Assessment of five statistical approaches in finding out the over- and under-represented plant species in traditional medicines in Nepal

Durga H. Kutal, Nabin R. Joshi, Chandra K. Subedi and Ripu M. Kunwar

Durga H. Kutal[1*], Nabin R. Joshi[2], Chandra K. Subedi[3] and Ripu M. Kunwar[3*]

[1]College of Science and Mathematics, Augusta University, Georgia, USA.
[2]Asia Network for Sustainable Agricultural Resources and Sustainability, Kathmandu, Nepal.
[3]Research Center for Applied Science and Technology, Tribuhvan University, Nepal.

*Corresponding Author: dkutal@augusta.edu, ripukunwar@gmail.com

Ethnobotany Research and Applications 27:51 (2024) - http://dx.doi.org/10.32859/era.27.51.1-17
Manuscript received: 14/08/2024 - Revised manuscript received: 02/12/2024 - Published: 04/12/2024

Research

Abstract

Background: In quantitative ethnobotany, different statistical tools and approaches have been used to compare medicinal floras with the overall flora of a given area and to investigate over- and under-represented medicinal plant families.

Methods: In this study, we analyzed a dataset of medicinal plant species of Nepal to evaluate their usefulness in traditional medicines in Nepal. We compared five different statistical methods (Bayesian, Binomial, Least-square, Log-transformed, and Negative-binomial) to test which plant families are more likely to harbor more medicinal plant species that are being used.

Results: Among the tested methods, least-square method was found more pertinent in the sense that the over-represented medicinal plant families of this approach and that being used in traditional medicines resembled the greater affinity. For small and community level datasets, negative-binomial analysis was found pertinent. Thus, it is particularly important to combine statistical approaches for small, and moderate-sized data to avoid inherent methodological biases. The combined approach of all five statistical methods generated the over-represented plant families in the following order Moraceae, Cucurbitaceae, Zingiberaceae, Rutaceae, Solanaceae, Euphorbiaceae, Lamiaceae, and Anacardiaceae, which is close to the result of 5% significance level of negative-binomial analysis.

Conclusions: This analysis allowed us to identify plant families which are apparently underutilized and which apparently are substantial sources of traditional medicines in Nepal. This study also contributed to the discussion of the methodology to test the non-random theory of medicinal plant selection. Similar assessments are needed for finding out the best methods for identifying the over- and under-utilized plant species.

Keywords: Statistical methods, regression, binomial, Bayesian, medicinal plants, over-represent, under-represent, Nepal.

Background

Selection of plants for traditional medicines follows a non-random hypothesis (Bennet & Husby 2008), where ethnopharmacological, geographical, socio-cultural, ecological, phylogenetic and plant therapeutic traits are taken care of (Farnsworth & Bingel 1977; Moerman 1979; Cox 1990; Kunwar *et al.* 2018; Kutal *et al.* 2021; Kunwar *et al.* 2022). Some studies also confirmed that closely related plant families are more likely to have similar medicinal uses than those that are phylogenetically distant (Saslis-Lagoudakis *et al.* 2014; Gaoue *et al.* 2021). The selection is also found taxonomically influenced since some certain plant families are more likely to be collected than others (Moerman 1996; Bennet & Husby 2008). It predicts that large families are more likely to be richer in medicinal plants than small-sized families (Moerman (1991, 1996). Given that plant family can become a determinant of plant use value (Phillips & Gentry 1993), identification of over- and under-represented families is a first step toward prioritization of research efforts for sustainable use, conservation and drug discovery (Saslis-Lagaudis *et al.* 2011; Muleba *et al.* 2020). Conservation of useful plant species and families can be maintained and enhanced once the complexities of a cultural landscape and knowledge, diversity, usefulness and richness of plants and the associated theory, principles and practices are tested (Ali *et al.* 2024). The use of plant databases and the associated knowledge of plant-uses to test theories and hypotheses in Nepalese ethnobotany is not yet a common practice despite the recent calls for more hypothesis-driven ethnobotanical research (Muleba *et al.* 2020). The paradigm shifts toward a more hypothesis- or theory-driven ethnomedicinal study is urged in Nepal (Kutal *et al.* 2021) to make ethnobotany a stronger scientific discipline with theories and hypotheses that can be used to predict new medicinal plant uses as well as better explain plant-human interactions (Gaoue *et al.* 2021).

To test the non-random hypothesis and appraise the influence of taxonomic (family) characteristics in traditional medicines, Moerman (1991, 1996) applied the use of linear regression analysis. Some other studies employed the binomial (contingency table) (Bennet & Husby 2008), least-squares regression (Douwes *et al.* 2008), Bayesian analysis (Weckerle *et al.* 2011; Kindscher *et al.* 2013; Medeiros *et al.* 2013), negative-binomial regression (Robles *et al.* 2020), and log-transformed linear regression (Muleba *et al.* 2020) to evaluate the relationships between the number of medicinal plants and total plants in a family. All these tools were useful for determining medicinal plant use differences among plant families, although the result varied by geography and the ethnic groups. A review of studies (Table 1) around the world reveals that the medicinal importance of plant families is only partly overlapping across space (Phumthum *et al.* 2019). However, such assessments in Nepal are scant since the hypothesis-driven ethnobotanical studies are limited (Kutal *et al.* 2021; Kunwar *et al.* 2022). In this gap, we analyzed and compared the medicinal usefulness of plant families across Nepal, considering the research question why some plants in a particular family are predominantly over-utilized in some traditional medicines and in some regions while other plants are underutilized?

Table 1. Important ethnomedicinal plant families identified from other parts of the world

	Lamiaceae	**Asteraceae**	**Fabaceae**	**Malvaceae**	**Apiaceae**	**Euhorbiaceae**	**Poaceae**	**Rosaceae**	**Ranunculaceae**	**Solanaceae**	**Rutaceae**
Campania, Italy	√			√				√			
Belize	√	√			√						
Ecuador[1]	√		√	√	√		√				√
Ecuador[1]		√								√	
Mexico	√	√	√			√					
North America		√			√			√	√		
Kansas, USA	√	√	√		√				√		
Hawaii			√	√			√				
Nepal[1]	√	√	√				√	√			
Nepal[2]		√	√				√				
India	√	√				√			√	√	
Pakistan	√							√			
Thailand	√	√	√	√						√	√
South Africa[2]	√		√								
South Africa[1]	√	√	√	√		√					
World	√		√	√	√	√			√		
	12	**10**	**10**	**6**	**5**	**4**	**4**	**4**	**4**	**3**	**2**

World (Kew 2017); India (Kapur *et al.* 1992); North America (Moermann 1996); Kansas USA (Kindscher *et al.* 2013); Mexico (Leonti *et al.* 2003); Belize (Amiguet et al. 2006); Ecuador[1] (Robles *et al.* 2020); Ecuador[2] (Bennett & Husby 2008); South

Africa[1] (Douwes *et al.* 2008); South Africa[2] (Muleba *et al.* 2020); Hawaii (Ford & Gaoue 2017); Thailand (Phumthum *et al.* 2019); Italy (Weckerle *et al.* 2011); Nepal[1] (Kutal *et al.* 2021); Nepal[2] (Kunwar *et al.* 2022); Pakistan (Khan *et al.* 2011)

Materials and Methods

In this paper, we used five different statistical approaches (least-square regression, log-transformed GLM regression, binomial analysis, negative-binomial regression, and Bayesian analysis) to test whether a family is over- or under-represented in an ethnomedicinal flora in comparison to overall local flora of Nepal. For this, we used the dataset collected and adapted from an online database (efloras.org) and other literature (Press *et al.* 2000; Manandhar 2002; Baral & Kurmi 2006; Kunwar *et al.* 2010; Kunwar *et al.* 2011; Kunwar *et al.* 2021; Shrestha *et al.* 2022; Kutal *et al.* 2022; Kunwar *et al.* 2022). The dataset contains total plant families (231), total number of plant species (6526), and total number of medicinal plant species (1506). The latter dataset was pooled from the resource of Kunwar *et al.* (2022) and the species recorded from at least two districts were considered for this study. Plant families follow the plant list theplantlist.org. The lowest taxon used for this study was species.

The number of medicinal plant species in a family used by ethnic communities is a response variable and the size of the family is an explanatory variable for regression analysis. To precisely identify the over-used and under-used plant species we used raw residuals and studentized residuals. While Moerman *et al.* (1999) used the raw residuals, we computed studentized residuals in order to compare them with the appropriate quantiles of the Student's t-distribution. A raw residual is the difference between the observed value and the predicted value for a data point. Studentized residuals are computed as regression model raw residuals divided by adjusted standard error of the raw residuals. Studentized residuals are more robust statistical measures to identify over-used and under-used species compared to raw residuals. Studentized residuals make the test easier to compare and interpret across different datasets and models. We applied graphs, raw residuals, and studentized residuals for least-square regression, log-transformed regression, and negative-binomial regression models to find the noticeable overused and underused medicinal plants. We also included binomial analysis and Bayesian analysis to identify over- or under-represented families. Binomial analysis is appropriate to analyze low-use and high-use of medicinal plant species since the distribution of species are not evenly distributed among the families in the dataset. We calculated the expected value from the number of medicinal plant species per family. Using the expected values (observed value - expected value), we identified which plant families are significantly overused and underused. Bayesian approach allows us to incorporate prior knowledge and new data information. It is particularly useful in ethnobotany since it can handle complex models, small sample size, and variability in a dataset.

Statistical Analysis

Statistical analysis in ethnobotany is crucial for understanding the relationships between plants and the cultural practices of different human communities. We fitted the least square regression model (LM) for medicinal species per family against total species. Using the general linear regression model, the total number of selected species *per* family was regressed on the total number of species in the same family in the area. The over or under-represented families were identified when the raw residuals and studentized regression residuals of the fitted line position in the extreme positive and negative residuals (Robles *et al.* 2020). Species with studentized residual falling into the lower or upper ±2 values indicate that less or more used respectively than expected based on the availability.

We performed the log-transformed GLM regression to test the association between the number of medicinal plant species per plant family and the total number of plant species per family after the log transform. These variables were $\boldsymbol{log1p}$ ($\boldsymbol{i.e.} \log(\boldsymbol{1}+\boldsymbol{x})$) transformed prior to see the association between these variables. This $\log(\boldsymbol{1}+\boldsymbol{x})$ transformed ensures the normal residuals and homogeneity of the variance (Phumthum *et al.* 2019). We identified over-utilized and under-utilized medicinal plant species based on the positive and negative residual values. Families with positive residuals were considered as over-utilized since they have a greater number of medicinal plant species than estimated from the general linear regression model. Families with negative residuals were considered under-utilized since this indicated that these families have less medicinal plant species than estimated from the predicted model.

The negative binomial regression model was most useful for our dataset since our datasets showed largest variance in compared to mean (mean (6.51) < variance (156.31)). The negative binomial regression model showed the positive non-linear association between total plant species family size and number of medicinal plant species per family (Muleba *et al.* 2020) in the population (β1 = 0.0160 ± 0.0009, Z = 16.59, $p < 0.00001$). The studentized residual followed a distribution with 228 degrees of freedom ($\boldsymbol{n}-\boldsymbol{k}-\boldsymbol{2}$) for the entire dataset. The critical value for the dataset $\boldsymbol{t_{\alpha/2,\ df}}$=1.97 at significance level 5%. Families with positive residuals are over utilized medicinal plant species and families with negative residuals indicate under-utilized medicinal plant species. The range of residual values in the negative binomial model is from -1.2528 to 4.2412. We fitted generalized linear model with negative-binomial following Robles *et al.* (2020) and Muleba *et al.* (2020).

The Bayesian analysis is based on the assumption that the overall average proportion of selected plants in the flora is not observed without error; this being especially relevant for small datasets (Weckerle *et al.* 2011). In this study we used non-informative prior distribution. To identify over-represented and under-represented medicinal plant families, we compared the 95% credible interval of the average proportion of overall dataset with the corresponding 95% credible interval for each family. If the 95% credible interval of a family fell outside (left/right) of the 95% credible interval of the overall dataset, those families are over-or under-represented medicinal plant families.

Binomial analysis is also appropriate to analyze over-used and under-used medicinal plant species since the distribution of medicinal plant species are not evenly distributed (not homogeneous since p-value < 0.0001 from Chi-square goodness of fit test) among the families in the dataset. The binomial test is based on the assumption that medicinal species are allocated within a family according to the proportion of medicinal species in the flora (Bennet & Husby 2008). Binomial analysis can be used to access whether medicinal plant species per family are being over-utilized or under-utilized compared to their observed frequency and expected frequency in an available data set. We tested whether the observed frequencies deviate significantly from the expected frequencies, and we calculated the p-value associated with observed frequencies under the null hypothesis. The positive deviation between observed and expected frequencies with low p-value indicated that the medicinal plant species is used significantly more frequently than expected. The negative deviation between observed and expected frequencies with low p-value indicated that the medicinal plant species is used significantly less frequently than expected.

We tested if the proportion selected within a family was proportionally more or less compared to the average proportion of the dataset. When the within-family proportion of selected species was statistically less (more) than the overall average proportion of the dataset, then the family was considered under-used (over-used). We computed the expected value of each family multiplying total number of plant species in a family and a total number of medicinal plant species used in an entire dataset divided by the total number of plant species in an entire dataset. Based on the deviation values of expected value and observed value, we obtained the over-used and under-used medicinal plant families. To identify which families are over- or under-used, we calculated the residuals and studentized residuals. We then compared the percentage agreement among these five approaches. This allowed us to investigate the agreement among the results under different methods (least square, log transform, negative binomial, binomial, and Bayesian). The application of these methods is based on the assumption that the main factor that drives the selection of medicinal plant species is phylogeny (plants in the same family have closer relationships and will all be preferentially selected/avoided). We also identified the minimum number of taxa within a family that would be required to identify over- and under-used given the five sets of analyses observed for the dataset.

Results

The total used medicinal species is around 23% (1506/6526=0.2307) and the 95% posterior credible interval of the overall proportion of species is between 0.2207 (22%) and 0.2412 (24%).

Log-transformed (Lt), least-square (Ls) and negative-binomial (Nb) regressions

The log-transformed regression model showed the positive linear association between total plant species family size and number of medicinal plant species per family in the population ($\beta1 = 0.6832 \pm 0.0236$, $t = 28.949$, $p < 0.00001$). The range of residual values in the log-transformed GLM was from -3.1614 to 1.8727. The log-transformed regression analysis is used to analyze the medicinal plant per family against the total plant species with an R^2 of 78.5%.

The least-square regression shows the medicinal plant species per family against the total plant species with an R^2 of 67.4%, which depicts that 67.4% of the variance in the number of medicinal plants used traditionally for medicine per family can be explained by the number of available plant species per family. The range of residual values in the least-square regression linear model was from -7.5262 to 5.5402.

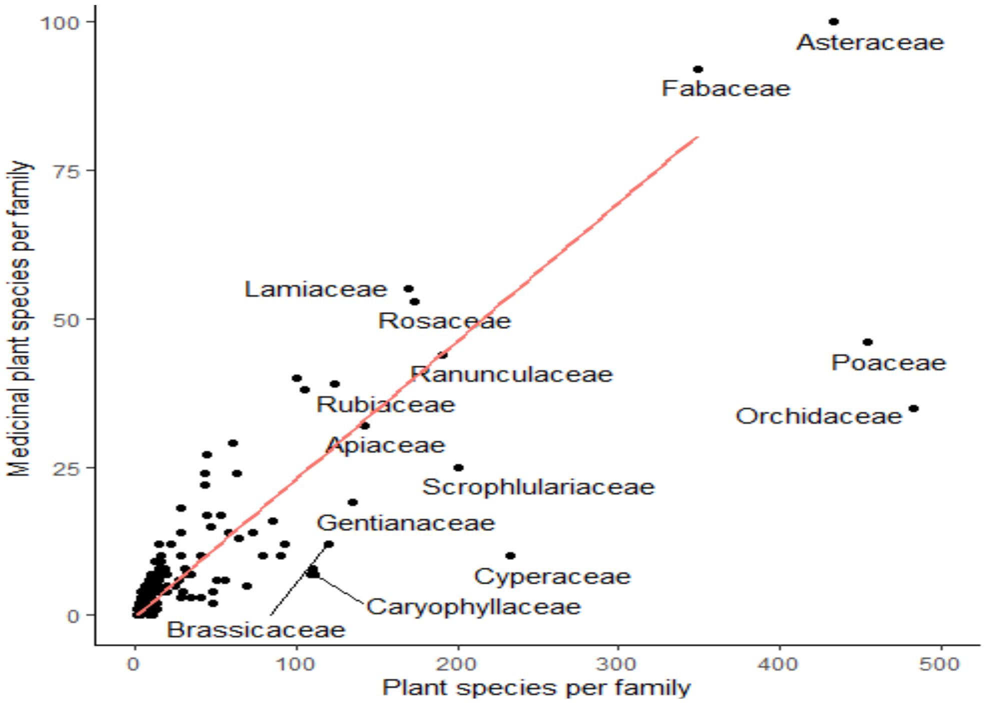

Figure 1. Log-transformed analysis of the number of medicinal plant species per family against the number of total number of plant species per family in Nepal

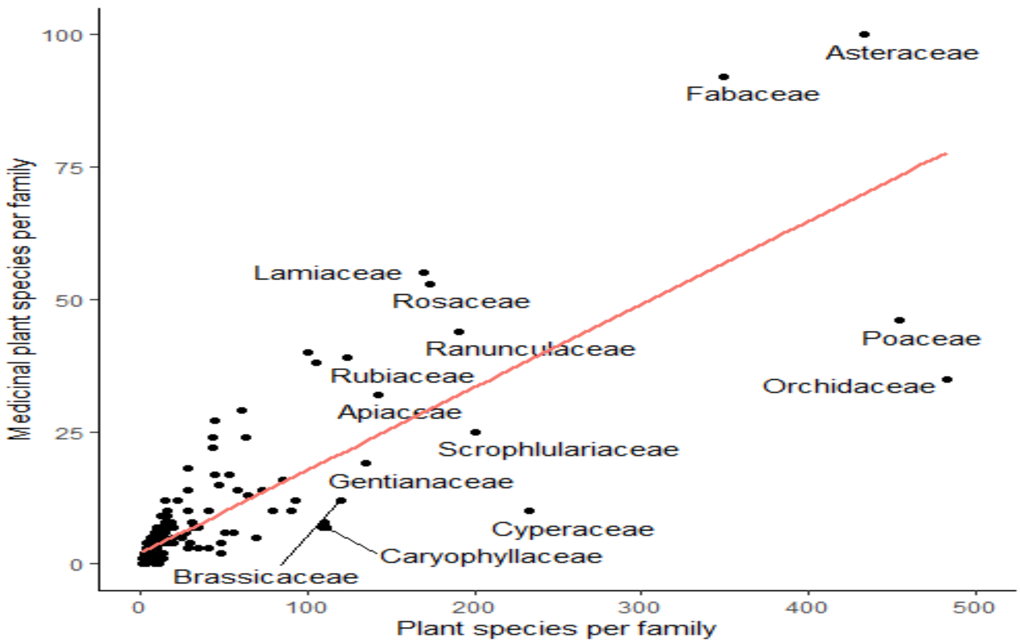

Figure 2. Least-square regression of the number of medicinal plant species per family against the number of total numbers of plant species per family in Nepal

The negative-binomial regression model showed the positive non-linear association between total plant species family size and number of medicinal plant species per family in the population (β1 = 0.0160 ± 0.0009, Z = 16.59, *p* < 0.00001). The studentized residual followed a distribution with 228 degrees of freedom $(n - k - 2)$ for the entire dataset. The critical value for the dataset $t_{\alpha/2,\ df}$=1.97 at significance level 5% was from -1.2528 to 4.2412.

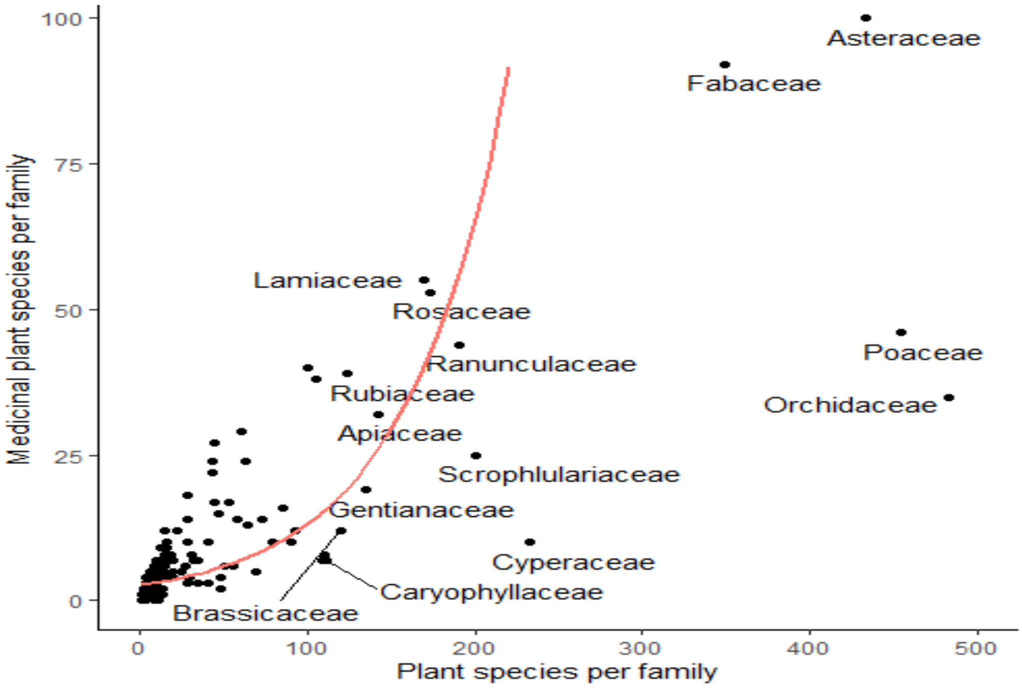

Figure 3. Negative-binomial regression of the number of medicinal plant species per family against the number of total numbers of plant species per family in Nepal

Binomial and Bayesian analyses

The distribution of medicinal plant species among families is not homogeneous since we obtained very small p-value (p-value<0.0001) from chi square goodness of fit test in Binomial regression. This indicated that medicinal plants are not evenly distributed among the corresponding families in the entire data set. Five-first over-represented families in Binomial regression are Euphorbiaceae, Moraceae, Lamiaceae, Solanaceae and Zingiberaceae and that in Bayesian analysis are Anacardiaceae, Balanophoraceae, Moraceae, Combretaceae and Rutaceae. In Bayesian analysis, the families Anacardiaceae (0.5435), Balanophoraceae (0.4782), Moraceae (0.4659), Combretaceae (0.4619), Rutaceae (0.4567) are superior having values greater than 0.2412 of 95% credible interval of overall families.

Table 2. The five-first and the five-last ranked families being used in traditional medicines in Nepal

Rank	Least-square	Log-transformed	Negative-binomial	Bayesian	Binomial
Overused 1	Fabaceae	Moraceae	Moraceae	Anacardiaceae	Euphorbiaceae
Overused 2	Asteraceae	Anacardiaceae	Zingiberaceae	Balanophoraceae	Moraceae
Overused 3	Lamiaceae	Rutaceae	Cucurbitaceae	Moraceae	Lamiaceae
Overused 4	Rosaceae	Zingiberaceae	Rutaceae	Combretaceae	Solanaceae
Overused 5	Euphorbiaceae	Cucurbitaceae	Solanaceae	Rutaceae	Zingiberaceae
Underused 1	Orchidaceae	Aquifoliaceae	Orchidaceae	Acanthaceae	Orchidaceae
Underused 2	Cyperaceae	Sabiaceae	Poaceae	Boraginaceae	Poaceae
Underused 3	Poaceae	Juncaceae	Asteraceae	Pteridaceae	Cyperaceae
Underused 4	Caryophyllaceae	Cyperaceae	Cyperaceae	Gentianaceae	Scrophlulariaceae
Underused 5	Saxifragaceae	Thelypteridaceae	Aquifoliaceae	Thelypteridaceae	Caryophyllaceae

A summary of studentized residuals of least-square, log-transformed and negative-binomial analyses are presented in Fig. 4.

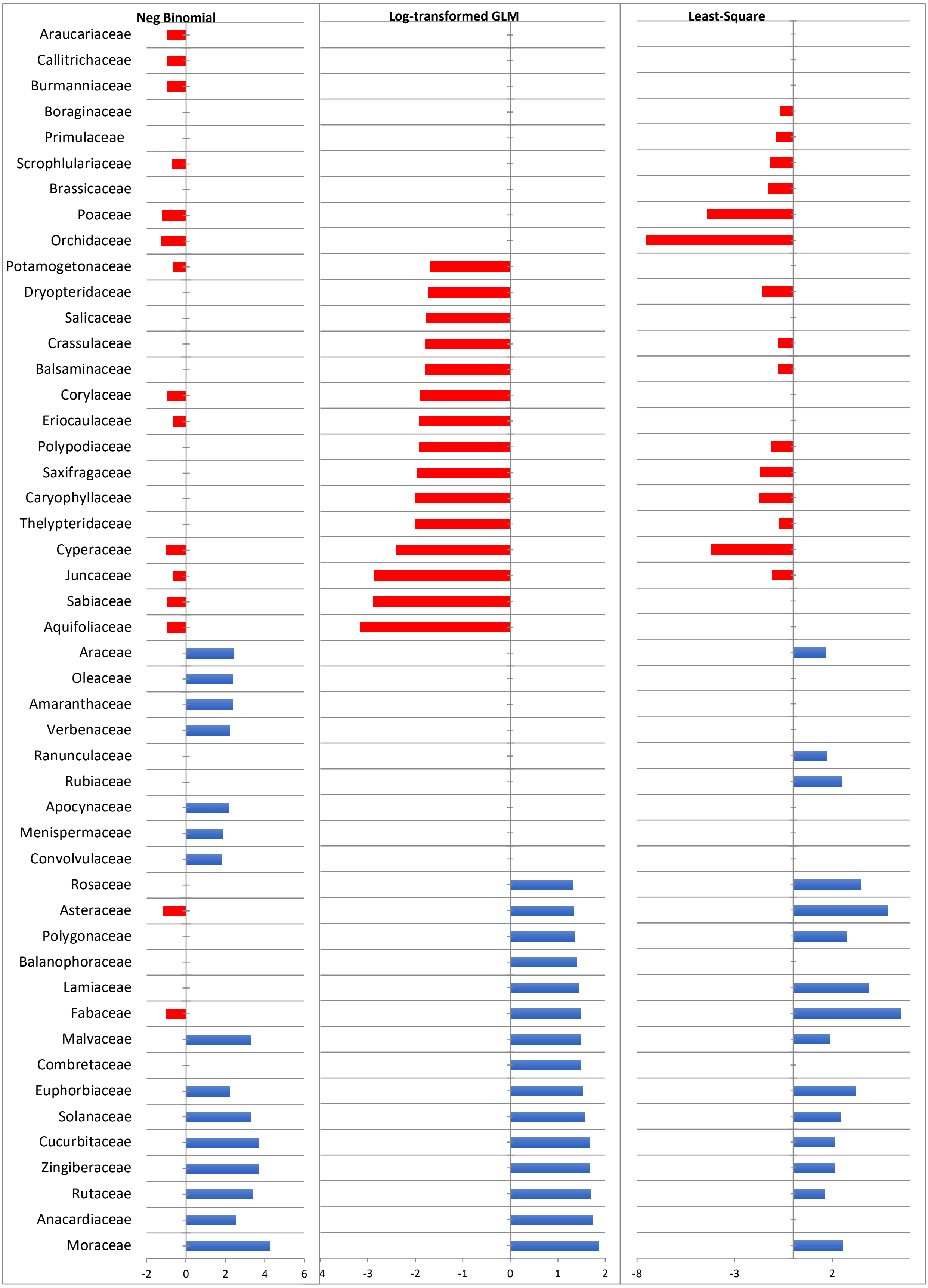

Figure 4. Studentized residuals of least-square, log-transformed and negative-binomial analyses

Table 3. Important ethnomedicinal plant families in traditional medicines in Nepal

District/ Family	Asteraceae	Lamiaceae	Fabaceae	Euphorbiaceae	Poaceae	Rosceae	Amaranthaceae	Solanaceae	Moraceae	Rutaceae	Zingiberaceae	Ranunculaceae	Apiaceae
Arghakhanchi	6	5			4	4	4						
Baglung	7	5		4		4			4				3
Baitadi	3			2				2		2	2		
Banke	2	3	4	5	2		2	2	2				
Bara	12	8	8	3			3	3					
Bardiya	3	4	8	3	3		4						
Bhaktapur	6	4	3		3			7		5	4		
Bhojpur	5	2	1			1				2	2		
Chitwan	3	4	4	3			3						
Dang	7	5	8	5				4					
Darchula	8		2		5	7							2
Dhading	7	3	7	7					3	3			
Dhankuta	2	4	1	1			1		1				
Dolakha	6	6			2	6			2	2	2		
Dolpa	3					4	2					2	2
Gorkha	6	4	6	3					7	3			
Gulmi		8	7	5	7						4	4	
Humla	9					13	4					12	6
Ilam						2				3		2	2
Jhapa	5	5	5	4				4					
Jumla	8	4				14						8	4
Kanchanpur	4	2	5	2	16		2						
Kapilbastu	4	4	6		2		2		4				
Kaski	9	5	7		9			7		4			
Kathmandu	5	2			2		3	2					2
Kavre	11	6	5	6			2						
Lalitpur	14	5	9		7						6		
Lamjung	6	5	6	4								3	
Makwanpur	4		18	5	5	6			6				
Manang	10	5				3						8	3

Morang		5	12					5	5				
Mustang	18	6	5			7						8	
Myagdi	5			7		4	3	4					
Nawalparasi	5	5	8	6									
Okhaldhunga		7	5			5		7		5	7		5
Palpa	4	3	10				2	2					
Panchthar	8	5	7		6	6			4	5			
Parbat	6	7	5	3									4
Parsa	3		10	2	5						4		
Ramechap	6	4	12		7				4		5		
Rasuwa	4					3				2		3	
Rukum		2	3	2		2		2				2	2
Rolpa	2	2	6			6					3	8	
Rupandehi	3	4	8	5					3				
Salyan	3	3		4	3	2	2	3					
Sankhuwasa	5	4	6		7			3		3			
Sindhupalchk		2	2	2		2					3	2	
Sindhuli	10	4	8	6			3	4					
Saptari			5	5	7				4				
Sunsari	4	5	5	2									
Syangja	5	4	7	4	7	3			5				
Tehrathum	5	5	8	3	3				5	3	3		
Frequency	45	42	39	29	21	21	16	16	15	13	12	12	11
Total species	271	185	252	113	112	104	42	61	64	42	45	62	35
Species/study	5.21	3.56	4.84	2.17	2.15	2.02	0.81	1.15	1.23	0.81	1.17	0.87	0.33

Arghakhanchi (Poudel *et al.* 2021); Baglung (Manandhar 1993); Bara (Singh 2017); Baitadi (Thapa *et al.* 2020); Banke (Manandhar 1998); Bardiya (Pariyar *et al.* 2021); Bhaktapur (Dulal *et al.* 2022); Bhojpur (Paudyal *et al.* 2021); Chitwan (Dangol & Gurung 1991); Dhading (Kunwar *et al.* 2006); Dolakha (Karki *et al.* 2023); Darchula (Joshi *et al.* 2021); Dhankuta (Subba *et al.* 2016); Dang (Manandhar 1985); Dolpa (Kunwar & Adhikari 2005); Gulmi (Acharya 2012); Gorkha (Tamang & Sedai 2016); Humla (Rokaya *et al.* 2010); Ilam (Bhattarai 2018); Jhapa (Rai 2004); Jumla (Manandhar 1986); Kathmandu (Silwal 2020); Kapilbastu (Mahara *et al.* 2022); Kaski (Adhikari *et al.* 2019); Kanchanpur (Bhatt & Kunwar 2021); Kavre (Manandhar 1991); Lamjung (Manandhar 1987); Lalitpur (Silwal *et al.* 2023); Manang (Bhattarai *et al.* 2006); Mustang (Bhattarai *et al.* 2010); Myagdi (Manandhar 1995); Makawanpur (Luitel *et al.* 2014), Morang (Baral & Bhagat 2018); Nawalparasi (Bhattarai *et al.* 2009); Okhaldhunga (Karki *et al.* 2023); Parbat (Malla *et al.* 2015); Panchthar (Bhandari *et al.* 2021); Palpa (Singh *et al.* 2018); Rasuwa (Shrestha *et al.* 2014); Rukum (Bhatta 1999); Rolpa (Budha-Magar *et al.* 2020); Rupandehi (Singh *et al.* 2011); Sunsari (Ale-Magar *et al.* 2022); Sindhupalchok (Gurung 2023); Saptari (Chaudhary *et al.* 2021); Salyan (Kurmi & Baral 2004); Syangja (Aryal & Thapa 2019); Sindhuli (Manandhar 1990); Sankhuwasawa (Dewan *et al.* 2023); Tehrathum (Rai 2003); Parsa (Singh 2017); Ramechap (Prashan *et al.* 2020).

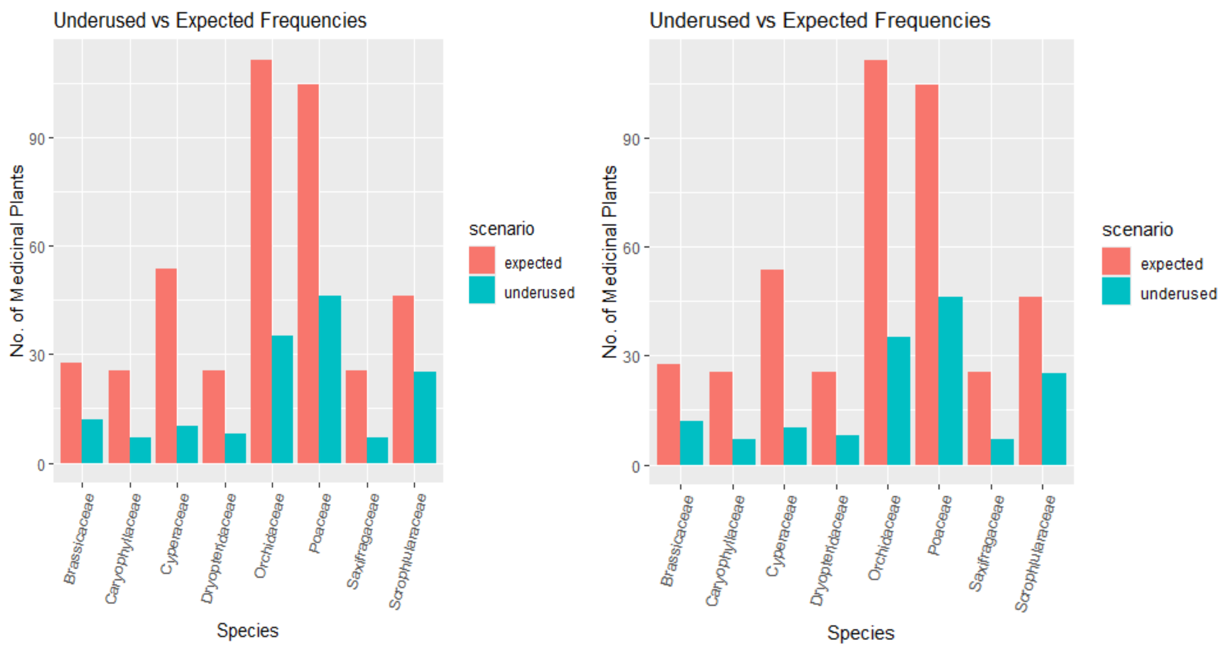

Figure 5. Side by side bar-plot of expected and observed frequency of over-used and under-used medicinal plant species

Discussion

Statistical analyses and most reported families

Total 27 plant families contributed half of the medicinal plant species (*n* = 753) of Nepal and the top five families namely Orchidaceae, Poaceae, Asteraceae, Fabaceae and Cyperaceae contributed 283 medicinal plant species (18.7%). Seven families (Lamiaceae, Asteraceae, Poaceae, Fabaceae, Malvaceae, Rutaceae, and Apiaceae) contributed nearly half of the total 216 introduced medicinal plants in Ecuador (Bennet & Prance 2000). Fabaceae, Asteraceae and Lamiaceae were also found overutilized in Thailand (Phumthum *et al.* 2019). The overuse of Asteraceae and Lamiaceae in Pakistan is a rather interesting result, given the extensive use of these families as medicinal in the country (Khan *et al.* 2011).

All five statistical analyses revealed that some plant families were over-represented, and some plant families were under-represented in traditional medicines of Nepal. Despite the fact that the five statistical approaches applied here to estimate medicinal importance of plant families all have their strength and weaknesses, our results show remarkable overlap in the results, particularly amongst Log-transformed, Binomial, Negative-binomial, and Bayesian analyses. The result of Least-square regression (Fabaceae, Asteraceae, Lamiaceae, Rosaceae, Euphorbiaceae, Polygonaceae, Moraceae, Rubiaceae, Solanaceae, Zingiberaceae, in descending order) is rather interesting since the extensive use of plants of Fabaceae, Asteraceae and Lamiaceae (highest residuals, used more often than predicted) as medicinal is frequently reported in several ethnobotanical studies in Nepal and abroad (Table 1 and 3, Scherrer *et al.* 2005; Saslis-Lagaudakis *et al.* 2011; Phumthum *et al.* 2019; Gras *et al.* 2021). The Asteraceae (n = 143) and Lamiaceae (n = 74) solely contributed about 11% of the total medicinal plant species of Nepal (Kutal *et al.* 2022).

A review of 52 district-level (small-scale) ethnomedicinal studies of Nepal showed that the families of high-use are in the following order: Asteraceae, Lamiaceae, Fabaceae, Euphorbiaceae, Poaceae, Rosaceae, Amaranthaceae, Solanaceae, Moraceae, Rutaceae, Zingiberaceae, Ranunculaceae, etc. The review shows that each family (Asteraceae, Lamiaceae, Fabaceae, Euphorbiaceae, Poaceae, Rosaceae) holds more than two medicinal plant species. This list was partially resembled however the order was different to the findings of least-square regression. This nuanced that the least-square regression hold closer affirmation to the small-scale ethnomedicinal studies. Plant families Orchidaceae, Poaceae, Asteraceae, Fabaceae, Cyperaceae, Lamiaceae, and Ranunculaceae are the largest plant families in Nepal (Shrestha *et al.* 2022). Species of families Poaceae, Asteraceae, and Cyperaceae are associated with anthropogenic landscape and disturbed habitats (Wohlwend *et al.* 2021). From this, the simple but clear idea can be deduced, that people use with preference (or at least importantly) the plants that they easily find not far from their place of daily life, as Johns *et al*. (1990) and Kadka *et al.* (2024) stated.

Over-utilization of medicinal plants of Asteraceae, Lamiaceae, Fabaceae families in Nepalese pharmacopoeias could infer that the traditional medicines of Nepal are not merely cultural constructs and embedded in belief, systems and indigenous plants, they are indigenous and influenced by alien plant species and sociocultural development. The families Asteraceae and Fabaceae contributed 56.6% of total 30 invasive alien plant species recorded in Nepal (FRTC 2021). Among 30 invasive alien plant species in Nepal, 12 belong to Asteraceae family and five belong to Fabaceae. There were 31 species from Asteraceae and 13 from Fabaceae, out of 190 invasive plant species in Indian Himalayan region (Sekar 2012). Among the 30 invasive species, despite they are colonized only for decades, 20 are being used in Nepalese pharmacopoeias (Kunwar *et al.* 2023), revealing that there is an increasing introduction of non-indigenous plant species (Asteraceae, Fabaceae) in indigenous pharmacopoeias in Nepal. This could be explained by the fact that many medicinal plants are weeds (Bennet & Prance 2000).

In the case of moderate sample size at smaller spatial scale like ours, it is particularly important to combine statistical approaches to avoid inherent methodological biases (Phumthum *et al.* 2019). The comprehensive analysis of the results of all five statistical methods yielded the families Moraceae, Cucurbitaceae, Rutaceae, Zingiberaceae, Solanaceae, Anacardiaceae, Euphorbiaceae, Lamiaceae, Fabaceae, Rosaceae, Asteraceae and Amaranthaceae repeatedly stood out as being over-represented. Of these families, Moraceae, Cucurbitaceae, Zingiberaceae, Solanaceae, Euphorbiaceae, and Amaranthaceae have previously been reported as over-utilized (Bennett & Husby 2008; Ford & Gaoue 2017; Robles *et al.* 2020; Kapur *et al.* 1992). The most over-used family Moraceae was consistent with the findings of Weckerle *et al.* (2011). The modest use of Asteraceae in our study and in Pakistan (Khan *et al.* 2011) is a rather interesting result, given the extensive use of Asteraceae and Lamiaceae as medicinal in abroad (Moerman 1991; Kindschaer *et al.* 2013). Asteraceae plant species are commonly consumed as food plants (Ghirardini *et al.* 2007) and Lamiaceae as aromatic (Kadereit 2004), and this could affect their selection and potential use in ethnomedicine. Regarding medicinal plants, there is a slight preference for collection for herbaceous plants. Most species of Asteraceae (sunflower family) are medicinal herbs (Barkley *et al.* 2006). Many of the Lamiaceae (mint family) plants are aromatic and they are widely used as culinary herbs for aroma (Kunwar *et al.* 2015). The well-known important presence and diversity of essential oil compounds in the Lamiaceae (Lawrence 1992) account—together with the size of the family, as already commented—for its relevance in many medicinal fields.

This list of over-represented families is partially resembled to the family order of 5% significance level of negative-binomial analysis (Moraceae, Zingiberaceae, Cucurbitaceae, Rutaceae, Solanaceae, Malvaceae, and Anacardiaceae). This result concurs to the earlier study of Nepal (Kutal *et al.* 2022) where Moraceae and Euphorbiaceae were over-represented. Moraceae, a common fodder tree family of Nepal (Panthi 2013) and Euphorbiaceae is rich in terpenoides (Douwes *et al.* 2008) could affect the representation in traditional medicines in Nepal. The negative-binomial regression model was most useful for our dataset since our datasets showed largest variance in compared to mean (mean (6.51) < variance (156.31). However, the significance of each analysis is based on the sensitivity of the approach. In general, the regression method is the least sensitive method, whereas the Bayesian method is sensitive to over-used species, and the binomial method is sensitive to under-used species. Binomial analysis is also appropriate to analyze over-used and under-used medicinal species since the distribution of medicinal species are not evenly distributed among the families in the dataset. The least-square, log-transformed, negative-binomial regression for over-used and under-used medicinal plant species are influenced by size of the plant family. Smaller and community-level data sets reveal a stronger fit to the Bayesian and Binomial analysis (Bennet & Husby 2008; Weckerle *et al.* 2011; Robles *et al.* 2021). We found Balanophoraceae (a small family, only four species in Nepal) as over-represented in Bayesian analysis.

Under-represented families

Our findings of significant low-use plant families were Cyperaceae, Caryophyllaceae, Poaceae, Orchidaceae, Scrophulariaceae, Asteraceae, etc., they significantly overlapped with the findings of other studies (Amiguet *et al.* 2006; Weckerle *et al.* 2011; Gras *et al.* 2021). Given that Poaceae and Cyperaceae often depend on resprouting and physical defenses rather than chemical defenses makes them less selective for traditional medicines (Ford & Gaoue 2017). The Cyperaceae and Poaceae families do not have high biological activity, but are mentioned as used for food (Amiguet *et al.* 2006). Interestingly, the most abundant families (Cyperaceae, Poaceae, Orchidaceae) are under-represented in the Nepalese ethnopharmacopeias, supporting the hypothesis that people select plants based on traditional knowledge and culture, not random. This does not imply that underutilized plant families are not important in ethnomedicine; it rather may be an expression of people's preferences for medicinal uses for a particular area and time.

Conclusions

Most of the over- and under-represented medicinal plant families we identified concur with the results from earlier similar studies. This study also contributes to the discussion of the methodology to test the non-random theory of medicinal plant selection. Despite the fact that the five statistical approaches applied here to estimate medicinal importance of plant families all have their strength and weaknesses, our results show remarkable overlap in the results. It should be noted that regression residuals are less sensitive to unequal family sizes. However, the least-square method was found more pertinent in the sense that the over-represented medicinal plant families of this approach and that being used in traditional medicines are greatly overlapped. Over-utilization of medicinal plants of Asteraceae, Lamiaceae, Fabaceae families could infer that the traditional medicines of Nepal are not merely cultural constructs and embedded in belief, systems and indigenous plants they are increasingly influenced by alien plant species. We consider these families particularly promising for the pharmacological industry because their widespread use is most probably due to their content of physiologically active compounds.

For small and community level datasets, negative-binomial analysis was found pertinent. Thus, it is particularly important to combine statistical approaches in small and moderate-sized samples to avoid inherent methodological biases. The combined approach of all five statistical methods yielded the over-represented families in the following order Moraceae, Zingiberaceae, Euphorbiaceae, Cucurbitaceae, Solanaceae, Rutaceae, Lamiaceae, and Anacardiaceae. The order is partially resembled to the family order of 5% significance level of negative-binomial analysis. Thus, the least-square regression and negative-binomial analysis could hold the better promise to find out the over- and under-represented plant families in traditional medicines in Nepal. Identification of over- and under-represented families is a first step toward prioritization of efforts for sustainable use, conservation and ethnopharmacology.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data are available in the manuscript.

Competing interests: The authors declare that there is no conflict of interest.

Funding: Partial funding for fieldwork was received from the Department of Mathematics, University of Wisconsin-Whitewater, Whitewater, WI, US.

Author contributions: DHK designed study and methodology, managed funding for this study, carried out formal analysis, wrote and reviewed drafts and final. NRJ and CKS reviewed literature and data, wrote and reviewed drafts and final. RMK designed study and methodology, carried out data analysis, wrote and reviewed drafts and final.

Acknowledgements

Author DHK extends his deep appreciation to the Department of Mathematics, University of Wisconsin-Whitewater, Whitewater, WI, USA for providing partial funding for this study.

Literature cited

Acharya R. 2012. Ethnobotanical Study of Medicinal Plants of Resunga Hill used by Magar Community of Badagaun, Gulmi District, Nepal. Scientific World 10: 54-65

Adhikari M, Thapa R, Kunwar RM, Devkota HP, Poudel P. 2019. Ethnomedicinal uses of plant resources in the Machhapuchchhre rural municipality of Kaski District, Nepal. Medicines 6:69. https://doi.org/10.3390/medicines6020069.

Ale-Magar R, Mallik AR, Chaudhary S, Parajuli S. 2022. Ethno-medicinal plants used by the people of Dharan, Eastern Nepal. Indian Journal of Traditional Knowledge 21: 72-80.

Ali M, Huang Z, Bai Y, Tng DY, Qin F, Fang Z. 2024. A multifaceted approach to expanding conservation efforts in the Pan-Himalayan landscape. Journal of Cleaner Production 476: 143783.

Amiguet VT, Arnason JT, Maquin P, Cal V, Sanchez-Vindas P, Alvarez LP. 2006. A regression analysis of Q'eqchi' maya medicinal plants from southern Belize. Economic Botany 60(1): 24-38.

Aryal P, Thapa CB. 2019. Ethno-medicinal uses of plants in Putali Bazar Municipality of Syangja district, Nepal. Himalayan Biodiversity 32-38.

Balick M, Cox P. 1996. Plants, people and culture: the science of ethnobotany. New York: Scientific American Library.

Baral BR, Bhagat I. 2018. Ethnomedicinal plants used by Dhimal community of Rajghat, Morang. Nepalese Journal of Biosciences 8(1): 36-47. https://doi.org/10.3126/njbs.v8i1.51724 36

Baral S, Kurmi P. 2006. A compendium of medicinal plants in Nepal. Kathmandu: Mrs Rachana Sharma Press.

Barkley TM, Brouillet L, Strother JL. 2006. Asteraceae. In Flora of North America Editorial Committee (ed.). Vol. 19. New York and Oxford: Oxford University Press.

Bennett BC, Husby CE. 2008. Patterns of medicinal plant use: an examination of the Ecuadorian Shuar medicinal flora using contingency table and binomial analyses. Journal of Ethnopharmacology 116:422-430. https://doi.org/10.1016/j.jep.2007.12.006.

Bennett BC, Prance GT. 2000. Introduced Plants in the Indigenous Pharmacopoeia of Northern South America. Economic Botany 54: 90-102.

Bhandari P, Gurung MB, Subedi CK, Chaudhary RP, Basnet K, Gurung J, Uprety Y, Neupane A, Shrestha KK. 2021. Traditional use of medicinal plants in the Chyangthapu-Phalaicha biological sub-corridor, Panchthar District, Kangchenjunga Landscape, Nepal. Ethnobotany Research and Applications 22: 1-43.

Bhatt MD, Kunwar RM. 2020. Distribution pattern and ethnomedicinal uses of plants in Kanchanpur district, Far-Western Nepal. Ethnobotany Research and Applications 20: 1-21.

Bhatta LR. 1999. Ethnobotanical study in a village at Rukum District, Nepal. Banko Janakari 9(2): 40-43.

Bhattarai KR. 2018. Ethnobotanical study of plants used by Thami community in Ilam District, eastern Nepal. Our Nature 16(1): 55-67.

Bhattarai S, Chaudhary RP, Quave CL, Taylor RS. 2010. The use of medicinal plants in the trans-himalayan arid zone of Mustang district, Nepal. Journal of Ethnobiology and Ethnomedicine 6: 1-11.

Bhattarai S, Chaudhary RP, Taylor RS. 2006. Ethnomedicinal plants used by the people of Manang district, central Nepal. Journal of Ethnobiology and Ethnomedicine 2: 1-8.

Bhattarai S, Chaudhary RP, Taylor RSL. 2009. Ethno-medicinal Plants Used by the People of Nawalparasi District, Central Nepal. Our Nature, 7(1): 82-99.

Budha-Magar S, Bhandari P, Ghimire SK. 2020. Ethno-medicinal survey of plants used by Magar (Kham) community, Rolpa district, Western Nepal. Ethnobotany Research and Applications 19: 1-29.

Chaudhary VK, Bhattarai S, Gautam B. 2021. Ethnomedicinal Plants of Kanchanrup Municipality Saptari, Nepal. Plant Resources 19:181-191.

Cox P. 1990. Ethnopharmacology and the search for new drugs. Ciba Foundation Symposium. Chichester: Wiley. pp. 40-47.

Dangol DR, Gurung SB. 1991. Ethnobotany of the Tharu tribe of Chitwan district, Nepal. International Journal of Pharmacognosy 29(3): 203-209.

Dewan K, Shrestha N, Sapkota RP, Rai J, Lama S, Shrestha B. 2023. Medicinal ethnobotany of the Yakkha community in eastern Nepal. Ethnobotany Research and Applications 26: 59. http://dx.doi.org/10.32859/era.26.59.1-34

Douwes E, Crouch NR, Edwards TJ, Mulholland DA. 2008. Regression analyses of southern African ethnomedicinal plants: informing the targeted selection of bioprospecting and pharmacological screening subjects. Journal of Ethnopharmacology 119: 356-364. https://doi.org/10.1016/j.jep.2008.07.040.

Dulal K, Chaudhary S, Uprety Y, Shrestha N, Shakya S, Munankarmi N. 2022. Ethnomedicinal plants used by the local people of Changunarayan Municipality, Bhaktapur, Nepal. Ethnobotany Research and Applications 23: 1-27.

Farnsworth N, Bingel A. 1977. Problems and prospects of discovering new drugs from higher plants by pharmacological screening. In: Heidelberg G, editor. New natural products and plant drugs with pharmaceutical, biological or therapeutic activity. Heidelberg: Springer.

Ford J, Gaoue O. 2017. Alkaloid-poor plant families, Poaceae and Cyperaceae, are over-utilized for medicine in Hawaiian Pharmacopoeia. Economic Botany 71: 123-132. https://doi.org/10.1007/s12231-017-9380-4.

FRTC. 2021. Building capacities to improve and sustain forest health to enhance the resilience of forests and livelihoods of forest-dependent communities in Nepal. (Final report) FAO, Nepal. Government of Nepal.

Gaoue O, Yessoufou K, Mankga L, Vodouhe F. 2021. Phylogeny reveals non-random medicinal plant organs selection by local people in Benin. Plants People Planet 66:1-11. https://doi.org/10.1002/ppp3.10202.

Ghirardini MP, Carli M, Del Vecchio N, Rovati A, Cova O, Valigi F, Pieroni A. 2007. The importance of a taste. A comparative study on wild food plant consumption in twenty-one local communities in Italy. Journal of Ethnobiology and Ethnomedicine 3: 1-14.

Gras A, Hidalgo O, D'Ambrosio U, Parada M, Garnatje T, Vallès J. 2021. The Role of Botanical Families in Medicinal Ethnobotany: A Phylogenetic Perspective. Plants 10: 163. https://doi.org/10.3390/ plants10010163.

Gurung M. 2023. Ethnomedicinal Study of Plants Used by Newar Community in Sindhupalchowk District, Nepal. Plant Resources 21(1): 32-39. https://doi.org/10.3126/bdpr.v21i1.57205

Johns T, Kokwaro JO, Kimanani EK. 1990. Herbal remedies of the Luo of Siaya District, Kenya: Establishing quantitative criteria for consensus. Economic Botany 44: 369-381.

Joshi, DR, Kunwar RM, Pokharel YR, Bhatt MD. 2021. Ecology and Ethnobotany in and around Api Nampa Conservation Area, Darchula, Nepal. Ethnobotany Research and Applications 22: 1-13.

Kadereit JW. 2004. Lamiales: introduction and conspectus. Berlin: Springer Verlag. p. 1-8.

Kapur SK, Shahi AK, Sarin YK, Daniel E. 1992. The medicinal flora of Majouri-Kirchi forests (Jammu and Kashmir State), India. Journal of Ethnopharmacology 36: 87-90.

Karki D, Khadka D, Kunwar RM, Aryal PC, Paudel HR, Bhatta S, Shi S. 2023. Ethnomedicinal plants in Champadevi rural municipality, Okhaldhunga district, Nepal. Journal of Ethnobiology and Ethnomedicine 19(1): 58.

Karki S, Dhital PA, Uprety Y, Ghimire SK. 2023. Medicinal plants and their use by an ethnic minority Jirel in Dolakha district, Central Nepal. Ethnobotany Research and Applications 25: 1-29.

Kew. 2017. State of the World's Plants 2017. Kew: Royal Botanic Gardens.

Khadka D, Kunwar RM, Baral B. et al. Prunus mira Koehne and Prunus armeniaca L. in Nepal Himalaya: distribution, use, and conservation. Genetic Resources and Crop Evolution 01923-6, https://doi.org/10.1007/s10722-024-01923-6

Khan SM, Harper D, Page SUE, Ahmad H. 2011. Residual value analyses of the medicinal flora of the western Himalayas: the Naran valley, Pakistan. Pakistan Journal of Botany 43: 97-104.

Kindscher K, Corbett S, Mcclure K, Kindscher K, Corbett S, Mcclure K. 2013. A statistical analysis of medicinal plants: a case study of plant families in Kansas and the Great Plains. Trans Kansas Acad Sci 116: 149-55.

Kunwar RM, Adhikari N. 2005. Ethnomedicine of Dolpa district, Nepal: the plants, their vernacular names and uses. Lyonia 8(1): 43-49.

Kunwar RM, Baral B, Luintel S, et al. 2022. Ethnomedicinal landscape: distribution of used medicinal plant species in Nepal. Journal of Ethnobiology and Ethnomedicine 18: 34. https://doi.org/10.1186/s13002-022-00531-x

Kunwar RM, Fadiman M, Cameron M, Bussmann RW, Thapa-Magar KB, Rimal B. 2018. Cross-cultural comparison of plant use knowledge in Baitadi and Darchula districts, Nepal Himalaya. Journal of Ethnobiology and Ethnomedicine 14:66.

Kunwar RM, Nepal BK, Sigdel KP, Balami N. 2006. Contribution to the Ethnobotany of Dhading District, Central Nepal. Nepal Journal of Science and Technology 7: 65-70.

Kunwar RM, Subedi BP, Baral SR, Maraseni T, LeBoa C, Adhikari YP, Bussmann RW. 2020. Ethnobotany of the Himalayas: the Nepal, Bhutanese, and Tibetan Himalayas. In: *Ethnobotany of the Himalayas,* Cham: Springer Publishing. pp. 65-103.

Kunwar RM, Thapa KP, Shrestha R, Shrestha PR, Bhattarai NK, Tiwari NN. 2011. Medicinal and aromatic plants network Nepal: an open access digital database. Banko Janakari 21: 48-50.

Kunwar RM, Thapa-Magar K, Subedi SC, Kutal DH, Baral B, Joshi NR, Bhandari AR. 2023. Distribution of important medicinal plant species in Nepal under past, present, and future climatic conditions. Ecological Indicators 146: 109879.

Kunwar RM, Shrestha K, Dhungana SK, Shrestha PR, Shrestha KK. 2010. Floral Biodiversity of Nepal an Update. Journal of Natural History Museum 25: 295-311.

Kurmi PP, Baral SR. 2004. Ethnomedical uses of plants from Salyan District, Nepal. Banko Janakari 14: 35-39.

Kutal D, Kunwar R, Baral K, Sapkota P, Sharma H, Rimal B. 2022. Factors that influence the plant use knowledge in the middle mountains of Nepal. PLoS ONE 66: 6-10.

Kutal DH, Kunwar RM, Uprety Y. et al. 2021. Selection of medicinal plants for traditional medicines in Nepal. Journal of Ethnobiology and Ethnomedicine 17: 59. https://doi.org/10.1186/s13002-021-00486-5

Leonti M, Fernando RR, Sticher O, Heinrich M. 2003. Medicinal flora of the Populaca, Mexico: a botanical systematical perspective. Economic Botany 57: 218-30.

Luitel DR, Rokaya MB, Timsina B, Münzbergová Z. 2014. Medicinal plants used by the Tamang community in the Makawanpur district of central Nepal. Journal of Ethnobiology and Ethnomedicine 10: 1-11.

Mahara S, Ojha P. Khan M, Bhujel A. 2022. Ethnomedicinal study of common medicinal plants of Kapilvastu district, Nepal. Journal of Medicinal Herbs 13: 19-27.

Malla B, Gauchan DP, Chhetri RB. 2015. An ethnobotanical study of medicinal plants used by ethnic people in Parbat district of western Nepal. Journal of Ethnopharmacology 165: 103-117.

Manandhar NP 1985. Ethnobotanical Notes on Certain Medicinal Plants Used by Tharus of Dang-Deokhuri District, Nepal, International Journal of Crude Drug Research 23(4):153-159, DOI: 10.3109/13880208509069022

Manandhar NP. 1986. Ethnomedicine of Jumla district Nepal. Intl Journal of Crude Drug Research 24: 81-89.

Manandhar NP. 1987. Traditional medicinal plants used by tribals of Lamjung District, Nepal. International journal of Crude Drug Research 25: 236-240.

Manandhar NP. 1990. Traditional phytotherapy of Danuwar tribe of Kamlakhinj in Sindhuli district Nepal. Fitoterapia LXI: 325-331.

Manandhar NP. 1991. Medicinal plant lore of Tamang tribe of Kabhrepalanchok district, Nepal. Economic Botany 45: 58-71.

Manandhar NP. 1993. Ethnobotanical note on folklore remedies of Baglung district Nepal. Contributions to Nepalese Studies 20: 183-196.

Manandhar NP. 1995. An inventory of some herbal drugs of Myagdi District, Nepal. Economic Botany 49: 371-379.

Manandhar NP. 1998. Ethnob0tanical census on herbal medicine of Banke, Nepal. Contribution to Nepalese Studies 25: 57-63.

Manandhar NP. 2002. Plants and people of Nepal. Portland: Timber Press; 2002. p. 599.

Medeiros D, Hayde A, De AUP. 2013. Does the selection of medicinal plants by Brazilian local populations suffer taxonomic influence? Journal of Ethnopharmacology 146: 842-852. https://doi.org/10.1016/j.jep.2013.02.013.

Moerman D, Pemberton R, Kiefer D, Berlin B. 1999. A comparative analysis of five medicinal floras. Journal of Ethnobiology 19: 49-67.

Moerman D. 1991. The medicinal analysis of Flora. Journal of Ethnopharmacology 31:1-42.

Moerman DE. 1979. Symbols and selectivity: a statistical analysis of Native American Medical Ethnobotany. Journal of Ethnopharmacolology 1: 111-119. https://doi.org/10.1016/0378-8741(79)90002-3.

Moerman DE. 1996. An analysis of the food plants and drug plants of native North America. Journal of Ethnopharmacology 52:1-22. https://doi.org/10.1016/0378-8741(96)01393-1.

Muleba I, Yessoufou K, Rampedi IT. 2020. Testing the non-random hypothesis of medicinal plant selection using the woody flora of the Mpumalanga. Environment, Development and Sustainability 23: 4162-4173. https://doi.org/10.1007/s10668-020-00763-5.

Panthi MP. 2013. Indigenous knowledge on use of local fodder tress in mid-hills of West Nepal. Tribhuvan University Journal 28: 171-80.

Pariyar D, Miya MS, Adhikari A. 2021. Traditional uses of locally available medicinal plants in Bardiya district, Nepal. Journal of Medicinal Herbs 12(2): 85-92.

Paudyal, SP, Rai A, Devdas B, Paudel N. 2021. Ethnomedicinal knowledge on Rai community of Ramprasadrai rural municipality, Bhojpur district, eastern Nepal. European Journal of Biological Research 11(3): 367-380

Phillips O, Gentry AH. 1993. The useful plants of Tambopata, Peru: I. Statistical hypotheses tests with a new quantitative technique. Economic Botany 47:15-32. https://doi.org/10.1007/BF02862203.

Phumthum M, Balslev H, Barfod AS. 2019. Important Medicinal Plant Families in Thailand. Frontiers in Pharmacolology 10:1125. doi: 10.3389/fphar.2019.01125

Poudel B, Bhandari J, Poudel A, Gautam D. 2021. Ethnomedicinal use of common garden species in Arghakhanchi district, Western Nepal. Asian Journal of Pharmacognocy 4: 31-65.

Pradhan SP, Chaudhary RP, Sigdel S, Pandey BP. 2020. Ethnobotanical Knowledge of Khandadevi and Gokulganga Rural Municipality of Ramechhap District of Nepal. Ethnobotany Research and Applications 20: 1-32.

Press JR, Shrestha KK, Sutton DA. 2000. Annotated checklist of flowering plants of Nepal. London: The Natural History Museum.

Rai MB. 2003. Medicinal Plants of Tehrathum District, Eastern Nepal. Our Nature 1: 42-48

Rai SK. 2004. Medicinal Plants used by Meche People of Jhapa District, Eastern Nepal. Our Nature 2:27-32

Robles Arias DM, Cevallos D, Gaoue OG, Fadiman MG, Hindle T. 2020. Non-random medicinal plants selection in the Kichwa community of the Ecuadorian Amazon. Journal of Ethnopharmacology 246: 112220. https://doi.org/10.1016/j.jep.2019.112220.

Rokaya MB, Münzbergová, Z, Timsina B. 2010. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. Journal of Ethnopharmacology 130(3): 485-504.

Saslis-lagoudakis CH, Hawkins JA, Greenhill SJ, Pendry CA, Watson MF, Tuladhar-Douglas W, et al. 2014. The evolution of traditional knowledge: environment shapes medicinal plant use in Nepal. Proceeding of Royal Society of Botany 66:1-7.

Saslis-Lagoudakis CH, Williamson EM, Savolainen V, Hawkins JA. 2011. Cross-cultural comparison of three medicinal floras and implications for bioprospecting strategies. Journal of Ethnopharmacolology 135: 476-487.

Savo V, Joy R, Caneva G, Mcclatchey WC. 2015. Plant selection for ethnobotanical uses on the Amalfi Coast (S Italy). Journal of Ethnobiology and Ethnomedicine 11: 58.

Scherrer AM, Motti R, Weckerle CS. 2005. Traditional plant use in the areas of monte vesole and ascea, Cilento national park (Campania, Southern Italy). Journal of Ethnopharmacology 97(1): 129-143.

Sekar KC. 2012. Invasive Alien Plants of Indian Himalayan Region— Diversity and Implication. American Journal of Plant Sciences 3: 177-184. doi:10.4236/ajps.2012.32021

Shrestha KK, Bhandari P, Bhattarai S. 2022. Plants of Nepal. Heritage publishers, Kathmandu, Nepal. 1088 p.

Shrestha N, Shreshta KK, Prasai DS, Shrestha S, Xhang ZC. 2014. Ethnomedicinal practices in the highlands of central Nepal: A case study of Syaphru and Langtang village in Rasuwa district, Nepal. Journal of Ethnopharmacology 155: 1204-1213.

Silwal-Gautam R, Shrestha SJ, Shrestha I. 2023. Ethnomedicinal Plant Resources of Tamang Community in the Konjyosom Rural Municipility, Central Nepal. Ethnobotany Research and Applications 25: 1-29.

Silwal-Gautam R. 2020. Medicinal plants and their traditional uses in Ramkot Village, Kathmandu Nepal. Patan Pragya 6(1): 180-190.

Singh A, Panthi M, Tewari DD. 2011. Ethno Medicinal Plants Used by the Tharu and Magar Communities of Rupandehi District, Western Nepal. Current Botany 2(2): 30-33. www.scholarjournals.org

Singh AG, Kumar A, Tewari DD, Bharati KA. 2018. New ethnomedicinal claims from Magar community of Palpa district, Nepal. Indigenous Journal of Traditional Knowledge 17(3): 499-511.

Singh S. 2017. Ethnobotanical Study of Wild Plants of Parsa District, Nepal. Ecoprint 24: 1-12. https://doi.org/10.3126/eco.v24i0.20641

Subba B, Srivastava C, Kandel RC. 2016. Scientific validation of medicinal plants used by Yakkha community of Chanuwa VDC, Dhankuta, Nepal. SpringerPlus 5:155. DOI 10.1186/s40064-016-1821-5

Tamang R, Sedai DR. 2016. Documentation of ethnomedicinal knowledge on plant resources used by Baram community in Arupokhari VDC, Gorkha District, Central Nepal. Plant Resources 38: 60-64.

Thapa CB. 2020. Ethnomedicinal practices by Tharu ethnic community in Rupandehi and Nawalparasi districts, Western Nepal. Journal of Institute of Science and Technology 25(2): 93-106.

Thapa S, Rawal S, Prasai A, Adhikari J, Bist S, Ghimire A. 2020. A case study of medicinal plants and their usage by the local community of Dilasaini Gaunpalika, Baitadi district, Nepal. Archives of Agriculture and Environmental Science 5(1): 50-54. https://doi.org/10.26832/24566632.2020.050107

Weckerle CS, Cabras S, Castellanos ME, Leonti M. 2011. Quantitative methods in ethnobotany and ethnopharmacology: Considering the overall flora—Hypothesis testing for over-and underused plant families with the Bayesian approach. Journal of Ethnopharmacolology 137: 837-843.

Wohlwend MR, Craven D, Weigelt P, et al. Anthropogenic and environmental drivers shape diversity of naturalized plants across the Pacific. Diversity & Distributions 27: 1120-1133. https://doi.org/10.1111/ddi.13260