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Nutritional and antinutritional composition of Kancolla seeds: an interesting and underexploited andine food plant

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Abstract

The quality of Kancolla seeds, a sweet variety of quinoa, an underexploited food plant, was determined by measuring proximate composition (carbohydrate, lipid, protein, fibre, essential amino acids and minerals), antinutritional factors (anions) and phytoecdysteroids. The results show that the kancolla seeds are nutritionally interesting and differ from other quinoa varieties, mainly in fibre and mineral contents. Results suggest a major alimentary use of kancolla seeds. They have promising economic value. The challenge is to find ways to incorporate them into existing food products, as well as to create new products from them.

Keywords: Proximate analysis; Phytoecdysteroids; Kancolla; Quinoa; NMR; Mass

1. Introduction

The greatest threat to the survival of humanity is the ever-increasing gap between population growth and food supply. FAO, in its annual report "The State of Food Insecurity in the World 2002", estimates that there were around 840 million undernourished people in 1998–2000, 799 million in the developing countries, 30 million in the countries in transition and 11 million in the industrialized countries (Diouf, 2002; Sartaj, 2001). In order to arrest the situation, much attention has been centred on the exploitation and utilization of unusual food plants, such as Andean pseudo-cereals. These food plants are often closer to the ideal protein balance than any other common grain, being at least equal to milk in protein quality. Particularly, they have a very high lysine content. Therefore, in order to obtain a suitable amino acid pattern to cover the needs of humans, it is not necessary to combine them with other crops, such as legumes, rich in lysine, but lacking in methionine and cystine, as happens for other cereals. Many Andine pseudo-cereals belong to the Chenopodiaceae family, noteworthy among which is quinoa (*Chenopodium quinoa*) (Ogungbenle, 2003; Ruales & Nair, 1992). The Food and Agriculture Organization (FAO) observed that quinoa seeds have high quality proteins and higher levels of energy, calcium, phosphorus, iron, fibre and B-vitamins than barley, oats, rice, corn or wheat (Koziol, 1992; Tapia, 2000). However, the seeds of most quinoa varieties contain saponins, located in the outer layers of the seed coat (Dini, Tenore, & Dini, 2002; Penafiel & Diaz Villar, 1988), most of which are bitter-tasting constituents. Because of this, they need to be washed or milled to remove the seed coat. The increased demand for quinoa has led researchers to produce several cultivars, selected and bred for their tolerance to heat and cold, resistance to disease, and for sweet taste. Perhaps the oldest and most widespread of the new varieties is kancolla (selected in 1950 in Perú). The aim of this work is a study of the nutritional quality of kancolla seeds. Kancolla

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seeds could be the best alternative, among quinoa varieties, for an equal or better nutritional value and for the presence of phytochemicals, which may provide health benefits. Previous work showed three flavonoid glycosides (Dini, Tenore, & Dini, 2004). The effects of dietary polyphenols are of great current interest due to their antioxidative and potential anticarcinogenic activities. In particular, we found mauritianin which may augment the immune resistance to cancer (Nishibe et al., 1996). We report here, for the first time, the isolation and characterization of two ecdysteroids from Kancolla seeds of which compound **2** is new. Their structures were determined using 1D NMR and MS spectra and verified by 2D NMR data. Phytoecdysteroids are plant steroids, which are analogues of vertebrate steroid hormones. Ecdysteroids are the steroid hormones of arthropods and possibly of other invertebrate phyla too. They also occur in certain plant species, where they are known as phytoecdysteroids and are believed to contribute to the deterrence of invertebrate predators (Dinan, 2001). In insects, they regulate moulting and metamorphosis and have been implicated in the regulation of reproduction and diapause (Simon & Koolman, 1989). Further, since the ecdysteroid receptor is specific to invertebrates, ecdysteroids appear to be non-toxic to vertebrates and plants (Sláma & Lafont, 1995). They have several pharmaceutical and medicinal properties, such as tonics, for growth promoting, as hypocholesterolaemic, hypoglycaemic, immunomodulatory, antioxidant and cellular proliferation and differentiation stimulating agents (Dinan, 2001; Lafont & Dinan, 2003; Simon & Koolman, 1989; Sláma & Lafont, 1995). Particularly, 20-hydroxyecdysone has significant spermicidal activity and influences the sexual activity of male rats (Dinan, 2001). A number of reports suggest that ecdysteroids may be potential cancer chemopreventative agents, may be effective in the control of diabetes, and may exhibit a hepatoprotective action (Dinan, 2001). They appear to be mildly anabolic, supposedly without the adverse side effects associated with vertebrate steroids or their analogues. A current, but unverified, assertion suggests that some athletes and sportsmen of various nations have taken advantage of this since the mid-1980s.

2. Materials and methods

2.1. Material

The plant material was collected in Perú in April, 1999, and identified by Dr. S.E. Jacobsen of the International Potato Centre (CIP), Lima, Perú. A sample used has been deposited in the Herbarium Neapolitanum of the Dipartimento di Biologia Vegetale, Università degli Studi "Federico II" of Naples. The collection number was NAP # A.C. 002. The seeds were reduced to a fine powder.

2.2. Oil analysis

Total fat content was obtained by the Soxhlet extraction method, using diethyl ether, as reported in AOAC methods 920.39 [Official methods of analysis of the AOAC (1980). Association of Analytical Chemists,(13th ed., pp. 7021–7024)].

2.3. Crude protein analysis

Proteins were determined by the Kjeldahl procedure, AOAC Method 920; the factor $N \times 6.25$ was used to convert nitrogen into crude protein.

2.4. Total amino acid analysis.

Total amino acid contents were determined after hydrolysis with 6 N HCl at 100 °C in vacuum hydrolysis tubes, following the Waters AccQ-Tag Method (1993) [Manual number WATO-52874 TP, Rev. April, 1993 WATERS]. The analysis was done by reverse-phase HPLC, using a Waters system with Alliance 2690 Separation Module pump, an auto sampler provided with injector programme, a fluorescence detector Mod. 474 ($\lambda_{ecc} = 250$ nm; $\lambda_{em} = 395$ nm) and AccQ-Tag 3.9×150 mm column thermostatted at 35 °C. The derivatization procedure was done with "AccQ-Fluor Reagent Kit Waters" (Borate buffer, AQC, CH_3CN; Cat. No. 052880). The eluents were a gradient of phosphate buffer pH 5.80 (A), CH_3CN (B) and H_2O (C) (flow-rate 1.0 ml/min) (Strydom & Cohen, 1994).

2.5. Free amino acid analysis

Free amino acid samples were prepared for HPLC analysis by flour (1 g) extraction with 10 ml. About 0.1 M HCl, then filtered by 0.45 μm filter (Millipore Millex-HV). 1 ml was applied to a cation-exchange (100×6 mm) column [AG 50W-X8 (H^+), Bio-Rad]. After the column was washed with 50 ml of milli-Q Water, the amino acids were eluted with 3.0 M NH_3 (about 10 ml). The sample was evaporated and recovered with 0.01 M HCl (9.2 ml) and internal standard (BABA 2.5 mM in 100 ml 0.1 M HCl). Free amino acids were determined using the same tools used to measure total amino acids.

2.6. Dietary fibre analysis

Total dietary fibre content was determined by AOAC method 960.52 and AOAC method 985.29.

2.7. Mineral analysis

Mineral elements (Na, K, Ca, Mg, Cu, Zn, Fe) were measured by atomic absorption after seed flour mineralization, performed with a sulfo-nitric mixture according to the National Italian Standards [Ministero dell'Agricoltura e delle Foreste. (1989). Official Italian Methods. "Metodi Ufficiali di Analisi per le Conserve Vegetali-parte generale" Gazzetta Ufficiale della Repubblica Italiana no 168 del 20-07-1989 (Rome, Italy: Istituto Poligrafico dello Stato)] using standard reference materials (BDH Chemical Ltd., Poole England).

2.8. Sugar analysis

Sugar contents (glucose, fructose, sucrose) were determined after extraction of the seed powder with warm H_2O for 2 h 30 min and centrifugation at 18,000*g*. The clarified extract was filtered through a 0.45 μm filter (Millipore Millex-HV). A Waters-Millipore Sugar-pak cartridge thermostatted at 85 °C was used. EDTA-Ca (50 mg/l), at a flow rate of 0.6 ml/min, served as the elution solvent. Individual sugars were identified by comparison with an internal standard (sucrose 0.1–0.2%; glucose 0.1–0.2%; fructose 0.05–0.1%; raffinose 0.02–0.04%). A Waters-Millipore 600 E liquid chromatograph was employed and a differentia1 refractometer (Model 410) was utilized as a detector for sugar analysis.

2.9. Anion analysis

Anion contents $(Cl^-, F^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, C_2O_4^{2-})$ were determined using the same tools used to measure sugars except for the detector, which was a Dionex Pulser electrochemical detector provided with Suppressed Conductivity ASRS-II™ Autosuppression™ Recycle Mode. The analyses were carried out on an Ion Pac AS4A-SC Analytical 4×250 mm column and samples eluted with buffer solution ($Na_2CO_3 = 1.7$; $NaHCO_3 = 1.8$ m M).

2.10. Phytoecdysteroids analysis

Optical rotations were determined on a Perkin–Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10-mm microcell.

The FTIR spectra were obtained on a Bruker IFS-48 spectrophotometer using a KBr matrix.

The NMR spectra were obtained in CD_3OD (**1**) and C_5D_5N (**2**) with a Bruker AMX 500 spectrometer (Dini et al., 2004).

Electrospray ionization ESI–MS spectra were recorded in CH_3OH on an AB Applied Biosystems mass spectrometer API 2000.

HPLC separations were performed on a Hewlett–Packard HP 1050 series apparatus with a Varian RI-4 refractive index detector, equipped with Waters μ-Bondapak C-18 column (7.8×300 mm).

2.11. Extraction and isolation of phytoecdysteroids

The whole flour from the seeds (709 g) was extracted with MeOH (2 L, four times). The MeOH extract (49.03 g) was partitioned between BuOH and H_2O. The butanol extract (26.2 g) was evaporated and defatted with $CHCl_3$. The residue fraction (10 g) was chromatographed on a Sephadex LH-20 column (100×5 cm), with MeOH as eluent. Fractions (9 ml) were collected and checked by TLC [Si-gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Fractions 1–36 (0.9 g), containing the crude mixture, were further separated by silica gel column (20 g), using $CHCl_3$/MeOH/H_2O (15:9:1.2) as eluent, giving 25 fractions (9 ml). Each fraction was checked by TLC [Si-gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Silica gel fractions 2–4 (13 mg) and 5–6 (4.3 mg) contained the phytoecdysteroids **1** (F_R, 0.90) and **2** (F_R, 0.79), respectively. Their structures were determined using 1D NMR and MS spectra and verified by 2D NMR data (HMQC and HMBC).

2.12. 20-Hydroxyecdysone (*1*)

Amorphous powder; $[\alpha]_D^{23} + 1.72°$ ($c = 0.001$ in MeOH); IR (KBr) ν = 3426, 1658, 1052 cm^{-1}; ESI–MS (negative ion): *m/z* 479 $[M - H]^-$; NMR data see Table 1.

2.13. Kancollosterone (*2*)

Amorphous powder; $[\alpha]_D^{23} + 25.1°$ ($c = 0.001$ in MeOH); IR (KBr) ν = 3426, 1658, 1052 cm^{-1}; ESI–MS (negative ion): *m/z* 491 $[M - H]^-$; NMR data see Table 1.

3. Results and discussion

3.1. General

Kancolla' s food value in the developed world lies primarily within its seeds, which are the main edible part. Previously phytochemicals present in these seeds have been studied, because they can provide health benefits and influence food taste (Horowitz & Gentili, 1979; Okubo et al., 1992). Now we have investigated chemical composition and phytoecdysteroids of kancolla seeds.

3.2. Proximate analysis

The proximate composition is shown in Table 2. These values were compared to corresponding data for several grains and local quinoa reported by Cardoza

Table 1
NMR spectral data of ecdysteroids

	Compound **1** (dissolved in CD_3OD)			Compound **2** (dissolved in C_5D_5N)		
	DEPT	^{13}C	^{1}H	DEPT	^{13}C	^{1}H
1	CH_2	37.2		CH_2	37.8	
2	CH	68.7	3.85 ddd, J = 12.0; 4.0; 3.2 Hz	CH	67.7	4.16 ddd, J = 12.0; 4.0; 3.2 Hz
3	CH	68.5	3.98 q 2.9	CH	66.6	4.22 q, J = 2.9 Hz
4	CH_2	32.9		CH_2	32.4	
5	CH	51.8		CH	51.4	
6		203.5			203.8	
7	CH	122.1	5.84 d, J = 2.6 Hz	CH	121.3	6.26 d, J = 2.6 Hz
8		167.9			166.5	
9	CH	35.0	3.18 ddd, J = 11.2; 7.0; 2.6 Hz	CH	34.5	3.59 ddd, J = 11.2; 7.0; 2.6 Hz
10		39.3			38.3	
11	CH_2	20.6		CH_2	21.1	
12	CH_2	32.5		CH_2	31.7	
13		48.0			48.1	
14		85.2			84.3	
15	CH_2	31.8		CH_2	31.9	
16	CH_2	21.5		CH_2	21.6	
17	CH	50.5		CH	50.3	
18	CH_3	18.0	0.92 s	CH_3	17.9	1.22 s
19	CH_3	24.4	0.99 s	CH_3	24.5	1.06 s
20		77.9			77.0	
21	CH_3	21.0	1.35 s	CH_3	21.7	1.58 s
22	CH	78.4	3.33 dd, J = 11.0; 1.7 Hz	CH	77.8	4.08 dd, J = 11.0; 1.7 Hz
23	CH_2	27.3		CH_2	27.6	
24	CH_2	42.6			150.2	
25		71.3		CH	42.7	
26	CH_3	29.7	1.23 s	CH_2	69.6	3.86 m
27	CH_3	29.0	1.24 s	CH_3	29.5	1.36 s
28				CH_2	109.9	5.65;5.27 d, J = 1.5 Hz

Table 2
Comparisons of the proximate analyses (% dry weight) of kancolla seeds with various grains and other quinoa varieties

Crop	Water	Crude protein (%)	Fat (%)	Carbohydrates (%)	Fibre (%)	Ash
Kancolla	16.6	13.4	5.9	51.7	12.3	1.7
[a]Quinoa	12.6	13.8	5.0	59.7	4.1	3.4
[a]Barley	9.0	14.7	1.1	67.8	2.0	5.5
[a]Buckwheat	10.7	18.5	4.9	43.5	18.2	4.2
[a]Corn	13.5	8.7	3.9	70.9	1.7	1.2
[a]Millet (pearl)	11.0	11.9	4.0	68.6	2.0	2.0
[a]Oat	13.5	11.1	4.6	57.6	0.3	2.9
[a]Rice	11.0	7.3	0.4	80.4	0.4	0.5
[a]Rye	13.5	11.5	1.2	69.6	2.6	1.5
[a]Wheat	10.9	13.0	1.6	70.0	2.7	1.8

[a] Cardoza and Tapia (1979) Reported by J. Risi and H.W. Galwey (1984). Analyses of the remaining crops reported by: Crampton and Harris (1969). *Applied animal nutrition*, (2nd ed.). San Francisco: W.H. Freeman and Co.,

and Tapia (1979). The protein level in kancolla is 13.4%, which is slightly lower than that in other quinoa varieties, lower than barley and buckwheat, higher than wheat, but much higher than corn, millet (pearl), oat, rice or rye. The protein content in most quinoa species is 13.8%. The amount of fibre present in quinoa seeds and the other grains is less than 4.2% while that in kancolla seeds is about three times higher, except for buckwheat, which is 18.2%. The total lipid content in kancolla seeds was found to be 5.9%, which is much higher than barley, rice, rye or wheat and slightly lower than that in other quinoa varieties, buckwheat, corn, millet and oat.

3.3. Amino acid analysis

Quantitative determination of amino acid concentrations was conducted by HPLC. Nine amino acids were detected and the separation of the amino acids in the samples was reasonably good. All of the essential amino acids, namely methionine, leucine, lysine, cystine, phenylalanine, tyrosine, isoleucine, threonine and va-

Table 3
Essential amino acid pattern of kancolla seeds compared to wheat, soy, skim milk, other quinoa varieties and the FAO/WHO (1990) for evaluating proteins

Amino acid	Amino acid content (g/100 g protein)					
	Kancolla (%)	[a]Quinoa (%)	[a]Wheat (%)	[a]Soy	[a]Skim milk	[b]FAO
Isoleucine	5.6 (C. I.140)	4.0	3.8	4.7	5.6	2.8
Leucine	9.4 (C. I.130)	6.8	6.6	7.0	9.8	6.6
Lysine	7.8 (C. I.141)	5.1	2.5	6.3	8.2	5.8
Phenylalanine	6.2 (C. I.172)	4.6	4.5	4.6	4.8	
Tyrosine	4.1 (C. I.172)	3.8	3.0	3.6	5.0	6.3
Cystine	2.7 (C. I.140)	2.4	2.2	1.4	0.9	2.5
Methionine	2.2 (C. I.140)	2.2	1.7	1.4	2.6	
Threonine	6.2 (C. I.155)	3.7	2.9	3.9	4.6	3.4
Valine	6.1 (C. I.122)	4.8	4.7	4.9	6.9	3.5

[a] *Source:* Johnson, R. & R. Aguilera (1980). Processing varieties of oilseeds (Lupine and Quinoa). In *Report to natural fibres and foods commission of Texas*, 1978–1980 (Reported by D. Cusack (1984). *The Ecologist* 14:21–31). C.I., Chemical Index.
[b] *Source:* FAO/WHO (1990) Protein quality evaluation in Report of Joint FAO/WHO expert consultation (p. 23) Rome: Food and Agricultural Organization of the United nations

line, were found to be present in kancolla seeds. Table 3 shows the essential amino acid pattern of kancolla seeds compared to wheat, soy, skim milk, other quinoa varieties and the FAO/WHO (1990) for evaluating proteins. The amino acid profile of kancolla showed that the essential amino acids, such as lysine and threonine, normally deficient in other grains, were at higher concentrations when compared with the reference pattern (FAO/WHO, 1990), while the other essential amino acids were adequate, with the exception of tyrosine, which is the limiting amino acid. Tyrosine and methionine were present in lower amounts, 4.1 and 2.2 g amino acid/100 g sample, than the other amino acids. Almost all of the essential amino acids studied in kancolla seeds had a high chemical score, which implied that the essential amino acids present had a high biological value. Of greater interest to individuals is the quality of kancolla seed proteins. In general, animal proteins (meat, fish, poultry, milk, cheese, and eggs), which contain ample amounts of all of the essential amino acids, are considered good sources of complete proteins. On the other hand, plant proteins (including those of cereals) are often called incomplete proteins, because they generally do not have enough of one or more of the essential amino acids. Despite this, kancolla seed proteins contained all of the essential amino acids. These are better than in other quinoa varieties and meet or exceed nutritional FAO standard requirements for all essential amino acids (Table 3). Moreover, grain proteins have high contents of lysine and sulphur amino acids, which are the limiting amino acids in other grains (Table 3).

3.4. Free aminoacid analysis

The free amino acids were found in highest amounts in kancolla seeds (Table 4). The free amino acids present in kancolla seeds contained large amounts of arginine (60 mg/100 g) and glutamic acid (58.0 mg/100 g), along with lesser amounts of aspartic acid (30.7 mg/100 g) and alanine (10.2 mg/100 g). Individual free amino acids have been reported to impart bitter, sour, and sweet tastes to foods (Kato, Rhue, & Nishimura, 1989; Macleod, 1998). However, free amino acids play an important role in the formation of colour and aroma during roasting (Basha & Young, 1985; Botta, Gianotti, Richardson, Suwanagul, & Sanz, 1994).

Table 4
Free amino acid pattern of kancolla seeds

Free amino acid	Kancolla (mg/100 g protein)
Isoleucine	4.6
Leucine	7.7
Lysine	5.8
Phenylalanine	6.9
Tyrosine	5.3
Cystine	Missing
Methionine	0.9
Threonine	4.4
Aspartic acid	30.7
Valine	6.7
Asparagine	4.1
Glutammic acid	58.0
Serine	4.8
Glycine	3.0
Glutamine	12.5
Hystidine	24.3
Arginine	60.0
Alanine	10.2
Proline	5.8

3.5. Dietary fibre analysis

Noteworthy is the dietary fibre content (12.3%) (Table 2). Dietary fibre (indigestible carbohydrate) is not a nutrient, but it still plays a very important role in maintaining good health (Anderson & Bridges, 1988; Johnson & Southgate, 1994; Kritchevsky, 1988). Diets rich in dietary fibre have been associated with beneficial

Table 5
Anion contents of kancolla seeds

Anions	ppm
Cl^-	474.0
F^-	34.7
NO_3^-	98.6
PO_4^{3-}	5099.0
SO_4^{2-}	763.0
$C_2O_4^{2-}$	1543.0

effects on human health and are sometimes considered to be useful for the prevention of obesity (Wisker, Daniel, & Felddheim, 1996).

3.6. Anion analysis

Oxalic acid ($C_2O_4^{2-}$) and PO_4^{3-} form water-soluble salts with Na^+, K^+ and NH_4^+ ions and also bind with Ca^{2+}, Fe^{2+} and Mg^{2+}, rendering these minerals biologically unavailable (Noonan & Savage, 1999). The anion content is shown in (Table 5).

3.7. Mineral analysis

Kancolla has higher levels of calcium (0.36%), potassium (0.96%), iron (108 ppm), and zinc (163 ppm) and a lower level of sodium (108 ppm) than wheat, barley, corn, and the other quinoa varieties (Table 6) (Konishi, Hirano, Tsuboi, & Wada, 2004). Calcium, iron and zinc bioavailabilities are low due to the high fibre content and low mineral/P ratio (Belitz & Grosh, 1999). A high potassium/sodium ratio makes kancolla interesting for diets with a defined electrolytic balance (Stamler, 1994). The high content of potassium can be utilized beneficially in the diets of people who take diuretics to control hypertension and suffer from excessive excretion of potassium. Minerals are also important as constituents of bones, teeth, soft tissues, haemoglobin, muscle, blood and nerve cells and are vital to overall mental and physical well being (MAFF, 1995; O'Dell & Sunde, 1997; Sardesai, 1998).

3.8. Free sugar analysis

Sugars are responsible for the sweetness of foods. Individual sugars possess different relative sweetness scores; fructose has been reported to be the sweetest sugar (1.1–1.8), followed by sucrose (1.0) and glucose (0.5–0.8) (Alexander, 1998). Therefore the yields of free sugars, such as glucose (2.93%), fructose (0.30%), and sucrose (1.85%) (Table 7) were also of importance.

Table 7
Composition of soluble sugars in kancolla seeds

Carbohydrate	g %
Sucrose	1.85
Glucose	2.93
Fructose	0.30

3.9. Isolation and identification of phytoecdysteroids

3.9.1. Compound *1*

The ESI–MS mass spectrum of compound **1** exhibited a quasi-molecular ion peak at *m/z* 479 $[M - H]^-$, indicating the molecular formula $C_{27}H_{44}O_7$, in accordance with ^{13}C NMR and ^{13}C DEPT NMR data. Its ^{13}C NMR spectrum contained 27 peaks (Table 1) and showed the presence of five tertiary methyls, while the ^1H NMR spectrum revealed three methine protons attached to two oxygen-bearing carbons and one proton attached to one double bond. These facts indicated that compound **1** was 20-hydroxyecdysone (Fig. 1) confirmed by 2D NMR (HMQC and HMBC) spectra (Table 1) and by comparison with literature data Zhu et al. (2001). It is the most common ecdysteroid found in the plant kingdom (Dinan, 2001).

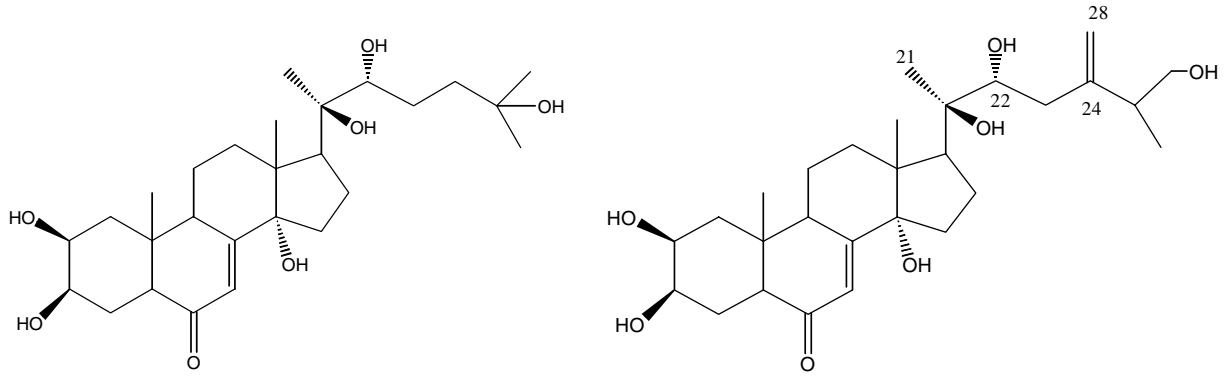
3.9.2. Compound *2*

This was obtained as an amorphous powder and gave a quasi-molecular ion peak at *m/z* 491$[M - H]^-$ in the ESI–MS spectrum, indicating the molecular formula $C_{28}H_{44}O_7$, in accordance with ^{13}C NMR and ^{13}C DEPT NMR data. The IR spectrum showed strong absorption of hydroxyl groups at 3426 cm^{-1} and carbonyl group at 1658. The ^1H-spectrum of **2** showed the presence of three tertiary methyl groups [δ 1.22 (3H, s, H-18), 1.06 (3H, s, H-19),1.58 (3H, s, H-21)], one secondary methyl group [δ 1.36 (3H, s, H-27)], three oxygenated methine protons [δ 4.16 (1H, ddd, J = 12.0; 4.0; 3.2 Hz, H-2), 4.22 (1H, q, J = 2.9 Hz, H-3), 4.08 (1H, dd, J = 11.0;

Table 6
Comparison of the mineral content of kancolla seeds with barley, yellow corn, wheat and other quinoa varieties (quinoa data are based on the average of 15 cultivars)

Crop	Ca (%)	P (%)	Mg (%)	K (%)	Na (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
Kancolla	0.36	N.D.	0.19	0.96	108	108	6.5	N.D.	163
[a]Quinoa	0.19	0.47	0.26	0.87	115	205	67	128	50
[a]Barley	0.08	0.42	0.12	0.56	200	50	8	16	15
[a]Corn	0.07	0.36	0.14	0.39	900	21	–	–	–
[a]Wheat	0.05	0.36	0.16	0.52	900	50	7	–	14

[a] E. Ballon (1987), personal communication, reported by Johnson (1990).



1: 20-Hydroxyecdysone **2:** Kancollosterone

Fig. 1. Structures of compounds: **1** and **2**.

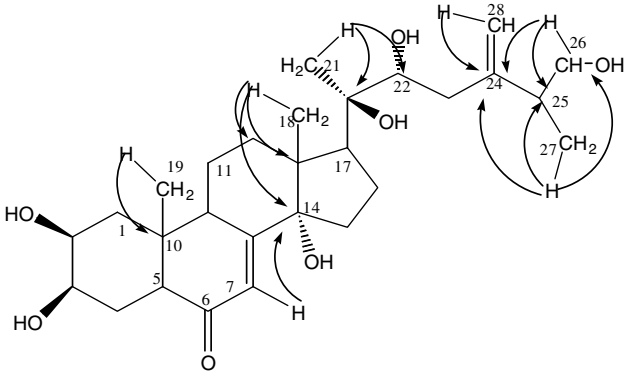


Fig. 2. Selected HMBC correlations in compound **2**.

1.7 Hz, H-22)], one oxygenated methylene proton [δ 3.86 (2H, m, H-26)], two methylene olefinic protons [δ 5.65 (1H, d, $J = 1.5$ Hz, H-28), 5.27 (1H, d, $J = 1.5$ Hz, H-28)], and a trisubstituted olefinic proton [δ 6.26 (1H, d, $J = 2.6$ Hz, H-7)]. The ^{13}C NMR (DEPT) spectrum (Table 1) of **2** contained 21 signals (C × 7, CH × 8, CH_2 × 9, CH_3 × 4). NMR spectra of **2** were similar to those of 24 (28)-dehydromakisterone A (Zhu et al., 2001) except for the absence of one CH_3 signal and the presence of one CH_2OH signal (Table 1). All proton resonances were correlated with those of their corresponding carbons from the HMQC experiment. The sequential arrangement of ecdysteroid moiety was deduced from the HMBC spectra. Key correlation HMBC cross-peaks were detected between the methyl proton (H-27) signal at δ 1.36 (3H, s) with signals at δ 150.2 (C-24), δ 42.7 (C-25) and δ 69.6 (C-26) and the methylene proton (H-26) signal at δ 3.86 (2H, m) with signals at δ 150.2 (C-24) and δ 42.7 (C-25). From all the evidence mentioned above, the structure of **2** was deduced to be 2β, 3β, 14α, 20R, 22R, 26-hexahydroxy-5 β-ergost-7,24(28)-dien-6-one and was named *Kancollosterone* (Fig. 2).

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