European Journal of Medicinal Plants
17(2): 1-7, 2016; Article no.EJMP.30144
ISSN: 2231-0894, NLM ID: 101583475

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Investigation of the Purity of Ursolic Acid Dietary Supplements

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Authors' contributions

This work was carried out in collaboration between both authors. Author SLU designed the study, performed the statistical analysis, revised the manuscript. Author ALS performed all experiments, analyzed the data and wrote the first draft of the manuscript. Authors SLU and ALS wrote all protocols and managed literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/30144
Editor(s):
(1) Shanfa Lu, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Foluso O. Osunsanmi, University of Zululand, South Africa.
(2) Łukasz Woźniak, Institute of Agricultural and Food Biotechnology, Poland.
(3) Mthokozisi Simelane, University of Zululand, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/17232

Short Research Article

Received 21st October 2016
Accepted 8th December 2016
Published 14th December 2016

ABSTRACT

Aims: Ursolic acid (UA) is a phytochemical found in fruits and herbs such as apples, cherries, rosemary and thyme. A multitude of rodent studies have shown UA's protective effects in cancer, cardiovascular disease, diabetes, obesity, and neurodegenerative diseases. This study aims to investigate if a high purity UA dietary supplement is available for use in future human studies.
Place and Duration of Study: Theoretical weight studies were performed at the University of Texas at San Antonio while UA quantification studies were performed in the Metabolomics Laboratory at The University of Texas Health Science Center at San Antonio between January 2015 and July 2015.
Methodology: Four oral UA supplements were analyzed for percent theoretical weight and UA concentration. UA supplement content was analyzed by high performance liquid chromatography–electrospray ionization mass spectrometry (HPLC-ESI-MS) and the UA concentration was calculated based on a standard curve. Additional high abundance compounds were identified using HPLC-ESI-MS.

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Results: All UA supplement capsules averaged over 200% theoretical weight. The UA concentration in the supplements ranged from 14-49% and 12 additional compounds were identified.
Conclusion: UA supplements provided more than 200% the stated quantity listed on the Supplement Facts Panel. However, UA supplements contained less than 50% UA and additional compounds identified were constituents of plants or commonly found in food products. A UA supplement with higher purity is needed to investigate its therapeutic effects in humans.

Keywords: Ursolic acid; supplement; quantification; purity.

1. INTRODUCTION

Ursolic acid (UA) is a phytochemical found in fruits and herbs such as apples, blueberries, cranberries, thyme, and rosemary and in many traditional herbal medicines [1]. UA's documented anti-oxidative, anti-inflammatory, and anticancer activities has led to an increase interest in studying the use of UA for the prevention and management of chronic diseases [1]. UA supplementation studies have demonstrated protective effects against obesity, heart disease, and diabetes through various metabolic effects and mechanisms of action resulting in reduced inflammation, oxidative stress, blood glucose, and blood cholesterol [2,3]. In addition, several studies have shown positive health benefits of UA in the setting of cancer, neurodegenerative, kidney, and liver diseases [4-7]. However, UA's potential for preventing and treating human diseases has not been thoroughly investigated, as most UA studies have been conducted using cell or rodent models of disease.

UA's documented beneficial health effects have led to an increase interest in its dietary supplementation, which primarily has been marketed to sell body-building and weight-loss supplements. Dietary supplements are regulated by Food and Drug Administration (FDA) as mandated in the Dietary Supplement Health and Education Act (DSHEA) of 1994. DSHEA defines dietary supplements, requires good manufacturing practices (GMP) to ensure quality and safety, and states a dietary supplement cannot be adulterated or misbranded. Based on DSHEA, dietary supplement manufacturers must ensure purity, strength, composition, and accurate labeling prior to release on to the market. Furthermore, since the DSHEA does not require the lengthy premarket evaluation used by the FDA for pharmacologic drugs, this calls into question the purity, effectiveness, and safety of many dietary supplements currently on the market. According to the FDA, weight-loss, body-building, and sexual enhancement dietary supplements are more likely to be tainted with pharmaceutical ingredients. Therefore, it is imperative to validate the concentration and purity of UA supplements prior to conducting human studies to attribute any beneficial effects to UA. Currently, there are three main suppliers of four unique UA supplements that can be purchased in capsule or powder form. The objective of this study was to determine the percent theoretical weight and UA concentration and identify non-UA compounds in commercially available UA dietary supplements.

2. MATERIALS AND METHODS

2.1 UA Supplements

Commercially available UA supplements were purchased from companies offering UA dietary supplements in capsule or powder form for purchase online. Hard shell capsule supplements will be referred to as dietary supplement 1-3 (DS1-3). Ursolic acid (≥90% purity as measured by the manufacturer) available for research use only was purchased from Sigma-Aldrich in powder form. UA in DS1-3 was extracted from rosemary and the powder was extracted from Loquat. Excipient ingredients for each supplement are listed: DS1 contains gelatin, microcrystalline cellulose, magnesium stearate, silicon dioxide, titanium dioxide, FD&C blue #1, FD&C red #3, FD&C red #40, FD&C yellow #6 and water; DS2 contains gelatin, microcrystalline cellulose, modified corn starch, silica, magnesium stearate; DS3 contains maltodextrin, gelatin, silica, magnesium stearate, titanium dioxide, sodium copper chlorophylin.

2.2 Percent Theoretical Weight

Three random UA capsules for DS1-3 were individually weighed and the powder contents of each capsule was removed and weighed. The powder weight was recorded as actual weight.

The amount listed on the Supplement Facts Panel for each UA supplement contents was recorded as theoretical weight. Percent theoretical weight was calculated by dividing the actual weight in grams by the theoretical weight in grams and multiplying by 100.

2.3 UA Quantification

UA from Sigma-Aldrich was dissolved in methanol (MeOH) to a concentration of 10 mM. DS1-3 and the powder were not soluble in MeOH and therefore were dissolved in dimethyl sulfoxide (DMSO) which required sonication for up to 10 min at 37℃. Samples dissolved in DMSO were then diluted with MeOH to a concentration of 10 mM. High performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) analyses were conducted on a Thermo Fisher Q Exactive mass spectrometer with on-line separation by a Thermo Fisher/Dionex Ultimate 3000 HPLC. HPLC conditions were: column, Kinetex, 2.6 µm, 100 x 2.1 mm (Phenomenex; Torrance, CA); mobile phase A, 10mM ammonium acetate in water; mobile phase B, methanol; flow rate, 250 µL/min; gradient, 20% B to 95% B over 2 minutes and held at 95% B for 6 minutes. MS detection conditions were: spray voltage, 3.2 kV; capillary temperature, 300℃; sheath gas flow rate, 50; aux gas glow rate, 10; probe heater temperature, 200 ℃; S-lens RF level, 50. Data-dependent analyses were conducted using one full MS scan (70,000 resolution) followed by six tandem MS scans with electrospray negative ion detection. A standard curve was generated for UA using UA from Sigma-Aldrich. Quantitative results were obtained by reference of the experimental peak area ratios to the standard curve.

2.4 Identification of Non-UA Compounds

Progenesis CoMet (Nonlinear Dynamics) was used to process the raw data files to detect non-UA compounds that exhibit significant differences in intensity among the supplements. Small molecules with an abundance ≥ 1.0 x 10^6 were identified with accurate mass searching using a 10-ppm mass tolerance. Interpretation and confirmation of MS/MS fragment patterns were performed through searching Metlin databases (Script Center for Metabolomics).

2.5 Statistical Analysis

Data were analyzed using ANOVA for multi-group comparisons. ANOVA and post hoc analyses were performed using the Least Significant Difference method (IBM SPSS statistics). All data are presented as mean ± SD of at least three independent experiments unless stated otherwise. Results were considered statistically significant at the $P < 0.05$ level and actual P values are stated when applicable.

3. RESULTS AND DISCUSSION

3.1 Theoretical Weight Analysis

Theoretical and actual weights for DS1-3 are listed in Table 1. Percent theoretical weights for DS1-3 were all above 200% indicating the contents measured in each supplement are higher than the listed UA contents on the Supplement Facts Panel. This may be due to the excipient ingredients listed in section 2.1. According to DSHEA, the manufacturer is responsible for verifying the supplement contents and accuracy of labeling, however FDA does not substantiate this information prior to release on to the market.

3.2 HPLC-ESI-MS Analysis

Mass spectrometry is an extremely sensitive technique that is able to detect and identify compounds by their mass-to-charge ratio, tandem mass spectrometry (MS/MS) fragments, and retention time. UA was detected at its predicted mass-to-charge ratio of 455.3535 (Fig. 1).

Table 1. Ursolic acid supplement weight, actual weights, and % theoretical weights

Dietary supplement	Theoretical weight (g)	Actual weight (g)	% Theoretical weight
DS1	0.3	0.66 ± 0.015	221 ± 5.09*
DS2	0.2	0.41 ± 0.025	207 ± 12.6*
DS3	0.05	0.47 ± 0.000	904 ± 0.00*

*Values are the mean ± SD (n=3), *P<0.001 compared to a theoretical weight of 100%*

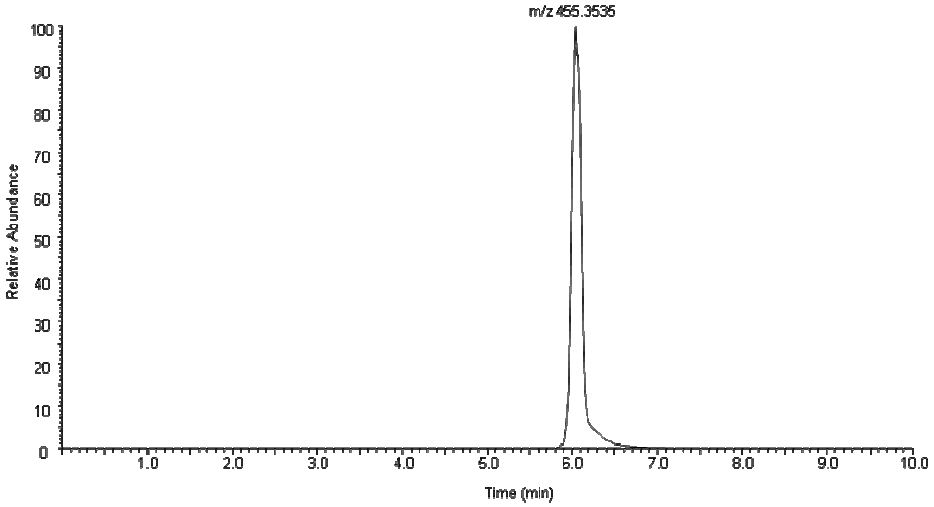

Fig. 1. Ursolic acid identification by HPLC-ESI-MS

Ursolic acid ($C_{30}H_{48}O_3$) was eluted at 6.04 mins at HPLC chromatography and detected in ESI negative mode with $[M-H]^-$ = 455.3535 (<5ppm mass error with the theoretical m/z of 455.3530)

Table 2. Ursolic acid supplement concentration

Dietary supplement	UA concentration (%)	% [UA] mass spectrometry/ % [UA] supplement
UA standard	94 ± 2.5	1.0
Powder	49 ± 3.4*	2.0
DS1	38 ± 2.6*	1.5
DS2	31 ± 1.5*	1.2
DS3	14 ± 1.3*	1.3

*UA concentration values are mean ± SD (n=3), *P<0.001 compared to the standard of 94%*

DS1-3 and the powder form contained less than 50% UA as analyzed by HPLC-ESI-MS and calculated using a standard curve with the UA standard (Table 2). All UA supplements provided more UA than indicated on the Supplements Fact Panel. For example, the powder states it contains 25% UA, while by our analysis it contains the highest amount of UA (49%) which is 2.0 times higher the estimated content of UA (Table 2).

Based on the data from the current study, there is not a high purity UA supplement available on the market as compared to UA purchased from a chemical company. Most studies that found positive health effects in rodents fed UA purchased from chemical companies, which are highly concentrated but not intended for human consumption. The chemical companies are not required to follow GMP for dietary supplements in order to be safe for human consumption and therefore may not have safe guards in place to prevent contamination from chemicals processed at their facilities. The only oral UA supplementation study conducted in humans resulted in a significant decrease in body fat and an increase in insulin-like growth factor-1 (IGF-1) and irisin among individuals undergoing resistance training and consuming UA supplements [8]. An analysis of the supplement purity is necessary to determine if these effects can be attributed solely to UA or if the other excipient compounds may provide benefits alone or synergistically with UA.

3.3 Non-UA Compounds

Mass spectrometry was used to detect and identify additional compounds present in the supplements by their unique mass-to-charge ratio, MS/MS fragments, and retention time. A total of 2,635 features (a feature is a specific m/z with its retention time) were detected by mass spectrometry analysis of all tested UA supplements (DS1-3, powder) and UA from Sigma-Aldrich as the standard. There were a total of 106 features with high abundance and 12

additional compounds were putatively identified (Table 3).

UA analogs, ursonic acid, corosolic acid, and feruloyleuscaphic acid, all belong to the triterpenoid family and have similar medicinal properties such as anti-oxidant and anti-inflammatory activities [9-12]. Ganoderiol, a triterpene identified, is a constituent of mushrooms and trees [13]. The identification of diterprenes carnosic acid and carnosol is not surprising since these compounds can be found in rosemary, which was used as the starting material to extract UA for DS1-3 supplements [14,15]. Palmitic acid, stearic acid, and hydroxy-9-triacontanone are saturated fatty acids found in animal and plant fats [16-18]. Foreign compounds included polysorbate 20 and citronellyl cinnamate. Polysorbate 20 is an emulsifier often used in pharmaceuticals and food to solubilize essential oils into water-based products [19]. Citronellyl cinnamate is often used in foods as food flavoring [20].

Limitations of the MS/MS analysis for non-UA compounds are the exclusion of less abundant compounds (mass abundance < 1.0×10^6), the limited MS and MS/MS database entries for small molecules, the lack of validation of the identified compounds with verified standards, and the limited solubility of the supplement contents. A major obstacle in identifying unknown compounds from natural products or synthesized drugs is the limited

Table 3. Putative identified compounds in ursolic acid supplements

Putative identified compounds	Chemical formula	Maximum abundance	Identified samples	Description
Ursolic acid	$C_{30}H_{48}O_3$	2.55×10^8	DS1-3 & Powder	Taraxastane, ursane, and bauerane triterpenoid found in various plants [12].
Carnosic acid	$C_{20}H_{28}O_4$	5.06×10^7	DS1-3 & Powder	Diterpenoid which is a major rosemary polyphenol [14].
Ursonic acid	$C_{30}H_{46}O_3$	4.60×10^7	DS1-3 & Powder	Taraxastane, ursane, and bauerane triterpenoid found in various plants [9].
Carnosol	$C_{20}H_{26}O_4$	3.65×10^7	DS1-3 & Powder	Phenolic diterpene lactone found in rosemary [15].
Palmitic acid	$C_{16}H_{32}O_2$	3.60×10^7	DS1-3 & Powder	Saturated fatty acid found in animal and vegetable fats and waxes [16].
Stearic acid	$C_{18}H_{36}O_2$	3.60×10^7	DS1-3 & Powder	Saturated fatty acid found in animal and vegetable fats [17].
3α-Corosolic acid	$C_{30}H_{48}O_4$	1.22×10^7	DS1-2 & Powder	Taraxastane, ursane, and bauerane triterpenoid found in herbs and spices [10].
Citronellyl cinnamate	$C_{19}H_{26}O_2$	9.09×10^6	DS1-3 & Powder	Commonly used in food flavoring [20].
Ganoderiol I	$C_{31}H_{50}O_5$	7.70×10^6	DS1-2 & Powder	Tirucallane and euphane triterpenoid found in mushrooms such as Ganoderma lucidum (reishi) [13].
3-O-trans-Feruloyleuscaphic acid	$C_{40}H_{56}O_8$	3.12×10^6	Powder	Taraxastane, ursane, and bauerane triterpenoid found in loquat fruit [11].
Polysorbate 20	$C_{26}H_{50}O_{10}$	2.07×10^6	DS1-2 & Powder	Emulsifier used in pharmaceuticals and food preparation [19].
(+)-11-Hydroxy-9-triacontanone	$C_{30}H_{60}O_2$	1.94×10^6	DS2 & Powder	Saturated fatty acid alcohol found in fruits, herbs, and spices [18].

MS and MS/MS metabolite databases, therefore, future structural identification of the unknowns will be necessary to obtain a more comprehensive picture. Standards for the identified compounds were not used to validate the mass spectra, thus providing a putative identification of all compounds. The contents of the UA supplements did not completely dissolve after the addition of MeOH and, therefore, DMSO was used to first solubilize precipitates in DS1-3 samples. However, a few samples did not fully dissolve and the precipitates were not analyzed. In addition, harmful contaminants were not identified in the UA supplements, but this does not exclude the possibility that contaminants could be detected using other analytical methods such as gas chromatography.

4. CONCLUSION

UA supplements in capsule form provided over 200% the theoretical weight on the Supplement Facts Panel, but this may be due to the inclusion of excipient ingredients. HPLC-ESI-MS analysis showed all UA supplements contained less than 50% total UA ranging from 14%-49%, but contained over 100% the listed UA amount in the dietary supplement. Additional compounds putatively identified in UA supplements are constituents of plants or commonly used in food products. No potentially harmful compounds were identified in our analysis. Future studies are needed to explore the possibility of developing a highly concentrated, pure UA supplement in order to investigate the therapeutic effects of UA in humans with accurate and interpretable results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This work was supported by the University of Texas at San Antonio's College of Education and Human Development Faculty Research Award 2014-2105 to SLU.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ikeda Y, Murakami A, Ohigashi H. Ursolic acid: An anti- and pro-inflammatory triterpenoid. Mol Nutr Food Res. 2008;52: 26–42.
2. Ullevig SL, Zhao Q, Zamora D, Asmis R. Ursolic acid protects diabetic mice against monocyte dysfunction and accelerated atherosclerosis. Atherosclerosis. 2011; 219(2):409-16.
3. Jayaprakasam B, Olson LK, Schutzki RE, Tai M-H, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). Journal of Agricultural and Food Chemistry. 2006;54(1):243-8.
4. Wang J, Liu L, Qiu H, Zhang X, Guo W, Chen W, et al. Ursolic acid simultaneously targets multiple signaling pathways to suppress proliferation and induce apoptosis in colon cancer cells. PLoS ONE. 2013;8(5):e63872.
5. Wang YJ, Lu J, Wu DM, Zheng ZH, Zheng YL, Wang XH, et al. Ursolic acid attenuates lipopolysaccharide-induced cognitive deficits in mouse brain through suppressing p38/NF-κB mediated inflammatory pathways. Neurobiol Learn Mem. 2011;96(2):156-65.
6. Pai PG, Chamari Nawarathna S, Kulkarni A, Habeeba U, Reddy C S, Teerthanath S, et al. Nephroprotective effect of ursolic acid in a murine model of gentamicin-induced renal damage. ISRN Pharmacology. 2012;2012.
7. Yang Y, Zhao Z, Liu Y, Kang X, Zhang H, Meng M. Suppression of oxidative stress and improvement of liver functions in mice by ursolic acid via LKB1-AMP-activated protein kinase signaling. Journal of Gastroenterology and Hepatology. 2015; 30(3):609-18.
8. Bang HS, Seo DY, Chung YM, Oh K-M, Park JJ, Arturo F, et al. Ursolic acid-induced elevation of serum irisin augments muscle strength during resistance training in men. The Korean Journal of Physiology & Pharmacology. 2014;18(5):441-6.
9. Ursonic acid. The Metabolomics Innovation Centre; 2012.
   Available:http://www.hmdb.ca/metabolites/HMDB36007
   (Accessed 17 December 2015)

10. 3alpha-Corosolic acid. The Metabolomics Innovation Centre; 2012.
Available:http://www.hmdb.ca/metabolites/HMDB41043
(Accessed 17 December 2015)
11. 3-O-trans-Feruloyleuscaphic acid. The Metabolomics Innovation Centre; 2012.
Available:http://www.hmdb.ca/metabolites/HMDB31837
(Accessed 17 December 2015)
12. Ursolic acid. The Metabolomics Innovation Centre; 2006.
Available:http://www.hmdb.ca/metabolites/HMDB02395
(Accessed 17 December 2015)
13. Ganoderiol l. The Metabolomics Innovation Centre; 2012.
Available:http://www.hmdb.ca/metabolites/HMDB37778
(Accessed 17 December 2015)
14. Jayaprakasam B, Olson LK, Schutzki RE, Tai MH, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). J Agric Food Chem. 2006;54(1):243-8.
PubMed PMID: 16390206. eng.
15. Carnosol. The Metabolomics Innovation Centre; 2006.
Available:http://www.hmdb.ca/metabolites/HMDB02121
(Accessed 17 December 2015)
16. Palmitic acid. The Metabolomics Innovation Centre; 2005.
Available:http://www.hmdb.ca/metabolites/HMDB00220
(Accessed 17 December 2015)
17. Stearic acid. The Metabolomics Innovation Centre; 2005.
Available:http://www.hmdb.ca/metabolites/HMDB00827
(Accessed 17 December 2015)
18. (+)-11-Hydroxy-9-triacontanone. The Metabolomics Innovation Centre; 2012.
Available:http://www.hmdb.ca/metabolites/HMDB29987
(Accessed 17 December 2015)
19. Polysorbate 20. The Metabolomics Innovation Centre; 2012.
Available:http://www.hmdb.ca/metabolites/HMDB37182
(Accessed 17 December 2015)
20. Lv Y-y, Jin Y, Han G-z, Liu Y-x, Wu T, Liu P, et al. Ursolic acid suppresses IL-6 induced C-reactive protein expression in HepG2 and protects HUVECs from injury induced by CRP. European Journal of Pharmaceutical Sciences. 2012;45(1–2): 190-4.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17232