Supplementary information

Title:
New insight from MALDI-TOF MS and multivariate data analysis on the botanical origin of polysaccharide-based paint binders in ancient Egypt

Authors:
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1. Table S1. List of the reference acacieae samples analyzed: reference sample acronym, reference sample number used in PCA analysis, reference sample catalog number, original and updated taxonomy, origin and supplier.

Sample	PCA No.	Collection No	Genus	Taxonomy (in collection)	Updated taxonomy/Synonym of[2]	Geographical Origin	Collection/ supplier*
DEA_A§	1	271012	Acacia	*Acacia dealbata* Link	Up to date	Australia (New South Wales)	FM
DEA_B§	2	78736	Acacia	*Acacia dealbata* Link	Up to date	Unknown	Kew
DEC_A§	3	271009	Acacia	*Acacia decurrens* Willd.	*Acacia decurrens* (J.C.Wendl.) Willd.	Australia (New South Wales)	FM, collected 1898
DEC_B§	4	58563	Acacia	*Acacia decurrens* Willd.	*Acacia decurrens* (J.C.Wendl.) Willd.	Australia (Tasmania)	Kew
MEA_A§	12	78735	Acacia	*Acacia mearnsii*	*Acacia mearnsii* De Wild.	Australia (New South Wales)	Kew
MEA_C	13	78707	Acacia	*Acacia mearnsii*	*Acacia mearnsii* De Wild.	South Africa	Kew
MIC_A§	14	271013	Acacia	*Acacia microbotrya* Benth.	Up to date	Australia (New South Wales)	FM, collected 1898
VES_A§	56	271016	Acacia	*Acacia vestita* Ker Gawl.	Up to date	Australia (New South Wales)	FM, collected 1898
PEN_A§	20	271014	Acacia	*Acacia penninervis* Sieber ex DC.	Up to date	Australia (New South Wales)	FM, collected 1898
PEN_B§	21	58701	Acacia	*Acacia penninervis*	*Acacia penninervis* Sieber ex DC.	Australia (New South Wales)	Kew
FER_A§	7	273717	Senegalia	*Acacia ferruginea*	*Senegalia ferruginea* (DC.) Pedley	India	FM, acquired 1912
FER_B§	8	58587	Senegalia	*Acacia ferruginea*	*Senegalia ferruginea* (DC.) Pedley	India (Madura)	Kew
MOD_A	15	78656	Senegalia	*Acacia modesta*	*Senegalia modesta* (Wall.) P.J.H.Hurter	India	Kew
POL_A§	22	78678	Senegalia	*Acacia polyacantha*	*Senegalia polyacantha* (Willd.) Seigler & Ebinger	Nigeria	Kew
POL_C	23	78672	Senegalia	*Acacia polyacantha* Willd. ssp. *campylacantha* (A. Rich.) Brenan	*Senegalia polyacantha* Willd. ssp. *campylacantha* (A. Rich.) Brenan	Nigeria	Kew

POL_D	24	78677	Senegalia	*Acacia polyacantha* Willd. ssp. *campylacantha* (A. Rich.) Brenan	*Senegalia polyacantha* Willd. ssp. *campylacantha* (A. Rich.) Brenan	Sudan	Kew
SEN_A[§]	25	na	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Unknown	Zecchi
SEN_B[§]	26	271356	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Pakistan (Balochistan)	FM, acquired 1907
SEN_C[§]	27	269622	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Sudan (Kordofan)	FM, acquired 1904
SEN_D[§]	28	269611	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Egypt	FM, acquired 1904
SEN_E[§]	29	273715	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	India	FM, acquired 1912
SEN_F[§]	30	na	Senegalia	*Gum arabic* (unspecified)	*Senegalia senegal* (L.) Britton	Sudan	JT
SEN_G[§]	31	na	Senegalia	*Gum arabic* (unspecified)	*Senegalia senegal* (L.) Britton	Unknown	TNN, collected 1999
SEN_H[§]	32	na	Senegalia	*Gum arabic* (unspecified)	*Senegalia senegal* (L.) Britton	Kenya	Unknown supplier
SEN_I[§]	33	na	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Western Sudan	GA, collected 1994
SEN_L[§]	34	na	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Southeast Sudan	GA, collected 1999
SEN_M[§]	35	na	Senegalia	*Acacia senegal* (L.) Willd.	*Senegalia senegal* (L.) Britton	Southeast Sudan	GA, collected 1994
DEP_A	5	78705	Vachellia	*Acacia drepanolobium*	*Vachellia drepanolobium* (Harms ex Y.Sjöstedt) P.J.H.Hurter	Tanzania	Kew
DEP_B	6	78662	Vachellia	*Acacia drepanolobium*	*Vachellia drepanolobium* (Harms ex Y.Sjöstedt) P.J.H.Hurter	Kenya	Kew
FIS_A	9	78650	Vachellia	*Acacia fischeri*	*Vachellia fischeri* (Harms) Kyal. & Boatwr.	Tanzania	Kew
HOR_A	10	58615	Vachellia	*Acacia horrida*	*Vachellia horrida (L.) Kyal. & Boatwr.*	South Africa	Kew
LEU_A	11	78698	Vachellia	*Acacia leucophloea*	*Vachellia leucophloea* (Roxb.) Maslin, Seigler & Ebinger	India (Mumbai)	Kew
SEY_A[§]	36	2108830	Vachellia	*Acacia seyal* Del.[$]	*Vachellia seyal* (Delile) P.J.H.Hurter	Unknown	FM, acquired 1995
SEY_B[§]	37	78686	Vachellia	*Acacia seyal* Del.	*Vachellia seyal* (Delile) P.J.H.Hurter	Niger	Kew

SEY_C§	38	78685	Vachellia	*Acacia seyal* Del.	*Vachellia seyal* (Delile) P.J.H.Hurter	Niger	Kew
SEY_D§	39	78780	Vachellia	*Acacia seyal* Del.	*Vachellia seyal* (Delile) P.J.H.Hurter	Niger	Kew
SEY_E§	40	78680	Vachellia	*Acacia seyal* Del.	*Vachellia seyal* (Delile) P.J.H.Hurter	Niger	Kew
SEY_F§	41	78648	Vachellia	*Acacia seyal* Del. var. *seyal*	*Vachellia seyal* var. *seyal*	Tanzania	Kew, acquired 1995
SEY_G§	42	na	Vachellia	*Acacia seyal* Del. var. *fistula*	*Vachellia seyal* var. *fistula* (Schweinf.) Kyal. & Boatwr.	Sudan (Khartoum)	GA, collected 1999
SEY_H§	43	na	Vachellia	*Acacia seyal* Del. var. *seyal*	*Vachellia seyal* var. *seyal*	Southeast Sudan	GA, collected 1997
SEY_I§	44	na	Vachellia	*Acacia seyal* Del. var. *fistula*	*Vachellia seyal* var. *fistula* (Schweinf.) Kyal. & Boatwr.	Unknown	CWSP
SEY_L§	45	na	Vachellia	*Acacia seyal* Del.	*Vachellia seyal* (Delile) P.J.H.Hurter	Unknown	AR
SEY_M	46	58333	Vachellia	*Acacia seyal*	*Vachellia seyal* (Delile) P.J.H.Hurter	Sudan	Kew, collected in 1911
SIE_A	47	78699	Vachellia	*Acacia sieberiana* DC.	*Vachellia sieberiana* (DC.) Kyal. & Boatwr.	Nigeria	Kew
STEN_A§	48	269608	Vachellia	*Acacia stenocarpa* Hochst. ex A. Rich.	*Vachellia seyal* var. *seyal*	Egypt	FM, acquired 1904
STEN_B	49	269609	Vachellia	*Acacia stenocarpa Hochst.* ex A. Rich.	*Vachellia seyal* var. *seyal*	Egypt	FM
STEN_C	50	269607	Vachellia	*Acacia stenocarpa Hochst.* ex A. Rich	*Vachellia seyal* var. *seyal*	Egypt	FM
STU_A	51	78649	Vachellia	*Acacia stuhlmannii* Taubert	*Vachellia stuhlmannii* (Taub.) Kyal. & Boatwr.	Tanzania	Kew
TOR_A§	52	78718	Vachellia	*Acacia tortilis* (Forssk.) Hayne ssp. *raddiana* (Savi) Brenan var. *pubescens*	*Vachellia tortilis* subsp. *raddiana* (Savi) Kyal. & Boatwr.	Unknown	Kew
TOR_B	53	78727	Vachellia	*Acacia tortilis* (Forssk.) Hayne ssp. *heteracantha* (Burch.) Brenan	*Vachellia tortilis* subsp. *heteracantha* (Burch.) Kyal. & Boatwr.	Unknown	Kew

TOR_C	54	78647	Vachellia	*Acacia tortilis* (Forssk.) Hayne ssp. *raddiana* (Savi) Brenan	*Vachellia tortilis* subsp. *raddiana* (Savi) Kyal. & Boatwr.	Sudan	Kew
TOR_D	55	78696	Vachellia	*Acacia tortilis* (Forsskal) Hayne ssp. *spirocarpa* (A. Rich.) Brenan	*Vachellia tortilis* (Forssk.) Galasso & Banfi	Tanzania	Kew
NIL_A[§]	16	58668	Vachellia	*Acacia nilotica*	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.	South Africa (Natal)	Kew
NIL_F	17	58646	Vachellia	*Acacia nilotica*	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.	India	Kew
NIL_G	18	58643	Vachellia	*Acacia nilotica*	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.	Pakistan	Kew
NIL_H	19	58644	Vachellia	*Acacia nilotica*	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb.	India	Kew

[*]Samples obtained from the following collections/suppliers: Kew=Royal Botanic Gardens, Kew, UK; FM=The Field Museum, Chicago, IL, USA; GA=Gandil Agricultural Co. Ltd., Karthoum, Sudan (A. Karamallah); Zecchi, Florence, Italy; CWSP=Centre for Water Soluble Polymers, Wrexham, UK; AR=Alland & Robert, Port-Mort, France; JT=Jack Thompson, OR, USA; TNN=Tessa Neylan Nolan, Nakuru, Kenya.
[§]Analyzed in previous work.[1]

2. MALDI-TOF MS analysis of pigmented *S. senegal* reference samples

S. senegal, prepared with 1 part gum and 2 parts water, was combined with various pigments (30% w/w) and brushed onto a glass slide. The paint was left to air dry for 1 week, sampled and analyzed within a month. The pigments used were: ivory black, minium (Pb_3O_4), iron oxide (Fe_2O_3), malachite ($Cu_2CO_3(OH)_2$), calcite ($CaCO_3$), cadmium orange (CdS), viridian (Cr_2O_3), cobalt blue ($CoO \bullet Al_2O_3$), orpiment (As_2S_3), zinc white (ZnO) and madder. Representative mass fingerprints of some of the mockups are reported in Figure S1. The PCA scores plot of the acacieae reference samples, together with the pigmented ones, is shown in Figure S2.

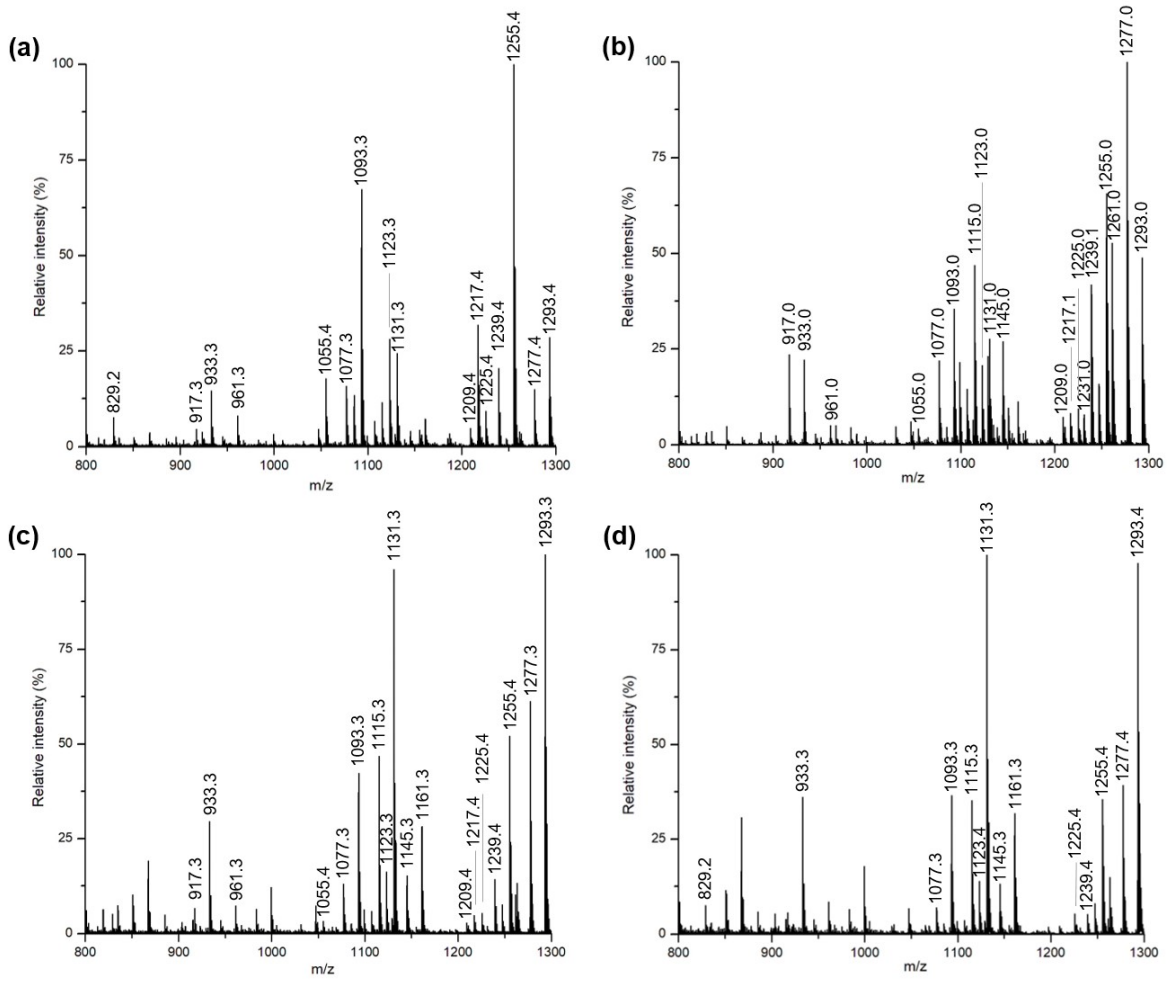

Figure S1
MALDI-TOF MS mass spectra of reference *S. senegal* combined with (a) calcite, (b) malachite, (c) iron oxide, and (d) orpiment, mass range 800-1300 Da. Only the *m/z* values of diagnostic ions are reported in the spectra. For the list of diagnostic ions of *S. senegal* see Granzotto et al. 2017.[3]

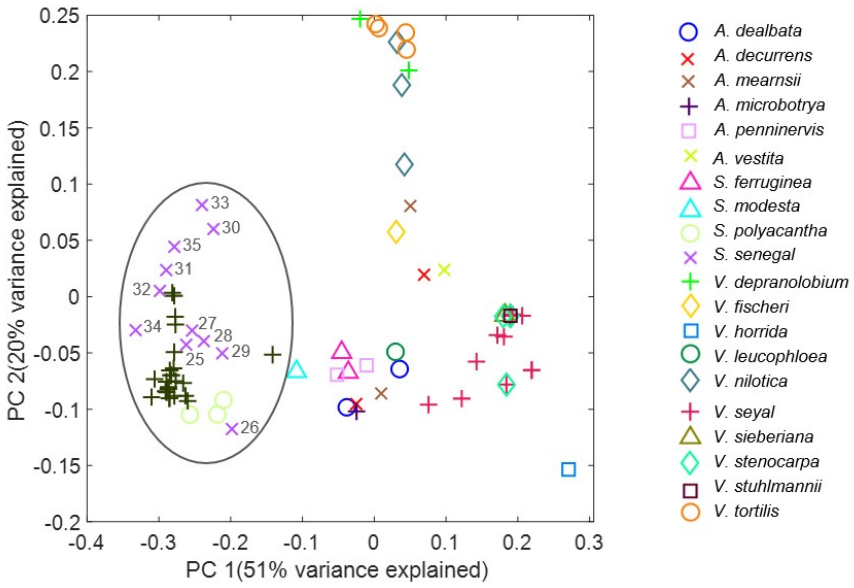

Figure S2
PCA scores plot (PC1 vs PC2) with legend of MALDI-TOS MS data of reference acacieae samples, including the pigmented *S. senegal* samples (dark green plus sign).

3. PC1 and PC3 scores plot of acacieae reference and historic samples, with legend

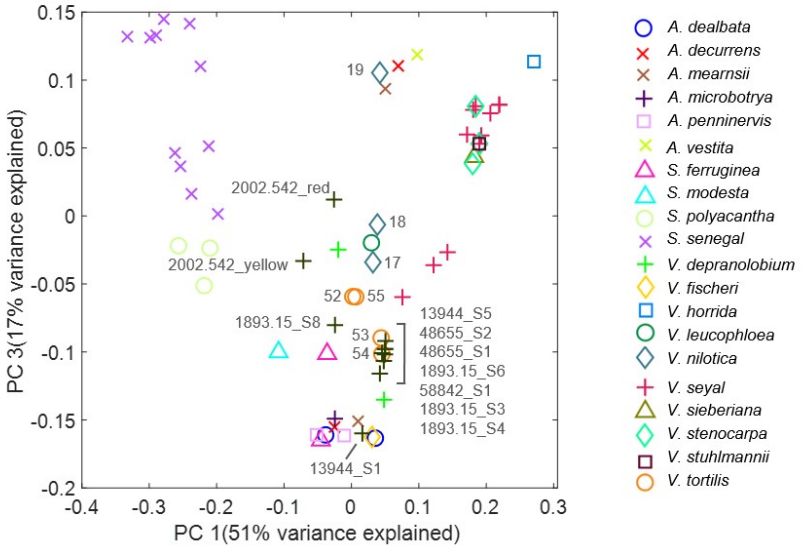

Figure S3
PCA scores plot (PC1 vs PC3) with legend of MALDI-TOF MS data of reference acacieae samples and historic samples. *V. tortilis*, *V. nilotica* and samples from artworks are labeled.

4. Mummy cartonnage (AIC 1893.15): analysis results and parameters.

The binding medium for all samples was identified as a gum from, most likely, the plant species *Vachellia tortilis* or *V. nilotica*, except in the case of the green paint, for which it was not determined.

Table S2. Results of pigment analysis

Color, layer	**Pigment ID and binding medium**
White, ground	Calcite ($CaCO_3$)[a,b] mixed with mammalian animal glue[e]
Yellow, paint	Goethite ($FeO(OH)$)[a,c] and possibly orpiment (As_2S_3)[b,c]
Blue, paint	Egyptian blue ($CaCuSi_4O_{10}$)[a-d]
Red, paint	Hematite (Fe_2O_3)[b,3]
Green, paint	Atacamite ($Cu_2Cl(OH)_3$) sometimes mixed with Egyptian blue[a,b,d]
Black, paint	Carbon-based black

Methods of analysis
[a]FTIR
[b]XRF
[c]HSI
[d]VIL
[e]Proteomics

Fourier transform infrared spectroscopy (FTIR)
A portion of each sample was mounted on a Specac diamond compression cell. Analysis was performed with a Bruker Hyperion 3000 FTIR microscope equipped with mercury cadmium telluride D315 detector interfaced to a Tensor 27 spectrometer bench. Data were collected in transmission mode between 4000 and 400 cm^{-1} at 4 cm^{-1} resolution and 256-512 scans per spectrum according to the response of the different samples. Data were interpreted with reference to published literature and databases.

X-ray fluorescence (XRF)
Analysis was performed non-invasively with an open-architecture Bruker Artax X-ray fluorescence spectrometer and a Mo excitation tube, radiation at 50 kV, 400 μA, with a 1.5 mm collimator, no filter used, acquiring each spectrum live-time for 180 seconds with a helium stream (60 L/min at the analyzed surface) for improved detection of light elements. The system is equipped with an X-Flash® X-ray detector, with energy resolution 160-165 eV for Mn-Kα at 10 kcps. Analysis was performed by positioning the instrument at the correct distance from the artwork's surface by using the integrated focusing system.

Hyperspectral imaging (HSI)
HSI was undertaken in the c. 400-900 nm range using a Resonon Pika II pushbroom camera and tungsten-halogen radiation sources.

Visible-induced luminescence (VIL)
VIL imaging was performed using a Nikon D850 modified camera and Nikon SB80DX flashes equipped with suitable filters.[4]

Proteomics
Protein extraction and enzymatic digestion with LysC and Trypsin was performed as described in Mackie et al. 2018.[5] The resulting peptides were desalted on C18 spin columns and analyzed by LC-MS/MS using

a DionexUltiMate 3000 Rapid Separation LC system and a linear ion trap - Orbitrap hybrid Elite mass spectrometer or QEHF (Thermo Fisher Scientific). The LC was equipped with a 75 µm × 10.5 cm PicoChip analytical column packed with 3 um ReproSil-Pur® beads. The flow rate was kept at 300 nL/min. Solvent A was 0.1% FA in water and Solvent B was 0.1% FA in ACN. The peptide was separated on a 120-min analytical gradient from 5% ACN/0.1% FA to 40% ACN/0.1% FA. The mass spectrometer was operated in data-dependent mode, source voltage was 2.40 kV and the capillary temperature was 275°C. MS1 scans were acquired from 400 to 2000 m/z at 60,000 resolving power. The top fifteen most abundant precursor ions in each MS1 scan were selected for fragmentation by collision-induced dissociation. Raw data were searched against a SwissProt database (January 2017), followed by other searches with different homemade databases downloaded from UniProt, and processed with MaxQuant software version 1.6.1.0. For specific parameters refer to Granzotto et al. 2021.[6]

5. Bibliography

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