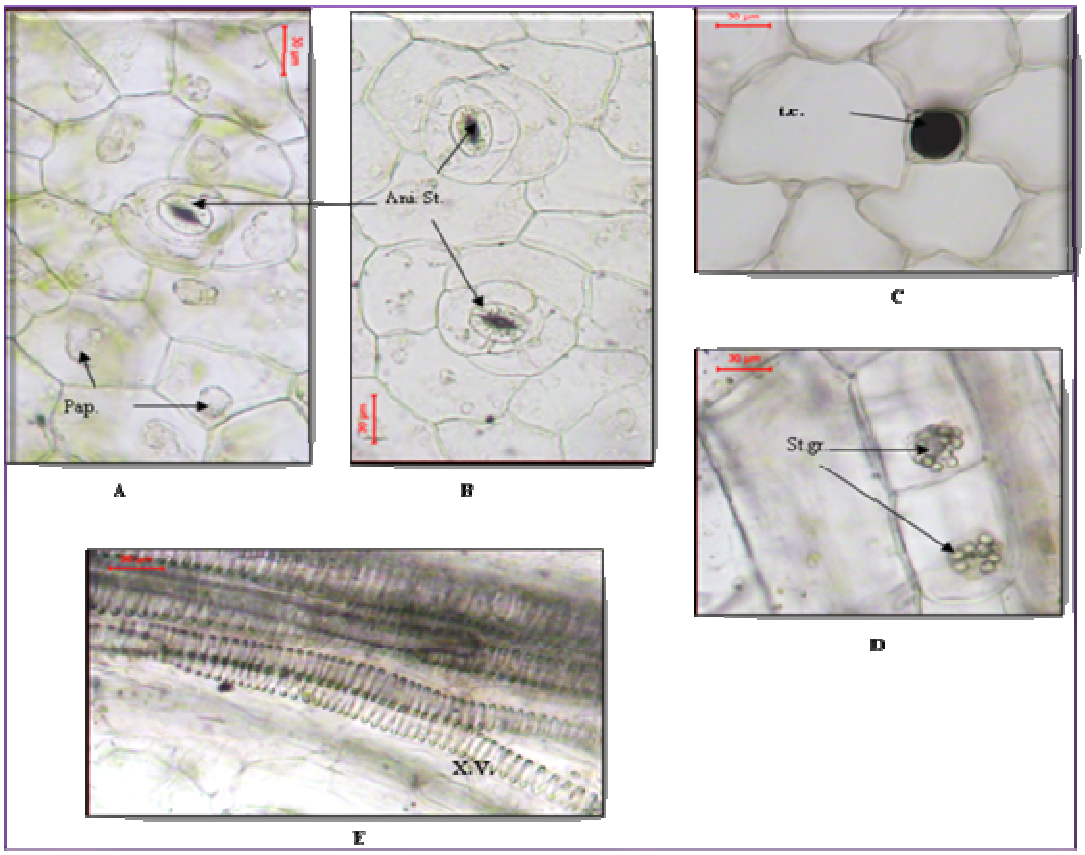
(A)Epidermal cells of the herbaceous stem (X 125.06)
(B) Cork cells of the woody stem (X 121.87)
(C) Parenchyma cells containing starch granules (X 231.11)
(D) Xylem vessels (X 428.07)
(E) Parenchyma cells containing prisms of calcium oxalate (X 260)
(F) Tannin cells (X 285.38)
ca.ox. pr: calcium oxalate prisms, st. gr: starch granules, t.c: tannin cells, x.v: xylem vessels

Figure 5: Micromorphology of the leaf of *Kalanchoe blossfeldiana* Poelln.



(A) Transverse sector of the lamina (X 10.44)
(B) Transverse sector of the lamina (X 53.45)
(C) Transverse sector in the petiole (X 10.44)
l.ep: lower epidermis, epi: epidermis, ph: phloem, sp.t: spongy tissue, t.c: tannin cells, u.ep: upper epidermis, v.b: vascular bundle, x.v: xylem vessels.

Figure 6: Powdered leaf of *Kalanchoe blossfeldiana* Poelln



(A) Upper epidermal cells (X 240)
(B) Lower epidermal cells (X 240)
(C) Fragments of parenchyma cells showing tannin cells (X 300)
(D) Fragments of parenchyma cells showing starch granules (X 300)
(E) Fragments of xylem vessels (X = 250)
Anis: st, anisocytic stomata, Pap: papillae, st.gr: starch granules, t.c: tannin cells, x.v: xylem vessels

3.5.2.2.3 The Powdered Leaf (fig. 7).
The powdered leaf of *K. blossfeldiana* Poelln. is greenish brown in color, has a faint characteristic odor and an astringent taste. Microscopically the following fragments were observed:

1-Fragments of the upper and the lower epidermises showing polygonal, papillosed cells with straight anticlinal walls, covered with smooth cuticle, showing anisocytic stomataand no trichomes.

2. Fragments of scattered thin-walled parenchyma cellsshowing rounded simple, rarely compound starch granules and small prisms of calcium oxalate.

3. Fragments of tannin cells with dark brown content (give blue with $FeCl_3$).

4. Fragments of lignified xylem vessels with spiral and annular thickenings.

Conclusion

The finding of this investigation have revealed that alcohol extract of *Kalanchoe blossfeldiana* Poelln. possesses significant antimicrobial and diuretic effects, this activity was reported previously for *K. pinnata* (Muhammad et al., 2012).The present report is considered the first report for Egyptian *Kalanchoe blossfeldiana* Poelln ,which may worth further investigation to correlate this activity with the elucidated compounds.

Acknowledgement

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