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Original Research Article

The commercial history of Cape herbal teas and the analysis of phenolic compounds in historic teas from a depository of 1933

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ABSTRACT

In an investigation into the historical use of various plant species as herbal teas in South Africa and the commercialization of rooibos (*Aspalathus linearis*), 37 tea samples from a depository of 1933 were analysed. These samples are known as the Nortier collection and were put in the depository by Benjamin Ginsberg. Nortier and Ginsberg were instrumental in the commercialisation of Rooibos tea. Liquid-chromatography–high resolution mass spectrometry (LC–HRMS) was used with electrospray ionisation (ESI) in negative mode to compare the flavonoids and phenolic acids and identify marker compounds in the samples. The positively identified marker compounds together with multivariate data tools were used to distinguish between the different teas and group similar ones together. The LC–HRMS chromatogram of the 1933 Nortier tea, the tea from which cultivated rooibos emanated, is virtually identical to a commercial cultivated tea from 2016. The selection of teas is an interesting look into the history of herbal tea in South Africa, the species used and the decision-making process of Nortier in developing the industry. The historic "reed tea" (*riettee*) was identified for the first time as *Thesium macrostachyum* and other *Thesium* species and is the same as the tea that is still used in the Wupperthal area as "*lidjiestee*".

1. Introduction

Rooibos (*Aspalathus linearis* (Burm.f.) R.Dahlgren, Fabaceae) is a plant endemic to the fynbos biome of the Cape Floral Kingdom. Its habitat is restricted to the western parts of the Cape Province of South Africa, with a centre of diversity around Citrusdal and Clanwilliam. There exists some uncertainty regarding the historical use of rooibos as a tea (Van Putten, 2000). It is known that from 1660 to 1663, a number of Dutch expeditions into the rooibos habitat (Cederberg area, North of Cape Town) documented their interactions with the indigenous Khoi people. The consumption of rooibos tea or traditional tea was never mentioned in their journals. The first settlers started farming in the area in the early 1700s and introduced the Khoi to tobacco, brandy, black tea and coffee. During that period, many fugitive slaves also sought sanctuary in the mountains in this area. The Dutch farmers started using rooibos as a substitute for black tea. However, it is unclear whether the Khoi consumed tea as a beverage historically or if it was influenced by the Dutch settlers. It might also be that the San (also known as the Bushmen), that were in the area for ages before the Khoi arrived, could have taught the Khoi about it, since the San had an extensive knowledge of plants (Van Putten, 2000).

In 1903 a Russian Jewish family, headed by Arend Ginsberg, moved into the area and started a trading store Ginsberg & Son at Rondeboschen. Ginsberg renamed it Blackhouse, which was quickly nicknamed *Blikhuis* (zinc/can house) by the local people, due to the type of buildings that he erected. His son, Benjamin, travelled in the area trading from his donkey-cart and befriended the local inhabitants. The Ginsberg family, direct descendants of the Russian Popoff family who had the sole distribution rights of black tea in Eastern Europe, became interested in rooibos after being offered rooibos by the inhabitants of the Clanwilliam area. They had knowledge of the Eastern black tea industry and used their knowledge to teach the local people how to improve the way in which they harvested and processed the tea. They began buying tea and selling it in their store. Rooibos tea was first presented in Europe at an exhibition of South African country products in London in 1907. However, it took another 70 years until it reached

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the international market place on a larger scale (Elkann, 2016).

Rooibos was initially commercialised from wild-harvested plant material. Benjamin Ginsberg convinced Pieter Lafras Nortier, a medical doctor (and layman horticulturalist) and Olof Bergh, a local farmer, to get involved in the industrial production (Van Putten, 2000). Olof Bergh harvested a large amount of rooibos in 1925 on his farm Kleinvlei, in the Pakhuis Mountains. Nortier collected seeds in the Pakhuis Mountains (Rocklands) and in a large valley called Grootkloof and these first selected seeds are known as the Nortier-type and Redtea-type. Poor germination yields hampered commercial production efforts, until Nortier found a way of scouring the seeds before planting them in trays. Nortier went into partnership with William Travers De Burgh Riordan in 1930. The new company established plantations at Varkenfontein and Klein Kliphuis and so the industry was born. At the same time another farmer Dirk Slabbert started experimenting and cultivating tea on his farm Olyfenboschkraal on his own (Van Putten, 2000), and he later marketed his tea under the Khoisan brand.

Reed tea or *riettee*, another herbal tea that was wild-harvested in the mountains and consumed by the inhabitants of the Cederberg region, was never commercialised (Van Wyk and Gorelik, 2017). The botanical identity of this tea has hitherto remained scientifically unknown.

Against the background of steady growth, quality remains an overarching concern for the rooibos industry. In this context, the collections of the Clanwilliam Museum serve as an excellent reference. Of prime importance is a salesman's case containing 37 historical tea samples from the 1930s. The samples of tea were collected in the Olifants River valley and western slopes of the Cederberg mountain region between 1930 and 1935 and were deposited in the Clanwilliam Museum by Benjamin Ginsberg, each with a handwritten note labelling its origin.

A small sample of each of these teas was supplied to us. Apart from the two well-known unique flavonoids in rooibos, aspalathin and phenylpropenoic acid glucoside (PPAG) that have been shown to have many health benefits, the other main flavonoids are orientin, isoorientin, vitexin, isovitexin and vicenin-2. In a study in 2017, we found that the relative concentrations of these compounds vary in wild populations of rooibos tea and that some of them even contain different flavonoids as main constituents, such as phloridzin and a sieboldin analogue (Stander et al., 2017).

Tracing the life-line of rooibos' "little brother", honeybush (*Cyclopia* spp.) is compounded by the fact that it took until the late 1990s for cultivation attempts to become successful, thus allowing commercialisation and marketing beyond the South African borders. The presence of honeybush in Nortier's sample case from the 1930s indicates that it was for all intents and purposes on the radar of Ginsberg & Co., only that volumes procured from the wild were too small and too unreliable for international commercialisation.

Looking further into honeybush's history, however, reveals the surprising fact that it was known to the world of science long before rooibos reached the same level of "intimacy" in the 1940s. A number of publications pre-date Greenish (1881a) who is generally recognised as having provided the first mention of a species in terms of its use as a tea (Joubert et al., 2011). Authoritative European compendia published prior to 1850. Kachler (1829), Geiger (1829) and Lindley (1838, 1849), for example, make no mention of either rooibos or honeybush. An excellent summary of early records on honeybush tea is provided by Van Wyk and Gorelik (2017), starting with Latrobe (1818) and Greenish (1881b). Rosenthal (1862) refers to the use of *Cyclopia* species (*C. galeoides* DC., *C. genistoides* Vent., *C. latifolia* DC., *C. sessiliflora* Eckl. & Zeyh., *C. intermedia* E.Mey. and *C. brachypoda* Benth.) as popular tea (surrogates), going by the name of "Honig- "or "Birsthee" but makes no mention of rooibos. Jackson (1873), in his paper on African tea plants, gives a general description for the genus *Cyclopia,* mentioning nine species as native to South Africa and emphasises the use of *C. genistoides* and *C. vogelii* Harv. leaflets for the making of bush tea.

The aim of this study is to analyse these nearly 100-year-old teas and compare them to the teas cultivated today, using liquid chromatography–high resolution mass spectrometry (LC–HRMS) and multivariate data analysis tools. We focused on the phenolic components in the tea, as these main compounds not only provide the perceived health benefits (Joubert et al., 2008) but are also commonly used in quality control of commercial rooibos tea.

Fig. 1. Collection of historical herbal tea samples of B. Ginsberg in the Clanwilliam Museum, Western Cape, South Africa, dating back to 1930–35.

2. Materials and methods

The same methods, equipment and standards were used as before (Stander et al., 2017) but the extraction was downscaled to use less of the precious samples.

2.1. Samples and sampling

The samples were stored in containers at room temperature at the museum. A small subsample (200–300 mg) was taken and extracted for LC–HRMS. The samples constituted a mixture of leaves, flowers and stems (Figs. 1 and 2). Table 1 contains the notes of Ginsberg and the subsequent notes on the appearance and identifications of the tea by a botanist (B.-E. Van Wyk).

2.2. Extraction

Approximately 0.2 g of dry plant material were extracted with 50% methanol in water containing 1% formic acid (2 mL) in a 15-mL polypropylene centrifuge tube by soaking it overnight, followed by extraction in an ultrasonic bath (0.5 Hz; Integral Systems, RSA) for 60 min at room temperature. The extracts were centrifuged (Hermle Z160 m; 3000*g* for 5 min) and transferred into vials.

2.3. Standards

Standards were obtained from Sigma-Aldrich and by kind donation by Dalene De Beer of the Agricultural Research Council, Stellenbosch, South Africa. Stock solutions were prepared quantitatively in cocktails ranging in concentration from 1 to 100 µg/mL. Four different cocktails were prepared at each level to enable isomers and compounds with similar elemental formulae to be distinguished. The solvent used for preparation of the cocktails was 50% methanol in water containing 1% formic acid, the same as that used for extraction of the samples.

Fig. 2. Selected historical samples of rooibos tea (*Aspalathus linearis*), honeybush tea (*Cyclopia* species) and other teas made from wild plants. The labels on the lids of the containers are in the handwriting of B. Ginsberg: 1, "Grandiflora Tea" (an early brand) – rooibos tea; 2, "Calvinia distr." – rooibos tea – probably Nieuwoudtville; 28, "Dr Nortier Season 1931" – a sample of some of the first cultivated rooibos tea; 14, "Swartbos var. Clanwilliam Distr." – black tea type – swartbos tea as opposed to rooibos tea; 29, "*Aspalathus corymbosa* Clanwilliam Distr." – an incorrectly named wild form of rooibos tea; 30, "*Aspalathus cedarbergensis* Clanwilliam Distr." – the Wupperthal tea type, formerly known as a separate species or subspecies; 36, "Vaal Brown tea" - grey tea type; 23, "Yellow tea" – probably *Aspalathus pendula*, which makes a bright yellow tea; 4, "*Cyclopia vogelii*" – a misapplied synonym of *C. intermedia* and *C. subternata*; 15, "Caspa tea" – an early brand of honeybush tea, probably made mainly from *C. intermedia*; 25, "Anysberg tea Montagu Distr." - tea made from the inland form of *C. intermedia*; 31, "Uniondale Distr." – tea made from *C. plicata* – the distinctive bracts of this species are visible in the lower right hand corner; 11, "Reed Tea Clanwilliam Distr." – *Thesium macrostachyum* or related species; 12, "Reed Tea Clanwilliam Distr." – *Thesium macrostachyum* or related species (note fruiting stalk on the left); 37, lid with no label – tea made from an as yet unidentified *Thesium* species (note distinctive floral bracts). Clanwilliam Museum, Western Cape, South Africa. For author citations see Table 1. Scale bars = 5 mm.

2.4. LC–MS analysis

A Waters Synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA) was used for high resolution UPLC-MS analysis. In short, electrospray ionisation in negative mode was used, a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column and mobile phase gradient of 0.1% formic acid (Solvent **A**) and acetonitrile containing 0.1% formic acid as solvent **B** (Stander et al., 2017). The samples were also all analysed under the same conditions in positive mode, but the negative mode data were mostly used since they contained the most information.

2.5. Data pre-processing and clustering

Direct statistical analyses of LC–MS data are not possible, as peak concentrations substantially vary among different components. Concentration is also not proportional to statistical importance in this case. Markerlynx (Waters) was used to align the data and convert it to retention time-mass pairs with a signal intensity for each peak. Selected mass peaks from the mass spectra were normalised to compensate for the variance in concentration and ensure equal representation in the dataset, thereby facilitating comparative analysis. Normalisation involves scaling each sample vector to a unit norm (L1 norm), independently of other samples.

Principal component analysis (PCA) was then performed on the dataset to reduce dimensionality and the number of PCA components chosen, such that the amount of variance that needs to be explained is greater than two times standard deviation (95.45%) data coverage. An unsupervised clustering analysis algorithm was then applied to the selected PCA components.

In traditional methods the PCA components are visualised in pairs while the loadings plot for all PCA components are displayed simultaneously. However, all the selected PCA components need to be considered collectively for meaningful discrimination of the dataset, whereas the loadings factors for each PCA component should be interrogated individually, to gain an understanding of the causative factors which contributed the most variation within the dataset.

In this experiment an implementation of the Mean Shift clustering algorithm was used as it holds no intrinsic hypothesis about the number of clusters, nor the shape thereof. It is a non-parametric density estimation based algorithm, where each point in the feature space corresponds to the initial centroid positions and the distribution distance of points in the feature space are used to estimate a bandwidth parameter, also known as the attractive interaction distance between samples. Iteratively, the mean of all additional points within the bandwidth of the initial centroids determine the new set of centroids (kernel density estimation) until convergence was achieved. The remaining set of centroids after convergence, being the cluster centres and the data points associated with the same centroid, are members of the same

Table 1
Historical tea samples from the Clanwilliam Museum (collection of B. Ginsberg, ca. 1930–35).

Sample number	Words / Data written on tin (in B. Ginsberg's hand)	Species / tentative identification, based on sample material	Notes
1	"Grandiflora" Tea	*Aspalathus linearis* (Burm.f.) R.Dahlgren	*Grandiflora Tea* was one of several early brand names for rooibos tea (tea type unknown)
2	Calvinia distr.	*Aspalathus linearis*	Almost certainly Nieuwoudtville (local wild type or cultivated type?)
3	Van Rhynsdorp distr.	*Aspalathus linearis*	Almost certainly Maskam / Gifberg (local wild type or cultivated type?)
4	*Cyclopia vogelii*	*Cyclopia* species	*C. vogelii* is a synonym for several species (*C. intermedia, C. subternata*, and others)
5	Van Rhynsdorp distr.	*Aspalathus linearis*	Same type as 3? Local wild type or cultivated type?
6	Van Rhynsdorp distr.	*Aspalathus linearis*	Same type as 3? Local wild type or cultivated type?
7	Van Rhynsdorp distr.	*Aspalathus linearis*	Same type as 3? Local wild type or cultivated type?
8	?dark Bossie Tea?	*Aspalathus linearis*	Provenance not indicated
9	no data	*Senna alexandrina* Mill.	Imported from Hamburg ("Hopfensack 9?")
10	Rooibos Sticks	*Aspalathus linearis*	"…showing how bushes are destroyed"
11	Reed Tea Clanwilliam distr.	*Aspalathus linearis* mixed with *Thesium macrostachyum* A.DC.	"Reed Tea" clearly refers to *Thesium macrostachyum*, which is still used as tea in the Wupperthal area
12	Reed Tea Clanwilliam distr.	*Thesium macrostachyum*	Reed Tea clearly refers to *Thesium macrostachyum*, which is still used as tea in Wupperthal area
13	Uniondale distr.	*Cyclopia* sp.cf. *intermedia* E.Mey.	Probably *C. intermedia*
14	Swartbos var. Clanwilliam distr.	*Aspalathus linearis*	Presumably the black tea type that was discontinued early on.
15	"Caspa Tea"	*Cyclopia* species	Finely chopped, difficult to identify the species
16	Clanwilliam distr.	*Aspalathus linearis*	Red type?
17	Clanwilliam distr.	*Aspalathus linearis*	Red type?
18	Clanwilliam distr.	*Thesium* species	Fruiting stalk, unidentified *Thesium* species
19	*Athrixia phylicoides*	*Athrixia phylicoides* DC.	"…Northern Transvaal"
20	*Cyclopia brachypoda*	*Cyclopia intermedia*	*C. brachypoda* is an old synonym. Flower and bract present (positive identification)
21	Heidelberg Tea *Cyclopia subternata*	*Cyclopia subternata* Vogel?	Probably correct, but identity not certain
22	Honey Flower Tea *Cyclopia genistoides* var.	*Cyclopia genistoides* (L.) R.Br.	Dried flowers with a few leaf fragments only
23	Yellow Tea	*Aspalathus pendula* R.Dahlgren?	No provenance indicated
24	Anysberg Tea Montagu distr.	*Cyclopia intermedia*	Very broad leaflets
25	Anysberg Tea Montagu distr.	*Cyclopia intermedia*	Flower present (positive identification)
26	Clanwilliam distr.	*Aspalathus linearis*	Red type?
27	Swartbos var. Clanwilliam distr.	*Aspalathus linearis*	Presumably the black tea type that was discontinued early on
28	Dr Nortier Season 1931	*Aspalathus linearis*	Presumably the red type / Rocklands type, grown at Klein Kliphuis
29	*Aspalathus corymbosa* Clanwilliam distr	*Aspalathus linearis*	Name unknown (an incorrect application of the name)
30	*Aspalathus cederbergensis*	*Aspalathus linearis*	Cederberg type (Wupperthal/Biedouw type)
31	Uniondale distr.	*Cyclopia plicata* Kies	Bracts present (positive identification)
32	Dr Nortier Season 1932	*Aspalathus linearis*	Presumably the red type / Rocklands type, grown at Klein Kliphuis
33	"Garfield" Tea from U.S. America	mixture of senna, couch grass, balmony and dandelion	Mostly *Senna alexandrina*, with chopped stems of dandelion and small amounts of *Chelone* and *Elymus*
34	Clanwilliam distr.	*Aspalathus linearis*	Red type?
35	no data	*Aspalathus linearis*	Red type? Material pulverised
36	Vaal Brown Tea	*Aspalathus linearis*	Wild type (grey resprouter?)
37	no data	*Thesium* species	See samples 10 and 11 (reed tea).

cluster. The choice of the bandwidth parameter has a significant impact on the performance of the mean shift algorithm. If the value is too small, convergence is slow, especially on large datasets; if it is too large, some clusters may be ignored. The bandwidth parameter was estimated for this feature space to be the average of the distance of each vector to their k nearest neighbours relative to furthest distance between the feature vectors. Setting the number of k nearest neighbours (kNN), to 10–13% of the total sample set, yielded optimal bandwidth setting results. The algorithm was implemented in the Python programming language.

3. Results and discussion

Electrospray ionisation in negative mode is best suited for phenolic compounds, including flavonoids and organic acids. The phenolic compounds can also be detected in positive mode, but the sensitivity is lower and organic acids (specifically the smaller ones) cannot be detected in positive mode under these conditions. Triterpenoids and alkaloids (including theobromine and caffeine) are best detected in positive mode and that is why we analysed all samples in this mode as well. None of the major peaks in all the tea samples could be allocated to alkaloids and no caffeine was detected in any of the samples. It was therefore decided to focus the study on the negative mode results, since no additional information was added by the positive mode data.

The most prominent phenolic compounds present in rooibos are aspalathin, orientin, isoorientin, vitexin, isovitexin, PPAG, hyperoside, (*S*) and (*R*)-eriodictyol-6-*C*-β-D-glucopyranoside and its 8-*C* equivalents (Figs. 3 and 4). See also Supplementary Fig. 1. Fig. 3 shows the control sample, which is a contemporary commercial fermented sample (Stander et al., 2017), compared to the Nortier season 1932 (OT28) and 1931 (OT32) on the same scale. They are remarkably similar. The 1931 sample has even a higher concentration of aspalathin (compound 32, m/z 451), which is believed to be unstable in solution.

The marker compounds for honeybush are mangiferin and isomangiferin (Fig. 4). Other main compounds in *Cyclopia subternata* Vogel are iriflophenone-3-*C*-β-glucoside; 3-hydroxyphloretin-3′,5′-di-*C*-hexoside; eriocitrin, luteolin-7-*O*-rutinoside (scolymoside) and phloretin-3′,5′-di-*C*-β-glucoside (De Beer et al., 2012), while iriflophenone-di-*O*,*C*-hexoside was detected in *Cyclopia genistoides* (Beelders et al., 2014).

The aim of the LC–MS analysis was to identify/confirm the identities of the different tea samples. The method was published previously (Stander et al., 2017) and the identification of peaks was done accordingly. We found previously that phenolic profiles cannot exclusively be used to distinguish between the different rooibos variants.

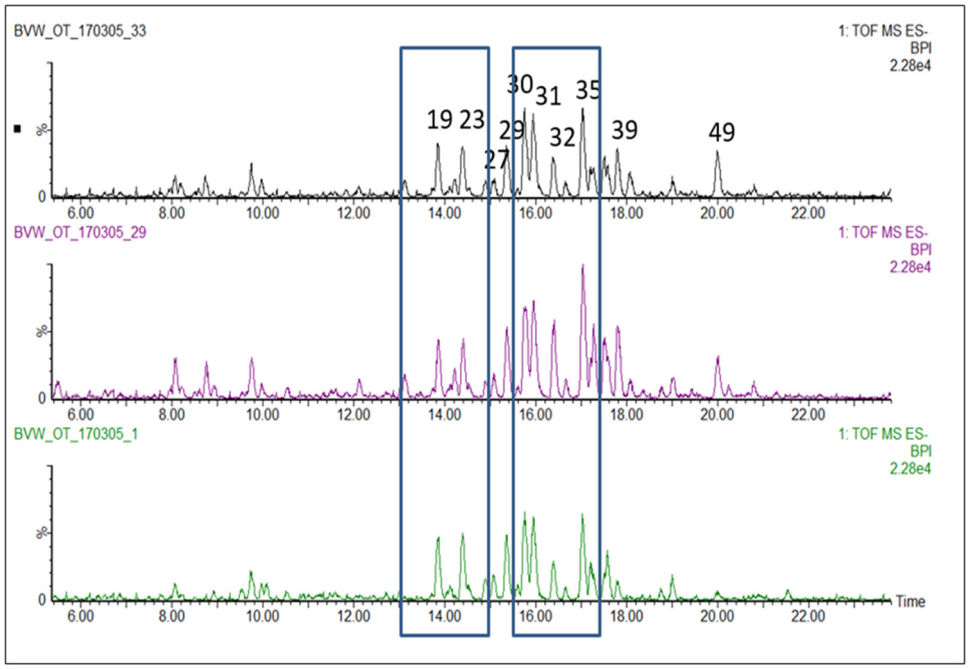

Fig. 3. Total ion chromatograms electrospray ionisation negative showing the extract of sample 28 (middle), the control (bottom) and 32 (top). All three are cultivated rooibos (*Aspalathus linearis*). These samples had an age difference of 83 years.

Geographically, northern region teas typically produce higher amounts of PPAG and the Wupperthal region teas produce phloridzin and derivatives, not found in cultivated teas. Initially a targeted approach was followed to identify the most prominent peaks in the chromatograms and to compare them between samples (Figs. 3 and 4). We focused on what was previously identified using this method (Stander et al., 2017) in rooibos and added the marker compounds of honeybush (De Beer et al., 2012; Beelders et al., 2014). The second approach was an untargeted one, where Markerlynx software, (an application manager of the Waters MS software, Masslynx) was used to perform principal component analysis on all the peaks detected in the samples. The latter could differentiate between honeybush and rooibos and clustered all the rest of the samples together, as shown in Supplementary Fig. 2.

The loadings plot (Supplementary Fig. 3), implies that the compounds that caused separation were mangiferin (*m/z* 421) for the honeybush versus piscidic acid (*m/z* 255), PPAG (*m/z* 325) and orientin (*m/z* 447) for the rooibos. The first two PCA components explained only 18% and 11% of the data variation. Similar results were obtained with the ESI positive data.

It was decided to export the aligned Markerlynx data to undertake

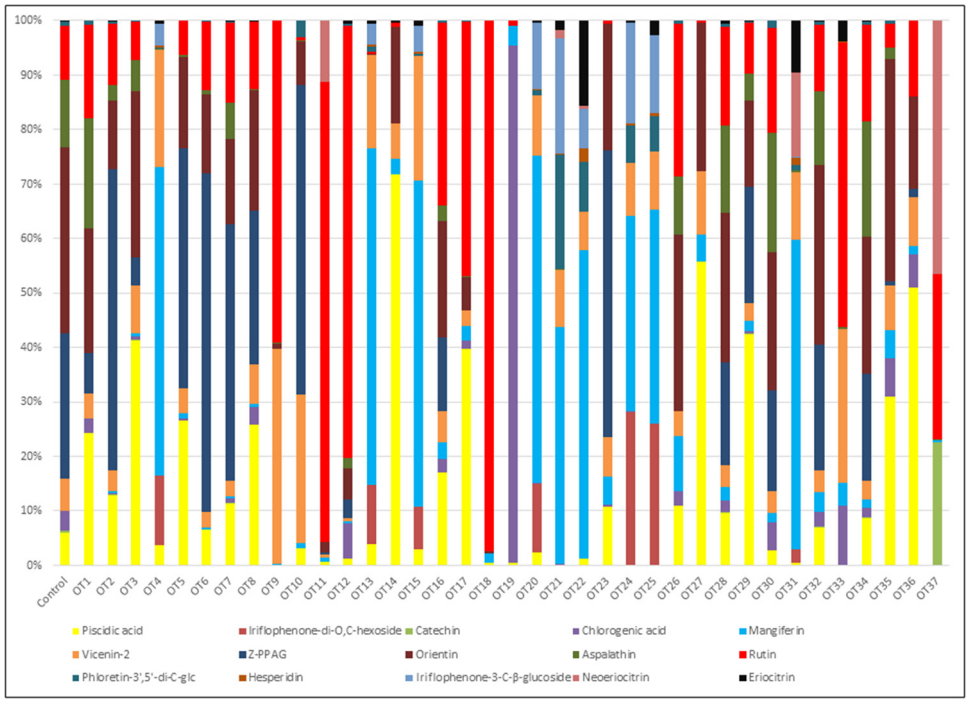

Fig. 4. Summary of relative concentrations of 15 important marker compounds in the 37 historical tea samples (with one contemporary rooibos tea sample as control), showing similarities and differences.

an experiment with the aim of explaining more of the sample variations and achieve a more detailed clustering by means of the hierarchical cluster analysis (HCA) algorithm. This technique was initially tested by subjecting two very similar samples to it to determine if it could discriminate between them. The same control sample extracted with 40% acetonitrile was compared to an extraction of 50% methanol with 1% formic acid. Supplementary Fig. 4 shows the correlation map between these samples and Supplementary Fig. 5 the cluster map. The loadings plot (not shown here) showed that the more polar compounds, such as piscidic acid, were extracted more effectively with the methanolic extraction and the non-polar, later eluting compounds, such as chrysoeriol, with acetonitrile, and the chromatograms looked very similar.

Subsequent to this validation, the HCA algorithm was applied to the historic tea data set. It required 19 PCA components to describe 98.31% of the data; this was done to achieve accuracy greater than 2 standard deviations. Two major clusters (rooibos and honeybush) were identified, along with 10 smaller clusters. The non-rooibos and non-honeybush tea samples are represented here in different clusters. The loadings plot of PC1 (Fig. 6), shows that the same compounds that caused the separation between rooibos and honeybush in the Markerlynx PCA caused the separation in PC1 here, as expected.

Table 1 contains the descriptions and notes on the individual samples and Table 2 the information on the identified compounds. Then mean shift clusters could be identified: Cluster 1 (orange) in Fig. 5 represents the rooibos samples. Cluster 2 (green) represents the honeybush samples. The rest of the samples form clusters on their own. Cluster 3: OT9, Cluster 4: OT10, Cluster 5: OT11, Cluster 6: OT12, Cluster 7: OT18, Cluster 8: OT19, Cluster 9: OT33 and Cluster 10: OT37.

Supplementary Fig. 6 is the hierarchical cluster map of the sample data and Supplementary Fig. 7 the correlation map. Sample OT18 clusters on its own and contains large amounts of rutin, citric acid, but no PPAG, aspalathin or other marker compounds for rooibos. It does not correlate to any of the other samples in the correlation map, but the high rutin level is typical of the *Thesium* samples (and it was identified as a *Thesium* species – see the fruiting stalk in Fig. 2). It was only labelled "Clanwilliam Distr." on its container. Sample OT10 also clusters on its own, but correlates weakly with the rooibos samples. It contains PPAG and very low amounts of aspalathin and the other marker compounds of rooibos, with no detectable non-rooibos phenolics. This sample contained more sticks than leaves, hence the low concentration of phenolics.

When the rooibos data are processed on its own (Supplementary Figs. 8 and 9), it is interesting to note that the samples OT 27, 14, 36 and 17 clustered together. All of these samples were referred to as black/brown tea on their tins. A brown/black version of *A. linearis* was once available but it was discontinued early on because of poor quality. This separation according to the loadings plot was on very low PPAG levels and high piscidic acid levels in these samples. The rest of the samples except for OT10 and 17, which were mostly sticks, cluster together, but according to the HCA the samples from the southern region [control, OT28, 32, 30, 34, 26 (Clanwilliam, Wupperthal, Cederberg, Kliphuis)] were similar and the samples from the northern, more arid region [OT2, 5, 6 and 8 (Calvinia, Van Rhynsdorp)] were similar.

Samples OT11, 12 and 37 are documented to contain "reed tea", now known to be *Thesium macrostachyum* A.DC., with OT12 being a mixture with rooibos. The rooibos components were detected in OT12. The correlations between these three samples are weak. All of them contain high concentrations of citric acid and rutin, but sample 37 also contains a large amount of catechin, quercetin and neoeriocitrin, which caused the variance. We have compared these chromatograms with authentic *Thesium* samples: *T. asterias* A.W.Hill, *T. costatum* A.W.Hill, *T. cupressoides* A.W.Hill, *T. procerum* N.E.Br., *T. macrostachyum, T. strictum* P.J.Bergius and *T. carinatum* A.DC. (from collections made by Natasha Visser, with voucher specimens housed in the Pretoria National Herbarium (PRE)). The comparisons confirmed that OT12 contained *T. macrostachyum*, but although OT11 and OT37 have some compounds in common with some of the *Thesium* species (e.g., OT11 with *T. strictum* and OT37 with *T. asterias*), there were compounds present in the one and not in the other. The confirmation of the reed tea as *Thesium* species tells us that the tea that is still used in the Wupperthal region and that is locally known as "*lidjiestee*" is the same species as the historical "reed tea" (Van Wyk and Gorelik, 2017).

If we look at the HCA clustering dendrogram of the LC–MS data and the correlation map of the *Cyclopia* samples on their own (Supplementary Figs. 10 and 11), we see that samples OT 4, 20, and 13 and 15 correlate. These samples were botanically identified as *C. intermedia*. Sample 4 was labelled as *Cyclopia vogelii*. This name is a synonym used for several *Cyclopia* species, including *C. subternata* and *C. intermedia*. Sample 20 was labelled *C. brachypoda* on its tin. This name is an old synonym for *C. intermedia* and sample 20 was positively identified by the presence of its flowers and the bract. Sample 15 was labelled "Caspa Tea". They all have similar LC–MS chromatograms and differ from the other honeybush samples by having lower phloretin-3′,5′-di-*C*-β-glucoside and eriocitrin; iriflophenone-di-*O*,*C*-hexoside was present but not as high as in some of the other samples. They were thus identified as *C. intermedia*. Samples 24 and 25 correlate strongly and have been documented as "Anysberg Tea, Montagu district". It contains, in addition to mangiferin and isomangiferin, also high concentrations of both iriflophenone-di-*O*,*C*-hexoside and iriflophenone 3-*C*-glucoside. These compounds are usually characteristic of *C. genistoides* (Beelders et al., 2014). The two samples could be positively identified as *C. intermedia* and represent a distinct form of the species, commonly known as the Anysberg form, which is found in the extreme North-Western part of the distribution range of the species. This is a good example that the chemistry in isolation cannot always be used to distinguish between *Cyclopia* species. The full range of chemical variation in the genus *Cyclopia* is as yet insufficiently known. Samples 21 and 22 do not correlate as strongly with the other *Cyclopia* samples and were grouped on their own. They contain no or very little iriflophenone-di-*O,C*-hexoside which is a marker compound for *C. genistoides* and can therefore not be *C. genistoides* (Beelders et al., 2014). Sample 21 and 22 contain very high concentrations of phloretin-3′,5′-di-*C*-β-glucoside and also showed large eriocitrin and scolymoside peaks, a combination that is typical of *C. subternata* (Beelders et al., 2014). Sample 21 was identified as *C. subternata*. Sample 22 contains larger amounts of iriflophenone-3-C-β-glucoside and neoeriocitrin compared to 21. This sample was positively identified as mostly *C. intermedia* flowers.

4. Conclusions

The previously unidentified "reed tea" (*riettee*) mentioned in the early Cape ethnobotanical literature was identified as *Thesium macrostachyum* and other *Thesium* species. Since *Thesium* species grow as semi-parasites on the roots of other plants, it is possible that the phenolic compounds may be influenced by their host plants. The species of *Thesium* are notoriously difficult to identity, so that the noteworthy chemical differences observed in this study indicate that a chemosystematic study may provide additional diagnostic characters.

Modern LC–HRMS is shown to be a powerful technique for the identification of complex mixtures of phenolic compounds in herbal teas, especially when used in combination with multivariate statistical techniques. Failure to positively identify some of the tea samples is not a shortcoming of the techniques that were used but is rather due to incomplete reference materials and an incomplete knowledge of the complete extent of chemical variation within the genera and species that were sampled. Detailed chemical variation studies of plant populations across the entire distribution ranges of the species are required to improve the interpretation of results.

Despite these limitations, it was possible to identify all of the historical tea samples to the generic level, and some of them even to species level. It is noteworthy that the historical rooibos tea samples dating from the 1930's are practically identical to contemporary

Table 2
Compounds detected in 37 historical herbal tea samples and a contemporary commercial rooibos tea reference sample.

No.	Compound Name	Trace *m/z*	[M-H]	Fragment ions	RT (min)	Stan-dard
1	Citric acid	191.019	$C_6H_7O_7$	191,111,87	3.10	Yes
2	8 C hexosyl derivative of aspalathin	613.1436	$C_{33}H_{25}O_{12}$	493,387,377,161	5.49	No
3	Fukiic acid	271.0448	$C_{11}H_{11}O_8$	181,123,109	6.65	No
4	501_7.5	501.127	$C_{21}H_{25}O_{14}$	381,291,261,205	7.55	No
5	Procatechuic acid (3,4 dihydroxy benzoic acid)	153.0192	$C_7H_5O_4$	109, 153,108	8.20	Yes
6	Piscidic acid	255.0515	$C_{11}H_{11}O_7$	165,193,179	8.30	No
7	Iriflophenone-di-*O*,*C*-hexoside	569.1499	$C_{25}H_{29}O_{15}$	287,449,569,479,317,167	8.65	No
8	355_8.7	355.0654	$C_{15}H_{15}O_{10}$	164.192	8.73	No
9	357_9	357.0817	$C_{15}H_{17}O_{10}$	105,123,151,195,313	9.02	No
10	205_9.7	205.0712	$C_8H_{13}O_6$	143,129,115	9.71	No
11	Unknown compound, erroneously identified as Esculin	339.0706	$C_{15}H_{15}O_9$	191,219,249	9.91	Yes
12	Caffeic acid glucoside	341.0873	$C_{15}H_{17}O_9$	179.135	9.91	No
13	Catechin	289.0705	$C_{15}H_{13}O_6$	205.137	11.36	Yes
14	Chlorogenic acid	353.0865	$C_{16}H_{17}O_9$	191,353	11.83	Yes
15	*E*-PPAG	325.0889	$C_{15}H_{17}O_8$	161.119	12.01	No
16	Luteolin 6,8 di-*C*-hexoside	609.1446	$C_{27}H_{29}O_{16}$	339,369,399,429,489,519,554	12.92	No
17	327.107; PPAG related?	327.107	$C_{15}H_{19}O_8$	147,165,103	13.04	No
18	Mangiferin	421.0753	$C_{19}H_{17}O_{11}$	421,301,331,271,259	13.75	No
19	(*S*)-Eriodictyol-6-*C*-β-D-glucopyranoside*	449.1079	$C_{21}H_{21}O_{11}$	329,193,135,449	13.83	No
20	Isomangiferin	421.0766	$C_{19}H_{17}O_{11}$	301,421,331,258,271	14.01	No
21	Luteolin *C*-glucoside-*C*-arabinoside (carlinoside)	579.1374	$C_{26}H_{27}O_{15}$	369.399	14.05	No
22	Apigenin-6,8-di-*C*-glycoside (vicenin-2)	593.1498	$C_{27}H_{29}O_{15}$	353,383,473,503	14.09	Yes
23	(*R*)-Eriodictyol-6-*C*-β-D-glucopyranoside*	449.108	$C_{21}H_{21}O_{11}$	329,193,135,449	14.37	No
24	Luteolin *C*-glucoside-*C*-arabinoside (carlinoside)	579.1377	$C_{26}H_{27}O_{15}$	369.399	14.49	No
25	Luteolin *C*-glucoside-*C*-arabinoside (carlinoside)	579.1348	$C_{26}H_{27}O_{15}$	369.399	14.88	No
26	(*S*)-Eriodictyol-8-*C*-β-D-glucopyranoside	449.1071	$C_{21}H_{21}O_{11}$	329,193,135,449	14.88	No
27	(*R*)-Eriodictyol-8-*C*-β-D-glucopyranoside	449.1077	$C_{21}H_{21}O_{11}$	329,193,135,449	15.07	No
28	*p*-Coumaric acid	163.0397	$C_9H_7O_3$	119, 163	15.35	Yes
29	*Z*-PPAG	325.0914	$C_{15}H_{17}O_8$	163,119,91	15.37	No
30	Luteolin-6-*C*-glucoside (isorientin)	447.0928	$C_{21}H_{19}O_{11}$	357,327,447,298	15.73	Yes
31	Luteolin 8-*C*-glucoside (orientin)	447.0931	$C_{21}H_{19}O_{11}$	357,327	15.95	Yes
32	Aspalathin (*C*-linked Luteolin glucoside)	451.123	$C_{21}H_{23}O_{11}$	331,361,209,167,451	16.26	Yes
33	Aspalallinin	449.1067	$C_{21}H_{21}O_{11}$	329,331,359,285	16.57	No
34	Ferulic acid	193.0495	$C_{10}H_9O_4$	134,193,178	17	Yes
35	Quercetin-3-*O*-robinobioside	609.1491	$C_{27}H_{29}O_{16}$	300,301,271,255,609	17.02	yes
36	Apigenin-8-*C*-glucoside (vitexin)	431.0958	$C_{21}H_{19}O_{10}$	341.311	17.2	yes
37	Quercetin-3-*O*-rutinoside (rutin)	609.1483	$C_{27}H_{29}O_{16}$	300,609, 301,271,255	17.26	yes
38	Quercetin-3-*O*-galactoside (Hyperoside)	463.0864	$C_{21}H_{19}O_{12}$	300,271,301,463,255,243	17.36	Yes
39	Apigenin-6-*C*-glucoside (isovitexin)	431.0972	$C_{21}H_{19}O_{10}$	341.311	17.57	yes
40	Quercetin-3-*O*-glucoside (Isoquercitrin)	463.0866	$C_{21}H_{19}O_{12}$	300,271,301,463,255,243	17.7	yes
41	Phloretin-3',5'-di-*C*-β-glucoside	597.1802	$C_{27}H_{33}O_{15}$	357,387,597,417,477,167	17.8	No
42	Luteolin-7-*O*-glucoside	447.094	$C_{21}H_{19}O_{11}$	285,447	18.04	yes
43	627_18.3	627.1934	$C_{28}H_{35}O_{16}$	387.417	18.35	No
44	Sieboldin-analog	451.1234	$C_{21}H_{23}O_{11}$	289,167,123	18.5	No
45	Nothofagin	435.1299	$C_{21}H_{23}O_{10}$	315.345	18.75	Yes
46	469_19 Coumaric acid diglucoside?	469.1327	$C_{21}H_{25}O_{12}$	325,163,119	18.97	No
47	Kaempferol glucoside	447.0927	$C_{21}H_{19}O_{11}$	447.285	19.33	No
48	579_19	579.2079	$C_{28}H_{35}O_{13}$	417,387,181,166	19.9	No
49	Hesperidin (Hesperetin 7-rutinoside)	609.1833	$C_{28}H_{33}O_{15}$	301,286,151	20.19	Yes
50	Phloridzin (Phloretin-2-β-D-glucopyranoside)	435.1274	$C_{21}H_{23}O_{10}$	167.273	20.57	Yes
51	Acetylglucose of asphalathin	493.1331	$C_{23}H_{25}O_{12}$	361,331,209,167	20.77	No
52	Secoisolaresinol	361.1643	$C_{20}H_{25}O_6$	331.346	21.49	No
53	Trilobatin (Phloretin-4-*O*-glucoside)	435.1274	$C_{21}H_{23}O_{10}$	167.273	22	Yes
54	Quercitin	301.0336	$C_{15}H_9O_7$	151.179	23.86	Yes
55	Luteolin	285.0399	$C_{15}H_9O_6$	285,133	24.05	Yes
56	Chrysoeriol (3'-*O*-Methylluteolin)	299.0551	$C_{16}H_{11}O_6$	284,299,256,183	24.38	Yes
57	Hesperetin	301.0711	$C_{16}H_{13}O_5$	299,284,237,193	24.42	Yes
58	Fisetin	285.2055	$C_{16}H_{29}O_4$	285,221,171	24.56	yes
59	Iriflophenone-3-*C*-β-glucoside	407.0986	$C_{19}H_{19}O_{10}$	287,407,317	11.23	No
60	343_13	343.1367	$C_{16}H_{23}O_8$	343,181,163,122	13.02	No
61	327_15	327.1439	$C_{16}H_{23}O_7$	327,165	15.11	No
62	357_16	357.1543	$C_{17}H_{25}O_8$	357,195,180	15.99	No
63	623_19	623.1601	$C_{28}H_{31}O_6$	623,315,299,271,243	19.38	No
64	Background ion	187.0969	$C_9H_{15}O_4$	187	20	No
65	Neoeriocitrin	595.1663	$C_{27}H_{31}O_{15}$	595,459,287,151,135	17.51	No
66	Eriocitrin	595.1647	$C_{27}H_{31}O_{15}$	595,287,151,135	16.99	No
67	Luteolin-7-*O*-rutinoside (Scolymoside)	593.1506	$C_{27}H_{29}O_{15}$	285	17.7	Yes
68	Neoponcirin	593.1520	$C_{26}H_{41}O_{15}$	515,273,167	20.66	No

* The absolute configuration of certain molecules has previously been described in rooibos (Stander et al., 2017) and honeybush tea extracts (De Beer et al., 2012; Beelders et al., 2014). During this study, previously analysed control tea samples were used to confirm the retention times of these compounds in the samples analysed.

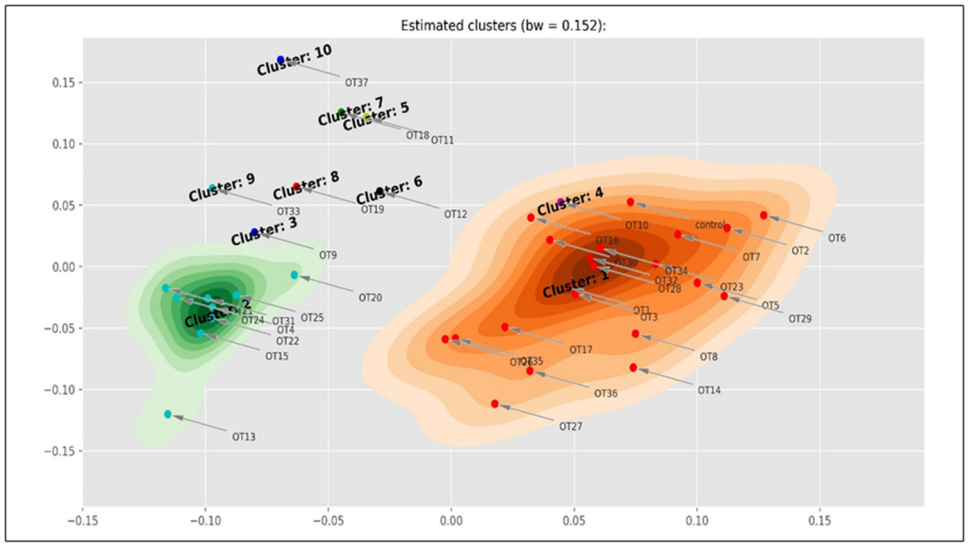

Fig. 5. Estimated clusters by HCA analysis of the LC–MS data of tea extracts showing in orange the cluster containing the rooibos samples and in green the cluster with the honeybush samples, with the rest of the tea samples clustering on their own (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

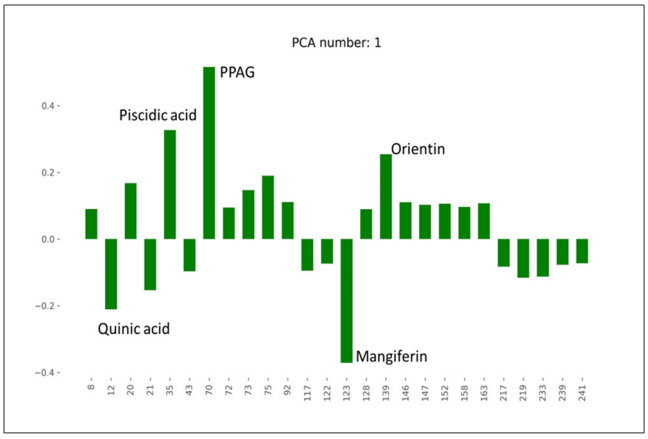

Fig. 6. Loadings plot for PC 1 with only the most significant contributors, showing the separation of rooibos from honeybush, mainly influenced by mangiferin that is only present in honeybush and PPAG, orientin and piscidic acid from rooibos.

commercial samples. This not only indicates that aspalathin and other phenolic compounds are stable when stored in a dry matrix, but that the phenotype of the red tea type has not changed since it was first cultivated on a commercial scale more than 80 years ago. The various rooibos tea types show considerable quantitative variation in their main phenolic compounds and a more detailed survey of geographical variation at the population level will yield interesting results. The same is true for the various species of the genus *Cyclopia,* which are known to be morphologically and chemically variable.

Conflict of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2018.11.001.

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