British Journal of Pharmaceutical Research
11(6): 1-8, 2016, Article no.BJPR.25437
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Quantitative Analysis of Acteoside in Batankor Syrup and *Plantago lanceolata L.* Collected from Different Areas of Rwanda by HPLC and UV-Vis Spectrophotometric Methods

Goretti M. Ingabire[1], Andre Ndagijimana[1,2], Marie Jeanne Mukazayire[1], George Nyombaire[1] and Justin N. Kabera[1*]

[1]National Industrial Research and Development Agency (NIRDA), P.O.Box 273, Kigali, Rwanda.
[2]JSS College of Pharmacy, Rocklands, Post Box No.20, Udhagamandalam 643001, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MJM designed the study, wrote the protocol and author AN wrote the first draft of the manuscript. Author JNK managed the literature searches and reviewed the first draft. Authors GMI and AN performed the spectroscopy and HPLC analysis and managed the experimental process. Author GN identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/25437
Editor(s):
(1) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) Zhi-guo Ma, Jinan University, China.
(2) Feyyaz Onur, Ankara University, Turkey.
(3) Monika Waksmundzka-Hajnos, Medical University of Lublin, Poland.
Complete Peer review History: http://sciencedomain.org/review-history/14793

Short Research Article

Received 2nd March 2016
Accepted 5th April 2016
Published 27th May 2016

ABSTRACT

A range of biological activities has been found from *Plantago lanceolata* L. extracts showing anti-inflammatory, analgesic and antioxidant activities. It is in this perspective that the plant was the target of our investigations in order to develop a standardized phytomedicine. Batankor syrup formulated from *P. lanceolata* L. is used to treat cough and other infections of the respiratory tract. Various active compounds such as flavonoids, glycosides and terpenoids have been identified from *Plantago* species. The present study was performed to estimate the acteoside content in

**Corresponding author: E-mail: jukabe5@gmail.com, kajust68@yahoo.fr;*

formulated Bantakor syrup and *P. lanceolata* leaves collected from four different region of Rwanda. During this investigation, both UV-Vis Spectrophotometric and HPLC methods were developed and validated for determination the content of acteoside in analysts. The chromatographic fingerprints of *P. lanceolata* extracts represent phyto-equivalence even thought, there are, some differences in their profiles. The retention time of acteoside in the samples was at 6.9 minutes compared to the peak of standard. The plant sample from Rwabuye showed highest yield of acteoside (1.837%) while sample from Ngoma showed less acteoside content (0.594%). The content of acteoside in 1ml of Batankor syrup was found to be 142.19 µg ±0.83. The intraday precision (% RSD) on the basis of content of acteoside was found to be 0.09–0.45 whereas interday precision (% RSD) on the basis of the content were found to be 0.35-0.83, showing the stability of the sample within five days. Also, LOD and LOQ values were 4.26 and 12.90 respectively.

Keywords: Plantago lanceolata; Batankor syrup; acteoside; quantification; chromatographic methods.

1. INTRODUCTION

Since ancient times humanity has used medicinal plants to treat and prevent diseases. It was proved that herbal drug given with right doses and formulations serve a number of therapeutic purposes [1-3]. The current demand for herbal drugs is rising, in both developed and developing countries. WHO reports 80% of world population relies on the herbal medicines due to various reasons [4-6]. The herbal medicines were proven to be less toxicity, better therapeutic effect, good patient compliance and cost effectiveness than synthetic drugs.

P. lanceolata extracts have showed potential inhibitory effects against pathogenic microorganism [2,3,7]. It is assumed that aucubigenin, acteoside and aucubin were responsible for the *in vitro* antibacterial activities of this plant. In other study, catalpol showed the activity against hepatitis B virus antigens (HBsAg) in HBsAg positive serum [7-9]. Acteoside (Fig. 1) was reported to have antioxidant, antihypertension and anti-inflammatory activities [10-14]. *P. lanceolata* methanolic extract showed growth inhibitory and cytotoxic effects *in vitro* on breast adenocarcinoma and melanoma tumoral cell lines and the luteolin-*7-O-β*-glucoside was attributed this activity [3]. The hepatoprotective effects were observed for aucubin, acteoside and catalpol compounds [3,7,15,16].

It was reported that *P. lanceolata* contains about 2-3% of iridoid glycosides (aucubin and catalpol) as well as phenylpropanoid glycoside especially acteoside and plantamajoside [3,7,16]. The total iridoid content in young leaves may reach up to 9%, while in the older ones iridoids are present only in traces [3,6,16]. Several research works have been conducted on the plant material containing acteoside manly for its isolation and purified [13,17,18]. Combination of HPLC fingerprint and quantitative analysis by HPLC-DAD-ESI-MS was also used to identify and quantify acteoside compound in different medicinal plants [19-21]. Li-hou X et al. [22] developed a Micellar electrokinetic chromatography method for evaluation of variation of acteoside content in wild and cultivated *Scutellaria baicalensis* roots. However, on our best on our knowledge, there is no paper has reported the simultaneous quantification of acteoside in raw *P. lanceolata* plant and its formulated herbal drug. In this paper, we report a simple HPLC and UV-Vis Spectrophotometric method for identification and quantification of acteoside in both *P. lanceolata* and Batankor syrup formulated on base of this plant.

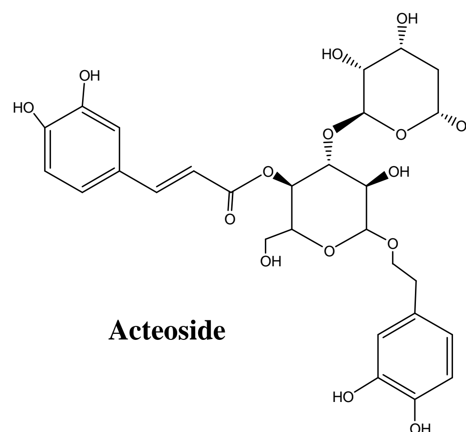

Fig. 1. Chemical structure of acteoside

2. EXPERIMENT PART

2.1 Equipment

An Agilent 1200 Series HPLC system equipped with a quaternary pump, a degasser, and a thermostatic auto-sampler and a photodiode array detector (DAD) with an Agilent extend-C18 column (150 mm x 4.6 mm, 5 µm) with C18 guard column, and UV-Vis Spectrophotometer, UV-1700 pharmaspec ShimaDzu, were the main equipment used in this study. Other equipment used, including ultrasound cleaner, electronic balance and Ultra-Water Purifier.

2.2 Chemicals and Reagents

HPLC grade acetonitrile was purchased from Aladdin Chemistry Inc. (Shanghai, China). Deionized water was obtained using a Milli-Q Water purification system (Millifore, MA, USA). Acteoiside reference was obtained from Chinese Food Medicine Identification Institute ≠ 111530-201007. All other organic solvents used in this study were of analytical grade and purchased from Shanghai Chemical Co. (Shanghai, China).

2.3 Plant Material

Four samples of *P. lanceolata* leaves were collected from three Provinces of Rwanda, Northern at Musanze District, Eastern at Ngoma District and Southern at Huye District (two samples from Mukoni and Rwabuye). The plant was authenticated as raw *P. lanceolata* by Professor Elias BIZURU from University of Rwanda and the sample was deposited in National Herbarium of National Industrial Research and Development Agency (NIRDA). Each plant sample was washed under running tap water to remove dust, dried at room temperature and then crushed into fine powder using electrical grinder. The obtained powdered samples were separately kept into polythene bags for the future use.

3. METHODS

3.1 Preparation of Crude Extract

200 g of powder of sample from Rwabuye were macerated two times with aqueous ethanol (2L, 70% v/v) at room temperature for 72 hours each time. The extraction mixture was filtered, combined and evaporated under reduced pressure at a temperature of 40ºC until total evaporation of the ethanol. The aqueous fraction was then used to formulate Batankor syrup.

3.2 UV-Vis Spectrophotometer Analysis

3.2.1 Preparation of sample

The samples were reparation according to the British Pharmacopoeia 2013. The amount of each plant sample (10 gr) was added in 90ml of ethanol (50% v/v) separately followed by macerating the solution to boil in water-bath under a reflux condenser for 30 minutes. The solution was allowed to cool and filtered. In 100 ml volumetric flask the filtered solution was completed to 100 ml with the same solvent. Then, in 10 ml volumetric flask, add 1 ml of the above obtained solution, 2 ml of 0.5M hydrochlolic acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water, and 2 ml of dilute sodium hydroxide solution. The obtained solution was completed to 10 ml with distilled water. The blank solution was prepared by adding in 10 ml volumetric flask, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water, 2 ml of 0.5 M hydrochlolic acid and 2 ml of dilute sodium hydroxide solution. The obtained solution was completed to 10 ml with distilled water.

3.2.2 Method

UV-Vis Spectrophotometric analysis was conducted by the measurement of the absorbance of analysts using a test solution at 525 nm using a blank solution. During this investigation the assay was done in triplicate. Taking the specific absorbance to be 185 for acteoside at 525 nm, the percentage content of total acteoside in each sample was calculated using the following expression:

$$\% = \frac{A x 1000}{185 x m}$$

A: Absorbance of the test solution at 525 nm
m: masse of the substance to be examined (g)

3.3 HPLC Analysis

3.3.1 Preparation of sample

The powder (1 gr) for each sample was accurately weighed, placed separately into a conical flask with stopper and soaked with 50 ml

of 70% methanol (v/v) for 30 min followed by ultrasonic extraction for another 30 min. The resultant mixture was adjusted to the original weight with extraction solvent, and the supernatant was removed through a 0.45 μm membrane filter. The standard solution was prepared by dissolving 5 mg of acteoside into 1ml of DMSO to obtain a stock solution. A serial of diluting ranging from 100 ng/ml to 900 ng/ml using methanol were prepared in order to obtain test solution used for calibration curve. Sample of Batankor syrup was prepared by diluting 1ml of formulated Batankor syrup into 20 ml of methanol. All samples used were sonicated, degassed and filtrated through a 0.45 μm membrane filter before HPLC injection.

3.3.2 Methods

The binary mobile phase consisted of acetonitrile (solvent A) and water containing 1% acetic acid (solvent B). The flow-rate was kept constant at 1.0 mL/min for a total run time of 16 min. The system was run with a gradient program: 0-11.5 min: 90% B to 60% B; 11.5-12.7 min: 60% B to 0% B; and 12.7-16min, 0% B to 90%B. The sample injection volume was 10 μL. Peak of interest was monitored at 320 nm by a DAD detector. The qualitative identification of acteoside in different crude extracts and Batankor syrup was achieved by comparing the retention times (RT) of characteristic peaks in different samples with those of the authentic acteoside standard. The quantitative analysis of acteoside in different samples was conducted using calibration curve method with external standard. This method is consisting of injecting an aliquot of the calibration (standard) solution of known concentration and measuring the peak area obtained. During this investigation the analysis was conducted in triplicate and the results were presented as mean ± Standard of Deviation.

3.3.2.1 Accuracy and precision

The repeatability of the concentration and measurement of peak area were carried out using three replicates of three different concentration of acteoside (200, 500 and 700ng/ml) which were expressed in terms of percentage relative standard deviation (% RSD) and standard error (SE). The intraday precision was determined at three different concentration levels of acteoside (200, 500, 700 ng/ml, six times on the same day, and the interday precision was determined at same the concentrations of marker, four times on five different interval days.

3.3.2.2 Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approaching the LOD according to the formulas: LOD= 3.3 (SD/S) and LOQ = 10 (SD/S). The SD of the response was determined based on the SD of y-intercepts of regression lines.

4. RESULTS AND DISCUSSION

4.1 UV-Vis Spectrophotometric

The precision test was investigated by analyzing sample from Ngoma 6 consecutive times. The result indicated that there was no obvious change of absorbance values obtained. The measured %RSD was smaller than 8%, showing the good precision of UV-Vis Spectrophotometer equipment. The stability test was conducted on sample from Ngoma at time points of 0, 2, 4, 8, and 10 hour. After UV-Vis spectrophotometer measurement, we got comparable values of absorbance. The measured RSD% was smaller than 8%, showing the stability of the sample within 10 hours.

The percentage of acteoside in each sample was calculated using the above mentioned formula. Results are presented as mean values ± standard deviation values (n=3). The measured absorbance was 0.034; 0,019; 0.017, and 0.011 respectively for samples from Rwabuye, Mukoni, Musanze and Ngoma. The measured %RSB values were smaller than 8% (Table 1). There is a high percentage of acteoside (1.84%) in the sample from Rwabuye, followed by Mukoni (1.01%) and Musanze (0.92%). The sample from Ngoma was found to have least percentage of acteoside (0.59%).

4.2 HPLC Analysis

The qualitative identification of acteoside in different crude extracts and Batankor was performed by comparing the retention times of characteristic peaks in different samples with those of the authentic acteoside standard. The crude extracts of *P. lanceolata* from 4 different locations and Batankor syrup were analyzed under the same HPLC conditions. The system was run with a gradient program: 0-11.5 min:

90% B to 60% B; 11.5-12.7 min: 60% B to 0% B; and 12.7-16 min, 0% B to 90% B. A volume of 2 µL of each crude extract and 10 µL of Bantankor syrup were injected for analysis and the peak of interest was monitored at 320 nm by a DAD detector for a total run time of 16 min. The Fig. 2 presents HPLC chromatograms of *P. lanceolata* samples and acteoside standard analyzed using same HPLC conditions.

The results given at Fig. 2 showed that the HPLC chromatograms of *P. lanceolata* extracts represent phyto-equivalence even thought, there are, some differences in the profiles taking into consideration the signal intensity, retention time , peak area and peak height of different peaks. The peaks with retention time of 6.97 min; 6.96 min; 6.97 min; 6.97 min and 6.89 min respectively for sample namely Mukoni, Rwabuye, Musanze, Ngoma and Batankor syrup were tentatively identified as the peak of acteoside compare to the one of standard which was obtained at 6.86 min. However, the peak height of acteoside standard was higher than those of crud extracts and Batankor syrup. The different concentrations of acteoside in the range 100-900 ng were used for calibration. At each concentration of acteoside and plant sample, the peak area was recorded. The calibration plots were linear within this range of acteoside, and the correlation coefficient (r) was 0.999 which indicate a good linear dependence of peak area on concentration. The repeatability of sample analyzed and measurement of peak area was expressed in terms of RSD % and the % RSD for intra- and interday analysis. As shown at Table 2, intraday precision (% RSD) on the basis of content of acteoside was found to be 0.09–0.45 whereas interday precision (% RSD) on the basis of the content were found to be 0.35-0.83, showing the stability of the sample within five days. Also, LOD and LOQ values were 4.26 and 12.90 respectively.

The quantitative analysis of acteoside in different samples was conducted using calibration curve method with external standard. The calibration curve was represented by the linear equation **y**= 1.1917 x - 0.7273, where **"y"** is the response as peak area and **"x"** is the concentration. According to the peak area obtained for each sample and above linear equation, the content of acteoside was calculated. Table 3 shows the content of acteoside in *P. lanceolat*a samples and Batankor Syrup. As shown at Table 3, the content of acteoside varied from 145.4 to 453.80 µg/g of dried plant sample according to the sample location. The content of acteoside in

Table 1. The % of acteoside in *P. lanceolata* samples (mean values n=3)

Sample name	Absorbance (mAU)	Acteoside content (%)	SD	%RSD	SE
Ngoma	0.011	0.594	0.004	7.231	0.0126
Mukoni	0.019	1.846	0.003	1.654	0.042
Rwabuye	0.034	1.009	0.003	3.354	0.0173
Musanze	0.017	0.918	0.005	5.448	0.0194

Table 2. Intra- and Interday precision of HPLC (n = 6)

Acteoside (ng/ml)	Intraday precision				Interday precision			
	Mean area	SD	% RSD	SE	Mean area	SD	% RSD	SE
200	238.10	0.23	0.09	0.09	234.23	1.96	0.83	0.80
500	605.69	2.75	0.45	1.12	527.79	1.871	0.35	0.76
700	833.37	1.56	0.19	0.63	643.89	2.87	0.44	1.17

Table 3. Acteoside content in different *P. lanceolat*a samples and Batankor syrup

Sample area	Absorbance (mAU)	Content of acteoside (µg/g of plant sample)	SD	% RSD	SE
Rwabuye	541.52	453.80	3.44	0.759	1.989
Mukoni	346.57	290.208	1.73	0.595	0.997
Musanze	304.14	254.603	0.834	0.329	0.483
Ngoma	174.03	145.432	0.852	0.586	0.492
Batankor	170.18	142.192 µg/ml	0.836	0.588	0.482

Fig. 2. Superposed HPLC chromatograms of *P. lanceolata* crude extracts and acteoside standard

(From the top to down: Acteoside standard, sample from Mukoni; sample from Rwabuye; sample from Musanze, sample from Ngoma and Batankor syrup)

Batankor syrup was found to be 142.192 µg/ml. The analytical results showed that the contents of the five analytes including acteoside in twenty-one commercial *Rehmannia glutinosa* samples from different markets in Korea and China vary significantly with sources [23].

5. CONCLUSION

Both obtained results by HPLC and UV-Vis Spectrophotometric methods proven that the amount of acteoside differed depending to the plant samples location. The obtained results from two quantification methods showed that sample from Rwabuye has a high amount of acteoside compound while sample from Ngoma showed less acteoside content. These findings are helpful for industrial scale production since are showed that samples from Southern Province contain a high content of acteoside. However, other factors such as use of fertilizers, climates etc could also be studied. These results indicated that it could be a great important to control the quality of *P. lanceolata* to ensure the therapeutic effects of Batankor syrup in clinical use. Therefore, setting a minimum limit on acteoside content could be useful to have standardized Batankor syrup and to ensure the quality and its clinical efficacy. The obtained results to this study suggested that the established and validated HPLC method could be used for the quality control of the plant materials containing *P. lanceolata* and other medicinal plants where acteoside related compounds are the dominant phytochemicals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the

appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mohammed AI. Antimicrobial activity of ethanolic extracts from some medicinal plant. Aust. J. Basic. & Appl. Sci. 2011; 5:678-83.
2. Haile AK. Antimicrobial activity and phytochemical screening of crude extracts of Medicinal plants grown in Eastern Ethiopia. Int J Pharm. Bio. Sci. 2013; 4:326-33.
3. Alice G, Misu M. Non-food crops-to-industry schemes in Eu27" Wp3. Bio-based products. National Institute for Chemical Pharmaceutical R&D Bucharest, Romania. 2012;1-94.
4. Andualem, Fisseha A, Berhanu. Antimicrobial potentials of different solvent extracts of *Justicia landonoides* and *Plantago lanceolata* against standard and drug resistant human bacterial pathogens. Int J Microbiol. Res. 2014;5:6-18.
5. Silva N, Fernandes J. Biological properties of medicinal plants: A review of their antimicrobial activity. J. Venom. Anim. Toxins incl. Trop. Dis. 2010;16:402-13.
6. Samuelsen AB. The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. J Ethnopharmacol. 2000;17:1-21.
7. Products Cohm. Assessment report on *Plantago lanceolata* L., folium. European medicines agency, Science Medicines Health. 2011;2-24.
8. Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia. 2011;1:98-106.
9. Omodamiro OD, Unekwe PC, Nweke IN, Jimoh MA. Evaluation of diuretic activity of ethanol extract and its fractions of *Agave sisalana* peak. J Pharm. Pharm. Sci. 2014; 2:200-204.
10. Marina Galvez CM, N-Cordero, Peter J. Houghton, and Mari´A Jesus Ayuso. Antioxidant activity of methanol extracts obtained from *Plantago* species. J Agr Food Chem. 2005;53:1927–1933.
11. Lien-Chain C. Immunomodulatory acivities of flavonoids,monoterpenoids, tritepenoids, irodoids glycosides. Planta Medecines. 2003;69:600-604.
12. Kartini A. Chromatographic fingerprinting and clustering of *Plantago major* L. from different areas in Indonesia. Asian J Pharm. Clin. Res. 2012;5:191-5.
13. Lekshmi VB, George KV, Solomon H. Identification of acteoside as the active antioxidant principle of *Premna serratifolia* root wood tissues. Phytopharmacology. 2013;4:228-36.
14. Chen CH, Lin YS, ChienMei- Y, Hou WC, Hu ML. Antioxidant and antihypertensive activities of acteoside and its analogs. Bot Stud. 2012;53:421-9.
15. Essam FAJ, Hassan AAR, Rana HR. Extraction and purification of tannins from *Plantago lanceolata* L. and assessment their antibacterial activity on pathogenesis of enteropathogenic *E. coli in vitro* and *in vivo*. Trends in Life Sciences. 2012;1: 17-21.
16. Kırmızıbekmez H. Phenylethanoid glycosides from *Digitalis viridiflora.* Rec Nat Prod. 2015;9:369-73.
17. Karel S, Pavla H, Ales Z, Jan M, Margita D. Antiradical activity of *Paulownia tomentosa* (Scrophulariaceae) extracts. Molecules. 2007;12:1210-9.
18. Han L, Lina J, Mavis BY, Wei L, Xinbo S, Xiumei G. Preparative isolation and purification of four compounds from *Cistanches deserticola* Y.C. Ma by High-Speed Counter-Current Chromatography. Molecules. 2012;17:8276-84.
19. Zhen-Ya Y, Dan-Yi L, Shun Y, Rong-Rong Z, Zheng-Jin J, Zhi-Guo M. Chemical fingerprint and quantitative analysis of *Cistanche deserticola* by HPLC-DAD-ESI-MS. J Food Drug Anal. 2013;21:50-7.
20. Shi HM, Wang J, Wang MY, Tu PF, Li XB. Identification of *Cistanche* species by chemical and inter-simple sequence repeat fingerprinting. Biol Pharm Bull. 2009;32: 142-6.
21. Zhao SY, He XY, JIa BG, Peng QY, liu X. Production of acteoside from *Cistanche tubulosa* by β-Glucosidase. Pak J Pharm Sci. 2011;24:135-41.
22. Li-hua X, Xuan W, Purusotam B, Naoko M, Seiichi Y, Dong-ye Y, et al. Evaluation of

variation of acteoside and three major flavonoids in wild and cultivated *Scutellaria baicalensis* roots by Micellar electrokinetic chromatography. Chem Pharm Bull. 2002; 50:896-9.

23. Tae Hyung W, Sung Kwang R, Sam Sik K, Jongheon S. Simultaneous analysis of bioactive metabolites from *Rehmannia glutinosa* by HPLC-DAD-MS/MS. Nat Prod Sci. 2010;16:116-22.

© 2016 Ingabire et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14793