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Quantitative Analytical Method Development for the Assessment of Bioactive Quinic Acid-type Esters and Free Quinic Acid in Dietary Supplements

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Abstract

In 1998 a general chemical class of bioactive ingredients were defined as carboxy alkyl esters (CAEs). They were identified mainly in Uncaria tomentosa (Cats Claw) water extracts such as AC-11. However, one primary bioactive CAE was identified in AC-11 as a bioactive quinic acid ester (QAE). Quinic acid in turn is a key metabolite in plants responsible for initiating most aromatic plant biosynthesis including tryptophan, phenylalanine and tyrosine via the shikimate pathway active in the human gastrointestinal (GI) tract. These metabolic facts have inspired us to search beyond Cat's Claw water extracts into other natural products including nutraceuticals. Here we report on estimating CAEs/QAEs and free quinic acid by several methods including a colormetric procedure (Bartos method), UV detection method validated against the Bartos reaction of esters, and by HPLC (high pressure liquid chromatography). All the various methods yielded about the same data which was between about 1.8 % - 4.6 % QAEs (avg 3.8 %). HPLC allowed the analysis of QAEs/CAEs and free QA as well, and both were found present in AC-11 extracts. Moreover, AIO a concentrated fruit puree also had QAEs and free QA, but at much lower levels than those found in AC-11, being only an avg of 28.9 % for QAEs (i.e.1.1 %/3.8 %) and an avg. of 24.1% (i.e. 0.7%/2.8%) for free QA that were found in AC-11. These data have confirmed our earlier studies collected over the years. In addition, they have also demonstrated the increased power of applying analytical HPLC to quantifying these type of compounds in crude natural product extracts.

Keywords: Free quinic acid, quinic acid esters, carboxy alkyl esters, dietary supplements AC-11, AIO, and HPLC

Introduction

Quinic acid (QA) is one of the most important metabolites found exclusively in plants. It is responsible for biosynthesis of all the aromatic amino acids tryptophan, phenylalanine and tyrosine found in nature via the shkimate pathway (1,2). This pathway does not exist in animals and humans, and thus the exogenous supply of aromatic amino acids are essential to life of animals. Rarely appreciated in earlier studies is the fact that gastrointestinal (GI) microflora are efficient metabolizers of QA to hippuric acid or aromatic amino acids (3). So, each individual has indeed a personal essential amino acid factory, that although outside the body can in fact make critical metabolites normally not available to human metabolism, become readily available via GI tract microflora metabolism.

Recently we have surveyed well known healthy food sources and found QA widely distributed and available in healthy diets at efficacious levels. Brightly colored foods (i.e. reds, oranges, greens, yellows) such as vegetables and fruits which are also well known to be exceptionally healthy food sources have efficacious levels of QA present (i.e. about 0.5% w/w per daily food consumption); for example, such as prune, kiwi, sea buckthorn, coffee, cranberry, lingonberry, blueberry, wortleberry, red/yellow tamarillo, sultana, quince, sunflower, nectarine, peach, pear, plum, honey, black currant, medlar, apricot, asparagus, mushroom and green olive (4-12).

The widespread occurrence of QA in combination with the delineation of QA as having many biological dietary properties leading to anti-aging properties such as DNA repair enhancement, anti-inflammation, immune function enhancement, anti-oxidation and neurogenic effects (13-16), has been a strong motivation to search in a variety of foodstuffs and natural nutraceutical products for the occurrence of CAEs, QAEs and QA. In order to accomplish this research goal we have developed a battery of new analytical procedures to be able to accomplish this research goal.

Materials and Methods

Sample preparation: AC-11 samples were Lot # 280809.1785 and AIO samples were Batch # 0129E1. AC-11 was a spray dried powder having a recommended daily dose of 250-350 mg/day, and AIO was a liquid concentrate having a daily dose recommendation of 2 to 4 fluid ounces per day. These products were compared directly by all the analytical procedures described herein. AC-11 and AIO were primarily soluble in water but could be additionally cleared of any particulate matter by increasing the ethanol content to above > 90% before analysis of CAES/QAEs when carrying out either the Bartos or UV methods. However, AIO was formulated already as a fruit puree liquid having more particulate matter in it, and there were also lower amounts of natural occurring CAEs/QAEs present. Hence, for AC-11 or AIO HPLC analysis of samples were routinely first centrifuged at 10,000 – 20,000 × G for 10 min to remove any particulate matter still found in the water samples.

Dry weight of AIO: To determine the dry weight of AIO, the product was aliquoted in 1.5 ml eppendorf tubes or 50 ml falcon tubes and air-dried for three weeks in a fume hood, or until all water had evaporated and weight remained constant. The dry

weight of AIO was determined to be 17.0 ± 0.4 % (w/w in a 95 % confidence interval). The density of AIO was determined to be 1.06 g/ml. This means that liquid AIO contains 180 mg/ml of the dry weight.

Unhydrolyzed AC-11: New procedures were developed in order to be able to subject the AIO and AC-11 products to comparable quantitative analysis procedures for QA related compounds. One of these procedures found to be particularly useful in clean-up and quantitative analyses was base hydrolysis presented here below. Hence we define here as 'unhydrolyzed' AC-11 to be that not yet exposed to 1M NaOH, and still in the form it was harvested from natural sources. The dry AC-11 was dissolved in water at 100 mg/ml for several hours in a 10 ml pyrex tube. The tube was centrifuged at 2000 × G for 10 minutes and the supernatant collected. This solution was diluted further in water for HPLC analysis or clean-up on ion exchange resin prior to HPLC. For Bartos reaction, the 100 mg/ml water extract was diluted to 2.0 , 4.0 and 10.0 mg/ml in 99.7 % ethanol. This sample was again centrifuged at 2000 × G for 10 minutes to remove precipitated material. The supernatant was collected and used for Bartos reaction. For UV-absorption the samples were diluted further to 25-200 μg/ml.

Hydrolyzed AC-11: The dry AC-11 was hydrolyzed in 1 M NaOH at a concentration of 100 mg/ml for 1-3 hours. The sample was then treated in the same way as unhydrolyzed AC-11. None of the analytical testing required neutralization of the NaOH before initiating the procedures.

Unhydrolyzed AIO: The liquid AIO product was centrifuged at 18 000 × G for 10 minutes. The supernatant was collected and diluted to 30 mg/ml and centrifuged again. The supernatant was collected and diluted further for direct HPLC analysis or clean-up on ion exchange resin prior to HPLC. For Bartos reaction, the AIO liquid product was diluted 1:1 in water (90 mg/ml) and extracted for several hours. This solution was centrifuged at 2000 × G for 10 minutes and the supernatant diluted to 2.0, 4.0 and 10.0 mg/ml in 99.7 % ethanol. This sample was again centrifuged at 2000 × G for 10 minutes to remove precipitated material. The supernatant was collected and used for Bartos reaction. For UV-absorption the samples were diluted further to 85-340 μg/ml.

Hydrolyzed AIO: Liquid AIO was hydrolyzed for 1-3 hours by diluting 1:1 in 2 M NaOH, thus giving a concentration of 1 M NaOH. The sample was centrifuged at 2000 × G for 10 minutes, supernatant collected, centrifuged again at 18000 × G for 10 minutes and the supernatant collected. This sample contained 90 mg/ml and was diluted further for HPLC analysis. For Bartos reaction and UV-absorption the water hydrolyzed samples were treated the same way as the the unhydrolyzed AIO was by diluted to > 90% with ethanol.

Bartos ester-reaction method: Ester determination in water soluble extracts of AC-11 and AIO were made by a method developed by Bartos (17). Briefly, it consists of

reacting the ester with hydroxylamine to produce a hydroxamic acid, which can be treated with a Iron(III) solution, producing a chromophore if esters are present with an absorption maxima around 505-520nm. Chlorogenic acid could not be analyzed by this method because it produces an interfering yellow color already upon addition of hydroxylamine solution.

The hydroxylamine solution was prepared the following way: 5.0 ml of 10% solution of hydroxylamine hydrochloride in methanol was neutralized (pH >8) with approximately 3 ml 10% solution of sodium hydroxide in methanol. Another 10.0 ml sodium hydroxide solution was added and the solution was filtered through a Munktell filter. This solution was always prepared fresh.

A 0.3 % solution of ferric chloride hexahydrate in 3 % v/v solution of 70% perchloric acid in ethanol was also prepared and stored at room temperature until used.

To 200 µl sample in ethanol, 100 µl hydroxylamine solution was added and incubated at room temperature for 30 minutes. 600 µl ferric chloride solution was added, the samples vortexed and were let stand for 15 minutes. 200 µl of the solution was transferred to a 96-well flat-bottom microtiter plate and analyzed with a Molecular Devices SpectraMax 340PC microplate spectrophotometer at 505 and 520 nm.

AC-11 and AIO samples were measured against standards of Dioctyl Phthalate (DOP) and Quinic Acid Lactone (QAL) with maximum absorbance at 505 and 520 nm, respectively.

UV-absorbance method: One method to determine the amount of esters in AC-11 was by direct UV-absorbance, proposed by Sheng et al (18). Samples were analyzed with a Beckman DU530 Life science UV-VIS Spectrophotometer.

High Performance Liquid Chromatography (HPLC):
Analytical separations were done on a Genesis® AQ Reversed Phase column, especially suited for hydrophilic and polar compounds. Particle size: 4µm. Length: 50 mm. Internal Diameter: 4 mm. The mobile phase contained 100 % 100 mM phosphate buffer (pH 2.15) and was delivered with a HP 1050 Series Pumping Symstem with an online ERC-3415 Degasser. The flow rate was 1.0 ml/min. Samples were injected with a HP 1100 Series Thermostatted Autosampler. The injection volume was 10 µl. Detection was done with a HP 1100 Series Diode Array Detector at 215 nm. Quinic Acid (QA) had a retention time of 0.95-0.98 minutes.

Sample-cleanup on Ion Exchange Resin for HPLC analyses: Preliminary HPLC analysis showed that AC-11 contained both QA and QAEs, and in AIO the HPLC chromatograms had so much background in the QA retention time area that no clear QA elution peak could be established. As a result, these early data demanded the development of a clean-up procedure that would permit the quantitative determination of both QA, QAEs and the total QA in AC-11, AIO or AC-11 + AIO mixtures. This has been accomplished as presented here. A batch clean-up method was developed for water soluble extracts of AC-11 and AIO. Water-extracts of unhydrolyzed or

hydrolyzed AC-11 and AIO were diluted to 30.0 mg/ml and 9.0 mg/ml respectively for preliminary clean-up. 10.0 ml of this sample was absorbed on 3.0 – 5.0 g BioRad AG 501-X8 Mixed Bed Resin. This resin consists of equivalent amounts of AG 50W-X8 strong cation exchange resin (H+ form) and AG 1-X8 strong anion exchange resin (OH- form). Unabsorbed substances were filtered off with a Munktell filter. The resin was recollected and eluted in 10.0-15.0 ml 4 M acetic acid to elute the quinic acid. The eluate was filtered off and the resin recollected for two more elutions with equal amounts of 4 M acetic acid. Three such fractions were collected for every sample and analyzed separately by direct injection on the HPLC system. Recovery of Quinic Acid was estimated by spiking samples of AC-11 and AIO with with 1.0-2.0 mg/ml Quinic Acid standard prior to clean-up.

Results

Peer-reviewed studies have been published that document the presence of CAEs such as quinic acid esters in water extracts of Uncaria tomentosa bark (see reviews by Pero ref. 15, 16). The primary bioactive components attributed to the antiaging properties of AC-11 , a water soluble Uncaria extract, were identified first in 2000 as CAEs (18-20, 22 – 24), then QAEs (17, 21) and finally as QA (17,25). The main objective of this study was to confirm the previously published analytical data on CAEs and its related identified compounds found in AC-11. Secondarily, to improve the CAE-related analytical procedures so that they may be utilized more reliably, and with greater precision, when AC-11 is combined with other natural products such as the broad spectrum neutraceutical, AIO.

CAE determination by the Bartos reaction: Standards of DOP (0-4000 μg/ml) and QAL (0-2000 μg/ml) had absorbance maxima at 505 and 520 nm respectively. Absorbance of AC-11 and AIO samples were therefore measured at these wavelengths. The standard curves of DOP and QAL showed dose dependent behavior within the concentrations measured. There is an appendix for standard curve data upon request. The amount of esters in each product are summarized below in Table 1.

Table 1: Summary of results analyzed for ester structures only by the Bartos reaction. Values are given in a 95 % confidence interval. Here both carboxy alkyl esters (CAEs) and quinic acid esters (QAEs) were estimated against two quite different standard esters: namely dioctyl phthalate for CAES and quinic acid lactone for QAEs.

Commercial products and treatment	DOP (505 nm) CAEs estimated as diesters (% w/w)	DOP (505 nm) QAEs estimated as monoesters (% w/w)	QAL (520 nm) Can only be used to estimate QAEs (% w/w)
AC-11	7.80 ± 0.54	3.90 ± 0.27	4.14 ± 0.96

AC-11 (hydrolyzed >1 hr, 1M NaOH	0.66 ± 1.06	0.33 ± 0.53	-2.97 ± 2.57
AIO liquid	3.59 ± 0.62	1.80 ± 0.31	2.00 ± 0.20
AIO liquid (hydrolyzed >1 hr, 1M NaOH	0.14 ± 0.82	0.07 ± 0.41	0.03 ± 0.08

UV-method for determination of CAEs: The UV-method has previously been evaluated against the Bartos reaction and been shown to give reliable measures of the amount of esters in AC-11 (17). These data were replicated by the results summarized on DOP in Table 2. Moreover, when a natural occurring Cat's claw ester (chlorogenic acid) was used as standard to quantify the esters in AC-11 the amount of esters were almost identical to those found with the DOP standard as well (Table 2).

UV absorbance maxima for DOP and chlorogenic acid were 205 and 219 nm, respectively. Hence, standard curves of DOP and chlorogenic acid could be easily generated at these wavelengths, and were determined to have a dose response relationship in the range used (0-20.0 µg/ml), thus both standards permitting a reliable estimate of QAEs in AC-11.

By comparison to the data generated by the Bartos reaction shown in Table 1, it was found that the UV method yielded a reliable surrogate estimate of CAEs or QAEs in AC-11 but not AIO, presumably due to the AIO color and/or unrelated background UV absorption in this product. However, both DOP and chlorogenic acid gave reliable calculation of CAEs by the UV method standardized to the DOP molecule by by correcting the UV absorption to equal 2.5 double bonds per ester linkage. This meant that DOP could be used to quantify CAEs containing 2 esters or one ester by dividing by 2. Although chlorogenic acid had only one ester linkage if the UV absorption was corrected to equal 2.5 double bonds per ester linkage instead of 5 by dividing by 2 then identical data were determined. This UV model was strongly supported by the fact, that QAL another model ester of quinic acid, could not be used by this UV method. Here the fact is there are no double bonds in QAL to be conjugated to the ester.

Table 2: Ester determination by UV-method against standards of dioctyl phthalate (DOP) and chlorogenic acid using AC-11 samples of 25-200 µg/ml and standard curve calculations between 0-20 µg/ml . Values are calculated as % (w/w) of the DOP or CA standard in AC-11, and given within 95% confidence intervals. In addition, the data are adjusted so that different standards can be directly compared to the model UV absorbing ester, DOP, by adjusting the UV absorption to equal 2.5 double bonds per each UV conjugated ester linkage.

Natural-occurring esters in AC-11 (Wt DOP or Chloro. Ac./ Wt AC- x 100 = % CAEs or QAEs	Diesters (CAEs)	Monoesters (QAEs)

Analytical standard		
Dioctyl phthalate (DOP) UV=205nm	9.0 ± 0.3	4.5 ± 0.2
Chlorogenic Acid (Chloro. Ac.) UV=219nm		4.6 ± 0.6

Determination of QA and QAEs in AC-11 and AIO using HPLC. The system robustness of HPLC technology for analysis of QA in natural products was evaluated by three serial injections of 1.0 mg/ml Quinic Acid standard. The relative standard error was only 0.50 % for peak height, suggesting that the system was very reproducible and accurate.

A standard curve of QA was produced in the range of 100-2000 μg/ml, showing a clear dose response relationship. Detection limits by this technique were approximately 100 μg/ml.

Direct injections of untreated extracts of AC-11 and AIO were done at concentrations of 5.0 mg/ml. For AC-11, a quinic acid peak was detected and identified. These preliminary data suggested that unhydrolyzed and hydrolyzed samples contained approximately 2.7% and 3.3% quinic acid respectively. No quinic acid peak could be identified in the untreated water extract of AIO (not cleaned on resin), likely due to the high background of UV absorbing materials present in AIO samples. The high content of UV absorbing materials in AIO caused interference because the HPLC was equipped with a UV detector for identification of HPLC analyzed compounds.

In order to improve on the quantitative determination of QA-type compounds by HPLC it was necessary to carry out a preliminary clean-up step of the neutraceutical samples by absorbing/desorbing them on an appropriate ion exchange resin that could selectively remove organic acids such as QA, but allow other types of structures not adsorbing to be separated. CAEs and QAEs are examples of compounds that do not absorb on ion exchange resins, and so QAEs can also be separated from QA using this type clean-up procedure in combination with ± base hydrolysis by 1M NaOH.

Unhydrolyzed and hydrolyzed samples of AC-11 could be satisfactorily cleaned at concentrations of 30.0 mg/ml without overloading the resin. Quinic acid eluted successfully by treatment with 4 M acetic acid and could be identified above background in three fractions collected. Recovery of quinic acid, determined by spiked AC-11 samples, was 111% and 95% for unhydrolyzed and hydrolyzed AC-11 respectively.

The concentration of AIO allowing successful clean-up without overloading the resin was 9.0 mg/ml. At this concentration, quinic acid could only be quantified above background in the first fraction. In the spiked sample, quinic acid eluted in two fractions and recovery was 108 % for both unhydrolyzed and hydrolyzed AIO.

By subtracting the amount of QA in hydrolyzed from unhydrolyzed sample, the amount of QA stored in QAE could be estimated. The analytical values determined by HPLC for QAEs and QA that were found in AC-11 and AIO have been estimated by using the molecular weight of chlorogenic acid as the generic QA ester candidate. The data are presented in Table 3. hromatograms produced are for

In addition, an example of the quality of the HPLC chromatograms being produced is shown in Figure 1.

Table 3: Determination of Quinic Acid (QA) in AC-11 and AIO. QAEs are estimated as the difference between the free QA (Naturally occuring in product) and total QA using the molecular weight of Chlorogenic Acid (Chloro. Ac.) as the model for the average molecular size of the unknown QAEs present in the natural products being analyzed.

HPLC analysis of AC-11 and AIO for QA ± 1M NaOH treatment (Wt QA or chloro. Ac./ Wt of AC-11 or AIO)	AC-11	AIO
	% (w/w)	% (w/w)
Free QA (Naturally occuring in product)	2.9%	0.7%
Total QA (>1hr in 1M NaOH)	3.8%	0.8%
QAE (Total QA – free QA)	1.8%	0.2%

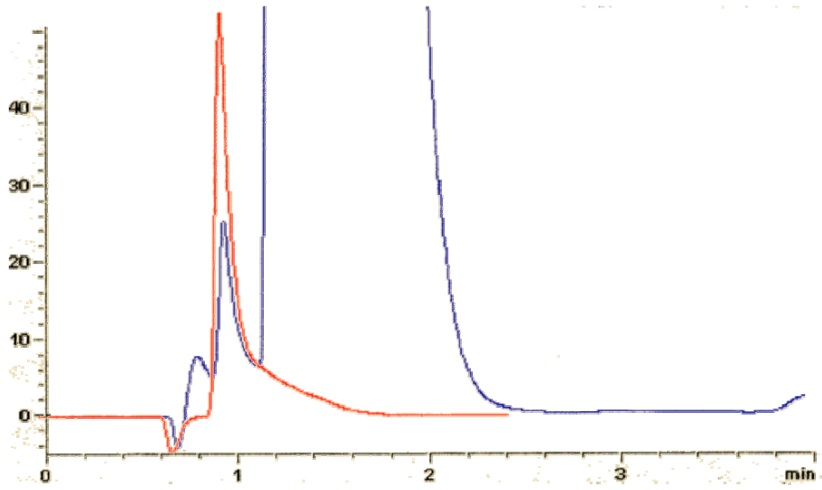

Figure 1: Overlay of chromatograms of eluate of AC-11 from BioRad AG 501-X8 Mixed Bed ion-exchange resin clean-up (blue) and standard of Quinic Acid at a concentration of 1600µg/ml (red). The large peak eluting right after the quinic acid in the eluate chromatogram is acetic acid, which was used for elution.

Discussion

An overall summary for the chemical procedures developed, improved and used for data colletion is this study are presented in Table 4. First of all it is important to understand what inspired such an effort. It is based on the fact that hippuric acid has no known function other than to facilitate excretion of benzoid-type of compounds. One natural source, and one major need is to excrete dietary quinic acid, so that benzoid-type toxic metabolites do not have the possibility accumulate and cause

health issues. However, in the early 2000's (13-15, 17-25) quinic acid and its esters (QAEs) were shown to have potent biological activity. As such it was quantified for marketing purposes as a dietary supplement, and sold as AC-11 where the active ingredients were standardized as CAEs (carboxy alkyl esters). CAEs are a generic way to characterize an active ingredient containing a QAEs, even though only half of the ester structure is known to be quinic acid. Hence, it was desirable to be able to determine by several prodecures exactly how much of the biological activity observed could be accounted by quinic acid or QAEs. For this purpose, we have developed the methods outlined colorimetric techniques, and by comparison to standard substrates that represent the portion, of CAEs/QAEs we have chemically identified. These standard substrates were qunic acid (QA), qunic acid lactone(QAL), Dioctyl Pthalate (DOP), and chlorogenic acid (Chloro.Ac.). The following analytical conclusions can be made for the data presented herein for the AC-11 and AIO dietary supplements:

1. AC-11 contains about 1.8 to 4.6 % (avg. 3.8%) QA-type esters. AIO contains about 0.2 to 2.0 % (avg. 1.1 %) QA-type esters (Table 4).
2. AC-11 contains about 2.9 % free, unesterified QA, and AIO contains 0.7 % free, unesterified QA (Table 3).
3. Hence, AC-11 contains 2.9/0.7 or 4.14 times more free QA than AIO does. Likewise, AC-11 contains 1.8 %/ 0.2 % or 9.0 times more QA-type esters than AIO does (Table 3).
4. AC-11 has 1.8 % of its total 3.8 % of QA present in the form of QA-type esters or about 47.4% of the total QA (i.e. 1.8/3.8% = 47.4%), and 76.3 % as free, unesterified QA (i.e. 2.9 / 3.8 = 76.3%).
5. AIO has 0.2 % of its total 0.8% QA present in the form of QA-type esters or about 25 % of its total QA (0.2/0.08 % = 25 %), and 87.8% as free QA (0.7/0.8% = 87.8%) (Table 3).
6. AIO or AC-11 could not be analyzed by HPLC without a preliminary clean up procedure using ion exchange resin chromatograhy for determination of pure, unesterified QA.

Table 4. Overall analytical summary for the determination of quinic acid and its analogs in AIO and AC-11 using the same natural extract samples but a variety of different chemical procedures. Data are from Tables 1-3 and procedures from Materials and Methods.

	Data in % QAEs or QA (w/w)	
Chemical procedure	Unhydrolyzed sample	Hydrolyzed sample
AC-11 extract application		
DOP colormetric procedure	3.90	0.33
QAL colormetric procedure	4.14	-2.97

UV detection procedure		
DOP substrate	4.50	-
Chloro. Ac.substrate	4.60	-
HPLC detection procedure		
Free QA (natural Occurring in product)	2.9	
Total QA	-	3.8
QAE (Total-free QA)	1.8	
AIO extract application		
DOP colormetric procedure	1.80	0.07
QAL colormetric procedure	2.00	0.03
HPLC detection procedure		
Free QA (natural Occurring in product)	0.7	
Total QA	-	0.8
QAE (Total-free QA)	0.2	

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Literature Cited

[1] Herrmann, KM. 1995. The shikimate pathway. Early steps in the biosynthesis of aromatic compouinds. The Plant Cell 7: 907-919.

[2] Herrmann KM Weaver LM. 1993. The shikimate pathway. Annual Review of Plant Physiology and Plant Molecular Biology 50: 473-503.

[3] Adamson RH, Bridges JW, Evans ME Williams, RT. 1970. Species differences in the aromatization of quinic acid in vivo and the role of gut bacteria. Biochemical Jour 116: 437-433.

[4] Mourgue, M, Lanet, J, Blanc A, Steinmetz, MD.1975.Quinic acid and ioschlorogenic acids in sunflower seeds (Helianthus annus Lin.). C R Seances Soc Biol Fil 169(5): 1256-1259.

[5] Englehardt, UH, Maier, HG. (1985). Acids in coffee. The proportion of individual acids in the total titratable acid. Z Lebensm Unters Forsch 18(1): 20-23

[6] Graham, HN. 1992. Green tea composition, consumption and polyphenol chemistry. Pre Med 21: 334-350.

[7] Romero Rodriguez MA, Vazquez Oderiz ML, Lopez Hernandez J, Simal Lozano J. 1992. Determination of vitamin C and organic acids in various fruits by HPLC. J Chromatogr Sci. Nov;30(11):433-7.

[8] Van Gorsel, H, Li, C, Kerbel, EL, Smits, M, Kadar, AA. (1992).Composition characterization of prune juice. J Agric Food Chem 40: 784-789.

[9] Lewis, J, Milligan, G, Hunt, A. 1995. NUTTAB95 Nutrient Data Table for Use in Australia. Canberra: Food Standards Australia New Zealand (FRANZ). Organic acid components of foods (g/100g edible portion). ORGAFOOD.TXT, COFA index number 2.

[10] Beveridge, T, Li, TSC, Oomah, BD, Smith, A.(1999). Sea buckthorn products: manufacture and composition. J Agri Food Chem 47: 3480-3488.

[11] Jensen, HD, Krogfelt, KA, Cornett, C, Hansen, SH, Cristensen, SB. (2002). Hydrophilic carboxylic acids and iridoid glycosides in the juice of American and European cranberries (Vaccinium macrocarpon and V. Oxycoccos), lingonberries (V. vitis-idaea) and blueberries (V. myrtillus). J Agri Food Chem 50(23): 6871-6874.

[12] Silva, BM, Andrade, PB, Mendes, GC, Seabra, RM, Ferreira, MA. 2002. Study of the organic acids composition of quince (Cydonia oblonga Miller) fruit and jam. J Agric Food Chem 50(8) : 2313-2317.

[13] Pero, RW, Lund, H, Leanderson, T. 2009a. Antioxidant metabolism induced by quinic acid. Increased urinary excretion of tryptophan and nicotinamide. Phytotherapy Research 23: 335-346

[14] Pero, RW, Lund, H. 2009b. DNA repair in serum correlates to clinical assessment of anti-aging lifestyle criteria in healthy volunteers treated with quinic acid ammonia chelate (QuinmaxTM,)) in drinking water. Inter Jour of Biotech and Biochem 5 (3): 293–305

[15] Pero, RW. In : DNA Repair Damage, Repair Mechanisms and Aging. 2010a Editor: Allison E. Thomas. Historical Development of Uncaria Preparations and their Related Bioactive Components. Chapter 9. Novascience (ISBN 978-1-61668-914-8). pp. (in press)

[16] Pero, RW. 2010b. Health Consequences of Catabolic Synthesis of Hippuric Acid in Humans. Current Clinical Pharmacology 5: 67-73.

[17] Bartos, J. 1980. Colorimetric determination of organic compounds by formation of hydroxamic acids. Talanta, Vol 27: 583-590

[18] Sheng, Y, Åkesson, C, Holmgren, K, Bryngelsson, C, Giampapa,V, Pero, RW. 2005. An active ingredient of Cat's Claw water extracts. Identification and efficacy of quinic acid. Journal of Ethanopharmacology 96(3): 577-584

[19] Sheng Y Bryngelsson C Pero RW. 2000A. Enhanced DNA repair, immune function and reduced toxicity of C-Med-100^{TM} , a novel aqueous extract from Uncaria tomentos. Journal of Ethanopharmacology 69: 115-126.

[20] Sheng Y Pero RW Wagner H. 2000B. Treatment of chemotherapy-induced leukopenia in the rat model with aqueous extract from Uncaria Tomentosa. Phytomedicine 7(2): 137-143.

[21] Sheng Y Li L Holmgren K Pero RW. 2001. DNA repair enhancement of aqueous extracts of Uncaria Tomentosa in a human volunteer study. Phytomedicine 8(4):275-282.

[22] Mammone T, Akesson C, Gan D, Giampapa V, Pero RW. 2006. A water soluble extract from Uncaria tomentosa (Cat's claw) is a potent enhancer of DNA repair in primary organ cultures of human skin. Phytotherapy Res 20: 178-183.

[23] Åkesson C Pero RW Ivars F. 2003A. C-Med-100, a hot water extract of Uncaria tomentosa, prolongs leukocyte survival in vivo. Phytomedicine 10: 25-33, 2003A

[24] Åkesson C Lindgren H Pero RW Leanderson T Ivars F. 2003B. An extract of Uncaria Tomentosa inhibits cell division and NF-kB activity without inducing cell death. International Immunopharmacology 3: 1889-1900.

[25] Åkesson C Lindgren H Pero RW Leanderson T Ivars F. 2005. Quinic acid is a biologically active component of the Uncaria tomentosa extract C-Med 100®. International Immunopharmacology 5: 219-22.