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EFFECT IN ACUTE INFLAMMATION OF SAPOGENIN EXTRACT AND ISOLATED SAPOGENINS FROM QUINOA WASTE (*CHENOPODIUM QUINOA* WILLD)

Maribel Lozano[a,b], Eduardo Gonzales[b], Yonny Flores[a], Giovanna R. Almanza[a,]*

[a] Laboratorio de Bioorgánica, Instituto de Investigaciones Químicas, Universidad Mayor de San Andrés, Calle 27 Cota Cota, P.O.Box. 303 La Paz-Bolivia; [b] Laboratorio de Farmacología, Instituto de Investigaciones Fármaco Bioquímicas, Universidad Mayor de San Andrés Av. Saavedra 1995, La Paz Bolivia

Keywords: Sapogenins, *Chenopodium quinoa*, acute anti-inflammatory activity, ear edema model, paw edema model.

ABSTRACT

A sapogenin extract was obtained from quinoa waste (*Chenopodium quinoa* Willd) and analyzed by HPLC, as well as other chromatographic and spectroscopic methods, determining four major constituents: Oleanolic acid **1**, methyl oleanate **2**, hederagenin **3** and phytolaccagenic acid **4**. The acute anti-inflammatory activity of both, isolated sapogenins and sapogenin extract, were evaluated in two animal models, carrageenan-induced paw edema and croton-induced ear edema, determining that the extract shows anti-inflammatory activity significant in ear edema model, greater than the compounds, suggesting a synergistic effect between the compounds, while in the paw edema model the isolated compounds show a significant anti-inflammatory activity while the extract shows only a moderate anti-inflammatory activity.

*Corresponding author: giovyalmanza@yahoo.com.ar

RESUMEN

A partir de residuos de quinua real (*Chenopodium quinoa* Willd) se obtuvo un extracto de sapogeninas el cual fue analizado por cromatografía HPLC, además de otros métodos cromatográficos y espectroscópicos, determinándose 4 constituyentes mayoritarios: ácido oleanólico **1**, oleanato de metilo **2**, hederagenina **3** y ácido fitolaccagenico **4**. La actividad antiinflamatoria aguda fue evaluada mediante dos modelos animales, modelo de edema de oreja de ratón inducido por aceite de croton y edema de pata inducido por carragenina, determinándose que el extracto muestra una actividad antiinflamatoria significativa en el modelo de edema de oreja, mayor que los compuestos, sugiriendo un efecto sinérgico entre ellos; mientras que en el modelo de edema de pata se observa una actividad antiinflamatoria significativa en los compuestos aislados y solo una actividad antiinflamatoria moderada en el extracto.

INTRODUCTION

Inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs exert their effects by inhibiting the metabolism of arachidonic acid, by both cyclo-oxigenase and lipoxygenase enzyme pathways.[1] Despite their widespread use, NSAIDs are often associated with severe adverse effects; the most common being gastrointestinal bleeding.[2] For this reason, safer compounds with less side effects are needed.

Bolivia is the first exporter of quinoa generating tons of quinoa waste each year. This quinoa waste does not have application and contents around 20% of saponins [12]. The hydrolysis of those saponins can produce sapogenins as oleanolic acid which have several studies about its anti-inflammatory effects [5]. The anti- inflammatory effect of Oleanolic acid was first reported in 1960s [3] where the inhibition of carrageenan-induced rat paw edema was showed and later was confirmed by other studies [4]. The other sapogenins have similar structures and can have similar anti-inflammatory effects than Oleanolic acid. In view of those antecedents, in the present work we have studied the activity of sapogenin extract and pure sapogenins in different inflammatory tests to observe the best administration vie and possible synergy among the compounds.

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RESULTS AND DISCUSSION

Isolated Compounds from quinoa waste

A sapogenin extract was obtained from an acid hydrolysis of saponin extract of quinoa waste. The sapogenin extract was subjected to diverse chromatographic separations giving four compounds. Compound **1** was identified as Oleanolic acid and isolated as white power of m.p 314 ^{o}C. The molecular formula $C_{30}H_{48}O_{3,}$ 456,70 g/mol was consistent with the ^{13}C NMR data (table1) which showed 30 signals, seven for methyl carbons C23 (δ 28,7), C24 (δ 16,7), C25(δ 15,6), C26 (δ 17,3), C27 (δ 26,1), C29 (δ 32,9), C30 (δ 23,8); one for a carbon hydroxyl substituted C3 (δ 77,3), one for a carboxyl group substituent C28 (δ 179,0) and two for a double bound in C12 (δ 122,0) and C13 (δ 144,3), all of them characteristic of an oleanane ring. Based on those data and previous reports we proposed the oleanoic acid structure for compound **1** which was confirmed by comparison with bibliographic data.[5] Compound **2** was identified as methyl oleanate, it has the molecular formula $C_{31}H_{50}O_3$, 470.73 g/mol, m.p 314-316^{o}C and was isolated as amorphous power. The ^{13}C NMR data (table 1) showed 31 signals, similar chemical shifts to compound **1** were observed, the unique difference is the signal C31 (δ 51,8) which was identified as a methyl ester located at C17 (δ 46,2) by HMBC correlation ^{2}J of H31 (δ_H 3.71) with C28 (δ_C 177.0), as well as ^{3}J coupling of H22 (δ_H 1.31) and H16 (δ_H 2.06) with C28, proposing for **2** a methyl oleanate structure. Compound **3** was identified as hederagenin by comparison of the ^{13}C chemical shifts with literature data.[6] It was isolated as white power of m.p. 314-316^{o}C, the molecular formula $C_{30}H_{48}O_4$, 472.36 g/mol was consistent with the ^{13}C NMR data which present 30 signals, similar to oleanolic acid only exception in C-23 by the hydroxyl substitution. The hydroxyl group was located in C23 (δ 70.3) by a HMBC analysis which revealed ^{3}J and ^{2}J couplings of H-24 (δ_H 0.65) with the carbons C-23 (δ_C 70.3), C3 (δ_C 75.6) and C4 (δ_C 41.5) confirming a hydroxyl group in C23.

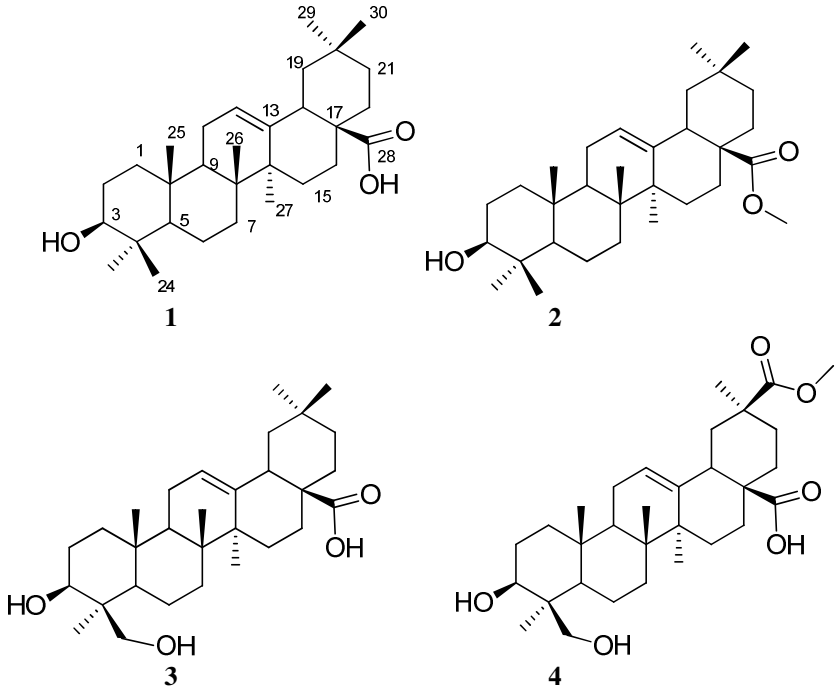

Figure 1. *Compounds isolated from quinoa waste*

Compound **4** was identified as phytolaccagenic acid; by comparison of experimental ^{13}C chemical shifts with literature data, it was also isolated as an amorphous white power, m.p 281 -284 ^{o}C, with a molecular formula, $C_{31}H_{48}O_{6,}$ 516.36 g/mol. The ^{13}C NMR spectrum showed 31 carbon signals (table1) similar to oleanolic acid except by the presence of an ester group located in C-30 confirmed by analysis of the HMBC spectrum.[5]

Table 1. *^{13}C NMR experimental data for compounds 1-4*

	1[a]	**2**[a]	**3**[b]	**4**[b]
1	37,0	38,4	37,9	38,0
2	27,5	27,1	27,4	27,5
3	77,3	79,0	75,6	75,8
4	38,5	38,7	41,5	42,4
5	55,3	55,2	49,4	49,3
6	18,5	18,3	18,1	18,2
7	32,6	32,6	32,1	32,3
8	38,9	39,2	39,0	39,0
9	47,6	47,6	47,6	47,5
10	33,8	37,1	36,6	36,7
11	23,1	23,1	23,2	23,0
12	122,0	123,4	122,0	122,8
13	144,3	142,9	143,7	143,1
14	41,8	41,4	41,4	41,4
15	27,7	27,7	29,4	25,6
16	23,4	23,4	22,8	23,2
17	46,7	46,2	46,2	45,6
18	41,3	41,3	41,0	42,4
19	45,9	45,9	45,8	42,0
20	30,9	30,3	30,4	43,7
21	33,3	33,5	33,6	30,2
22	30,9	28,4	32,7	33,5
23	28,7	28,7	70,3	70,6
24	16,7	15,5	11,3	11,3
25	15,6	15,3	15,4	15,4
26	17,3	17,1	16,6	16,6
27	26,1	26,1	25,8	26,0
28	179,0	177,0	180,7	180,0
29	32,9	32,9	32,3	28,2
30	23,8	23,8	25,6	177,6
31		51,8		51,6

[a] ^{13}C NMR data for compound 1-2 measured in DMSO at 300 at 300MHz
[b] ^{13}C NMR data for compound 3-4 measured in $CDCl_3$ +CD_3OD at 300 MHz

Anti- inflammatory activity

Ear edema test

Compounds **1**-**4** and sapogenin extract were submitted to the croton oil ear test, at the dose of 2 and 56 mg/ear, to evaluate the topical anti-inflammatory effect. As showed in figure 2, all pure compounds **1**-**4** exerted some anti-inflammatory activity, while sapogenin extract showed strong active, inducing 68,2% edema inhibition at the highest administered dose (56 mg/ear). Consequently, the contribution to the activity of the extract could be very interesting for mew pharmaceutical formulations by topical application.

Paw edema test

The development of edema induced by carrageenan corresponded to the events in the acute phase of inflammation mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclooxygenase.[7] Sapogenin extract and compounds **1**-**4** had an anti-inflammatory effect at 636mg/kg po and 100mg/po, respectively, observable to 3 ($p< 0,05$), 5($p< 0,01$) and 7($p<0,01$) hours, respectively (figure 3). In this test, the compounds **1**-**4** had higher anti-inflammatory between 5-7 hours after treatment, while the activity of the sapogenins extract was less respect to compounds. The strong activity of oleanolic acid and phytolaccagenic acid could be employed for anti-inflammatory drugs of oral administration

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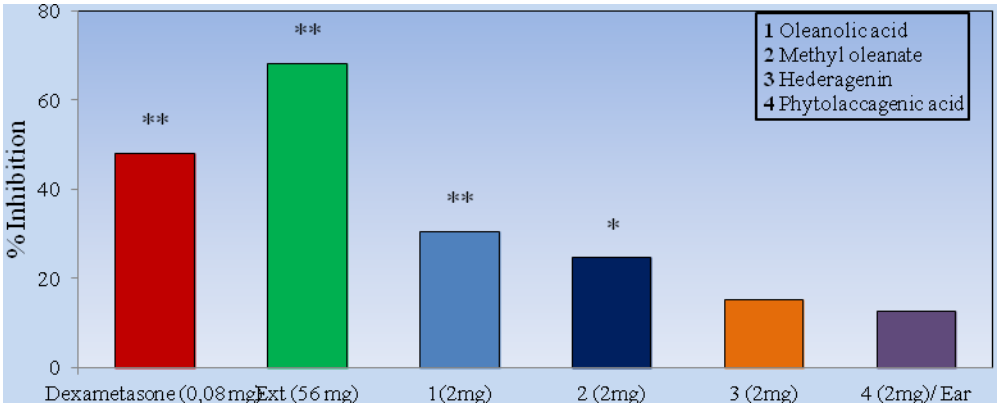
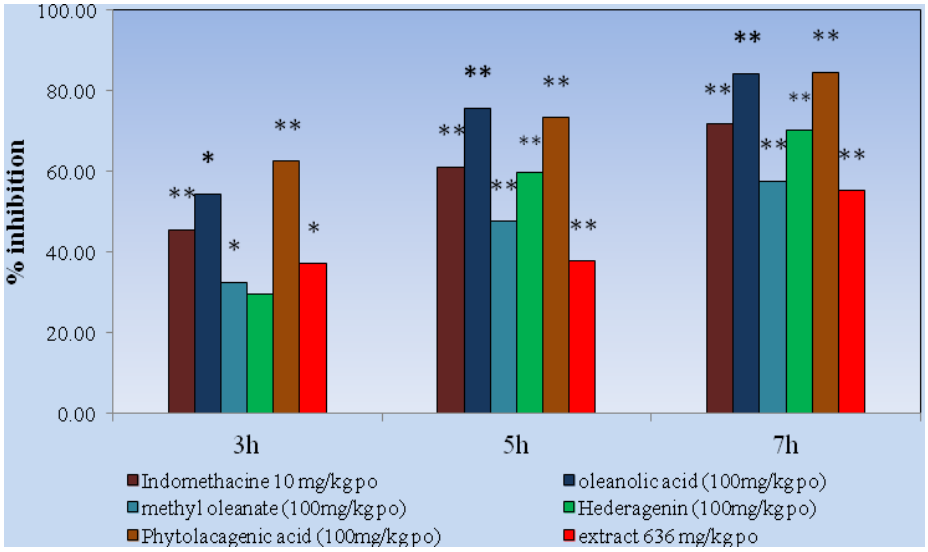


* significative difference regarding to control ($p < 0,05$)
**significative difference regarding to control ($p < 0,01$)

Figure 2. *Topical anti-inflammatory effect of compounds* ***1-4*** *and sapogenin extract versus croton oil-induced imflammation of the mouse ear. All values were significantly different from the negative control considered as 100% inflammation*



* Diferencia significativa frente al control ($p < 0,05$)
**Diferencia significativa frente al control ($p < 0,01$)

Figure 3 *Anti- inflammatory activity of isolated compounds* ***1-4*** *respect of negative control; Paw edema test using carrageenan as irritant agent.*

HPLC analysis of sapogenins

The HPLC analysis revealed the presence of four triterpenic compounds in quinoa waste identified by external standard and the purity peaks (purified compounds **1**-**4**) verified by UV (DAD, 210 nm) spectra (figure 4). Oleanolic acid (13,3 min), methyl oleanate (6,2 min) , hederagenin (5,4 min) and Phytolaccagenic acid (2,8 min). A subsequent HPLC quantification of sapogenin extract, give the follow yields for the pure compounds: 24% Oleanolic acid, 12% methyl oleanate, 28% hederagenin and 27% Phytolaccagenic acid. The extract contents 91% of sapogenins (Figure 5). This composition could be used for the analysis of the strong topical anti- inflammatory activity of the extract.

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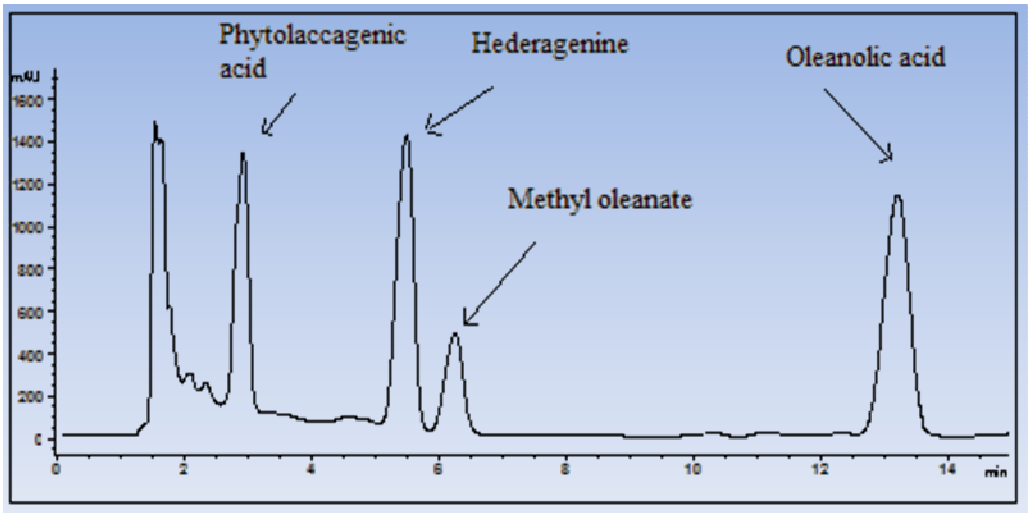

Figure 4*.HPLC Chromatogram of sapogenins of quinoa waste at 210 nm*

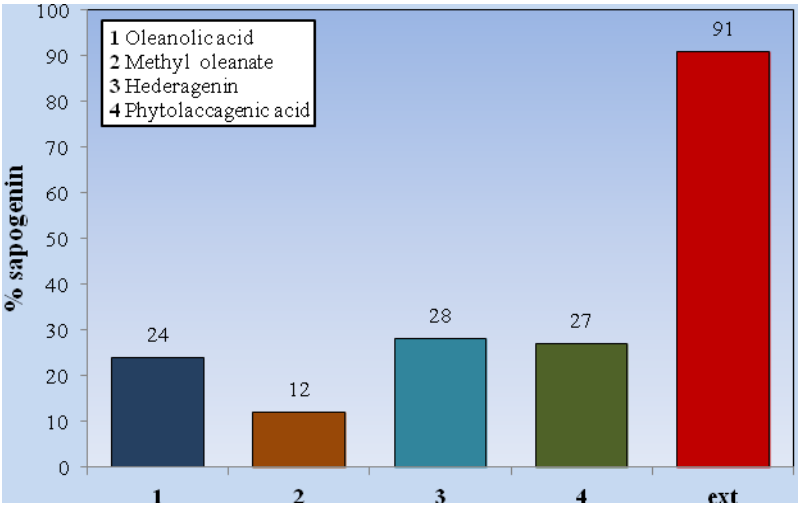

Figure 5. *Quantitative HPLC analysis of pure compounds in the crude sapogenin extract from quinoa waste; HPLC analysis using external standards*

CONCLUSION

The quinoa waste is generally considered for its higher content in saponins while triterpenoid sapogenins are not equal considered. An interesting pharmacological activity was observed for the sapogenin extract and isolated sapogenins, which can be considered of therapeutical relevance for quinoa waste in pharmaceutical formulations against inflammatory disorders. The HPLC analytical method set up here was fit for the determination of sapogenins in the extract, as a mean to control the quality of bioactive extract which could be obtained in big quantities from quinoa waste. Furthermore, the quinoa waste could also be a cheap primary material to obtain sapogenins extract or individual sapogenins with anti-inflammatory propierties.

EXPERIMENTAL

Extraction and isolation

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The modified method of San Martin and Briones [8] was used for saponins extractions; first 500 g of ground sample was extracted with an aqueous solution of ethanol 50% v/v (ratio 1:9) for 3 h under constant stirring at 200 rpm. The extract was then filtered and ethanol was removed using a rota-evaporator and the water residue was dried by frizzed. 20g of saponin extract was hydrolyzed with 200ml of 2N HCl in 50% aqueous ethanol under reflux for 3h at 85^{o}C, and therefore sugars and aglicones were separated. The solid phase sapogenins were washed with H_2O and $NaHCO_3$ at 5% m/v, this residue was dried using high vacuum pump by 7h.[9], [10]

6,2g was subjected to Vacuum Liquid Chromatography (VLC) silica gel chromatography using mixtures of petroleum ether/EtOAc (EtOAc: 10, 20, 30, 40, 50, 70, 80, 100 %, v/v) and EtOAc/ MeOH (MeOH: 5% v/v) as a gradient solvent system to give ten fractions. The fraction 2 consist of pure compound oleanolic acid 1 and fraction 9 pure phytolaccagenic acid 4. The pure compounds 2 and 3 were obtained by further silica gel flash chromatography using ether/EtOAc 90% and40 % v/v.

HPLC chromatographic analysis of sapogenins

Quantification of oleanolic acid **1**, methyl oleanate **2**, hederagenin **3** and phytolaccgenic acid **4** were performed on an HPLC system (Agilent, 1100 series) equipped with quaternary pump, DAD detector and Eclipse Plus C_{18} column (125x4,6, 5µm). All these four compounds were detected at 210 nm at room temperature with an eluent flow rate of 1.0 mL/min. The mobile phase consisted of formic acid (0,1%) (A) and methanol (B) with a ratio of 15:85 (A:B, v/v) and isocratic elution.[11]

Animals

Experiments were performed on females *Swiss* mice (24 -26g), housed in controlled room temperature (20 ±2 ^{o}C) under a 12: 12h light-dark cycle (lights on 7 a.m.). Animals were kept in groups of 6 in light cages and had a free access to standard laboratory diet (pellets) and tap water in their cages.

Administration of extracts, fractions, isolated compound and drugs

Croton-induced ear edema

The topical anti-inflammatory activity was evaluated as inhibition of the croton oil-induced ear edema in mice (CYTED, 1995ref) at doses of 2 mg/ear for the pure compounds and 56 mg/ear for the sapogenin extract, to the right ear of each mouse was administered mean dose and after mean hour was administered the other mean dose. Inflammation was induced on the inner surface of the right ear of mice, by application of 20µL of Croton oil, suspended in the appropriated vehicle, In the left ear was only applied the vehicle. Control animals received only the irritant solution, whereas the other mice received both the irritant and the test substances: At the maximum of the edematous response, 4 h later, mice were sacrificed and a plug (6mm Ø) was removed from both the treated (right) and the untreated (left) ears. The edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percentage of the edema reduction in treated mice in comparison to control mice. As a reference, the non-steroidal anti-inflammatory drug (NSAID) Dexamethasone (0.04 mg/ear) was used.

Carrageenan-induced paw edema

Pure compounds and sapogenin extract in 100 mg/kg p.o. and 636 mg/kg p.o., indomethacin in 10 mg/kg p.o. doses were given to rats orally by feeding tube. One hour after of administration, 0,05 ml (1% w/v) carrageenan solution was subcutaneously injected into the plantar surface of the left hind paw. The paw volume was measured with bernier, at 1, 2, 3, 5 y 7 hours after carrageenan administration . The anti-inflammatory activity in animals of pure compounds and sapogenins extract were compared with that of indomethacin and control groups.

Statistical analysis

Values are presented as mean ± S.E.M. Independent samples *t*-test and analysis of variance (ANOVA, Dunnett method) were used for the evaluation of data and P005 was accepted as statistically significant

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REFERENCES

[1] INSEL, P.A.,
Analgesic, Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout. McGraw-Hill, New York1996, pp. 617–657. In: J.G. Hardman, Limbird, L.E. (Eds.),
[2] FUNG, H.B., KIRSCHENBAUM, H.L.,
Clinical Therapeutics, 1999, 21, 1131–1157.
[3] GUPTA, M.B., BHALLA, T.N., GUPTA, G.P., MITRA, C.R., BHARGAVA K.P.
European Journal of Pharmacology, 1969, **6**, 67–70
[4] JIE L.
Journal of Ethnopharmacology, 1995, **49**; 57-68
[5] FLORES, Y., DÍAZ, C., GARAY, F., COLQUE, O., STERNER, O., ALMANZA. G.R.,
Revista Boliviana de química, 2005, **1**, 22
[6] MEHTA, B. K, MEHTA, D., AMRITA, I.,
Carbohydrate Research, 2004, 339; 2871–2874
[7] NSONDENTANDOU, G.F, BANZOUZI, J.T., MBATCHIA, B., ELION-ITOU, R.D.G., ETOU-OSSIBI, A.W., RAMOS, S., BENOIT-VICAL F., ABENA, A.A., OUAMBA, J.M.,
Journal of Ethnopharmacology 2010, 127; 108–111
[8] SAN MARTÍN, R., BRIONES, R.
Journal of the Science of Food and Agriculture, 2000, **80**: 2063- 2068.
[9] TIWATT, K., THONGPHASUK, P., CHAMULITRAT, W., WINK, M..
Phytochemistry 2008, **69**; 1919–1926
[10] DICK M.H., LIMBURG, H., GILLES, T., MARVIN H.J.P.
J Sci Food Agric, 2000, **80**:152-156
[11] CHUNHUA, Z., CHEN, K., SUN, C., CHEN, Q., ZHANG, W., LI, X.
Biomed.Chromatogr, 2007, **21**, 755–761.