

Ultrasound-assisted Extraction of Ursolic Acid from the Flowers of *Ixora coccinea* Linn (Rubiaceae) and Antiproliferative Activity of Ursolic Acid and Synthesized Derivatives

Silvana Amadeu Ferreira Alves Monteath, Maria Aparecida M. Maciel¹, Raquel Garcia Vega², Heloisa de Mello³, Carolinna de Araújo Martins⁴, Andressa Esteves-Souza, Cerli Rocha Gattass⁴, Aurea Echevarria

Departamento de Química, ICE, Universidade Federal Rural do Rio de Janeiro, Seropédica-RJ, ¹Departamento de Química, Universidade Federal do Rio Grande do Norte, CEP, Natal-Rio Grande do Norte, ²Laboratório de Ciências Químicas-Setor de Química de Produtos Naturais, Universidade Estadual do Norte Fluminense, Campos dos Goitacázes-RJ, ³Universidade Federal de Sergipe, Campus Prof. Alberto Carvalho, Itabaiana-SE, ⁴Instituto de Biofísica, CCS, Universidade Federal do Rio de Janeiro, Rio de Janeiro-RJ, Brazil

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ABSTRACT

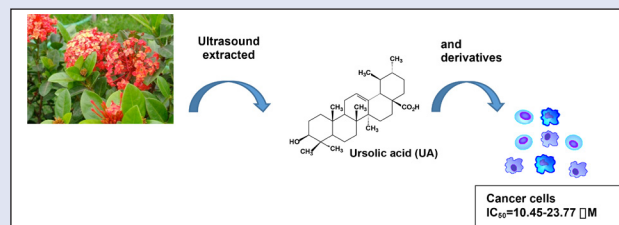
Background: *Ixora coccinea* Linn (Rubiaceae) is an evergreen shrub with bright scarlet colored flowers found in several tropical and subtropical countries. It is used as an ornamental and medicinal plant. Phytochemical studies revealed that its major special metabolites are triterpene acids, such as ursolic and oleanolic acid. **Objective:** To evaluate the isolation of ursolic acid (UA) (1) from methanol extracts of *I. coccinea* flowers through two methodologies, to prepare four derivatives, and to evaluate the cytotoxic effect against six cancer cell lines. **Materials and Methods:** The UA was isolated from vegetal material by percolation at room temperature and by ultrasound-assisted extraction. The preparation of derivatives was performed according to literature methods, and the cytotoxic effects were evaluated using the MTT (3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) assay. **Results:** The most efficient extraction was achieved through ultrasound irradiation with a yield of 35% after KOH-impregnated silica in chromatography column. Furthermore, four derivatives (3, 5, 6, 7) of UA were prepared and evaluated, including 1, against two lung cancer (A549 and H460) and four leukemia (K562, Lucena, HL60, and Jurkat) cell lines. Generally, results showed that 1 and 7 were the most active compounds against the assayed cell lines. Also, the cytotoxic effects observed on terpenes 1 and 7 were higher when compared with cisplatin, used as positive control, with the exception of Jurkat cell line. **Conclusion:** The efficiency of such an alternative extraction method led to the principal and abundant active component, 1, of *I. coccinea*, thus representing a considerable contribution for promising triterpenoid in cancer chemotherapy.

Key words: Cytotoxic activity, *Ixora coccinea*, ultrasound-assisted extraction, ursolic acid

SUMMARY

- The ultrasound-assisted extraction of *Ixora coccinea* flowers improved of the ursolic acid isolation

- Methanolic extract from flowers of *I. coccinea* provided, by ultrasound irradiation, after KOH-impregnated silica in chromatography column, the ursolic acid in 35% yield
- The ursolic acid and four derivatives were prepared and assayed against two lung cancer and four leukaemia cell lines
- The ursolic acid and their 3-oxo-derivative, in general, were more cytotoxic when compared to cisplatin, used as positive control



Abbreviations used: MTT: 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, RP: reverse phase, TLC: thin layer chromatography, KOH: potassium hydroxide, IR: infrared, DMF: dimethylformamide, DMSO: dimethyl sulfoxide, TEA: triethylamine, RT: room temperature, EtOAc: ethyl acetate, MeOH: methanol, *i*-PrOH: *iso*-propanol, NMR: nuclear magnetic resonance, MDR: multiple drug resistance, RPMI: Roswell Park Memorial Institute

Correspondence:

Dr. Aurea Echevarria,
Departamento de Química, ICE,
Universidade Federal Rural do Rio de Janeiro,
Seropédica-RJ, Brazil.
E-mail: echevarr@ufrj.br
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INTRODUCTION

The *Ixora* genus of the tribe Ixoreae in the subfamily Ixoroideae (Rubiaceae) is represented by ca. 150 species widespread in tropical areas of Asia, Africa, and South America.^[1] Many of them are extensively used in folk medicine and Ayurveda, the traditional Indian system of medicine, in the treatment of several diseases, such as dysentery, dysmenorrhea, hypertension, bronchitis fever, chronic ulcers, and skin diseases, among others.^[2] *Ixora coccinea* Linn species is a small evergreen shrub that shows eye-catching and colorful flowers, which are used in the treatment of dysentery, leucorrhea, dysmenorrhea, bronchitis, and microbial infections.^[2-5] The hexane extract of these flowers has displayed antiproliferative activity against different types of leukemia cell lines and enhanced the survival of mice inoculated with Dalton lymphoma and Ehrlich carcinoma.^[6]

Phytochemical studies have shown that the major metabolites present in *I. coccinea* flowers are ursolic acid (UA), oleanolic acid, stearic acid, oleic acid, linoleic acid, lupeol, and sitosterol.^[7] Literature has also reported the presence of ixoroid, D-mannitol, 5-O-caffeoyl-quinic acid,

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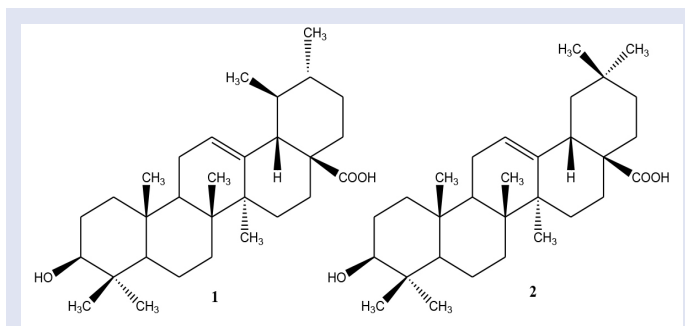


Figure 1: Chemical structures of UA (1) and oleanolic acid (2).

rutin, leucocyanadin glycoside, cyanadin-3-rutinoside, and delphinidin monoglycoside in these flowers.^[8,9] The study of *I. coccinea* leaves showed the presence of proanthocyanidin epicatechin-(2 β -O \rightarrow 7, 4 β -8)-epicatechin-(5 \rightarrow O \rightarrow 2 β , 6 \rightarrow 4 β)-epicatechin (named ixoratannin A-2), epicatechin, procyanidin A2, cinnamtannin B-1, kaempferol-7-O- α -l-rhamnanside, kaempferol-3-O- α -l-rhamnanside, quercetin-3-O- α -l-rhamnanside, and kaempferol-3,7-O- α -l-dirhamnanside.^[10]

Studies of UA (1), 3-hydroxy-urs-12-en-28-oic acid, which is an ursane-type pentacyclic triterpenic acid, have shown it can be isolated from many types of medicinal plants, including *I. coccinea*. It can also be found in the protective wax-like coating of several fruits.^[11] The various biological activities of UA, such as anti-inflammatory activity,^[12] analgesic and antioxidant,^[13] trypanocidal,^[14] and antibacterial^[15] have been described in literature. Recent studies showing the effects of UA in retarding invasion and metastasis of lung cancer cells,^[16] and in inhibiting the initiation, promotion, and metastasis of other types of cancer^[17] attracted the attention to the therapeutic potential of such triterpene.

Since UA is largely distributed in plants, great attention has been dedicated to improve methodologies for isolating it from these sources. Generally, the methodologies used to isolate UA from plants result in a mixture of UA (1) and oleanolic acid (2), an olean-type pentacyclic triterpene, which is difficult to separate [Figure 1]. Recently, a mixture of UA, oleanolic acid, and oridon (a diterpenoid compound) was extracted from *Rabdosia rubescens* using three methodologies: Ultrasound irradiation, shaking extraction, and heat-reflux extraction. Then, the three terpenoid compounds were isolated by RP-high performance liquid chromatography (HPLC) technique.^[18] The ursolic and oleanolic acids were also obtained from *Ligustrum lucidum* Ait through ultrasound-assisted extraction, and the effects on the extraction efficiency due to parameters, such as temperature, nature and concentration of solvent, and standard extraction time^[19] were studied.

In this paper, the UA was isolated from extracts of *I. coccinea* Linn flowers obtained through two methodologies: (a) percolation at room temperature and (b) ultrasound-assisted extraction. The extraction efficiency (yield) of UA was evaluated by (a) silica gel column chromatography and (b) KOH-impregnated silica gel flash column filter monitored by HPLC analysis. In addition, four UA derivatives were synthesized (3, 5, 6, and 7) and their antiproliferative activity against six cancer cell lines was evaluated.

MATERIAL AND METHODS

Plant material

I. coccinea flowers were collected in the botanical garden of Universidade Federal Rural do Rio de Janeiro and identified by the Botanic Department

of this institution, where a voucher specimen (no. 4243e) has been deposited.

Preparation of the crude extract and UA isolation

Methodology A-percolation method and traditional silica gel column chromatography for UA isolation

The methanol extract (ME-F; 26.3 g; 8.4%) from *in natura* flowers of *I. coccinea* (315 g) was prepared by percolation at room temperature. The obtained crude extract was submitted to chromatographic separations by classical phytochemical procedures.^[20] ME-F extract afforded 340 mg (0.12%) of a rich mixture of several nonpolar constituents (hydrocarbons and steroids), which was obtained from the fraction groups F1-19 (eluted with Hexane: EtOAc 10:0 - 9:1); 570 mg (2.17%) of UA [from F20-39 eluted with Hexane:EtOAc (8:2-5:5)] and 143 mg (0.04%) of the sugar mannitol [from F40-46 eluted with EtOAc:MeOH (100:0 - 50:50)].

Methodology B-ultrasound-assisted extraction and KOH-impregnated silica gel column flash filter for UA isolation

The methanol crude extract was prepared from 400 g of fresh flowers subjected to solvent-soaking for 30 min; then, the tube with the mixture was immersed into the water bath of the ultrasonic device and irradiated for 40 min. After the extraction, the sample was concentrated under vacuum, and the methanol extract was compared with a standard sample of UA by CCDA.

KOH-impregnated silica gel column flash filter was used for the isolation and purification of the UA.^[21] The column preparation was carried out as follows: 100 g of silica gel (230–400 mesh) in *i*PrOH (1000 mL) were mixed with 200 mL of a saturated solution of KOH (25 g) for 10 min. This mixture was then transferred into a glass column and washed with 400 mL of hexane. Crude extract (388.1 mg) was applied to the top of this column and successively eluted with hexane, CH₂Cl₂ and MeOH in order of increasing polarity. The fractions were combined in eight groups using analytical thin-layer chromatography (TLC) compared with standard UA. The detection and quantification of this natural product in these fractions as well as the extract was conducted by HPLC.

Instrument and chromatographic conditions

The ultrasound bath (Cleaner-Unique USC 2500) with ultrasonic power of 155 Watts RMS, operating at a frequency of 60 Hz with controlled time and temperature, was used to obtain the crude extract from *I. coccinea* flowers.

HPLC consisted of Shimadzu LC-10A pump with UV detector (205 nm)-a loop of 20 μ L was used for injection. The standard UA and fractions were analyzed by RP-18 column 250 \times 46 mm \times 5 μ m and acetonitrile (isocratic) was used as mobile phase at a flow rate of 1.7 mL min⁻¹.

Preparation of the standard solution and validation of HPLC method

A stock solution in acetonitrile containing 1 mg/mL of UA was prepared in order to obtain the standard curve. A total of 20 μ L of each of the successive dilutions, 10, 15, 25, 50, and 70 μ g/mL in acetonitrile was used to obtain the standard curve and the area corresponding to each concentration. The analyses were performed in triplicate. After plotting the peak area versus concentration of analyte, the linear regression treatment provided the correlation coefficient and the standard deviation values. Each sample of extract was analyzed in quintuplicate, and the average area of these measurements replaced the value of *y* in Equation 1.

$$y = 67,285.32 + 2,358.93 \times (1)$$

Synthesis

Chemicals

All commercially available reagents were used without further purification unless otherwise stated. The progress of the reactions was monitored by analytical TLC performed on Marchery–Nagel silica gel 60 F254 plates, and the visualization, using anisaldehyde. The purification of derivatives was done using column chromatography with silica 60 matrix (70–270 mesh). Melting points were determined on melting point PFM-II Tecnopon apparatus, but they were not corrected. IR spectra through the range of 4000 to 600 cm⁻¹ were run on a Jasco FT/IR – 4200 spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AC500 spectrometer operating at 500 and 125 MHz, respectively, with tetramethylsilane as internal standard, using deuterated chloroform. Chemical shifts were expressed as values in parts per million (ppm), and the coupling constants (*J*) were given in hertz (Hz). Yield values correspond to purified compounds and were not optimized.

Acetylation of UA (1)

UA (0.22 mmol) was treated with acetic anhydride and pyridine (1:1) at room temperature for 30 min and was worked up as usual to get acetyl UA. Compound 3 (3-*O*-acetyl UA) was obtained as a white powder in 75% yield with melting point of 168–171°C, in accordance to literature.^[22]

General synthetic procedure for *N*-(substituted)-3-*O*-acetylursolamides

For the solution of 3-acetyl UA (0.1 mmol) in CH₂Cl₂ (0.5 mL) and DMF (0.01 mL), oxalyl chloride (0.2 mmol) was added. The reaction was stirred at room temperature for 2 h. Then, the oxalyl chloride was removed under vacuum evaporation. Chloride compound 4 was not isolated. After that, TEA (0.15 mmol) and appropriate amine (0.3 mmol) were added, and the solution was stirred for 5 h at room temperature. The reaction mixture was concentrated to dryness under reduced pressure and treated with 0.1 M HCl in CH₂Cl₂ at room temperature. The aqueous phase was extracted with CH₂Cl₂ (2 × 15 mL). The organic phases were concentrated under vacuum to afford 5 as a solid in 70% yield. The resultant crude products were purified by silica gel column chromatography (CH₂Cl₂/hexano 60:40) to provide 6 as a solid in 39% yield.

N-[phenyl]-3-*O*-acetylursolamide (5): Yield: 70%. Mp: 214–216 °C. IR (KBr) cm⁻¹: 3036, 1528 (NH), 1714, 1620 (C=O). ¹H-NMR (CDCl₃): 9.41 (1H, s), 7.12–7.77 (5H, m), 5.35 (1H, m), 4.53 (m, 1H), 2.11 (1H, d), 2.07 (2H, s), 0.82–1.65 (2H, m), 0.73–1.55 (7H, m). ¹³C-NMR (CDCl₃): 176.3, 171.1, 140.2, 138.2, 128.9, 129.0, 126.1, 123.4, 119.6, 119.3, 80.8, 82.4, 54.4, 55.3, 47.5, 42.7, 39.9, 39.6, 38.4, 38.3, 39.1, 32.7, 30.9, 37.1, 27.9, 28.1, 25.1, 23.5, 23.3, 21.3, 18.1, 17.3, 16.9, 16.7, 15.6.

N-[4-toluy] -3-*O*-acetylursolamida (6): Yield: 39%; Mp: 228–230 °C. IR (KBr) cm⁻¹: 3211 (NH); 1779, 1700 (C=O). ¹H-NMR (CDCl₃): 7.57 (2H, d), 7.21 (2H, d), 5.27 (1H, s), 4.51 (1H, m), 2.38 (3H, s), 2.24 (2H, d), 1.92 (2H, d), 1.81 (2H, m), 1.81/1.38 (2H, m), 1.69 (2H, m), 1.68 (2H, m), 1.65 (1H, t), 1.54 (2H, m), 1.52/1.33 (2H, m), 1.42 (2H, m), 1.33 (1H, m), 1.28 (2H, m), 1.09 (3H, s), 0.97 (6H, s), 0.89 (3H, s), 0.88 (3H, s), 0.77 (7H, s). ¹³C-NMR (CDCl₃): 173.1, 171.0, 54.3, 140.2, 135.6, 133.5, 129.4, 129.3, 125.9, 119.7, 119.6, 80.9, 80.8, 55.3, 54.3, 47.5, 42.6, 39.9, 39.6, 39.5, 39.1, 38.4, 38.3, 36.8, 32.7, 30.9, 31.0, 28.1, 27.9, 26.3, 23.5, 23.3, 21.3, 21.2, 18.2, 17.3, 16.8, 16.7, 15.6

3-Oxo-urs-12-en-28-oic acid^[7]

UA (200 mg, 0.43 mmol) in acetone (20 mL) was treated with pyridinium chlorochromate (PCC) (280 mg). After being stirred at room temperature for 48 h, the mixture was treated using the reported procedure to afford 2.^[23]

3-Oxo-urs-12-en-28-oic acid (7): Yield: 48%; Mp: 265–270 °C (267–268 °C^[23]). IR (KBr) cm⁻¹: 3380 (OH); 2944, 2871 (CH₂, CH₃) 1703, 1696 (C=O). ¹H-NMR (CDCl₃): 5.30 (1H, dd), 3.43 (1H, dd), 2.58 (2H, d), 2.24 (1H, d), 2.05 (1H, m), 2.01–1.94 (2H, m), 1.88 (2H, d), 1.70–1.72 (2H, m), 1.55–1.49 (2H, m), 1.55–1.33 (2H, m), 1.50 (1H, m), 1.12 (3H, s), 1.11 (2H, m), 1.08 (3H, s), 1.05 (3H, s), 0.98 (3H, s), 0.89 (3H, s), 0.86 (3H, s). ¹³C-NMR (CDCl₃): 183.6, 173.6, 138.1, 125.8, 55.3, 52.7, 47.4, 46.8, 42.1, 39.1, 38.8, 36.7, 32.5, 30.6, 28.0, 26.6, 24.1, 23.5, 21.5, 21.1, 19.6, 17.0, 16.9, 15.2.

Cell culture

A549, H460 (lung cancer), JURKAT, HL60, K562, and Lucena1 (a MDR vincristine derivative of K562^[24]) cells were maintained in RPMI 1640 medium supplemented with fetal calf serum, 50 μM of 2-mercaptoethanol, 100 IU/mL of penicillin and 100 μg/mL of streptomycin. The cultures were incubated at 37 °C in humidified atmosphere with 5% CO₂, and the medium changed twice a week.

Drugs and MTT assay

Stock solutions of compounds 1, 3, 5, and 6 were prepared in DMSO. The maximum DMSO percentage in assays was 0.5% (v/v) in saline solution. Drug cytotoxicity assays were performed using MTT for viable cell measurements.^[25] Aliquots of 104 cells/mL were seeded onto 96-microtiter flat well plates and incubated for 24 h. After that, cells were treated with medium, different concentrations of the drugs (6.25, 12.5, 25, or 50 μM), or the DMSO. 48 h later, the cultures were treated with MTT (5 mg/mL), incubated for 4 h in the dark, the formazan produced by live cells solubilized with DMSO, and the absorbance was read at 570 nm. Cisplatin was used as positive control. Results represent mean ± standard deviation of at least three experiments performed in triplicate. IC₅₀ values were obtained by a linear regression analysis of the absorbance percentage *versus* the log of the drug concentration.

RESULTS AND DISCUSSION

Extraction, isolation, and purification of UA from *I. coccinea* flowers

Not only did the present study investigate the effects of two methods in the extraction efficiencies of UA content from *I. coccinea* flowers, but it also evaluated their isolation technique. Initially, the methanol extract was prepared by percolation at room temperature, and it afforded 8.4% of the crude extract. The traditional isolation of UA by silica gel chromatographic column furnished 2.2% of triterpenic acid, characterized by melting point, IR, NMR ¹H, and ¹³C spectroscopies. However, the crude extract was obtained in 19.4% yield using the technique of ultrasonic-assisted extraction. Interestingly, the UA isolated from the crude extract of the ultrasound-assisted method proved very efficient for obtaining this bioactive natural product with high yield after the use of KOH-impregnated silica gel column chromatography; it furnished pure 1, which represents 35.0%. This methodology resulted in an increase of the isolation yield of the bioactive natural product as well as in the decrease of the organic solvent usage and the amounts of time; it also simplified the work up.

The detection and quantification of UA from *I. coccinea* extracts by HPLC indicating the presence of a large amount of 1 in the flowers and the minor concentration of oleanoic acid when compared with others studied species which were detected [Figure 2]. These data indicate that *I. coccinea* has great potential as a source to obtain this active principle; it becomes even more important when considering that the species is highly resistant enduring high temperatures and that it blooms all year.^[26]

Synthesis of UA derivatives

In this work, three UA derivatives (5, 6, and 7) were prepared in an attempt to search for more active compounds. The structure modification of the UA was done at the carbinolic carbon atom (C-3) and carboxylic carbon atom (C-28).

Initially, the UA was acetylated with anhydride acetic and pyridine to afford its acetyl ester 3. This compound (3) was treated with oxalyl chloride to obtain the corresponding acid chloride 4 as intermediate, which was not isolated. After this, a condensation reaction with 4 and aniline as well as with *p*-toluidine, each separately, was performed. The obtained derivatives were purified by silica gel chromatography column and afforded compounds *N*-[phenyl]-3-*O*-acetylursolamide (5) and *N*-[4-toluy]-3-*O*-acetylursolamide (6), respectively [Figure 3].

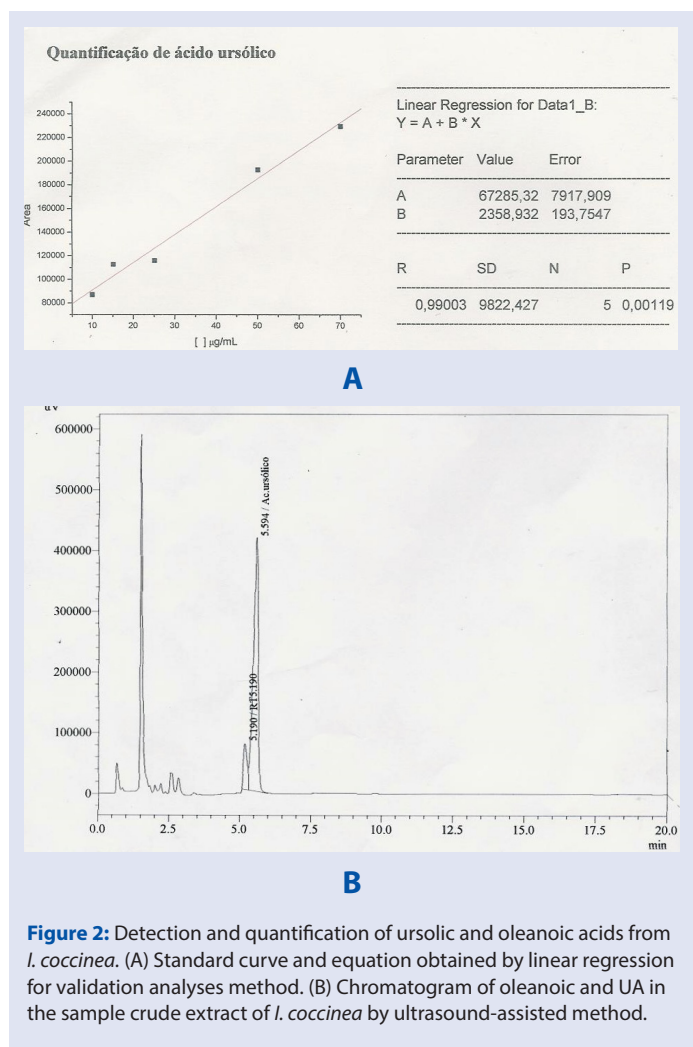


Figure 2: Detection and quantification of ursolic and oleanolic acids from *I. coccinea*. (A) Standard curve and equation obtained by linear regression for validation analyses method. (B) Chromatogram of oleanolic and UA in the sample crude extract of *I. coccinea* by ultrasound-assisted method.

In addition, the UA was submitted to an oxidation reaction with PCC oxidant agent, thus furnishing its derivative 3-oxo, compound 7.

Antiproliferative assays

MTT procedure,^[25] with minor modifications, was used to evaluate the antiproliferative activity of 1, 3, 5, 6, and 7 against the two lung cancer (A549 and H460) and four leukemia (K562, Lucena, HL60, and Jurkat) cell lines, and IC₅₀ values were determined in M, at least in three independent experiments. Table 1 shows the IC₅₀ values observed to each cell line.

Generally speaking, the results indicated that the UA and the oxidized derivative were the most active compounds against the assayed cell lines. For the A549 lung cancer cell line, the best result was observed to 1 (IC₅₀ = 13.12 μM), while for the H460, to 7 (IC₅₀ = 17.58 μM). The results for leukemia cell lines presented similar values of IC₅₀ for 1 and 7, with the exception of HL60, against which derivative 7 was more active. Furthermore, the cytotoxic effects observed to terpenes 1 and 7 were higher when compared with cisplatin, a well-known chemotherapeutic agent used in clinic treatment, with the exception of Jurkat cell line.

The results suggested the importance of structure modification in C-3 position and the maintenance of the carboxylic acid group that keeps the acid character of compounds 1 and 7 when compared with 5 and 6 with amide moieties. In addition, we calculated the lipophilic parameter log *P* by ACD Labs software package (version 12.0) for all assayed terpenes, and the values indicated the possible reason for this difference in the cytotoxicity observed. Terpenes 1 and 7, the most active compounds, presented log *P* 8.52 ± 0.37 and 7.94 ± 0.42, respectively, corresponding to the most hydrophilic compounds, whereas 3, 5, and 6 presented higher log *P* values, 9.41, 10.78, and 11.24, respectively, thus reinforcing the importance of the polar carboxylic group in the structures.

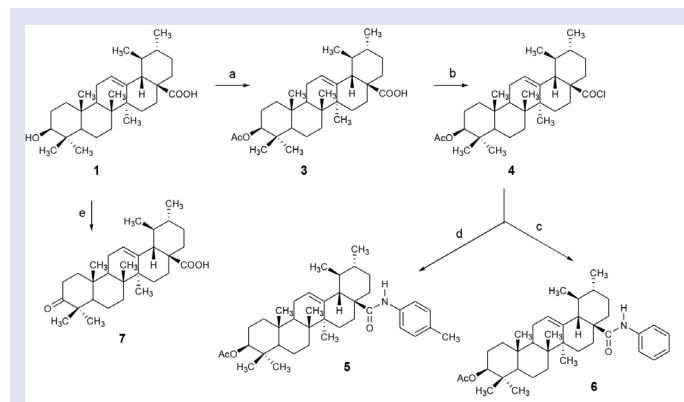


Figure 3: Synthetic route of UA derivatives. Reaction conditions: (A): (Ac)₂O, pyridine, RT, 30 min; (B): (COCl)₂, DMF, RT, 2 h; (C): aniline, TEA, reflux, 5 h, CH₂Cl₂, HCl; (D): *p*-toluylaniline, TEA, reflux, 5 h, CH₂Cl₂, HCl; and (e) PCC, acetone, RT, 48 h.

Table 1: IC₅₀ in μM values of 1, 5, 6, and 7 against two lung cancer (A549 and H460) and four leukemia (K562, Lucena, HL60, and Jukart) cell lines

Cell line	IC ₅₀ (μM)					CIS*
	1	5	6	7		
A549	13.12 ± 0.36	31.70 ± 2.33	18.71 ± 1.33	23.56 ± 1.9	25.00 ± 2.12	
H460	21.04 ± 0.98	45.71 ± 3.69	33.57 ± 3.00	17.58 ± 0.59	33.33 ± 1.87	
HL60	23.77 ± 1.36	40.46 ± 3.69	29.99 ± 1.99	12.82 ± 0.98	36.65 ± 2.34	
JUKART	22.91 ± 0.33	52.36 ± 0.56	99.54 ± 1.36	23.93 ± 2.10	< 17.00	
K562	10.45 ± 0.69	>100	92.26 ± 0.96	11.99 ± 0.36	22.27 ± 0.86	
LUCENA	13.09 ± 0.45	45.29 ± 1.26	47.64 ± 2.56	12.59 ± 1.25	18.32 ± 0.76	

*CIS = Cisplatin used as positive control

CONCLUSION

This study investigated the different extraction methods for the isolation of triterpene UA from *Ixora coccinea* flowers, a species widely used in traditional medicine, and verified that the ultrasound-assisted extraction is the best methodology with a remarkable 35% yield. Ursolic derivatives were prepared and characterized at reasonable yields, and the cytotoxic effects against two lung cancer (A549 and H460) and four leukemia (K562, Lucena, HL60, and Jurkat) cell lines showed that the most active compounds were the UA and the oxidize derivative, thus indicating the importance of hydrophilic moieties. Finally, we concluded that among the semisynthetic derivatives, 7 improved or was similar to UA 1 antiproliferative effects in leukemia and lung cancer cell lines. Furthermore, the importance of using alternative methods of extraction led us to the principal and abundant active component, 1, of *I. coccinea*, which represents a considerable contribution for drug discovery in cancer.

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Conflicts of interest

There are no conflicts of interest

REFERENCES

- Arnaud-Mouly A, Razafimandimbison SG, Khodabandeh A, Bremer B. Phylogeny and classification of the species-rich pantropical showy genus *Ixora* (Rubiaceae-Ixoreae) with indications of geographical monophyletic units and hybrids. *Am J Bot* 2009;96:686-706.
- Baliga MS, Kurian PJ. *Ixora coccinea* Linn: Traditional uses, phytochemistry and pharmacology. *Chin J Integr Med* 2012;18:72-9.
- Sankaranarayanan S, Bamal P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshmi M, *et al.* Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamilnadu, India. *J Med Plants Res* 2010;4:1089-101.
- Annapurna J, Amarnath PVS, Kumar DA, Ramakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* leaves. *Fitoterapia* 2003;74:291-3.
- Momin FN, Kalai BR, Shikalgar TS, Naikwade NS. Cardioprotective effect of methanolic extract of *Ixora coccinea* Linn. leaves on doxorubicin-induced cardiac toxicity in rats. *Indian J Pharmacol* 2012;44:178-83.
- Latha PG, Panikkar KR. Cytotoxic and antitumor principles from *Ixora coccinea* flowers. *Cancer Lett* 1998;130:197-202.
- Ayyanar M, Ignacimuthu S. Herbal medicines for wound healing among tribal people in Southern India: ethnobotanical and scientific evidences. *Int J Appl Res Nat Prod* 2009;2:29-42.
- Latha PG, Nayar MNS, Singh OV, George V, Panikkar KR, Pushpangadan P. Isolation of antigenotoxic ursolic acid from *Ixora coccinea* flowers. *Actual Biol* 2001;23:21-4.
- Versiani MA, Ikram A, Khalid S, Faizi S, Tahiri IA. Ixoroid: a new triterpenoid from the flowers of *Ixora coccinea*. *Nat Prod Commun* 2012;7:831-4.
- Idowu TO, Ogundaini AO, Salau AO, Obuotor EM, Bezabih M, Abegaz BM. Doubly linked, a-type proanthocyanidin trimer and other constituents of *Ixora coccinea* leaves and their antioxidant and antibacterial properties. *Phytochemistry* 2010;71:2092-8.
- Ngo SN, Williams DB, Head RJ. Rosemary and cancer prevention: preclinical perspectives. *Crit Rev Food Sci Nutr* 2011;51:946-54.
- Zhang P, Cheng Y, Duan RD. Ursolic acid inhibits acid sphingomyelinase in intestinal cells. *Phytother Res* 2013;27:173-8.
- Taviano MF, Miceli N, Monforte MT, Tzakou O, Galati EM. Ursolic acid plays a role in *Nepeta sibthorpii* Benthams CNS depressing effects. *Phytother Res* 2007;21:382-5.
- Ferreira DS, Esperandim VR, Toldo MPA, Kuehn CC, Prado-Júnior JC, Cunha WR, *et al.* *In vivo* activity of ursolic and oleanolic acids during the acute phase of *Trypanosoma cruzi* infection. *Exper Parasitol* 2013;134:455-9.
- Fontanay S, Grare M, Mayer J, Finance C, Duval RE. Ursolic, oleanolic and betulonic acids: antibacterial spectra and selectivity indexes. *J Ethnopharmacol* 2008;120:272-6.
- Huang CY, Lin CY, Tsai CW. Mei-Chin. Inhibition of cell proliferation, invasion and migration by ursolic acid in human lung cancer cell lines. *Toxicol In vitro* 2011;25:1274-80.
- Shanmugam MK, Dai X, Kumar AP, Tan BKH, Sethi G, Bishayee A. Ursolic acid in cancer prevention and treatment: Molecular targets, pharmacokinetics and clinical studies. *Biochem Pharmacol* 2013;85:1579-87.
- Yang YC, Weib MC, Huang TC. Optimisation of an Ultrasound-assisted extraction followed by RP-HPLC separation for the simultaneous determination of oleanolic acid, ursolic acid and oridonin content in *Rabdosia rubescens*. *Phytochem Analysis* 2012;23:627-36.
- Xia EQ, Yu YY, Xu XR, Deng GF, Guo YJ, Li HB. Ultrasound-assisted extraction of oleanolic acid and ursolic acid from *Ligustrum lucidum* Ait. *Ultrason Sonochem* 2012;19:772-6.
- Maciel MAM, Martins JR, Pinto AC, Kaiser CR, Esteves-Souza A, Echevarria A. Natural and semi-synthetic clerodanes of *Croton cajucara* and their cytotoxic effects against Ehrlich carcinoma and human k562 leukemia cells. *J Braz Chem Soc* 2007;18:391-6.
- Pinto AC, Braga WF, Rezende CM, Garrido FMS, Veiga-Jr VF, Bergter L, *et al.* Separation of acid diterpenes of *Copaifera cearensis* Huber ex Ducke by flash chromatography using potassium hydroxide impregnated silica gel. *J Braz Chem Soc* 2000;11:355-60.
- Gnoatto SCB, Dassonville-Klimpt A, Nascimento S, Galera P, Boumediene K, Gosmann G, *et al.* Evaluation of ursolic acid isolated from *Ilex paraguariensis* and derivatives on aromatase inhibition. *Eur J Med Chem* 2008;43:1865-77.
- Ma CM, Cai SQ, Cui JR, Wang RQ, Tu PF, Hattori M, *et al.* The cytotoxic activity of ursolic acid derivatives. *Eur J Med Chem* 2005;40:582-9.
- Rumjanek VM, Trindade GS, Wagner-Souza K, Meletti-de-Oliveira MC, Marques-Santos LF, Maia RC, *et al.* Multidrug resistance in tumor cells: characterization of the multidrug resistant cell line K562-Lucena 1. *Ann Acad Bras Ci* 2001;73:57-69.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
- Nagaraj S, Baskar L, Papiya MM, Muthukumar K, Simon SJ, Ashok KP. Evaluation of wound healing and antimicrobial potentials of *Ixora coccinea* root extract. *Asian Pac J Trop Med* 2011;4:959-63.