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Artigo

1-Octen-3-*O*-α-L-arabinopyranosyl-(1→6)-β-glucopyranoside, a minor substance from the leaves of *Kalanchoe pinnata* (Crassulaceae)

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RESUMO: "1-Octeno-3-*O*-α-L-arabinopiranosil-(1→6)-β-glicopiranosídeo, uma substância minoritária das folhas de *Kalanchoe pinnata* (Crassulaceae)". A partir das folhas de *Kalanchoe pinnata* (Crassulaceae), uma planta medicinal amplamente utilizada contra processos inflamatórios e que apresenta importante atividade imunossupressora e anti-leishmania, foi isolado um álcool vinílico diglicosilado minoritário, caracterizado como 1-octeno-3-*O*-α-L-arabinopiranosil-(1→6)-β-glicopiranosídeo baseado em RMN mono e bi-dimensional e em CG-EM, após sucessivos processos cromatográficos em coluna. Esta molécula é um derivado hidrossolúvel da aglicona 1-octen-3-ol, molécula que atua como atrativo para polinizadores e sinalizador de defesa contra herbivoria.

Unitermos: *Kalanchoe pinnata*, Crassulaceae, extrato etanólico, 1-octeno-3-*O*-α-L-arabinopiranosil-(1→6)-β-glicopiranosídeo, defesa da planta, atrativo para polinizadores.

ABSTRACT: From the leaves of *Kalanchoe pinnata* (Crassulaceae), a medicinal plant widely used against inflammatory processes which exhibit a important immunosuppressive and anti-leishmanial activities, was isolated a minor vinylic aliphatic alcohol diglycoside which structure was proposed as the known 1-octen-3-*O*-α-L-arabinopyranosyl-(1→6)-β-glucopyranoside based on ^{1}H and ^{13}C mono and bi-dimensional NMR experiments and GC-MS analysis, after successive chromatographic column procedures. This molecule is a water-soluble derivative of the volatile aglicone 1-octen-3-ol that appears to be attractant of pollinators and signalling of defence against herbivores.

Keywords: *Kalanchoe pinnata*, Crassulaceae, ethanolic extract, 1-octen-3-*O*-α-L-arabinopyranosyl-(1→6)-β-glucopyranoside, plant-defence, pollinator attractant.

INTRODUCTION

Plants synthesise and emit a large variety of volatile organic compounds where terpenoids and fatty acid derivatives constitute the dominant classes. Floral volatiles serve as attractants for species-specific pollinators; whereas, the volatiles emitted from vegetative parts appear to protect plants by deterring herbivores and by attracting the enemies of herbivores. Plant volatiles are herbivores-induced and these volatiles activate the defense of the neighbouring plants (Pichersky; Gershenzon, 2002).

In general, plants provide shelter for insects, permitting them to mature, digest and gestate under equitable conditions, also provide places protected from direct sunlight for larval deposition. The ability of some insects to respond to olfactory stimuli can explain their propensity to find suitable cover under a specific group of plant (Syed; Guerin, 2004).

It is interesting to note that aliphatic secondary alcohols are components of several aggregation pheromones of important beetle and weevil pests. Some of these pheromones are used frequently for the monitoring and mass trapping of the relevant insects (Walton et al., 2004), and especially the aglycone 1-octen-3-ol plays an important role as insect attractant (Zada et al., 2002).

The genus *Kalanchoe* (Crassulaceae) comprises about one hundred species, most of them native from Madagascar. Succulent plants belonging to this genus can reproduce sexually during the blooming season or by leaf-plantlet development (Allorge-Boiteau, 1996). *Kalanchoe pinnata* (Lamarck) Persoon (=*Bryophyllum pinnatum*) is a perennial medicinal herb, popularly used in Brazil and other parts of the world to treat various inflammatory diseases (Hema et al., 1986 and Rossi-

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Bergmann et al., 1994). Several biological activities have been reported for *K. pinnata* such as immunosuppressive effects (Rossi-Bergmann et al., 1994), hepatoprotective activity (Yadav; Dixit, 2003), acetylcholinesterase inhibition (Barbosa-Filho et al., 2006), besides an important protection against progressive infection with *Leishmania amazonensis* (Da-Silva et al., 1995 and 1999; Muzitano et al., 2006). *Kalanchoe brasiliensis* - another medicinal species popularly known by the same name (saião) and widely used against rheumatism and inflammatory processes - showed protection against the local effects (edema, hemorrhage and necrosis) of *Bothrops alternatus* venom in mice (Fonseca et al., 2004) and anti-inflammatory activity (Mourão et al., 1999; Ibrahim et al., 2002; Falcão et al., 2005; Costa et al., 2006)." Our results showed that an enriched saturated fatty acid fraction from *K. pinnata* (KP) could play an important role on its immunosuppressive *in vivo* effect (Almeida et al., 2000).

Previous studies on the chemical composition of *K. pinnata* showed that bufadienolides (Yamagishi et al., 1989), terpenoids and flavonoids (Costa et al., 1995) are the main constituents of this species. Here we describe the identification of a rare vinylic *O*-glycosylated chain from *Kalanchoe pinnata*, until now only reported for a Fabaceae species.

MATERIAL AND METHODS

General experimental procedures

Reverse phase column chromatographies were carried out on silanized silica gel RP-2 (70-230 mesh, Merck) and RP-8 using a gradient of water/methanol. Sephadex LH-20 gel (25-100 mm, Sigma) column was also used. TLC plates [Silica 60 F_{254} Merck; n-butanol/acetic acid/water 8:1:1 (v/v)] were revealed with a spray of ceric sulfate solution. ^{1}H (200MHz; DMSO-d_6; δ 2.49 as internal reference) and ^{13}C NMR (50MHz) spectra were recorded in a Varian Gemini 200. Optical rotation was measured on a Perkin-Elmer 243 B Polarimeter using a sodium lamp (589 nm).

A Finnigan model GCQ (MS) and a model 9001 (gas chromatography) were used in the analysis of the per-acetylated derivatives. The capillary column was a J & W DB-5MS (30 m; 0.25 mm ID; 0.25 μm film). Injection of 2 μL was used for all the samples (helium carrier gas velocity was 40 cm/min). The temperature was programmed at a rate of 65 ºC/min; 40 ºC/min until 100 ºC; 12 ºC/min from 100 ºC until 290 ºC; 290 ºC during 15 min. The eluent used was ethyl acetate and the voltage was 70 eV. These analyses were carried out by Dr. Arthur de Lemos Scofield (Departamento de Química, Pontifícia Universidade Católica, PUC, Rio de Janeiro, Brazil).

Plant material

Kalanchoe pinnata was collected during the autumn season, out of flowering time; at the garden of Universidade Federal do Rio de Janeiro campus (Brazil), where the plant was cultivated under direct sunlight and water stress. A voucher specimen number 292.697 is deposited at the herbarium of Rio de Janeiro's Botanical Garden.

Extraction and isolation

Fresh leaves (3.1 kg) were dried on a ventilated oven (30 ºC). Previously powdered dried leaves (278 g) were macerated in ethanol at room temperature. The ethanolic extract (EE) was concentrated until dryness under vacuum at 65-70 ºC yielding a dark-green syrupy material (9 g).

The crude EE (9 g) was exhaustively washed with water, at room temperature. The water-soluble fraction (EE-1; 4.3 g, 47.8% of EE) was chromatographed on a RP-2 column (29.5 x 4.0 cm) yielding twelve fractions (KP1 to KP12). Fraction KP7 (474 mg), eluted with water/ methanol 1:1 (v/v), was further purified.

A sample (449 mg) of KP7 was washed with ethanol (12 mL), yielding a main soluble fraction (KP7A; 410 mg). KP7A (410 mg) was chromatographed on Sephadex LH-20 (16.0 x 2.5 cm) with ethanol giving four fractions. A sample (355 mg) of the major fraction KP7A2-3 (362 mg) was chromatographed on a reverse phase column RP-8 (1.2 x 19.5 cm; water/methanol gradient). Seven sub-fractions were obtained considering TLC analyses: A (20 mL, MeOH 50%, 35 mg); B (10 mL, MeOH 50%, 1.3 mg); C (2 mL, MeOH 50%, 20 mg); D (5 mL, MeOH 50%-70%, 267 mg); E (2 mL, MeOH 70%, 20 mg) and F (15 mL, MeOH 100%, 0.3 mg). The KP7A2-3E fraction (20 mg) showed a light-purple spot after ceric sulfate (R_f = 0.38) and was successively chromatographed on Sephadex LH-20 using ethanol as eluent. Compound **1** (5 mg), not visible under UV light, was obtained as a light-yellowish oily material (R_f = 0.38).

Hydrolysis of compound 1

A sample of **1** (0.9 mg) was submitted to a hydrolysis reaction, with trifluoroacetic acid solution 4 M (1.5 mL), during 2 h (100 ºC). To the hydrolysed material were added 6 ml of methanol (2 x 3 mL) and the resulting solution dried under vacuum at 65-70 ºC. The dry material was analyzed on TLC [n-BAW 8:1:1 (v/v)] where was observed, after $CeSO_4$ revelation, a large gray spot (R_f= 0.33), relative to the sugar portion of the molecule. Another spot was visualized (R_f = 0.75), with a discrete blue fluorescence (UV) that became gray after revelation with ceric sulfate. The hydrolyzed material and L-arabinose and D-glucose standard samples (Fluka) were submitted to the acetylation reaction.

Acetylation of compound 1 hydrolysate and sugar commercial samples

For each 0.9 mg (hydrolyzed material from **1**, L-arabinose and D-glucose) it was utilized 2 mL of pyridine and 2 ml of anhydride acetic. The resulting solution was heated under agitation for 30 minutes. The acetylated material was dried under vacuum. These acetylated fractions were analyzed separately by gas chromatography coupled to mass spectrometry (GC/MS).

RESULTS

In this study we used an ethanolic extract (EE) obtained from *K. pinnata* leaves collected during the autumn season, in a region at sea level, where the plant grew under direct sunlight and without watering.

Successive chromatographic procedures of the water-soluble fraction (EE-1) obtained from the *K. pinnata* crude extract afforded compound **1** (0.0019% from dried leaf) as an oily material. The optical rotation value $[\alpha]_D$ = -38.3 (MeOH, c 0.15) measured for **1** is here reported for the first time.

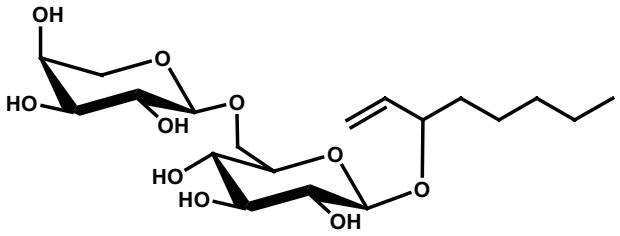
The ^{1}H NMR of **1** (DMSO-d_6, 200 MHz) exhibited signal pattern for an aliphatic chain bearing a terminal vinyl group and a disaccharide moiety (Table 1 – *J* data were listed with basis on the apodized spectra). The olefinic protons H-1_a (*J* 17.0 Hz) and H-1_b (*J* 10.3 Hz) were assigned to be *trans* and *cis* in relation to H-2, respectively.

The ^{13}C NMR data (DMSO-d_6, 50 MHz) for compound **1** confirmed the aliphatic chain and defined its length. The chain was composed by eight carbons (C-1, 115.0 ppm; C-2, 139.7 ppm; C-3, 79.4 ppm; C-4, 33.9 ppm; C-5, 23.8 ppm; C-6, 31.3 ppm; C-7, 22.0 ppm and C-8, 13.3 ppm). The signals attributed to the aliphatic chain are in agreement with the literature data (Yoshikawa et al., 1998; Wang et al., 1998 and Kanchanapoom et al., 2001). Our assignment was confirmed unambiguously by 2D-NMR APT, HETCOR and COSY ^{1}H, ^{1}H data. In the reported study by Yamamura et al. (1998), C-1 and C-2 signals of the aliphatic chain were wrongly interchanged, because of the absence of 2D-NMR experiments.

The presence of a down-field signal at 79.4 ppm indicated the attachment point of the disaccharide unit (64.4-103.0 ppm) to the aliphatic chain. From the ^{13}C NMR data (APT, HETCOR and COSY ^{1}H, ^{1}H), this disaccharide unit is composed by a β-glucopyranosyl inner moiety linked to an α-arabinopyranosyl unit, by a

Table 1. ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) data obtained for compound **1** in DMSO-d_6 and expressed in δ ppm (*J* Hz).

1-Octen-3-ol		Glucose		Arabinose	
^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
H-1_a, 5.16, dd, *J* 1.1, 17.0 H 1_b, 5.04, dd, *J* 1.1, 10.3	C-1, 115.0	H-1', 4.15, d, *J* 7.7	C-1', 101.7	H-1", 4.25, d, *J* 6.13	C-1", 103.0
H-2, 5.80, ddd, *J* 6.2, 10.3, 17.0	C-2, 139.7	H-2', 2.95, dd, *J* 7.8, 7.7	C-2', 73.6	H-2", 3.32, m	C-2", 70.5
H-3, 4.04, d, *J* 6.2	C-3, 79.4	H-3', 3.08, m	C-3', 76.7	H-3", 3.26, m	C-3", 72.3
H-4, 1.46, m	C-4, 33.9	H-4', 3.02, m	C-4', 70.1	H-4", 3.60, m;	C-4", 67.0
H-5, 1.15-1.33, m	C-5, 23.8	H-5', 3.26, m	C-5', 75.6	H-5_a", 3.65, m H-5_b", 3.25, m	C-5", 64.4
H-6, 1.15-1.33, m	C-6, 31.3	H-6a', 3.85, dd, *J* 3.1, 10.1 H-6b', 3.64, dd, *J* 6.0, 11.0	C-6', 67.8		
H-7, 1.15-1.33, m	C-7, 22.0				
H-8, 0.85, t, 6.2	C-8, 13.3				



Compound **1**

1→6 interglycosidic bound. Table 1 shows all the ^{1}H and ^{13}C NMR data for compound **1**.

A sample of **1** was submitted to a hydrolysis reaction, with trifluoroacetic acid solution (4 M). The hydrolyzed material as well as standard samples of L-arabinose and D-glucose was separately submitted to the acetylation reaction for posterior analysis in GC-MS.

The GC-MS analysis of the acid hydrolysate of compound **1**, after acetylation, confirmed the presence of glucose and arabinose in its structure. Two peaks were detected for the carbohydrate residues present in the per-acetylated hydrolysate. The peak observed at t_{R1} 11.28 min that showed $M^{+\cdot}$ ion peak at *m/z* 259 (diagnostic ions at *m/z* 199, 170, 157, 128, 115, 100, 86, 73) was identified as the corresponding per-acetylated arabinose by comparison with the per-acetylated L-arabinose authentic sample. A second peak at t_{R2} 13.34 min, and $M^{+\cdot}$ ion at *m/z* 331 (diagnostic ions at *m/z* 273, 242, 228, 211, 199,182, 168, 157, 140, 126, 115, 98, 85, 73, 57) exhibited the same values found for the per-acetylated D-glucose authentic sample.

Therefore, compound **1** was identified as 1-octen-3-*0*-arabinopyranosyl-(1→6)-β-glucopyranoside.

DISCUSSION

The glycoconjugate 1-octen-3-*0*-arabinopyranosyl-(1→6)-β-glucopyranoside was first isolated from leaves of *Trifolium subterraneum* (Fabaceae) (Wang et al., 1998). The chemical structure was based on NMR data using CD_3OD as solvent and no biological activity was atributed to this compound. Our NMR data obtained using DMSO-d_6 are very close to that observed previously.

As far we know, only four other glycosides of 1-octen-3-ol were reported: the 1-octen-3-*0*-glucopyranosyl-(1→6)-β-glucopyranoside (Yoshikawa et al., 1998); the (3*R*)-1-octen-3-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside (Yamamura et al., 1998; Kanchanapoom et al., 2001) and two triglycoside: (3*R*)-1-octen-3-*O*-β-D-xylopyranosyl-(1"'→6')-*O*-[β-D-glucopyranosyl-(1"→2')]-*O*-β-D-glucopyranoside (Kanchanapoom et al., 2001) and 1-octen-3-yl-*O*-β-apiofuranosyl-(1→6)-*O*-[β-D-glucopyranosyl-(1→2)]-β-glucopyranoside (Çaliş; Kirmizibekmez, 2004).

The 1-octen-3-ol glycosides reported until now have been isolated from plants belonging to few families such as Acanthaceae (Kanchanapoom et al., 2001), Fabaceae (Wang et al., 1998), Lamiaceae (Yamamura et al., 1998; Çaliş and Kirmizibekmez, 2004) and Orchidaceae (Yoshikawa et al., 1998). At our knowledge, there is no biological activity correlated with any of these compounds.

The aliphatic alcohol 1-octen-3-ol is a component of flavour mixtures of some mushrooms (Wood et al., 2001) and plants, especially *Lamiaceae* species (Mastelic; Jerkovic, 2003). It was demonstrated that this volatile alcohol has antifeedant properties (Wood et al., 2001). In Basidiomycetes, 1-octen-3-ol is produced by the action of a specific hydroperoxide lyase on 10-hydroperoxy-8 (*E*), 12 (*Z*)-octadienoic acid (Delcarte et al., 2000).

The alcohol 1-octen-3-ol was also identified as the major volatile component from the millipede *Niponia glandulosa* secretion (Ômura et al., 2002).

In plants, the alcohol volatile compounds have been identified as free compounds or their glycoconjugated compounds (Jerkovic; Mastelic, 2001).

It is interesting to note that α-L-octil-arabinopyranosyl-(1→6)-β-D-glucopyranoside, a rare glycoside, was isolated from the traditional Chinese medicine *Rhodiola quadrifida* which is also a Crassulaceae member (Yoshikawa et al., 1995). Despite the lack of the vinyl group, this glycoside could be originated as compound **1** from a similar pathway.

This is the second report on the isolation of 1-octen-3-*0*-arabinopyranosyl-(1→6)-β-glucopyranoside that is here described for the first time in Crassulaceae. This rare glycoconjugated from 1-octen-3-ol may play an important role of storage of the volatile aglycone in the plant.

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REFERENCES

Allorge-Boiteau L 1996. Madagascar Centre de Spéciation et d'Origine du Genre *Kalanchoe* (Crassulaceae). In: Lourenço, W.R. (org.) Biogéographie de Madagascar. Paris: Editions de l'ORSTOM, p.137-145.

Almeida AP, Da Silva SAG, Souza MLM, Lima LMTR, Rossi-Bergmann B, Gonçalves De Moraes VL, Costa SS 2000. Isolation and chemical analysis of a fatty acid fraction of *Kalanchoe pinnata* with a potent lymphocyte suppressive activity**.** *Planta Med 66*: 134-137.

Barbosa-Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde-Filho PF, Silva MS, Cunha EVL, Almeida JRGS, Quintans-Júnior LJ 2006. Natural products inhibitors of the enzyme acetylcholinesterase. *Rev Bras Farmacogn 16*: 258-285.

Çaliþ Ý, Kirmizibekmez H 2004. Glycosides from *Phlomis lunariifolia*. *Phytochemistry 65*: 2619-2625.

Costa SS, Jossang A, Bodo B 1995. Propriétés biologiques et phytochimie des *Kalanchoe*. In: Boiteau, P.; Allorge-Boiteau, L. (org.) *Kalanchoe* (Crassulacées) *de Madagascar, Systématique, écophysiologie et phytochimie*. Paris: Edition Karthala, p.219-235.

Costa SS, Souza MLM, Ibrahim T, Melo GO, Almeida AP, Guette C, Férézou JP, Koatz VLG 2006. Kalanchosine dimalate, an anti-inflammatory salt from *Kalanchoe*

brasiliensis. *J Nat Prod 69*: 815-818.

Da-Silva SAG, Costa SS, Mendonça SCF, Silva EM, Moraes VLG, Rossi-Bergmann B 1995. Therapeutic effect of oral *Kalanchoe pinnata* leaf extract in murine Leishmaniasis. *Acta Trop 60*: 201-210.

Da-Silva SAG, Costa SS, Rossi-Bergmann B 1999. The antileishmanial effect of *Kalanchoe* is mediated by nitric oxide intermediates. *Parasitology 118*: 575-582.

Delcarte J, Fauconnier M, Hoyaux P, Jacques P, Thonart P, Marlier M 2000. Revue Bibliographique: L'hydroperoxyde Lyase. *Biotechnology, Agronomy, Society and Environment 4*: 157-167.

Falcão HS, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, Batista LM 2005. Review of the plants with anti-inflammatory activity studied in Brazil. *Rev Bras Farmacogn 15*: 381-391.

Fonseca FV, Melo MM, Silva J, Pereira GP, Dantas-Barros AM 2004. Extratos de *Curcuma longa* L. e *Kalanchoe brasiliensis* Camb. no tratamento local do envenenamento por *Bothrops alternatus. Rev Bras Farmacogn 14(Supl. 1)*: 26-29.

Hema D, Tidjani M, Bassene E, Pousset JL, Giono-Barber H 1986. Plantes médicinales Africaines XXXIV Étude de l'action anti-inflammatoire de *Bryophyllum pinnatum* (Crassulacées). *Plantes Médicinales et Phytothérapie 20*: 231-235.

Ibrahim T, Cunha JM, Madi K, da Fonseca LM, Costa SS, Gonçalves Koatz VL 2002. Immunomodulatory and anti-inflammatory effects of *Kalanchoe brasiliensis. Int Immunopharmacol 2*: 875-883.

Jerkovic I, Mastelic J 2001. Composition of free and glycosidically bound volatiles of *Mentha aquatica* L. *Croat Chem Acta 74*: 431-439.

Kanchanapoom T, Kasai R, Picheansoonthon C, Yamasaki K 2001. Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. *Phytochemistry 58*: 811-817.

Mastelic J, Jerkovic I 2003. Gas Chromatography-Mass Spectrometry analysis of free and glycoconjugated aroma compounds of seasonally collected *Satureja montana* L. *Food Chem 80*: 135-140.

Mourão RH, Santos FO, Franzotti EM, Moreno MP, Antoniolli AR 1999. Antiinflammatory activity and acute toxicity (LD50) of the juice of *Kalanchoe brasiliensis* (Camb.) leaves picked before and during blooming. *Phytother Res 13*: 352-354.

Muzitano MF, Cruz EA, de Almeida AP, da Silva SA, Guette C, Kaiser CR, Rossi-Bergmann B, Costa SS 2006. Quercitrin: an antileishmanial flavonoid glycoside from *Kalanchoe pinnata. Planta Med 72*: 81-83.

Ômura H, Kuwahara Y, Tanabe T 2002. 1-Octen-3-ol together with geosmin: New secretion compounds from a polydesmid millipede, *Niponia nodulosa*. *J Chem Ecol 28*: 2601-2612.

Pichersky E, Gershenzon J 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Curr Opin Plant Biol 5*: 237-243.

Rossi-Bergmann B, Costa SS, Borges MBS, Da Silva SAG, Noleto GR, Souza MLM, Moraes VLG 1994. Immunosuppressive effect of the aqueous extract of *Kalanchoe pinnata* in mice. *Phytother Res 8*: 399-402.

Syed Z, Guerin PM 2004. Tsetse flies are attracted to the invasive plant *Lantana camara*. *J Insect Physiol 50*: 43-50.

Walton VM, Daane KM, Pringle KL 2004. Monitoring *Planococcus ficcus* in South African vineyards with sex pheromone-baited traps. *Crop Prot 23*: 1089-1096.

Wang S, Ghisalberti EL, Ridsdill-Smith J 1998. Bioactive isoflavonols and other components from *Trifolium subterraneum*. *J Nat Prod 61*: 508-510.

Wood WF, Archer CL, Largent DL 2001. 1-Octen-3-ol, a banana slug antifeedant from mushrooms. *Biochem Syst Ecol 29*: 531-533.

Yadav NP, Dixit VK 2003. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. *J Ethnopharmacol 86*: 197-202.

Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH 1989. Bryophyllin B, a novel potent cytotoxic bufadienolide from *Bryophyllum pinnatum. J Nat Prod 52*: 1071-1079.

Yamamura S, Ozawa K, Ohtani K, Kasai R, Yamasaki K 1998. Antihistaminic flavones and aliphatic glycosides from *Mentha spicata*. *Phytochemistry 48*: 131-136.

Yoshikawa M, Murakami T, Kishi A, Sakurama T, Matsuda H, Nomura M, Matsuda H, Kubo M 1998. Novel indole S,*O*-bisdesmoside, Calanthoside, the precursor glycoside of tryptanthrin, indirubin, and isatin, with increasing skin blood flow promoting effects, from two *Calanthe* species (Orchidaceae). *Chem Pharm Bull 46*: 886-888.

Yoshikawa M, Shimada H, Shimoda H, Matsuda H, Yamahara J, Murakami N 1995. Rhodiocyanosides A and B, new antiallergic cyanoglycosides from Chinese natural medicine "Si Lie Hong Jing Tian", the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey. *Chem Pharm Bull 43*: 1245-1247.

Zada A, Soroker V, Harel M, Nakache J, Dunkelblum E 2002. Quantitative GC analysis of secondary alcohol pheromones: determination of release rate of red palm weevil, *Rhynchophorus ferrugineus*, pheromone from lures. *J Chem Ecol 28*: 299-306.