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BOSWELLIC ACID ISOLATION FROM BOSWELLIA SERRATA OLEO GUM RESIN USING QBD APPROACH

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Abstract:
This study employs a Quality by Design (QbD) approach to isolate boswellic acid from Boswellia serrata oleo gum resin. Solubility studies using ten different solvents, analyzed via thin layer chromatography (TLC), indicated that all solvents except water were effective for extraction. Among various extraction methods evaluated, maceration proved to be the most effective, though it required multiple extractions with fresh solvent to achieve complete extraction of boswellic acids. The extracted material was then concentrated, leading to significant evaporation of volatile oils. To isolate total acids, the resin was treated with alkali to convert the acids into their salt forms, followed by precipitation using a mineral acid, resulting in a white precipitate. The efficiency of KOH and NaOH as basifying agents was compared, with 2% KOH yielding the highest amount of isolated acid. This allowed for the successful isolation of acetyl keto β-boswellic acid (AKBA) and a mixture of α and β boswellic acids.
The identities of the isolated compounds were confirmed through spectroscopic analyses,This study demonstrates a systematic and efficient approach to the extraction and isolation of bioactive compounds from Boswellia serrata, highlighting its potential therapeutic applications.
Keywords: Boswellic acid, Boswellia serrata, Quality by Design, extraction, isolation, maceration, TLC, spectroscopic analysis.

Introduction:

Boswellia serrata, commonly referred to as Indian frankincense, has been revered for centuries in traditional Ayurvedic and Unani medicine due to its potent anti-inflammatory, analgesic, and anti-arthritic properties.[1] The oleo gum resin extracted from Boswellia serrata is particularly valuable because it contains a complex mixture of bioactive compounds, notably boswellic acids. These

acids, including β-boswellic acid and acetyl-11-keto-β-boswellic acid (AKBA), have been extensively studied for their pharmacological benefits, such as reducing inflammation and potentially treating chronic diseases like arthritis and inflammatory bowel disease.[2]

In modern pharmaceutical research, the Quality by Design (QbD) approach has gained prominence as a systematic methodology that emphasizes designing processes with predefined objectives and an understanding of variability sources. This approach ensures consistent quality and efficiency, making it highly relevant for the extraction and isolation of complex natural products like boswellic acids. Implementing QbD principles can enhance the reproducibility, scalability, and overall success of isolating high-purity compounds from natural sources.[3,4]

The primary objective of this study is to develop a QbD-based protocol for the efficient extraction and isolation of boswellic acids from Boswellia serrata oleo gum resin. This involves several key steps:

1. **Selection of Suitable Solvents:** Conducting solubility studies with various solvents to determine the most effective medium for extracting oleo gum resin.
2. **Optimization of Extraction Methods:** Comparing different extraction techniques, with a focus on maceration, to ensure complete and efficient extraction of boswellic acids.
3. **Isolation of Total Acids:** Employing alkali treatment to convert the acids into their salt forms, followed by precipitation using mineral acids, and evaluating the impact of different basifying agents.
4. **Isolation of Individual Boswellic Acids:** Using column chromatography with distinct solvent systems to separate and purify individual boswellic acids, including AKBA and mixtures of α and β boswellic acids.
5. **Spectroscopic Analysis:** Confirming the identity and purity of the isolated compounds through comprehensive spectroscopic techniques.

Through this structured and systematic approach, the study aims to provide a robust and reproducible method for isolating high-purity boswellic acids from Boswellia serrata. This not only enhances the therapeutic potential of these compounds but also contributes to the broader field of natural product extraction and pharmaceutical development.[5-11]

Materials and Methods

1. Selection of Suitable Solvent

To determine the most appropriate solvent for extracting oleo gum resin, solubility studies were conducted using ten commonly available solvents, including water. Each solvent was tested, and thin layer chromatography (TLC) was used to analyze the extracts. All solvents produced identical TLC patterns except for water, indicating that any solvent other than water could be effectively used for extraction.[12]

2. Selection of Extraction Method for Oleo Gum Resin

Various extraction methods were evaluated to isolate oleo gum resin, which comprised polysaccharides, volatile oils, and resin containing boswellic acids. Soxhlet extraction and other heat-based methods resulted in clogging and melting issues. Maceration was found to be effective, albeit requiring multiple extractions with fresh solvent each time to ensure complete extraction of boswellic acids. TLC was used to verify the presence of boswellic acids in each extract, with 4-5 repetitions necessary for complete extraction. Post-extraction, the material was concentrated, leading to significant evaporation of volatile oils. (A) and with Anisaldehyde sulphuric acid (ASA) (B). Photograph 2 displayed the TLC fingerprints confirming the completeness of extraction.[13]

3. Isolation of Total Acids (Boswellic Acid)

Boswellic acids, the oleo gum resin's active anti-inflammatory ingredients, were isolated by treating the resin with an alkali to change the acids into their salts, then precipitating the salts with a mineral acid. We looked at the effects of changing the KOH and NaOH concentrations used as basifying agents. A mobile phase comprising toluene, ethyl acetate, n-heptane, and formic acid in an 8:2:1:0.3 ratio was used for the TLC. The stationary phase was a precoated silica gel plate with a fluorescent indicator (F254) from Merck PSGF254. Anisaldehyde sulphuric acid (ASA) (B) and UV light at 254 nm (A) were used for the detection. The TLC fingerprints for the impact of KOH and NaOH, respectively, on the acid fraction separation were shown in photographs 3.1 and 3.2.[14]

4. Isolation of Individual Boswellic Acids

The goal was to separate a combination of α and β boswellic acids as well as acetyl keto β-boswellic acid (AKBA). Two distinct solvent systems were used in column chromatography. Toluene, n-heptane, formic acid, and ethyl acetate were employed in the first system, while Toluene, ethyl acetate, and methanol (8:2:1) were used in the second. Using TLC and spectroscopic analysis, fractions comprising of α and β boswellic acids and pure AKBA were detected. A mobile phase comprising toluene, ethyl acetate, n-heptane, and formic acid in an 8:2:1:0.3 ratio was used for the TLC. The stationary phase was a precoated silica gel plate with a fluorescent indicator (F254) from Merck PSGF254. At 254 nm, detection was done in the presence of UV radiation. The TLC fingerprints of the fractions from column chromatography were shown in photos 4.1 and 4.2.[15]

β-Boswellic Acid:

The chemical name of β-boswellic acid is 3a-hydroxy-urs-12-en-23-oic acid, with a molecular formula of C30H48O3. The compound exhibited a melting point of 226-228°C. The Fourier-transform infrared (FTIR) spectroscopy analysis, conducted in potassium bromide (KBr), showed significant absorption bands at 3500 cm^{-1}, indicating the presence of hydroxyl groups, and at 1699 cm^{-1}, corresponding to carbonyl groups. Ultraviolet (UV) spectroscopy in methanol revealed a maximum absorption at 208 nm. Gas chromatography-mass spectrometry (GC-MS) analysis produced peaks at m/z 394, 218, 203, 189, 175, and 161. Proton nuclear magnetic resonance (1H-NMR) spectroscopy in deuterated chloroform (CDCl3) showed chemical shifts (δ) at 11 (OH of COOH), 5.29 (vinyl proton, C=C), 3.58 (CH-OH), and multiple shifts between 2-1 (methylenes and methines) and 1-0.9 (methyls).[16]

Acetyl-11-keto-β-boswellic Acid:

The chemical name of acetyl-11-keto-β-boswellic acid is 3a-Acetoxy-urs-12-en-11-keto-23-oic acid, with a molecular formula of C32H48O5. The compound had a melting point of 271-274°C. FTIR spectroscopy in KBr indicated absorption bands at 1740 cm^{-1} (acetyl group), 1701 cm^{-1} (carboxyl group), and 1647 cm^{-1} (α, β unsaturated carbonyl group). UV spectroscopy in methanol showed a maximum absorption at 250 nm. GC-MS analysis yielded peaks at m/z 394, 218, 203, 189, 175, and 161. The 1H-NMR spectroscopy in CDCl3 displayed chemical shifts (δ) at 5.5, 3.58, 2.5, and multiple shifts between 2-1 (methylenes and methines) and 1-0.9 (methyls). The spectra

confirmed the presence of specific functional groups and structural features consistent with the literature.[17]

Results and discussion

Selection of suitable solvent:

The solubility of oleo gum resin was investigated in several solvents. For the extraction process, a total of 10 readily accessible solvents were utilized, which included water. All of them exhibited an identical thin-layer chromatography (TLC) pattern, with the exception of water. Any solvent, with the exception of water, can be utilized for the extraction of oleo gum resin.

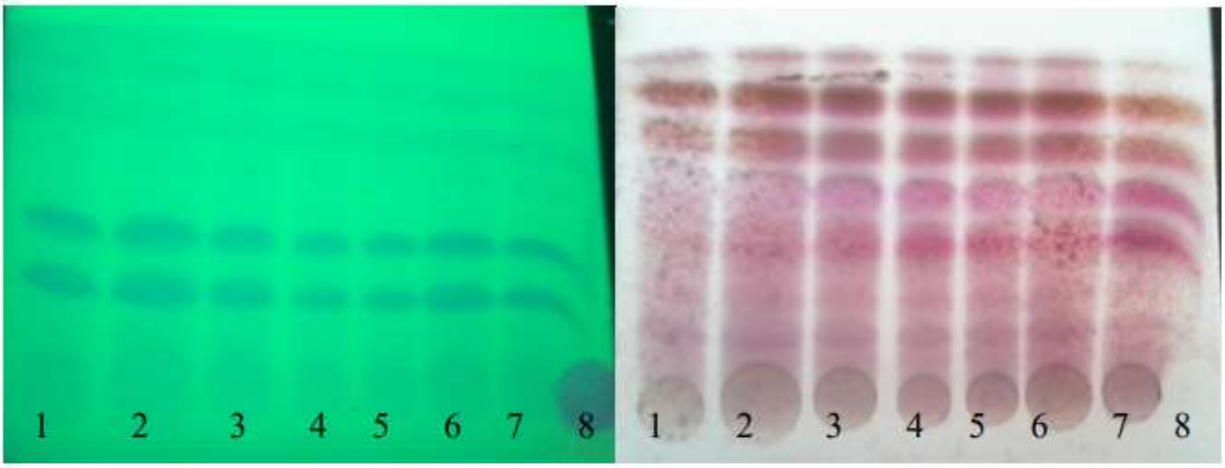


Fig 1: TLC fingerprint for effect of solvents

Selection of extraction method

Choosing the most appropriate extraction method extraction process with oleo gum resin technique

Oleo gum resin underwent a variety of extraction processes. Polysaccharides, gum, volatile oil, and resin come together to form oleo gum resin. Boswellic acids are present in the resin component. The soxhlet extraction process, like other heat-based extraction processes like decoction, is hindered by the potential for material blockage and melting. Maceration does not need heating the substance, although it does require repeated extraction with new solvent. The use of solvents becomes more important in this process. To make sure the extraction was thorough,

TLC was used to look for boswellic acids in the extract at each step. To fully extract the boswellic acids by maceration, it was necessary to repeat the extraction process four or five times. Material concentration was performed after extraction. The volatile oil evaporates significantly during the concentration process.

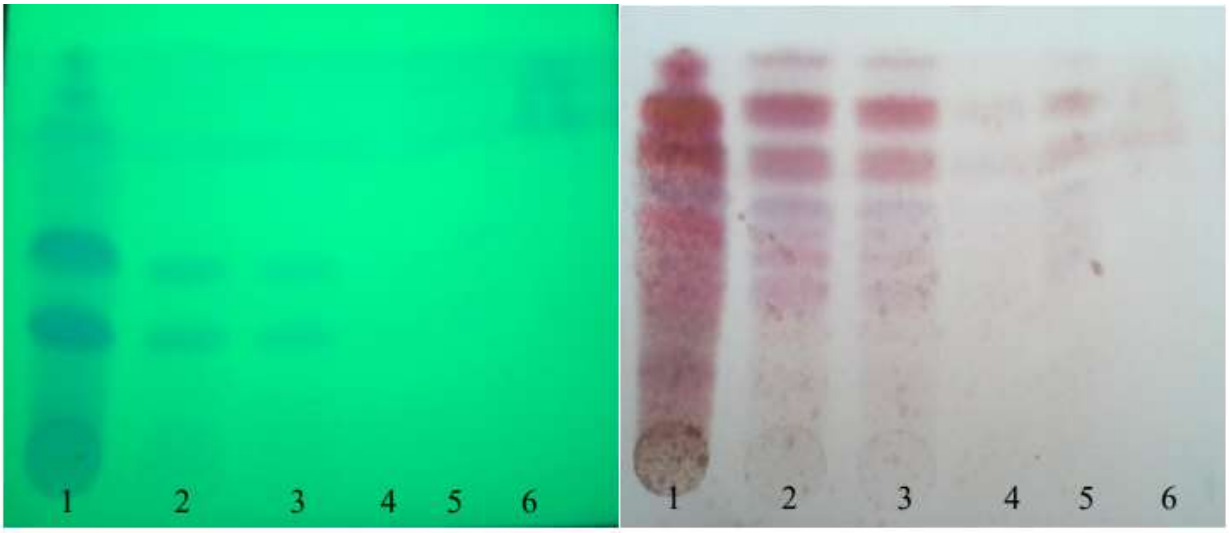


Fig 2: TLC fingerprint for completeness of extraction

Isolation of total acids (boswellic acid)

The acid fraction was isolated in the experiment as a white precipitate by treating the resin with an alkali to change the acid to salt form. This is a typical isolation process. After that, a mineral acid was used to precipitate the acid salt. The quantity of acid fraction produced on a weight basis was determined by separating, drying, and weighing the isolated white precipitate. The acid fraction in the sample may be successfully isolated and quantified using this approach.

Effect of KOH:

Here are some things to keep in mind while using KOH as a basifying agent:

Table 1: The acid fraction's response to varying concentrations of KOH as a basifying agent

Sr. No.	**1**	**2**	**3**	**4**
Percentage of KOH	2% KOH	5% KOH	10% KOH	20% KOH

Amount of acid	1.42g	1.03g	0.61g	0.12g

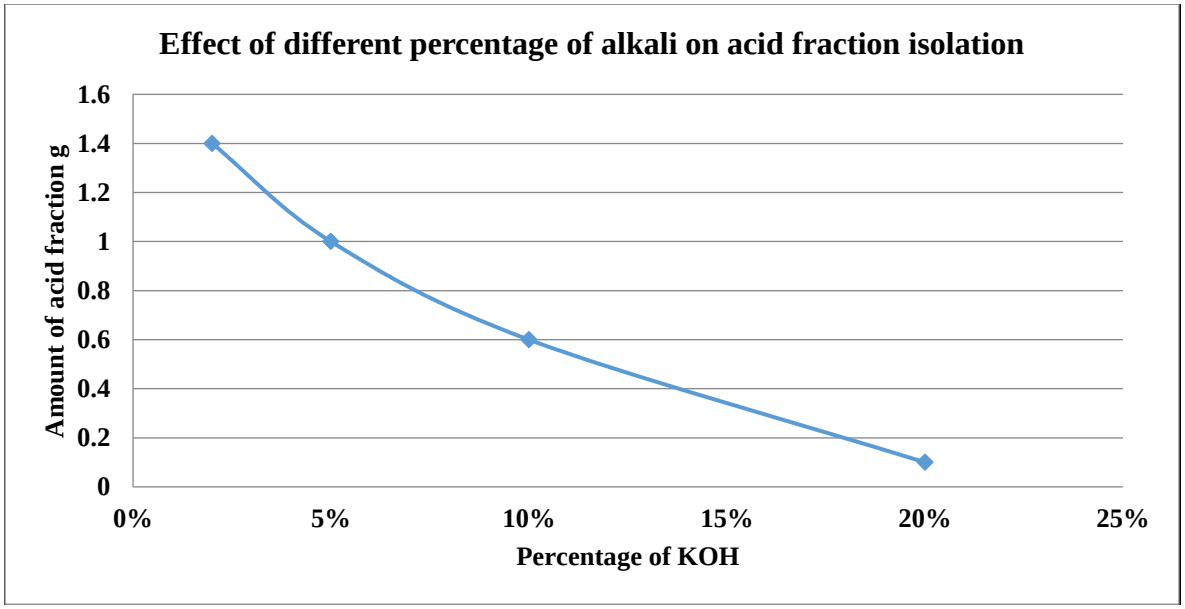


Fig 3: Effect of different percentage of alkali on acid fraction isolation

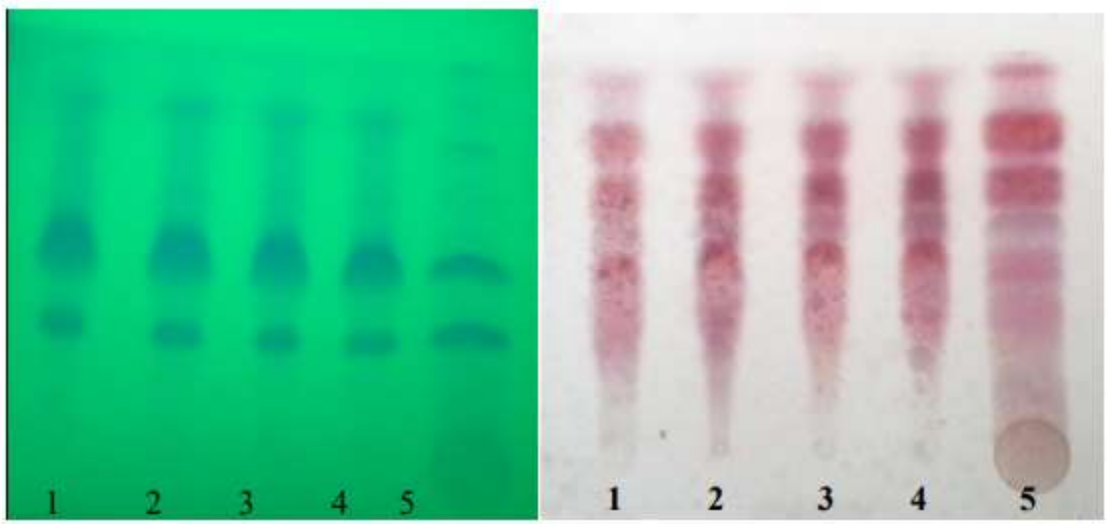


Fig 4: TLC fingerprint for effect of KOH

Effect of NaOH:

Table 2: Effect of percentage of NaOH as basifying agent on acid fraction

Sr. No.	**1**	**2**	**3**	**4**
Percentage of NaOH	2% NaOH	5% NaOH	10% NaOH	20% NaOH
Amount of acid	1.0g	0.9g	0.5g	0.1g

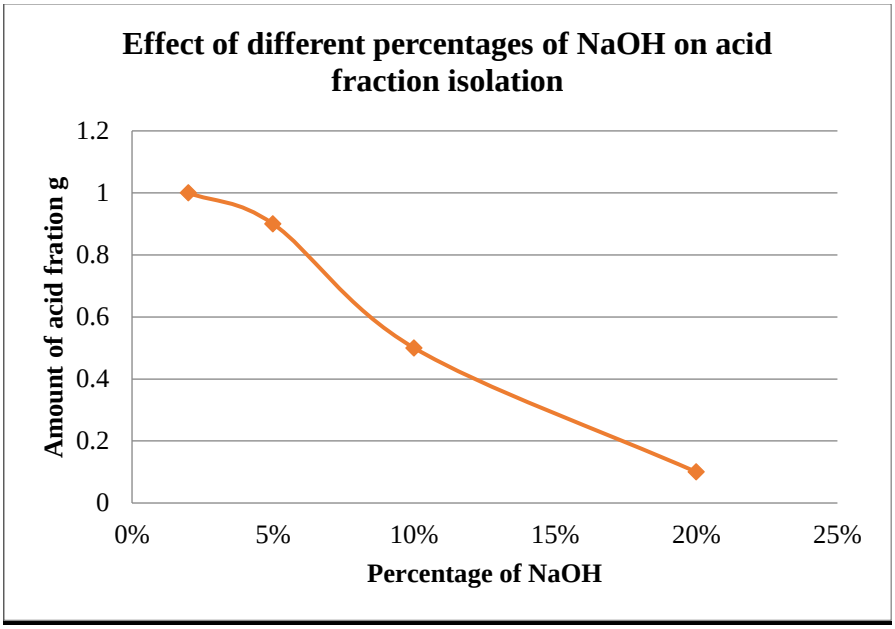


Fig 5: Effect of percentage of NaOH as basifying agent on acid fraction

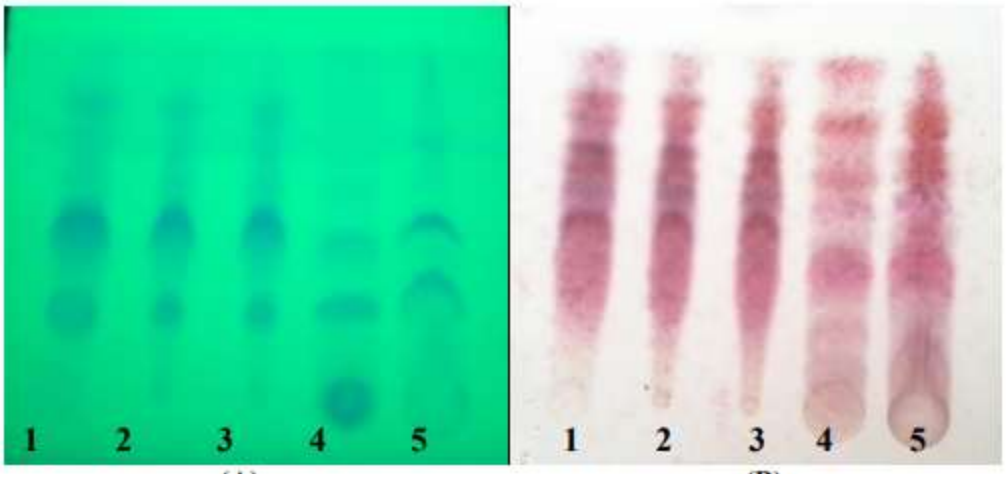


Fig 6: TLC fingerprint for effect of NaOH

Effect of Change in mineral acid

Table 3: Effect of Change in mineral acid on acid fraction

Sr.No.	**Percentage of Alkali**	**Conc. HCl**	**Conc. H_2SO_4**
1.	2% KOH	1.4g	1.2g
2.	5% KOH	1.0g	0.8g
3.	10% KOH	0.6g	0.5g
4.	20% KOH	0.1g	0.1g
5.	2% NaOH	1.0g	1.0g
6.	5% NaOH	0.9g	0.7
7.	10% NaOH	0.5g	0.4
8.	20% NaOH	0.1g	0.1

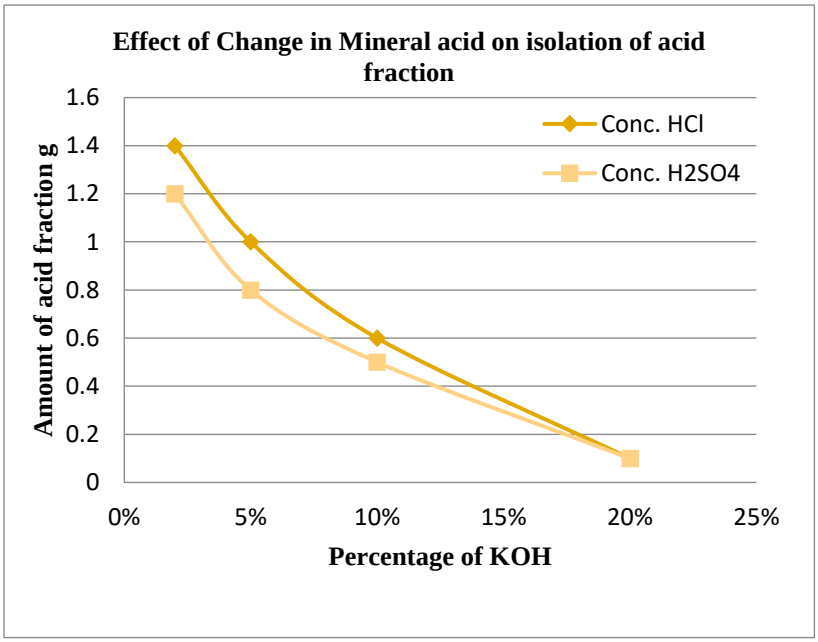


Fig 7: Effect of Change in Mineral acid on isolation of acid fraction (KOH)

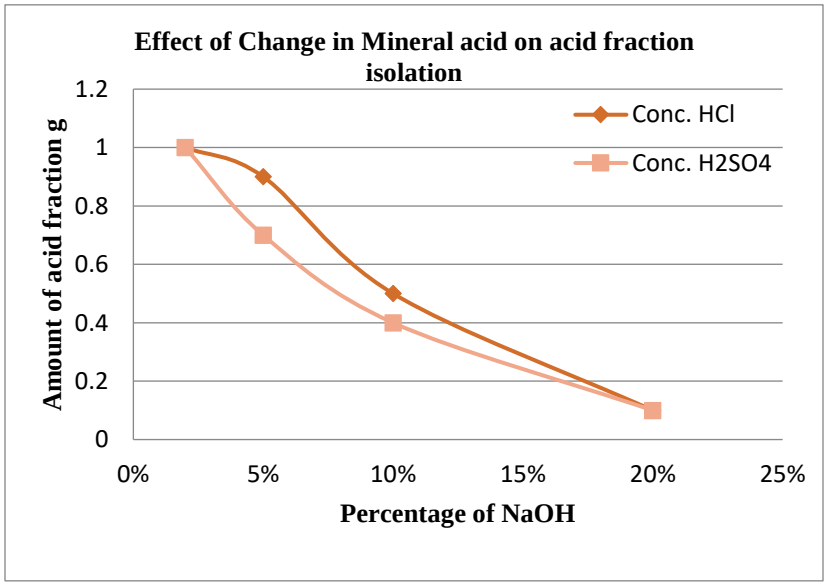


Fig 8: Effect of Change in Mineral acid on isolation of acid fraction (NaOH)

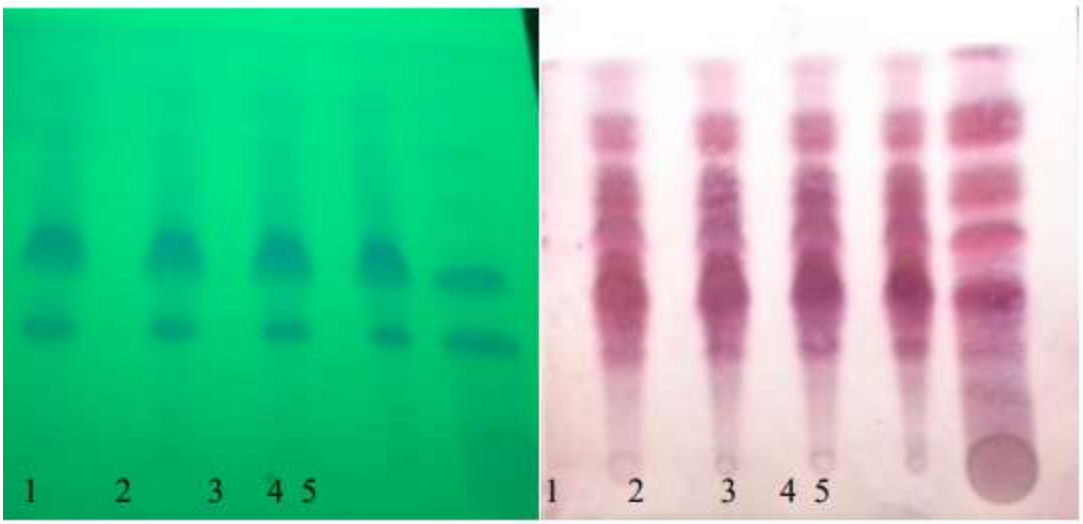


Fig 9: TLC fingerprint for effect of Change in mineral acid on acid fraction (KOH)

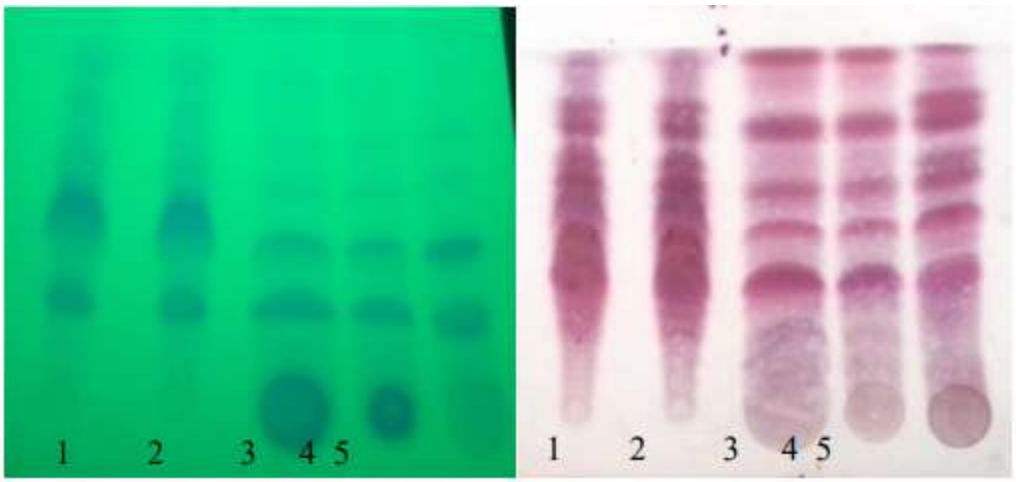


Fig 10: TLC fingerprint for effect of Change in mineral acid on acid fraction (NaOH) (1. 2% NaOH 2. 5 % NaOH 3. 10% NaOH 4. 20 % NaOH 5. Whole extract)

Effect of Change in strength of mineral acid

Table 4: Effect of strength of mineral acid on acid fraction

Sr. No.	**Percentage of Alkali**	**Dil HCl**	**Conc. HCl**	**Dil H_2SO_4**	**Conc. H_2SO_4**
1.	2% KOH	1.5	1.4g	1.0	1.2
2.	5% KOH	1.0	1.0g	0.8	0.8
3.	10% KOH	0.4	0.6g	0.3	0.5
4.	20% KOH	0.05	0.1g	0.1	0.1
5.	2% NaOH	0.8	1.0g	1.0	1.0
6.	5% NaOH	0.6	0.9g	0.6	0.7
7.	10% NaOH	0.5	0.5g	0.3	0.4
8.	20% NaOH	0.1	0.1g	0.05	0.1

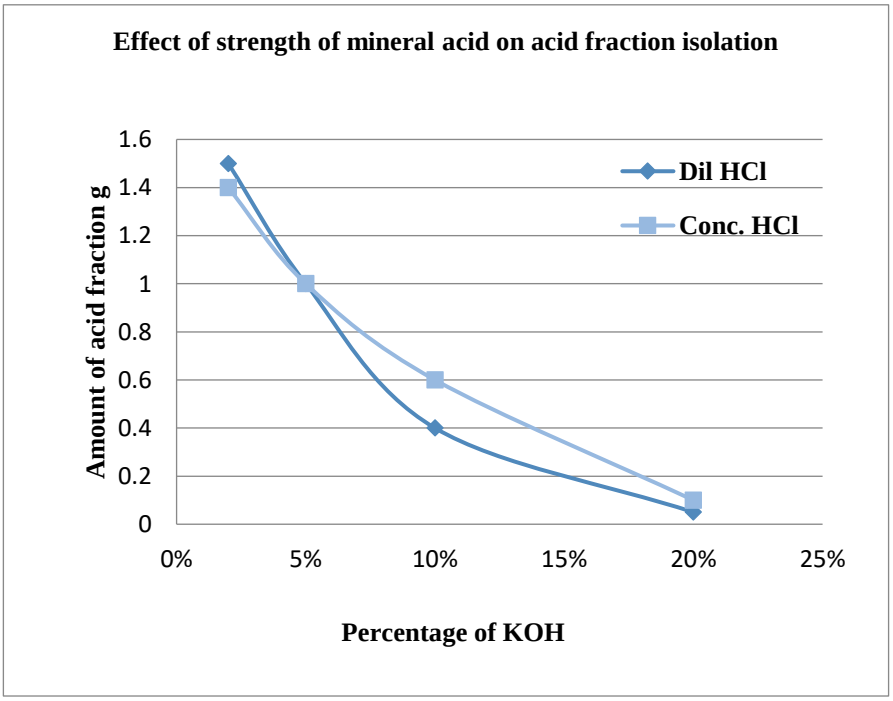


Fig 11: Effect of strength of mineral acid on isolation of acid fraction (HCl + KOH)

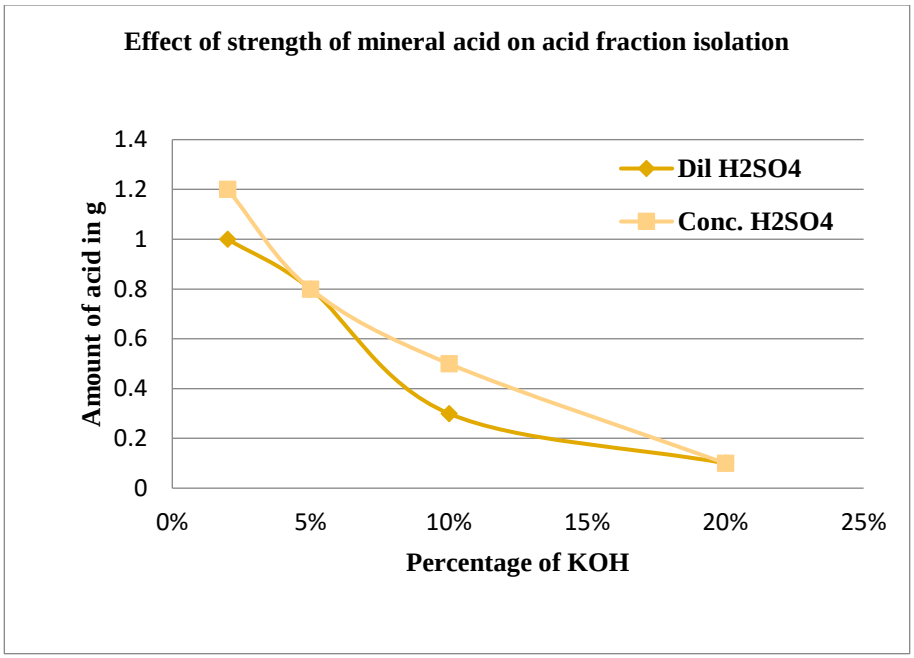


Fig 12: Effect of strength of mineral acid on isolation of acid fraction

(H_2SO_4 + KOH)

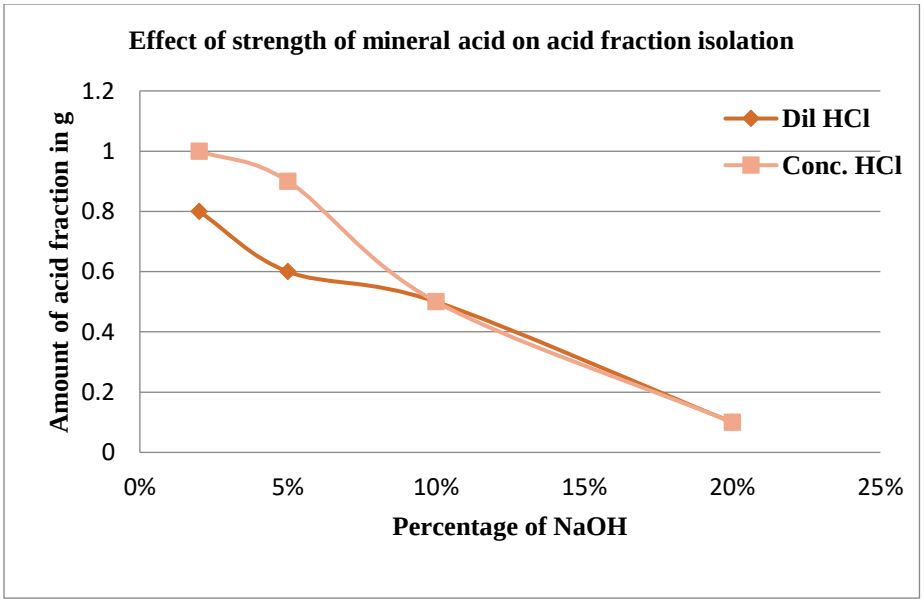


Fig 13: Effect of strength of mineral acid on isolation of acid fraction

(HCl + NaOH)

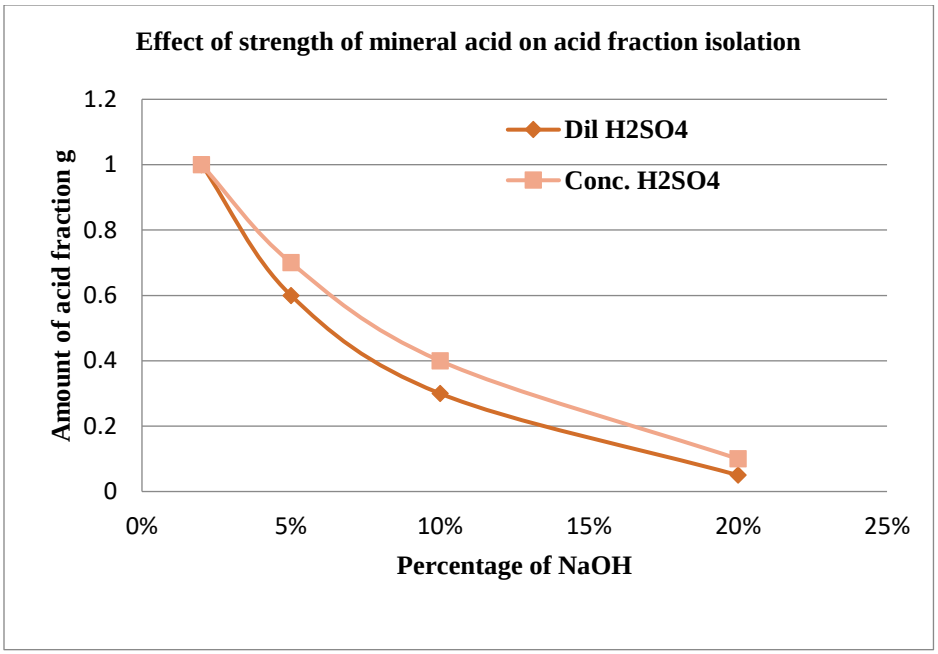


Fig 14: Effect of strength of mineral acid on isolation of acid fraction

(H_2SO_4 + NaOH)

Isolation of individual boswellic acids

Column Chromatography1:

The mobile phase Ethyl acetate: n heptane Toluene : Formic acidwas used for separation of individual acids

Pure AKBA and α and β boswellic acid can be isolated from column chromatography. These compounds were isolated and confirmed by R_f values as well as by spectroscopic studies.

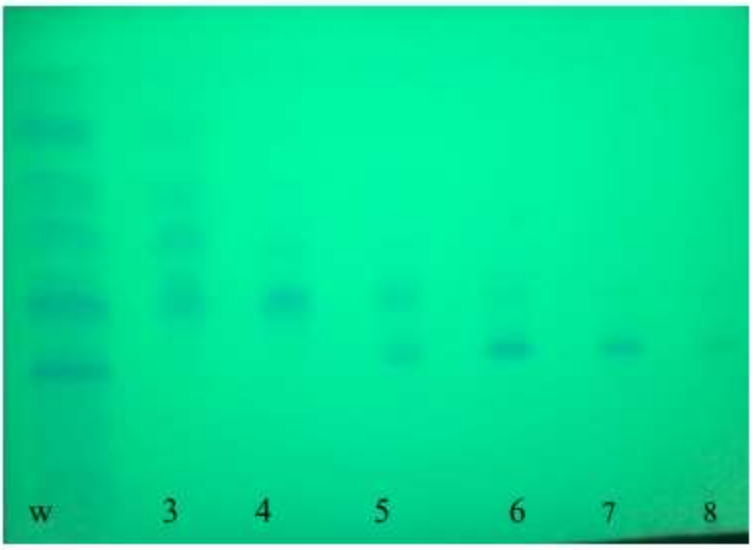


Fig 15: TLC fingerprint of fractions of column 1

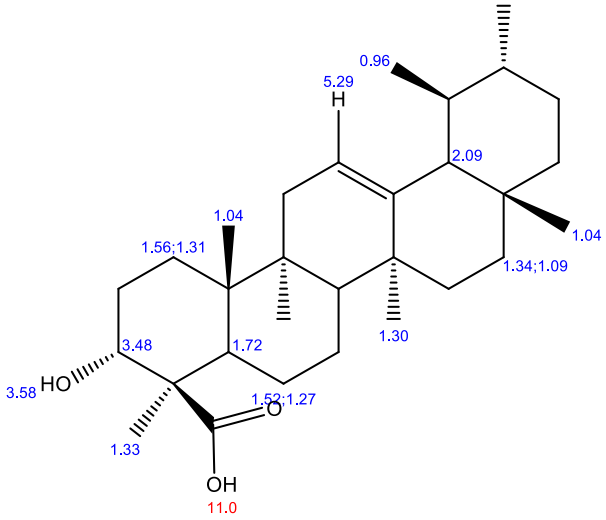
w- Whole Extract, Numbers corresponds to fraction numbers

Column Chromatography 2

Isolation of pure AKBA and α and β boswellic acid can also be done by different solvent systems in column chromatography. Procedure was repeated twice and nearly same fractions yield pure compounds. AKBA was isolated from fractions 6-9 and mixture of α and β boswellic acid was obtained from fractions 10-13. Identity of these two was confirmed by TLC running them with standard.

4.3 Spectroscopic studies:

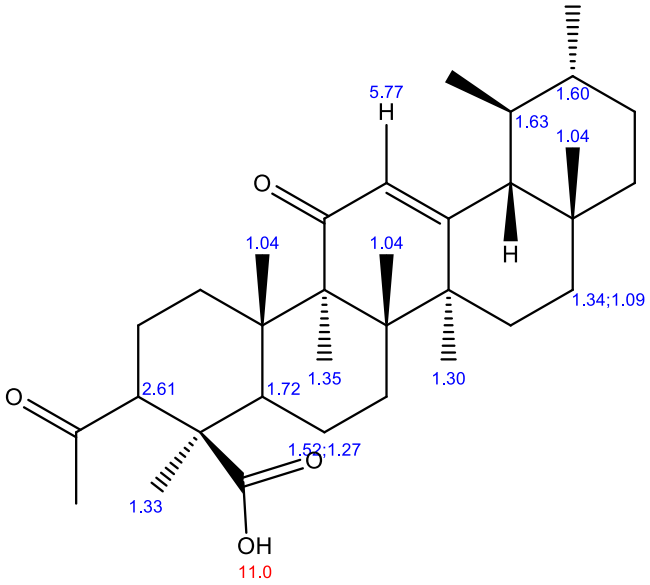
β-Boswellic acid:



- **Chemical Name:** 3a-hydroxy-urs-12-en-23-oic acid
- **Molecular Formula:** $C_{30}H_{48}O_3$
- **Melting Point**: 226-228^{0}C
- **FTIR(in KBr) cm^{-1}**:3500, 1699
- **UV (methanol):** maxima at 208nm
- **GC MS:** 394, 218, 203, 189, 175, 161
- **HNMR ($CDCl_3$)**: 11, 5.29, 3.58, 2-1, 1-0.9

In above spectroscopic data FTIR values 3500 stands for OH group and 1699 stands for COOH.Molecular ion peak of 394 is due to loss of CO_2(-44) andH_2O(-18). 218 is base peak. Proton NMR δ values of 11 indicates OH of COOH. 5.29 indicates presence of C=C i.e. vinyl proton. 3.58 indicates CH-OH. Other values from 2 to 1stands for methylenes and methines 23 protons and from 1-0.9 stands for methyls i.e 21 protons.NMR values are mentioned in structure.

Acetyl-11-keto-β-boswellic acid:



- **Chemical Name:** 3a-Acetoxy-urs-12-en-11-keto-23-oic acid
- **Molecular Formula:** $C_{32}H_{48}O_5$
- **Melting Point**: 271-274^{0}C
- **FTIR(in KBr) cm^{-1}**: 1740, 1701, 1647
- **UV (methanol):** maxima at 250nm
- **GC MS:** 394, 218, 203, 189, 175, 161
- **HNMR ($CDCl_3$)**: 5.5, 3.58, 2.5, 2-1, 1-0.9

In the above spectroscopic data IR values of 1740 indicates presence of acetyl group, 1701 is for COOH and 1647 for α, β unsaturated carbonyl. Molecular ion peak of 408 indicates loss of CO_2 (-44)and H_2O(-18). NMR δ values of 11 indicates OH of COOH. 5.29 indicates presence of C=C i.e. vinyl proton. 3.58 indicates CH-OH. Other values from 2 to 1stands for methylenes and methines 21 protons and from 1-0.9 stands for methyls i.e 21 protons. Other NMR values are mentioned in structure.

Conclusion

This study successfully demonstrates the application of a Quality by Design (QbD) approach for the efficient extraction and isolation of boswellic acid from Boswellia serrata oleo gum resin. The solubility studies indicated that any solvent except water could be used for extraction, with maceration being the most effective method, albeit requiring multiple extractions. The subsequent isolation of boswellic acids using alkali treatment and mineral acid precipitation was optimized by comparing different basifying agents, with 2% KOH found to be the most effective. Column chromatography using two distinct solvent systems allowed for the isolation of pure acetyl keto β-boswellic acid (AKBA) and a mixture of α and β boswellic acids. The identities of these compounds were confirmed through comprehensive spectroscopic analyses. This systematic approach not only enhances the efficiency of extracting and isolating bioactive compounds from Boswellia serrata but also underscores its potential for therapeutic applications, paving the way for further research and development in this field.

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