Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information for

Direct Analysis of Alkaloids in Natural *Cinchona* Bark and Commercial Extracts using Time-of-Flight Secondary Ion Mass Spectrometry

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Experimental Section

Bark sample preparation. *Cinchona* bark with a dimension of ca 40 x 10 cm^2 was obtained from a forest region, Southwest of Cameroon. The bark was sun dried to enhance preservation. The bark was further dried at 80 °C to a constant weight using laboratory oven set at 80 °C. The dried sample was milled using a milling pot. The powder was passed through a mesh sieve to remove ungrounded fibres and larger particles.

A 100 mg of the powder was extracted with 100 mL ethanol in the ultrasonic bath at 60 °C for 30 minutes. After sedimentation, a clear supernatant was filtered with a syringe filter (pore size 0.2 µm) and kept for analysis

Results and Discussion Section

Table S 1: Surface roughness parameters for *Cinchona* bark obtained from 3D laser microscope.

Roughness parameters		**Value (µm)**
Sa	Average roughness	5.78
Sq.	Root mean square roughness	7.66
Ssk	Skewness	0.41
Sku	kurtosis	4.68
Sv	Maximum profile valley depth	98.089
Sp	Maximum profile peak height	97.70
Sz	Ten-spot average roughness	193.79

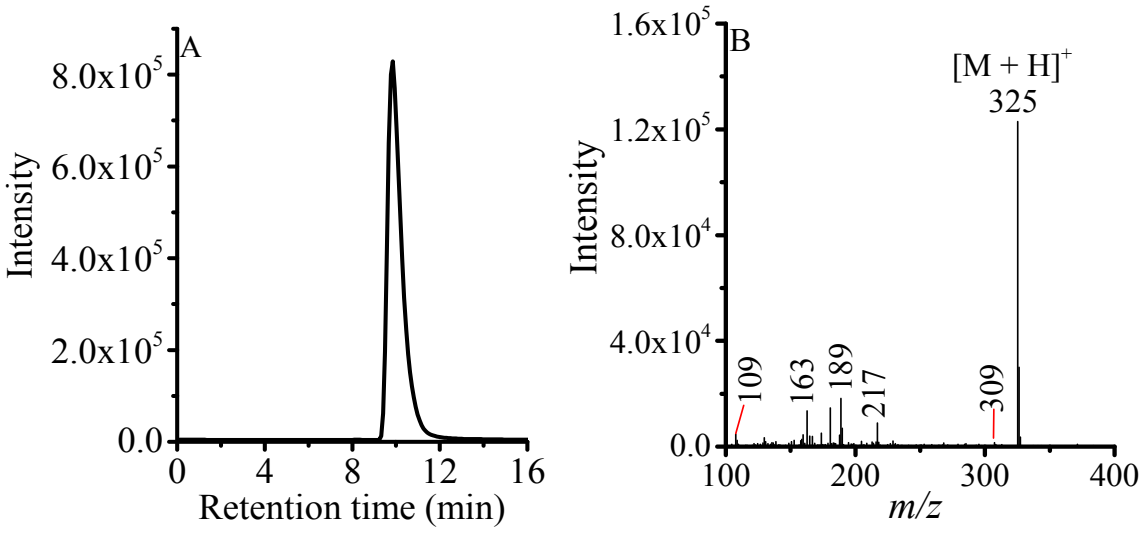

Figure S 1: Liquid chromatography-mass spectrometry of Qn. A) Mass chromatogram, B) mass spectra. Column: XBridge C_{18}, 3.5 µm, 150 x 4.6 mm Mobile phase: 10% MeOH/ 90% H_2O with 0.1 % FA, pH= 3.0 .Column temperature=80 °C, flow rate: 1.0 ml/min with post-column splitting. Injection volume: 20 µl. Detection: Micromass Quattro LC MS in positive electrospray (ESI+) mode; capillary voltage: 3.5 kV; cone voltage: 50 V

Heterogenous deposit. As can be seen from Figure S 2, the ion image of protonated molecular ion that results from different concentrations of Qn on silicon wafer shows variability in the spot size, shape and homogeneity.

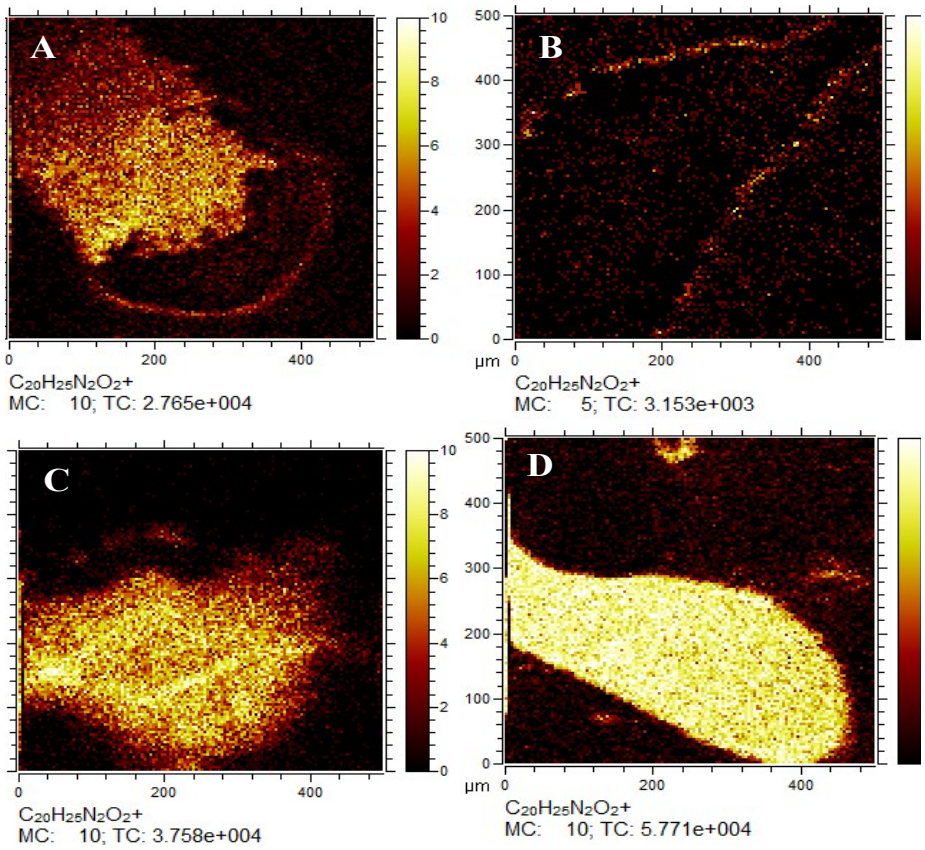

Figure S 2: Coffee-stain effect seen on the SIMS image of protonated molecular ion of Qn. A= 0.1 ng/µl, B= 1 ng/µL, C= 100 ng/µL and D=1000 ng/µl of Qn standard on silicon wafer.

This is as a result of the nature of the deposit formed after blow-drying the solution on the silicon wafer. As is evident from Figure S 2B, the protonated molecular ion of Qn is found at the edges of the "coffee stain ring". This is because in the course of blow-drying with nitrogen, the solution was pushed towards the edge. The coffee stain artefact does not only affects the ion image and the signal intensity from the spot but also goes further to pose challenges in quantification and data interpretation. The coffee stain effect can be minimised by controlling the solution deposition and the drying process. Even then, the nature of the monolayer bonding will still have an effect on the intensity of the ejected secondary ions.

LC-MS of extracted bark powder. To elucidate the intense peak at *m/z* 338 and 352, seen in the ToF-SIMS spectra, 100 mg bark powder was extracted in a 100 mL ethanol. Soxhlet

extraction, a reference method for extraction of quinoline alkaloids from *Cinchona* bark powder after treatment with alkali could not be used because large sample amount and long operation time required.

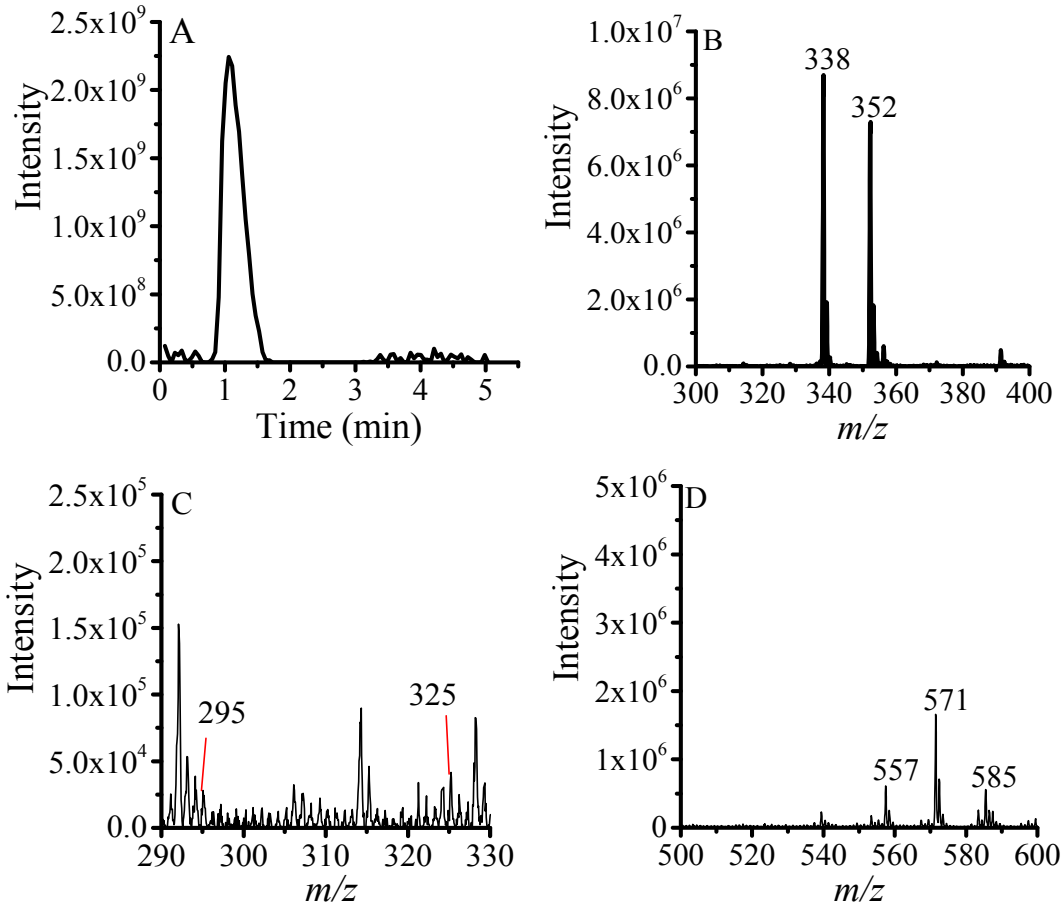

Figure S 3: LC-MS analysis of ethanol extract of *Cinchona* barks powder. A) Mass chromatogram, B-D) Mass spectra at different *m/z* intervals. LC condition: Instrument: Accela LC system; flow injection: 150 µl/min; mobile phase: 50% aqueous MeOH with 0.1% FA. Detector: Quattro LC mass spectrometer operated in positive ESI mode.

HPLC-MS analysis of *Cinchona* extract. The mass chromatogram shows the separation of quinine and derivatives after a high temperature liquid chromatography on an Xbridge C_{18} column. The standard mixture used in the analysis contains 6.7 mg/l each of Cn, Cdn, Qdn and Qn. The broader nature of peak 6 in the mass chromatogram is due to co-elution of hydrocinchonidine and quinine. Selected ion recording (SIR) chromatogram for *m/z* 297.4 shows a peak at same retention time as quinine. The *Cinchona* extract shows peak of the

Cinchona alkaloid standards. In addition to the known peaks, there are other minor peaks before the retention time, 8 minutes and after the elution of Qdn, which can probably be attributed to some other impurities with same mass-to-charge as those in the SIR scan mode.

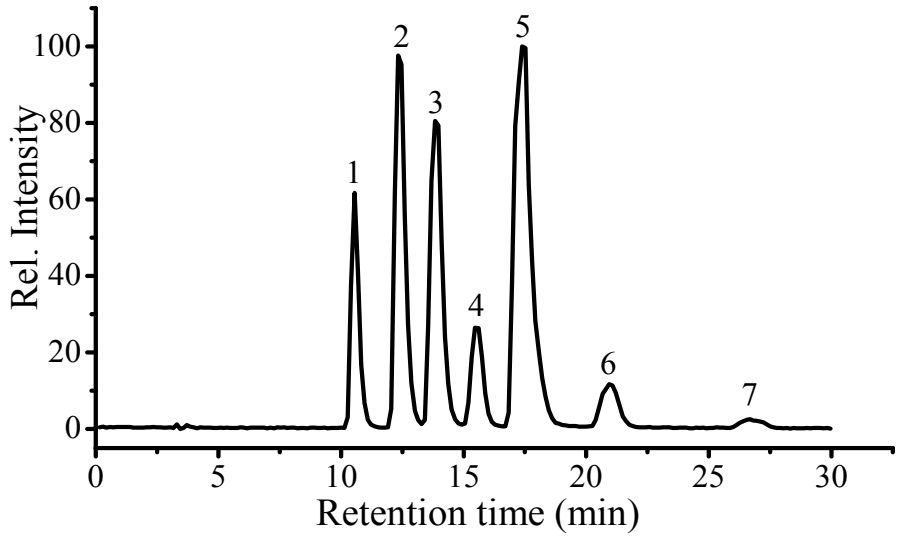

Figure S 4: SIR mass chromatogram of protonated molecular ion of Qn and derivatives standard: 1) Cn (*m/z* 295.14), 2) Cdn (*m/z* 295.14), 3) Qdn (*m/z* 325.18), 4) DCn (*m/z* 297.14), 5) DCdn (*m/z* 297.18)/Qn (*m/z* 325.18), 6) DQdn (*m/z* 327.18), 7) DQn (*m/z* 327.18). Column: XBridge C_{18}, 150 x 4.6 mm, 3 µm particles; Column temperature 80 °C. Mobile phase: 10% MeOH/90% water (0.1% FA, pH 3.0). Detector: Quattro LC mass spectrometer.

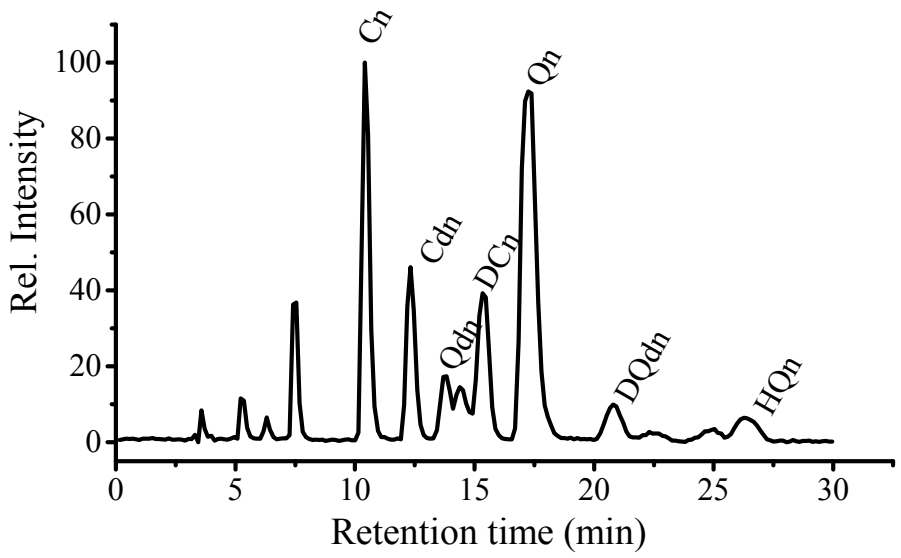

Figure S 5: SIR mass chromatogram of protonated molecular ion of Qn and derivatives in *Cinchona* extract. The peak identities are shown on the chromatogram. The LC and MS conditions are same as in Figure S 4.