Advances in Pharmacoepidemiology and Drug Safety

OPEN ACCESS Freely available online

Research Article

Quantification of 3α-Acetyl-11-Keto-β-Boswellic Acid and 11-Keto-β-Boswellic Acid in the Resin of Boswellia species by HPTLC

Sisay Awoke*

Department of Chemistry, Wollo University, Dessie, Ethiopia

ABSTRACT

In this article, the amount of the most potent anti-inflammatory and anti-cancer compounds found in the resin of B. papyrifera was quantified and compared with the well-known species, *B. serrata* and *B. sacra* (Synonyms B. carteri), using CAMAG HPTLC scanner IV equipped with a sample applicator Limonat V at wavelength 254 nm without derivation. The two most active ingredients, 11-keto-boswellic acid and 3-acetyl-11-keto-boswellic acid, in the EtOH extract of *B. papyrifera* resin were determined as 14.0% and 8%, respectively.

Keywords: Boswellia; papyrifera; KBA; AKBA; Kebtele; Ethiopia; Kebtele

INTRODUCTION

The genus Boswellia comprises more than 30 species [1-3]. Among them are Boswellia serrata, B. sacra Flück (syn: B. carterii Birdw. and B. papyrifera Hochst., are rich in boswellic acids [4]. The main constituents of Boswellia species are volatile oils, composed of monoterpenes and sesquiterpenes, diterpenes including incensole, incensole acetate, serratol, and verticilla-4(20),7,11-triene; tetracyclic triterpenic acids such as lupeolic acids, tirucallic acids; and pentacyclic triterpene acids such as -boswellic acids and -boswellic acids [5].

Among triterpenoids, the major bioactive boswellic acids, 11-keto-b-boswellic acid (1), 3--acetyl-11-keto--boswellic acid (2), -boswellic acid (3), -boswellic acid (4), 3-acetyl--boswellic acid (5) and 3-acetyl--boswellic acid (6) are of particular interest [6]. The analysis of these triterpenes is performed by different analytical methods including high performance thin layer chromatography (HPTLC) [7], although the most used methods are based on HPLC coupled to both photodiode array detection and mass spectrometry detection [8]. In accordance with the spectral properties of the boswellic acids, their analysis is performed at three different wavelengths, 210 nm for - and -boswellic acids, 250 nm for AKBA and KBA (Figure 1).

Among all boswellic acids, AKBA and KBA are the most active ingredients found in the species for their anti-inflammatory and cytotoxic activities. An article reported by Mannino et al. in 2016 indicated that the amount of AKBA in B. sacra (syn. B. carteri) is higher than B. serrata resin, whereas, the amount of KBA is vice versa. However, the amount of these compounds in the resins of B. papyrifera (Kebtele) and B. papyrifera are not yet detected and compared with the well-known B. serrata and B. carteri. The aim of this article is to determine the amount of AKBA and KBA in these two endemic resins in Ethiopia [9].

MATERIALS AND METHODS

For the quantitative analysis of Boswellia species towards potent anti-inflammatory compounds, 3-acetyl-11-keto--boswellic acid (AKBA) and 11-keto--boswellic acid (KBA), only four species were selected, B. papyrifera (Kebtele), B. papyrifera, B. sacra, and B. serrata, based the result obtained from qualitative analysis of resin.

To prepare resin samples, 20 g of coarsely powdered resins were stirred in 60 mL EtOH using magnetic stirrer for 4 h. 50 mg crude extract of each sample was dissolved in CHCl3 up the mark in 10 ml volumetric flask to make 5 mg/ml stock solution for quantification of AKBA and KBA. AKBA and KBA used as a standard for HPTLC quantitation were isolated from B. papyrifera resins by column chromatography and purified by PTLC [10]. The purities were confirmed by UV, IR and NMR spectroscopic data. Standard solutions were prepared as 1 mg/ml and kept in a freezer until analysis (Figure 2).

Correspondence to: Sisay Awoke, Department of Chemistry, Wollo University, Dessie, Ethiopia, E-mail: sisay.awoke@wu.edu.et

Received: January 28, 2021, **Accepted:** February 12, 2021, **Published:** February 19, 2021

Citation: Awoke S, (2021) Quantification of 3α-Acetyl-11-Keto-β-Boswellic Acid and 11-Keto-β-Boswellic Acid in the Resin of Boswellia species by HPTLC. Adv Pharmacoepidemiol Drug Saf. 10:240.

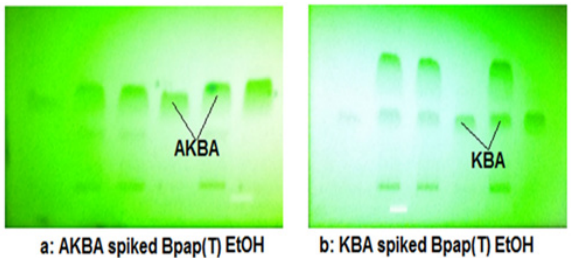

Figure 1: TLC chromatogram of spiked Bpap samples AKBA and KBA).

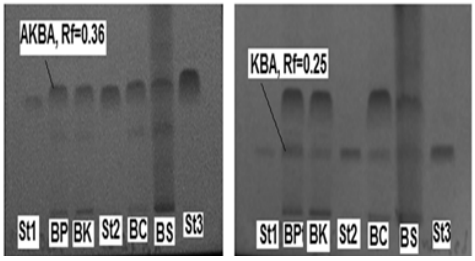

Figure 2: Three-dimensional spectra and spectral comparison.

A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm2) with SS lid, TLC Scanner IV and Wincats an integrated Software 4.02 (Switzerland). HPTLC Glass pre-coated plate with Silica gel F254 (20x10 cm2; 0.2 mm thick) was used with optimized mobile phase containing hexane: chloroform: ethyl acetate (1:2:1) v/v gave a good resolution. Well-defined spots were obtained when the developing chamber was saturated for 10 min at room temperature.

EtOH extract of samples and standard solutions (AKBA and KBA) were applied on a plate by using Linomat V applicator. HPTLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature (Figure 3).

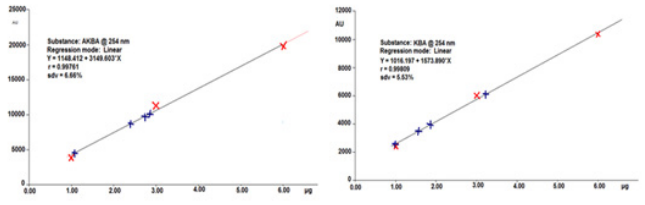

Figure 3: TLC chromatogram and a calibration curve of AKBA and KBA.

AKBA and KBA are UV active compounds that make the quantitative analysis easy. HPTLC fingerprint profile was snapped by Huawei G730-U10 Mobile Camera under UV 254 nm. For the quantification of AKBA and KBA the TLC plate was scanned immediately using CAMAG TLC Scanner IV at wavelength 254 nm without derivation. In order to establish linearity, standard solutions of AKBA and KBA (1 mg/ml) were applied to TLC plate, 1.0 µl, 3.0 µl, 6.0 µl on track 1, 4 and 7, respectively, and for AKBA assay, 4.0 µl of EtOH extract of BpR, BpKR, BsaR, and BsR were applied on track 2, 3, 5 and 6, respectively. However, 8 µl of each sample was applied on respective tracks for the quantification of KBA. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data from peak area and height (Figures 4 and 5).

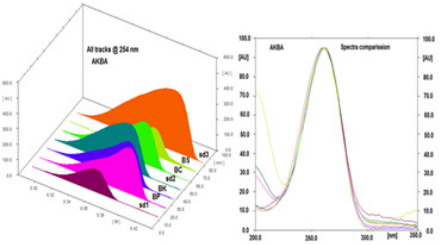

Figure 4: Three-dimensional spectra and spectra comparison of AKBA.

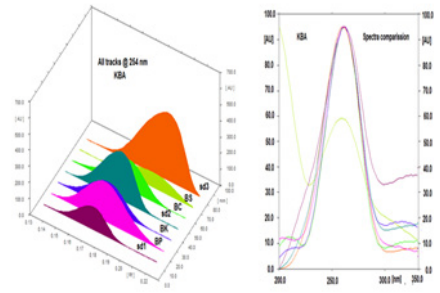

Figure 5: Three-dimensional spectra and spectra comparison KBA.

RESULTS AND DISCUSSION

The TLC procedure was optimized with a view to quantifying AKBA and KBA in four Boswellia resin extracts. The mobile phase consisting of solvent system hexane: chloroform: ethyl acetate (1:2:1) gave a good resolution and a sharp and well-defined peak at Rf = 0.36 for AKBA and 0.25 for KBA in the standard as well as in the extracts. Well-defined spots were obtained when the developing chamber was saturated for 10 min at room temperature that enabled to accurately quantify AKBA and KBA in the diluted solutions.

To prepare the calibration, from the standard solution 1.0, 2.0, 3.0, 4.0 and 5.0 l were spotted. Track 1, 3 and 5 were considered as standards and the remaining tracks 2 and 4 were considered as unknown samples. The developed TLC method for estimation of AKBA and KBA showed a good correlation coefficient (rAKBA = 0.99, rKBA = 0.98) in the concentration range of 1 to 5 g spot-1 with respect to the peak area scanned at 254 nm. The amount of AKBA on Track 2 and 4 were quantified as 2.2 and 4.0 µg, whereas, the amount of KBA were 1.7 and 3.8 for 2.0 and 4.0 µg, respectively.

Precision, repeatability, of the sample application and measurement of peak areas were carried out using three replicates of the same spots (4.0 g) of AKBA and KBA. The percent relative standard deviation (% R.S.D) for repeatability of sample application was found as 5.5% and 3.0% for AKBA and KBA, respectively. The recovery study or the accuracy of the quantification was assessed in a recovery study. One of the samples analyzed here was spiked with extra 0.5, 1.0 and 1.5 g of both standards AKBA and KBA separately and reanalyzed as shown on the TLC below. The proposed method, when used for estimation of AKBA, afforded recovery of 94.8 to 100%, and for estimation of KBA 94.2 to 98.8%, which were acceptable values.

EtOH extract of each of these samples analyzed three times using HPTLC and results are summarized . A single spot at

Rf = 0.25 and 0.36 were observed in the chromatogram of KBA and AKBA isolated from the extract along with other components. There was no interference in analysis from other components present in the extracts. These components appeared in the chromatogram at significantly different Rf values on TLC and the curves of estimation of AKBA and KBA are indicated in Figure 3. The three-dimensional spectra and spectral comparison of four samples also indicated.

CONCLUSION

The amount of AKBA found in 20 µg of EtOH extract of BpR, BpKR, BsaR, and BsR was 2.8 µg (14.0%), 2.4 µg (12.0%), 2.7 µg (13.5%), and 1.4 µg (7.0%), respectively. Whereas, the amount of KBA quantified was 1.6 µg (8.0%), 0.8 µg (4.0%), 1.0 µg (5.0%) and 0.7 µg (3.5%), respectively. The amount of most potent anti-inflammatory and anti-cancer compounds found in the resin of B. papyrifera is higher than found in the well-known Indian species B. serrata and Somalian species B. sacra, which is reliable with the literature.

REFERENCES

1. Paul M. Chemotaxonomic investigations on resins of the frankincense species Boswellia papyrifera, Boswellia serrata and Boswellia sacra, respectively, Boswellia carterii: a qualitative and quantitative approach by chromatographic and spectroscopic methodology 2012.
2. Awoke S, and Dagne E. Chemical Studies on the Resins of Boswellia pirottae and B. papyrifera. PhD Thesis, Addis Ababa University, Ethiopia 2018;4(4):316-320.
3. Ganzera M, Khan IA. A reversed phase high performance liquid chromatography method for the analysis of boswellic acids in Boswellia serrata. Planta medica. 2001;67(8):778-780.
4. Krohn K, Rao MS, Raman NV, Khalilullah M. High-performance thin layer chromatographic analysis of anti-inflammatory triterpenoids from Boswellia serrata Roxb. Phytochemical Analysis: J. Plant Nutr Soil Sci. 2001;12(6):374-376.
5. Pozharitskaya ON, Ivanova SA, Shikov AN, Makarov VG. Separation and quantification of terpenoids of Boswellia serrata Roxb. extract by planar chromatography techniques (TLC and AMD). J Sep Sci. 2006;29(14):2245-50.
6. Büchele B, Zugmaier W, Simmet T. Analysis of pentacyclic triterpenic acids from frankincense gum resins and related phytopharmaceuticals by high-performance liquid chromatography. Identification of lupeolic acid, a novel pentacyclic triterpene. Journal of Chromatography B. 2003;791(12):21-30.
7. Frank A, Unger M. Analysis of frankincense from various Boswellia species with inhibitory activity on human drug metabolising cytochrome P450 enzymes using liquid chromatography mass spectrometry after automated on-line extraction. Journal of Chromatography A. 2006;1112(1-2):255-262.
8. Banno N, Akihisa T, Yasukawa K, Tokuda H, Tabata K, Nakamura Y et al. Anti-inflammatory activities of the triterpene acids from the resin of Boswellia carteri. Journal of ethnopharmacology. 2006;107(2):249-53.
9. Mannino G, Occhipinti A, Maffei ME. Quantitative determination of 3-O-acetyl-11-keto-βboswellic acid (AKBA) and other boswellic acids in Boswellia sacra Flueck (syn. B. carteri Birdw) and Boswellia serrata Roxb. Molecules. 2016;(10):1329.
10. Büchele B, Zugmaier W, Genze F, Simmet T. High-performance liquid chromatographic determination of acetyl-11-keto-α-boswellic acid, a novel pentacyclic triterpenoid, in plasma using a fluorinated stationary phase and photodiode array detection: Application in pharmacokinetic studies. Journal of Chromatography B. 2005;829(12):144-148.