Comparative analysis of four medicinal floras: phylogenetic methods to identify cross-cultural patterns

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RESEARCH ARTICLE

Comparative analysis of four medicinal floras: Phylogenetic methods to identify cross-cultural patterns

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Societal Impact Statement

Plants are living repositories of pharmacologically active chemicals and help to meet society's health care needs directly, or by providing natural products for drug development. We describe phylogenetic approaches to compare medicinal floras from different cultures in distinct regions of the world, and consider how these findings can improve knowledge of how plants have been selected for medical purposes. Greater insight into how people have selected plants for medicinal use will benefit healthcare and drug discovery strategies, and ultimately contribute to the future health and well-being of society.

Summary

- Four medicinal floras were compared using phylogenetic methods, to test whether there are shared patterns in medical plant use at the level of the whole medicinal floras, or for specific therapeutic applications.
- Checklists of the native plants and medicinal plants of Oman were compiled, and analyzed alongside existing checklists for Nepal, the Cape of South Africa and New Zealand. We reconstructed a plant phylogeny at generic level for Oman, and a new, more inclusive phylogeny to represent the genera found in all four local floras. Methods from community phylogenetics were used to identify clustering and overdispersion of the plants used. The impacts of using local or more inclusive phylogenies and different null model selections were explored.
- We found that Omani medicinal plant use emphasizes the same deep lineages of flowering plants as the other three medicinal floras, most strongly when comparing Omani and Nepalese medicinal plants. Drivers of this similarity might be floristic composition, opportunity for exchange of knowledge and shared beliefs in the causation of illness. Phylogenetic patterns among therapeutic applications are cross-predictive within and between cultures, and must be interpreted with care since inappropriate use of null models can result in spurious similarity. High levels of cross-predictivity suggest that targeting plants used for specific therapeutic applications to identify specific bioactives may have limited value.

- We outline the questions that might be addressed using a global phylogeny and medicinal plant checklists, suggest the best methods for future studies and propose how findings might be interpreted.

KEYWORDS
cross-cultural, ethnobotany, medicinal flora, methods, phylogeny, therapeutic application

1 | INTRODUCTION

The plants used for medicine, referred to as the medicinal flora of a culture (Ellen & Puri, 2016; Moerman, Pemberton, Kiefer, & Berlin, 1999), or sometimes as its plant ethnopharmacopoeia (Cox, Sperry, Touminen, & Bohlin, 1989; Saslis-Lagoudakis et al., 2012), are documented in the scientific literature by ethnobotanists. Ethnobotanical research has entered a phase of hypothesis testing using this body of published data (Albuquerque & Muniz de Medeiros, 2012; Gaoue et al., 2017). Whether there is a "global pattern of human knowledge" (Moerman et al., 1999) is one question posed by ethnobotanists and ethnopharmacologists, and relevant to healthcare practice and policy, and to bioprospecting (Albuquerque & Muniz de Medeiros, 2012; Saslis-Lagoudakis et al., 2012; Waldstein & Adams, 2006). Although there has been no global study to date, it is known that selection of plants for medicine is not random: some plant families are preferred (Leonti et al., 2013; Moerman, 1979, 1991). People of different ethnolinguistic cultural groups (cultures hereafter) may prefer the same families (Moerman, 1979, 1991; Saslis-Lagoudakis, Williamson, Savolainen, & Hawkins, 2011; Weckerle, Cabras, Castellanos, & Leonti, 2011), but not always (Ford & Gaoue, 2017). Shared patterns might be attributed to common selection criteria (Leonti, Ramirez, Sticher, & Heinrich, 2003), independent discovery of efficacy (Saslis-Lagoudakis et al., 2012) or transmission of knowledge (Hawkins & Teixidor-Toneu, 2017; Teixidor-Toneu, Jordan, & Hawkins, 2018). Differences could reflect adaptations to different floristic environments, or different healthcare practices, health needs or belief systems. The explanations of common patterns, or of deviations from them, are relevant to healthcare and drug discovery (Teixidor-Toneu et al., 2018).

Phylogenetic approaches offer insights distinct from the widely used taxon-based cross-cultural investigations. In one application, phylogenetic approaches are able to identify and quantify shared preferences for plants overall (Saslis-Lagoudakis et al., 2014; Teixidor-Toneu et al., 2018; Thompson et al., unpublished). In cross-cultural studies such as these, metrics describe the relatedness of pairs of medicinal floras, rather than the component lineages, families or genera; the relatedness of the medicinal floras can be used as the comparative basis for identifying drivers of overall similarity in medicinal floras. For example, when relatedness of floristic environment is significantly correlated with relatedness of medicinal floras, this suggests adaptation to the floristic environment is driving medicinal plant selection (Saslis-Lagoudakis et al., 2014). However, other studies have shown a significant effect of cultural ancestry (Thompson et al., unpublished).

Although phylogenetic methods are a powerful tool to explore global patterns in medicinal plant use, the scope of this approach is still poorly understood. For instance, there are no accounts yet that explore the outcomes of adding further local floras and medicinal floras to an existing phylogenetic analysis of three floras (Saslis-Lagoudakis et al., 2012). This study was among the first to use community phylogenetic tools to explore patterns of medicinally used plants. Saslis-Lagoudakis et al. (2012) revealed clustering of medicinal floras within the floras they were drawn from. They also revealed shared phylogenetic patterns across the floras: medicinal floras were more closely related than would be expected if there were not shared preferences. The finding was taken to indicate independent discovery of plant efficacy, an interpretation supported by significant over-representation of proven bioactive species in shared lineages. In the present study, we compare the medicinal flora of Oman to those of three medicinal floras contrasted in the previous study by Saslis-Lagoudakis et al. (2012), the medicinal floras of New Zealand, South Africa and Nepal. These four represent different ecoregions. Oman, located in the Southeast of the Arabian Peninsula at the meeting point between Africa and Asia, has over 1,200 vascular plant species over half of which are annuals, flowering irregularly from year to year according to the timing and amount of rain (Ghazanfar, 2003). Mountainous areas are especially diverse floristically and include endemic species (around 5% of the species in Dhofar, southern mountains; Ghazanfar, 2003). New Zealand comprises an archipelago of three main islands in the southern Pacific Ocean, and has a flora of approximately 1900 species, 45% of which are endemic (Wilton & Breitwieser, 2010). The Cape of South Africa, located at the south and south-eastern tip of Africa has over 9,000 species of which 70% are endemic (Goldblatt & Manning, 2002). The flora of Nepal, a country spanning from lowland plains (Terai) to the highest Himalayan peaks, is estimated to have between 6,000 and 6,600 species of flowering plants (Press, Shrestha, Sutton, & Carneiro, 2000), of which around 4% are endemic (Shrestha & Joshi, 1996).

The medical tradition of Arabic regions is a pluralistic system that includes Prophetic medicine, concerned with spirit aetiologies, and Galenic humoral medicinal, concerned with environmental factors (Greenwood, 1981). The Galenic humoral system or "*Unani tibb*" underlies traditional medicine in northern and central Oman (Ghazanfar & Al-Sabahi, 1993) and is also part of the pluralistic medical system in southern Oman (Miller & Morris, 1988). Knowledge about herbal medicines in Oman is not traditionally written down but is passed orally from one generation to the next (Ghazanfar & Al-Sabahi, 1993). Until

recently, traditional medicine was the only available healthcare strategy in Oman (Ghazanfar, 1994; Ghazanfar & Al-Sabahi, 1993), but treatments using traditional medicines have become less popular in the past two decades because of the establishment of hospitals. However, minor ailments such as headaches, colds, fever, and stomach upsets, are still treated using medicinal plants (Ghazanfar & Al-Sabahi, 1993). Nepal is a mosaic of cultures with over 75 ethnolinguistic groups (mainly of Tibetan-Burman or Indio-European origin), resulting in diverse healthcare strategies (Gaenszle, Turin, Tuladhar-Douglas, & Chhetri, 2015). As in Oman, in Nepal scholarly, written medical systems are used alongside oral folk medicine. Unani medicine is one of these scholarly medicines, together with the more popular Ayurveda and Tibetan medicine (Gewali, 2008). Moreover, in Nepal shamanistic medicine is practiced when a person's illness is believed to be caused by a spirit possession (Gewali, 2008). Formal, written medical systems were not traditionally used in New Zealand or the Cape South Africa. The native people of New Zealand, the Māori, are of Polynesian origin and had developed an independent ethnopharmacopoeia due to their isolation between settlement (around 1,300 S.D; Wilmshurst, Anderson, Higham, & Worthy, 2008) and European colonization (18th century). Since contact with Europeans it is likely that their medical system was influenced by newcomers, but the precolonial medicinal flora is well documented. The Cape of South Africa is populated by various ethnic groups, but according to Kale (1995), their traditional medicine is essentially similar and based on supernatural belief. Different traditional healers play a key role: "isangomas" are mostly female spiritual healers that also address social causes of illness and "inyangas" are mostly male and use herbs and medicines to treat people (South African History Online, 2018).

The primary objective of this study is to determine whether the medicinal plants of Oman, (a) overall and (b) for specific therapeutic applications, are more closely related to those of New Zealand, the Cape of South Africa and Nepal than expected by chance. The floras of New Zealand, the Cape of South Africa and Nepal were selected for the original study to represent cultures with negligible pre-colonial contact (Saslis-Lagoudakis et al., 2012). By introducing Oman, patterns of putatively shared knowledge across disparate floras but culturally connected people are characterized for the first time. A further objective is to consider the questions that might be addressed by a global phylogenetic survey, and to outline methods appropriate to address these questions.

2 | MATERIALS AND METHODS

2.1 | Data collection

A checklist list of the plant species of Oman was compiled from the four volumes of the Flora of Oman (Ghazanfar, 2003, 2015, 2018; Ghazanfar & Patzelt, 2007; Kearse et al., 2012). As in other cross-cultural studies (i.e., Moerman et al., 1999), our checklist only includes angiosperms because gymnosperm data were not readily available. A checklist of the native medicinal species of Oman was compiled from the medicinal flora of northern Oman (Ghazanfar & Al-Sabahi, 1993) and the medicinal flora of southern Oman (Miller & Morris, 1988). The applications of medicinal plants were classified into 12 therapeutic applications (gastro-intestinal, general, gynaecology/fertility, dentistry/mouth, musculoskeletal, neurology, ophthalmology, otorhinolaryngology, other, respiratory/pulmonary, skin, and urinary) following Saslis-Lagoudakis et al. (2012) and others cited therein. The cardiovascular/blood purity therapeutic application included in the study of Saslis-Lagoudakis et al., (2012) was not represented among the applications of the native Omani medicinal flora and was not included further.

Floristic checklists and medicinal floras for Nepal, New Zealand, and the Cape of South Africa were sourced from Saslis-Lagoudakis et al., (2012). The number of genera shared between Oman and each of the other three floras, and between the Omani medicinal floras and each of the other three medicinal floras were recorded.

2.2 | DNA sequences and phylogenetic reconstruction

A phylogeny for the Omani flora was reconstructed at the genus level, following Saslis-Lagoudakis et al., (2012). A list of the 452 genera present in Oman was prepared and a single exemplar *rbcL* sequence downloaded from Genbank for each genus using the Geneious software (Kearse et al., 2012). The plastid DNA marker *rbcL* was used because of the availability of data for this marker, its successful amplification among plant lineages, and its ability to resolve phylogenetic relationships in large-scale studies (Chase et al., 1993; Forest et al., 2007; Savolainen et al., 2000) . Where possible, we selected a species present in the Omani flora, but in cases where a DNA sequence for Omani species was not available, a Saudi Arabian or other species was selected; 361 exemplar sequences were downloaded from Genbank, therefore we compiled genetic data for ~80% of the genera present in the flora. When the Genbank names did not correspond to those in the flora of Oman, a recognized synonym from the Plant List (The Plant List, 2013) was used. Outgroup sampling comprised the following 16 species (as in Saslis-Lagoudakis et al., 2012): *Abies homolepis*, *Araucaria bidwillii*, *Cedrus deodara*, *Cryptomeria japonica*, *Cupressus sempervirens*, *Cycas circinalis*, *Ephedra gerardiana*, *Ginkgo biloba*, *Gnetum montanum*, *Juniperus communis*, *Larix occidentalis*, *Picea smithiana*, *Pinus wallichiana*, *Podocarpus neriifolius*, *Taxus wallichiana* and *Tsuga dumosa*. Alignment of the *rbcL* sequences was performed in BioEdit v. 7.0 using CLUSTAL W (Hall, 1999) and adjustments were made manually. A phylogenetic tree of relationships of Oman flora was reconstructed under maximum likelihood criterion using RAxML (Stamatakis, Hoover, & Rougemont, 2008).

Data for the reconstruction of a combined phylogeny including the floras of the Cape of South Africa, Nepal and New Zealand were those used by Saslis-Lagoudakis et al. (2012). The sequence alignments were combined with the Omani sequences to construct a phylogenetic tree representing genus-level plant relationships among all four floras. For this analysis, when a genus was present in more than one flora it was included only once in the

analysis. The alignment of the combined matrix was performed in MAFFT v.7 (Katoh, 2013) with manual adjustments perfomed in BioEdit v. 7.0 (Hall, 1999). Sequence data were analyzed under the maximum-likelihood (ML) criterion implemented in RAxML (Stamatakis et al., 2008). Rate smoothing was implemented in the ape package (Paradis, Claude, & Strimmer, 2004), using the chronoMPL function. This model-free method applies the mean path length for each node to all descendants for dating (Britton, Oxelman, Vinnersten, & Bremer, 2002). Where this method introduces very short negative branches, they were converted to zero-length branches.

2.3 | Data analysis

PHYLOCOM version 4.2 (Webb, Ackerly, & Kembel, 2008) was used for phylogenetic interpretation. To run an analysis, a phylogeny written in Newick format and a sample file are needed. For ecological applications, the sample files typically describe presence or absence of taxa in different communities. In our work, the sample files can describe different categories, such as presence or absence of a genus in a local flora, presence or absence of the medicinal use of a genus, or presence or absence of use of genera for specified therapeutic applications.

Three functions in PHYLOCOM, comstruct, comdist, and comdistnt, were employed to calculate two phylogenetic metrics, mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD). Comstruct is used to test whether a category specified by a sample file has phylogenetic structure. Comdist and Comdistnt are used to test for phylogenetic relatedness between categories specified by a sample file, either at deep (Comdist) or shallow (Comdistnt) levels. In general, MNTD is influenced by patterns near the tips, whilst MPD is informative of phylogenetic structure deeper in the phylogeny. Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) are the standardized effect size (z-scores) of MPD and MNTD respectively according to the equations described in PHYLOCOM user's manual 4.2:

$$\beta\mathrm{NRI}_{i,j} = -1 \times \frac{\mathrm{MPD}_{\mathrm{observed}} - \mathrm{MPD}_{\mathrm{random}}}{sd(\mathrm{MPD}_{\mathrm{random}})},$$

$$\beta\mathrm{NTI}_{i,j} = -1 \times \frac{\mathrm{MNTD}_{\mathrm{observed}} - \mathrm{MNTD}_{\mathrm{random}}}{sd(\mathrm{MNTD}_{\mathrm{random}})}.$$

In these equations, $\mathrm{MPD}_{\mathrm{random}}$ and $\mathrm{MNTD}_{\mathrm{random}}$ represent values calculated for null communities, where null communities are random samples. Sampling to generate the null communities can be implemented in different ways, according to different null models. There are two null models that we use here (Table 1). For example, under null 0 which samples any plant in the phylogeny to make a null community, and where the phylogeny only comprises genera in the local flora, comstruct analysis can be used to test whether medicinal genera (a category in the sample file) are a phylogenetically structured subset of plants in the local flora. If the phylogeny includes genera that are not in the local flora, the same question can be asked if the sample file specifies plant genera in the local community as a category. This is because, under null model 1 the null community is sampled from genera that appear in any category in the sample file (Figure 1).

In these analyses, NRI and NTI values are outputs along with their rankLow and rankHigh values. The rankLow/High values describe the number of actual comparisons for which the observed distance in the sample is shorter/longer than the null community. From rankLow/High values, two-tailed p-values are calculated. Positive NRI/NTI values represent phylogenetic clustering, negative values indicate phylogenetic over-dispersion. NTI or NRI values > 1.96 or <−1.96 are considered significant at an alpha threshold of $p < .05$ (in a two-tailed p test, corresponding to $p < .025$ and $p > .975$ respectively).

Here, we carry out six tests; their purpose and the metric and function, phylogeny and null model are described in full in Table 1. Tests 1 and 2 have equivalent aims. In both cases the analysis sets out to determine whether the medicinal flora (in this case the medicinal genera of Oman) is clustered relative to the local flora (in this case the generic-level flora of Oman). In the first test the local flora is delimited by the phylogeny. In other words, the sequences used to reconstruct the phylogeny represent only the genera from the local flora. Therefore, a null model that draws from the whole phylogeny to make a null community is used (equivalent to null model 0 in Phylocom; Webb et al., 2008). In the second test, the phylogeny included genera that are not in the local flora. Therefore, the sample file must be used to delimit the local flora and the medicinal flora, and the null community is drawn from the local flora by specifying a null model that will draw the null community from the sample file. This is Phylocom's null model 1, specifically "for each sample, species are drawn without replacement from the list of all species actually occurring in at least one sample" (page 20, Webb, Ackerly, & Kembel, 2011). Figure 1 explains the relationship between null models and sample files for local and combined phylogenies. Comparison of tests 1 and 2 can be made to determine the influence of a phylogeny built to represent a local flora, and one that is more inclusive.

Tests 3 to 6 depend on measures of inter-sample phylogenetic distances, where the samples are the whole medicinal floras, or the subset of the medicinal flora with specific therapeutic applications (Table 1). For tests 3 to 6, all four local floras are represented in the phylogeny. The aim of test 3 is to determine whether the whole medicinal flora of Oman is drawn from the same deep lineages as the medicinal floras of other areas. Tests 4, 5, and 6 are different methods of investigating the relatedness of the plants used for specific therapeutic applications. In test 4, using null 0, all tips in the phylogeny could be included in the null. In test 5, we use null 1 to sample from the medicinal plants for any therapeutic application and across all medicinal floras. If medicinal plants are themselves clustered, test 4 could be influenced by the overall pattern, whereas test 5 asks whether from among the medicinal plants only there is evidence of relatedness.

TABLE 1 Summary of tests

Test	Phylogeny	Metric; Function	Null Model	Supplementary data number: Sample(s)
Test 1 QUESTION: Is the Omani medicinal flora a phylogenetically clustered subset of the Omani flora?				
1	Oman	Mean pairwise phylogenetic distance (MPD); comstruct	0	S3: Sample = all Omani medicinal plants
Test 2 QUESTION: Is the Omani medicinal flora a phylogenetically clustered subset of the Omani flora?				
2	Combined	Mean pairwise phylogenetic distance (MPD); comstruct	1	S4: Sample 1 = all Omani plants; sample 2 = all Omani medicinal plants
Test 3 QUESTION: Is the medicinal flora of Oman as a whole is drawn from the same deep lineages as the medicinal floras of Nepal, The Cape of South Africa and New Zealand?				
3	Combined	Mean pairwise phylogenetic distance (MPD); comdist	0	S5: Four samples, representing each of the four local medicinal floras
Test 4 QUESTION: Are the plants used for specific therapeutic applications drawn from the same deep lineages, where comparisons include same therapeutic application between floras and different therapeutic applications within and between floras				
4	Combined	Mean pairwise phylogenetic distance (MPD); comdist	0	S6: Thirteen therapeutic applications from four local medicinal floras[a]
Test 5 QUESTION: Are the plants used for specific therapeutic applications drawn from the same deep lineages, where comparisons include same therapeutic application between floras and different therapeutic applications within and between floras				
5	Combined	Mean pairwise phylogenetic distance (MPD); comdist	1	S6: Thirteen therapeutic applications from four local medicinal floras[a]
Test 6 QUESTION: Are medicinal genera for specific therapeutic applications nearest phylogenetic neighbours, either from the same therapeutic application between floras or different therapeutic applications within and between floras				
6	Combined	Mean nearest taxon distance (MNTD); comdistnt	1	S6: Thirteen therapeutic applications from four local medicinal floras[a]

Note: We carry out six analyses, for different combinations of phylogeny, function, null, and sample. For phylogeny, Oman refers to a generic level phylogeny of the native angiosperms of Oman, reconstructed using *rbc*L, and Combined includes generic level sampling to represent four local Angiosperm floras, Oman, Nepal, the Cape of South Africa and New Zealand. The functions and null models are as described in the Phylocom manual (Webb et al., 2011); samples describe the taxonomic composition of "clumps" sensu Webb et al. (2011).

[a]Although there are 52 samples possible, only 51 samples were included, because there were no cardiovascular disorder applications for Oman. Comparison of Tests 1 and 2 reveals the effect of a phylogeny sampling a local flora and being more inclusive. Comparison of Tests 4 and 5 reveals the effect of null models selection with the same phylogeny, function, and sample.

Test 6 considers shallow, tip-level relatedness between therapeutic applications; for every combination of place and therapeutic application, it is tested whether the closest relatives of medicinal genera are used significantly more often than in a null sampled from all medicinal genera.

3 | RESULTS

The Omani phylogeny (Dataset S1) and combined phylogeny for four floras (Dataset S2) are provided as Supporting Information. The combined phylogeny reconstructed comprised sequences representing 2,982 genera in total, 361 to represent Oman (~80% of the flora), and for the three original floras Nepal—1,335(>85%); South Africa Cape—792 (~80%); New Zealand—494 (>88%). The four Phylocom sample files (Dataset S3 to S6), phylocom instructions and commands (Dataset S7) and output values from phylocom (Dataset S8) are also provided as Supporting Information.

The two literature sources indicated 106 species in 76 genera with medicinal use, representing documented use of 17% of the 452 genera of Oman. Only 12 of the 13 therapeutic applications were represented in the Omani data: there were no reports of use for

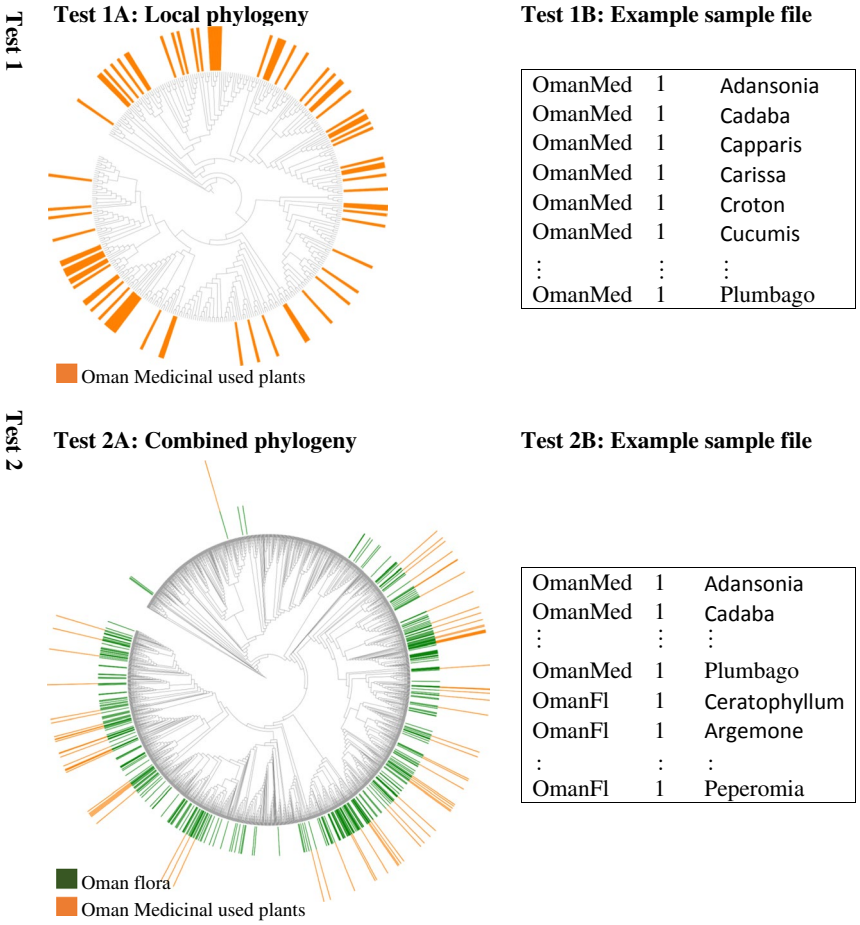

FIGURE 1 The relationship between null models and sample files for local and combined phylogenies. Test 1: Using null model 0 to test the structure of Omani medicinal genera in Omani flora, the Oman flora was all included in the local phylogeny. Test 1A: Local phylogeny represent the Oman flora; each orange colour bar indicates the position of medicinal used genus. Test 1B: A part of the sample file used for test 1. The first column is the sample, the second is the abundance (in this study it was all set to 1), the third is the species code which should be identical with the tip label in the phylogeny. Test 2: Using null model 1 to test the structure of Omani medicinal genera in the combined phylogeny. Test 2A: combined phylogeny including Oman flora; each orange colour bar indicates the position of medicinal used genus. Green bars indicate genera in the Omani flora. Test 2B: A part of the sample file used for test 2. The first column is the sample, the second is the abundance (in this study it was all set to 1), the third is the species code which should be identical with the tip label in the phylogeny. Tree figures prepared using iTOL (https://itol.embl.de/itol.cgi)

cardiovascular disorders. Classification of therapeutic applications revealed the largest number of genera was used for skin problems (29 genera) and gastro-intestinal disorders (27 genera). The fewest genera were used for urinary and dentistry/mouth-related disorders, with only one taxon used for each of these therapeutic applications. In total, there were 114 combinations of use and therapeutic application for the 76 medicinal genera of Oman.

Tests 1 and 2 address the same question, whether the Omani medicinal flora is a phylogenetically clustered subset of the Omani flora. They differ in the phylogeny used, so comparison of the two tests shows the implications of using a local (Test 1) or more inclusive phylogeny and appropriate null (Test 2). Our community phylogenetic analysis performed using the Oman phylogeny (Test 1) did not reveal significant clustering or overdispersal of medicinal plants [NRI = 1.6; MPD.rankLow = 9,467; runs = 9,999, *P* value > 0.025 and < 0.975 where significant values are $p < .025$ (overdispersal) and $p > .975$ (clustering)]. However, our community phylogenetic analysis performed using the combined phylogeny (Test 2) revealed phylogenetically structured medicinal plants [NRI = 2.5; MPD.rankLow = 9,965, runs = 9,999; *p* value = >.975].

A taxonomic comparison between Oman and the other three floras showed the Omani flora and medicinal plants to be most similar to those of Nepal (Table 2). Phylogenetically (Test 3), the Omani medicinal flora is clustered with the other three medicinal floras (NRI values positive and > 1.96; Table 2).

The comparisons between medicinal floras at the generic level for the 13 therapeutic applications (Tests 4, 5 and 6) are presented in Figure 2 (a-c respectively). Test 4 shows that under null 0, use for a therapeutic application in one flora predicts often use for the same therapeutic application in other floras. However, similarly high levels of cross-predictivity between therapeutic applications at deep phylogenetic levels are also revealed (Figure 2a). Using null model 1 rather than null model zero, so that the null is sampled from the medicinal plants rather than the whole phylogeny (the flora), the

TABLE 2 Taxonomic and phylogenetic comparison between Omani, Nepali, South African Cape, and New Zealand floras and medicinal floras

Comparison	Flora Number of genera shared	Medicinal plants Number of genera shared	Medicinal plants NRI values
Oman/Nepal	202	39 (19%)	5.6
Oman/South Africa Cape	125	18 (14%)	3.7
Oman/New Zealand	56	2 (3.5%)	3.3

Note: For taxonomic comparison, the number of genera shared between Oman and the other three floras and number of medicinal genera shared is reported. For phylogenetic comparison (Test 3), we report NRI values. NRI values that are positive and > 1.96 are indicative of significant phylogenetic clustering. The total numbers of genera in each of the three floras is as follows: Oman—361; Nepal—1,335; South Africa Cape—792; New Zealand—494.

frequency of predictivity is strongly reduced (Test 5; Figure 2b). The highest frequency of significant clustering is between therapeutic applications in Oman, and within and between therapeutic applications in the comparisons between Oman and Nepal. At tip-levels in the phylogeny comparisons between local medicinal floras reveal a high frequency of overdispersal, but within local floras there is a high frequency of clustering between therapeutic applications (Test 6; Figure 2c).

4 | DISCUSSION

Phylodiversity metrics, first used in conservation biology then in macroecology and community ecology, have become a critical component of modern ecology (Tucker et al., 2016). Increasingly complete phylogenetic hypotheses and better understanding of the power and reach of the approaches have enabled a diversity of applications. The development of the field has led to a proliferation in phylodiversity metrics, with at least 70 metrics available (Tucker et al., 2016). Borrowing the appropriate metric from ecology to address questions in a different field demands good understanding of the metrics available. Since one of our goals here is to make phylogenetic study of medicinal floras more accessible to researchers with backgrounds in ethnobotany, we begin here by introducing the metrics we have chosen to test our hypotheses.

The metrics we use here are divergence metrics sensu Tucker et al. (2016). Divergence metrics are used to measure the overall evolutionarily dissimilarity of samples (in ecological applications referred to as groups, assemblages or communities, though we use "sample" here to indicate plants chosen for medicinal use). Dissimilarity can be calculated for the sample of taxa sampled from a pool, as a measure alpha-phylodiversity or community structure. Alpha-phylodiversity metrics indicate whether the community sampled from a pool of taxa is significantly more clustered, or significantly more dispersed, than a random sample of the local species pool. In ecology, significant phylogenetic clustering is interpreted as the result of environmental filtering of lineages that possess specialized adaptations to counter extreme environments (e.g. Fine & Kemble, 2013). Where alpha phylodiversity measures are available for multiple environments, environments with and without environmental filtering may be identified. In ethnobotany, clustering points toward preference for specific lineages for medicinal use (Saslis-Lagoudakis et al., 2012; Souza, Williamson, & Hawkins, 2018); a metric used in ecology to investigate environmental filtering is appropriate here since filtering is analogous to the selection by people for medicine. Dissimilarity can be also calculated between pairs of samples, and we make use of this approach too. Comparisons between samples, describing the dissimilarity of pairs of samples, are measuring beta phylodiversity. We might imagine an ecological scenario where beta-phylodiversity is used as follows: there are multiple mountains for which species lists for both low elevation and high elevation sites are available. Is any community from high elevation more similar to other high altitude communities, or are more similar communities found at low and high elevations within mountains? Considering medicinal floras, an analogous question might be whether medicinal floras are more similar if the people adopting each medicinal flora share more recent ancestry, or if they live in closer proximity to each other and can share knowledge (Saslis-Lagoudakis et al., 2014; Teixidor-Toneu et al., 2018). Here beta-phylodiversity is considered in the light of opportunity for exchange of knowledge and background floristic similarity.

Having identified divergence metrics as appropriate for our study, we selected Mean Pairwise Phylogenetic Distance (MPD) and the related metric Mean Nearest Taxon Distance (MNTD) as our metrics of choice. These divergence metrics measure pairwise distances, using either all pairwise distances for a sample ((MTD, Tests 1 and 2), or a pair of samples of taxa (MTD, tests 3 to 5, Table 1), or the subset of shortest distances between a pair of samples (MNTD, Test 6, Table 1). Thus, we apply these divergence methods to assess alpha phylodiversity or sample structure (Tests 1 and 2, Table 1), but also beta phylodiversity or the dissimilarity between samples (Tests 3 to 6, Table 1). We choose these metrics because of the simplicity of using the same metric for alpha and beta-phylodiversity, and also because MPD is an "anchor" metric, one with well-known properties that lies at the centre of a constellation of less well-known, similar metrics (Tucker et al., 2016).

The study we present here draws out methodological issues. Firstly, we investigate whether there is clustering of medicinal plants overall, within a local flora. Local phylogenies were used to test for clustering of medicinal plants in local floras in the first study of this

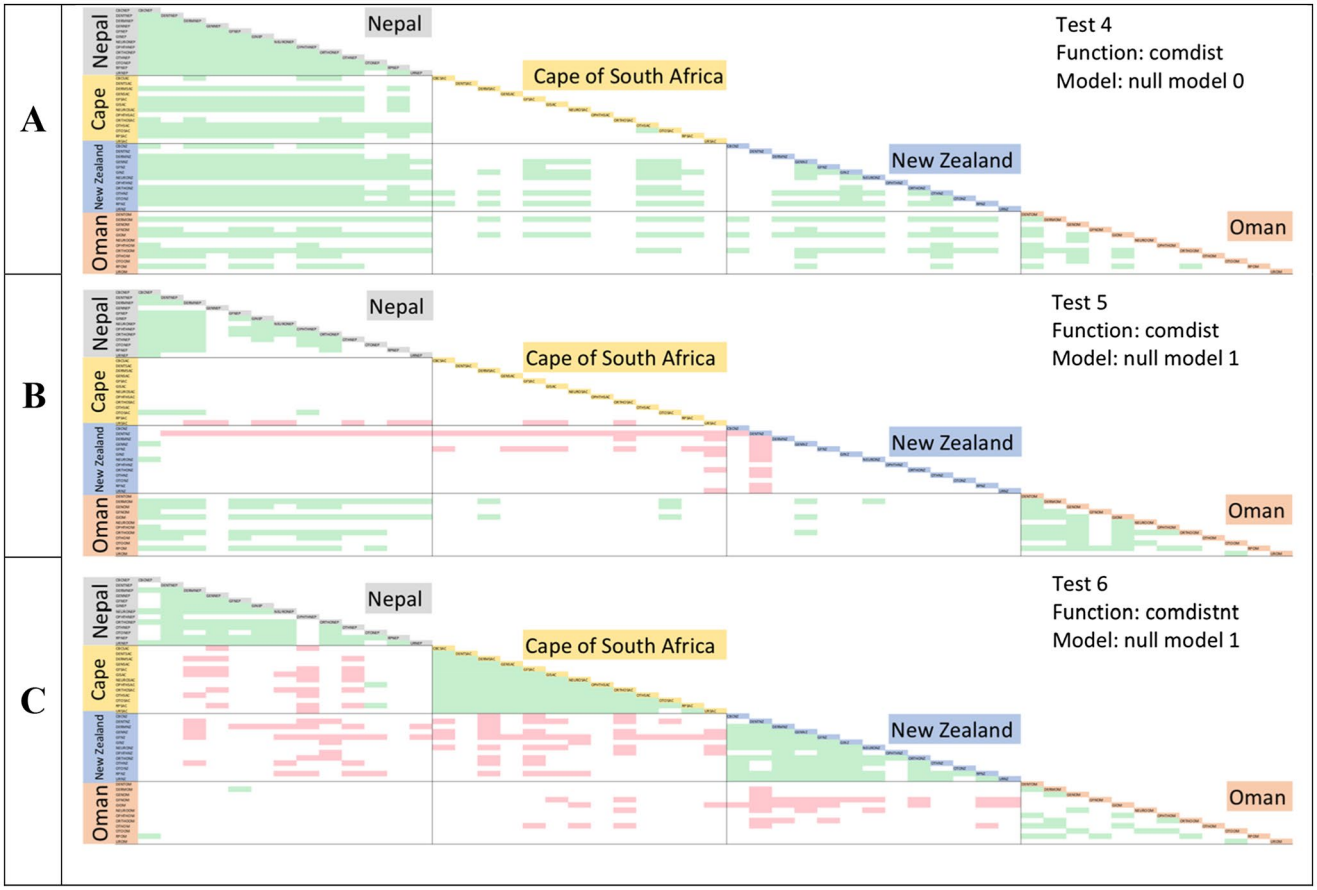

FIGURE 2 Cross-cultural tests of relatedness of therapeutic applications. Heat maps show clustering (green) and overdispersal (red) for all pairs of comparisons. Comparisons are for the four cultures, Nepal (grey), the Cape of South Africa (yellow), New Zealand (blue), and Oman (orange). The blocks of the heatmap are as follows: block A for test 4, using comdist and null model 0; block B for test 5 using comdist and null model 1; block C for test 6 using comdistnt and null model 1. Block A is mostly green (significantly similar lineages) with no red (significantly different lineages). There are some parts of block A where almost all cells are green, for example, comparing different therapeutic applications within Nepal. There are some parts of block A where few cells are green, for example comparing different therapeutic applications within Cape of South Africa. In this case any plant present in the phylogeny can be included in the null, and the large number of green cells points to lineages that, relative to plants as a whole, are used for multiple therapeutic applications. Block B has many green cells (clustering) for comparisons between therapeutic applications within Nepal, also within Oman, and for many Nepal-Oman comparisons. Green cells are few between other pairs of cultures, and there is overdispersal for some therapeutic applications. Since the null is sampled from medicinal plants, the effect of the overall clustering of medicinal plants is removed and there is much less evidence of the same lineages used for different therapeutic applications than is apparent in block A. Block C has only has green cells when within-flora comparisons are made. Many comparisons between floras show overdispersal (red cells). The closest relative of a genus with any medicinal use is most likely found in the flora in which that genus is found, regardless of therapeutic application. The effect of floristic composition is strong when investigating tip-level relationships

kind (Saslis-Lagoudakis et al., 2012). Here, we found differences when using more inclusive, combined rather than local phylogenies, despite the use of null models to make comparable comparisons between the Omani medicinal flora and its whole flora. Significant clustering was revealed for Oman only when we used a combined phylogeny, this can be attributed solely to the phylogenetic framework. We suggest that it is best practice to use a more inclusive phylogeny and appropriate null model as we do in Test 2, rather than local phylogenies. In the case of this characterization of the Omani medicinal flora in the context of the Omani flora, when the combined phylogeny is used the whole flora was included in the sample file and the null that draws from the sample file to assemble null communities was used. A global phylogeny would provide the most robust estimate of plant relationships, particularly when considering small local floras where local approaches might introduce Long Branch Attraction and other topological anomalies (Park, Worthington, & Xi, 2018). It may not be possible to generate a fully sampled phylogeny using published and new sequence data to reconstruct the phylogeny. An alternative to building bespoke phylogenies from sequence data is to use existing mega-trees and to insert missing species or genera at the nodes shared with their closest taxonomic relatives (Jin & Qian, 2019). An advantage of the latter approach is that the mega-trees are reconstructed from sequence data representing multiple gene regions, whereas the phylogenies we use here are reconstructed using just one gene region, *rbc*L. We follow Park et al.'s (2018) recommendation for community phylogenetics that an

effort should be made to generate a better phylogeny at least by including non-local taxa as placeholders.

Secondly, we highlight the importance of choice of null model in cross-cultural analysis. Null model 0 is used to test whether plants of one medicinal flora are clustered with those of another, where the null is drawn from the whole phylogeny. Null model 1 is used to test whether, from within the sample of medicinal plants, plants of one medicinal flora are clustered with those of another. The selection of the null model therefore frames the question as well as influencing the results. Here, we used null 0 to test whether a pair of therapeutic applications is a phylogenetically structured subset of all plants. We found many were more closely related than expected by chance (Figure 2a), consistent with the findings of the previous study that we extend here (Saslis-Lagoudakis et al., 2012). However, when we tested whether a pair of therapeutic applications are a phylogenetically structured subset of the medicinal plants there was less evidence of relatedness. When considering therapeutic applications under null 0, it is not surprising that so many therapeutic applications are clustered, since we know that medicinal plants overall are clustered (Table 2). By selecting null model 1, we remove the influence of the relatedness of medicinal plants overall, allowing more critical evaluation of how different therapeutic application behave. Null model 0, because of the strong influence of relatedness of medicinal plants overall, recovers spurious similarity attributed to plants for specific therapeutic applications being a subset of the phylogenetically clustered medicinal plants. Using the more conservative null, we show that aside from the Nepal—Oman comparison, there is little evidence that people in different localities are using the same lineages of plants for the same therapeutic applications.

A third important finding related to methodological approach, and with implications for interpretation of findings, is that therapeutic applications within cultures are often cross-predictive. In other words, we find that the same plant lineages are used for different therapeutic applications. We found many cases of cross-predictivity for therapeutic applications in Nepal and in Oman, the largest and smallest medicinal floras respectively. Cross-predictivity was rare for Cape of South Africa (under null 0 and 1) and New Zealand (under null model 1). Cross-predictivity might be attributed to plants with shared bioactivity finding use for different therapeutic applications, as might be expected if for example many therapeutic applications of plants depend on anti-inflammatory properties (Ernst et al., 2016; Saslis-Lagoudakis, Klitgaard, et al., 2011) or other properties such as antibiotic properties. Cross-predictivity suggests that targeting plants for screening based on their specific therapeutic applications, in order to identify lead compounds with properties relevant to that specific application, may not be an effective approach. Reverse ethnopharmacology has shown few positive associations between the clinical use of proven bioactives and traditional therapeutic applications, and that cross-over relationships where traditional use and biomedical therapeutics point to different applications is more numerous (Leonti et al., 2017). In this context, it might be more interesting when different lineages are used for different therapeutic applications, as evidenced by overdispersal between therapeutic applications. For example, Souza et al. (2018) found that plants used by women represented different phylogenetic lineages of Brazilian Leguminosae. Reverse ethnopharmacology highlighted an association between plants used by women and anticancer drugs (Leonti et al., 2017); Souza et al. (2018) suggested that the drugs used by women included plants of strong effect used to terminate unwanted pregnancies. Plants of strong effect might be expected to appear in different lineages to the more frequently used and often interchangeable plants of mild effect. We find some overdispersal under null 1 in the case of a few therapeutic applications, including use for dentistry/mouth in New Zealand (19 genera), and for urinary complaints in the Cape of South Africa (45 genera). Why different lineages are used for different therapeutic applications in these two local medicinal floras, but not in Oman or Nepal, could be investigated further. One hypothesis to test is that cross-predictivity is associated with humoral medicine, the shared beliefs about illness causation found in Oman and Nepal (Durkin-Longley, 1984; Ghazanfar & Al-Sabahi, 1993).

Our final test, using the comdistnt measure of nearest taxon distance, investigated relatedness of plants with different specific therapeutic applications within or between areas, or with different therapeutic applications within an area. We showed that the closest relative of a medicinal plant with a specific therapeutic application in an area is mostly likely found within that same area. This was true for all four medicinal floras. This may reflect the structure of the combined phylogeny at tip level (sister genera would be expected to be found within floras). The genera of the Cape of South Africa have the strongest pattern of tip-relatedness, as might be expected since it comprises a Floral Kingdom composed of many radiating or endemic taxa. Oman has the fewest instances of closest relatives within Oman. This could be due to the low number of reports for Oman: in total there were 114 combinations of use and application for 76 medicinal genera, compared to 1872 for the 563 medicinal genera of Nepal, 896 for the 198 medicinal genera of Cape of South Africa and 416 for the medicinal 97 genera of New Zealand. Generally, deeper measures of relatedness are more informative in studies of medicinal plant use. Preferences for lineages at deep levels can be associated with shared bioactivity (Saslis-Lagoudakis et al., 2012; Zhu et al., 2011).

There are three methodological issues that we do not explicitly explore here: the completeness of sampling, the hierarchical level of sampling, and the rate-smoothing approach taken. Considering completeness of sampling, in the case of Oman and Cape of South Africa our phylogenies include approximately 80% of the genera found the local flora, and our best represented flora is New Zealand with more than 88% of genera in the phylogeny. Although Tests 1 and 2 explore taxon sampling effects due to the use of local versus more inclusive phylogenies, we did not consider the effects of missing genera. Others have explored this, for example Jantzen et al. (2019) made a test of the effects of taxon sampling on phylodiversity metrics. They showed that sampling higher proportions of local species increases the likelihood of finding significant phylogenetic patterns when they exist. Jantzen et al. (2019) caution that, for small or undersampled

communities, there may not be enough statistical power to detect nonrandom patterns even when they may exist in nature. The tests in our study which did not reveal significant clustering but for which some values were close to the threshold for significance were tests 4, 5, and 6, tests which examined smaller samples because they consider the subsets of genera used for specific therapeutic applications. There might be more significant relationships between pairs of therapeutic applications if we had more complete sampling, more frequent reporting of phylogenetic structure. This would not refute our most important finding with respect to therapeutic applications, that there is a great deal of cross-predictivity between therapeutic applications. Nor would we expect that the patterns revealed by Test 6 to change, we would still expect that most often the closest relatives of medicinal genera are found in the same flora.

The second methodological issue not explored here relates to the hierarchical level of sampling. Whether, and if so when, it is more appropriate to use species-level phylogenies should be the focus of future study. In reconstructing the phylogeny and in scoring the presence or absence of plant use at the generic level, we follow the study that we extend here (Saslis-Lagoudakis et al., 2012). To date, published studies using species-level phylogeny to explore medicinal plant use are generally limited to studies of genera (e.g. Saslis-Lagoudakis, Klitgaard, et al., 2011; Ernst et al., 2016, but see Souza et al., 2018). We expect that generic level is most appropriate if the study includes all flowering plants and calculates NRI, as ours does. This is because NRI values describe a deep relationship in the phylogeny, and generic-level data could provide evidence for clustering of interest at tribal or sub familial levels. If the study is directed toward understanding tip-level, shallow relationships these might be best investigated in a species-level study of a genus of family. The phenomenon of generic complexes, where different scientific species with the same vernacular name are used interchangeably, is well known in ethnobotany (Linares & Bye, 1987). Although generic complexes may include unrelated species, the prevalence of use of clusters of close relatives and dispersed deep linages might contribute to a pattern of overdispersed clusters when species-level analyses are performed (Souza et al., 2018). Ultimately, calculating metrics for phylogenies at family, genus and species levels might reveal test whether patterns recovered are caused by the concentration of useful species in particular taxonomic units.

The third methodological issue not explored here relates to rate-smoothing or time calibration of the phylogeny used. Our analyses were performed on a rate-smoothed but not time-calibrated phylogeny. The community phylogenetics literature includes metrics calculated using phylograms and chronograms (Li et al., 2019). This choice can lead to significantly different results in some cases (Allen et al., 2019; Jantzen et al., 2019; Li et al., 2019). We found rate smoothing to reduce the strong effect of taxa on long branches, and we considered this desirable, especially when some samples are small.

A final consideration is whether there should be an adjustment to the threshold for significance when many tests are carried out, as is the case here. The Bonferroni correction is sometimes used to adjust the alpha threshold for each comparison, so that the study-wide error rate remains at 0.05 (Cabin & Mitchell, 2000). The correction provides a conservative estimate of significance, minimizing type I errors but inflating type II errors. Saslis-Lagoudakis et al. (2012) highlighted results remaining significant after Bonferroni correction in their results tables. This approach has the advantage of allowing the reader to decide what is worse, false positives or negatives, when considering individual values. However, where the interest is in overall pattern rather than individual comparisons as is the case with the heat map presented here (Figure 2), we argue against Bonferroni correction.

Whether different people share preferences for medicinal plants is a question that has been addressed many times, using different methods applied at different spatial scales and at different taxonomic levels. Since independent discovery of plant properties is considered to point toward efficacy (Bletter, 2007; Moerman, 2007; Trotter & Logan, 1986), discovering shared preferences may be informative (Saslis-Lagoudakis et al., 2012). Here, our approach is phylogenetic, and we consider distantly related cultures exposed to very different floristic environments. By showing significant phylogenetic clustering of medicinal genera between cultures, we demonstrate that Omani medicinal use of plants emphasizes the same deep lineages of flowering plants as the Nepalese, South African Cape and Māori uses. This finding points toward a global pattern of preferred plant uses, relevant to the interpretation of the pre-history of human–plant interactions and the history of medicine.

Phylogenetic investigation of whole medicinal floras opens up several lines of research. One depends on the identification of "hot nodes", nodes on the phylogeny that include significantly more plants traditionally used in medicine (Saslis-Lagoudakis, Klitgaard, et al., 2011; Saslis-Lagoudakis et al., 2012). These hot node methods allow the taxa belonging to lineages used more than expected to be enumerated. How these hot node methods might be applied in bioprospecting is a matter of ongoing research (Souza et al., 2018). Another approach depends on the spatial mapping of phylogenetic diversity (PD), a diversity measure predictive of feature diversity that can have conservation applications (Faith, 1992; Forest et al., 2007). Neither hot node nor PD mapping approaches are applied here. Instead, in this study we use cross-cultural comparisons that are directed toward better understanding of the cultural factors underlying the use of plants as medicines. For example, it is possible to evaluate whether relatedness of medicinal floras is predicted by relatedness of floras as would be the case when plants from the local environment are selected in the absence of significant alternative drivers of plant selection, such as cultural ancestry or migration history. The influence of cultural ancestry has been investigated (Saslis-Lagoudakis et al., 2014; Thompson et al., unpublished). However, although it has been proposed that plant preferences could reflect migration history, rather than the current floristic environment in which people are currently settled (Leonti et al., 2003; Moerman et al., 1999), this hypothesis has not been tested phylogenetically. In the present study, we find the Omani medicinal flora is most similar to the Nepalese one. One driver of this relationship could be floristic environment since we also find that the Omani flora is also more

similar taxonomically to the Nepalese flora than to any of the other floras considered. That the flora—a proxy for plant availability—is the main determinant of plant use in our study, was also revealed by another phylogenetic study (Saslis-Lagoudakis, Klitgaard, et al., 2011). Secondly, other drivers of relatedness of medicinal floras can be explored. These could include shared beliefs about illness causation, use of a shared scholarly medical system, or transmission of traditional knowledge of plants. Cultural contact between Nepal and Oman would be expected to be greater than contact between Oman and the other cultures in our study, through the movement of people and ideas. Oman was an important medieval trading post with many products entering its ports, including Indian plant drugs also important in Nepal (Amar & Lev, 2017). In terms of beliefs, the Galenic humoral system found in northern and central Oman (Ghazanfar & Al-Sabahi, 1993) also contributes to Nepalese practice through historical regional (Yoeli-Tlalim, 2013) and modern local pluralistic plant medicine (Durkin-Longley, 1984).

Were a wider set of medicinal floras under investigation, it would be possible to account for Galton's problem - that relatedness accounts for cross-cultural similarity - and formally test the extent to which overall similarity in composition of medicinal floras was due to present geographic proximity (as a proxy for the likelihood of knowledge exchange through cultural diffusion), health needs or theory of disease causation (Teixidor-Toneu et al., 2018). Whether the folk knowledge of Oman and Nepal reflect the influences of transmission of knowledge could also be tested further by focusing on the plant names, therapeutic applications, and plant parts used at species level for shared species, using phylogenetic comparative methods and ethnolinguistic phylogenies. An approach of this kind could also tease apart Tibeto-Burman and Eurasian influences on the medicinal flora of Nepal. Our study represents a step toward using a global phylogeny and a global estimate of the relatedness of cultures, using macro-evolutionary methods.

5 | CONCLUSION

Phylogenetic methods are a powerful tool to better understand medicinal plant use. Here, we focus on cross-cultural patterns in the use of medicinal plants. We show that a more inclusive phylogeny of plants provides an optimal framework, as long as appropriate null models are selected. We highlight the best practice for cross-cultural study, particularly in the interpretation of shared therapeutic applications. We contribute to the emerging picture of the same plant lineages being used for multiple therapeutic applications. The first study to use a phylogeny of plants to explore cross-cultural patterns at the level of whole medicinal floras, in the way we do here, identified lineages that were putatively independently discovered (Saslis-Lagoudakis et al., 2012). Here, we incorporate the medicinal flora of a cultures likely to have had opportunity for transmission of traditional knowledge with another, if not directly then through multiple intermediaries across wide distances. Ultimatel, we may be able to identify drivers of overall similarity in medicinal floras using the methods outlined here, alongside those of evolutionary anthropology (Teixidor-Toneu et al., 2018). Including cultures with more or less similar floras, health needs, theories of disease causation, and opportunity for knowledge exchange, we can more rigorously test the hypothesis that lineages were independently discovered by identifying factors contributing to knowledge transfer (Teixidor-Toneu et al., 2018). Interdisciplinary study combining plant phylogenies, evolutionary anthropology, and ethnobotanical data have great promise, but only if methods are robust (Hawkins & Teixidor-Toneu, 2017); here, we take steps to promote these methods.

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AUTHOR CONTRIBUTIONS

JAH and DL designed and conceptualized the project; TA-J, supported by SAG, collected data, further data were provided by CHSL; TA-J and DL performed analyses; JAH, IT-T and DL wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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