San and Nama indigenous knowledge: The case of |*nhora* (*Pteronia camphorata*) and its medicinal use

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A hitherto unidentified medicinal plant is here identified for the first time as *Pteronia camphorata* (L.) L., an aromatic shrub of the Asteraceae family endemic to the western and southern coastal region of South Africa. The plant was described in this journal by Laidler[1] in 1928 as '*D/nhora* buchu', and is one of the important types of buchu used by the Nama people. We report the traditional medicinal uses among San and Nama people, based on our interviews with rural participants. These include the treatment of colds, influenza, chest ailments and tuberculosis, as well as convulsions, haemorrhoids and inflammation of the neck. The major and minor chemical compounds of the essential oil that is produced by the plant are identified, together with the site of accumulation of this volatile oil within the leaf. We also investigated the plant's antimicrobial activity against a selection of a yeast and two Gram-negative and one Gram-positive bacteria, all of which are associated with respiratory infections. *P. camphorata* is scientifically poorly known but is an important San and Nama traditional remedy. Our study not only prevents the potential loss of historically important indigenous knowledge, but also provides the first scientific evidence to validate the traditional use of |*nhora* against upper and lower respiratory tract infections, including tuberculosis. This detailed study has wider application in demonstrating the fragility of the oral–traditional knowledge of a scientifically neglected indigenous group. It also highlights the scientific and practical importance of preserving traditional plant-use knowledge within a botanically diverse region.

Significance:

- Reveals the botanical identity of |*nhora*, an important Nama medicinal plant.
- Presents scientific evidence to validate the traditional uses.
- Contributes to the cultural heritage of a scientifically neglected indigenous group.
- Demonstrates the fragility of oral–traditional knowledge.

Introduction

The traditional Khoi and San practices of plant use in the botanically rich Cape region of South Africa are poorly documented. They are also rapidly diminishing because of the fragility of orally transferred indigenous knowledge, and the fact that modern medicine has almost completely replaced the traditional health care system. Because the Khoi and San peoples are ancestral to the rest of humanity, their ethnobotanical knowledge is of global significance. It provides insights into the early history and origins of medicinal plant use. We therefore hope to prevent the loss of profound ethnobotanical information relating to an important fynbos-endemic medicinal plant. Fynbos is a natural shrubland or heathland vegetation located in the south-western part of South Africa.

The hitherto unidentified plant described by Laidler[1] in 1928 as '*D/nhora'*, one of the important types of buchu used by the Nama people, is here identified for the first time as *Pteronia camphorata* (L.) L. (Asteraceae). 'Buchu' (*boegoe*) refers to aromatic plants that are used, often in powdered form, for medicinal and cosmetic purposes. Laidler used *D/* to denote the voiceless (tenuis) dental click, but we use the pipe symbol (|) in accordance with the International Phonetic Alphabet. Some linguists still prefer the (ǀ) symbol of the Doke / Beach convention.

The cultural and historical importance of this species came to light gradually, mainly because of ethnobotanical field survey work in Namaqualand.[2] Only ten elderly persons, two of whom are now deceased, had traditional knowledge about *P. camphorata* and its use in San and Nama medicine. *Pteronia* L. is a genus of roughly 70 small shrubs subendemic to southern Africa[3] and has been the focus of recent ethnobotanical studies[4–6]. Although these aromatic plants are of considerable local importance as sources of traditional medicine, their ethnobotany, leaf anatomy, essential oil chemistry and antimicrobial activity had previously not been studied systematically. *P. camphorata* is a small shrub of up to 0.8 m in height; it has a wide distribution in the Northern, Western and Eastern Cape provinces of South Africa. Four varieties have been distinguished (Figure 1), based on leaf arrangement (alternate or opposite) and the presence and density of white hairs (cilia) on leaf surfaces and margins.[3]

We wish to dispel the myth that indigenous knowledge is mainly of cultural and symbolic value, with limited practical use in health care. Our study explores the scientific rationale for the main use of |*nhora* in treating respiratory infections, including tuberculosis. For this purpose we conducted a detailed scientific study of the ethnobotany, leaf anatomy, essential oil chemistry and antimicrobial activity of *P. camphorata.*

Materials and methods

Materials studied

Three fresh samples of *P. camphorata* var. *armata* were collected at Kleinvlei, close to Wupperthal (32° S, 19° E), for the purpose of essential oil analysis and antimicrobial studies. Samples of all four varieties of *P. camphorata* were collected at various localities for anatomical study. Exact localities, voucher specimen details and authorities for names are shown in Table 1.

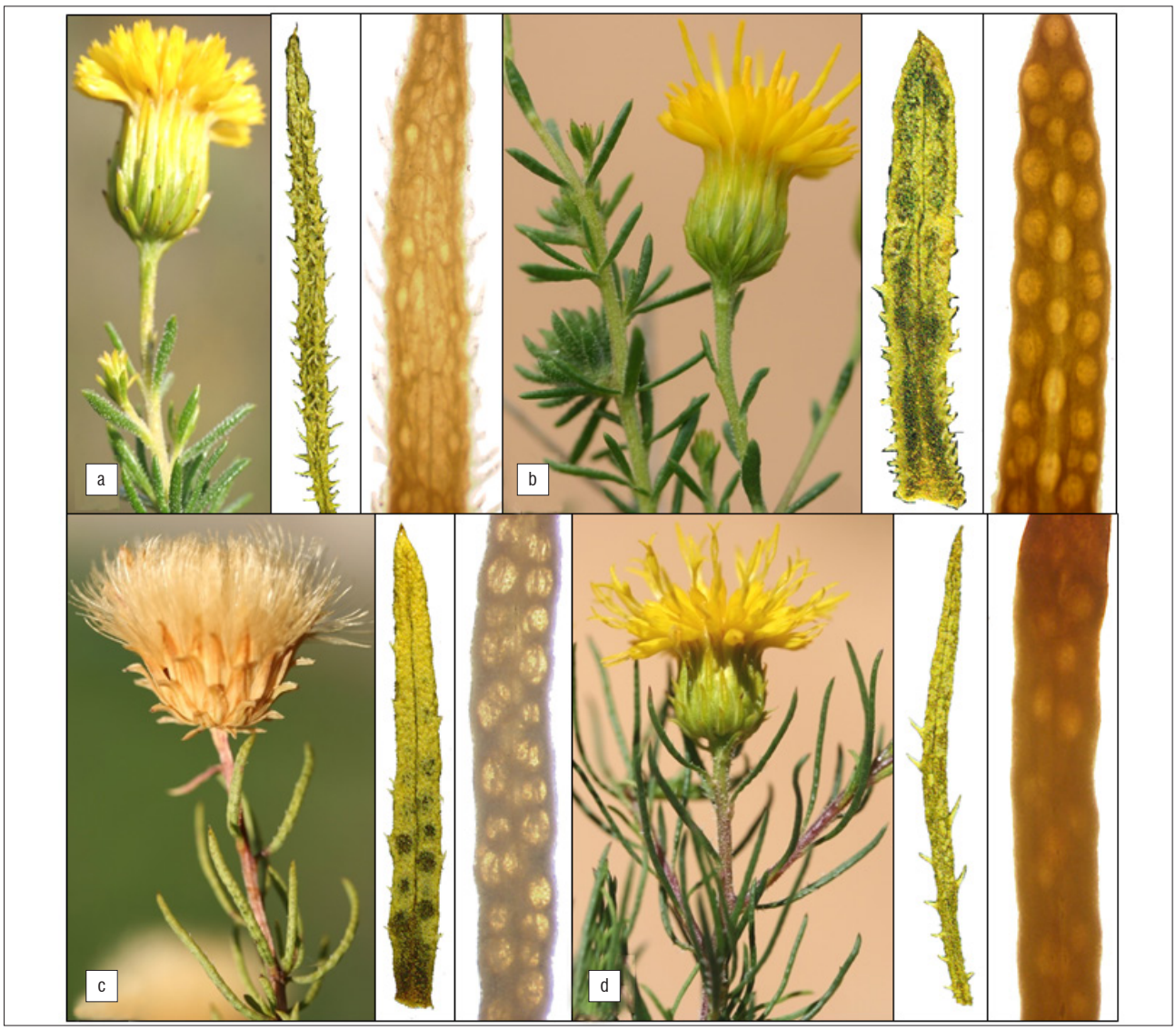

Figure 1: The morphology of flower-heads and leaves of four varieties of *Pteronia camphorata*. Specimens were (a) *P. camphorata* var. *armata* at Kleinvlei near Wupperthal, (b) *P. camphorata* var. *camphorata* at Dasklip Pass near Porterville, (c) *P. camphorata* var. *laevigata* at Nieuwoudtville, and (d) *P. camphorata* var. *longifolia* from Paarl Mountain. Note the distribution of cilia on leaves and presence of translucent oil glands. Photographs by B.E. Van Wyk.

Table 1: Voucher specimen details of the plant materials of *Pteronia camphorata* that were studied

Variety	Locality	Date collected	Voucher specimens (all housed in JRAU)	Anatomy (A) Extracts for antimicrobial (MIC) and chemical composition (GC–MS)
P. camphorata (L.) L. var. *armata* Harv.	Kleinvlei, Wupperthal	4 October 2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4443*	MIC, GC–MS, A
P. camphorata (L.) L. var. *camphorata*	Dasklip Pass, Porterville	3 October 2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4428*	A
P. camphorata (L.) L. var. *laevigata* Harv.	Nieuwoudtville, Oorlogskloof	19 August 1997	*B.-E. Van Wyk 3702*	A
P. camphorata (L.) L. var. *laevigata* Harv.	Nieuwoudtville, Oorlogskloof	11 December 2008	*B.-E. & M. Van Wyk 4287*	A
P. camphorata (L.) L. var. *longifolia* Harv.	Paarl Mountain	3 October 2009	*B.-E. Van Wyk, I.M. Hulley & P.M. Tilney 4427*	A

Ethnobotanical interviews

The recording of ethnobotanical data strictly adhered to all the ethical principles in the Code of Ethics[7] of the International Society of Ethnobiology. These include the concepts of traditional resource rights, educated prior informed consent, and respect for the privacy and customs of rural people. Formal approval was also obtained (by B.E.V.W. and J.M.N.) from the ethics committee of the Faculty of Science at the University of Johannesburg. The data presented here form part of two extensive ethnobotanical surveys in Namaqualand and the Cederberg region. The ethical clearance reference numbers are UJ Protocol No. 13 January 2015 (B.E.V.W., Cederberg survey and other studies); UJ Protocol No. 20 May 2011 (J.M.N., Kamiesberg survey, for MSc) and UJ Protocol No. 7 July 2015 (J.M.N., Namaqualand survey, for PhD).

Interviews were conducted in the local language, Afrikaans, and we used the matrix method as previously described[2]. An extensive network of local participants was interviewed: close to 100 people in Namaqualand and more than 30 people in the Cederberg region, including several local experts (Table 2). Our paper provides a firm starting date and geographical localities for the traditional knowledge associated with *P. camphorata*, including the identities of the knowledge holders.

Anatomical procedures

Dried leaf material of *P. camphorata* var. *armata* was rehydrated and placed in formaldehyde-acetic acid–alcohol (formaldehyde [40%] : glacial acetic acid : ethanol [96%] : distilled water, 2:1:10:7) together with fresh leaf material from the remaining three varieties, for 24 h. Thereafter the material was treated according to the methods described previously[4–6] (dehydration, infiltration with and embedding in glycol methacrylate, sectioning, staining and data capturing). Unstained slides were viewed under a polarising microscope to observe whether any crystals were present.

Distillation and analysis of essential oil

Leaves and twigs (roughly 300 g dry weight) from three different plants of *P. camphorata* var. *armata* (Table 3) were air-dried for 9 days, then subjected to hydro-distillation for 180 min using a Clevenger-type apparatus. The oils were weighed and stored in sealed vials in the dark at 4 °C before analysis.

The oils (20% diluted in hexane) were analysed by a gas chromatography–mass spectrometry system using standardised settings, as described previously[4]. Compounds were identified by their mass spectral data and retention indices, as well as library searches of the NIST®, Mass Finder® and Flavour® libraries.

Antimicrobial studies

Various extracts (Table 4) and the oil samples listed in Table 3 were investigated for antimicrobial activities, using the minimum inhibitory concentration (MIC) microtitre plate method as previously described[4]. A yeast (*Cryptococcus neoformans*, American type culture collection [ATCC] 90112) as well as two Gram-negative bacteria (*Moraxella catarrhalis* ATCC 23246 and *Klebsiella pneumoniae* ATCC 13883) and one Gram-positive bacterium (*Mycobacterium smegmatis* ATCC 14468) were selected for the study. All cultures were selected on the basis of their respiratory pathogenesis, with the exception of *M. smegmatis*, which is a non-pathogenic *Mycobacterium* strain with a faster growth rate than that of *M. tuberculosis* – hence its ease of use. *Cryptococcus neoformans* is associated with lung infection, and *M. catarrhalis* is known to cause bronchitis, sinusitis and laryngitis. *Klebsiella pneumoniae* commonly causes pneumonia. The preparation of bacterial cultures and the methodology followed, including the preparation of extracts, were exactly the same as described previously.[4–6] MIC assays were undertaken in duplicate or triplicate on separate occasions, and the mean results are presented.

Results and discussion

Ethnobotany

A historically important ethnobotanical paper by Laidler[1], published in this journal in 1928, gave the names of several species of buchu used in Namaqualand. The use of buchu (powdered aromatic bushes, usually stored in tortoise shells) as a topical treatment of the skin is of San origin, and is well described in the literature.[4,8] Buchu, sab or *P/nkaou* (Laidler used *P/* to denote the palatal or palato-alveolar click, ǂ) is said to have two varieties. The first is *D/nhora* (|*nhora*), *D/khonsa* (|*khonsa*) or *haas buchu*, and the other is *P/kabourie* (ǂ*kabourie*). The botanical identities of these two plants have remained unknown, perhaps because Laidler was not a botanist or he saw only the powdered plant material. He described the use of the two buchu types as follows[1]:

> *Considered by Hottentot to possess great virtues in curing disorders, and rarer forms are valuable to the Native. In powder used for dusting, for fire and sunburns Baby powder made of D/nhora kept in tortoise shell puff box, roasted with fat and dropped in ear for earache. Powder also mixed with C/ghoonabi, and acacia thorn sucker, and then considered a high class powder. The second rate powder used for babies was mixed with ground acacia thorn bark, and is named O/kai. D/nhora Buku is roasted with oil and fat, mixed with mother's milk and dropped into the ear for earache. P/nkaou is rare and valuable, and a thimbleful is worth from a sheep to an ox. It is obtained in mountainous districts and when used is pounded and tied in a cloth over which hot water is poured.*

According to Laidler[1], the name *P/nkaou* was given to him for what was known as 'Buchu barosma' or 'Letulina' at the time. This clearly refers to round-leaf buchu, *Agathosma betulina* (P.J. Bergius) Pillans, a species confined to the mountainous region of the Bokkeveld, Cederberg and Grootwinterhoek. It is understandable that this plant would be considered rare and valuable, because it was not available locally but had to be transported over a considerable distance from the Cederberg to Namaqualand; the Kamiesberg is roughly 300 km north of the Cederberg. *P/kabourie* (ǂ*kabourie*) is not mentioned again, and the reader is left with the impression that this name also refers to *P/nkaou*.

The identification of *D/nhora* (|*nhora*) as *P. camphorata* is the result of a few anecdotes spread over more than a decade. The first and most convincing was the explicit information given by Mr Willem 'Blikkies' Steenkamp at Nieuwoudtville, an area where the Namaqualand variety of the species (var. *armata*) also occurs naturally. All available ethnobotanical information on *P. camphorata* is shown in Table 2. Original anecdotes, recorded in Afrikaans, have been retained to ensure that subtle nuances about the plant and its uses are not lost in translation. *P. camphorata* shares with several other species the Afrikaans name *gombossie*.[9–11]

The first reference to *P. camphorata* as a medicinal plant appears on a herbarium specimen (*E.B. Watermeyer 6350* in PRE). According to the label information, the specimen was collected in January 1925 and the plant is known as *koortsbos* (meaning *koorsbos*, i.e. fever bush). Unfortunately, the locality is given imprecisely as 'Little Namaqualand'. The first-ever published ethnobotanical use for *P. camphorata*, namely the treatment of boils, was reported by Watt and Breyer-Brandwijk[12], who cite Kling[13] as the source of their information. Curiously, the species was not included in Rev. Kling's[13] booklet of 1923, so the origin of the information remains a mystery and appears to be based on a misinterpretation – uncritically cited by Arnold et al.[14]

The only other published ethnobotanical anecdote for the species was reported by Van Wyk and Gericke[15], who cited Willem 'Blikkies' Steenkamp, an elderly man of Khoisan descent from the farm Oorlogskloof near Nieuwoudtville in the Northern Cape province of South Africa. The origin of the knowledge is Mr Steenkamp's grandfather, who was ethnically pure San. The plant is regarded as the most important of all the traditional medicines in the area, and is known locally as *norraboegoe*.

Table 2: Summary of vernacular names and ethnobotanical anecdotes for *Pteronia camphorata*

Vernacular names	Anecdotes and their origin
koortsbos: Herbarium specimen label (January 1925, *E.B. Watermeyer 6350* [PRE])	–
–	Watt and Breyer-Brandwijk, 1962[12]: 'A plaster, made from *P. camphorata* L., is used in the Western Cape for drawing boils'. This is an error and cites Kling, 1923[13] as the source.
gombossie: Marloth, 1932[9]; Smith, 1966[10]	–
gombos, ghombossie: Burman and Bean, 1985[11]	–
norraboegoe: Van Wyk and Gericke, 2000[15], citing Willem 'Blikkies' Steenkamp	*'Getrek in kookwater vir verkoue en griep; vir grootmense, gebruik soos dit is (baie bitter); vir kinders, gooi suiker by en kook tot dit stroperig is.'* [Infused in boiling water for colds and influenza; for adults, use as is (very bitter); for children, add sugar and boil until syrupy.]
t/gôrroboegoe: Anna Brand (Nourivier, Kamiesberg)	–
t/ôrro: Elizabeth Kardinaal (Leliefontein, Kamiesberg)	*'Hy is 'n boegoe, gebruik vir kinders met steek in die oor saam met moedersmelk, gooi in die oor en maak toe met 'n pluisie (watte); word gemeng met melk ook vir grootmense met steek in die oor.'* [It is a buchu, used as medicine for earache for infants, mixed with mother's milk as ear drops (cover with cotton wool); mixed with milk and used by adults to treat ear infection.]
koorsbos: Gertruida Brand (Paulshoek, Kamiesberg)	*'Trek hom af vir rumatiek.'* [An infusion used for rheumatism.]
t/ôrroboegoe: Jakobus Brand (Nourivier, Kamiesberg)	*'As poeier gebruik vir kinders se steek in die oor.'* [Used as powder for earache in children.]
t/ôrro: Jan van der Westhuizen (Garies, originally from Paulshoek but also lived in Leliefontein)	*'Die blaar word poeier gemaak en dan tee, dit is bitter, en gebruik vir winde.'* [Powdered leaf used as bitter infusion for flatulence.]
t/ôrro: Pieter Dirkse (Paulshoek, Kamiesberg)	–
wakkerbos: Sors Cloete (Paulshoek, Kamiesberg)	–
t/nouroeboegoe, t/nôrraboegoe, t/nôrro: Anna Stewe (Leliefontein, Kamiesberg)	(1) '*Die plant het smal blaartjies. Gebruik as medisyne, snuif, saam met ander kruie; vir tandpyn (trek die takkie in melk of water); vir steek in die oor (vat die droë blare en maak `n poeier wat op watte in die oor gesit word) – dit het `n pyndodende effek; trek ook daai wind uit.'* [The plant has narrow leaves. Use as medicine, snuff, with other herbs; for toothache (steep a twig in milk or water); for pain in the ear (take the dry leaves and make a powder that is placed on cotton wool in the ear) – it has an analgesic effect; it also 'pulls out that wind' (relieves flatulence).] (2) '*Org word gebruik vir TB. Dit word saam met jantjie-bêrend en t/nouroeboegoe gebruik. Kookwater word op die blare gegooi en laat trek, soos `n tee. Drink drie maal per dag so 'n kelkie vol. Dit is die beste medisyne vir TB. `n Ou vrou het dit gebruik, as die TB pasiënte huis toe gestuur word, maar hulle is nog nie heeltemal gesond nie. Dit het hulle gehelp om aan te sterk'.* [*Org* (*Notobubon pearsonii*) is used as medicine for TB. It is used in combination with *jantjie-bêrend* (*Sutherlandia frutescens*) and *t/nouroeboegoe* (*Pteronia camphorata*). Boiling water is poured on the leaves and allowed to steep, like a tea. Drink a small glassful three times per day. It is the best medicine for TB. An old lady used it when TB patients were sent home before they were fully recovered. It helped them to regain their strength.]
t/nôrro, t/nôrra, t/gôrrôboegoe: Gert Dirkse (Paulshoek, Kamiesberg)	'*Dis `n bos. Die wortel maal dit fyn, vir babatjies, stuipe en vir grootmense. Gebruik vir vuil bloed en siek voel, ook vir grootmens stuipe. Die blare word droog en fyn gebruik. Vir siek voel, meng met baarbos en slangbos. Dit word gekook. Baie goed*'. [It is a bush. The root, grind it to a powder, for babies, convulsions and for adults. Use for impure blood and when feeling ill, also for adult convulsions. The leaves are used dried and powdered. For 'feeling ill', mix with *baarbos* (*Limeum africanum*) and *slangbos* (*Stoebe plumosa*). It is boiled (a decoction). Very good.]
t`ôrrôboegoe* or *t`ôrrôbosboegoe: Sarah Fortuin (Spoegrivier, Namaqualand)	'*t`ôrrôboegoe of t`ôrrôbosboegoe – die t`ôrrô verwys na die nek; dit word gebruik veral vir kinders vir ontsteking om en agter in die nek (as dit so rooi word). Dit is ook `n bors medisyne; hierdie bos is baie goeie medisyne*.' [The *t`ôrrô* refers to the back of the neck; it is used especially for children, to treat inflammation around and behind the neck (when it turns red). It is also a chest medicine; this shrub is very good medicine.]
aambeiebos: Corneels 'Kaldei' Christiaan (Spoegrivier, Namaqualand)	'*Hy groei meer in die nat wêreld en word gebruik vir aambeie*' [It grows in moist places and is used to treat haemorrhoids.]
t`ôrrôbos* or *t`ôrrôboegoe: Esau Flink (Vanrhynsdorp)	'*Trek af vir griep die blare en die stokkies*' [Use the leaves and the twigs as an infusion for the treatment of influenza.]

'–' indicates no information

Table 3: Compounds of six samples of *Pteronia camphorata* as identified by GC–MS (percentage area)

	Locality	**Wupperthal**			**Montagu Pass**		
	Samples	**1**	**2**	**3**	**1**	**2**	**3**
	Yield (% dry weight)	**0.53**	**0.59**	**0.71**	**NA**	**NA**	**NA**
RRI	**Major compounds**						
1000	Decane	0.3	0.5	1.9	–	–	–
1032	α-Pinene	0.3	0.6	0.5	0.5	0.2	0.7
1118	β-Pinene	2.1	3.1	2.0	1.1	0.7	0.9
1132	**Sabinene**	**3.5**	**3.6**	**5.1**	**9.1**	**7.1**	**12.7**
1174	Myrcene	6.0	1.6	2.5	–	–	–
1176	α-Phellandrene	–	–	–	1.6	1.7	5.5
1188	α-Terpinene	0.3	0.2	0.3	–	0.1	0.3
1195	Dihydro1,8-cineole	–	–	–	–	0.1	0.1
1203	**Limonene**	**9.9**	**17.1**	**2.0**	**5.0**	**3.8**	**7.7**
1213	**1,8-Cineole**	**22.3**	**17.2**	**26.5**	**42.7**	**40.4**	**42.6**
1246	(*Z*)-β-Ocimene	4.6	5.5	4.6	1.0	0.8	1.5
1255	γ-Terpinene	0.8	0.4	0.6	1.2	0.4	0.7
1266	(*E*)-β-Ocimene	2.6	2.7	2.3	0.2	0.03	0.1
1280	***p*-Cymene**	**3.5**	**15.7**	**25.6**	**17.1**	**21.1**	**10.0**
1290	Terpinolene	–	–	–	0.3	–	0.1
1400	Tetradecane	0.9	5.4	4.1	–	–	–
1450	*trans*-Linalool oxide (furanoid)	–	–	–	–	–	0.1
1474	*trans*-Sabinene hydrate	–	–	–	0.4	0.8	0.6
1450+	*cis*-Linalool oxide (furanoid)	–	–	–	–	0.1	0.1
1500	Pentadecane	–	5.7	4.2	–	–	–
1512	Dillether	–	–	–	–	0.04	–
1553	Linalool	–	–	–	0.4	2.8	3.1
1571	*trans*-*p*-Menth-2-en-1-ol	–	–	–	0.3	0.1	0.2
1586	Pinocarvone	–	–	–	0.1	–	–
1600	Hexadecane	1.0	5.4	4.2	–	–	–
1611	**Terpinen-4-ol**	**5.3**	**2.3**	**3.6**	**5.8**	**3.1**	**2.4**
1629	*cis*-α-Bisabolene	0.2	0.3	–	–	–	–
1632	*cis*-*p*-Menth-2-en-1-ol	–	–	–	–	–	0.2
1639	*trans*-*p*-Menth-2,8-dien-1-ol	–	–	–	0.4	0.4	–
1648	Myrtenal	–	–	–	0.1	0.2	0.1
1651	Sabinaketone	–	–	–	0.3	0.2	0.2
1661	*trans*-Pinocarveol	–	–	–	0.2	0.2	0.1
1662	Estragol	1.0	0.7	0.4	–	–	–
1671	**Methyl chavicol (estragol)**	–	–	–	**4.5**	**6.8**	**2.9**
1682	α-Terpineol	–	–	–	2.3	4.8	3.9
1700	Limonene-4-ol	–	–	–	0.05	0.4	–
1700	Heptadecane	1.0	4.3	3.4	–	–	–
1751	Carvone	0.2	0.7	–	1.2	1	0.7
1755	Bicyclogermacrene	1.0	0.6	0.4	–	–	–
1765	Geranyl acetone	–	–	–	–	0.1	0.1
1773	δ-Cadinene	–	–	–	0.1	–	0.1
1776	γ-Cadinene	–	–	–	–	0.1	–

Table 3 continued

	Locality	Wupperthal			Montagu Pass		
	Samples	**1**	**2**	**3**	**1**	**2**	**3**
1797	*p*-Methyl-acetophenone	–	–	–	0.5	0.5	–
1800	Octadecane	0.3	1.4	1.2	–	–	–
1802	Cumin aldehyde	–	–	–	0.4	0.4	0.2
1804	Myrtenol	–	–	–	–	0.2	0.1
1814	*p*-Mentha-1,5-dien-7-ol	–	–	–	0.2	–	–
1834	*trans*-Carveol	–	–	–	0.4	0.3	0.2
1854	Germacrene B	–	0.4	–	–	–	–
1864	*p*-Cymen-8-ol	–	0.1	0.4	0.2	0.4	0.2
1882	*cis*-Carveol	–	–	–	0.1	–	0.1
2008	Caryophyllene oxide	–	–	–	0.3	0.3	0.4
2012	1-Allyl-2,4-dimethoxybenzene	–	–	–	0.3	–	–
2030	**Methyl eugenol**	**23.4**	0.5	–	–	0.4	0.2
2202	Germacrene D-4-ol	–	–	–	–	–	0.1
2081	Humulene-epoxide III	–	–	–	–	0.04	–
2113	Cumin alcohol	–	–	–	0.2	0.1	0.1
2144	Spathulenol	–	–	–	0.1	–	0.1
2187	T-Cadinol	0.2	–	–	–	–	0.04
2228	Himachalol	–	–	–	0.1	0.1	0.2
2255	α-Cadinol	–	–	–	0.2	–	–
Total		**90.7**	**96.0**	**95.8**	**99.4**	**99.3**	**99.7**

Note: *Three essential oil samples of P. camphorata from Kleinvlei, Wupperthal (P. camphorata var. armata) are compared with published results for three samples of P. camphorata var. camphorata from Montagu Pass.[16] The three specimens from each population are shown as 1, 2 and 3.*

Table 4: Minimum inhibitory concentrations for extracts and essential oils of three specimens of *Pteronia camphorata* var. *armata*

Extract	**Sample number**	**Minimum inhibitory concentration (mg/ml)**			
		***Moraxella catarrhalis* ATCC 23246**	***Mycobac-terium smegmatis* ATCC 14468**	***Crypto-coccus neoformans* ATCC 90112**	***Klebsiella pneumoniae* ATCC 13883**
H_2O extract	1	> 8	3.0	**0.5**	8.0
H_2O extract	2	> 8	2.0	> 8	8.0
H_2O extract	3	> 8	1.5	> 8	8.0
MeOH:H_2O extract	1	> 8	8.0	**0.2**	> 8
MeOH:H_2O extract	2	> 8	8.0	**0.1**	> 8
MeOH:H_2O extract	3	> 8	1.5	**0.1**	> 8
MeOH:CH_2Cl_2 extract	1	4.0	**0.5**	**0.8**	4.0
MeOH:CH_2Cl_2 extract	2	> 8	**0.3**	**0.8**	4.0
MeOH:CH_2Cl_2 extract	3	> 8	**0.5**	**0.8**	1.5
Essential oil	1	> 16	**1.0**	**0.3**	4.0
Essential oil	2	> 16	**1.0**	**0.3**	4.0
Essential oil	3	> 16	**1.0**	**0.5**	4.0
Positive control (ciprofloxacin/amphotericin B)		0.313 μg	0.313 μg	2.5 μg	0.078 μg

Note: Concentrations were tested on a selection of pathogens associated with respiratory infections, including a yeast (*Cryptococcus neoformans*), two Gram-negative bacteria (*Moraxella catarrhalis* and *Klebsiella pneumoniae*) and one Gram-positive bacterium (*Mycobacterium smegmatis*).

This word appears to be derived from a corruption of the word '*|nhora*' (| denoting the dental click) and *boegoe* (the common Khoisan term for aromatic bushes). It was explained to B.E.V.W. that the term *|nhora* in the San culture refers to the 'life force' in human beings, which is believed to be situated in the nape of the neck. Directly translated the name means 'life force buchu', reflecting the perceived importance of this plant species.

Recent enquiries in various parts of the Western and Northern Cape provinces (including the Cederberg region, where the species is quite common) revealed only a few known contemporary uses. In the Kamiesberg area of Namaqualand in Northern Cape, we located two herbalists with original knowledge about the species and its uses in the Nama culture. The late Anna 'Boom' Stewe was a herbalist from Leliefontein in the Kamiesberg, who obtained her medicinal knowledge from the late Jan 'Bordhoed' Beukes, a local *bossiedokter* – a term of honour for an acknowledged herb doctor. The plant, which Anna Stewe called *|nôrraboegoe*, is used in a mixture with *org* [*Notobubon pearsonii* (Adamson) Magee] and *jantjie-bêrend* [*Sutherlandia frutescens* (L.) R. Br. which is] [=*Lessertia frutescens* (L.) Goldblatt & J.C. Manning] to treat tuberculosis. A small glass (*kelkie*) of a hot water infusion (tea) of the leaves of the three species is taken three times a day, and is considered to be the best medicine for tuberculosis. This medicine was used by an elderly lady, whose name is no longer recalled, for convalescent tuberculosis patients. Powdered leaves mixed with other herbs can also be used medicinally as snuff, and infusions of a leafy twig in hot milk or water are used to alleviate toothache. Powdered leaves are applied to a piece of cotton wool and inserted into the ear for relief of earache (*dit het 'n pyndodende effek* – 'it has a painkilling effect'). The mixture can also be used for flatulence ('to remove wind'). The use against earache and flatulence was confirmed by three other local inhabitants of Kamiesberg, and another person added that an infusion can be used to treat rheumatism.[8]

The second local expert in the Kamiesberg was the late Gert 'Joelk' Dirkse, a herbal doctor from Paulshoek and one of the last professional *bossiedokters* in Namaqualand. He independently supplied information about *P. camphorata*. Powdered root is used to treat febrile convulsions in infants, and powdered leaves are used to treat convulsions and epilepsy in adults as well as for blood purification. Gert Dirkse stated that a decoction of the leaves, mixed with *baarbos* (*Limeum africanum* L.) and *slangbos* [*Stoebe plumosa* (L.) Thunb.] is taken orally and is very effective in treating general malaise (*siek voel*).

We also heard recent anecdotes from the small Namaqualand village of Spoegrivier. An experienced and highly knowledgeable midwife, Ouma Sarah 'Toesie' Fortuin, still remembered *|ôrrôboegoe* or *|ôrrôbosboegoe*. According to Sarah Fortuin, *|ôrrô* refers to the back of the neck, and the plant is used to treat inflammation and redness of the neck in children. It is also a medicine for chest ailments, and is considered highly effective (*hierdie bos is baie goeie medisyne* – 'this shrub is very good medicine'). Corneels 'Kaldei' Christiaan from the same village reported that the plant grows only in moist areas and is used for the treatment of haemorrhoids – hence his vernacular name for the plant, *aambeiebos* (haemorrhoids bush). Further south, near Vanrhynsdorp not far from Nieuwoudtville, Esau Flink reported that he uses a decoction of the leaves and twigs to treat influenza. He referred to it as *t`ôrrôbos.*

Given its obvious medicinal importance, it is surprising that the uses of *P. camphorata* have remained almost unknown to science until recently. We were fortunate to have the opportunity to record, and preserve for posterity, the rich knowledge of two very knowledgeable and experienced herbalists, Anna Stewe and Gert Dirkse. Sadly, neither of them had any apprentices to whom they could pass on their knowledge.

Anatomy

The leaves of *P. camphorata* are amphistomatic. They have a thin cuticle and the outer periclinal cell walls of the epidermal cells are markedly thickened. The mesophyll is composed of usually two layers of palisade parenchyma, more highly developed adaxially than abaxially, which surround the central spongy parenchyma (Figure 2). Secretory structures that can be referred to as oil glands or cavities occur in the spongy parenchyma adjacent to the phloem of some vascular bundles. Macroscopically they are visible as translucent dots (Figure 1). Secretory trichomes are situated in the medial adaxial groove, but can sometimes also be found in the corresponding position on the abaxial surface.

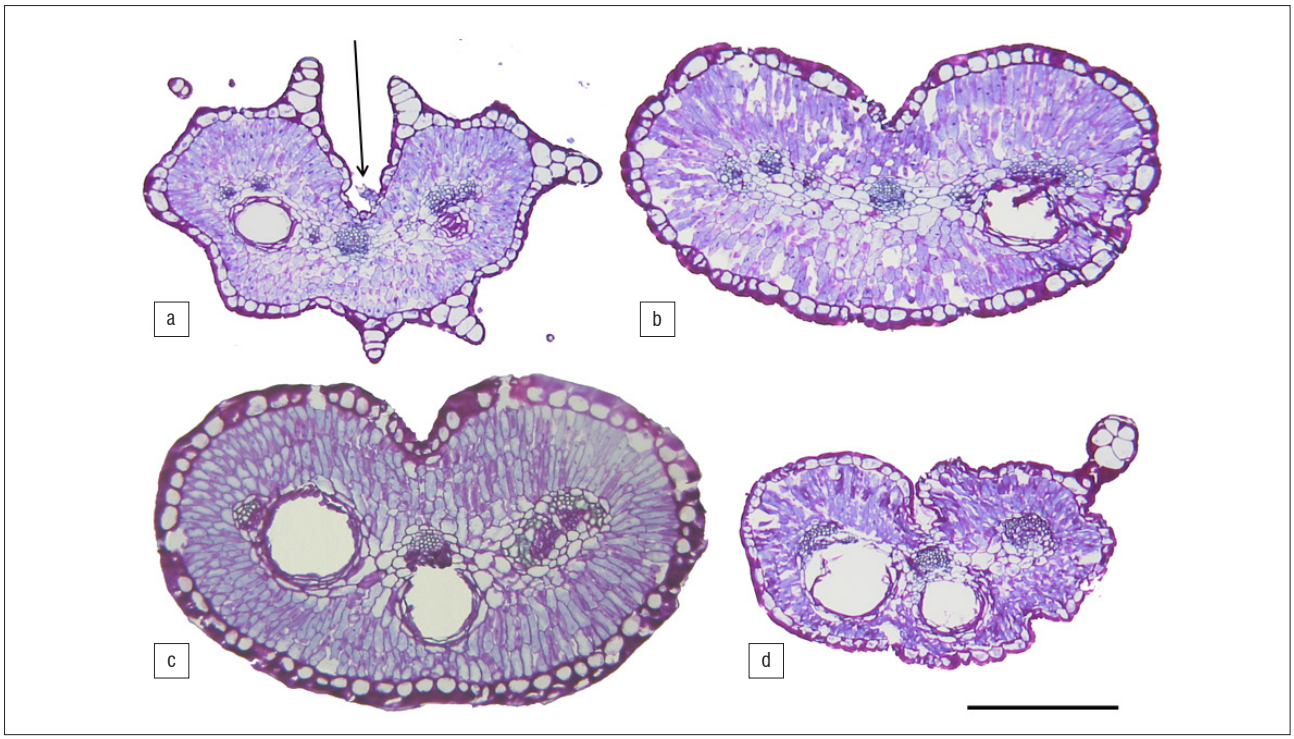

Figure 2: Transverse sections of leaves from four varieties of *Pteronia camphorata*. (a) *P. camphorata* var. *armata*, (b) *P. camphorata* var. *camphorata*, (c) *P. camphorata* var. *laevigata*, and (d) *P. camphorata* var. *longifolia*. Oil glands are present in all, and secretory trichomes in (a) and (d).

Key: The arrow indicates a secretory trichome; scale bar = 0.4 mm.

It appears that three types of secretory structures are present in the genus *Pteronia*, and the ethnobotanically relevant species can be classified into four groups based on their combinations. The structures are as follows: (1) globose oil glands or cavities, similar to those in *Citrus,* which are present in *P. onobromoides* D.C.*, P. stricta* Aiton and *P. succulent* Thunb.; (2) oil ducts associated with the vascular bundles, mainly the midrib, which are present in *P. adenocarpa* Harv.; (3) oil ducts associated with the vascular bundles as well as secretory trichomes, which occur in *P. cinerea* L.f., *P. divaricata* Less., *P. incana* D.C. and *P. lucillioides* D.C.; and (4) globose oil glands and secretory trichomes mainly in the medial grooves – this type is present in *P. camphorata*. The leaf anatomy of *Pteronia* species appears to be of considerable value in providing diagnostic characters, and has the potential to contribute to a better understanding of the infrageneric taxonomy and relationships of the genus.

Essential oil composition

We identified 21 to 25 volatile components in the three samples of *P. camphorata* var. *armata* that we analysed from Wupperthal (Table 3). A similar combination of main compounds was found in *P. camphorata* var. *camphorata* from Montagu Pass in the southern Cape, as studied by Coovadia[16] and reported by Viljoen et al.[17] The major compounds in one or both of the varieties are several monoterpenes as well as sesquiterpenes (Table 3). The two varieties agree in the presence of sabinene, limonene, 1,8-cineole, *p*-cymene and terpinene-4-ol as main compounds. In addition, (*Z*)-β-ocimene is a major constituent in Wupperthal samples but a minor constituent in Montagu Pass samples. Estragol occurs at relatively high levels at Montagu but only as minor compounds at Wupperthal. Methyl eugenol was a major compound in one of the Wupperthal samples. Both varieties have smaller amounts of α-pinene, β-pinene, γ-terpinene and (*E*)-β-ocimene.

A number of non-volatile diterpenes and other phenolic compounds have been isolated from *P. camphorata* and other *Pteronia* species.[18] These include 3,4-dimethoxypropiophenone, eugenol methyl ether and several other more widespread compounds. The biological, chemotaxonomic and medicinal significance of these compounds are as yet unknown.

We compared the main essential oil compounds in *P. camphorata* with those of four other ethnobotanically relevant species for which data are available.[16] The results showed the following general patterns: (1) sabinene, limonene, 1,8-cineole, *p*-cymene and myrcene are main constituents in most of the species, and the same pattern is evident for *P. camphorata*; (2) β-pinene is a major compound in *P. adenocarpa* and *P. incana* and is present as a minor compound in all other species; and (3) sesquiterpenoids appear to be more restricted in their distribution. There might well be a link between the essential oil composition and antimicrobial activity. A previous study showed that cineole and limonene may interact synergistically to exert enhanced antimicrobial activity.[19]

Antimicrobial activity

The results of our antimicrobial study on the extracts of *P. camphorata* are shown in Table 4, with noteworthy activity highlighted in bold text. Noteworthy activities for extracts were considered where MIC values were below 1.0 mg/ml[20–21] and for essential oil samples where MIC values were below 2.0 mg/ml[22]. The methanol-to-dichloromethane (MeOH : CH_2Cl_2) extracts were the most active against *Mycobacterium smegmatis*, with MIC values as low as 0.3 mg/ml to 0.5 mg/ml. The methanol-to-water (MeOH : H_2O) extracts were the most active against *C. neoformans*, with MIC values as low as 0.1 mg/ml to 0.2 mg/ml. The essential oils also showed strongest antimicrobial activity against *C. neoformans,* with the most noteworthy having an MIC value of 0.3 mg/ml.

Water and methanol-to-water extracts generally showed poor to no activity at the highest concentration tested, against all organisms studied except for *C. neoformans*. In the latter case, the mean MIC value was 0.5 (H_2O extract), with MIC values ranging from 1.5 mg/ml to 3.0 mg/ml (H_2O extracts) for the various *Mycobacterium* species tested.

Conclusion

P. camphorata is poorly known as a medicinal plant, but available information and recently recorded ethnobotanical data suggest it was once an important Khoisan remedy. Almost no local knowledge or local users could be found in most of the rural localities where the plants grow. The traditional uses include treatment of respiratory conditions (colds, fever, influenza, chest ailments and tuberculosis) and inflammation of the neck, convulsions and haemorrhoids. These uses suggest the plant has antimicrobial, anti-inflammatory and antipyretic properties.

The globose oil glands found in *P. camphorata* are similar to those of *P. onobromoides*, *P. stricta* and *P. succulenta* but not those of any other species. *P. camphorata* differs from the others by the presence of additional glandular trichomes in the medial grooves. The leaf anatomy appears to have some diagnostic value at the species level, and holds considerable potential as a source of taxonomic characters.

P. camphorata essential oil has a similar combination of main compounds as that found in other *Pteronia* species: sabinene, limonene, 1,8-cineole, *p*-cymene and terpinene-4-ol are the main constituents. Smaller amounts of α-pinene, β-pinene, (*Z*)-β-ocimene, γ-terpinene, (*E*)-β-ocimene and myrcene also occur in all or most of the samples of this species. The plant has small amounts of sesquiterpenes, such as bicyclogermacrene – which is a main compound in *P. divaricata* and a minor compound in *P. incana*.

Most of the extracts, as well as the essential oil of the plant, display activity against *C. neoformans*. The dichloromethane extracts and essential oil samples were active against *M. smegmatis*. The level of activity against these respiratory pathogens seems to support the reported efficacy of the traditional treatment against tuberculosis, colds and influenza.

Authors' contributions

I.M.H. prepared the samples and performed the experiments; P.M.T. provided anatomical know-how and infrastructure, and prepared the anatomical descriptions; S.F.V.V. was responsible for the experimental design of antimicrobial tests, and provided the microbial samples and infrastructure; G.P.P.K. performed the GC–MS analyses and calculations; J.M.N. contributed original ethnobotanical data; A.M.V. made conceptual contributions, guided the essential oil analyses and provided the analytical infrastructure; and B.E.V.W. was the project leader, conceptualised the study, wrote the first draft and contributed taxonomic and ethnobotanical data.

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