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Characterization and analysis of a *Commiphora* species germinated from an ancient seed suggests a possible connection to a species mentioned in the Bible



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A seed recovered during archaeological excavations of a cave in the Judean desert was germinated, with radiocarbon analysis indicating an age of 993 CE– 1202 calCE. DNA sequencing and phylogenetic analysis identified the seedling as belonging to the angiosperm genus *Commiphora* Jacq., sister to three Southern African *Commiphora* species, but unique from all other species sampled to date. The germinated seedling was not closely related to *Commiphora* species commonly harvested for their fragrant oleoresins including *Commiphora gileadensis* (L.) C.Chr., candidate for the locally extinct “Judean Balsam” or “Balm of Gilead” of antiquity. GC-MS analysis revealed minimal fragrant compounds but abundance of those associated with multi-target bioactivity and a previously undescribed glycolipid compound series. Several hypotheses are offered to explain the origins, implications and ethnobotanical significance of this unknown *Commiphora* sp., to the best of our knowledge the first identified from an archaeological site in this region, including identification with a resin producing tree mentioned in Biblical sources and possible agricultural relationship with the historic Judean Balsam.

The germination of ancient seeds derived from archaeological sites¹, permafrost² and historical and botanical collections³ with verifiable provenance and properly dated radiocarbon analysis include; ~30,000 year-old *Silene* sp. from Siberian permafrost²; 2000 year-old date seeds^{4–6}, live callus from 1600 year-old *Anagyris foetida* seeds⁷, 1,300 year-old lotus seeds⁸, 680 year-old peatland *Sphagnum* spores⁹ and 151 year-old acacia seeds¹⁰.

Reviving historic seeds has generated considerable interest because of its potential applications to many fields as a way to bring back lost taxa and identify earlier and extinct phenotypes^{11,12}; contribute to a better understanding of crop domestication, evolution and improvement of current

crops^{4–6}; circumvent sequencing errors arising from degraded ancient DNA through the emerging field of “resurrection genomics”⁶; reinforce populations of rare species and revive species extinct in the wild^{11,12}; provide valuable information on seed longevity, resilience and stress tolerance with implications for agriculture, biodiversity conservation and seed banking; discovery of compounds of potential pharmaceutical interest; enable a better understanding of ancient societies through insights into past environments, ethnobotany, economies, society and material culture^{4,5}.

In the current study, we report on the germination and growth of an ancient seed identified as belonging to the *Commiphora* genus recovered

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during archaeological excavations of a natural cave in the northern Judean desert.

The *Commiphora* genus (Gr: “kommi” gum bearing), a species-rich member of the Frankincense and Myrrh family (Burseraceae) predominantly distributed in Africa, Madagascar and the Arabian Peninsula¹⁴, has been valued throughout history for its economic and ethnobotanical uses because of the aromatic gum resins or oleoresins produced by members of this family^{14–16}. Since the 18th century, *Commiphora gileadensis* (L.) C.Ch., an extant, highly aromatic species native to the Arabian Peninsula and northeast tropical Africa, and one of at least 25 *Commiphora* species whose oleoresins are used ethnomedicinally¹⁷, has been considered a candidate for the historic “Judean Balsam” or “Balm of Judea”, cultivated in this region for at least 1000 years exclusively at oasis sites around the Dead Sea basin^{18–21}. The most valuable export of ancient Judea (modern day Israel and Palestine) and described extensively by writers in antiquity, Judean Balsam, was highly prized for its fragrant aromatic resin “*opobalsamum*” (Gk: “sap of balsam”) and its many economic uses^{18–22}.

Judean Balsam however, disappeared from the region by the 9th century CE, leading to an extended, unresolved debate in published literature regarding its scientific identity and if it had survived elsewhere^{19,23–25}. Nevertheless, identification of Judean Balsam with *Commiphora gileadensis* is contestable due to morphological differences between the two¹⁹, lack of verified archaeobotanical remains of any *Commiphora* species in the Southern Levant (modern day Israel, Palestine and Jordan)^{20,24} and the absence of a native, extant *Commiphora* species in the region today²⁵.

Adding to this uncertainty because of the life-history and remote location of most *Commiphora* species, the taxonomy of contemporary *Commiphora* species is not well-resolved^{26,27}. New species are routinely discovered while circumscriptions of known species continue to shift to reflect new knowledge. For instance, *Commiphora gileadensis* (L.) C.Ch. is inclusive of and synonymous with 21 other taxa including *C. opobalsamum* Engl²⁸. Thus, if Judean Balsam survives today as an extant *Commiphora* species there remains the possibility that scientists have not yet recognized it.

In the current study, we used DNA sequencing, phylogenetic and phytochemical analysis together with archaeological and historical source material and phytogeographic data to explore various hypotheses that could identify and explain the presence of this unknown *Commiphora* species in the region approximately 1000 years ago. We questioned if it could be a candidate for the valuable Judean Balsam of antiquity or whether it may represent an extinct (or at least extirpated) species of *Commiphora* once native to the region suggested by early Biblical texts, and if so whether its presence may have been associated with cultivation, commerce and trade.

In reviving an unknown *Commiphora* species, this study provides a unique opportunity to rediscover the origins of a population that once existed in the southern Levant and provide insights into its possible historical and economic role in cultivation and commerce.

Results

Seed germination

A seed of unknown identity was recovered during archaeological excavations (1986–87) of a natural cave in Lower Wadi el-Makkuk (31°53′27.92″N, 35°21′2.88″E), by the Archaeology Dept. of the Hebrew University of Jerusalem, during surveys of caves in the northern Judean desert carried out from 1986–89²⁹. The seed, which formed part of the archaeological finds, was stored at the Dept. of Archaeology of the Hebrew University until it was selected from other archaeobotanical material by Dr Sallon.

The well preserved seed, 1.8 cm in length, weighing 0.565 g (Fig. 1) (identification code: HULMKG1), was planted in 2010 at the greenhouse facility of the Center for Sustainable Agriculture (CSA) with seedling emergence ≈ 5 weeks later.

Morphological features

Growth of the seedling, informally named “Sheba”, displayed morphological features typical of the *Commiphora* genus (Fig. 1) Currently 14 years old, ≈ 3 m high, its bark is pale green-brown peeling in thin, papery sheets revealing

a dark green under bark. Leaves are alternate, imparipinnately compound with three to five leaflets and a fine, velutinous pubescence becoming sparse to subglabrous at maturity covering leaves and young and emergent stems. The tree is deciduous, shedding leaves during the cooler months of December–April (mean local temperature 24.8 °C). Wounding the bark produces a small amount of clear oleoresin. Minimal to no fragrance is detected from leaves, bark or resin. Since “Sheba” has not flowered, we do not have reproductive material to attempt a species description at this time.

Radiocarbon dating

Radiocarbon ages of the ancient seed were obtained from the operculum of the woody endocarp encasing the seed. The operculum is a hatch-like portion of the endocarp that opens and detaches when the seed germinates. The operculum did not germinate and therefore should not have incorporated young and fresh carbon during the experiment. Nonetheless, we made a scenario calculation to see what would have happened if the operculum had incorporated 1, 2 or 3% of modern carbon during seedling growth. Two different mathematical approaches were used to calculate the age change with an addition of 1 – 3% modern carbon (see ‘Material and Methods’). Both approaches gave identical results. (The calibrated radiocarbon ages (OxCal 4.4) with error ranges are shown in Supplementary Tables 1 and 2).

Although these scenarios are very unlikely, these radiocarbon ages were recalculated to take into account such a potential contamination, previously shown to reduce measured radiocarbon age⁴⁵. Under the assumption the seed fragment was not affected by modern germination, which is very likely since woody endocarp tissue does not grow or photosynthesize, the origin of “Sheba” has an age between 1026–1202 cal CE. A less likely scenario is that the seed fragment might have absorbed some fresh carbon during germination, based on our previous studies of germinating ancient date seeds⁴⁵. In such a case, the value is around 1% (to max. 3%) of new carbon⁴⁵. Assuming between 0% and 3% of modern carbon was absorbed, the age range extends to 993 CE– 1202 calCE. (Fig. 2).

Phytochemical analysis

Early leaf and stem material collected in 2010 was analyzed by Headspace Solid Phase Microextraction (SPME) in combination with gas chromatography-mass spectrometry (SPME/GC-MS). Only minor amounts of volatile compounds, mainly mono-terpenes α and β -pinene, were detected, consistent with the specimen’s general lack of fragrance. Heating leaf and stem material to investigate if more abundant volatile compounds were released did not show distinctive compounds except for common pyrolysis products including phenolics and furans (Comparison of volatile compounds by SPME/GC-MS analysis from “Sheba’s” plant material is shown in Supplementary Fig. 1).

Leaf and stem material was extracted using solvents of varying polarity. Hexanes produced the most concentrated extract indicating many non-polar compounds including squalene (over 30% of extracted), various hydrocarbons (C₂₅–C₃₁), fatty acids e.g. hexanoic and linolenic acids, α -tocopherol, pentacyclic triterpenoids α - and β -amyrin and some phytosterols. Additional triterpenoids were also detected and confirmed by GC-high resolution-MS but remained unidentified by comparison with commercial mass spectral libraries (Table 1 and Fig. 3).

In 2013, a resin sample obtained from “Sheba” (age 3 years) was extracted using different solvents (hexane, acetone, methanol). Although the majority of the resin material remained mostly undissolved in all solvents tested, trace amounts of monoterpenes in the hexane extract were detected including α - and β -pinene and several pentacyclic triterpenes including α - and β -amyrin, lupeol and two similar unidentified triterpenes having mass spectra resembling lanosterol but eluted separately when compared by GC co-injection experiments using a lanosterol reference compound. These may correspond to the related euphol and/or tirucalol whose mass spectra are similar to lanosterol, but reference standards of these two higher plant triterpenoids were not available at the time of testing (A list of major compounds detected in “Sheba’s” resin is shown in Supplementary



Fig. 1 | Morphological features of “Sheba” at different ages. (a) ancient seed prior to planting (b) developing seed at 5 weeks showing epicotyl and developing cotyledons covered by seed coat (c) seedling (6 months) (d) peeling bark (12 years)

(e) leaves showing fine hairs (12 years) (f) mature tree (12 years). Permission for the use of pictures shown in Fig. 1 (a) and (c-f) was provided by Mr Guy Eisner and for Fig. 1 (b) by Dr Elaine Solowey.

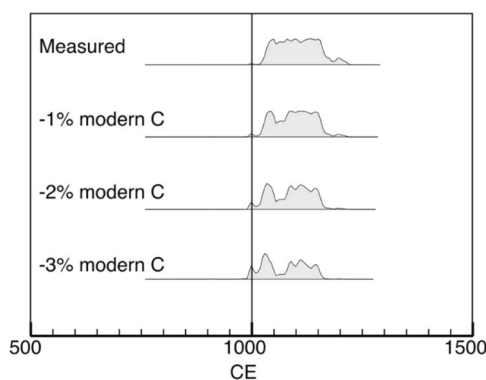


Fig. 2 | Original and recalculated radiocarbon age distributions (scenario calculations) of the germinated seed taking into account absorbed percentage (0%, 1%, 2% and 3%) of modern carbon (pMC).

Table 3 and Supplementary Fig. 2. Mass spectra of the 2 unknown triterpenoids are shown in Supplementary Fig. 3).

Incremental heating of the resin sample to determine if any unique volatiles were released on burning showed chromatographic profiles of increasing complexity as heating increased (Comparison of volatiles emitted from heating “Sheba’s” resin is shown in Supplementary Fig. 4). Major compounds detected included various furans, pyrans, phenols and

pyridines, common pyrolysis products of polysaccharides and proteins³⁰, consistent with the predominant constituents of other *Commiphora* resins³¹.

In 2023, resin and leaves were re-sampled from “Sheba” (age 13 years) for further characterization of lipid content. These were extracted by sonication with CH₂Cl₂ and MeOH (1:1, v/v) and total lipid extracts (TLE) directly acetylated and methylated to lower polarity and improve GC lipid analysis. Derivatized TLE were separated by column chromatography on silica gel into apolar and polar fractions (F1 and F2, respectively), the former being analyzed by GC-Flame ionization detector (FID) and GC-MS.

Investigation of fraction F1 from leaf extract by GC-MS revealed predominance of sugar derivatives and triterpenoids α - and β -amyrin and the phytosteroid sitosterol (Fig. 4a).

Straight-chain lipids corresponding to the wax coating of leaves were also identified comprising predominantly C₂₈-C₃₄ *n*-alcohols (C₃₀ > C₃₂ > C₂₈), C₁₄-C₃₄ *n*-acids (C₁₆ predominant) and C₂₇-C₃₃ *n*-alkanes (C₂₉ predominant). Most of these compounds (except sugars) were also present in earlier extracts from 2010 and 2013.

Surprisingly, a series of late eluting compounds (I-III) was observed at the end of the gas chromatogram obtained by GC-FID. The same compound series was shown to represent the sole lipids from the apolar fraction of the resin extract, compound II making > 80% of the whole fraction (Fig. 4b). The presence of these high molecular weight compounds went undetected in the earlier phytochemical studies as the extracts were not derivatized.

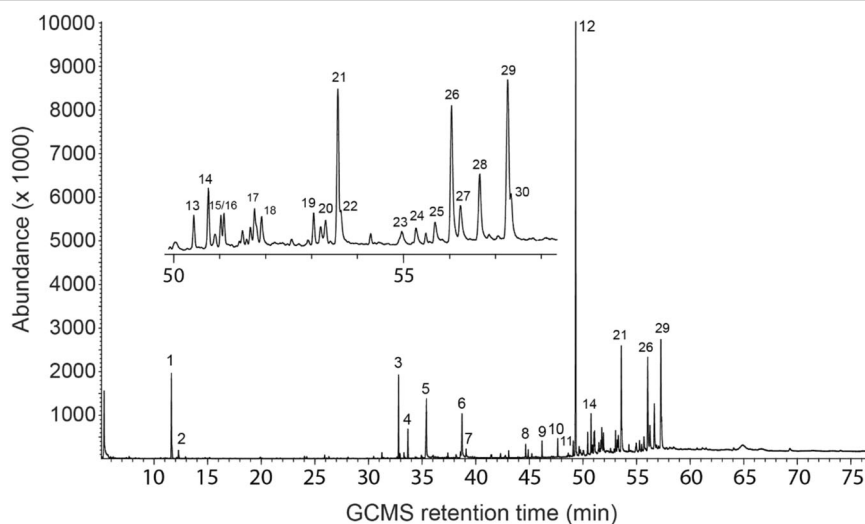
Investigation of the Electron Ionization (EI) mass spectrum of the predominant compound II, showed no molecular ion and a complex

Table 1 | Initial identification of major compounds detected in the hexane extract of “Sheba” plant material (leaf and stem) using GC-MS (Fig. 3), showing relative and % abundance compared to internal standard

Number	RT	Compound	RI	Lit. RI	Amount ^a	Amount (%)
1	11.62	Isobutyl benzene (IS) ^b	1010		50.00	
2	12.28	limonene ^b	1032		5.61	0.64
3	32.81	<i>neophytadiene isomer 1</i>	1836	1839 ^c	38.76	4.45
4	33.69	<i>neophytadiene isomer 2</i>	1879	1881 ^c	14.64	1.68
5	35.41	hexadecanoic acid ^b	1965		46.93	5.39
6	38.72	linolenic acid	2143	2143 ^a	33.25	3.82
7	39.10	octadecanoic acid ^b	2164		5.02	0.58
8	44.67	pentacosane	2499	2500	6.31	0.73
9	46.19	hexacosane ^b	2597		7.97	0.92
10	47.65	heptacosane	2698	2700	9.01	1.04
11	49.09	octacosane ^b	2797		8.40	0.97
12	49.34	squalene ^b	2816	2814 ^a	270.17	31.04
13	50.44	nonacosane	2897	2900	10.96	1.26
14	50.76	<i>Unknown 1 (C₃₀H₄₈)</i>	2921		21.28	2.45
15	51.03	<i>Unknown 2 (C₃₀H₅₀O)</i>	2941		9.69	1.11
16	51.10	<i>Unknown 3 (C₃₀H₅₀O)</i>	2946		11.09	1.27
17	51.76	triacontane ^b	2995		16.49	1.89
18	51.91	<i>Unknown 4 (C₃₀H₅₀O)</i>	3007		16.45	1.89
19	53.05	hentriacontane	3097	3100	10.95	1.26
20	53.31	<i>Unknown 5</i>	3118		11.37	1.31
21	53.57	α -tocopherol ^b	3138		44.97	5.17
22	53.64	<i>α-tocopherolquinone</i>	3144		14.62	1.68
23	54.97	<i>campesterol</i>	3253		10.35	1.19
24	55.28	<i>stigmasterol</i>	3279		9.70	1.11
25	55.69	<i>n-triacontanol</i>	3313		13.10	1.51
26	56.05	<i>γ-Sitosterol</i>	3344	3351 ^b	66.49	7.64
27	56.24	<i>isofucosterol</i>	3360	3343 ^a	20.93	2.40
28	56.66	β -amyrin ^b	3395		34.04	3.91
29	57.27	α -amyrin ^b	3444		80.58	9.26
30	57.34	α -tocopherol derivative	3450		21.16	2.43

^a relative to IS; ^b confirmed with commercial standard. Compounds in italics tentatively identified based on mass spectra and retention indices (RI) values sourced from: a. (ref. ⁷⁷), b. (ref. ⁷⁸), c. (ref. ⁷⁹). Retention times correlate with Fig. 3

Fig. 3 | GC-MS chromatogram of hexanes extract of “Sheba” plant (leaf and stem) material. Detailed analysis of major compounds corresponding with numbers are shown in Table 1.



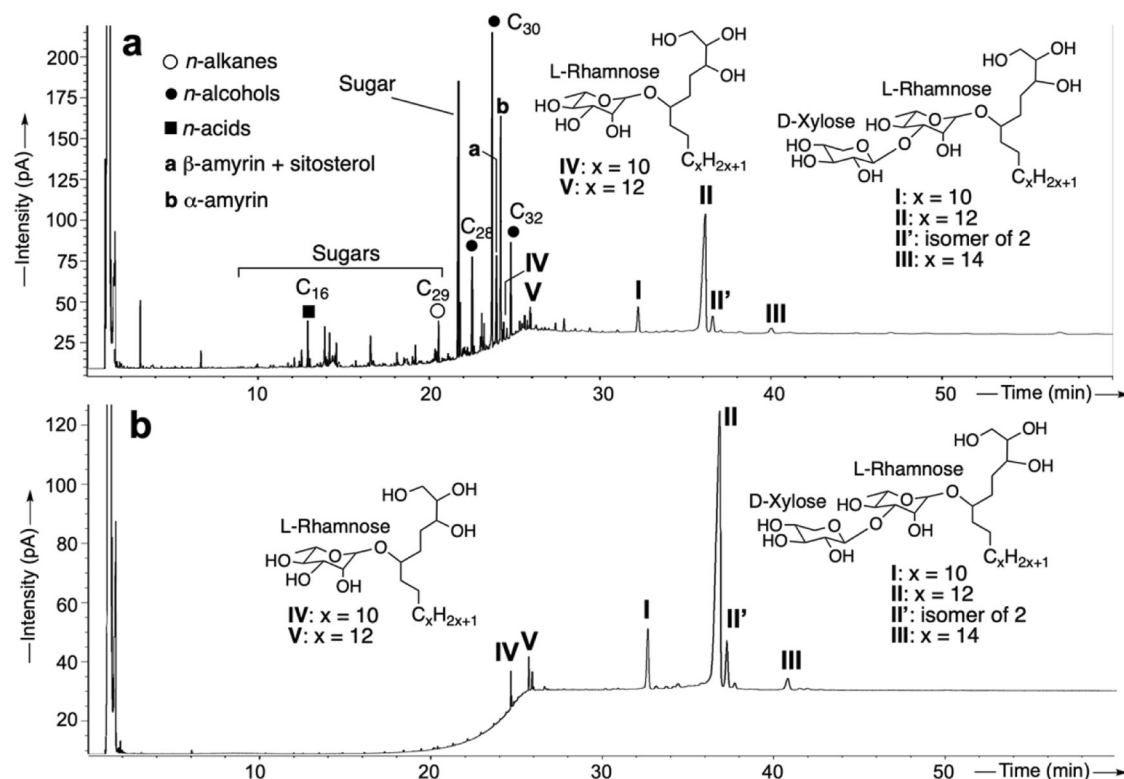


Fig. 4 | Gas chromatograms (GC-FID) showing lipid distribution of the apolar fraction F1 from. a. “Sheba” leaves; b. “Sheba” resin. Alcohols are analyzed as acetate derivatives and acids as methyl ester derivatives.

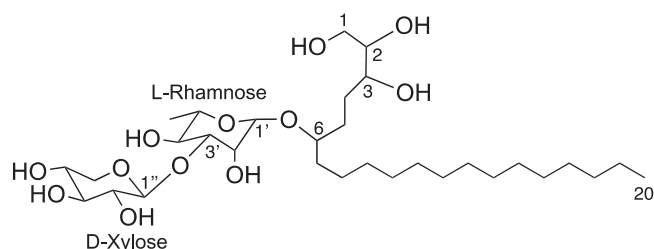


Fig. 5 | Structure and carbon numbering of the predominant glycolipid II identified in the resin and leaf extracts from “Sheba”.

fragmentation pattern that could not be interpreted except for two fragments at m/z 259 and m/z 489, likely corresponding to a pentose (m/z 259) and a pentose + deoxyhexose (m/z 489) moiety. In the absence of a molecular ion under EI conditions, high resolution mass spectrometry using electrospray source in positive mode was performed on the acetylated derivative, yielding a $[M+Na]^+$ ion at 983.4844, corresponding to the molecular formula $C_{47}H_{76}NaO_{20}$. Isolation of compound II with a purity > 85% (as the acetate derivative) and identification by 1D and 2D NMR spectroscopy and chemical degradation (methanolysis and oxidative cleavage using periodic acid), determined Compound II corresponded to a glycolipid with an unusual C_{20} straight-chain hydrocarbon skeleton bearing 3 hydroxy groups at C-1, C-2 and C-3 positions and a disaccharide moiety attached to an additional OH group at C-6 consisting of rhamnose and xylose (terminal group) between positions C-3' and C-1' (Fig. 5, see Supplementary Table 4 and Supplementary Figs. 5-11 for NMR data. See Supplementary Data for raw NMR data used to prepare Supplementary Table 4 and Supplementary Figs. 6-11). Given the natural occurrence of these sugars in living plants, we propose they belong to the L- and D-series respectively. Full structural identification of this novel compound will be reported in a future publication.

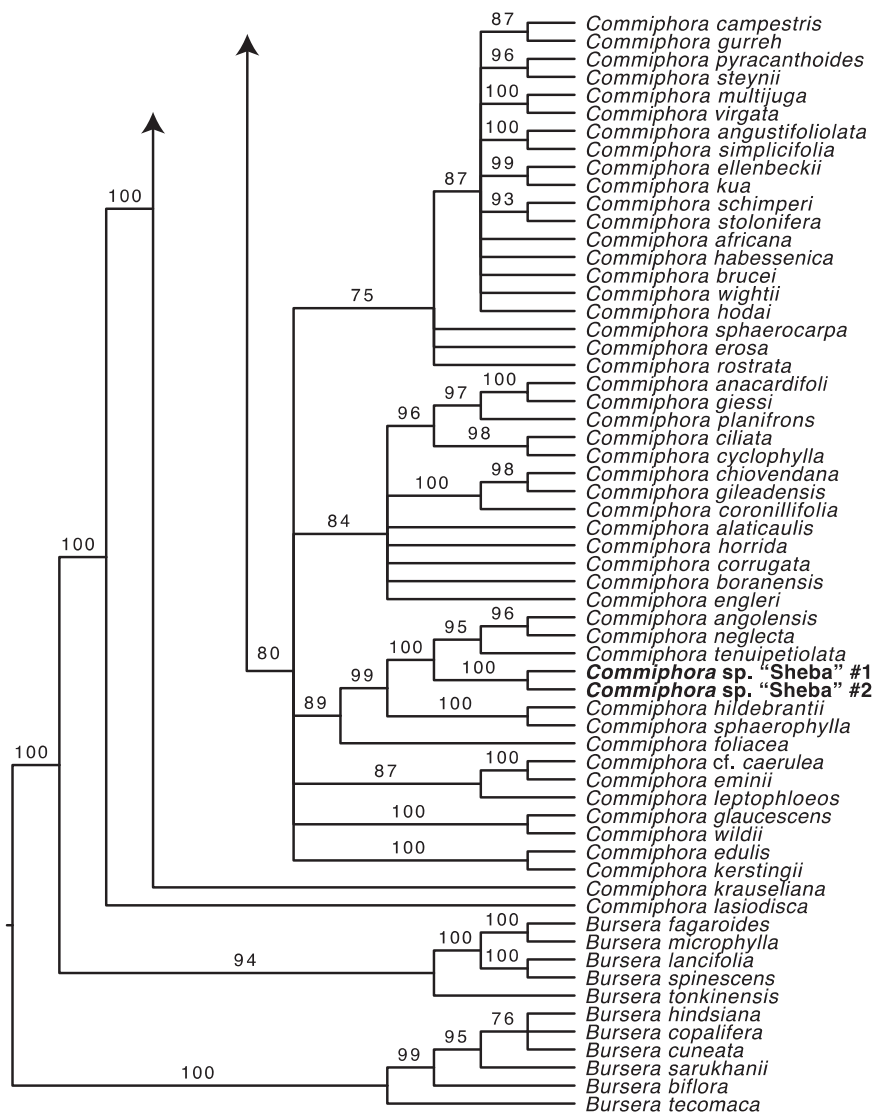
By analogy with the mass spectrum of compound II and relative retention times in GC, we concluded compounds I and III are homologues of II, having respectively a C_{18} and a C_{22} straight-chain hydrocarbon skeleton. Similarly, the first eluting compounds IV and V (Fig. 4) correspond to monosaccharide derivatives of I and II respectively, bearing a L-rhamnose moiety likely attached at C-6. To the best of our knowledge compounds with such a structure have not been reported elsewhere. Other tetrol compounds known as “guggultetrols” however, have been identified from resin of *Commiphora wightii* (syn. *C. mukul*), (identified as octadecan-1,2,3,4-tetrol, nonadecan-1,2,3,4-tetrol and eicosan-1,2,3,4-tetrol)^{32,33}, sharing some structural similarities with aglycones from “Sheba’s” compound series. These have the same C_{18} and C_{20} straight-chain hydrocarbon skeleton and four hydroxy groups although located at a different position for one of the hydroxy group (at C-6 in “Sheba” vs. C-4 for the guggultetrols). C_{23} and C_{24} unsaturated homologues of guggultetrols isolated from *C. wightii* have shown significant cytotoxic activity and moderate scavenging effect against DPPH radicals³⁴, while C_{18} tetrol glycolipid derivative with a terminal rhamnose moiety isolated from *C. opobalsamum* has exhibited moderate anti-proliferative effects against PC3 cells³⁵.

DNA sequencing and phylogenetic analysis

Herbarium voucher specimens (Sarah Sallon s.n.) of “Sheba” were deposited in HUJ (Hebrew University of Jerusalem), (Accession 134891), TELA (Tel Aviv University (Accession 4350) and MO (Missouri Botanical Gardens), (Accession Number 7012950, Barcode Number 3363152).

The chloroplast gene *rbcl* was amplified and sequenced using Sanger chemistry to produce a 1455 bp DNA barcode for BLAST query (Genbank accession number OP963950) to test family and generic level taxonomic placement. The five highest bit-scores with E values of 0.0 that were returned belonged to Burseraceae with the highest being *Commiphora habessinica*. Because the *rbcl* gene did not provide sufficient nucleotide variation among species within *Commiphora*, *nrETS* and *psbA-trnH* loci were used to create multiple sequence alignments for the genus. These alignments containing

Fig. 6 | Placement of “Sheba” within *Commiphora* species phylogeny; arrows lead to 59 other *Commiphora* species within the remaining topology. “Sheba” is nested within the “Spinescens” clade, which encompasses *C. campestris* through *C. kerstingii*. Tree topology is a 75% majority rule consensus cladogram of 1000 ultrafast bootstrap replicate trees from maximum likelihood analysis of nuclear and chloroplast DNA sequence data. “Sheba” 1 and “Sheba” 2 were obtained from separate DNA extraction.



415 bp and 565 bp respectively included, DNA sequences from “Sheba” of nrETS (Genbank accession numbers OP96395, OP963952) and *psbA-trnH* (Genbank accession numbers OP963953, OP963954). Sequences of “Sheba” were generated twice from separate DNA extractions.

Phylogenetic position of “Sheba” and relationships to other species of *Commiphora*

Phylogenetic reconstruction was carried out using maximum likelihood in IQ-Tree³⁶ for separate and combined nuclear and chloroplast DNA sequence datasets. Results of each analysis are summarized by 75% majority rule consensus trees with branch support values. (Branch-length proportional phylograms are shown in Supplementary Figs. 12–14). The cladogram of the combined analysis is shown in Fig. 6. The aligned matrix of sequence data for the nrETS and *psbA-trnH* loci, analysis scripts and resulting trees have been deposited in the Dryad Digital Repository³⁷. Results from phylogenetic analyses corroborate those recovered in previous studies^{27,38,39}, including low phylogenetic signal from the chloroplast genome (Supplementary Fig. 13) and corresponding greater resolution of the combined nuclear and chloroplast datasets (Fig. 6) (Supplementary Fig. 14). The combined analyses indicate that “Sheba” is nested within the species-rich and geographically widespread “Spinescens” clade³⁸, with strong support. Within the “Spinescens” clade, “Sheba” is sister to a subclade comprising three southern African *Commiphora* species (*C. angolensis*, *C. neglecta*, and *C. tenuipetiolata*).

Discussion

In the current study, an unidentified seed previously recovered from archaeological excavations of a natural cave in the northern Judean desert²⁰ was germinated, with radiocarbon dating suggesting an age between 993 CE–1202 CE. Morphological features identified the germinated seedling “Sheba” as belonging to the *Commiphora* genus, a finding confirmed by DNA sequencing and phylogenetic analysis that showed it to be unique from all other species sampled to date.

The most species-rich member of the Burseraceae family, the genus *Commiphora*, comprises some 200 trees and shrubs distributed across Africa, Madagascar, Arabian Peninsula, India, Sri Lanka and South America with species diversity concentrated in the *Acacia-Commiphora* woodland of tropical East Africa and Madagascar’s western dry, deciduous forests^{26,38}.

Our comparative phylogenetic study of 109 *Commiphora* species indicates “Sheba” is closely related to *C. angolensis*, *C. neglecta* and *C. tenuipetiolata*, part of the species rich, geographically widespread “Spinescens” clade containing all *Commiphora* species that produce true thorns (e.g. spine-tipped branches) and most *Commiphora* species whose oleoresins are harvested commercially³⁸. Species sampling of the present study did not include *Commiphora myrrha*, one of the species that produces myrrh for contemporary commercial markets. However, “Sheba” is unlikely to be closely related to it, as in a previous study the subclade of *C. angolensis*, *C. neglecta* and *C. tenuipetiolata* is only distantly related to *C. myrrha*³⁸.

In the current study, we attempted to identify this unknown *Commiphora* species, explain its presence in a cave in the northern Judean desert ca.1000 years ago and suggest what could be the significance and implications of these findings.

Our initial hypothesis was that “Sheba” might be a candidate for the historical “Judean Balsam” or “Balm of Judea” (Greek: *opobalsamum*, Latin: *balsamum*; Arabic: *balsan*, Hebrew *bosem/ besem/ balsam /afarsemon*), a highly fragrant tree or shrub cultivated exclusively in this region during antiquity. Extensively described by writers and commentators in Hellenistic, Roman-Byzantine and Post-Classical periods from the 4th century BCE to the 8th century CE (Ancient sources discussing Judean Balsam are shown in Supplementary Table 5), Judean Balsam’s oleoresin (“*opobalsamum*”)^{23,40} was the most valuable commodity of ancient Judea^{16,18–22}, a mountainous, partly desert region in the southern Levant. Highly prized in the ancient world and exported throughout the Roman empire, Judean Balsam’s oleoresin was used as perfume, incense, medicine, treatment for cataracts, embalming, antidote to poisons/snake venom and ritual/ceremonial purposes^{16,18–22,25,40,41}.

Cultivated only in garden oasis sites around the Dead Sea basin, Judean balsam was considered unique to ancient Judea^{16,18–21,24,25}. However, it was not considered native to the region. Classical commentators including *Strabo* and *Josephus Flavius* (Historical sources discussing Judean Balsam are shown in Supplementary Table 5) attribute its origins to the ancient kingdom of Saba, encompassing parts of Ethiopia, Eritrea and Southern Arabia, known for its aromatic trees and involvement in the spice trade⁴². Trade links between Southern Arabia and the Israelite Kingdom chronologically dated to this period (Iron Age 1, Southern Levant) suggest Judean balsam was introduced to Judea in the 10th century BCE⁴³ or following Assyria’s 8th century BCE conquest of Israel with establishment of Dead Sea oases as centers for its cultivation⁴⁴. By the 9th century CE, despite its economic importance, Judean balsam had disappeared from the region with “true Balsam” according to Arab geographers, found only in the gardens of Ayn Shams (*Matariyya*), Heliopolis, Egypt, where a cultivated, sterile strain, allegedly originating in Judea was reportedly still extant in the 16th century CE¹⁹. Botanical identification of Judean Balsam has long been contestable^{19,23}. Since the 18th century, *Commiphora gileadensis* (L.) C. Chr. (Syn: *Amyris opobalsamum* L., *Amyris opobalsamum* L., *Balsamodendrum opobalsamum* (L.) Kunth ex DC., *Commiphora opobalsamum* (L.) Engl. etc.) has been considered the most likely candidate⁴⁵. Commonly known as “Balm of Gilead”, *Commiphora gileadensis* (L.) C. Chr. is an aromatic resin producing tree or shrub in the Burseraceae family native to the Arabian peninsula and NE Tropical Africa^{15,46}. Few images of Judean balsam exist from antiquity, although shrub like bushes with trifoliate leaves depicted in a 6th century CE mosaic, thought to represent Judean Balsam plantations, have been considered typical of *Commiphora* species¹⁸. Nevertheless, several other resin producing trees have also been cited as potential candidates for Judean Balsam including *Balanites aegyptiaca* (L.) Delile and members of the *Pistacia* and *Liquidambar* genus^{23,47}. *Commiphora* species vary considerably in their morphology and fragrance with oleoresin production ranging from “gummy... liquid.... volatile.. copious” to “scanty or hardly apparent” and almost odorless¹⁴. Additionally members of the Burseraceae family including some aromatic *Commiphora* species whose oleoresins contain a high proportion of benzoic or cinnamic acids with medicinal and antiseptic activity, are regarded as “True Balsams”^{15,19}. Based on our sampling strategy, we can confirm “Sheba” is not closely related to *Commiphora* species harvested or utilized commercially for their fragrant aromatic resinous exudate including: *C. gileadensis* (L.) C. Chr., *C. africana* (A. Rich) Engl., *C. schimperii* Engl., *C. habessinica* (Berg) Engl. and *C. wightii* (Arn.) Bandari^{15,28,48}. “Sheba’s” position within the Spinescens clade (Fig. 6), distant from *Commiphora myrrha*, was discussed above. Phytochemical analysis of Sheba’s resin and leaves also show lack of volatile aromatic compounds even when burned, suggesting that unlike other contemporary, commercially valuable species of *Commiphora*, it was not used by local communities for this purpose. However, the presence of pentacyclic triterpenes associated with low toxicity, multi-target bioactivity including wound healing, anti-

inflammatory, anti-bacterial, antiviral, hepatoprotective and anti-tumoral activity^{22,35,49,50} and the high relative abundance of a series of glycolipids, which, to the best of our knowledge, have not been reported elsewhere and may correspond to potential pharmacological activity^{32–34}, suggest “Sheba” could have been valuable for its medicinal and/or other uses.

Based on the above findings, we refuted our initial hypothesis that “Sheba” is the historical Judean Balsam cultivated in this region during antiquity and considered a 2nd hypothesis to explain the identity of the ancient germinated *Commiphora* seedling.

Using results of DNA sequencing, phylogenetic and phytochemical analysis in conjunction with historical and archaeological source material and phytogeographic data, we suggest “Sheba” may represent an extinct (or at least extirpated) species of *Commiphora*, once native to the region, whose resinous extract “*tsori*” (Hebrew: *flow/drip*) mentioned in Biblical texts, was considered a valuable substance associated with healing but not described in these sources as fragrant²³.

First mentioned in early Biblical sources (*Genesis 37:25*, *Gen 43:11*) dated to the 18th-16th centuries BCE (Middle Bronze Age)⁵¹ and later writings (*Jeremiah. 8:22*, *46:11*, *51:8*, *Ezekiel 27:17*) dated to Iron Age II (7th- 6th centuries BCE)⁵², the identity of Biblical “*tsori*” (translated in English as “balm”) has long been open to debate. While there are opinions that identify it with the Judean balsam²³, existing evidence has been insufficient to prove this connection⁴⁰.

Biblical “*tsori*”, most likely the product of a local species, was associated with the historical region of Gilead in the Dead Sea-Jordan Rift valley, a mountainous, richly forested area in antiquity with a lower fertile valley (*ghor*) intensively cultivated throughout history⁵³. Located on the east bank of the Jordan river between the Yarmuk river and northern end of the Dead Sea⁵³, Gilead today occupies the northwest region of the Hashemite Kingdom of Jordan.

“Sheba’s” identification with Biblical “*tsori*” and therefore probably native to the region, is supported by its discovery in a cave in the Dead Sea-Jordan Rift valley, a region where currently ca.14.5% of the 800 native flora are classified as *Sudanian /Sudano-Zambesian* in origin. This phyto-geographical zone of which the Dead Sea-Jordan Rift valley is its northern-most extent, includes many *Commiphora* species and extends through the arid/semi-arid regions of subtropical Africa Arabia, Thar Desert of India and West Pakistan^{54,55}. Containing taxa thought to have migrated from Africa along the Rift Valley to the Dead Sea region during the post-Pleistocene interglacial periods 12000-17000 years ago, it is characterized by native plants of tropical origin acquiring one or more adaptations to relatively hot and arid conditions yet retaining higher demands for moisture than true desert species⁵⁴. Many of these plants are evergreen and spiny with small compound xeromorphic leaves multi stemmed and with sparse open canopies⁵⁴.

“Sheba’s” phylogenetic position within the Spinescens clade also supports the hypothesis that it was likely native to the region, as this species rich, geographically widespread clade contains many taxa found outside of continental Africa including India, Socotra, Madagascar and South America³⁸. “Sheba’s” identification with Biblical “*tsori*”, associated with healing in these texts, is further suggested by phytochemical analysis of its leaves and resin which show lack of aromatic compounds but many known to be medicinal. Amongst these, pentacyclic triterpenoids (e.g. α - and β -amyrin) have been detected in other *Commiphora* species including *C. confusa*⁵⁶, *C. holzianna*⁵⁰ and members of the *Burseraceae* family while their presence in archaeological sites has been linked to embalming and other burial practices⁴¹. High levels (30%) of squalene, the main component of skin surface polyunsaturated lipids, present in Sheba’s leaves and stems and widely distributed in nature have also been associated with positive effects on skin physiology including emollient, antioxidant, hydration and anti-tumor activity⁵⁷. The glycolipid compound series identified from “Sheba’s” resin which, to the best of our knowledge has not been reported elsewhere, will be tested for biological activity in future assays, however other tetrol compounds, “guggultetrols”, identified from the resin of *Commiphora wightii*, (syn. *C. mukul*), have shown potential anti-cancer activity^{34,35}.

We believe these findings support our 2nd hypothesis, that “Sheba”, an unknown *Commiphora* species with a unique genetic fingerprint, may represent an extinct (or at least extirpated) taxon once native to this region, whose resin “*tsori*” mentioned in Biblical texts was valuable, associated with healing but not described as fragrant.

The second question raised by our research is why the ancient *Commiphora* seed was deposited in a cave in the Judean desert and whether this could be related to historical interest in the taxon. Our hypotheses are that: (i) it may have been deposited in the cave by an animal or bird or (ii) it was deliberately stored there by human intervention because of its value in commerce and/or in agriculture.

“Sheba’s” deposit in the cave by an animal or bird is supported by evidence that small rodents store *Commiphora* seeds⁵⁸ and its ripe fruits are eaten by birds including pigeons and doves⁵⁹, fauna whose remains have been found in Judean desert archaeological excavations⁶⁰ and are still extant in the region today. The small number of seeds found in the cave also suggests that it was carried into the cave by animals.

Nevertheless “Sheba” may have been deliberately stored in the cave through human intervention.

The age of the seed (993-1202 CE), some time after the reported disappearance of Judean Balsam from the region in the 9th century CE¹⁹, was marked by considerable political and social upheaval⁶¹. Struggle for domination between the early Fatimid rulers of Palestine (970-1073 CE) and Seljuk Turks (1073-1098), continued with arrival of the First Crusade in 1099, territorial expansion wars of the newly founded Kingdom of Jerusalem during the early 12th century and its fall in 1289⁶¹. Marked by uprisings, economic hardship, and frequent fighting between the local population and their rulers, archaeological discoveries have shown evidence that some caves in the Judean desert during this period were used as safe storage areas for local goods such as textile fragments, although a general lack of other artifacts suggests they were not used for dwelling purposes⁶².

As the seed from a surviving member of a native species possibly associated with commerce, “Sheba”, may have been considered valuable enough to deliberately sequester in a cave. If human intervention was involved, it may also have been brought from outside the region, possibly with the intension of reintroducing a once valuable *Commiphora* species. We find this hypothesis less likely however because of the absence of material finds in the cave with similar dates to “Sheba”, which might imply a more prolonged human presence at this period, although artifacts from other periods were discovered at this site including human skeletons from the Chalcolithic (5th millennium BP), date seeds from the 1st- 4th century BCE⁵ and Roman period remains²⁰.

Could the discovery of “Sheba”, possibly a native *Commiphora* species that may have been the source of a valuable, resinous material mentioned in the Bible, solve some of the many controversies, contradictions and linguistic misinterpretations that have historically surrounded both Biblical “*tsori*” and Judean Balsam^{23,40}?

While there are no descriptions in Biblical sources of the tree which produced “*tsori*”, there are many descriptions of Judean Balsam from writers in antiquity. If both were indeed members of the *Commiphora* genus, the use of a native species such as “Sheba” as a rootstock in grafting to the scion of the aromatic, non-native Judean balsam could explain the latter’s morphological differences with *Commiphora gileadensis* (a spineless tree or shrub reaching 6 m)⁴⁸ and its changing historical descriptions over the centuries including : a tallish tree similar to pomegranate (4th century BCE, *Theophrastus*); small “shrub” sized plant (1st century CE, *Strabo*, *Dioscorides*); and vine trellised over hillsides, (1st-6th century CE, *Pliny*, *Pompeius Tragus*, *Bede*) (Ancient sources discussing Judean Balsam are shown in Supplementary Table 5). These changes, previously explained by Judean Balsam as a variant or cultivar of *C. gileadensis* subject to centuries of domestication^{16,20}, could have been due to rootstock-induced reduction in scion vigor causing dwarfing with decrease in tree volume, height, canopy, diameter and circumference⁶³. Grafting may also explain why *Commiphora* seeds have not been identified from excavation sites associated with Judean Balsam cultivation,

although it’s dried fruits (*carpobalsamum*) were considered valuable in antiquity (*Pliny*) (Supplementary Table 5), since grafting is associated with rootstock induced seed abortion and/or parthenocarpy causing seedless fruits⁶⁴. Grafting, developed around 1800 BCE, well established in Greece by 5th century BCE and commonly used in the Roman era⁶³ was likely familiar to Judean farmers, subordinate to their rule from the 4th century BCE⁶⁴. Agricultural advantages of grafting the rootstock of a native species such as “Sheba” in cultivation of Judean Balsam, could have included: reduced need for pruning⁶³; better adaptation to the stressful, xeric, oligotrophic conditions of the Dead Sea region⁶⁵; and increased resistance to local biotic and abiotic stresses including soil borne pests/pathogens, soil pH, salinity, drought and flooding⁶⁶. As noted in our previous studies on the germination of ancient seeds from this region^{4,5}, low precipitation and humidity in the Dead Sea Basin may have enabled the seed to remain in a dry quiescent state⁶⁷, conditions fostering seed dispersion in species like *Commiphora* whose distribution includes extreme desert conditions. Seed longevity may also be associated with this region’s unique environmental conditions: 415 m below mean sea level, the thickest atmosphere on Earth, a unique radiation regime and a complex haze layer associated with the chemical composition of the hypersaline Dead Sea⁶⁸.

The current study, which confirms the long-term survival of *Commiphora* seeds, is important in its resurrection of a species that may have been of significance to the ancient cultures of this region. Nevertheless, there are two limitations of the current study in determining a Latin species name for “Sheba”. Firstly, the phylogenetic analysis does not sample all known *Commiphora* species. The genus comprises ca. 200 species, many of which are distributed in remote locations. The current phylogeny containing 109 species is the most densely sampled to date (e.g. 38). Therefore it is not possible to determine if the unique genetic fingerprint of “Sheba” matches another extant, but un-sampled, species. Future phylogenetic studies that samples *Commiphora* exhaustively and compares more variable genomic regions (e.g., 39) would resolve this question.

Secondly, “Sheba” has not yet produced flowers and fruit that would provide distinguishing morphological features for comparison with extant species or that would support a diagnosis as part of a new species description. Its vegetative features alone do not provide enough characters for a diagnostic taxonomic identification. For instance, Sheba’s trifoliolate leaves are similar to those of many extant *Commiphora* species in both size and shape, including those outside of the “*Spinescens*” clade” and those un-sampled by the present phylogeny.

It is unknown why Sheba remains non-reproductive or when flowering might occur in the future. Perhaps it’s taxon naturally reproduces at a more advanced age, or the artificial greenhouse environment where it is currently located is not conducive to its transition from a vegetative to reproductive state.

Despite these limitations, the germination of an ancient *Commiphora* seed from the Judean desert shows evidence for the first time of its presence in this region approximately 1000 years ago and possible identification with a native tree or shrub whose valuable resin “*tsori*” was associated with medicinal use in the Bible, but whose identity has long been debated.

Insights into the origins of “Sheba”, its relationship to other species of *Commiphora*, and the broader archaeological, historical and ethnobotanical significance of this discovery require further investigation. This includes analysis of compounds in “Sheba” for biological activity, re-examination of archaeobotanical material from the Dead Sea region for evidence of *Commiphora* species in antiquity and further evaluation of a possible relationship through grafting with the historic Judean Balsam that may have contributed to the economic success of the latter in antiquity.

Materials and Methods

Origin and selection of the ancient seed from a cave in the northern Judean desert

The ancient seed in the current study was recovered during the 1986-87 archaeological excavations of Wadi el-Makkuk²⁹, a canon-like winter water

channel in the Northern Judean desert surrounded by steep cliffs where 374 caves have been previously documented during surveys carried out between 1982–1986⁶⁹. Consisting of hard limestone and dolomite rocks, the depth of the canyon relative to the edge of the plateau through which it cuts is ca. 200 m. The caves are karstic, most lying along the cliff line at a max height of 25 m, above which is a soft limestone layer where soil formed, facilitating agriculture. Surveys have shown that very few of the caves were converted into human dwellings, with signs of habitation mostly during the Byzantine period (313–636 CE) when some served as monks cells and during the early Roman period as “Caves of Refuge” during Judea’s wars against Rome (66–135 CE)⁶⁹. Very few remains have been found from the Middle Ages. The seed in the current study was found in Cave 1 at the top of an ca. 8 m high cliff, in an area designated Lower Wadi Makukk, lying on the southern bank of the wadi. The cave with a depth of ca. 6 m contained two natural holes used for burials but showed signs of theft by grave robbers with considerable disruption to the site²⁹. Contents of the cave included a Chalcolithic burial site (5th millennium BP) with 35 adult and child skeletons, ceramics from this period and Roman period remains including a number of beads, pieces of cloth and woven ropes. Botanical material discovered at the site consisted of ca. 12 seeds including specimens identified as date seeds (*Phoenix dactylifera*) and *Balanitis aegyptica*.

The site was excavated under the direction of Prof J. Patrich in conjunction with B. Agor and B. Arabas on behalf of The Department of Archaeology, Hebrew University, Jerusalem, during surveys of caves in the northern Judean desert from 1986–89. Permission was provided by the Civil Administration, Office of Archaeology (Permit numbers : L-398/1986 and L-426/1987) and was funded by the Ministry of Science of the State of Israel. The seed in the current study, whose identity was initially unknown and formed part of the archaeological finds from the 1986–87 excavations²⁹, was stored since discovery at room temperature in the Department of Archaeology at the Hebrew University, Jerusalem. It was provided to author Sarah Sallon (SS) in 2009 by Prof Patrich for germination experiments after selection by SS, together with two date seeds also from Cave 1, one of which was also germinated with radiocarbon dating to the 1st- 4th century BCE⁵.

Selection for germination was based on the specimen’s appearance as a visually intact whole seed in a good condition and without holes. Seeds selected above were identified by a code number, photographed and measurements of weight and length were made prior to planting.

Germination of the ancient seed in a quarantine site following a preparatory process

Prior to planting, the ancient seed was subject to a preparatory process to increase likelihood of seed germination using the following established methods to sprout delicate germplasm⁷⁰: the seed was initially soaked in water for 24 h and with gibberellic acid (5.19 mM) (Ortho-Grow, USA) for 6 h, to encourage embryonic growth. This was followed by Hormoril T8 solution (5 g/L) (Asia-Riesel, Israel) for 6 h to encourage rooting and KF-20 organic fertilizer (10 ml/liter) (VGI, Israel) for 12 h. All solutions were maintained at 35 °C. Following the above procedure, the ancient seed was potted in new sterile potting soil, one cm below the surface and placed in the locked greenhouse facility of the Center for Sustainable Agriculture, (CSA) of the Arava Institute of Environmental Sciences, (AIES) Kibbutz Ketura. Eight weeks following germination and periodically afterwards, KF-20 (10 ml/liter) was added to the seedling. Irrigation used desalinated water, rather than the region’s highly mineralized water based on our previous germination studies of ancient seeds^{4,5}.

Radiocarbon dating

Radiocarbon age in the current study was obtained on a fragment of the operculum (a cap-like covering that detaches at maturity) emerging from the germinating embryo. Radiocarbon age of this fragment was recalculated to take into account the possibility of modern carbon incorporated during seedling growth^{4,5}.

Non-organic carbon (carbonates) was removed from the sample with 10% HCl under reduced pressure followed by repeated washes in deionized

water until neutral (pH 7). Organic acids formed during the rotting process were removed with 10% NaOH followed by repeated washes (as above). To prevent absorption of atmospheric CO₂, the sample was placed again in 10% HCl and then washed in de-ionized water until neutral. To remove chemicals used in the germination process, a 7 mm long shell fragment from the germinated seed weighing 80 mg was cut into 6 cubes of 8 mm³ and subject to an additional series of 4 boil washes. The sample was heated in an evacuated sealed quartz tube with CuO as an oxygen source. The resulting CO₂ was mixed with hydrogen in the ratio 2.5:1 and catalytically reduced over cobalt powder at 550 °C to elemental carbon (graphite). This mixture was pressed into a target and the ¹⁴C:¹²C ratio (for radiocarbon age) measured by Accelerator Mass Spectrometry at the Institute for Particle Physics of the Swiss Federal Institute of Technology Zurich (ETHZ).

Calendar age was obtained using the OxCal 4.4 calibration program based on the latest IntCal 20 calibration curve⁷¹. Calibrated calendar ages can be found with a probability of 68.3% in the 1-sigma range and with a probability of 95.4% in the 2-sigma range (Calibration of radiocarbon data using OxCal 4.4, is shown in Supplementary Table 1 and Supplementary Table 2). The probability distribution *P* of individual ages is given for each sigma range. The ¹⁴C activity is reported as pMC (percentage of modern carbon) and corresponds to the ratio of the activity of the sample to the corrected activity of the oxalic acid standard which has an age of 0 yBP (Before the Present).

The growing shoot appeared 5 weeks after the seed was planted, pushing out the operculum fragment (sample) as it grew. Although this woody endocarp tissue does not grow or photosynthesize, some ‘fresh and modern carbon’ potentially might have been absorbed in the sample. The endocarp tissue, however, remained hard and brown with no traces of algae or similar organism detected. Nonetheless, the fragment was cleaned from possible contamination during the preparation for radiocarbon dating. The effect of contamination by modern carbon incorporated during seedling growth was hypothesised to be max. 2–3% modern carbon, as previously shown in our first germination experiment of an ancient date seed⁴. As no suitable control sample was available for radiocarbon dating in the current study, a modelling approach had to be used to estimate the potential contamination with modern carbon and its effect on age. The age obtained for the germinated seed is a minimum age. However, due to the relatively short growth period and the state of the seed, we must assume that only little, if any, fresh carbon was absorbed. Based on this, we modelled the effect of fresh carbon on the obtained radiocarbon age. This modelling is based on the assumption that: 0%, 1%, 2% and 3% of the total carbon in the shell is additional fresh and modern carbon. We choose a twofold approach to calculate the effect of modern carbon on the radiocarbon age of the sample. One is directly based on the pMC values (percent of modern carbon) and the other one on an iterative, mixing model.

1. The ¹⁴C content of the sample of interest is a mixture of old and new carbon. The pMC value of a sample is given by⁷²

$$pMC_{tot} = x \cdot pMC_a + y \cdot pMC_b$$

$$x + y = 1$$

with pMC_{tot} as the measured value of the sample, pMC_a as the value of the fresh carbon, pMC_b as the value of the unaffected sample and x and y as the proportion of the amount of fresh carbon and unaffected carbon, respectively.

2. If the degree of contamination of a sample with fresh carbon is known, then the age difference (Δt) of the measured to the unaffected sample can be measured according to:

$$\Delta t = -\frac{1}{\lambda_{14}} \ln \left(1 + \frac{\nu}{100} (e^{-\lambda_{14}(t_C - t_S)} - 1) \right)$$

where λ_{14} is the decay constant (1/yr)⁷³, ν = degree of contamination (relative value, in %), t_C = age of the contaminated substances, t_S = age of the unaffected sample. The age of the unaffected sample, however, is not known,

but only the degree of contamination. To solve this equation, an iterative (mixing model) procedure was necessary.

Phytochemical analysis

Solvent extractions and chromatographic separation were performed as follows: In 2013 one sample (ca. 150 mg) of air-dried leaf and stem material from “Sheba” (aged 3 years) was ground in a mortar and pestle with liquid N₂ to a fine powder. A sample of the ground powder (100 mg) was extracted with hexanes (2 mL) containing 50 µg/mL of isobutyl benzene as internal standard. The extracts were magnetically stirred for 2 h, then filtered and analyzed by GC-MS.

In 2023, air-dried leaves and resin (not weighed) from “Sheba” (aged 13 years) were extracted by sonication (30 min, x 2) using a mixture of MeOH and CH₂Cl₂ (1:1 v/v; 100 mL). After removal of the solvent under reduced pressure, the crude extracts were directly acetylated (Ac₂O/Pyr, 60 °C, 1 h). After removal of the excess of reagent under a stream of argon, the acetylated extracts were methylated using N,N-dimethylformamide-dimethyl acetal in toluene (4 h, 70 °C). Excess of reagents and solvent were removed under a stream of argon yielding the derivatized solvent extracts. The latter were adsorbed onto silica gel and loaded on the top of a chromatographic column filled with silica gel. A first elution with a mixture of EtOAc/CH₂Cl₂ (8:2, v/v, 2 dead volumes (Dv)) led to the obtention of an apolar fraction (F1), whereas the more polar part of the solvent extract (fraction F2) was recovered by elution with a mixture of MeOH/CH₂Cl₂ (1:1, v/v, 2 Dv). Fraction F1 was investigated by GC-FID and GC-MS, whereas fraction F2 was not investigated further at this stage.

Isolation of compound **II** for NMR structural characterization was performed as follows: the apolar fraction F1 recovered from the resin was re-separated on a silica gel column. A first elution using CH₂Cl₂ (1Dv) yielded an apolar fraction, whereas elution with a mixture of CH₂Cl₂/EtOAc (8:2, v/v, 2 Dv) led to the obtention of ca. 50 mg of a fraction containing compound **II** with a purity > 85%, the other 15% corresponding to the homologues of **II** (i.e., compounds **I**, **II'** and **III**; cf. Figure 4 for the structures of compounds **I**, **II**, **II'** and **III**). An aliquot of ca. 15 mg of this fraction was used for NMR characterization of **II**.

In 2010 and 2013, solid-phase microextraction (SPME) was conducted using a fiber consisting of divinylbenzene, carboxen and polydimethylsiloxane phase (p/n 57348-U, StableFlex, Sigma-Aldrich, USA). Samples (100 mg) of ground air-dried leaf and stem material of “Sheba” were placed in a 20 mL headspace vial with PTFE septum. After activating the SPME fiber as per manufacturer’s instructions, the fiber was inserted in the vial and volatiles were absorbed for 1 h before analysis by GC-MS.

To evaluate the effect of burning on plant and resin material, the following procedures were carried out: Samples (100 mg) of ground air-dried leaf and stem material of “Sheba” were placed in a test tube and heated with a Bunsen burner to pyrolyse the sample. A SPME fiber consisting of divinylbenzene, carboxen and polydimethylsiloxane phase (p/n 57348-U, StableFlex, Sigma-Aldrich, USA) was placed at the entrance of the test tube and exposed to the resulting vapors for 2 min. The SPME fiber was then desorbed and analyzed by GC-MS. For the “Sheba” resin, a small sample (ca. 20 mg) was also burned in a test tube using a Bunsen burner with low heat for 1 min, increased to medium heat for 2 min and finally at high heat for 5 min. A SPME fiber was exposed to the resulting vapors for each time period to compare the released volatile compounds.

In 2010 and 2013 SPME or solvent injections (1 µL) were analyzed by GC-MS using an Agilent 5973 mass selective detector connected to an Agilent 6890 GC equipped with a Varian factor four column VF-5ms (30 m x 0.25 mm id. x 0.25 µm film thickness, Varian, USA). Separations were achieved in splitless injection mode (1 min) using UHP helium as the carrier gas (1 mL/min). For method A (longer method), the initial oven temperature was set to 40 °C for 1 min, before increasing at 5 °C/min to 320 °C which was held for 20 min (inlet temperature 320 °C; transfer line 320 °C). For method B (shorter method), the initial oven temperature was set to 40 °C for 1 min, before increasing at 7 °C/min to 320 °C which was held for 10 min (inlet temperature 320 °C; transfer line 320 °C). For SPME methods,

the initial oven temperature was set to 40 °C for 2 min before increasing at 5 °C/min to 250 °C which was held for 10 min (inlet temperature 250 °C; transfer line 250 °C). For SPME, the fiber was activated in the inlet for a further 5 min following the 2 min sampling time. The ion source was set to 200 °C, and the mass range to 35–600 amu.

Structural characterization of compounds was done firstly by (a) comparison of mass spectra with MS databases (Wiley Registry 12th edition and NIST mass spectral library 2020), (b) retention indices with those present in the Wiley12 and NIST2020 libraries or (c) literature sources, and (d) comparison of mass spectra and retention times with standards for compounds of interest. Retention indices (RI) were calculated using the linear gradient method with comparison to an *n*-hydrocarbon mixture containing C₉ to C₃₆ *n*-alkanes (Sigma-Aldrich, p/n 46827-U).

Gas chromatography with high resolution mass spectrometry (GC-HRMS) was recorded on a Waters GCT Premier TOF-MS using a DB-5ms column (30 m x 0.25 mm id. x 0.25 µm film thickness, J&W Scientific, USA) and method conditions as described above for method B.

In 2023 the following examinations were performed:

GC with flame ionization detection (GC-FID) analyses of derivatized apolar (F1) fraction dissolved in EtOAc were carried out on a Agilent Technologies 7890 A gas chromatograph equipped with an on-column injector, a flame ionization detector and a HP-5 fused silica capillary column (30 m x 0.32 mm; 0.25 µm film thickness). H₂ was used as carrier gas (constant flow, 2.5 mL min⁻¹), and the oven programmed as follows: 70 °C–320 °C (10 °C min⁻¹), 60 min isothermal at 320 °C.

GC-MS analyses of fraction F1 were carried out using a Thermo Trace gas chromatograph (Thermo Scientific) coupled to a Thermo Scientific TSQ Quantum mass spectrometer equipped with a programmed temperature vaporizing (PTV) injector. The temperature of the source was set at 220 °C. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV and scanning *m/z* 50 to 850. Gas chromatographic separations were performed using a HP5-MS column (30 m x 0.25 mm; 0.1 µm film thickness) with He as carrier gas (constant flow rate of 1.2 mL min⁻¹). The oven temperature was programmed as follows: 70 °C (1 min), 70–320 °C (10 °C min⁻¹), 40 min isothermal at 320 °C.

High resolution mass spectrometry (HRMS) analysis of compound **II** was performed on a micrOTOF-Q II[™] ESI-Qq-TOF (Bruker Daltonics) in positive electrospray ionization mode. Compound **II**: *m/z* 983.4844 [M+Na]⁺ (calculated for C₄₇H₇₆NaO₂₀: 983.4822).

Nuclear Magnetic Resonance (NMR) analyses on compound **II** (as an acetate derivative) were performed on a Bruker Avance I – 500 MHz spectrometer (Bruker) operating at an observation frequency of 500 MHz (¹H) and 125 MHz (¹³C). The chemical shifts are reported in ppm relative to tetramethylsilane with carbon atoms and residual protons of the deuterated solvent used as internal standards (CDCl₃; δ¹H 7.26 ppm; δ¹³C 77.2 ppm). NMR analyses comprised 1D (¹H, ¹³C, DEPT) as well as 2D homo- (¹H-¹H COSY, ¹H-¹H-NOESY) and heteronuclear (¹H-¹³C-HSQC, and ¹H-¹³C-HMBC) experiments. ¹H and ¹³C chemical shifts are presented in Supplementary Table 4. 1D ¹H, and ¹³C-spectra and 2D ¹H-¹³C-HSQC, ¹H-¹³C-HMBC, ¹H-¹H-NOESY and ¹H-¹H-COSY correlation patterns are shown in Supplementary Figs. 5–11).

DNA sequencing and phylogenetic analysis

DNA was extracted from dried leaf tissue of “Sheba” using the FastDNA[®] Spin Kit (BIO101 Systems, La Jolla, CA) and 1 µL of whole DNA was used as template to amplify the nuclear ribosomal external transcribed spacer (ETS) and the chloroplast intergenic spacer region of *trnH* and *psbA* genes (*psbA-trnH*)²⁷. The chloroplast ribulose biphosphate 1',5' carboxylase large subunit gene (*rbcL*) was amplified for “Sheba” only⁷⁴ and PCR products cleaned enzymatically and sequenced by Psomagen (Rockville, Maryland, USA) using ABI 3730x1 DNA Analyzers (Applied Biosystems, Foster City, California USA). The phylogenetic placement of “Sheba” among other species of *Commiphora* was tested using DNA sequence data³⁷ and comprised multiple sequence alignments from two loci, including the nuclear ribosomal external transcribed spacer region (nrETS) and the chloroplast

psbA-trnH spacer from 109 *Commiphora* species and 11 outgroup species of *Bursera* Jacq. Sequence data from “Sheba” was manually added to the alignments³⁸.

Phylogenetic inference was performed using a partitioned, concatenated alignment from both loci using maximum likelihood as implemented by IQ-Tree (multicore version 1.6.12) web-interface³⁶. Within IQ-Tree, the best-fitting model of sequence evolution for each locus was estimated and applied to the partitioned matrix using ModelFinder⁷⁵ and maximum likelihood analysis was carried out using 1000 ultrafast^{76–78} and 100 standard maximum likelihood bootstrap replicates using 1000 iterations and a 0.99 minimum correlation coefficient. Tree search parameters included a perturbation strength of 0.5 and an IQ-stopping rule of 100. The majority rule consensus tree output from the IQ-Tree analysis is provided in Fig. 6 which includes branch support values from the 1000 ultrafast bootstrap replicates.

Statistics and Reproducibility

Statistical analysis was not used on any data in this study. Sequences of “Sheba” were generated twice from separate DNA extractions. Results of phylogenetic analyses can be replicated by rerunning the aligned DNA matrix using parameters indicated by the log files archived in “Weeks and Gostel (2024)”.

Phytochemical analyses involved only qualitative identification of compounds with no quantification and the different fractions were only processed once. For radiocarbon analysis, only one sample was analysed with no replicates available due to the small sample size with all data produced shown in Fig. 2 and Supplementary Tables 1 and 2.

Data availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors. DNA sequences generated for this project were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession numbers OP963950, OP96395, OP963952, OP963953, OP963954. The aligned matrix of DNA sequences created for phylogenetic analyses, all analysis log files and their corresponding output files were deposited in DRYAD (<https://doi.org/10.5061/dryad.hqbkzkh1n5> as a single, publicly available archive (Weeks and Gostel, 2024)³⁷.

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Author contributions

Sarah Sallon (SS) initiated, coordinated and supervised the project, selected the seed, collected and analyzed textual sources, organized and interpreted the data and wrote the paper with the assistance of Morgan Gostel (MG), Andrea Weeks (AW), Gavin Flamatti (GF), Björn Bohman (BB), Philippe Schaeffer (PS), Pierre Adam (PA), Marcus Egli (ME) and Elaine Solowey (ES). The ancient seed was germinated by ES who was responsible for its growth since 2010. ME carried out radiocarbon testing. AW and MG conducted molecular genetic laboratory work, performed phylogenetic analyses and interpreted their significance. Chemical experiments were carried out by GF and BB (2010, 2013) and PS and PA (2023) who prepared text, figures, reviewed the manuscript and contributed resources. This work is consistent with the general policies on research, ethics and reporting standards outlined by Nature portfolio journals according to inclusion & ethics in global research.

Competing interests

The authors declare no competing interests.

Additional information

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