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USE OF FINGERPRINTING AND MARKER COMPOUNDS FOR THE IDENTIFICATION AND STANDARDIZATION OF *BOSWELLIA SERRATA* GUM EXTRACT

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ABSTRACT

Extracts of *Boswellia serrata* gummy exudate have been traditionally used in the field of medicine as an antiarthritic, astringent, stimulant, expectorant, and antiseptic agent. In this research we made an attempt to establish a comprehensive approach for the complete standardization of this important medicinal plant. The gum exudates were subjected for both physical and chemical standardization. Various physico-chemical parameters along with TLC fingerprint profiling have been carried out in triplicate. A rapid and simple high-performance liquid chromatographic method has been developed for simultaneous determination Keto beta boswellic acid (KBBA), acetyl keto beta boswellic acid (AKBBA), Beta boswellic acid (BBA), and acetyl beta boswellic acid (ABBA). Separation of boswellic acids was achieved on a C_{18} column with a mobile phase of acetonitrile and 0.05% acetic acid in the ratio of 90:10, v/v in gradient elution mode with a run time of 40 minutes. Detection carried out at 210 nm BBA and ABBA; 254 nm for KBBA and AKBBA. Results from linear regression analysis were indicative of a good linear relationship ($r^2 \geq 0.9957$). The recovery of the method was found in the range of 99.6-102.1, 97.5-102.5, 98.0-101.4 and 98.5-103.0 % for KBBA, AKBBA, BBA and ABBA, respectively. The LOD found to be 0.4 and 1.5 µg/mL for KBBA and AKBBA while 1.0 µg/mL for both BBA and ABBA, respectively. The LOQ found to be 1.0 µg/mL for KBBA and 5.0 µg/mL for AKBBA, BBA and ABBA. Intra-day, inter-day, and inter-analyst precisions were

satisfactory, with relative standard deviation in the range (0.35-2.03). The robustness of the method also found satisfactory (%RSD ≤ 1.98). The findings of the research would be a useful guideline for quality control and standardization of gum extracts for a good quality herbal raw material.

Key words: *Boswellia serrata*, Physico-Chemical Standardization, HPTLC Fingerprint, HPLC-PDA

INTRODUCTION

Boswellia serrata (Indian Frankincense) resin dry extract is traditionally used for the treatment of various diseases in Ayurveda, Unani and other folk medicine as analgesic and anti-arthritic (1-3). *Boswellia serrata* gum is used in raw form and also used as an ingredient in various traditional polyherbal formulations. Gum resin extracts of *Boswellia serrata* have been found to represent a promising supplemental herbal remedy which is in great demand in current time. *Boswellia serrata* is a tree of moderate height, which grows in hilly areas of India. The therapeutic value of dried resinous gum, derived from tapping the Boswellia tree has been known since antiquity. *Boswellia serrata* is mainly used in rheumatic disorders, to improve appetite and general weakness. *Boswellia serrata* resins contain different types of secondary metabolites, such as essential oils, sesquiterpenoids, diterpenoids and triterpenoids (4). The gum resin of the *Boswellia serrata* have been shown to be effective against rheumatoid arthritichronic colitis, ulcerative colitis, skin allergies, ulcers, peritumoral brain edema and osteoarthritis (5-9).

From *Boswellia serrata* gum resin, 4 main compounds belonging to alpha-amyrin class have been isolated viz., Keto-beta-boswellic acid (KBBA), Acetyl keto beta boswelic acid (AKBBA), Beta-boswellic acid (BBA), and Acetyl-beta-boswellic acid (ABBA), which are responsible for 5-lipoxigenase inhibitory activity (10).

The literature survey revealed that there are various methods reported on the quantification of boswelic acids in crude drug, formulations and plasma. Non-aqueous titrimetric method for the quantification of total boswellic acid is the most commonly used conventional method for the quality control of *Boswellia serrata* (11). Other than this there are various HPLC (12-14), HPTLC (15-16), LC-MS (17) methods were also reported for the quantification identified boswellic acids. Most of these reported methods mainly deals with the quantification

of only AKBA and KBA. There are only few methods available for the quantification of other identified boswellic acid than AKBA and KBA (18).

The aim of the present study is to standardize *Boswellia serrata* extract using conventional physic-chemical quality control parameters including HPTLC fingerprints and also to develop a rapid, reliable and validated HPLC-PDA method for the simultaneous quantification of 4 major identified boswellic acids KBBA, AKBBA, BBA and ABBA. As far as our knowledge no attempts were made till now for the simultaneous quantification these four pharmacologically active pentacyclic triterpenic boswellic acids using HPLC-PDA.

EXPERIMENTAL

Materials and Methods

The gum exudate of *Boswellia Serrata* was procured from the Local Market of Al-Kharj and Riyadh. The voucher specimens were deposited at Department of Pharmacognosy, College of Pharmacy, Salman Bin Abudlaziz University.

All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (USA) and Merck (Germany). HPLC-grade acetonitrile, methanol and acetic acid were purchased from Sigma Chemical Co. (USA) and Merck (Germany). Milli-Q water used throughout the experiment was prepared using a Millipore water purification system.

Physico-chemical evaluation

Various physico-chemical evaluations such as organoleptic evaluations like colour, odour, taste and consistency; physico-chemical evaluation like loss on drying, moisture content by Karl Fischer method, total ash, acid insoluble ash, water soluble ash, pH of 1 & 10 % solution, extractive values, water soluble matter, alcohol soluble matter etc. were carried out in triplicate as per the procedure mentioned in Pharmacopoeia.

HPTLC Analysis

HPTLC instrumentation

The samples were applied in triplicate (8.0 µL), the band width was kept to 5.0 mm and distance between tracks was 13 mm on precoated silica gel 60 F_{254} plates (E. Merck, 0.20 mm thickness) using Linomat V (HPTLC sample applicator). After sample application, the plates were developed up to 80 mm in development chamber saturated with the respective solvent system. The chromatograms were scanned at 254 and 366 nm wavelength followed by spectral analysis. Reprostar was used for taking photographs of the HPTLC plates. Plates were also scanned at visual range after spraying with visualizing reagent.

Preparation of sample solution for HPTLC analysis

High Performance Thin Layer Chromatography was performed to develop fingerprint profiles of gum exudate of *Boswellia Serrata*. Methanol, petroleum ether and chloroform extracts were used for the fingerprint development. The methanol (160 mg/mL), chloroform (160 mg/mL) and petroleum ether (200 mg/mL) extracts were prepared by sonicating 0.8, 0.8 and 1.0 g of the sample in 20 mL of respective solvent for 30 mins, followed by centrifugation to get the supernatant, which was concentrated under nitrogen and further the volume was adjusted to 5 mL using respective solvents.

HPLC Analysis

HPLC Instrumentation

The HPLC analysis has been carried out on a Waters Alliance e2695 separating module (Waters Co., MA, USA) using UV detector (Waters 2998) with autosampler and column oven. The instrument was controlled by use of "BREEZE" software installed with equipment for data collection and acquisition. Compounds were separated on a C_{18} reverse phase column (150 x 4.6mm, particle size 5 µm, Merck, Germany) maintained at room temperature.

Calibration curve for boswellic acids

The calibration curve of boswellic acid was made by dissolving 20 mg of mix standard in 50 mL of methanol. It was then diluted to seven concentrations for constructing of calibration plots in the ranges of 1.3-133, 6.4-640, 5.0-508 and 3.7-377 µg/mL for KBBA, AKBBA, BBA and ABBA, respectively. Further dilutions with the lowest concentrations in the calibration curves were carried out to afford a series of standard solutions for evaluating the LOD and LOQ of the compounds. The stock and working solutions were stored at 4°C. The calibration graph was plotted using peak area versus drug concentration to obtain linearity, least square regression equation and the correlation coefficient.

Preparation of sample solution for HPLC analysis

Accurately weighed 2.0 g of the powdered sample, which was extracted with methanol (10 mL) by refluxing for 30 minutes at 60°C, it was filtered and filtrate obtained was evaporated to dryness on water bath. The residue obtained was re-constituted in HPLC grade methanol and volume was adjusted to 25.0 mL, which was filtered through 0.22 µm syringe filter before injecting to HPLC column.

Validation of the method

Recovery studies may be performed in a variety of ways depending on the composition and properties of the sample matrix. In the present study, three different solutions were prepared with a known added amount of pure boswellic acid standards to give a concentration range of 50 – 150% of that in a test preparation. The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day and inter-analyst variation). Intra-day was examined by analysing three determinations of the same batch of each component at 100% of the test concentration. Intermediate precision (inter-day variation) was studied by assaying three batches of samples containing the nominal amount of boswellic acids on different days. Inter-analyst precision was done by repeating same procedure using same HPLC system but by a different analyst. Solutions corresponding to each concentration level were injected in triplicate. The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of boswellic acids. Robustness of the particular method was determined by changing the mobile phase compositions from 90:10 to 88:12 and 92:8, v/v and by changing the detection wavelength from 254 to 252 and 256 nm. LOD and LOQ were calculated by measuring the standard deviation of analytical background response of six blank standard samples at the corresponding Rt of each analyte. The signal to noise ratio (S=N) of 3:1 (peak area ratio of the analyte vs baseline noise) and 10:1 were used for the calculation of the LOD and LOQ, respectively.

RESULTS & DISCUSSION

Organoleptic and physico-chemical evaluations

The results of organoleptic and physico-chemical evaluations was given in **Table 1**

HPTLC Analysis

HPTLC Fingerprint Analysis

The methanolic extract of the sample has given nine peaks when scanned at 450 nm after spraying with anisaldehyde-sulphuric acid spraying reagent at R_f 0.11, 0.12, 0.19, 0.26, 0.38, 0.45, 0.61, 0.78 and 0.87 using toluene: ethyl acetate: formic acid (5:4:1 v/v/v) as mobile phase **(Figure 1)**.

The chloroform extract of the sample has given nine peaks when scanned at 450 nm after spraying with anisaldihyde-sulphuric acid spraying reagent at the R_f 0.11, 0.12, 0.17, 0.24, 0.40, 0.45, 0.61, 0.78 and 0.89 using petroleum ether: di ethyl ether (1:1 v/v) as mobile phase **(Figure 2)**.

The petroleum ether extract of the sample has given nine peaks when scanned at 450 nm

after spraying with anisaldehyde-sulphuric acid spraying reagent at R_f 0.11, 0.13, 0.17, 0.39, 0.45, 0.62, 0.77, 0.86 and 0.95 using petroleum ether: di ethyl ether (1:1 v/v) as mobile phase **(Figure 3)**.

HPLC Analysis

HPLC Optimization of Chromatographic Conditions

HPLC method for the quantification of four boswellic acid was done by modifying the method, which reported by Shah *et. al*., 2008 (18). The method was developed for the quantification two boswellic acids simultaneously (KBBA and AKBBA). The same mobile phase was optimized further for the simultaneous estimation of four boswellic acids (KBBA, AKBBA, BBA and ABBA). The reported isocratic method was found not enough for the satisfactory separation of four boswellic acids. So, the elution pattern was changed from isocratic to gradient elution. The mobile phase of acetonitrile and 0.05% acetic acid in the ratio of 90:10, v/v in gradient elution mode with a run time of 40 minutes was used for analysis (Table 2). Standard and sample solutions were filtered through 0.22 µm syringe filter before injection and the separation was achieved by using a C_{18} column (250 x 4.6 mm, particle size 5.0 µm). The retention times of KBBA and AKBBA were found 6.323 and 10.406 min at 254 nm while for BBA and ABBA eluted at 15.933 and 30.983 min at 210 nm.

Calibration Curve for Boswellic Acids

The working solutions containing all four standard compounds were prepared to construct a calibration curve. Solutions corresponding to each concentration level were injected in triplicate and linear regression analysis of the boswellic acids peak area (y) versus boswellic acids concentration (x) were calculated. The correlation coefficients ($r^2 \geq 0.9957$) obtained for each boswellic acid for the regression line demonstrates that there is a strong linear relationship between peak area and concentration of boswellic acids **(Table 3 & Figure 4)**.

Analysis of Samples

The *Boswellia Serrata gum* was analyzed for the content of four different boswellic acids. Samples were confirmed for its presence of four boswellic acids. The method found to be capable enough to separate the four boswellic acids in standard and samples. The peak areas of triplicate samples were analysed by regression equation obtained from calibration plot to get the content of boswellic acids in samples. It was found to contain 0.796, 0.820, 0.619 and 0.356% w/w of KBBA, AKBBA, BBA and ABBA respectively in *Boswellia Serrata* gum samples. The sample and

standard chromatograms are given in **Figure 5 & 6**.

Validation

The proposed method was validated as per the ICH guidelines for different parameters like accuracy, precision, robustness, LOD and LOQ. The recovery of the method was found in the range of 99.6-102.1, 97.5-102.5, 98.0-101.4 and 98.5-103.0 % for KBBA, AKBBA, BBA and ABBA, respectively **(Table 4)**. The intermediate precision has been evaluated by carrying out intra-day, inter-day, and inter-analyst precisions. The low values of % RSD (0.35-2.03) indicated the reproducibility of the method, which can be adopted in any laboratory for the routine analysis boswellic acids **(Table 5)**. The robustness was studied by varying small changes in flow rates and detection wavelengths. The percentage standard deviations of area and retention time was monitored and found satisfactory. The results of the experiments were listed in **Table 6 & 7**. The LOD and LOQ were determined by signal to noise ratio method. The LOD found to be 0.4 and 1.5 µg/mL for KBBA and AKBBA while 1.0 µg/mL for both BBA and ABBA, respectively. The LOQ found to be 1.0 µg/mL for KBBA and 5.0 µg/mL for AKBBA, BBA and ABBA.

CONCLUSION

Being the first report on the physical and chemical specifications of *Boswellia serrata* gum exudates this information would be useful as a guideline for quality control and standardization of raw materials used drugs and cosmetics. Standardization of herbal drugs was always an issue since the herbal products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant. The strategy applied here for the standardization can be successfully adopted for the quality control/ standardization similar herbal materials. The work has given emphasis on the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles along with the conventional parameters followed for the standardization of *Boswellia serrata* and its formulations.

REFERENCES

[1] Gupta I, Parihar A, Malhotra B, Singh GB, Ludtke R, Safayhi H, Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis, Eur. J. Med. Res. 37, 1997, 43-46.

[2] Pandey RS, Singh BK, Tripathi YB. Extract of gum resins of *Boswellia serrata* L. inhibits lipopolysaccharide induced nitric oxide production in rat

macrophages along with hypolipidemic property, Indian. J. Exp. Biol. 43, 2005, 509–516.

[3] Chevrier MR, Ryan AE, Lee DY, Zhongze M, Wu-Yan Z, *Boswellia carterii* extract inhibits TH1 cytokines and promotes TH2 cytokines *in vitro*, Clin. Diagn. Lab. Immunol. 12, 2005:, 575–580.

[4] Ajmeera RR, Ciddi V, Asres K, *In Vitro* and *In Vivo* inhibitory activities of four Indian medicinal plant extracts and their major components on rat aldose reductase and generation of advanced glycation endproducts, Phytother. Res. 27, 2012, 753–760.

[5] Chatterjee GK, Pal SD, Indian Drugs 21, 1984: 431.

[6] Kirtikar KR, Basu BD, Indian Medicinal Plants, Vol. I, 1935, 521–529.

[7] Ammon HP, Boswellic acidinchronic inflammatory diseases, Planta. Med., 72, 2006, 1100–1166.

[8] Poeckel D, Werz O, Boswellic acids: biological actions and molecular targets. Curr. Med. Chem. 13, 2006, 3359–3369.

[9] Khadem H, El-Shafei Z, El-Sekeily M, Abdel Rahman MM, Derivatives of boswellic acids, Planta Med., 22, 2009, 157–159.

[10] Safayhi H, Mack T, Sabieraj J, Anazodo MI, Subramanian LR, Ammon HP, Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase, J. Pharmacol. Exp. Ther., 261, 1992, 1143-1146.

[11] Gupta RK, Gupta VN, Gupta VK and Atal CK, Non-aqueous method for estimation of total triterpene acids in Salai guggal and its different forms, Indian Drugs, 21, 1984, 523–525.

[12] Mona AT, Astrid K, Bahr U, Karas M, Wurglics M, Manfred SZ, Development of a high-performance liquid chromatographic method for the determination of 11-keto-β-boswellic acid in human plasma, J. Chromatogr. B, 761, 2001, 221–227.

[13] Ganzera M, Khan IA, A reversed-phase high-performance liquid chromatography method for the analysis of boswellic acids in *Boswellia serrata*, Planta Med., 67, 2001, 778–780.

[14] Berthold B, Waltraud Z, Felicitas G, Thomas S, 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, J. Chromatogr. B, 829, 2005, 144–148.

[15] Krohn K, Rao MS, Raman NV, Khalilullah M, HPTLC analysis of anti-inflammatory triterpenoids from *Boswellia serrata* Roxb. Phytochem. Anal., 12, 2001, 374–376.

[16] 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. Separation Sci., 29, 2006, 2245–2250.

[17] Gerbeth K, Meins J, Kirste S, Momm F, Manfred SZ , Mona AT, Determination of major boswellic acids in plasma by high-pressure liquid chromatography/mass spectrometry, J. Pharm. Biomed. Anal., 56, 2011, 998–1005.

[18] Shah SA, Rathod IS, Suhagia BN, Pandya SS, Parmar VK, A simple high-performance liquid chromatographic method for the estimation of boswellic acids from the market formulations containing *Boswellia serrata* extract, J. Chromatogr. Sci., 46, 2008, 735-738.

Table 1: Physico-Chemical Parameters of the *Boswellia serrata* Extract

S. No.	Parameters	Observations	Limits (Lower-Upper)
1.	Colour	Off white	
2.	Odour	Characteristic	
3.	Taste	Bitter	
4.	Loss on drying at 105°C (% w/w)	6 ± 0.01	6.0-7.0
5.	Moisture content by Karl Fischer method (% w/w)	5.9 ± 0.15	5.5-7.0
6.	Total ash (% w/w)	1.2 ± 0.19	1.0-1.5
7.	Acid insoluble ash (% w/w)	0.06 ± 0.015	0.050-0.063
8.	Water soluble ash (% w/w)	0.22 ± 0.02	0.19-0.25
9.	pH of 1 % suspension	4.8 ± 0.017	4.5-5.5
10.	pH of 10 % suspension	4.45 ± 0.05	4.2-5.2
11.	Petroleum ether extractive value (% w/w)	1.86 ± 0.12	1.5-2.0
12.	Chloroform extractive value (% w/w)	3.76 ± 0.16	3.0-4.5
13.	Acetone extractive value (% w/w)	3.9 ± 0.10	3.5-4.8
14.	Methanol extractive value (%)	12.6 ± 0.40	12.0-13.0
15.	Water soluble matter (% w/w)	26.4 ±1.70	25.0-28.0
16.	Alcohol soluble matter (% w/w)	14.3 ± 0.94	13.5-15.0

Table 2: HPLC Gradient Elution Programme for the Analysis of Boswellic Acids

Time in minutes	Gradient flow (mL/min)
1	1
5	1.5
10	2
30	2
32	1

Table 3: Linearity of the HPLC Analysis of Boswellic Acids

Compound	Retention time ± SD (min)	Linear range (µg/mL)	Linear regression equation	Correlation coefficient (r^2)
KBBA	6.323±0.09	1-100	y = 15774x - 10558	0.9978
AKBBA	10.406±0.11	5-600	y = 15472x - 10642	0.9986
BBA	15.933±0.10	5-300	y = 12425x + 18172	0.9984
ABBA	30.983±0.21	5-300	y = 10228x + 18993	0.9957

Table 4: Accuracy of the HPLC Analysis of Boswellic Acids (n=3)

% of Standard spiked to the sample	Theoretical content (µg/mL)	Amount of drug recovered (µg ± SD)	% Drug recovered	% RSD
KBBA				
0	220.8	219.9±1.90	99.6	0.87
50	331.2	330.7±1.97	99.8	0.60
100	441.6	446.9±1.96	101.2	0.44
150	552.0	563.3±4.16	102.1	0.74
AKBBA				
0	304.0	311.5±4.71	102.5	1.51
50	456.0	454.6±4.04	99.7	0.89
100	608.0	592.5±6.48	97.5	1.09
150	760.0	772.4±4.57	101.6	0.59
BBA				
0	181.6	178.0±1.50	98.0	0.84
50	272.4	270.6±5.62	99.4	2.08
100	363.2	367.8±1.53	101.3	0.42
150	454	460.1±5.00	101.4	1.18
ABBA				
0	94.4	93.0±1.41	98.5	1.52
50	141.6	142.9±2.51	100.9	1.76
100	188.8	194.5±2.50	103.0	1.29
150	236	236.4±3.09	100.2	1.31

Table 5: Precision of the HPLC Analysis of Boswellic Acids (n=3)

Conc (µg/mL)	Interday precision		Intraday precision		Inter analyst precision	
	Mean peak area ± SD	%RSD	Mean peak area ± SD	%RSD	Mean peak area ± SD	%RSD
KBBA						
20	319646±8517	2.7	329713±1978	0.6	317180±5700	1.8
50	722778±1609	0.22	723672±4546	0.63	724092±3453	0.48
100	1597765±5312	0.33	1614551±32063	1.99	1594195±4270	0.27
AKBBA						
100	1592254±6241	0.39	1585329±22303	1.41	1561661±5436	0.35
200	3058360±55573	1.82	3056802±24527	0.80	3059116±28835	0.94
300	4849606±39741	0.82	4849614±35062	0.72	4867795±53248	1.09
BBA						
100	1348477±34875	2.59	1381200±17091	1.24	1356936±6389	0.47
200	2577128±12441	0.48	2561825±36558	1.43	2597129±8991	0.35
300	3638464±77445	2.13	3680395±53421	1.45	3614721±51166	1.42
ABBA						
100	1095758±20315	1.85	1097841±10091	0.92	1105034±22428	2.03
200	2189341±12557	0.57	2160975±26938	1.25	2179556±19415	0.89
300	2951726±37859	1.28	2982973±4056	0.14	2956535±45652	1.54

Table 6: Robustness of the HPLC Analysis of Boswellic Acid by Changing Mobile Phase Composition (n=3)

Mobile phase composition (v/v)	KBBA		AKBBA		BBA		ABBA	
	Retention time (%RSD)	Peak area (%RSD)	Retention time (%RSD)	Peak area (%RSD)	Retention time (%RSD)	Peak area (%RSD)	Retention time (%RSD)	Peak area (%RSD)
88:12	1.12	1.55	0.71	1.87	1.91	0.69	1.40	0.29
90:10	0.66	0.70	0.38	1.63	0.80	0.14	1.83	0.39
92:8	0.81	0.77	0.49	1.72	0.62	1.32	0.39	0.22

Table 7: Robustness of the HPLC Analysis of Boswellic Acid by Changing Mobile Phase Composition (n=3)

Components	Detection wavelength (nm)	Retention time (%RSD)	Peak area (%RSD)
KBBA	256	0.55	0.43
	254	0.87	0.91
	252	1.25	0.74
AKBBA	256	1.72	0.77
	254	1.11	0.61
	252	0.55	0.19
BBA	212	0.65	0.39
	210	1.65	0.90
	208	1.98	1.56
ABBA	212	0.84	1.51
	210	0.98	1.66
	208	1.33	0.76

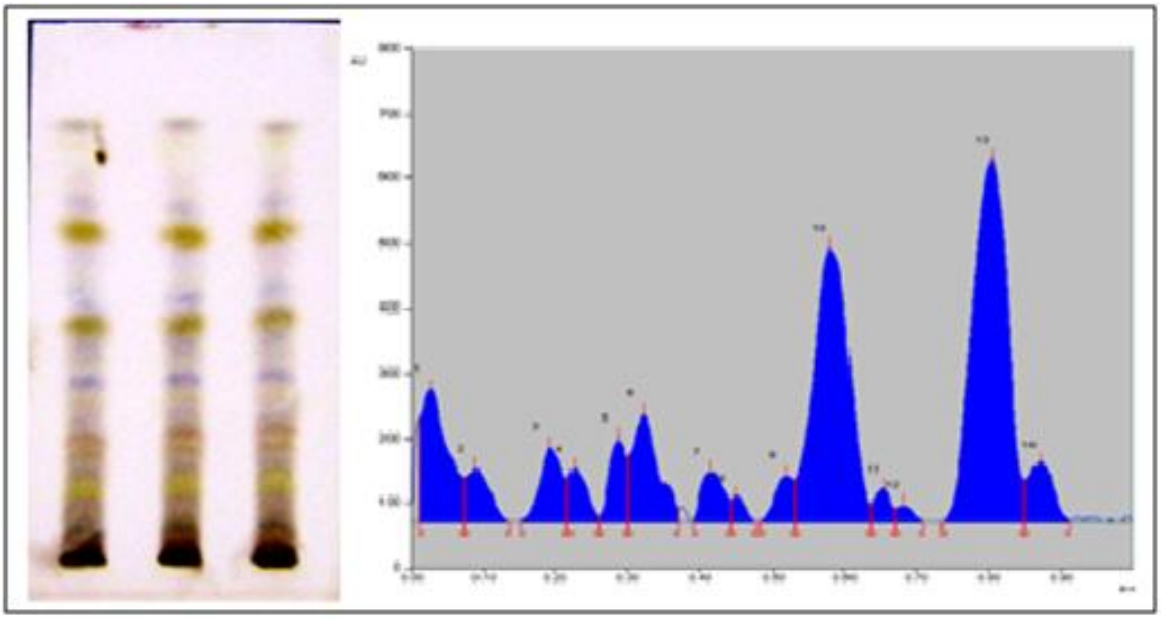

Figure 1: Developed TLC plate of Methanolic Extract at day Light After Derivatization and HPTLC Chromatogram Scanned at 450 nm

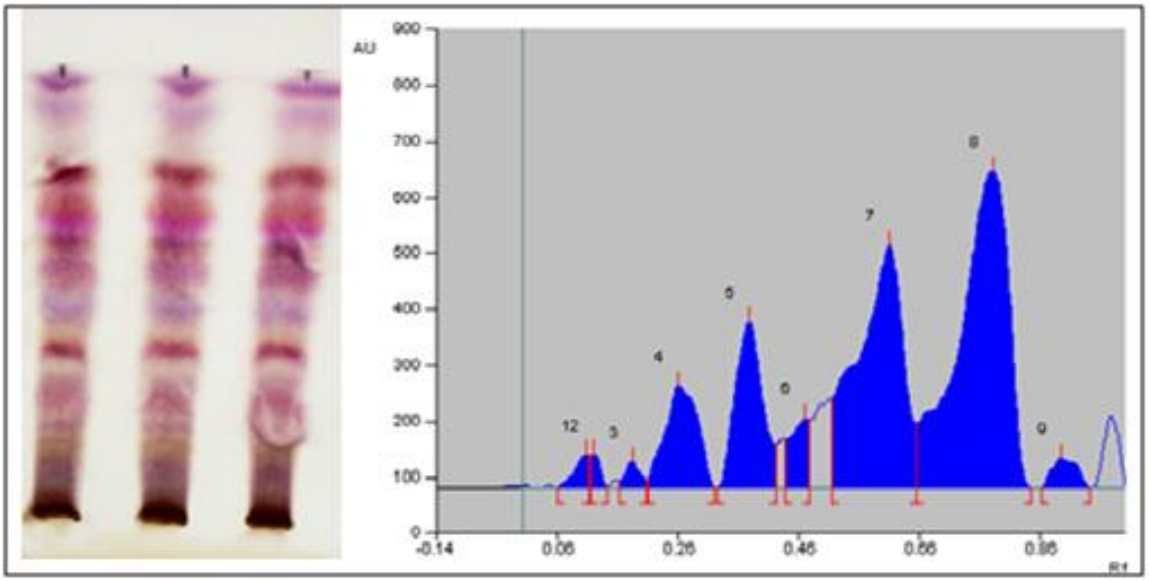

Figure 2: Developed TLC Plate of Chloroform Extract at Day Light After Derivatization and HPTLC Chromatogram Scanned at 450nm

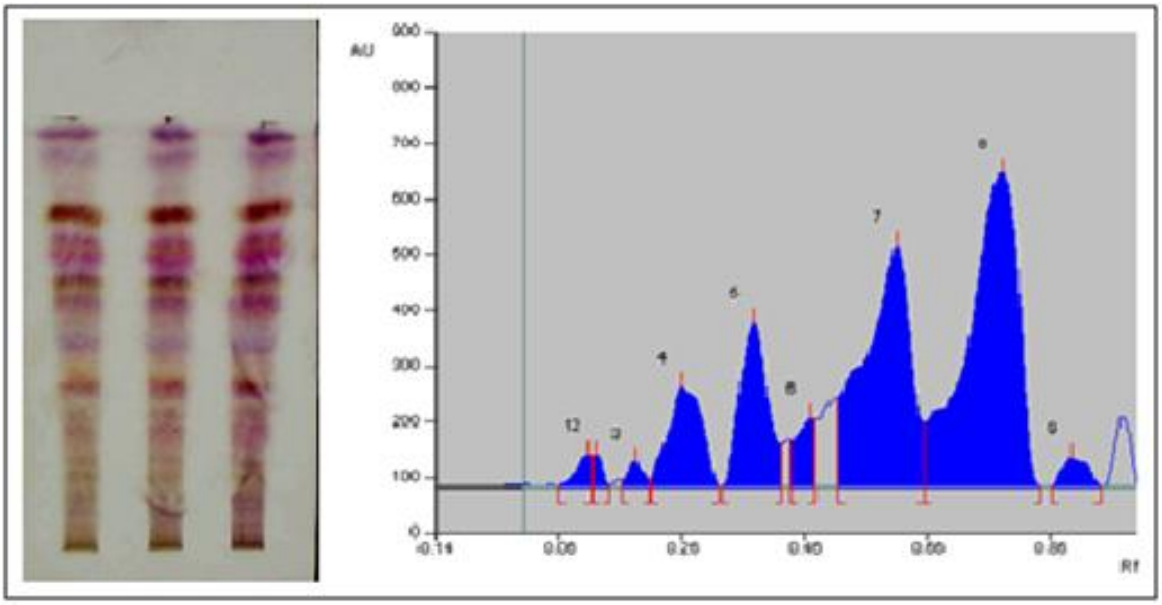

Figure 3: Developed TLC Plate of Petroleum Ether Extract of at Day Light After Derivatization and Scanned at 450nm

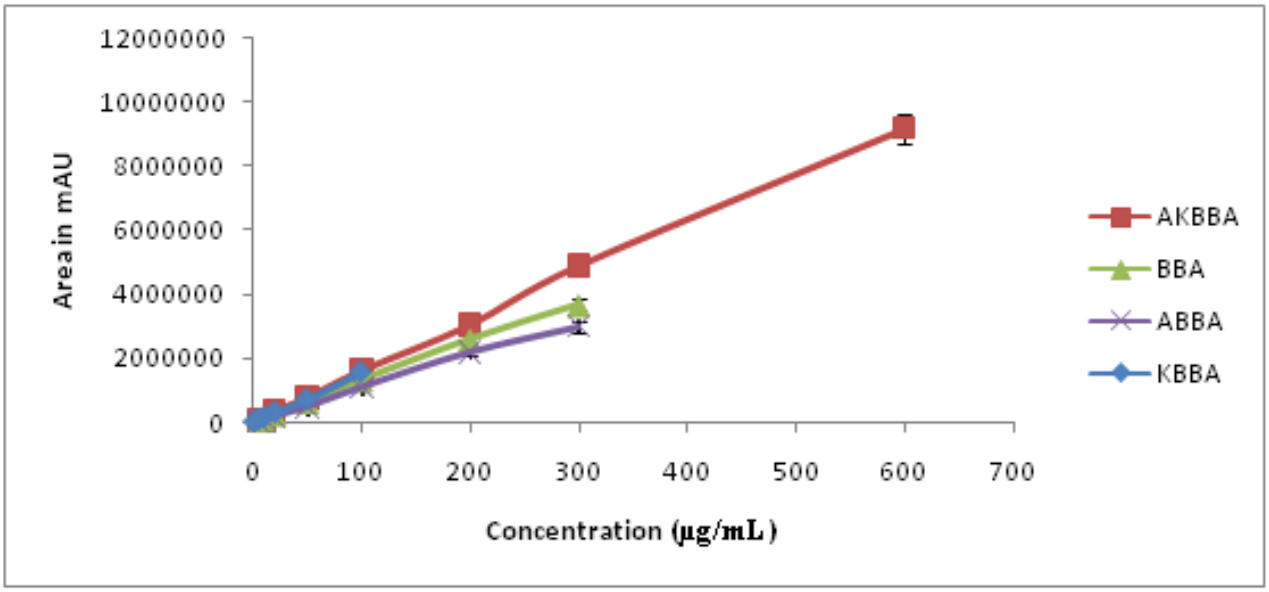

Figure 4: Linearity Curve Plotted Using Four Boswellic Acids

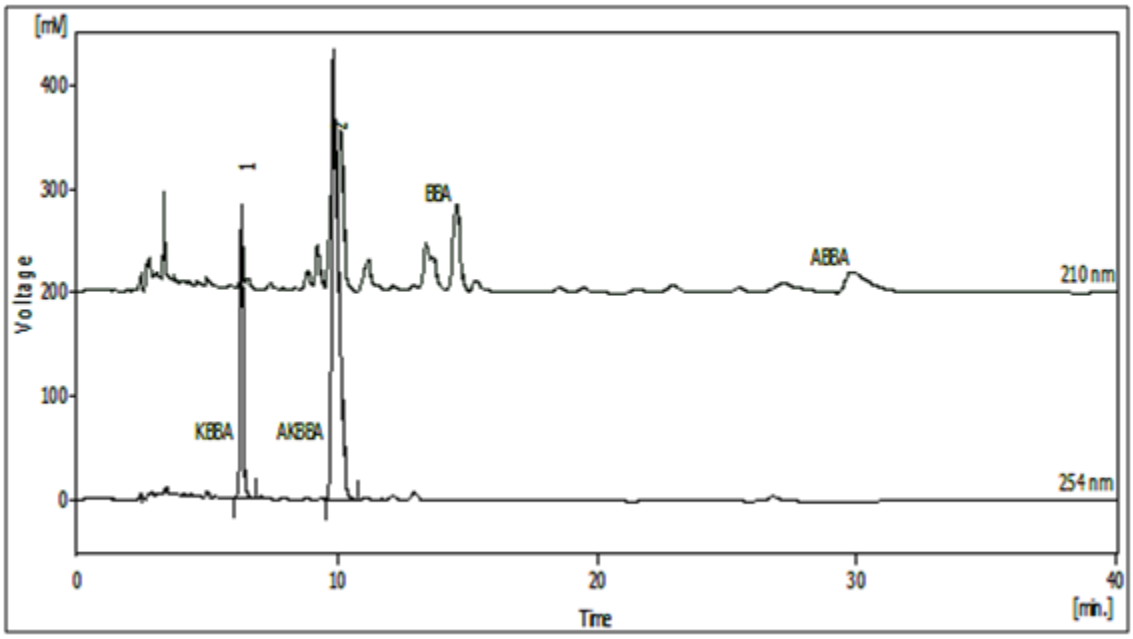

Figure 5: HPLC Chromatogram of standard KBBA, AKBBA at 254 nm and BBA, ABBA at 210 nm

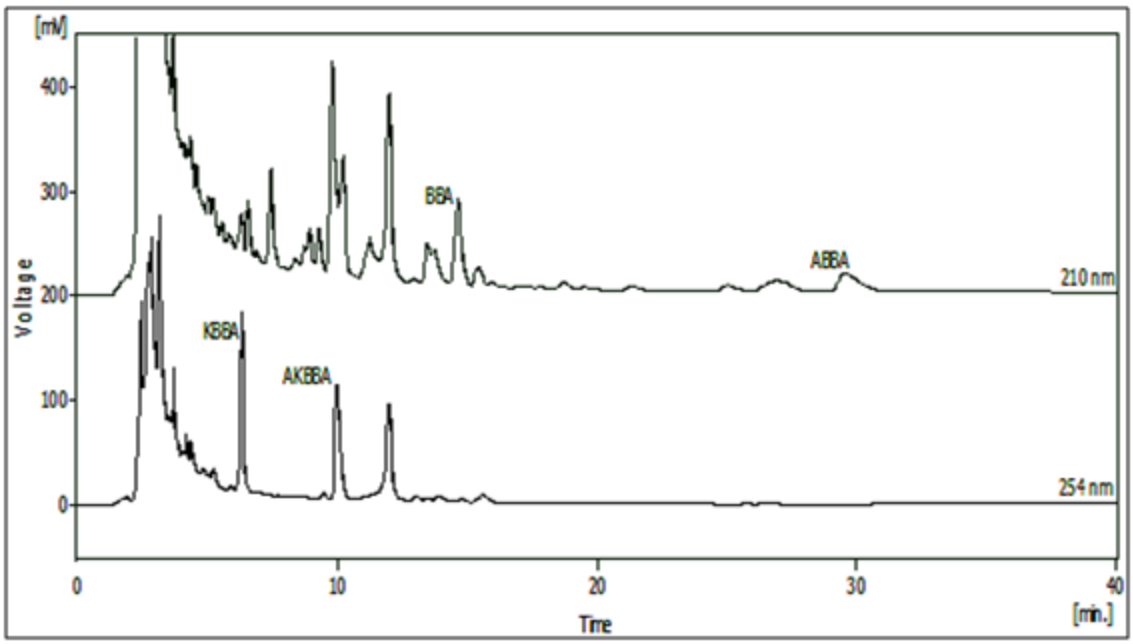

Figure 6: HPLC Chromatogram of KBBA, AKBBA at 254 nm and BBA, ABBA at 210 nm Crude Drug