WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 9, Issue 3, 1243-1275 Research Article ISSN 2278 – 4357

A PHAMACOGNOSTICAL APPROACH IN ISOLATION OF BOSWELLIC ACIDS FROM BOSWELLIA SERRATE

Arshiya Zulfeen Mohd Fahim*[1], Mohammed Shakir Ghouse[2], Praveen S. Wathore[3], Syed Qumarul Islam[4]

Aayan Education and Welfare Trust's Aurangabad Pharmacy College.

Article Received on
08 Jan. 2020,

Revised on 28 Jan. 2020,
Accepted on 18 Feb. 2020

DOI: 10.20959/wjpps20203-15641

***Corresponding Author**
Arshiya Zulfeen Mohd Fahim
Aayan Education and Welfare Trust's Aurangabad Pharmacy College.

ABSTRACT

Olibanum also known as "Dhup", Indian Frankincense is an oleo gum resin of *Boswellia species.* In India it is obtained from *Boswellia serrata. Boswellia serrata* (Burseraceae), much branched, deciduous tree that grows abundantly in the dry, hilly parts of India. In India, the gum resin exudates of *Boswellia serrata*, known in the vernacular as "Salai guggal", has been used in the Ayurvedic system of medicine in the managements of several inflammatory conditions and as a topical anti-inflammatory agent. The major use of *Boswellia serrata* in contemporary medicine is as an anti-arthritic and anti-inflammatory pharmacological agent. The anti-inflammatory properties of the gum resin are attributed to the presence of "boswellic acids". In 1992, the active principles within the multi-component mixture of resin were identified, resulting in recognition of Boswellic acids. The most important are Acetyl 11-Keto β-Boswellic Acid (AKBA) and 11- Keto β-Boswellic Acid (KBA). **Boswellic acids were found to inhibit two pro-inflammatory enzymes,** 5-lipoxygenase (which generates inflammatory leukotrienes) and Human Leukocyte Elastase (HLE). HLE is a serine protease that initiates injury to the tissue, which in turn triggers the inflammatory process. This dual inhibitory action on the inflammatory process is unique to boswellic acids. ***Boswellia*** species are trees or shrubs with an outer bark often peeling in parchment like flakes, inner bark greenish, with watery aromatic resin, wood with milky latex. The leaves are imparipinnate, mostly congested at the end of the branches. Flowers are bisexual in panicles or racemes. *Boswellia* trees are found at areas from the sea level up to 1000 meters, usually in rocky slopes and gullies, often on limestone boulders, more rarely on vertical rock-faces, growing to a height of 3 up to 12 meters. The four major pentacyclic triterpenic acids present in the acidic extract of *Boswellia serrata* gum resin.

- β-Boswellic Acid
- Acetyl-β-Boswellic Acid
- 11-keto-β-Boswellic Acid
- Acetyl-11-keto-β-Boswellic Acid

Drug occurs in globular, transparent, tears forming agglomerates of various shapes and sizes, brownish-yellow, upto 5 cm long, 2 cm thick, fragrant, fracture brittle; fractured surface waxy and translucent; burns readily and emanates an agreeable characteristic, balsamic resinous odor; taste, aromatic and agreeable.

KEYWORDS:- Boswellic acid, olibanum, Human Leukocyte Elastase, 11- Keto β-Boswellic Acid, anti-inflammatory, hilly parts of India, inflammatory leukotrienes, Burseraceae.

INTRODUCTION To MATERIAL

Olibanum also known as "Dhup", Indian Frankincense is an oleo gum resin of*Boswellia species.* In India it is obtained from *Boswellia serrata. Boswellia serrata* (Burseraceae) is a large, much branched, deciduous tree that grows abundantly in the dry, hilly parts of India. It is or Indian Olibanum. Since ancient times, resins have been important in the preparation of incense, medicines, cosmetics and perfumes. The Egyptians, Hindus, Persians, Israelites, Greek, Romans and the Europeans of Queen Victoria's times greatly valued these materials. Olibanum, the resin from the *Boswellia* species has been used as incense for centuries. However, its major use today is as a fixative in perfumes, soaps, creams lotions and detergents.

In India, the gum resin exudates of *Boswellia serrata*, known in the vernacular as "Salai guggal", has been used in the Ayurvedic system of medicine in the managements of several inflammatory conditions and as a topical anti-inflammatory agent. The major use of *Boswellia serrata* in contemporary medicine is as an anti-arthritic and anti-inflammatory pharmacological agent.

The anti-inflammatory properties of the gum resin are attributed to the presence of "boswellic acids".

It known as Gajabhakshya in Sanskrit, implying its ingestion by elephants has been used in the Ayurvedic medicine since antiquity. The interest in this herb was aroused by the fact that such a heavy animal carried its weight on its limbs for so long, yet lived longer than humans.

This stimulated effort to find the ingredients in its diet, where Boswellia was found to be one. Boswellia has been mentioned in the ancient Indian Ayurvedic texts – the Sushruta Samhita and Charak Samhita. Boswellia is a tree of moderate height, which grows widely on dry hills of northwest India. In Ayurveda the oleogum resin of BSE is known as 'Salai Guggul' or 'Sallaki Guggul'. It has been used in the treatment of rheumatism, nervous diseases and as a topical anti-inflammatory agent.[2]

Preparations from the gum of *Boswelliaserrata* Extract (BSE) have been used in traditional/folk medicinefor treatment of inflammatory diseases. On stripping the bark, it yields gummy oleoresins, which containoils, terpenoids and gums. Upto 16% of the resin is essential oil, the majority being α thujene and p-cymene. Four pentacyclic triterpene acids are also present, with β-boswellic acid being the major constituent. BSE showed anti-inflammatory and antibacterial activity while the non-phenolic fraction of gum resin exhibited sedative and analgesic effects when tested in rats. Animal and in vitro studies suggest its usefulness in many inflammatory and broncho-constrictive conditions. Animal studies performed in India show that ingestion of defatted alcoholic extract of BSE decreases polymorpho-nuclear leucocyte infiltration and migration, decreased antibody synthesis and caused almost total inhibition of the classical complement pathway. Recently, it has been shown to be the inhibitor of 5-lipoxygenase and also human leucocyte elastase and consequently it has been proposed in the treatment of various inflammatory conditions.

In 1992, the active principles within the multi-component mixture of resin were identified, resulting inrecognition of Boswellic acids. The most important are Acetyl 11-Keto β-Boswellic Acid (AKBA) and 11- Keto β-Boswellic Acid (KBA).[2]

Boswellic acids were found to inhibit two pro-inflammatory enzymes, 5-lipoxygenase (which generates inflammatory leukotrienes) and Human Leukocyte Elastase (HLE). HLE is a serine protease that initiates injury to the tissue, which in turn triggers the inflammatory process. This dual inhibitory action on the inflammatory process is unique to boswellic acids.

Botanical Aspects of Boswellia Species

The botanical origin of *Boswellia* species has been characterized as:

- **Division:** *Spermatophyta*
- **Subdivision:** *Angiospermae*
- **Tribe**: *Rosopsida*

- **Subtribe:** *Rosidae s. lat.*
- **Overclass:** *Rutanae*
- **Class:** *Anacardiales*
- **Family:** *Burseraceae*
- **Genus:** *Boswellia*

The family of *Burseraceae* is represented in the plant kingdom with 17 genera and 600 species, widespread in all tropical regions. The species are often a predominant component of the vegetation in dry, lowland areas. Some species of the two most important genera of this family, *Commiphora* and *Boswellia*, produce resins that are of considerable commercial value as raw materials of balm, myrrh and frankincense.

INTRODUCTION

Boswellia species are trees or shrubs with an outer bark often peeling in parchment like flakes, inner bark greenish, with watery aromatic resin, wood with milky latex. The leaves are imparipinnate, mostly congested at the end of the branches. Flowers are bisexual in panicles or racemes. *Boswellia* trees are found at areas from the sea level up to 1000 meters, usually in rocky slopes and gullies, often on limestone boulders, more rarely on vertical rock-faces, growing to a height of 3 up to 12 meters.

Olibanum; gum olibanum, incense or frankincense, (in German Weihrauch, Gummiresina, Kirchenharz); are the common names given to the oleo-gum resin that exudes from incisions in the bark of trees of *Boswellia* (*Burseraceae*). There are about 25 species known belonging to this genus that are widespread in India, Arabia and the northeastern coast of Africa. Since ancient times, three of these species have been considered as the "true Frankincense" producing trees. The first species grows in South Arabia. *Boswellia sacra* Flueck.is known by the Arabians as "maghrayt d'sheehaz" and the resin it produces as "lubãn dhakar".

The second one grows in Somalia, known as *Boswellia carterii* Birdw., and in native language it is called "moxor". Recently, *Boswellia bhau-dajiana* Birdw.has been identified as identical to *B. carterii*. Generally, the resins that both species produce are called "lobãn dakar" or more commonly as "beeyo" quality.

The third important olibanum is from another Somalian species, *Boswellia frereana* Birdw. Ornatively as "jagcaar". The resin produced by this species is called "lobãn majdi" or commonly"maydi". It is the most expensive brand of olibanum on the market.

Boswellia papyriferaHochst.(*B. papyrifera* Rich.) produces another olibanum quality which is known as "boido" in Somalia, Ethiopia, especially in Eritrea, in Sudan and in the other east African countries. ***Boswellia neglecta***S. Moore (*B. hildebrandtii* Engl., *B. multifoliolata* Engl.) in Kenia and Ethiopia, *Boswellia rivae* Engl. (*B. boranensis* Engl) in Ethiopia, *Boswellia odorata* Hutch. And***Boswellia dalzielli***Hutch in tropical regions of Africa produce resins similar to olibanum.

Another resin producing species with a similar fragrance to frankincense is known as "Indian olibanum" or in botanical terms as *Boswellia serrata* Roxb. (syn.: *B. thurifera* Roxb.,*B.thurifera*Colebr., *B. serrata* Stachh., *B. glabra* Roxb., *Canarium balsamiferum* Willd.). *B. serrata*, "salpha tree", is found in the middle and northern parts of East India producing olibanum resin with various qualities which are commonly known as "salai guggul".

The *Boswellia* tree contains resin channels on the bark. When the bark is incised, a white emulsion exudes and dries into globular, pear or club shaped light yellow to dark brown tears. The resin is generally harvested all through the summer and autumn after the tree has been wounded in March or April. It is supposed that a *Boswellia* tree can produce this exudate in good quality only for three subsequent years. After this period, the quality of the collected resin decreases considerably. Therefore, even in the ancient records, it has been recommended that the tree should be left to rest for some years after this harvesting period.

Chemical History of Olibanum

Although the oil of olibanum had occupied the shelves of the 16th century pharmacies as "*oleum thuris*", the first investigation on its chemical composition was performed in 1788 **by *Johann Ernst Baer***at the University of Erlangen. Following his work, the first elementary analysis was carried out by ***F.W. Johnston* in 1839.** The constituents of the essential oil were first investigated by ***J. Stenhouse* in 1840,** and he identified depending on the origin of the resin fourteen monoterpenoic constituents including pinene, dipentene, phellandrene and cadinene. **In 1898, *A. Tschirch* and *O. Halbey*** published for the first time that olibanum had an acidic constituent, boswellic acid, with a molecular formula of $C_{32}H_{52}O_4$ but they could not suggest a structure at that time.

At the beginning of the 1930's, the olibanum resin was investigated in more detail. The study of ***A. Winterstein*** **and** ***G. Stein*** **in 1932** drew the attention to the resin acids, the pentacyclic triterpenoic α- and β-amyrin like skeletons with different functional groups, which were attempted to be isolated and identified with the analytical methods possible for that time. Nevertheless, by the 1960´s several of these acids such as α- and β-boswellic acids, 11α-hydroxy-β-boswellic acid and 3-*O*-acetyl-11-hydroxy-β-boswellic acid were identified by various derivatisation methods.

In 1967, ***G. Snatzke*** **and** ***L. Vértesy***published the structures of acetyl-11-keto-β-boswellic acid as well as *epi*-α- and *epi*-β-amyrin and their acetates, α- and β-amyrenone and viridiflorol from the neutral fraction of olibanum, adding that it is composed of 5-9 % essential oil, 15-16 % resin acids, 25-30 % of material insoluble in ether containing the polysaccharides and 45-55 % ether soluble compounds.

In 1978 ***R.S. Pardhy*** **and** ***S.C. Bhattacharya***identified tirucallic acids as well as β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic acid from *B. serrata* Roxb.and a diterpenoic cembrene derived alcohol, "serratol".

Studies on the isolation and identification of the boswellic acids with modern analytical techniques and on their pharmacological effects are still going on. Therefore these topics will be further discussed in the following parts of this work.

The first important and comparative study on the essential oil of olibanum of different origins was performed by ***H. Obermann*** **from Dragoco (Holzminden, Germany) in 1977.** He investigated two different commercial brands of olibanum, "*Eritrea*" and "*Aden*" by GC-MS, which corresponded to *B. carterii* and *B. serrata* resins, respectively. As a result of this investigation it was reported that not only the fragrance of these two qualities but also the composition of the constituents in the oils were different.

The "*Eritrea*" oil was reported to have octylacetate as the major constituent (52 %) as well as α-pinene, camphene, p-methoxytoluol, hexyl acetate, limonene, 1,8-cineole, octanol, linalool, bornyl acetate, cembrene A, incensole, incensyl acetate and an unknown diterpenoic constituent. In contrast, "*Aden*" oil was found to contain α-pinene as the major constituent (43%), camphene, β-pinene, sabinene, o-cymol, limonene, 1,8-cineole, p-cymol, campholenaldehyde, verbenone, octyl acetate and cembrenol, a diterpene alcohol with

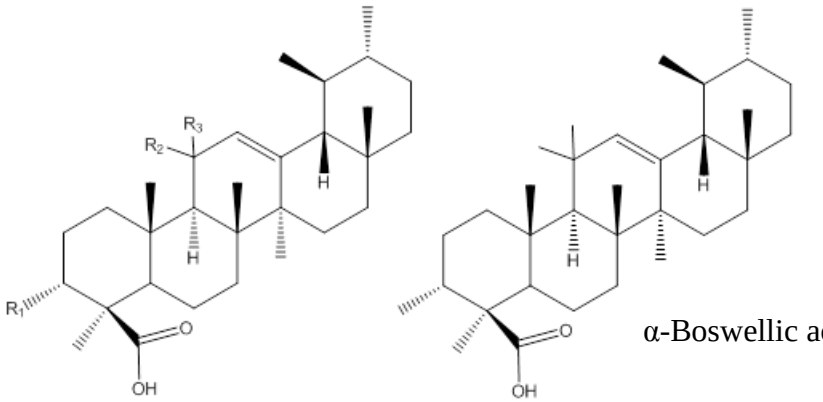
cembrene skeleton which was identified later by the same group, which was expected not be different than "serratol" described before.

In 1985 a detailed review was published by *P. Maupetit* on the "*Aden*" brand of olibanum. He reported 47 new constituents identified in the resinoid and in the oil of olibanum in addition to 169 formerly identified substances including the pyrolysis products. Recent studies by ***Verghese* on *B. serrata* oil and by *A. M. Humprey et al.*** comparing *B. carterii* oil with cumin, ginger, rosemary oil, were reinvestigations of known facts. These studies pointed to the difficulties in the identification of the origin of olibanum resin as well as in the determination of standard olibanum oil.

The gum resin of ***Boswellia serrata*** is known to contain:

- Monoterpenes (α thujene)
- Diterpenes (macrocyclic diterpenoids such as incensole, incensole oxide, inoincencole oxide, a diterpene alcohol (serrtol))
- Triterpenes (such as α- and β-amyrins)
- Pentacyclic triterpenic acids (boswellic acids)
- Tetracyclic triterpenic acids (tirucall-8, 24-dien-21-oic acids)

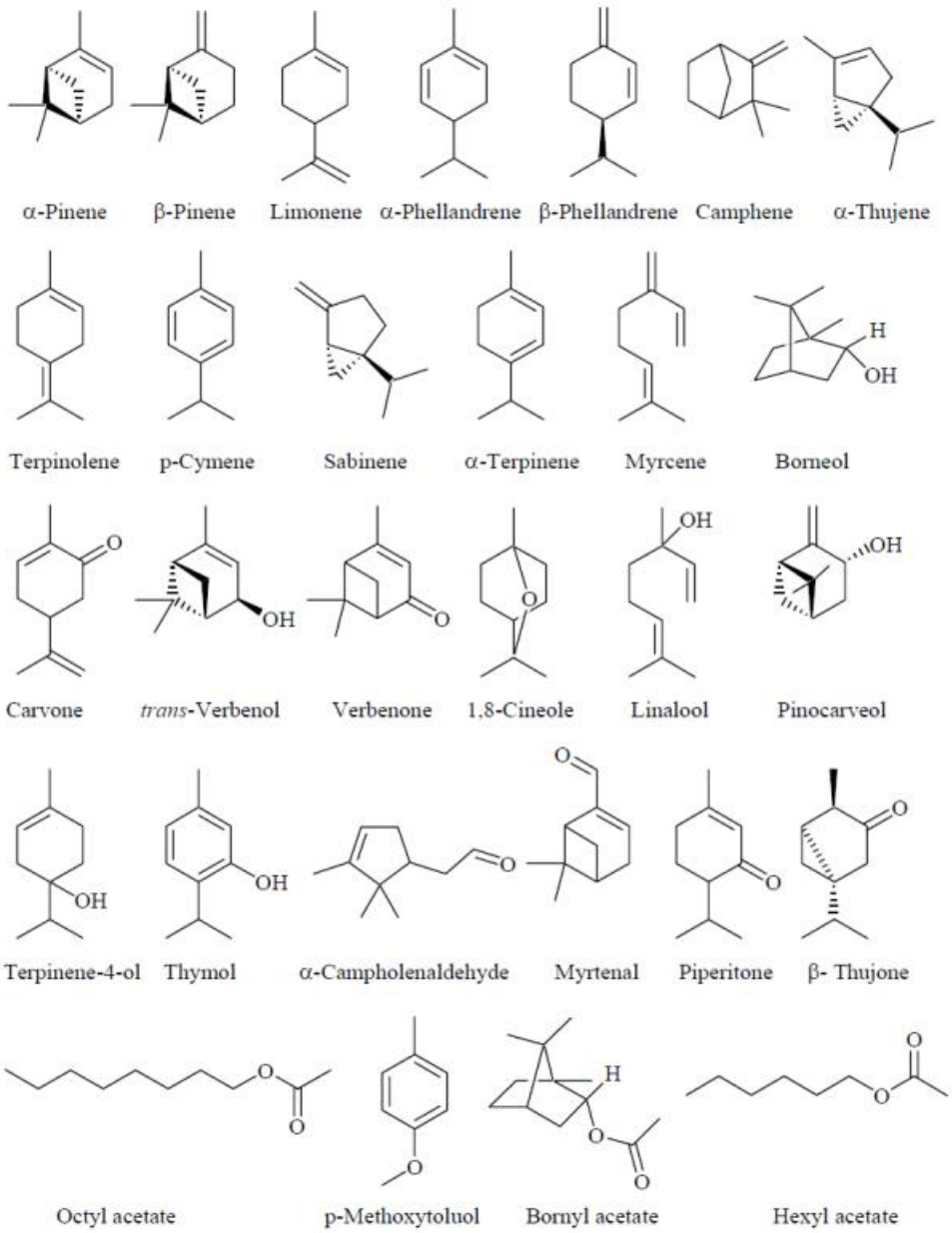
A complete list of major constituents of olibanum resin hitherto described are given in following figure,

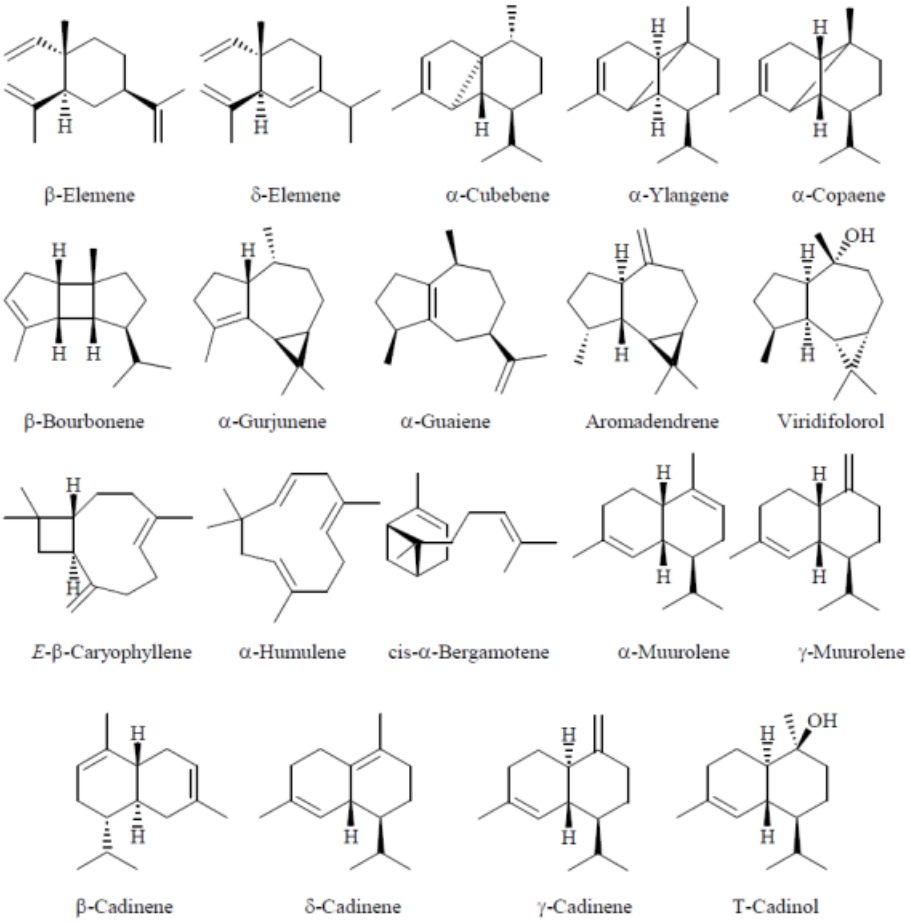


α-Boswellic acid

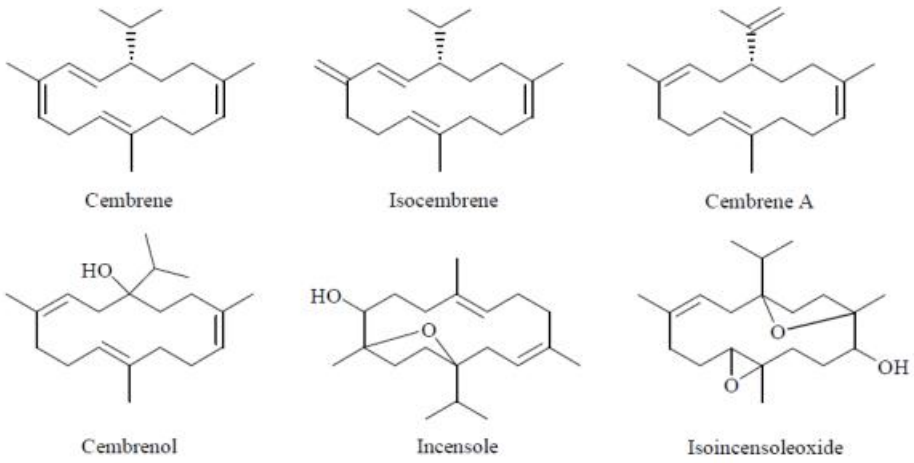
Compound	R_1	R_2	R_3
β Boswellic acid	OH	H	H
11-hydroxy- β-Boswellic acid	OH	OH	H

3-O-acetyl-11-hydroxy-β-Boswellic acid	Ac	OH	H
3-O-acetyl-β-Boswellic acid	Ac	H	H
11-keto-β-Boswellic acid	OH	O	O
3-O-acetyl-11-keto-β-Boswellic acid	Ac	O	O





Sesquiterpenes



Diterpenoids

Table 1: Literature Survey: Phytochemical and Pharmacological.

Sr.no	Activity	Journal	Author
1	Hepatocellular Carcinoma	Biomed Res Int. 2014;2014:294143	Khan MA1, Singh M2, Khan MS2, Najmi AK1, Ahmad S2.
2	Pediatric headache	Neurol Sci. 2014 May; 35 Suppl 1:145-8.	Dalla Libera D1, Colombo B, Pavan G, Comi G.
3	Cardioprotective and antioxidant effects	Chin J Nat Med. 2014 May;12(5):345-50.	Zaki AA1, Hashish NE2, Amer MA2, Lahloub MF2.
4	anti-inflammatory	J Nat Prod. 2014 Jun 27;77(6):1445-51	Verhoff M1, Seitz S, Paul M, Noha SM, Jauch J, Schuster D, Werz O.
5	Prevention of intestinal adenomatous polyposis	Drug Discov Ther. 2014 Feb;8(1):25-32.	Wang R1, Wang Y, Gao Z, Qu X.
6	Hypocholesteromic	J Diabetes Metab Disord. 2014 Feb 4;13(1):29.	Ahangarpour A, Heidari H1, Fatemeh RA, Pakmehr M, Shahbazian H, Ahmadi I, Mombeini Z, Mehrangiz BH.
7	Neurorecovery following injury	Brain Inj. 2013;27(12):1454-60	Moein P1, Abbasi Fard S, Asnaashari A, Baratian H, Barekatain M, Tavakoli N, Moein H.
8	Urease inhibitory activities of β-boswellic acid	Daru. 2013 Jan 2;21(1):2	Golbabaei S1, Bazl R, Golestanian S, Nabati F, Omrany ZB, Yousefi B, Hajiaghaee R, Rezazadeh S, Amanlou M.
9	Anti-inflammatory, antinociceptive and antioxidant	Food Chem Toxicol. 2011 Oct;49(10):2594-9.	Mothana RA.
10	inhibition human monocytic (THP-1) cell activation and platelet aggregation.	J Ethnopharmacol. 2011 Sep 1;137(1):893-901	Kokkiripati PK1, Bhakshu LM, Marri S, Padmasree K, Row AT, Raghavendra AS, Tetali SD.
11	Anticancer (Boswellic acid)	Int J Cancer. 2012 May 1;130(9):2176-84.	Yadav VR1, Prasad S, Sung B, Gelovani JG, Guha S, Krishnan S, Aggarwal BB.
12	Antiinflammatory activity (preclinical)	Clin Pharmacokinet. 2011 Jun;50(6):349-69.	Abdel-Tawab M1, Werz O, Schubert-Zsilavecz M.
13	Antitumor (Boswellic acid)	Food Chem Toxicol. 2011 Sep;49(9):1924-34.	Agrawal SS1, Saraswati S, Mathur R, Pandey M.
14	Effect on spatial memory retention	J Nat Med. 2011 Jul;65(3-4):519-25.	Mahmoudi A1, Hosseini-Sharifabad A, Monsef-Esfahani HR, Yazdinejad AR, Khanavi M, Roghani A, Beyer C, Sharifzadeh M.

15	Anticancer (Acetyl-11-keto-β-boswellic acid)	Int J Cancer. 2011 Jul 1;129(1):23-33.	Park B1, Sung B, Yadav VR, Cho SG, Liu M, Aggarwal BB.
16	Antistaphylococcal and biofilm inhibitory activities (acetyl-11-keto-β-boswellic acid)	BMC Microbiol. 2011 Mar 16;11:54.	Raja AF1, Ali F, Khan IA, Shawl AS, Arora DS, Shah BA, Taneja SC.
17	Immunological adjuvant	Int Immunopharmacol. 2011 Aug;11(8):968-75.	Gupta A1, Khajuria A, Singh J, Singh S, Suri KA, Qazi GN.
18	Anticholinesterase activity (Ethnobotanical)	Afr J Tradit Complement Altern Med. 2011;8(3):296-9.	Bakthira H1, Awadh Ali NA, Arnold N, Teichert A, Wessjohann L.
19	for inflammatory bowel disease.	World J Gastroenterol. 2010 Sep 28;16(36):4504-14.	Rahimi R, Shams-Ardekani MR, Abdollahi M.
20	Immunomodulator	Phytomedicine. 2010 Sep;17(11):862-7.	Ammon HP.
21	Asthma	Prim Care Respir J. 2010 Dec;19(4):307-14.	Clark CE1, Arnold E, Lasserson TJ, Wu T.
22	Antiplasmodial	J Ethnopharmacol. 2010 Jul 6;130(1):143-50.	Nicolas JP, De Mol P, Nikiéma JB, Frédérich M. Jansen O1, Angenot L, Tits M,
23	Memory deficit	Arch Pharm Res. 2010 Mar;33(3):463-8.	Hosseini M1, Hadjzadeh MA, Derakhshan M, Havakhah S, Rassouli FB, Rakhshandeh H, Saffarzadeh F.
24	Antioxidant activity of essential oil	Nat Prod Res. 2010;24(2):140-51.	Yang SA1, Jeon SK, Lee EJ, Shim CH, Lee IS.
25	Inhibitiion of vascular smooth muscle cell migration and proliferation in response to platelet-derived growth factor.	Korean J Physiol Pharmacol. 2009 Apr;13(2):107-13	Choi OB1, Park JH, Lee YJ, Lee CK, Won KJ, Kim J, Lee HM, Kim B.
26	inhibition of nitric oxide production in macrophages.	Chem Pharm Bull (Tokyo). 2009 Sep;57(9):957-64.	Yoshikawa M1, Morikawa T, Oominami H, Matsuda H.
27	inhibition of prostate tumor growth (Acetyl-11-keto-beta-boswellic acid)	Cancer Res. 2009 Jul 15;69(14):5893-900.	Pang X1, Yi Z, Zhang X, Sung B, Qu W, Lian X, Aggarwal BB, Liu M.
28	Antimalarialactivity	Evid. Based Complement Alternat. Med. 2009 Dec;6(4):453-6.	Alshawsh MA1, Mothana RA, Al-Shamahy HA, Alsllami SF, Lindequist U.

29	Prevention of colonic fibrosis	Eur J Clin Invest. 2008 Jun;38(6):410-20.	Latella G1, Sferra R, Vetuschi A, Zanninelli G, D'Angelo A, Catitti V, Caprilli R, Gaudio E.
30	Antiandrogen acetyl-11-keto-beta-boswellic acid	Biochem Pharmacol. 2008 Jun 1;75(11):2112-21.	Yuan HQ1, Kong F, Wang XL, Young CY, Hu XY, Lou HX.
31	The gastric ulcer protective effect (boswellic acids)	Phytomedicine. 2008 Jun;15(6-7):408-15.	Singh S1, Khajuria A, Taneja SC, Khajuria RK, Singh J, Johri RK, Qazi GN.
32	Immunomodulatory activity of biopolymeric fraction BOS 2000	Phytother Res. 2008 Mar;22(3):340-8.	Khajuria A1, Gupta A, Suden P, Singh S, Malik F, Singh J, Gupta BD, Suri KA, Srinivas VK, Ella K, Qazi GN.
33	Antiinflammatory and antiatherogenic effects acetyl-11-keto-beta-boswellic acid	Arterioscler Thromb Vasc Biol. 2008 Feb;28(2):272-7.	Cuaz-Pérolin C1, Billiet L, Baugé E, Copin C, Scott-Algara D, Genze F, Büchele B, Syrovets T, Simmet T, Rouis M.
34	anti-inflammatory - Incensole acetate novel antiinflammatory compound From boswelia resin	Mol Pharmacol. 2007 Dec;72(6):1657-64.	Moussaieff A1, Shohami E, Kashman Y, Fride E, Schmitz ML, Renner F, Fiebich BL, Munoz E, Ben-Neriah Y, Mechoulam R.
35	vaccine adjuvant (BOS 2000)	Vaccine. 2007 Jun 6;25(23):4586-94.	Khajuria A1, Gupta A, Malik F, Singh S, Singh J, Gupta BD, Suri KA, Suden P, Srinivas VK, Ella K, Qazi GN.
36	Modulation of Pgp function by boswellic acids.	Planta Med. 2006 May;72(6):507-13.	Weber CC1, Reising K, Müller WE, Schubert-Zsilavecz M, Abdel-Tawab M.
37	liver damage	Y J1, Kamath JV, Asad M.	Pak J Pharm Sci. 2006 Apr;19(2):129-33.
38	Osteoarthritis of knee	Phytomedicine. 2003 Jan;10(1):3-7.	Kimmatkar N1, Thawani V, Hingorani L, Khiyani R.
39	Crohn disease	Z Gastroenterol. 2001 Jan;39(1):11-7.	Gerhardt H1, Seifert F, Buvari P, Vogelsang H, Repges R.
40	hepatitis C virus (HCV) protease inhibitory	Phytother Res. 2000 Nov;14(7):510-6.	Hussein G1, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, Shimotohno K.
. 41	cyclooxygenase enzyme inhibition	Nat Prod Commun. 2013 Oct;8(10):1365-6.	Ali SI1, Zhang CR1, Mohamed AA1, El-Baz FK1, Hegazy AK2, Kord MA2, Nair MG3.

Table 2: Literature Survey: Formulation.

Sr.no	Formulation/attempt	Journal	References
1	Transdermal microemulsions *Boswelliacarterii*	Drug Deliv. 2014 Apr 14.	Mostafa DM1, Ammar NM, Basha M, Hussein RA, El Awdan S, Awad G.
2	Investigating permeability related hurdles in oral delivery of 11-keto-β-boswellic acid.	Int J Pharm. 2014 Apr 10;464(1-2):104-10.	Bagul P1, Khomane KS1, Bansal AK2.
3	Enhanced absorption of boswellic acids by a lecithin delivery form (Phytosome) of Boswellia extract.	Fitoterapia. 2013 Jan;84:89-98.	Hüsch J1, Bohnet J, Fricker G, Skarke C, Artaria C, Appendino G, Schubert-Zsilavecz M, Abdel-Tawab M.
4	Complexation with phosphatidyl choline as a strategy for absorption enhancement of boswellic acid.	Drug Deliv. 2010 Nov;17(8):587-95.	Sharma A1, Gupta NK, Dixit VK.
5	Topical Boswellic acids for treatment of photoaged skin.	Calzavara-Pinton P1, Zane C, Facchinetti E, Capezzera R, Pedretti A.	Dermatol Ther. 2010 Jan-Feb;23

Boswellia and Boswellic acids

The four major pentacyclic triterpenic acids present in the acidic extract of *Boswelliaserrata* gum resin.

- β-Boswellic Acid
- Acetyl-β-Boswellic Acid
- 11-keto-β-Boswellic Acid
- Acetyl-11-keto-β-Boswellic Acid

(See structures above)

Apart from this oleogum resin of boswellia also contains monoterpenes, Diterpenes and tetracyclic triterpenes (described above). These compounds are responsible for anti-inflammatory activities of resin.

Activities of Boswellic acids

Sr.no	Disease	Role
1	Cancer	(i) Anti-carcinogenicity in mice with ehrlic ascites carcinoma and sarcaoma-180, found inhibition of tumor growth. (ii) Anti-proliferative and apoptotic effect on colon cancer. Induced anti-edema effect in glioblastoma patients.
2	Arthritis	(i) Decreases infiltration of leukocytes into knee joint and pleural cavity and inhibits the migration ofpolymorph nuclear leukocytes. (ii) Decreases severity of pain and disability in osteoarthritic patients.
3	Inflammation	(i) Decreases galactosamine/endotoxin induced hepatitis in mice. (ii) Decreases inflammatory features in indomethacin-induced ileitis in rats. (iii) Decreases experimental murine colitis. (iv) Inhibits the synthesis of 5-LOX products. (v) Inhibits topoisomerase, elastase and C-3 convertase enzymes.
4	Hypolipidemia	(i) Decreases cholesterol and increases HDL in rats. (ii) Induced nitric oxide production in rat macrophages. Asthma Increases stimulation of mitogen activated protein kinase MAPK and mobilization of intracellular Ca^{++} Immunomodulatory Anti-anaphylactic and mast cell degranulation
5	Autoimmune encephalitis	(i) Inhibited ionophore stimulated release of leukotrienes from PMNL's. (ii) Decreases symptoms of AE.
6	Crohn's disease	Decreases activity index
7	Analgesic	Decreases motor activity and ptosis in rats

Loban/Kunduru

The oleo gum resin used in this study is also called as loban. In Ayurveda it is described asKunduru. According to Ayurvedic Herbal Pharmacopoeia Kunduru consists of exudate of *Boswelliaserrata* Roxb. (Fam. Burseraceae), amoderate sized, deciduous tree, upto 18 m in height and upto 2.4 m in girth, commonlyfound in the dry forests from Punjab to West Bengal and in peninsular India.

Synonyms

- **Sanskrit :** sáallaki
- **Assamese :** Sallaki
- **Bengali :** Luban, Salai, Salgai
- **Gujrati :** Shaledum, Saleda, Saladi, Gugal, Saledhi
- **Hindi :** Salai, Labana
- **Kannada :** Madimar, Chilakdupa, Tallaki, Maddi

- **Kashmiri :** Kunturukkam, Samprani
- **Marathi :** Salai cha dink
- **Punjabi :** Salai Gonda
- **Tamil :** Parangi Sambrani
- **Telugu :** Parangi sambrani, Anduga, Kondagugi tamu
- **Urdu :** Kundur

Description

a) Macroscopic

Drug occurs in globular, transparent, tears forming agglomerates of various shapes and sizes, brownish-yellow, upto 5 cm long, 2 cm thick, fragrant, fracture brittle; fractured surface waxy and translucent; burns readily and emanates an agreeable characteristic, balsamic resinous odor; taste, aromatic and agreeable.

Chemical Constituents

Essential oil 8-12 %, Polysaccharides (45-60%), Higher terpenoids (25-35%).

oswellia serrata tree

Boswellia serrata trunk

Boswellia serrata

branches and leaves

Boswellia serra

oleo gum

Experimental Evaluation

Procurement of Material

Oleogumresin of *Boswellia serrata* (Commonly known as Loban) was purchased from Medical Shop of crude drugs at Gulmandi, Aurangabad. 500g of material was purchased. It was available as yellowish brown to blackish masses, opaque and transparent.

Identification test

- **General Test:** (90%) a tear of' Kunduru is not altered much in form but becomes almost opaque and white; when a drop of con. H_2SO_4 is added on a freshly fractured surface, it

becomes cherry red which, when washed with water changes to a white emulsion, then turn to a buff colour.

- **Fluorescence Test -** Brownish-yellow colour in day light; aqueous extract under U.V. light (366 nm) light green and in (254 nm) shows dark blue colour; alcoholic extract under U.V. light (366 nm) is colourless and in (254 nm) shows light green colour.

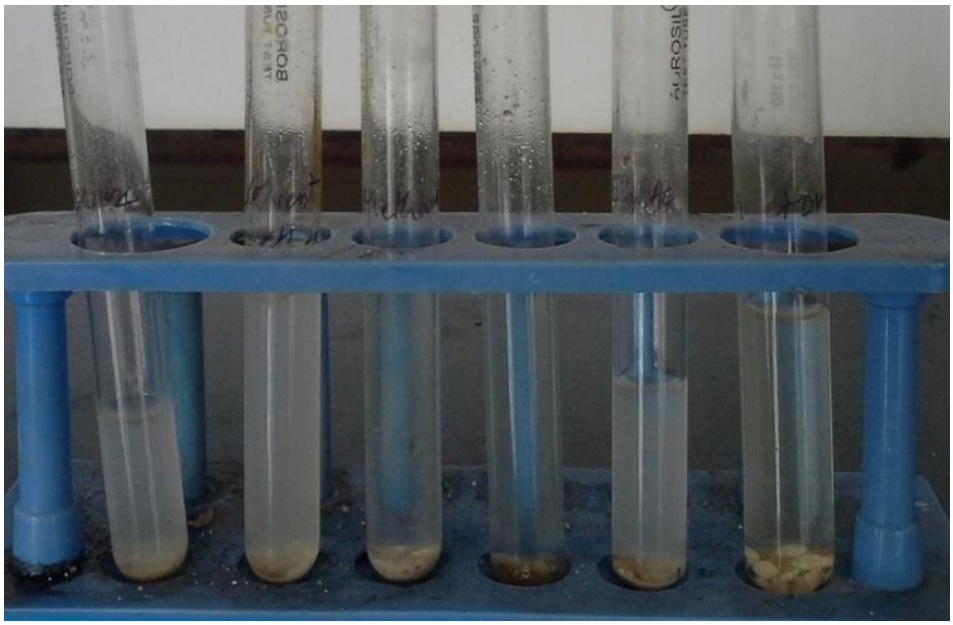
***Positive identification tests are given by oleogumresin**

Solubility studies of Oleogumresin:

For solubility study gum was crushed by mortor and pestle and its solubility was tested in different solvents. Following are the solvents used with observation. These solutions were subjected to TLC studies.

Table 3: Solubility studies of Oleogumresin.

Sr. no	Solvent	Appearanceof solution	Residue, comments
1.	Acetone	Milky (slight cloudy)	White
2.	Ethyl acetate	Milky (slight cloudy)	Yellowish
3.	Toluene	Transperant, colourless	Yellowish
4.	Methanol	Colourless	White
5.	Ether	Milky	White yellowish
6.	n Hexane	Slight cloudy	Brown
7.	Ethanol	Clear yellow transperant	Amorphus white
8.	Water	Yellowish turbid solution	Waxy lumps of white colour



Thin Layer Chromatography(TLC)

- **Sample Preparation**

In case of dried extract dissolve a small quantity of the sample in the least polar solvent in which it is soluble. Alternatively sample can also be prepared by extracting the small amount of crude drug material (say 1g) with solvent.

- **Sample Application**

Sample thus prepared is applied in very small amount (in microlitres) as spot or as lane on TLC plate just 1cm-2cm above the base of it. This is called as solute front.

- **Development of TLC Plate**

After the TLC plate is spotted it has to be transferred to the development tank. This tank holds the mobile phase. The solute front is not allowed to sink in to the solvent system otherwise the compound will diffuse in to the solvent system. Large variety of development tanks are commercially available, the cheapest way is to use a beaker with a watch glass as lid. To saturate the inside atmosphere of the tank, the tank can be lined with filter paper.

- **Visualization of TLC Plate/ Detection of Spots**

It is easy to visualize coloured compounds but all compounds are not coloured. There are numerous reagents available to visualise TLC plates. Even when the material being analyzed is coloured it is necessary to treat the TLC plate to visualize any no-coloured spots that may be present in the sample. The two most useful means of analysis are ultraviolet-light and iodine vapour. The TLC plate can be dipped into a stock solution of the reagent or the plate can be sprayed with a diffuser. Commonly used reagents are anisaldehyde sulphuric acid reagent, vanillin sulphuric acid reagent, antimony trichloride reagent. After the plate has been sprayed, it is heated for 10 minutes at 110^{o}C. The compounds produce spots visible under ultraviolet light 360 nm and 254nm.

- **Stationary phase:** Pre coated silica gel plate with fluorescent indicator (F_{254}) from Merck $PSGF_{254}$
- **Mobile Phase:** Toluene: Ethyl Acetate: Methanol (8:2:1)
- **Detection**

1. UV 254nm
2. Anisaldehyde sulphuric acid (ASA) (Heat treatment is required after TLC plate is sprayed with ASA).

Extraction Studies

Crude drug material is subjected for extraction studies by employing different methods of extraction commonly available in laboratory.

Following methods were tested

1. Maceration
2. Hot continuous extraction (Soxhlet extraction)
3. Steam distillation and reflux
4. Direct heating (Decoction)

Maceration: In this method material 250g of oleogumresin is subjected for maceration for 20 hrs in ethanol (500ml) with continuous shaking at 100 rpm on shaker. This process is repeated successively for three times.

Hot continuous Extraction: This is also called as hot continuous percolation or soxhlet extraction. Crude drug material is placed in body of extractor. Heating is applied.

Steam distillation or reflux: In this method oleogumresin is placed in RBF fitted with condenser and heat is applied to RBF.

Direct Heating: In this method oleogum resin is placed in a glass container and direct heat is applied to container.

Isolation of Boswellic Acid by Column Chromatography

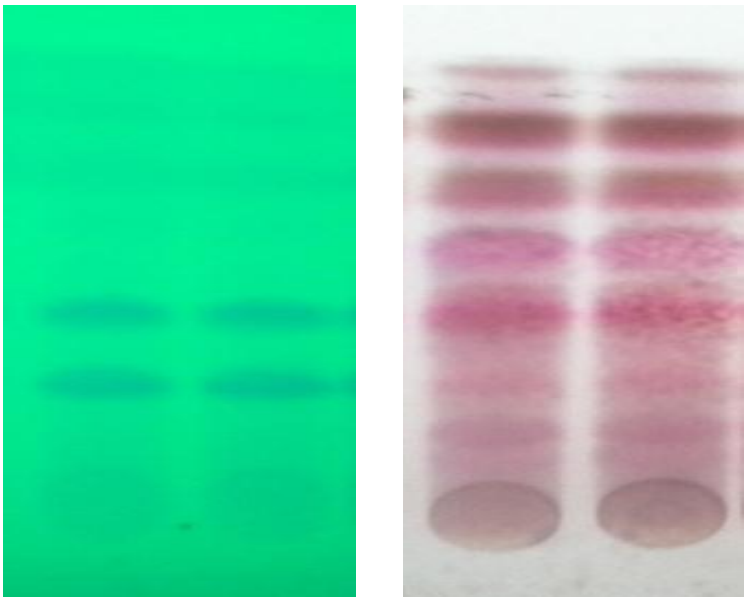
For running of column optimization of solvent system is carried out by Thin Layer Chromatography.

- **Thin Layer Chromatography**

  Thin layer chromatography is carried out under following conditions
- **Mobile Phase:** Toluene: Ethyl acetate: n heptane: Formic acid (8:2:1:0.3)
- **Stationary phase:** Precoated silica gel plate with fluorescent indicator (F254) from Merck $PSGF_{254}$
- **Detection**

A. UV 254 nm
B. Anisaldehyde sulphuric acid (ASA)

Heat treatment is required after TLC plate is sprayed with ASA



A **B**

Photograph 4.1: TLC of Boswellia extract

(Toluene: Ethyl acetate: n heptane: Formic acid)

Column Chromatography

Procedure

In column chromatography, the mobile phase is again a solvent, and the stationary phase is a finely divided solid, such as silica gel or alumina. Chromatography columns vary in sizes. There is an element of trial and error involved in selecting a suitable solvent and adsorbent for the separation of the constituents of a particular mixture. A small volume of the sample whose constituents are to be separated is placed on top of the column. The choice of the eluting solvent should ensure that the sample is soluble. However, if the sample was too soluble the mobile phase (solvent) would move the solutes too quickly, resulting in the non-separation of the different constituents. Usually, one should start with a less polar solvent to remove the less polar compounds, and then slowly increase the polarity of the solvent to remove the more polar compounds.

Photograph 4.2: Steps in Column Chromatography.

Column Chromatography 1

- **Stationary Phase:** Silica 230-330 mesh size.
- **Mobile phase:** Toluene: Ethyl acetate: n heptane (8:2:1)
- **Column:** Glass column
- **Column diameter** (internal diameter of column):1.5cm
- **Height of column:** 10.5cm
- **Height of extract loaded:** 1.5cm

  (Height of column refers to height at which silica, the stationary phase is filled)
- **Number of fractions collected**: 32 fractions of 20ml each.

  Fraction 4, 5 shows single spot corresponds to Acetyl keto β boswellic acid

  Fraction 8-9 yields single spot corresponds toα and β boswellic acid

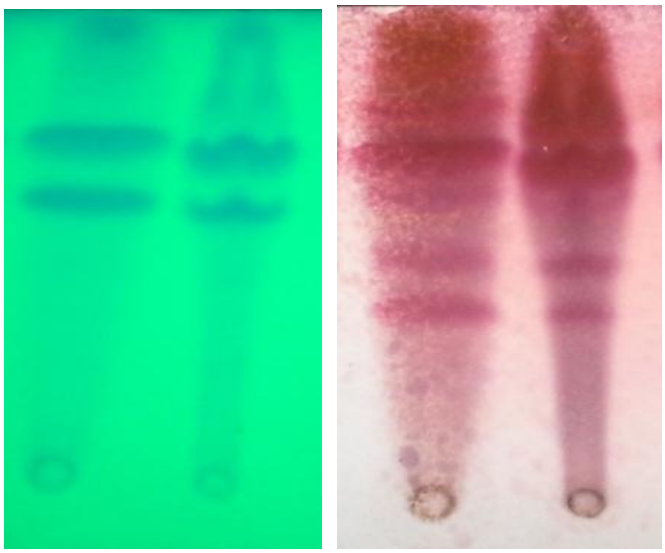
Column Chromatography 2

This column was run on Toluene: Ethyl acetate: Methanol (8:2:1). This mobile phase was optimized with TLC.

- **Thin Layer Chromatography**

  Thin layer chromatography is carried out under following conditions
- **Mobile Phase:** Toluene: Ethyl acetate: methanol (8:2:1)
- **Stationary phase:** Precoated silica gel plate with fluorescent indicator (F254) from Merck $PSGF_{254}$
- **Detection**
  - Visible (Vis.)-without any chemical treatment-
  - Anisaldehyde sulphuric acid (ASA)-Plate 2
  - UV 254 nm

- Heat treatment is required after TLC plate is sprayed with ASA



Photograph 4.3:TLC of Boswellia extract

(Toluene: Ethyl acetate: methanol)

Column Chromatography

- **Stationary Phase:** Silica 230-330 mesh size.
- **Mobile phase:** Toluene: Ethyl acetate: n heptane: Formic acid (8:2:1:0.3)
- **Column:** Glass column
- **Column diameter** (internal diameter of column):1.5cm
- **Height of column:** 10.5cm
- **Height of extract loaded:** 1.5cm
  (Height of column refers to height at which silica, the stationary phase is filled)
- **Number of fractions collected**: 32 fractions of 10ml each.

Fraction 4, 5 shows single spot corresponds to Acetyl keto β boswellic acid

Fraction 8-9 yields single spot corresponds to α and β boswellic acid

Isolation of acid fraction

Following general procedure is employed for isolation of boswellic acid from oleo gum resin

(a) Crushing the lumps of the gum resin of *Boswellia serrata* and extracting the crushed lumps with ethanol.

(b) Removing insoluble material from above extract;

(c) Concentrating the extract till a reddish brown syrupy mass is obtained;

(d) Basifying the syrupy mass with an aqueous solution of an alkali to provide a solution having a pH in the range of 9 to 10.

(e) Extracting the solution with a solvents to provide an aqueous layer, and acidifying the aqueous layer with mineral acid to a pH in the range of 3-5 to provide a precipitate comprising boswellic acids;

(f) Washing the precipitate with water to provide said fraction being neutral to litmus;

(g) Separating individual boswellic acids from said fraction.

Checking effect of alkali

In the procedure mentioned above step (d) which indicates use alkali for basification. The aim of the present study is to check effect of different concentrations of alkali (NaOH and KOH) on separation of acid fraction.

Koh

Acid separation by using 2% koh

10g of sample dissolved in ethanol. This solution was treated with 2% KOH solution (pH=10) and acidified with Conc. HCl.

Acid separation by using 5% koh

10g of sample dissolved in ethanol. This solution was treated with 5% KOH (pH 10.5) and acidified with conc. HCl. If no Precipitate is formed and extract remain as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethyl acetate. It gave a soluble portion and insoluble precipitate. Insoluble precipitate was separated and dissolved in ethanol.

Acid separation by using 10% koh

10g of sample dissolved in ethanol. This solution was treated with 10% KOH (pH 10.5) and acidified with conc. HCl. If no Precipitate is formed and extract remain as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethyl acetate. It gave a soluble portion and insoluble precipitate. Insoluble precipitate was separated and dissolved in ethanol.

Acid separation by using 20% koh

10g of sample dissolved in ethanol. This solution was treated with 10% KOH (pH 10.5) and acidified with conc. HCl. If no Precipitate is formed and extract remain as such without getting dissolved as viscous orange mass. This orange mass was very less which separated and dissolved in ethanol and this solution was treated with Conc. HCl. No precipitate was formed.

Naoh

10g of sample dissolved in ethanol. This solution was treated with 2% NaOH (pH 10.5) and acidified with conc. HCl. If no Precipitate is formed and extract remain as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethanol.

5% Naoh

10g of sample dissolved in ethanol. This solution was treated with 5% NaOH and acidified with conc. HCl. The extract remain as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethanol.

10% Naoh

10g of sample dissolved in ethanol. This solution was treated with 10% NaOH and acidified with conc. HCl. The extract remain as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethanol.

20% Naoh

10g of sample dissolved in ethanol. This solution was treated with 10% NaOH and acidified with conc. HCl. The extract remained as such without getting dissolved as viscous orange mass. This orange mass was separated and dissolved in ethanol and this solution was treated with Conc. HCl. Precipitate is formed which is separated and dissolved in ethanol

Checking effect of acid

In this parameter same above procedures were repeated for NaOH and KOH. Conc. H_2SO_4 was used instead of Conc. HCl.

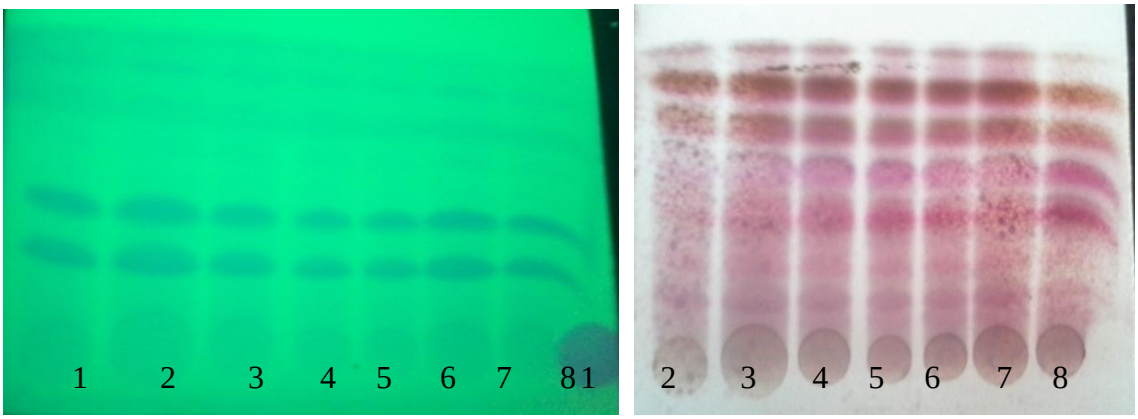
Effect of strength of acid

Here same procedures were repeated dilute acid was used instead of concentrated acid.

OBSERVATION AND RESULTS

Selection of suitable solvent

As mentioned above in experimental work done, oleo gum resin was subjected for solubility studies in different solvents. In this 10 commonly available solvents were used for extraction including water. All of them showed same TLC pattern except water. This indicates that any solvent can be used for extraction of oleo gum resin except water (Use of any solvent here is recommended if the aim of extraction is isolation of Boswellic acid). Here one important point of cost can be considered with respect to solvent which will influence choice of solvent for extraction.



(A) **(B)**

Photograph 5.1: TLC fingerprint for effect of solvents

1. Ac- acetone
2. Etac-ethyl acetate
3. Tol- Toluene
4. Met-Methanol
5. Eth-Ethanol
6. Hex-Hexane
7. Chl- Chloroform
8. Wat- water

Details of TLC

- **Thin Layer Chromatography**

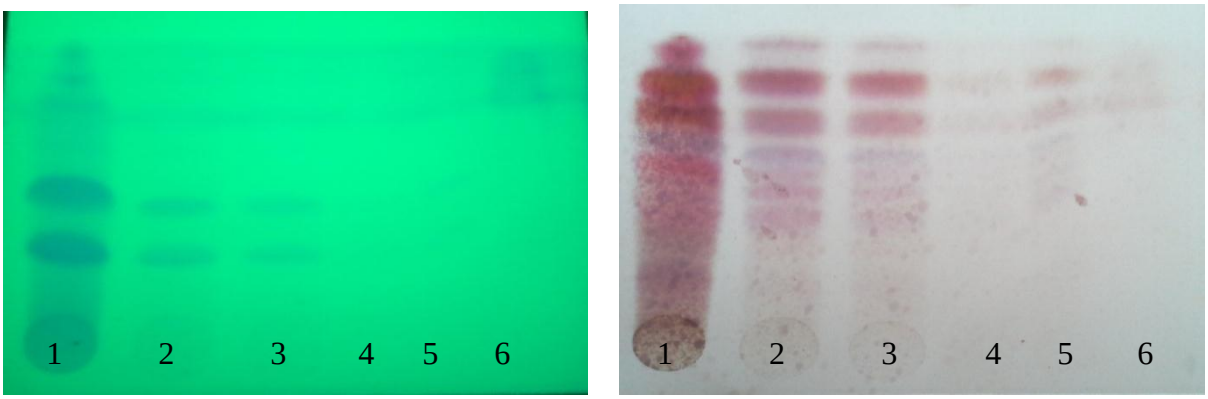
  Thin layer chromatography is carried out under following conditions
- **Mobile Phase:** Toluene: Ethyl acetate: n heptane: Formic acid (8:2:1:0.3)
- **Stationary phase:** Precoated silica gel plate with fluorescent indicator (F_{254}) from Merck PSGF254
- **Detection**
  - (A)-UV 254 nm
  - (B)- Anisaldehyde sulphuric acid (ASA)
- Heat treatment is required after TLC plate is sprayed with ASA

Selection of extraction method of oleogum resin and completeness of extraction

As mentioned in experimental conditions above different extraction methods were used for oleogumresin. Oleogum resin is mixture of polysaccharides gum, volatile oil and resin. Resin portion contains boswellic acids.

In case of soxhlet extraction and other methods of extraction employing heat like decoction problem of clogging and melting of material arises, which hampers process of extraction.

In case of maceration no heat is employed, but the material has to be extracted multiple times with fresh solvent each time. In this method solvent requirement increases. Each time the extract was checked for presence of boswellic acids by TLC to ensure complete extraction. It took 4-5 times repeated extraction for complete extraction of Boswellic acids by maceration. Afterextraction concentration of material was carried out. During concentration much of the volatile oil evaporates.



(A) **(B)**

Photograph 5.2: TLC fingerprint for completeness of extraction

Thin Layer Chromatography

Thin layer chromatography is carried out under following conditions

- **Mobile Phase:** Toluene: Ethyl acetate: n heptane: Formic acid (8:2:1:0.3)
- **Stationary phase:** Precoated silica gel plate with fluorescent indicator (F_{254}) from Merck PSGF254
- **Detection**
  - (A)-UV 254 nm
  - (B)- Anisaldehyde sulphuric acid (ASA)

    Heat treatment is required after TLC plate is sprayed with ASA

Isolation of total acids (boswellic acid)

Literature survey reveals that anti-inflammatory activities associated with this resin are completely restricted to presence of Boswellic acids. So focus in experimental work done is placed on isolation of acid fraction of oleo gum resin.

As mentioned in the procedures above acid fraction was obtained as white precipitate. This white precipitate was separated, dried and weighed.

Amount of acid fraction obtained was determined on weight basis.

In the experimental work done isolation of acid fraction was carried out by procedures mentioned in section above. This is common procedure which uses treatment of resin with alkali to convert acid into its salt and then precipitating salt of acid by using mineral acid.

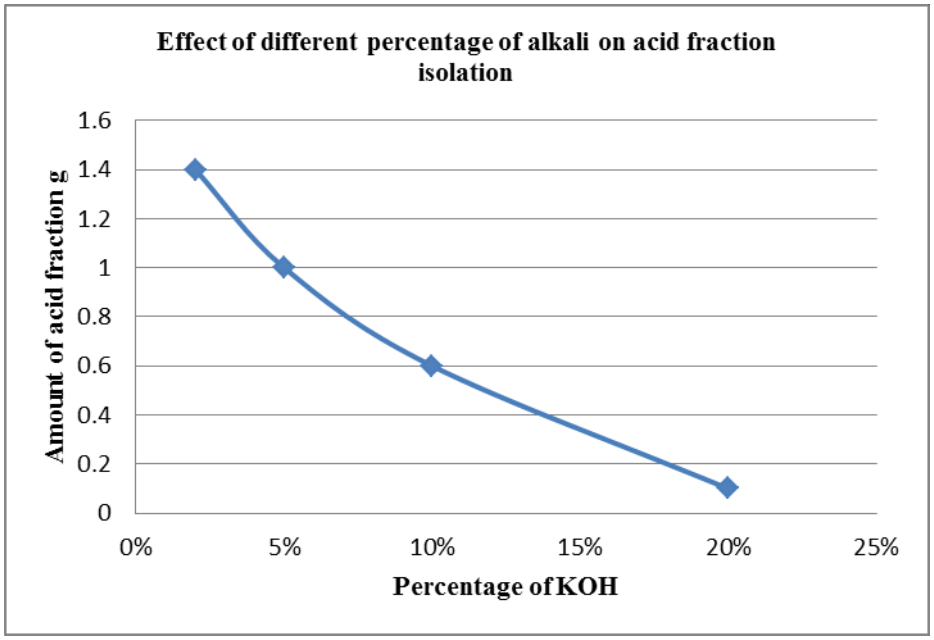
Studies were carried out to check variations in amount of acid portion obtained when these parameters were altered.

Effect of koh

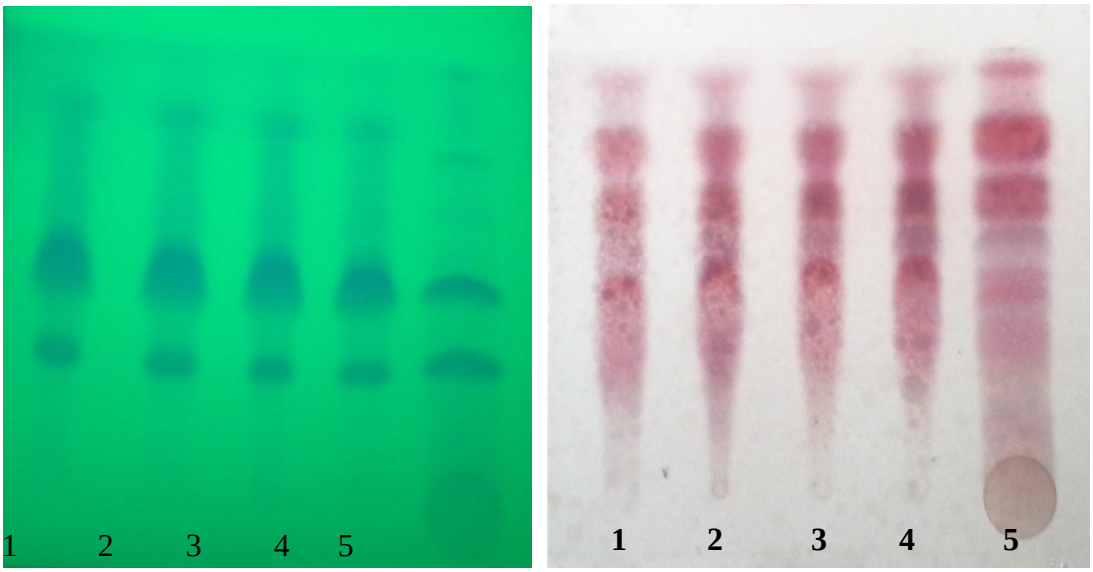
Following are observations when KOH is used as basifying agent.

Table 5.1: Effect of percentage of KOH as basifying agent on acid fraction.

Sr.no	Percentage of KOH	Amount of acid
1	2% KOH	1.4g
2	5% KOH	1.0g
3	10% KOH	0.6g
4	20% KOH	0.1g



Graph 5.1: Effect of different percentage of alkali on acid fraction isolation.



(A) **(B)**

Photograph 5.3: TLC fingerprint for effect of KOH

1. 2% KOH
2. 5 % KOH
3. 10% KOH
4. 20 % KOH
5. Whole extract

Thin Layer Chromatography

Thin layer chromatography is carried out under following conditions

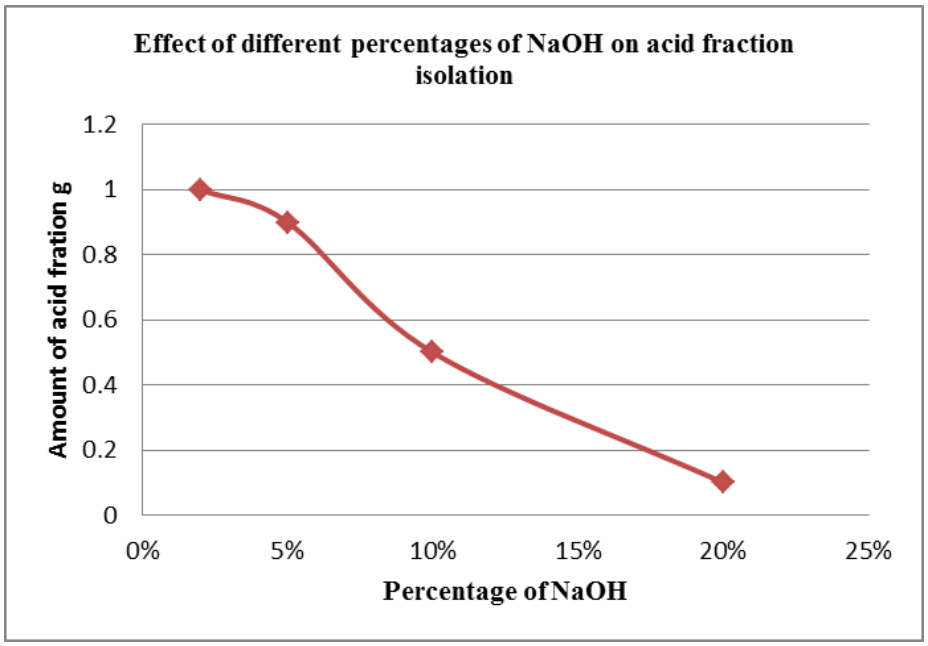
- **Mobile Phase:** Toluene: Ethyl acetate: n heptane: Formic acid (8:2:1:0.3)
- **Stationary phase:** Precoated silica gel plate with fluorescent indicator (F_{254}) from Merck $PSGF_{254}$
- **Detection:**
  - (A)-UV 254 nm
  - (B)- Anisaldehyde sulphuric acid (ASA)
- Heat treatment is required after TLC plate is sprayed with ASA

Effect of Naoh

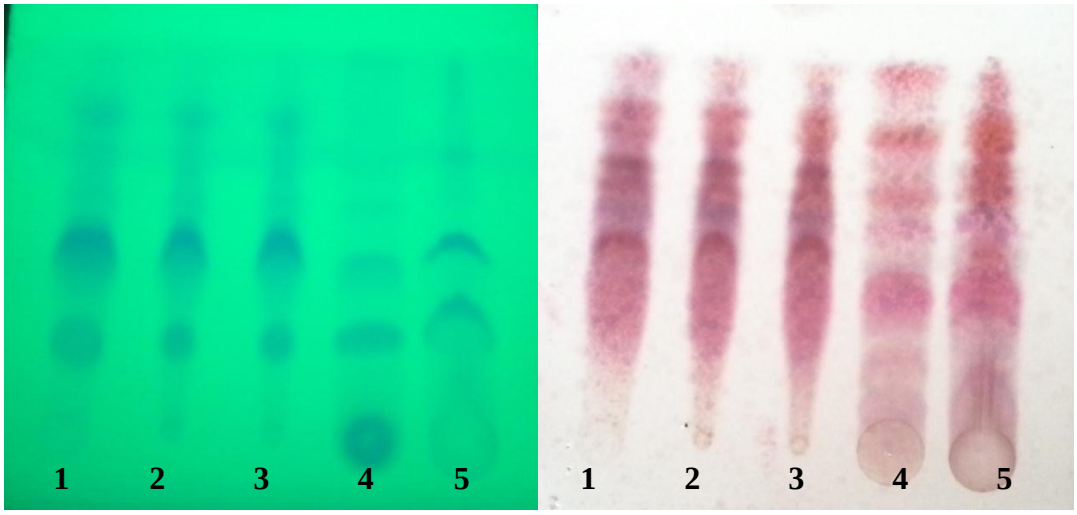
Following are observations when NaOH is used as basifying agent.

Table 4: Effect of percentage of NaOH as basifying agent on acid fraction.

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



Graph 5.2: Effect of different percentages of NaOH on acid fraction isolation.



(A) **(B)**

TLC fingerprint for effect of NaOH

1. 2% NaOH
2. 5 % NaOH
3. 10% NaOH
4. 20 % NaOH
5. Whole extract

REFERENCES

1. Rueda DC1, Raith M1, De Mieri M1, Schöffmann A2, Hering S2, Hamburger M3. Identification of dehydroabietc acid from Boswellia thurifera resin as a positive GABAA receptor modulator. Fitoterapia., 2014; 5: S0367-326X(14)00238-X.
2. Khan MA1, Singh M2, Khan MS2, Najmi AK1, Ahmad S2. Caspase Mediated Synergistic Effect of *Boswellia serrata* Extract in Combination with Doxorubicin against Human Hepatocellular Carcinoma. Biomed Res Int., 2014; 2014: 294143.
3. Kessler CS1, Pinders L, Michalsen A, Cramer H, Ayurvedic interventions for osteoarthritis: a systematic review and meta-analysis, .Rheumatol Int., 2014; 26.
4. Jalili C1, Salahshoor MR1, Moradi S2, Pourmotabbed A3, Motaghi M2, The therapeutic effect of the aqueous extract of *Boswellia serrata* on the learning deficit in kindled rats, Int J Prev Med., 2014; 5(5): 563-8.
5. Thummuri D1, Jeengar MK1, Shrivastava S1, Areti A1, Yerra VG1, Yamjala S1, Komirishetty P1, Naidu VG2, Kumar A1, Sistla R3, Boswellia ovalifoliolata abrogates ROS mediated NF-κB activation, causes apoptosis and chemosensitization in Triple Negative Breast Cancer cells,

6. Dalla Libera D1, Colombo B, Pavan G, Comi G, Complementary and alternative medicine (CAM) use in an Italian cohort of pediatric headache patients: the tip of the iceber Neurol Sci., 2014; 35(1):145-8. doi: 10.1007/s10072-014-1756-y.g,
7. Zaki AA1, Hashish NE2, Amer MA2, Lahloub MF2, Cardioprotective and antioxidant effects of oleogum resin "Olibanum" from Bos Boswellia carteri Birdw. (Bursearceae). Chin J Nat Med., 2014; 12(5): 345-50. doi: 10.1016/S1875-5364(14)60042-X.
8. Cameron M1, Chrubasik S, Oral herbal therapies for treating osteoarthritis, .Cochrane Database Syst Rev., 2014; 22(5): CD002947. doi: 10.1002/14651858.CD002947.pub2.
9. Verhoff M1, Seitz S, Paul M, Noha SM, Jauch J, Schuster D, Werz O, Tetra- and pentacyclic triterpene acids from the ancient anti-inflammatory remedy frankincense as inhibitors of microsomal prostaglandin E(2) synthase-1, .J Nat Prod., 2014; 27,77(6): 1445-51. doi: 10.1021/np500198g. Epub 2014 May 20.
10. Wang H1, Zhang C, Wu Y, Ai Y, Lee DY, Dai R, Comparative pharmacokinetic study of two boswellic acids in normal and arthritic rat plasma after oral administration of *Boswellia serrata* extract or Huo Luo Xiao Ling Dan by LC-MS, Biomed Chromatogr., 2014; 8. doi: 10.1002/bmc.3182.,
11. Mostafa DM1, Ammar NM, Basha M, Hussein RA, El Awdan S, Awad , Transdermal microemulsions of Boswellia carterii Bird: formulation, characterization and in vivo evaluation of anti-inflammatory activity, .Drug Deliv., 2014; 14.
12. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M, Frankincense (rǔ xiāng; boswellia species): from the selection of traditional applications to the novel phytotherapy for the prevention and treatment of serious diseases, .J Tradit Complement Med., 2013; 3(4): 221-6. doi: 10.4103/2225-4110.119723
13. Dey D1, Chaskar S, Athavale N, Chitre D, Inhibition of LPS-Induced TNF-α and NO Production in Mouse Macrophage and Inflammatory Response in Rat Animal Models by a Novel Ayurvedic Formulation, BV-9238, Phytother Res., 2014; 7. doi: 10.1002/ptr.5151. [Epub ahead of print]
14. Ahmed M1, Ali D2, Harrath AH2, Hussain T3, Al-Daghri N3, Alokail MS3, Aladakatti RH4, Ghodesawar MA5, Ultrastructural and hormonal changes in rat cauda epididymal spermatozoa induced by Boswellia papyrifera and Boswellia carterii, C R Biol., 2014; 337(4): 250-7. doi: 10.1016/j.crvi.2014.01.007.
15. Schrott E1, Laufer S2, Lämmerhofer M2, Ammon HP3, Extract from gum resin of *Boswellia serrata* decreases IA(2)-antibody in a patient with "Late onset Autoimmune

Diabetes of the Adult" (LADA), Phytomedicine., 2014; 15.21(6): 786. doi: 10.1016/j.phymed.2014.02.004,

16. Umar S1, Umar K2, Sarwar AH3, Khan A4, Ahmad N5, Ahmad S5, Katiyar CK6, Husain SA7, Khan HA8, *Boswellia serrata* extract attenuates inflammatory mediators and oxidative stress in collagen induced arthritis, Phytomedicine., 2014; 15.21(6): 847-56. doi: 10.1016/j.phymed.2014.02.001.
17. Wang R1, Wang Y, Gao Z, Qu X, The comparative study of acetyl-11-keto-beta-boswellic acid (AKBA) and aspirin in the prevention of intestinal adenomatous polyposis in APC(Min/+) mice. Drug Discov Ther., 2014; 8(1): 25-32.
18. Hartmann RM1, Fillmann HS, Morgan Martins MI, Meurer L, Marroni NP, *Boswellia serrata* has Beneficial Anti-Inflammatory and Antioxidant Properties in a Model of Experimental Colitis, Phytother Res., 2014; 28(9): 1392-8. doi: 10.1002/ptr.5142. Epub 2014 Mar 12.
19. Hosseini-Sharifabad M1, Esfandiari E, Effect of *Boswellia serrata* gum resin on the morphology of hippocampal CA1 pyramidal cells in aged rat, Anat Sci Int., 2014; 11.
20. Ahangarpour A, Heidari H1, Fatemeh RA, Pakmehr M, Shahbazian H, Ahmadi I, Mombeini Z, Mehrangiz BH, Effect of *Boswellia serrata* supplementation on blood lipid, hepatic enzymes and fructosamine levels in type2 diabetic patients, J Diabetes Metab Disord., 2014; 4.13(1): 29. doi: 10.1186/2251-6581-13-29.
21. Stürner KH1, Verse N, Yousef S, Martin R, Sospedra M, Boswellic acids reduce Th17 differentiation via blockade of IL-1β-mediated IRAK1 signaling,Eur J Immunol., 2014; 44(4): 1200-12. doi: 10.1002/eji.201343629. Epub 2014 Feb 16.,
22. Bagul P1, Khomane KS1, Bansal AK, Investigating permeability related hurdles in oral delivery of 11-keto-β-boswellic acid, Int J Pharm., 2014; 10.464(1-2): 104-10. doi: 10.1016/j.ijpharm.2014.01.019. Epub 2014 Jan 22.
23. Nikam TD1, Ghorpade RP2, Nitnaware KM1, Ahire ML1, Lokhande VH1, Chopra A3, Micropropagation and non-steroidal anti-inflammatory and anti-arthritic agent boswellic acid production in callus cultures of *Boswellia serrata* Roxb, Physiol Mol Biol Plants., 2013; 19(1): 105-16. doi: 10.1007/s12298-012-0137-3.
24. Ali SI1, Zhang CR1, Mohamed AA1, El-Baz FK1, Hegazy AK2, Kord MA2, Nair MG3, Major constituents of Boswellia carteri resin exhibit cyclooxygenase enzyme inhibition and antiproliferative activity, .Nat Prod Commun., 2013; 8(10): 1365-6.

25. Wang YG1, Ren J, Wang AG, Yang JB, Ji TF, Ma QG, Tian J, Su YL, Hepatoprotective prenylaromadendrane-type diterpenes from the gum resin of Boswellia carterii, J Nat Prod., 2013; 2276(11):2074-9. doi: 10.1021/np400526b. Epub 2013 Nov 6.
26. Moein P1, Abbasi Fard S, Asnaashari A, Baratian H, Barekatain M, Tavakoli N, Moein H, The effect of *Boswellia serrata* on neurorecovery following diffuse axonal injury, Brain Inj., 2013; 27(12): 1454-60. doi: 10.3109/02699052.2013.825009. Epub 2013 Oct 2.
27. Rahimi R1, Nikfar S, Abdollahi M, Induction of clinical response and remission of inflammatory bowel disease by use of herbal medicines: a meta-analysis. World J Gastroenterol., 2013; 14.19(34): 5738-49. doi: 10.3748/wjg.v19.i34.5738
28. Saxena S1, Uniyal V, Bhatt RP, Inhibitory effect of essential oils against Trichosporon ovoides causing Piedra Hair Infection. Braz J Microbiol., 2012; 43(4): 1347-54. doi: 10.1590/S1517-838220120004000016. Epub 2012 Jun 1.
29. Zhang Y1, Ning Z, Lu C, Zhao S, Wang J, Liu B, Xu X, Liu Y, Triterpenoid resinous metabolites from the genus Boswellia: pharmacological activities and potential species-identifying properties, Chem Cent J., 2013; 12.7(1): 153. doi: 10.1186/1752-153X-7-153.
30. Ahmed M1, Al-Daghri N, Harrath AH, Alokail MS, Aladakatti RH, Ghodesawar MA, Alwasel S, Potential ultrastructural changes in rat epididymal cell types induced by Boswellia papyrifera and Boswellia carterii incense, C R Biol., 2013; 336(8): 392-9. doi: 10.1016/j.crvi.2013.04.004.