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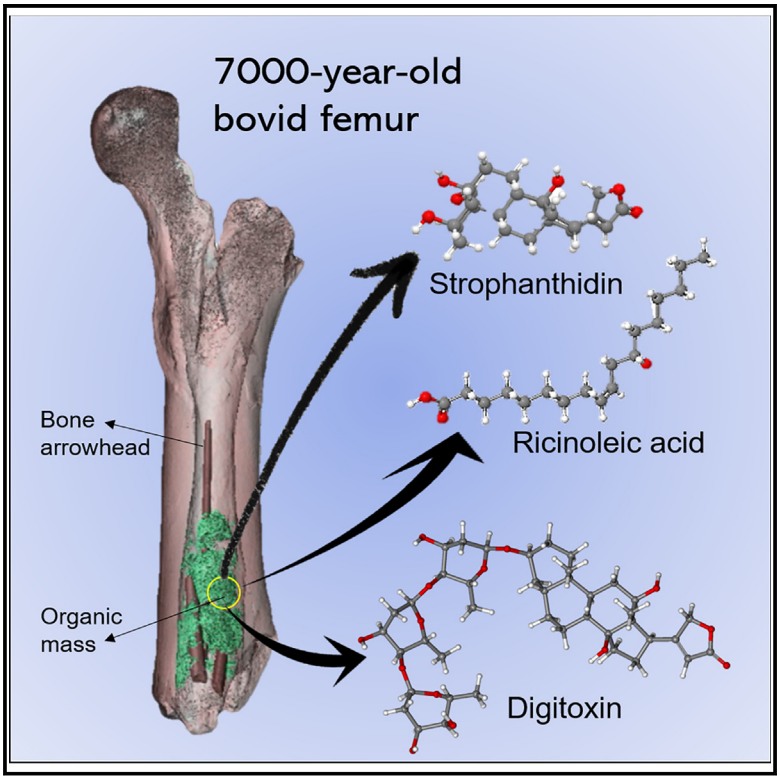
Graphical abstract



Highlights

- GC-MS and UHPLC-MS results of the contents of a 7,000-year-old artifact are presented
- The contents of the femur contain three different plant-based toxins
- The presence of toxins indicates that the substance is a poison
- The toxins derive from different plant taxa, indicating possible long-distance acquisition

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In brief

Social sciences; Archeology; Research methodology social sciences.

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A 7,000-year-old multi-component arrow poison from Kruger Cave, South Africa

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SUMMARY

We present the results of a GC-MS and UHPLC-MS analysis of residue recovered from the marrow cavity of a 7,000-year-old bovid femur from Kruger Cave, South Africa. The femur was filled with an unknown substance into which were embedded three bone arrowheads, indicating that the femur served as a quiver. Our results reveal the presence of digitoxin and strophanthidin, both cardiac glycosides associated with hunting poisons. These two compounds, and others identified, do not occur in the same plants and thus indicate a multi-taxa recipe. This is the oldest unequivocal complex hunting poison recipe yet identified, notwithstanding the many chemically unsupported assertions of older examples. Furthermore, the identification of ricinoleic acid points to the possibility of ricin as a third toxin and lends credence to the 2012 interpretation of this compound's presence on a 24,000-year-old wooden applicator at Border Cave, South Africa.

INTRODUCTION

The use of poison as a hunting aid when applied to spears and arrows signals an evolutionary advancement in the development of hunting technology. Ethno-historical records demonstrate that in most parts of the world hunters relied on toxic compounds derived from plants and animals to increase the effectiveness of their weapons.[1–10] Indeed, the knowledge of poisons, as with medicines, was well articulated within most human cultures.[11] In southern Africa, a great variety of plants and animals are known to have been used by different groups of hunters to tip their arrows. These poisons were often combined in complex recipes using a variety of preparatory procedures.[12–15]

The application of poison to hunting weapons is thought to have originated between 70,000 and 60,000 years ago, concomitant with the invention of projectile technology in Africa, based on the presence of adhesive residues on purported arrowheads and their small tip cross-sectional area, which would have been incapable of inflicting sufficient damage to kill a large animal without the addition of poison.[16–19] The evidence for poison at this period is, however, tentative at best and is yet to be verified chemically. The earliest molecular evidence for poison comes from a 24,000 calBP wooden spatula at Border Cave, South Africa, where gas chromatography combined with mass spectrometry (GC-MS) detected traces of ricinoleic acid (*cis*-12-hydroxy-9-octadecenoic acid) and its *trans* isomer, ricinelaidic acid, from the castor oil plant, *Ricinus communis* L. (Euphorbiaceae).[20] However, even this evidence is disputed, as ricinoleic acid is also found in castor oil, which is not toxic and is widely used medicinally and in the treatment of leather garments.[21,22] Be that as it may, if the Border Cave molecular evidence does indeed point to a poison, it is probably a single-component poison and not a complex recipe as is commonly seen in the ethno-historic period. Putative arrow poison has been found on bone arrowheads reported at Kuumbi Cave, Zanzibar, from 13,000 calBP deposits, although no chemical or other scientific tests were undertaken to verify this interpretation.[23] Similarly, the presence of Diodontidae dermal spines in 13,000 calBP deposits at Mindoro in the Philippines has been used to argue for the early processing of poisons, if not its active use in hunting.[24] Finally, poison from a 1,000-year-old arrow from Kruger Cave, South Africa, was recently analyzed, and short-chain cardenolide residues (the oxidative by-products of cardiac glycosides) were positively identified. This specimen was unfortunately too degraded for the specific glycoside to be annotated.[25] It is only in the comparatively recent period that we find verifiable evidence of complex hunting poisons, the oldest example coming from arrows found at Naga ed Der in Egypt, dating to 2,481–2,005 BC.[26]

The ability to mix together complex recipes, whether for poison, adhesive, or medicinal purposes, speaks directly to the cognitive capacities and traditional pharmacological knowledge of their makers.[12,27] Neanderthals used complex recipes and production procedures 200,000 years ago in their manufacture of hafting adhesives through the distillation of birch bark.[28] In southern Africa, adhesives were made with conifer resin, and ochre and fat mixtures date to at least 60,000 years ago.[29,30] The manufacture of complex adhesives has been shown to be not only persistent, with people prepared to travel upward of 70 km to collect the correct ingredients, but also highly malleable and adaptable.[31–33] Knowledge of the medicinal properties of

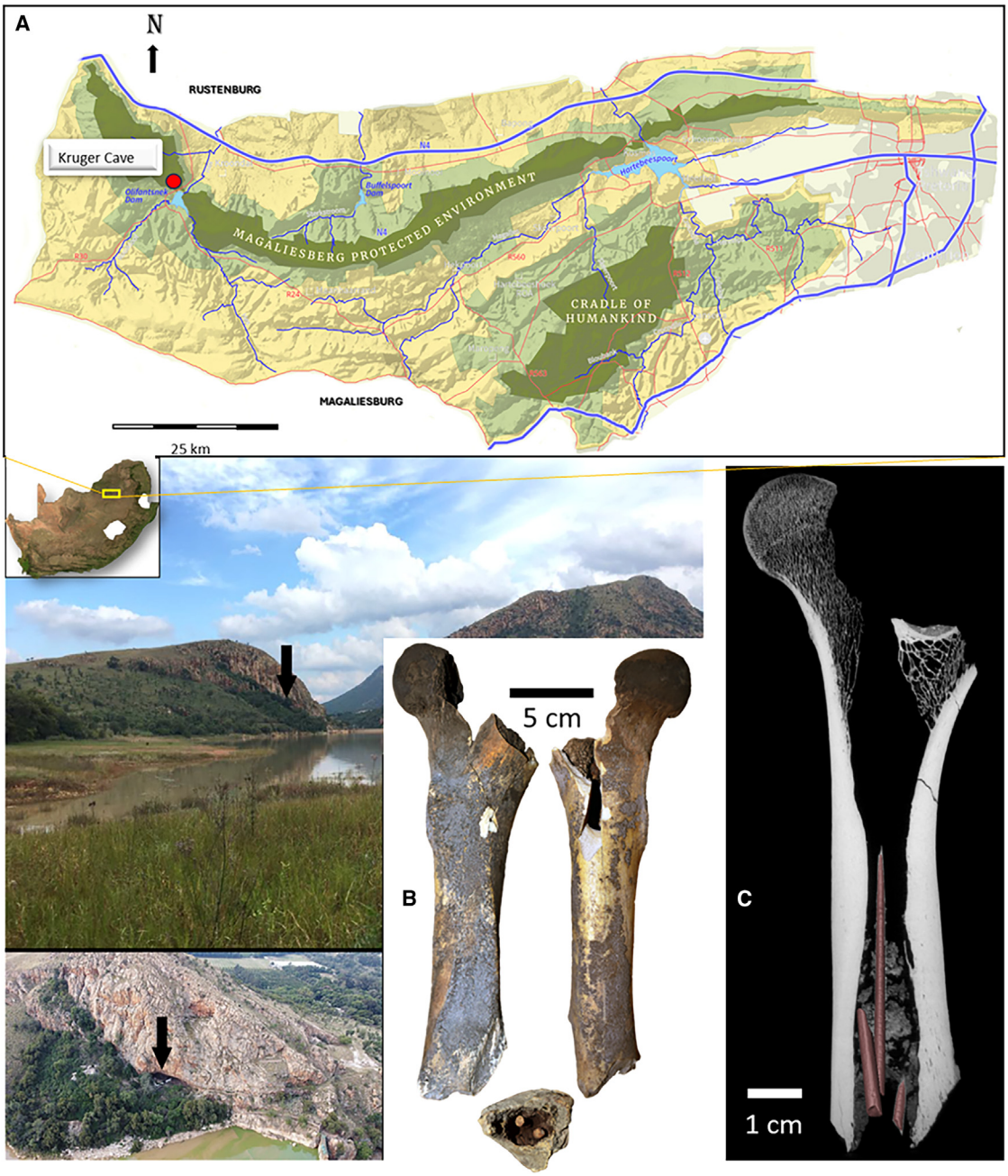

Figure 1. The location and situation of Kruger Cave and bovid femur containing the bone arrowheads and poison

(A) The location of Kruger Cave within the Magaliesberg Biosphere Reserve (map adapted from Carruthers[41]), showing a picture of the site taken from across the dam and an aerial view of the cave entrance.

(B) The bovid femur seen from three angels: in anterior and posterior perspective with the bottom inset showing the superior perspective in transverse plane.

(C) Micro-computed tomography (CT) segmented scan of the femur quiver clearly showing the three bone arrowheads embedded in the poison residue in the marrow cavity.

plants has a similar antiquity in southern Africa,[34] although the oldest confirmed medicine that combines more than one ingredient is only 500 years old.[35] Bushmen hunters of the historic period displayed a sophisticated understanding of how different plant toxins affected the animals they hunted and would sometimes use different poisons depending on which animal they were hunting.[36] For example, coniine (a piperidine alkaloid) can only be used to hunt mammals, as it corrupts the meat of fowl, rendering it unsafe for human consumption.[37] Similarly, the lethal dose of some toxins, like ricin, differs between animal taxa, meaning that certain poisons are more effective for hunting specific animals as opposed to others.[5,22]

Table 1. List of small-molecule organic compounds identified by gas chromatography coupled to a mass spectrometer detector (GC-MS)

Rt (min)	Annotation/putative identification	CAS Registry Number	NIST Entry Number	SI (%)	Empirical Formula	Mol Weight (g/mole)
8.303	2-Heptanone, 6-methyl	928-68-7	6211	84	$C_8H_{16}O$	128
9.047	Limonene	5989-27-5	7903	81	$C_{10}H_{16}$	136
11.623	Undecane	1120-21-4	12474	65	$C_{11}H_{24}$	156
12.837	n-Pentadecanol	629-76-5	26270	59	$C_{15}H_{32}O$	228
13.210	Isomenthone	491-07-6	11902	64	$C_{10}H_{18}O$	154
13.500	Dimethylbenzaldehyde (2,5-)/(2,4-)	5779-94-2 (2,5-) 15764-16-6 (2,4-)	7354 7352	79	$C_9H_{10}O$	134
11.030	Hexadecanoic (palmitic) acid	57-10-3	151973	95	$C_{16}H_{32}O_2$	256
11.675	Octadecanoic (stearic) acid	57-11-4	290961	86	$C_{18}H_{36}O_2$	284
15.480	26-Hydroxycholesterol[a]	13095-61-9	252033	85	$C_{27}H_{46}O_2$	402
16.480	Lupane derivatives ● Lupeol ● Betulin	545-47-1 473-98-3	38102 583653	88 82	$C_{30}H_{50}O$ $C_{30}H_{50}O_2$	426 442
21.027	Digitoxin	71-63-6	39215	62	$C_{41}H_{64}O_{13}$	764

CAS registry number: unique identification number, assigned by the Chemical Abstracts Service (CAS), the SI (similarity index) percentage indicates the match to the NIST17 mass spectral library.
[a]Present in acetone extract.

To date, there have been several attempts to identify putative poisons on stone and bone arrowheads with varying degrees of confidence.[25,38] Because organic molecules are subject to biodegradation, which increases with time, we are often left only with the constituent molecular components that make up the parent compound. This can make reconstruction of the original compound(s) challenging. Using an attenuated total reflectance Fourier transform infrared spectroscopy (ATR FTIR spectroscopy) and GC-MS method, Isaksson and colleagues[25] were able to identify the presence of lipids, terpenoids, and short-chain cardenolide residues, which are the oxidative by-products of cardiac glycosides, from a 1,000 CalBP arrow poison from Kruger Cave, South Africa. The identification of these residues on the Kruger Cave specimen means that they can be used as a biomarker of cardiac glycosides on archaeological arrow tips thought to have been poisoned.

Building on these studies, we present the MS results of a residue found in a bovid femur quiver from Kruger Cave dating to 6,690 ± 50 BP (Figure 1). Kruger Cave is a painted rock shelter and living heritage site in the western Magaliesberg, South Africa.[39] The site underwent a large-scale excavation in the early 1980s to document the plant material that people were using in the region between 10,000 and 1,000 BP.[40] A single bovid proximal femur shaft was recovered from the 6,222–3,901 BC occupation phase and represents a unique find (Figures 1B and 1C). The marrow cavity was filled with a dark, sediment-like substance. X-rays of the femur revealed the remains of three bone arrowheads inserted into the marrow cavity.[40]

The results of our chemical analysis of this sediment-like substance indicate that it is composed primarily of plant-based ingredients and that two distinct types of cardiac glycosides are present in the mixture, as well as an unsaturated omega-9 hydroxy fatty acid that may point to the presence of ricin. These results suggest that the substance in the Kruger Cave femur quiver is a multi-component plant-based arrow poison. Although by no means the oldest use of poison, this is the earliest confirmed use of a mixture comprising two or more plant toxins specifically applied to arrowheads and adds to our understanding of the complexity of traditional pharmacological knowledge systems. The results of our MS analyses in combination with gas chromatography and/or ultra-high performance liquid chromatography are presented below.

RESULTS

Our GC-MS analysis detected a number of small molecules derived from oils (e.g., limonene and isomenthone), waxes of plants and beeswax (e.g., undecane and pentadecanol), and fatty acids of plant or animal origin (palmitic acid and stearic acid) (Table 1). The detection of digitoxin is of great interest. Digitoxin is a well-known cardiotoxin derived from the *Digitalis* genus, most notably *D. purpurea*. The MS data and associated structure of digitoxin are shown in Figure S1.

In order to verify the presence of digitoxin in the extracts with a greater level of confidence and to search for related compounds, the samples were next analyzed by reverse phase UHPLC with high-definition, accurate mass quadrupole time-of-flight (qTOF)-MS detection. The chromatographically distinct base peak intensity (BPI) chromatograms of the extract (Figure 2) provide a chromatographic fingerprint and visual presentation of the composition thereof and reflect the complexity of the analyte profile. Figure 2 indicates that methanol was an efficient extraction solvent, suitable to solubilize a wide spectrum of metabolites with varying levels of polarity. Although the UHPLC is extremely useful in separating the analytes based on their polarity, high-definition MS enables accurate mass determination in order to generate empirical formulae to aid in analyte annotation. Based on initial optimization

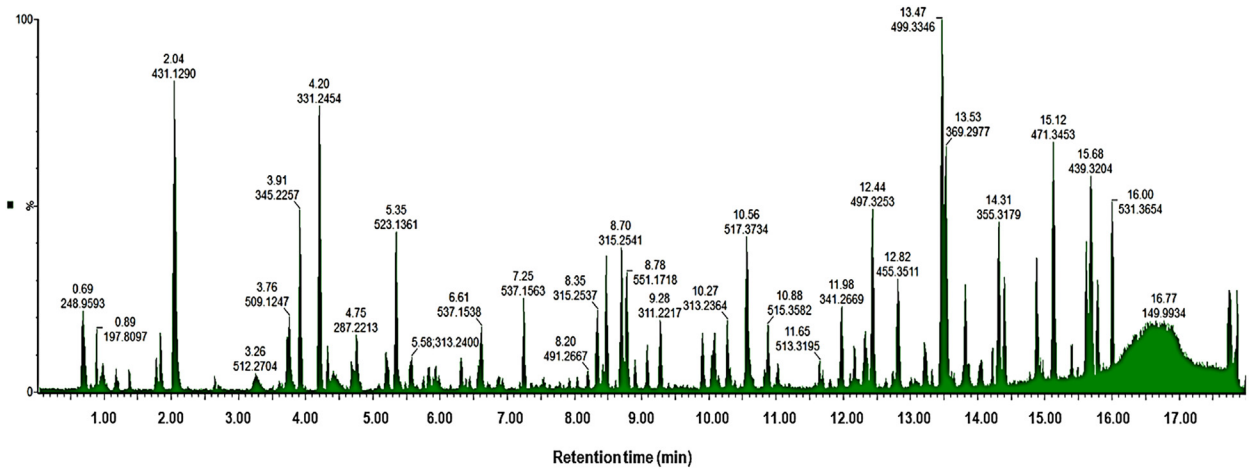

Figure 2. UHPLC-qTOF-MS analysis of the methanolic extract from sample derived from bone arrowheads stored in the femur shaft container

Shown is the base peak intensity (BPI) mass chromatogram in negative electrospray ionization (ESI) mode. Peak intensities are expressed as percentage values of that of the most intense peak. Sample preparation and conditions of analysis are described under materials and methods.

experiments, electrospray ionization (ESI) in negative mode showed better ionization efficiency.

The acquired ESI(–) data were then further analyzed. Digitoxin was again found to be present in the sample, together with traces of another cardiac glycoside and known arrow poison, strophanthidin. In addition, ricinoleic acid and ricinelaidic acid were identified in the sample, both of which are found in the castor bean plant (*R. communis* L) and can occur during the oxidation of ricin (Table 2). The spectra of these compounds of interest are illustrated in Figures 2, 3, 4, and 5.

DISCUSSION

Kruger Cave and the archaeological context of the bone quiver

The Kruger Cave deposit was excavated in 10 cm spits and sieved through an 8 mm mesh.[42] Approximately 460 kg of sediment (about 3% of the total excavated material) was floated and sieved through a 2 mm mesh to retrieve small, light-weight botanical remains.[43] Five phases of occupation were recognized, of which only three were dated. The earliest phase of occupation calibrates to 10,751–7,956 BC using the latest ^{14}C curve for the Southern Hemisphere.[44] This phase is characterized by a large, macrolithic technocomplex, called the Oakhurst.[45] The second occupation phase, in which the femur quiver was found, spans the period 6,222–3,901 BC and is characterized by a slightly smaller lithic variant than the earlier deposit, and exceptional organic preservation, including layers of grass bedding. The final dated occupation phase (Phase 4) lasts from AD 641 to AD 1217 and occurs only in the fore of the shelter, abutting the NE wall, and cuts into the older deposits in this area (Mason 1988). Fish and freshwater shellfish supplement the diet at this period.[46] Kruger Cave underwent remedial action in 2022 to protect the remaining deposit from destruction.[47]

The archaeobotanical remains from Kruger Cave were identified based on preserved seeds and pieces of wood and are presented in Table 3. Although the charcoal remains are yet to be analyzed, the archaeobotanical data show that the Kruger Cave deposit is dominated by *Mimusops zeyheri*, *Strychnos pungens* and *Sclerocarya caffra*. All the identified plants currently occur in the area, and given that there is almost no change in species representation over time, we can conclude that there was very little climatic variation over the course of the 10,000-year occupation of the site. Most of the plants identified have known nutritional uses[48] and were probably brought into the site to consume.

The bone arrowheads recovered from Kruger Cave, including those present in the quiver, all conform to Type A arrowheads, which is a simple bone lanceolate point and the most common type of bone arrowhead found in southern Africa.[49,50] The femur quiver, however, is unique in the southern African archaeological record. The artifact is unaccessioned except for its provenience information (EF 21-23M, 10–30 cm). A radio carbon date from charcoal associated with the femur provided a date of 6,690 ± 50 BP, which calibrates to 5,659–5,480 BC. This femur quiver had been placed vertically in the deposit, possibly to prevent the contents from spilling out. Unlike the arrowhead sampled in the Isaksson[25] study, which was an isolated arrowhead recovered from the archaeological sediment, it is probable that in the present case the femur quiver protected the poison from the worst effects of oxidation and biodegradation.

An assessment of the chemical results

Most of the organic compounds detected in the 7,000-year-old sample from Kruger Cave occur naturally in the waxy cuticles and oils of a wide range of plants (PubChem database, https://pubchem.ncbi.nlm.nih.gov/; Table S1). Given how arrow poisons were prepared, often by taking whole parts of plants and mashing them together, these residues are unsurprising.[12,15] Of more particular interest to our study is the identification of two potent cardiotoxins, digitoxin and strophanthidin, the latter of which is a well-known arrow poison.[6,12] Another unexpected

Table 2. Analytes of interest detected in methanolic extracts of poison arrowheads by UHPLC-qTOF-MS

Rt (min)	Analyte	Mass ESI(–)	Calculated mass	Δ mDa	Δ ppm	DBE	i-Fit	Formula
4.81	Strophanthidin	403.2102 335.2234[a]	403.2121	−1.9	−4.7	8.5	35.4	$C_{23}H_{32}O_6$
7.48	Digitoxin	763.4318	763.4269	3.3	4.3	10.5	20.6	$C_{41}H_{64}O_{13}$
9.90	Ricinoleic acid	297.2429	279.2430	−0.1	−0.3	2.5	149.3	$C_{18}H_{34}O_3$
10.04	Ricinelaidic acid	297.2436	279.2430	0.6	2.0	2.5	113.2	$C_{18}H_{34}O_3$

[a]Base peak, ppm = parts per million, mDa = milliDalton, DBE = double bond equivalence, i-Fit = the reliability of the candidate formula calculated by the consistency between the mass shift and the isotope intensity ratio.

compound is ricinoleic acid, which, although non-toxic and occurs in a variety of plant taxa (see Table 3), is the basis for the putative identification of the toxin ricin on a wooden spatula from 24,000 calBP deposits at Border Cave.[20]

Digitoxin is a glycoside linked to three molecules of the sugar digitoxose. It acts by inhibiting Na+/K+-ATPase associated with the membrane-bound sodium pump. It causes an alteration of cardiac electrical function, resulting in premature atrial beats, atrial fibrillation, atrioventricular block, ventricular tachycardia, and ventricular fibrillation. Symptoms include arrhythmia, anorexia, nausea, vomiting, diarrhea, and disorientation. The steroid nucleus and lactone ring of the aglycone are necessary for activity, whereas other components like the attached sugar residues influence pharmacokinetic variables.[51,52] Digitoxin is known from its main source, the foxglove plant *Digitalis purpurea*. Other poisonous constituents associated with *Digitalis* (digitoxigenin, digitonin, and digitalin) were not detected. Digitoxin has been recorded in three native African taxa (Table 3). Both Dornan[53] and Watt and Breyer-Brandwijk[3] recorded the use of *Digitalis* varieties, most likely *Digitalis lanata*, as arrow poison ingredients in southern Africa. Although these taxa are not considered indigenous to South Africa, two are listed as naturalized exotics.[53] Naturalization occurs when an exogenous species is introduced into an ecosystem and manages to establish itself and propagate independently. Such introductions may occur either through human agency or naturally via wind, current, or the slow creep of ecosystem change.[54] Establishing precisely when such introductions occurred is not an easy task.

Strophanthidin, the aglycone of the glycoside strophanthin, was the second toxin detected in the UHPLC column.

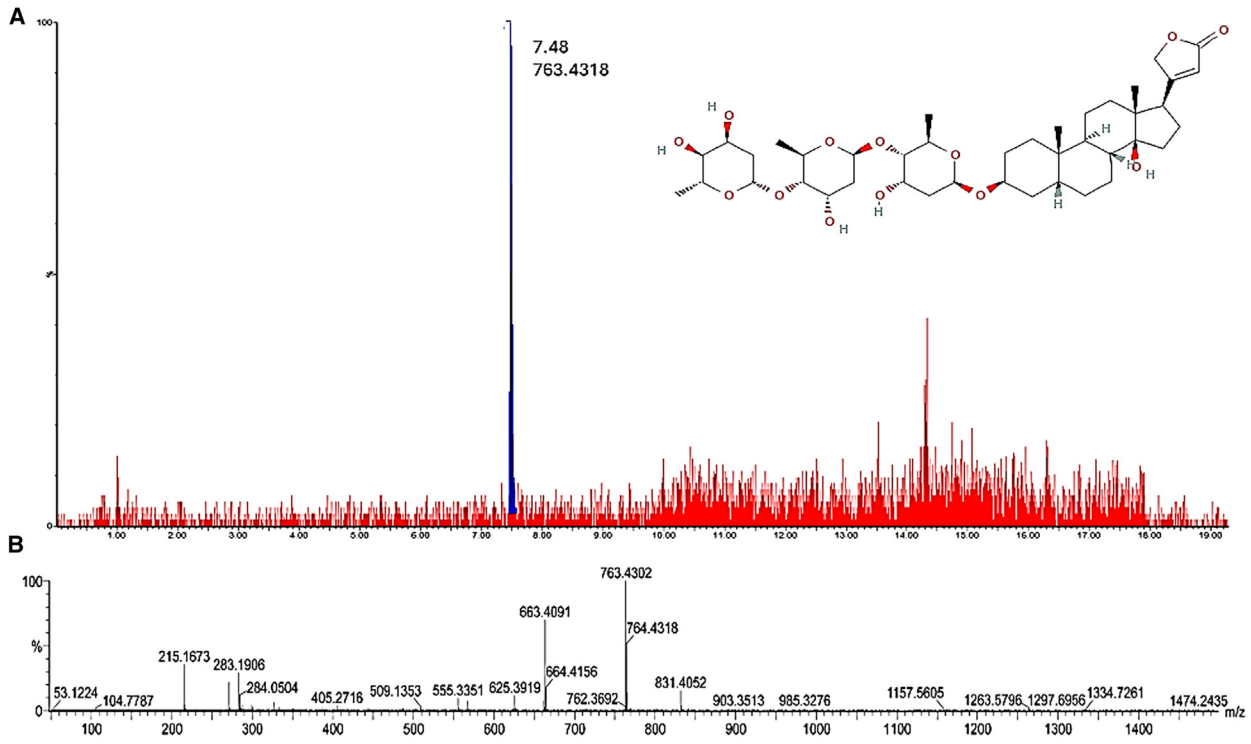

Figure 3. Extracted ion chromatogram (XIC) for digitoxin

Showing the diagnostic peak at retention time 7.48 min and *m/z* = 763 in ESI(–) mode following UHPLC separation and MS analysis (A), and mass fragmentation spectrum (B) The associated mass spectrometric data is reported in Table 2.

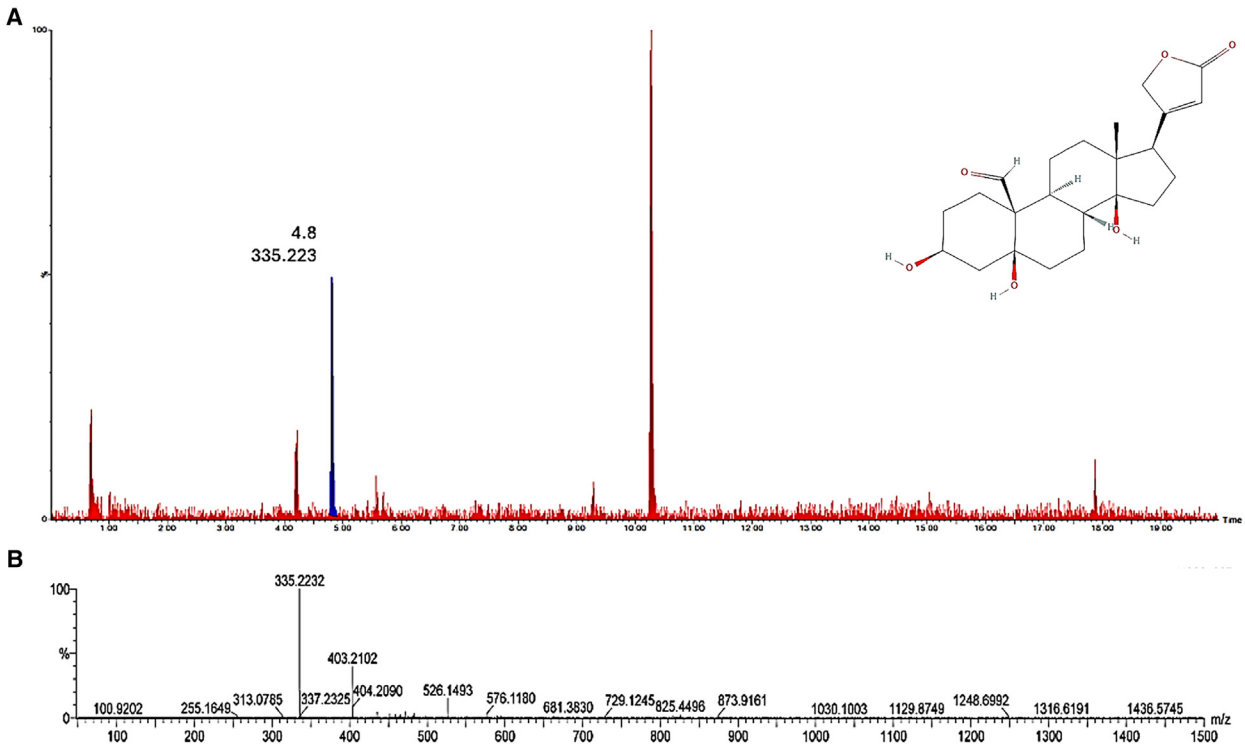

Figure 4. Extracted ion chromatogram (XIC) for strophanthidin

Showing the diagnostic peak at retention time 4.81 min and *m/z* = 403 (base ion *m/z* = 335) in ESI(–) mode following UHPLC separation and MS analysis (A), and associated mass fragmentation spectrum (B). The associated mass spectrometric data are reported in Table 2.

k-Strophanthin is most notably found in the ripe seeds of *Strophanthus* sp. The mechanism of action of strophanthidin and strophanthin is similar to ouabain (obtained from the seeds of *Strophanthus gratus*), digitalis, digitoxigenin, and digitoxin.[6,55] It specifically inhibits the membrane protein Na+/K+-ATPase in muscle tissue of the heart, which can lead to calcium overload, diastolic dysfunction, arrythmias, and ultimately to heart failure and death.[8] *Strophanthus* sp. are the most widely used poisonous plants in Africa and occur as a base ingredient in many hunting poison recipes throughout the continent.[5,6,56] Two species, namely the *Strophanthus kombe* (Oliver) and *Strophanthus Speciosus*, occur in eastern and southern Africa, from south-eastern Kenya and eastern Tanzania to eastern Namibia (Caprivi Strip), Botswana, Zimbabwe, Mozambique, and south-eastern South Africa. Neither species occurs in the vicinity of Kruger Cave. Ju/wasi informants described how crushed seeds were used in hunting poisons.[7,53] The same use and method of preparation was practised in KwaZulu-Natal by the Zulu.[3] *Corchorus olitorius* is the only other taxon in the Magaliesberg region known to contain strophanthidin, but it has not been recorded as an arrow poison.

Ricinoleic acid has been recorded in a number of plants and at least one fungus (*Claviceps purpurea*), in South Africa (Table 4). Two of those taxa, including the well-known *R. communis*, are considered to be naturalized exotics[57] but have been in South Africa for a sufficiently long time to be heavily articulated within the traditional pharmacological knowledge systems of many Bantu-speaking groups.[3] The castor oil plant contains a complex cocktail of toxic substances.[58] Ricinoleic acid and its *trans* isomer, ricinelaidic acid, occur in seeds and oils extracted from the seeds. The occurrence of both *cis* and *trans* isomers of unsaturated carboxylic acids, as found both in this study and that of the Border Cave wooden applicator, suggests that the material was subjected to a heating step during preparation.[59] All six plant taxa containing ricinoleic acid are widely distributed in South Africa; although, according to the distribution maps published by the South African National Biodiversity Institute (https://www.sanbi.org/), only *R. communis* is currently found in the vicinity of Kruger Cave and Border Cave.[55]

Most of the compounds identified from the contents of the femur quiver, including the two cardiac glycosides, do not occur in the same plant taxon. This indicates the presence of a multi-ingredient recipe. Several plants were collected, mixed together, probably underwent a heating stage, and the resultant mixture finally placed into the bovid femur containing three bone arrowheads. This correlates with what we know of how arrow poisons were prepared in the ethno-historic period.[12,13] Bushmen groups would often mix together different plant and arthropod ingredients into their poisons. Although some of these ingredients would have been to aid adhesion, others would have had no effect and likely represent placebic ingredients that were added because of some inherited belief in their efficacy.[51,60] An alternative option to account for multiple active ingredients could be

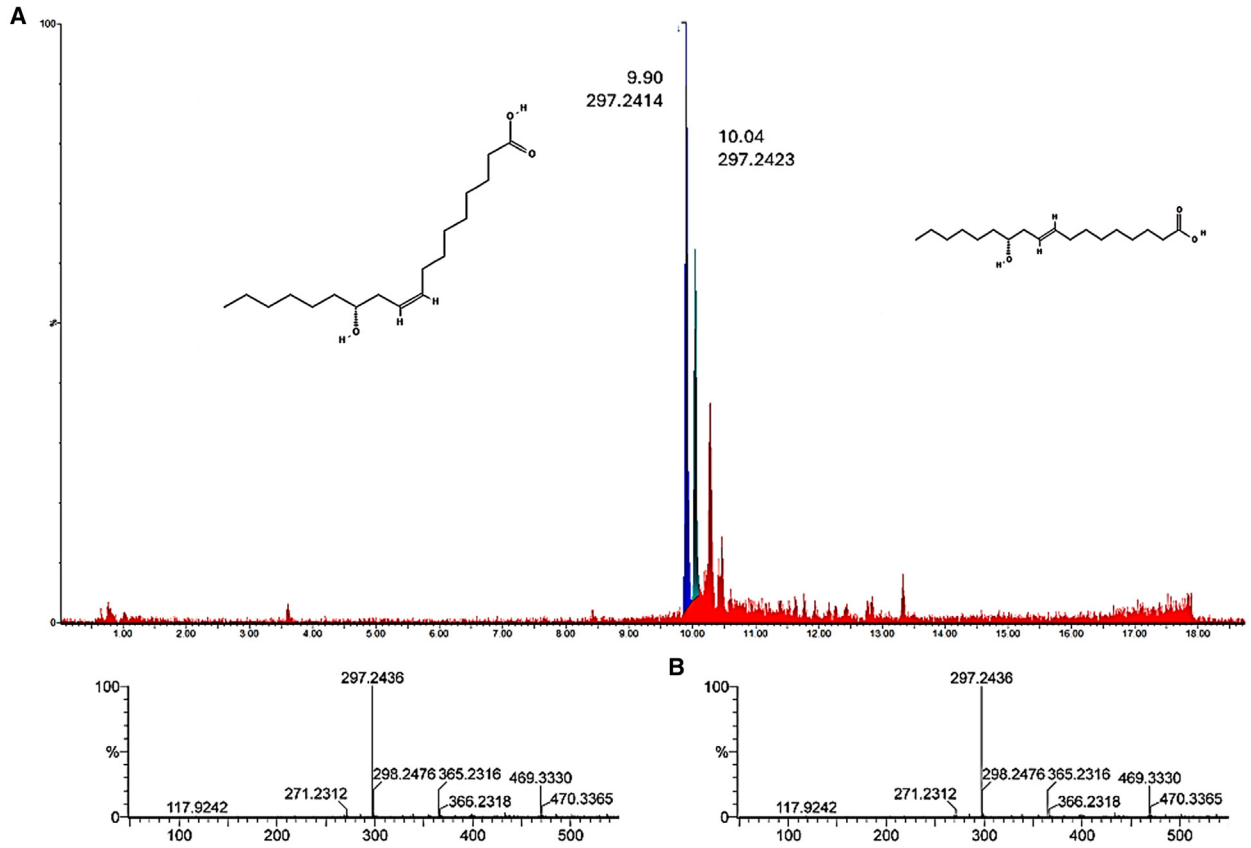

Figure 5. Integrated extracted ion chromatograms (XIC) for ricinoleic acid (cis-12-hydroxy-9-octadecenoic acid) and its trans isomer, ricinelaidic acid

Showing peaks at retention times 9.90 and 10.04 min and *m/z* = 297.2414 and 297.2423 in ESI(–) mode following UHPLC separation and MS analysis (A), and associated mass fragmentation data (B). The two isomers share a near-identical mass fragmentation pattern. The associated mass spectrometric data are reported in Table 2.

that the hunter desired to increase the range of game that could be hunted. Because certain toxins affect different animals differently,[12] it is plausible that a multi-toxin poison would have had greater versatility. The fact that during excavation the femur was found placed in a vertical position suggests that the contents were originally in a liquid or gelatinous state. There are no correlates in the ethno-historical literature of bovid femurs being filled with poisons and used to store arrowheads. In this sense, the specimen from Kruger Cave is unique.

Table 3. Archaeobotanical identifications from from Friede showing the minimum number of individual specimens identified from each of the sampled occupation phases

Taxonomic name	Common name	Phase 1	Phase 2	Phase 4
Acacia karoo	Sweet thorn	60	0	0
Cyprus sp.	–	3	0	0
Grewia sp.	–	17	1	37
Hypoxis	African potato	2	0	0
Mimusops zeyheri	Red milkwood	122	206	42
Sclerocarya caffra	Marula	18	8	2
Strychnos pungens	Monkey orange	75	25	22
Ziziphus sp.	–	7	12	0

The list of plant taxa from which the identified compounds could derive indicates few species that currently occur in the vicinity of Kruger Cave. Nor were any of the potential plant taxa identified from Friede's analysis[43] of macro-botanical remains at the site. This suggests that either (1) people were traveling long distances to acquire their ingredients or (2) there were established long-distance exchange networks through which poisonous plant items moved, or (3) there has been an ecological shift over the last 7,000 years and species that once grew locally subsequently went extinct in that area. Of these, the first two are most plausible. The macro-botanical analysis, although incomplete, did not reveal any indication of climatic shift in the region that would cause localized plant extinction.[40,43] On the other hand, there is ample archaeological evidence of long-distance movements of artifacts, such as shells, that would support either of the first two scenarios.[61,62] Caution must, however, be applied, as complete pharmacological profiles have only been obtained from a small proportion of South African flora.[63,64]

The presence of ricinoleic aid and ricinelaidic acid does not necessarily indicate the presence of a toxin. These two

Table 4. Taxa in which the identified compounds of interest have been found and their purported native distribution in Africa

Compound	Taxa	Native location
Digitoxin	*Digitalis purpurea*	North Africa[a]
Digitoxin	*Digitalis obscura*	North Africa
Digitoxin	*Digitalis lanata*	Southern & Eastern Africa[a]
Strophanthidin	*Antiaris toxicaria*	Central Africa
Strophanthidin	*Corchorus capsularis*	Tropical Africa
Strophanthidin	*Corchorus olitorius*	Tropical Africa[a]
Strophanthidin	*Cryptolepis nigrescens*	Tropical West Africa
Strophanthidin	*Strophanthus kombe*	South Africa
Strophanthidin	*Strophanthus speciosus*	South Africa
Ricinoleic acid	*Ricinus communis*	North-eastern Africa[a]
Ricinoleic acid	*Trichodesma zeylanicum*	South Africa
Ricinoleic acid	*Hevea brasiliensis*	Amazon basin via Central Africa
Ricinoleic acid	*Ganoderma lucidum*	Sub-tropical Africa
Ricinoleic acid	*Crotalaria retusa*	Sub-tropical Africa
Ricinoleic acid	*Cordia sinensis*	South Africa
Ricinoleic acid	*Claviceps purpurea*[b]	South Africa
Ricinoleic acid	*Cephalocroton cordofanus*	North Africa
Ricinoleic acid	*Catharanthus roseus*	Madagascar[a]
Ricinoleic acid	*Azima tetracantha*	South Africa[a]

Data for the table comes from Watt & Breyer-Brandwyk,[3] Neuwinger,[6,56] Van Wyk et al.,[55] and LOTUS—the natural products occurrence database accessible on PubChem.
[a]denotes occurrence in South Africa but considered a naturalized exotic by the South African National Biodiversity Institute (SANBI).
[b]denotes a fungus.

compounds are in themselves non-toxic, and their presence in a variety of plant taxa that do not contain ricin or ricinine cautions against hasty inferences. However, given the context, (1) their association with two cardiac glycosides in a container that holds arrowheads, (2) the occurrence of *R. communis* in the vicinity of Kruger Cave, and (3), their earlier identification on a wooden spatula at Border Cave that closely resembles ethnographic poison applicators, it is not unreasonable to suppose that these two acids indicate the presence of a toxin. Indeed, the presence of these compounds in two separate assemblages, both associated with hunting material culture, lends credence to this interpretation in both contexts.

Despite the reports of purported arrow poisons from much older contexts, the residue from the Kruger Cave bovid femur quiver is the oldest chemically confirmed poison associated with arrow hunting equipment. Its recovery from a level dated to 6,690 ± 50 BP makes it at least 3,500 years older than the oldest chemically confirmed poison from Egypt.[26] Furthermore, our results show that the arrow poison was a complex recipe of several plant ingredients containing at least two potent cardiac glycosides and quite possibly also the lectin ricin. In the case of *Strophanthus* sp. and *Ricinus* sp., the toxic compounds can only be isolated from processing the seeds, indicating a relatively sophisticated botanical knowledge and understanding of plant pharmacology by southern African Bushman groups 7,000 years ago.

Limitations of the study

By combining GC-MS and LC-MS analytical techniques, our study was able to detect both polar and non-polar compounds. However, larger proteins and polysaccharides break down into constituent components with age, which means that there is a chance of additional compounds being missed in the interpretation of results. Despite the relatively good preservation of the femur contents, we do not know how much was not detectable due to the age of the sample. The relatively low concentrations (ppm) of the identified toxic compounds also pose a challenge to interpretation. We cannot know categorically whether the toxins are incidental or were intended to be the main property of the substance. In this, we were aided by the cultural context of the find, considered holistically.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Justin Bradfield (justinb@uj.ac.za).

Materials availability

This study did not generate new unique reagents or materials.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.B.; methodology, I.A.D. and P.A.S.; investigation, I.A.D and P.A.S.; writing—original draft, J.B. and I.A.D.; writing—review & editing, J.B. and I.A.D.; visualization, J.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Lewin, L. (1912). Neue Untersuchungen über die Pfeilgifte der Buschmänner. Z. Ethnol. *44*, 831–837.
2. Schapera, I. (1925). Bushmen arrow poisons. Bantu Studies *2*, 190–214.
3. Watt, J., and Breyer-Brandwijk, M. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa (E&S Livingstone).
4. Bisset, N.G. (1981). Arrow poisons in China. Part II. Aconitum – botany, chemistry and pharmacology. J. Ethnopharmacol. *4*, 247–336.
5. Bisset, N.G. (1989). Arrow and dart poisons. J. Ethnopharmacol. *25*, 1–41.
6. Neuwinger, H. (1996). African Ethnobotany: Poisons and Drugs (Chapman and Hall).
7. Nadler, O. (2005). Die Pfeilgifte der !Kung-Buschleute (Heidelberg: Oserna-africana-Verlag).
8. Philippe, G., and Angenot, L. (2005). Recent developments in the field of arrow and dart poisons. J. Ethnopharmacol. *100*, 85–91.
9. Borgia, V., Carlin, M.G., and Crezzini, J. (2017). Poison, plants and Palaeolithic hunters. An analytical method to investigate the presence of plant poison on archaeological artefacts. Quat. Int. *427*, 94–103.
10. Marshall-Thomas, E. (2006). *The Old Way* (Picador).
11. Wexlar, P. (2019). Toxicology in Antiquity (Academic Press).
12. Bradfield, J., Wadley, L., and Lombard, M. (2015). Southern African arrow poison recipes, their ingredients and implications for Stone Age archaeology. South. Afr. Humanit. *27*, 29–64.
13. Wadley, L., Trower, G., Backwell, L., and d'Errico, F. (2015). Traditional glue, adhesive and poison used for composite weapons by Ju/'hoan San in Nyae Nyae, Namibia. Implications for the evolution of hunting equipment in prehistory. PLoS One *10*, e0140269.
14. Chaboo, C.S., Biesele, M., Hitchcock, R.K., and Weeks, A. (2016). Beetles and plant arrow poisons of the Ju|hoan and Hai||om San peoples of Namibia (Insecta, Coleoptera, Chrysomelidae; Plantae, Anacardiaceae, Apocynaceae, Burseraceae). ZooKeys *558*, 9–54.
15. Chaboo, C., Hitchcock, R., Bradfield, J., and Wadley. (2019). L. Beetle and plant arrow poisons of the San people of southern Africa. In Toxicology in Antiquity, P. Wexler, ed. (Elsevier), pp. 11–72.
16. Backwell, L., d'Errico, F., and Wadley, L. (2008). Middle Stone Age bone tools from the Howiesons Poort layers, Sibudu Cave, South Africa. J. Archaeol. Sci. *35*, 1566–1580.
17. Backwell, L., Bradfield, J., Carlson, K.J., Jashashvili, T., Wadley, L., and d'Errico, F. (2018). The antiquity of bow-and-arrow technology: evidence from Middle Stone Age layers at Sibudu Cave. Antiquity *92*, 289–303.
18. Bradfield, J., Lombard, M., Reynard, J., and Wurz, S. (2020). Further evidence for bow hunting and its implications more than 60 000 years ago: Results of a use-trace analysis of the bone point from Klasies River Main site, South Africa. Quat. Sci. Rev. *236*, 106295.
19. Lombard, M. (2020). Testing for poisoned arrows in the Middle Stone Age: A tip cross-sectional analysis of backed microliths from southern Africa. J. Archaeol. Sci.: Reports *34*, 102630.
20. d'Errico, F., Backwell, L., Villa, P., Degano, I., Lucejko, J.J., Bamford, M.K., Higham, T.F.G., Colombini, M.P., and Beaumont, P.B. (2012). Early evidence of San material culture represented by organic artifacts from Border Cave, South Africa. Proc. Natl. Acad. Sci. USA *109*, 13214–13219.
21. Evans, A.A. (2012). Arrow poisons in the Palaeolithic? Proc. Natl. Acad. Sci. USA *109*, E3290–E3292.
22. Yeboah, A., Ying, S., Lu, J., Xie, Y., Amoanimaa-dede, H., Boateng, K.G.A., Chen, M., Yin, X., and Yin, X. (2021). Castor oil (*Ricinus communis*): a review on the chemical composition and physicochemical properties. Food Sci. Technol. *41*, 399–413.
23. Langley, M.C., Prendergast, M.E., Shipton, C., Quintana Morales, E.M., Crowther, A., and Boivin, N. (2016). Poison arrows and bone utensils in late Pleistocene eastern Africa: Evidence from Kuumbi Cave, Zanzibar, Tanzania. Azania Archaeologocal Research in Africa *51*, 155–177.
24. Boulanger, C., Pawlik, A., O'Connor, S., Sémah, A.-M., Reyes, M.C., and Ingicco, T. (2023). The exploitation of toxic fish from the terminal Pleistocene in maritime Southeast Asia: A case study from the Mindoro archaeological sites, Philippines. Animals. *13*, 2113. https://doi.org/10.3390/ani13132113.
25. Isaksson, S., Högberg, A., Lombard, M., and Bradfield, J. (2023). Potential biomarkers for southern African hunter-gatherer arrow poisons applied to ethno-historical and archaeological samples. Sci. Rep. *13*, 11877.
26. Stanley, P., Philips, J., and Clark, J.D. (1974). Interpretations of prehistoric technology from ancient Egyptian and other sources. Part I: ancient Egyptian Bows and arrows and their relevance for African prehistory. Palaeorient *2*, 323–388.
27. Wadley, L. (2013). Recognizing complex cognition through innovative technology in Stone Age and Palaeolithic sites. Camb. Archaeol. J. *23*, 163–183.
28. Mazza, P.P.A., Martini, F., Sala, B., Magi, M., Colombini, M.P., Giachi, G., Landucci, F., Lemorini, C., Modugno, F., and Ribechini, E. (2006). A new Palaeolithic discovery: tar-hafted stone tools in a European Mid-Pleistocene bone-bearing bed. J. Archaeol. Sci. *33*, 1310–1318.
29. Wadley, L., Hodgskiss, T., and Grant, M. (2009). Implications for complex cognition from the hafting of tools with compound adhesives in the Middle Stone Age, South Africa. Proc. Natl. Acad. Sci. USA *106*, 9590–9594.
30. Charrié-Duhaut, A., Porraz, G., Cartwright, C.R., Igreja, M., Connan, J., Poggenpoel, C., and Texier, P.J. (2013). First molecular identification of a hafting adhesive in the late Howiesons Poort at Diepkloof rock shelter (western Cape, South Africa). J. Archaeol. Sci. *40*, 3506–3518.
31. Veall, M.A., and Mitchell, P.J. (2023). Hafting adhesives from the final Later Stone Age layers at Sehonghong, Lesotho: a case study and commentary on the use of imported resources by nineteenth-century San. Azania *58*, 596–619.
32. Aleo, A., Jerardino, A., Chasan, R., Despotopoulou, M., Ngan-Tillard, D.J., Hendrikx, R.W., and Langejans, G.H. (2024). A multi-analytical approach reveals flexible compound adhesive technology at Steenbokfontein Cave, Western Cape. J. Archaeol. Sci. *167*, 105997.
33. Fajardo, S., Zeekaf, J., van Andel, T., Maombe, C., Nyambe, T., Mudenda, G., Aleo, A., Kayuni, M.N., and Langejans, G.H. (2024). Traditional adhesive production systems in Zambia and their archaeological implications. J. Anthropol. Archaeol. *74*, 101586.
34. Wadley, L., Sievers, C., Bamford, M., Goldberg, P., Berna, F., and Miller, C. (2011). Middle Stone Age bedding construction and settlement patterns at Sibudu, South Africa. Science *334*, 1388–1391.
35. Bradfield, J., Woodborne, S., Hollmann, J., and Dubery, I. (2023). A 500-year-old medicine container from the Eastern Cape. South Afr. J. Sci. *119*, 13011.
36. Stow, G.W. (1905). The Native Races of South Africa: A History of the Intrusion of the Hottentots and Bantu into the Hunting Grounds of the Bushmen, the Aborigines of the Country (Swan Sonnenschein & Co., Ltd).
37. Lopez, T., Cid, M., and Bianchini, M. (1999). Biochemistry of hemlock (Conium maculatum L.) alkaloids and their acute and chronic toxicity in livestock. A review. Toxicon *37*, 841–865.
38. Wooding, M., Bradfield, J., Maharaj, V., Koot, D., Wadley, L., Prinsloo, L., and Lombard, M. (2017). Report on biochemical detection methods of

some plant-based arrow poisons used by San hunter-gatherers from southern Africa. South Afr. J. Sci. *113*, 1–10.

39. Bradfield, J., and Lotter, M. (2021). The current occupation of Kruger Cave, A Later Stone Age site, South Africa. Journal of Contemporary Archaeology *8*, 1–20.
40. Mason, R. (1988). Kruger Cave Late Stone Age (*Magaliesberg* (University of the Witwatersrand Press).
41. Curruthers, V. (2019). *Cradle of Life: The Story of the Magaliesberg and the cradle of Humankind* (Struik Nature).
42. Steele, R. (1987). Kruger Cave 35/82 excavation. In Kruger Cave Late Stone Age, Magaliesberg, R. Mason, ed. (University of the Witwatersrand Press), pp. 103–132.
43. Friede, H. (1987). Kruger Cave plant identification. In Kruger Cave Late Stone Age, Magaliesberg, R. Mason, ed. (University of the Witwatersrand Press), pp. 133–151.
44. Hogg, A.G., Heaton, T.J., Hua, Q., Palmer, J.G., Turney, C.S., Southon, J., Bayliss, A., Blackwell, P.G., Boswijk, G., Bronk Ramsey, C., et al. (2020). SHCal20 Southern Hemisphere calibration, 0–55,000 years cal BP. Radiocarbon *62*, 759–778.
45. Lombard, M., Bradfield, J., Caruana, M., Dussledorp, G., Kramers, J., Tebogo, and Wurz, S. (2022). SALSA update. S. Afr. Archaeol. Bull. *77*, 172–212.
46. Brown, A. (1987). The faunal remains from Kruger Cave 35/83, Olifantsnek, Rustenberg District. In Kruger Cave Late Stone Age, Magaliesberg, R. Mason, ed. (University of the Witwatersrand Press), pp. 152–188.
47. Bradfield, J., Cleminson, E., Hodgeson, C., Naudé, R., Senyane, L., Spruit, B., Taylor, N., Tsakatsi, L., Woodborne, S., and Lotter, M. (2022). The remediation of Kruger Cave: A Later Stone Age and living heritage site in the western Magaliesberg. Southern African Field Archaeology *1*, 1361.
48. Van Wyk, B.-E., and Gericke, N. (2000). People's Plants: A Guide to Useful Plants of Southern Africa (Briza Publications).
49. Goodwin, A.J.H. (1945). Some historical Bushmen arrows. South Afr. J. Sci. *41*, 429–443.
50. Bradfield, J. (2015). Identifying bone-tipped arrow types in the archaeological record of southern Africa: the contribution of use-wear studies. J. African Arch. *13*, 135–147.
51. Van Wyk, B.-E., Oudtshoorn, B., and Gericke, N. (2002). Medicinal Plants of South Africa (Briza Publications).
52. Hodgson, E. (2012). Toxins and venoms. In Progress in Molecular Biology and Translational Science, E. Hodgson, ed. (Elsevier), pp. 373–415.
53. Dornan, S. (1925). *Pygmies and Bushmen of the Kalahari* (Seeley Service).
54. Rana, S., Singh, S., and Bisht, A. (2022). Digitalis purpurea L: an overview on important medicinal plant. In Advances in Agriculture for Doubling Farmers' Income, W. Hasan, M. Lnu, H. Singh, and K. Chakrapani, eds. (Publications Private Limited: Lucknow)), pp. 190–194.
55. Pyšek, P., Hulme, P., Kühn, I., Wild, J., Arianoutsou, M., Bacher, S., Chiron, F., Didžiulis, V., Essl, F., Genovesi, P., et al. (2010). Disentangling the role of environmental and human pressures on biological invasions across Europe. Proceedings of the National Academy of Sciences of the United States of America *107*, 12157–12162.
56. Neuwinger, H. (1998). Alkaloids in arrow poisons. In Alkaloids: Biochemistry, Ecology and Medicinal Applications, M.F. Roberts and M. Wink, eds. (Plenum Press), pp. 45–86.
57. Henderson, M. and Anderson, J. (1966). Common Weeds in South Africa (Memoirs of the Botanical Survey of South Africa 37).
58. Worbs, S., Köhler, K., Pauly, D., Avondet, M.-A., Schaer, M., Dorner, M.B., and Dorner, B.G. (2011). *Ricinus communis* intoxications in human and veterinary medicine—a summary of real cases. Toxins *3*, 1332–1372.
59. Walton, T.J. (1990). Lipids, membranes and aspects of photobiology. In Methods in Plant Biochemistry, L. Harwood and J.R. Bowyer, eds. (London: Academic), pp. 105–158.
60. Marshall, L. (1969). The Medicine Dance of the !Kung Bushmen. Africa: Journal of the International African Institute *39*, 347–381.
61. Mitchel, P. (1996). Prehistoric exchange and interaction in southeastern southern Africa: marine shells and ostrich eggshell. Afr. Archaeol. Rev. *13*, 35–76.
62. Mitchel, P. (2024). The Archaeology of Southern Africa (Cambridge University Press).
63. Semenya, S., and Maroyi, A. (2019). Ethnobotanical survey of plants used by Bapedi traditional healers to treat tuberculosis and its opportunistic infections in the Limpopo Province, South Africa. South Afr. J. Bot. *122*, 401–421.
64. Twilley, D., Rademan, S., and Lall, N. (2020). A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents. J. Ethnopharmacol. *261*, 113101.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Shimadzu GC-MS Solutions software	Shimadzu, Kyoto, Japan	
MassLynx 4.1 (SCN 872) software for UHPLC-MS analysis	Waters Corporation, Milford, MA, USA.	

METHOD DETAILS

Kruger Cave excavation and sample collection from the bone quiver

The Kruger Cave material is housed in the Archaeological Collections storeroom of the University of the Witwatersrand, Johannesburg, South Africa. We collected into a sterile plastic vial approximately 100 mg of the consolidated 'sediment-like' matrix surrounding the bone arrowheads inside the marrow cavity of the femur quiver. The sampling was done under permits ID6839 and ID7010, granted by the South African Heritage Resources Agency.

Extraction and sample preparation

Fifty milligrams of the collected sample material was weighed in a sterile microcentrifuge tube and suspended in 0.5 mL analytical grade methanol (100%) as a mid-polar extraction solvent (Romil, SpS, Cambridge, UK) in an m/v ratio of 1:10. Where indicated with an asterisk in Table 1, extractions were also performed with acetone. The soluble material was extracted over a period of 24 h with frequent vortexing and sonication in an ultrasonic water bath. The suspension was centrifuged at 13 000 g for 20 min in a benchtop centrifuge to separate the supernatant from the insoluble material. The supernatant was transferred to chromatography vials and stored at 5°C before analysis.

GC-MS analysis: Analyte separation and mass detection

A Shimadzu series 2050 GC-MS combined with a Shimadzu 20i autosampler (Shimadzu Corporation, Kyoto, Japan) was used. GC conditions: the split/splitless injection mode was set at a 1:10 ratio. The GC midpolar column was a Shimadzu-5MS (30 m length by 0.25 mm internal diameter and 0.25 μm film thickness). The sample volume injected onto the column was 1–2 μL. The injection port temperature was 270°C and the carrier gas was helium (purity 99.999%) at a flow rate 1 mL per min. The column temperature: a gradient of 45°C–300°C in 15 °C/min, initial time 3 min, final time 5 min. The MS conditions were scan mode; electron impact (EI) ionisation with the ionisation energy as 70 eV; ion-source temperature was 220°C and the capillary direct interface heated at 260°C. Data analysis was done on Shimadzu GC-MS Solutions Chromatography Software. The MS spectra were searched against the Wiley Registry/NIST Mass Spectral Library 2023 (Wiley Science Solutions, Marlborough, MA, USA).

UHPLC-qTOF-MS: Analyte separation and mass detection

An ultra-high performance liquid chromatography (UHPLC) system coupled in series to an SYNAPT G1 HDMS mass spectrometer (Waters Corporation, Milford, MA, USA) was used to generate accurate mass data. Optimisation of the chromatographic separation was done utilising a reverse phase Waters HSS T3 C18 column (150 mm × 2.1 mm, 1.8 μm) and the column temperature controlled at 60°C. A binary solvent mixture was used consisting of water (Eluent A) containing 10 mM formic acid (natural pH of 2.4) and acetonitrile (Eluent B) containing 10 mM formic acid. Various methods were developed, with the initial conditions ranging from 100% A to 70% A at a flow rate of 0.4 mL/min and were maintained for 1 min, followed by a linear gradient to 1% A at 16 min. The conditions were kept constant for 1 min and then changed to the initial conditions. The runtime was 20 min and the injection volume varied between 1 and 5 μL. Samples were kept cool at 8°C in the Waters sample manager during the analysis.

For the quadrupole time-of-flight (qTOF) mass spectrometric analysis, the SYNAPT G1 mass spectrometer (Waters Corporation, Milford, MA, USA) was used in V-optics and operated in electrospray mode to enable detection of all electrospray ionisation (ESI)-compatible compounds. Leucine enkephalin (50 pg/mL) was used as reference calibrant (Lock Mass) to obtain typical mass accuracies between 1 and 5 mDalton (mDa). The mass spectrometer was operated in both ESI positive and negative modes with a capillary voltage of 2.5 kV, the sampling cone at 30 V and the extraction cone at 4.0 V. The scan time was 0.2 s covering the 50 to 1500 Da mass range with an interscan time of 0.02 s. The source temperature was 120°C and the desolvation temperature was set at 450°C. Nitrogen gas was used as the nebulisation gas at a flow rate of 550 L/h and cone gas was added at 50 L/h. The software used to control the hyphenated system and do all data processing was MassLynx 4.1 (SCN 872) (Waters Corporation, Millford, MA, USA).

QUANTIFICATION AND STATISTICAL ANALYSIS

For GC-MS analysis, the vendor-specific Shimadzu GC-MS Solutions software (Shimadzu, Kyoto, Japan) was used for method development, data acquisition and data analysis. For UHPLC-MS analysis, the MassLynx software package (MassLynx 4.1 SCN 872 software, Waters Corporation, Milford, MA, USA) was used to acquire, analyze, and manage nominal and exact mass, MS and MS/MS data.