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Ahmed Al-Harrasi
University of Nizwa
Nizwa, Oman

Abdul Latif Khan
University of Nizwa
Nizwa, Oman

Sajjad Asaf
University of Nizwa
Nizwa, Oman

Ahmed Al-Rawahi
University of Nizwa
Nizwa, Oman

ISBN 978-3-030-16724-0 ISBN 978-3-030-16725-7 (eBook)
https://doi.org/10.1007/978-3-030-16725-7

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Frankincense is precious. It is a resin that is used worldwide in cultural and religious ceremonies, not only in the past but still today. It has been traded for the last 5000 years and has supported happiness and prosperity in many cultures and regions.

Who has never heard of the ‘Frankincense Trail’, long distance camel transport routes from southern Arabian Peninsula, and possibly the Horn of Africa, towards the large cities in the Middle East, Greek and Roman Classical Empires and the Indian civilizations? Frankincense was a main product transported along these trails and traded for other precious goods. Who has never heard of ‘gold, frankincense and myrrh’, the famous precious gifts that were brought by the three kings to the newborn Christ just over two thousand years ago? Who has never heard of frankincense being an essential part of many worldwide used perfumes and an essential part of traditional medicines both in their source countries and in faraway countries like China?

Frankincense is collected from trees and shrubs in the dry tropics of the Arabian Peninsula, the sub-Sahel region in Africa, the Horn of Africa and the Indian subcontinent. The genus thus has a very wide distribution, and of the 19 species, there are five that are produced for local, regional, national and international markets. The species taxonomy is still debated, the distribution of *Boswellia* trees and their abundances are known for some geographical locations but not for others, the resin chemistry is now more understood but not the genetics. So far, almost all of the resin is being collected from the wild populations in far away and mostly environmentally harsh locations. These populations are under increasing threats due to unkind cut and maltreatment by unprofessional harvest of the resin. In addition, the young trees are being eaten by cattle, goats, sheep and camels as well as are being burnt by frequent fires. This needs urgent attention.

My personal involvement as a biologist with frankincense started over 20 years ago when an Eritrean student asked me to be his PhD advisor. Since then I have been working much in Eritrea and Ethiopia on the biology and sustainable management of frankincense plants and the vegetation in which they thrive. As so little was known, all results were new and exciting, and I kept on working and getting more and more involved in frankincense. For years I looked forward to the possibility to visit Oman, the world-famous land of frankincense, where *Boswellia sacra* grows. In late 2018 the great opportunity emerged as some great Omani frankincense researchers organized the first international frankincense scientific symposium in Muscat. And the book you are reading now is written by these great researchers.

I visited Dhofar, the land of frankincense, and travelled to several frankincense-growing locations, some along the coast and others in the interior and on the mountains. The *Boswellia sacra* trees are impressively green and healthy with fascinating white-pinkish flowers. The landscape is open, dry, rocky and harsh for sure. Several large-sized trees had toppled over, probably the result of strong winds during the past rainy season. Small plants were growing here and there; they did not abound but maybe enough to replace the older ones. I was excited to see all this and discuss it with my fellow travellers, both frankincense researchers as I am and frankincense collectors and producers. Lots of exciting questions remain to be addressed, but we all agreed that we need to make sure that this species will be treated with respect, that we need to help to make long-term sustainability possible.

This new book is the first scientific book on biology of genus *Boswellia*, with its in depth information focusses on many undiscovered aspects of *Boswellia sacra* and its resin. It highlights the genetics and genomics, and much of the details that the Omani group is concentrating on, but not only that. I think it is very important and informative that these specialty topics are embedded in a broad framework of species descriptions (look at the great pictures in this book) and species ecology. Much emphasis is put to the collection of the resin: how the plants are being tapped, what are the biological consequences thereof and how much resin is collected. Production and trade, not only nowadays, but also in the past, is brought to the front.

For me, a person with a very broad interest in all aspects of frankincense, this is really exciting and rewarding. And I am convinced that many people are interested in several of the topics tackled in this book. I commend the authors with such a detailed book reflecting their exciting and new work on this famous species and its highly valued resin frankincense.

Wageningen University & Research,
Wageningen, The Netherlands
January 2019

Frans Bongers

Introduction

Frankincense has long been associated with different cultures, civilizations and religions. In the Arabian culture, particularly in Oman and Yemen, frankincense is popular in social and religious celebrations, while in western cultures, its characteristic smell is associated with the Catholic church. Frankincense is burned on hot charcoal, and incense smoke is emitted. In Christianity, frankincense is mentioned 22 times in the Bible (Duke, 2008). The ancient Roman, Egyptian and Greek civilizations knew about the therapeutic importance of frankincense, and very early in human history, frankincense trading routes were established starting from the southern part of the Arabian Peninsula, primarily from Dhofar in southern Oman and Hadramaut (Yemen), to the capitals of the western world (Al-Ghassany, 2008).

The word 'frankincense' is derived from the old French word franc-encens, meaning 'pure incense' (Skeet, 1963), or, literally, 'free lighting' (Walker, 1957), and it has appeared in different civilizations under different names. The Arabic word for frankincense is *Luban*, which is derived from the Semitic root denoting whiteness and implying purity. The Hebrew name is *Lebona*, the Greek is *Libanos* or *libanotos* and the Latin is *tus* (Groom, 1981; Walker, 1957). The ancient Egyptian name was *neter-sent* (Tucker, 1986). In India, it is called *salai gugga*; in Socotra, which is one of the centres of endisms of *Boswellia*, which hosts seven rare species, it is called *am eiro*. In Somalia, frankincense from *Boswellia carterii* is known as *moxor*, while frankincense from *Boswellia frereana* is known as *jagcaar*, and frankincense from *Boswellia papyrifera* is known as *boido*.

The famous frankincense trade was well documented because of its importance in linking different civilizations during ancient times. The earliest reports concerning the frankincense trade route go back to the old Babylonians, when the caravan roads crossed between India, Arabia and Syria. In the Babylonian settlement of Sippar (ca. 2250 BC), the merchants exchanged goods for natural products, including frankincense and myrrh. Both frankincense and myrrh resins were used by old Egyptians for fumigation, as revealed by an analysis of archaeological samples. Between the third century BCE and the second century CE, the incense trade flourished from South Arabia to the Mediterranean through Incense Road. Frankincense and myrrh were carried on camels from southern Oman (Dhofar), Yemen and Hejaz to the Mediterranean ports of Gaza.

The exceptional importance of frankincense explains the interest of ancient civilizations in this material. Among several other uses, its pharmacological use rises to the top. Frankincense was connected to the Indian philosophy of Ayurveda, and it comes from *Boswellia serrata* and was used to treat a variety of diseases (Ammon, 2006). Furthermore, the scripts of the Ebers Papyrus mention frankincense as a drug.

There have been 19 reported species in the *Boswellia* genus since the discovery of the first species by the Scottish botanist John Boswell in 1807 until the last discovery of *Boswellia bullata* in 2001 on Socotra island. There has been much controversy in the literature with regard to the number of species and confusion about the original species and synonyms. For example, *Boswellia carterii* has long been confused with *Boswellia papyrifera*, and *Boswellia carterii* was assumed to be different from *Boswellia sacra*. This confusion is likely due to the use of commercial samples that were purchased from local markets without proper taxonomic identification. In this book, we adopt the species listed by Eslamieh in his book *The Genus Boswellia*. *Boswellia carterii* is a synonym of *Boswellia sacra*, but *Boswellia microphylla* is a different species from *Boswellia neglecta*, and *Boswellia popoviana* is different from *Boswellia nana* (Eslamieh, 2010). In this book, we have also omitted *Boswellia madagascariensis* from the list of *Boswellia* species because it is now considered independent.

Unfortunately, a similar observation was noted with regard to the chemical components of resins from different species, in particular the volatile constituents. Misleading results have been encountered in the past literature and have been quoted and requoted, leading to confusion.

The natural habitat of *Boswellia* trees (trees and shrubs) varies between dry regions as represented by Sudan, Ethiopia, Somalia, Yemen and Oman and humid habitats as represented by India and Sri Lanka. In southern Oman (Dhofar), the resin produced by the *Boswellia sacra* tree varies depending on the geographical location (mountainous and coastal areas) of the tree. This variation is reflected in the commercial grades of the frankincense produced from this tree.

Frankincense is obtained from frankincense trees through careful incisions into the trunk of the tree in which a small strip of the bark is peeled off (cutting should be performed through the cambium) to allow the milk-like substance to ooze out from the trunk and be solidified by exposure to air (Fig. 1). These exudates are likely to be a result of a defensive mechanism through which the tree heals wounds from insect attacks and possibly helps to reduce water loss. The best quality frankincense is harvested after the second incision.

Unkind cuts (wide and deep) and over-tapping (excessive incisions) will render the tree vulnerable to insects and will eventually kill the tree. Interestingly, for a few species, including the coastal *Boswellia sacra* tree, and on certain occasions, the resin oozes out naturally due to the expansion of the trunk and the cracking of the cambium, which will ultimately lead to a rupture in the resin canals. Despite the geographical locations and the habitat of the tree, the method for collecting the resin is the same in all frankincense-producing countries. There are 19 species of

Fig. 1 Stages of harvesting frankincense and different grades of resin obtained from *Boswellia sacra*

frankincense that are geographically distributed throughout South Arabia, Africa and India, as will be discussed in Chap. 2.

The chemical profile of the resin varies significantly from one species to another. While some species are rich in monoterpenes, others are rich in diterpenes and triterpenes. The resin generally possesses fascinating structural diversity. In the essential oil of *Boswellia* from all the species, there have been more than 300 components identified to date (Mertens, Buettner, & Kirchhoff, 2009), and the resin contains more than 100 diterpenes and triterpenes (Al-Harrasi et al., 2018). The botanical origin, geographical location, time of harvest and other environmental factors contribute collectively to the overall composition and hence the biological activity of the resin.

With recent advances in modern medicine, the pharmacological importance of frankincense as a drug has declined, whereas its social and religious values have been maintained. This trend was observed by the disappearance of the term 'Olibanum' from pharmacopoeias in the middle of the twentieth century. However, with the development of preclinical and clinical studies at the end of the last century, frankincense has regained its value when some studies supported its potency in treating a variety of diseases, including rheumatoid arthritis, ulcerative colitis, bronchial asthma and multiple sclerosis (Al-Harrasi et al., 2018).

The frankincense extracts or pure compounds isolated from various *Boswellia* species, in particular triterpenes and diterpenes, have demonstrated superior anti-inflammatory and anticancer activities (Al-Harrasi et al., 2018). The majority of the reported activity of frankincense is due to its rich content of boswellic acids. Another fascinating class of cembranoids, primarily incensole and incensole acetates, displays exceptional anti-inflammatory and anti-depression activities due to their ability to activate ion channels in the brain to alleviate anxiety or depression. In parallel to the advances in clinical studies, several pharmaceutical and cosmetic frankincense-based products have appeared on the market. With the development of olfactometry science, a remarkable achievement has been made with regard to the identification of the fragrant components of frankincense, which has in turn facilitated the development of frankincense-based fragrances.

This is the first book on the biology of the frankincense tree. It is noteworthy that the chemistry and bioactivity of boswellic acids and other terpenoids from the *Boswellia* genus have been covered in our recently published book (Al-Harrasi et al., 2018), and the applications of frankincense in western medicine have been discussed in detail by Hermann P. T. Ammon in his book *Weihrauch-Anwendung in der westlichen Medizin*' (Ammon, 2006). Furthermore, the horticulture of the genus *Boswellia* was well described in a book by Eslamieh (Eslamieh, 2017).

Chapter 1 describes the human uses of frankincense during ancient times and in different civilizations. This chapter also highlights the frankincense trading route and its importance in cultural, civilizational and economic activities across different continents. Chapter 2 emphasizes the *Boswellia* species, their taxonomic identifications and details about the *Burseraceae* family. Chapter 3 reports the production of the resin inside the tree and its transportation to different parts of the stem. Chapter 4 explains the physiological tapping or wounding of the *Boswellia* tree and its ensuing physiochemical-molecular responses via phytohormones, essential biochemicals and metabolites. Chapter 5 illustrates the recent trends in the ex situ conservation of *Boswellia* through tissue culture and bud propagation methods. Chapter 6 describes the recently reported trends in genetic diversity assessments of various populations of *Boswellia sacra* and *Boswellia papyrifera*. It also explains the conservation threats confronted by these populations using various molecular markers and detailed phylogenetic differentiation. Chapter 7 presents the genomics of *Boswellia sacra* and its gene map. This chapter provides detailed information on designing molecular markers for understanding and assessing population gene flow and diversity. Chapter 8 describes the bacterial and fungal rhizosphere communities living with both cultivated and wild *Boswellia sacra* tree populations using next-generation sequencing approaches. Chapter 9 explains the diversity and abundance of endophytes (bacteria or fungi), which provide a diverse hub of bioactive secondary metabolites, phytohormones, extracellular enzymes and essential nutrients. This information not only assists in understanding the role of associated microorganisms but also helps in understanding the tree life and evolution. Chapter 10 summarizes the chemical composition of resins harvested from the 19 species in the *Boswellia* genus, which will be attractive to biologists, chemists, pharmacologists and medicinal chemists due to their fascinating structural diversity.

Nizwa, Oman

Ahmed Al-Harrasi
Abdul Latif Khan
Sajjad Asaf
Ahmed Al-Rawahi

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Contents

About the Authors

Ahmed Al-Harrasi received his BSc in Chemistry from Sultan Qaboos University (Oman) in 1997. Then, he moved to the Free University of Berlin from which he obtained his MSc in Chemistry in 2002 and then his PhD in Organic Chemistry in 2005 as a DAAD fellow under the supervision of Prof. Hans-Ulrich Reissig. His PhD work was on New Transformations of enantiopure 1,2-oxazines. Then, he obtained the Fulbright Award in 2008 for his postdoctoral research in chemistry for which he joined Prof. Tadhg Begley's group at Cornell University where he worked on the synthesis of isotopically labelled thiamine pyrophosphate. After a postdoctoral research stay at Cornell University in 2009, he started his independent research at the University of Nizwa, Oman, where he founded the Chair of Oman's Medicinal Plants and Marine Natural Products merging chemistry and biology research. He is currently a professor of organic chemistry and the vice chancellor for Graduate Studies, Research and External Relations at the University of Nizwa. He is also the founder and chairperson of the Chair of Oman's Medicinal Plants and Marine Natural Products. The budget of his interdisciplinary-funded projects exceeds seven million USD. He was a chair and invited speaker in many international conferences and is a referee for more than 15 international chemistry and biotechnology journals. He has authored and co-authored over 300 scientific papers, 1 book and 6 book chapters and taught many chemistry courses both at MSc and BSc levels.

Abdul Latif Khan received his BSc and MSc degree in distinction from the University of Peshawar, Pakistan. Later, he joined the Department of Chemistry, Kohat University of Science and Technology, Kohat, Pakistan, to complete his MPhil in Phytochemistry and JASSO Research Fellowship by the Gene Research Center University of Tsukuba, Japan. He was selected as honorary scholar by Kyungpook National University, South Korea, for his PhD degree in Plant Physiology. After his PhD, he remained as a postdoctorate fellow at the School of Applied Biosciences. He also worked as research professor at the Institute of Agricultural Science and Technology, Kyungpook National University, South Korea. He has published more than 150 research articles as a principal author and co-author in journals of international repute and of impact factor. His research focuses on plant molecular physiology during the activities of environmental and microbial antagonists and plant genome sequencing. His current projects include draft and chloroplast genomes of date palm, *Boswellia sacra*, pomegranate and various endemic medicinal plants of Oman. He is currently working as associate professor at the Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, Oman.

Sajjad Asaf completed his Master in Botany (MPhil) from Kohat University of Science and Technology (KUST), Kohat, Pakistan, in 2012. He received a KNU Honour Scholarship for PhD studies in South Korea and completed his PhD (2017) from the Laboratory of Crop Physiology at Kyungpook National University, South Korea. His PhD research work was based on the physiology and genomics of plant growth-promoting endophytic bacteria and its role in environmental stress tolerance. Little is known about bacterial endophytes from arid land plants. Beyond his PhD research project, he is deeply committed to genome sequencing and the analysis of plant organelles and their comparison with nuclear genomes. He has been fortunate to obtain a wide range of experience in the field of chloroplast, mitochondria and microbial genomics. Moreover, he pursued postdoctoral research for 1 year in plant physiology and genomics at Kyungpook National University, South Korea. Currently, he is working as assistant research professor at the University of Nizwa, Oman. He is currently involved on the whole plant genome sequencing analysis, as he has experience in plant, chloroplast, mitochondrial and microbe's genome sequencing and data analysis. Therefore, comparative genomic analysis is the main focus of his current interest. Furthermore, he has the potential to analyse the data through different bioinformatics tools responsible for NGS data processing.

Ahmed Al-Rawhi earned his MSc and PhD in Plant Pathology from the University of California at Berkeley in 1992 and 1995, respectively. Earlier (1988), he had completed with Honours his BSc in Biological Science at North Carolina State University (Raleigh, USA). Upon his return to Oman, he became a lecturer at Sultan Qaboos University where he taught courses in the field of plant pathology and microbiology. He also conducted research in soil-borne pathogens, biocontrol and disease management programs and published articles in high-impact international journals. From December 1997 until May 2001, he had the honour of serving as minister of Agriculture and Fisheries and played, as such, a major role in developing various strategies and development plans for these vital sectors of the Omani economy. He served as member of the State Council from 2001 to 2011 and led important studies and reports for the government of Oman. He was also chairman of the Academic Foundation Committee for the University of Nizwa Project from 2000 to 2004, the year he was appointed as chancellor of the newly founded university. Throughout his tenure as chancellor, he embarked on bringing the project to reality and creating a functioning academic institution that adheres to institutionalization, quality standards, ethics and procedures. Under his dynamic leadership, thousands of Omani students graduated with bachelor's and higher degrees, and the university contributed strongly to community services and produced innovative research relevant to the needs of Oman. Ultimately, he was promoted to the academic position of founding professor by a scholarly independent Academic Committee in December 2006. Professor Ahmed Al Rawahi is a member of various national academic committees including the Education Council and Research Council. He was also a member of the American Phytopathological Society. In November 2000, he was greatly honoured to receive from HM Sultan Qaboos bin Said Oman Medal of Merits (2nd degree, civil). In December 2013, the University of Wisconsin Oshkosh awarded him the Honorary Doctorate Degree in recognition of his role as “a distinguished and visionary academic leader with an international voice and perspective”.

Chapter 1
Frankincense and Human Civilization: A Historical Review

Etymology of Frankincense

In Arabic, the *Boswellia* tree and the resin it produces are called '*luban*' or '*loban*', which literarily describes the clear white exudate resin that is secreted by tapped trees and equates it with the '*laben*', or the 'milk' of a tree, hence, the origin of the 'libanum' and 'olibanum' used in ancient trade. '*Luban*' or '*loban*' for the plural and '*lebanah*' for a single tear droplet are not far from the Hebrew name '*levonah*' or '*lebonah*', which also indicate '*lavan*' or 'white' in Hebrew (Ben-Yehoshua, Borowitz, & Ondrej Hanuš, 2012; Coder, 2011). One of the important uses of frankincense since ancient times is as burning incense; thus, its English name 'frankincense' reflects these properties. The origin of this name comes from the Old French, with '*franc*' meaning pure or noble and '*encens*' meaning incense. Two types of Somalian frankincense, namely, '*Maidi*' and '*Bayo*', are the best.

Oleoresin (Tears of the Sun)

Frankincense gum resin is harvested by incising and scraping a piece of the periderm with a tool called a '*menguf*', which is an oval-shaped, scalpel-like instrument. The exposed surface is tapped to induce wounding, allowing the resin to ooze out. As it oozes out to the surface, the exudate appears to be similar to white milk in colour but is viscous as honey. Upon exposure to the air, it hardens, and its colour is transformed into a translucent to light amber, and the resin solidifies as pear-like tears or droplets known as 'tears of the sun'. After 2–3 weeks, those clumps of droplets are gently scraped off the tapped area and allowed to harden more for a few weeks before being marketed. During these collections, the tapped areas of the stem are re-tapped to induce further secretions from the tree. The resin is harvested during the dry season from October to December and from

A. Al-Harrasi et al., *Biology of Genus Boswellia*,
https://doi.org/10.1007/978-3-030-16725-7_1