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Ethnobotanical Knowledge on Medicinal Plants Used for Cutaneous Leishmaniasis in Northeastern Algeria: Application of Multivariate Approach

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Abstract: Algeria ranks second after Afghanistan for the incidence of cutaneous leishmaniasis worldwide. As a result, it is critical to expand the traditional knowledge. Interviews were conducted with 21 herbalists in Annaba and El-Tarf provinces including 26 variables. The multiple correspondence analysis and the hierarchical clustering analysis were applied using the FactoMineR package. Nineteen species were reported and derived from thirteen plant families. Among them, the most important families were the Lamiaceae with five species. According to the Relative Frequency Citation value, the most famous species are the aerial parts of *Teucrium polium* L. (57.14%). Secondly, a data matrix for the species generated showed higher contribution values in the first dimension for the lack of the topical administration form, the presence of preparation's mode by decoction and cataplasm, and the association of medicinal plants specifically with olive oil and honey, respectively. The analysis of the first dimension demonstrates a high association for three variables: Ta_F, Oa_F, and Dec_MP, with correlation ratios η^2 of 6.261E-01, 5.501E-01, and 4.772E-01 ($p<0.05$), respectively. Finally, the cluster analysis of species identified three clusters discriminated by the administration form *per os* with a *p-value* of 7.49E-05 ($p<0.05$).

Keywords: leishmaniasis; FactoMineR; contribution.

1. Introduction

Leishmaniasis is a significant and devastating public health issue ranked top among notifiable parasite infections [1]. It is caused by flagellate protozoa of the genus *Leishmania*, which are transmitted by phlebotomy insects [2]. Leishmaniasis is widespread in about 100 countries, and the population at risk is estimated to be around 350 million [3]. There are three clinical types of Leishmaniasis, depending on the species: localized cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). CL is the most common clinical manifestation worldwide, affecting 12 million people and adding about 2 million new cases annually [4]. Furthermore, Afghanistan, Algeria, Brazil, Iran, Pakistan, Peru, Saudi Arabia, and Syria account for 90% of all cases worldwide [5]. In Algeria, three common species are encountered: *L. infantum*, which causes the CL and VL forms of the disease in the northern part of the country; *L. killicki*, which causes the CL form in Central Southern Algeria (Ghardaïa region), and *L. major*, which is spread in the Central Eastern region of Algeria, with the M'Sila province being the most affected [6].More than a quarter of a million (252,659) cases of CL

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were registered in the country between 1982 and 2017 in five provinces (Bechar, El Oued, Batna, Biskra, and M'Sila), accounting for more than 70% of the total number of cases [6]. Given the epidemiology of leishmaniasis, the scarcity of treatment and the severe side effects of existing drugs such as amphotericin B and pentamidine, the lack of successful vaccines, and the lack of interest from major pharmaceutical companies, finding new molecules with leishmanicidal activity has become an urgent challenge [7]. As a result, a global program for the prioritized assessment of natural products against the various forms of leishmaniasis is in demand [3]. Ethnopharmacological knowledge is a holistic systems approach that can create better, safer, more accessible, and sustainable medicines [8, 9]. It is also worth noting that despite numerous published works over the last decade exploring the diversity of ethnopharmacological research in Algeria [10-27], only three papers reported on the medicinal plants used for the treatment of cutaneous leishmaniasis in Algeria [28-30]. Besides, the antileishmanial activities of medicinal plants from Morocco and Tunisia have shown that natural products may inhibit the growth of several *Leishmania* species, such as *L. major* (cutaneous leishmaniasis) and *L. infantum* (visceral leishmaniasis) [31-36].

In the last decades, the use of multivariate analyses in various ethnosciences has risen significantly, allowing for quantitative examinations of various topics linked to traditional knowledge and its applications [37]. In 1979, Moerman outlined the first strategy, which was used for Native American medicinal plant utilization [37]. The author proposed the first statistical multiple linear regression model between two variables, tested the hypothesis that medicinal usage is a function of generic and particular availability, and found significant selectivity in plant use. The multivariate approaches are particularly beneficial for understanding the exploitation of biodiversity in ethnomedicinal systems, demonstrating that selecting valuable taxa may be explained by objective criteria rather than being driven by chance [38]. Höft *et al.* (1999) described the application of multidimensional approaches in the ethnobotanical area for the first time. The study's objective is to determine these statistical methodologies to identify the similarity of patterns between indigenous people and local plants [39]. These methodologies have been investigated to analyze the ethnobotanical data set, including the description of traditional knowledge and the relevance of use by different ethnic, social, or gender groups [39]. Seven years later, this novel strategy was utilized to determine the specificity of the link between the plant species employed for treating type 2 diabetes by the Cree Nation, the native people in Quebec [40]. There is a constant increase in studies focusing on using these methodologies in ethnobotany and ethnopharmacology [41-44].

According to the reviews conducted by this group, no studies have highlighted the multidimensional elements of CL correlating with ethnopharmacology and ethnobotany data in Algeria. As a result, there is a clear need to develop a new approach to ethnobotanical and ethnopharmacological research in Algeria.

Consequently, the objectives of this study were (a) to use multivariate analyses to investigate the association between medicinal plants used by herbalists, (b) to evaluate the most frequently used species, and (c) to investigate factors associated with the CL's traditional knowledge in northeastern Algeria.

2. Materials and Methods

2.1. Description of the Study Sites

Interviews were held with established herbalists in two districts situated in Northeastern Algeria namely Annaba and El Tarf as shown in figure 1. The Annaba station (36°53'59'' North, 7°46'00'' East) is 533 km from Algiers (1439 Km^2 area). The El Tarf station (36°75'58'' North, 8°22'12'' East) is 589 km from Algiers (3339 Km^2 area). According to the Köppen and Geiger climate classification, the climate of the two study locations is warm and classified as «Csa» hot-summer Mediterranean climat , with average temperature values (A: 18.4°C, T: 18.3°C) and average annual rainfall values (A: 712 mm, T: 694 mm) [45].

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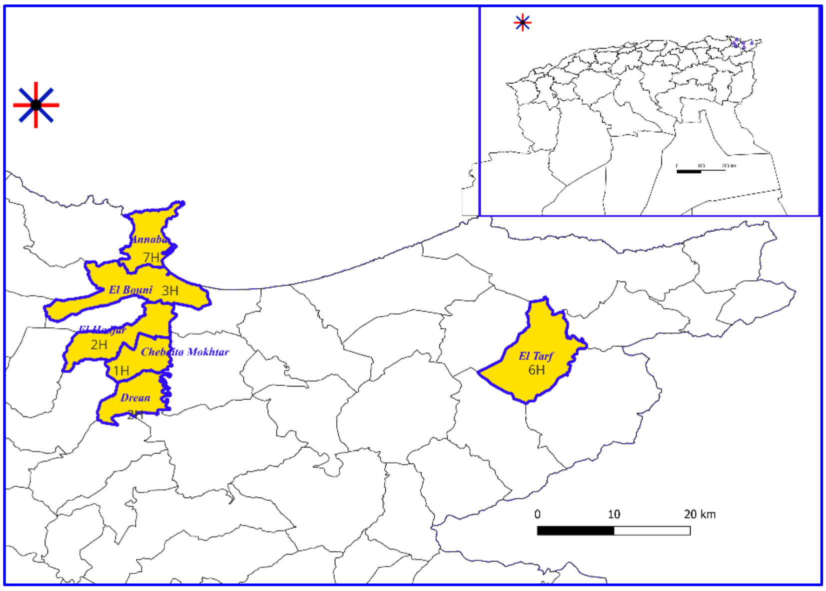

Figure 1. Geographical position of districts in the study area map.**H:** herbalists

2.2. Data Collection

2.2.1. Qualitative Variables

The ethnobotanical approach was based on describing the use of the traditional treatment. The questionnaire addressed traditional treatments used in the treatment of CL and included closed questions that required a 'yes' or 'no' response. As a result, the questions were semi structured [46]. The interviews were conducted entirely in Arabic.

The questionnaire was prepared according to the model described by Chassagne et al. (2022) [47]. Due to the absence of an ethics committee in Algeria, the ethical guidelines provided by the International Society of Ethnobiology (http://www.ethnobiology.net/) were strictly followed [48].

The questionnaire was focused on five aspects of use: 1) the part of the plant used, 2) the mode of preparation, 3) the form of administration, 4) the possibility of association with various natural products, and 5) the frequency of use. Each item was subdivided into qualitative variables (25 qualitative variables): 1-a: aerial part, 1-b: bark, 1-c: bulb, 1-d: flower, 1-e: fruit, 1-f: leaves, 1-g: roots, 1-h: resin, and 1-i: seeds; 2-a: decoction, 2-b: infusion, 2-c: cataplasm, 2-d: juice, 2-e: latex, 2-f: powder, and 2-g: oil; 3-a: topical administration or 3-b: oral administration; 4-a: honey, 4-b: olive oil, 4-c: cow's milk, or 4-d: plant; 5-a: once a day, 5-b: twice a day, or 5-c: thrice a day.

2.2.2. Quantitative Variables

A supplementary quantitative variable (V26) was determined in this survey, represented by the relative frequency of citation percentage. The Relative Frequency Citation (RFC) index indicated the local importance of each plant species based on the number of citations by the informants. Based on the number of citations by informants, the Relative Frequency Citation (RFC) index determined the local relevance of each plant species [49]. This index was calculated by making specific changes to the formula used in prior research

$$RFC = FC \div N$$

$$0 < RFC < 1$$

Where *FC* indicates the number of citations given by the total number of informants showing the use of each species, and *N* denotes the total number of informants interviewed in the study.

2.3. Authentication of Medicinal Plants

Each voucher specimen was identified by comparing it with the morphological characteristics described in the Algerian flora [50]. The scientific name of each species was checked and asserted based on their identification in the international database the plant list [51].

2.4. Multivariate Analysis

This research used new statistical methods to evaluate the traditional knowledge for treating CL in North East Algeria. The multivariate analyses were performed with the assistance of r software (4.2.2) [52], an open-source programming language and

environment for statistical and graphical techniques, with the assistance of the package "FactoMineR"[53], using the graphical interface "Factoshiny" dedicated to multivariate data analysis taking and into account the main features: different types of variables (active and supplementary elements), the structure of the data (a partition on the variables and the individuals), and a hierarchical structure on the variables [54].

2.4.1. Structure of the Data

The variables of a multivariate matrix could be classified as multistate or binary. The 25 qualitative variables for this dataset were considered "binary variables". It is noteworthy to codify the qualitative variables data into a binary data set with the possibilities of states or modalities as: "no"="0" or "yes"="1". The resulting "*descriptive"* data on traditional knowledge was transformed into an analytical "*statistical"* table known as a "*Complete Contingency Table"*.

2.4.2. Description of the Dimensions

Each dimension of a multivariate analysis can be described by the active variables (quantitative and/or categorial). The description of the individuals (=species) and the descriptors (=variables) is an essential step for defining the type of multivariate analysis. The multivariate matrix was used to characterize the comparison between the identified species and the correlation between the descriptors [37].

2.4.3. Multiple Correspondence Analysis (MCA)

According to the nature of the variables, the data set of this study was submitted to Multiple Correspondence Analysis (MCA). The data frame was constructed with 19 rows and 26 columns. Rows represented objects or species, while columns represented the 25 actives qualitative variables and one supplementary quantitative variable.

The qualitative matrix was used in MCA to generate two quantitative matrices. The output matrix for «*items*» comprises 19 rows and 25 columns (coordinates against each of 25 axes), with columns arranged in decreasing order of explained variance from the source matrix. The «*variable*» output matrix has 50 rows (variable modalities) and 19 columns (coordinates against the same 25 axes).

For one qualitative variable, in the first step, a descriptive analysis was obtained from the frequency derived from the data set described in the section on data collection. Then, in the second step, the eigenvalue, the contribution, and the correlation for the main features were derived. Understanding and interpreting the data in the results section is essential to clarifying the distinctions between these parameters.

The eigenvalues in the MCA reproduce, in descending order, the highest variation of inertia among the observations; the total number of eigenvalues is determined by considering the dimension of the dataset as indicated in Equation (1):

$$p = n - k$$

Where k is the number of variables included in the MCA and n is the total number of possible values the variables may assume. This contribution indicates which variables best explain the variations in the data set and are essential in the axis' development. Conversely, the correlation reflects the relationship between two variables or the degree of impact of one variable over the other [57]. As a result, a one-way analysis of variance was done with the coordinates of the individuals on the axis explained by the categorical variable. The student *t-test* was then used to compare the category average to the overall average [54].

2.4.4. Hierarchical Clustering Analysis (HCA)

The MCA was applied in the HCA analysis using Ward's minimum variance approach [55]. Ward's linkage method attempts to group observations to reduce cluster variation. As a result, an observation is deemed a cluster member if its inclusion in that cluster results in a minimal increase in the error sum of squares [56]. The distance of the

Ward's method (D_{AB}) is calculated using Equation (2):

$$D_{AB} = \frac{\|\underline{X_A} - \underline{X_B}\|^2}{\frac{1}{N_A} + \frac{1}{N_B}}$$

Where A and B represent the set of clusters, N_A and N_B are the number of observations in clusters A and B, respectively; $\underline{X_A}$ and $\underline{X_B}$ demonstrate the mean vectors representing clusters A and B and $\|\underline{X_A} - \underline{X_B}\|^2$shows the squared Euclidean distance between the vectors $\underline{X_A}$ and $\underline{X_{B.}}$

Cluster analysis was used to *i)* characterize the partition of classes or groups of species used in traditional cutaneous leishmaniasis treatment (in terms of quantitative variables) and *ii)* characterize the parameters that best describe the partition using the Chi2 test (by increasing the *p*-value) between the qualitative variable and the class variable. Based on their ethnopharmacological applications in CL, our research produced a dendrogram representing the number of species in each class.

3. Results

3.1. Sociodemographic Features of Informants

A total of 21 herbalists were interviewed, with 57.10% belonging to Annaba and 42.90% from El Tarf provinces. The average age of all participants was 53.4 ±16.7 years old, ranging from 20 to 60 years old. The vast majority of participants (95.2%) were men. A substantial percentage of participants (76.20%) stated that their professional experience had made them the most informed in traditional medicine (Table 1).

Table 1. Sociodemographic features of interviewees.

Characteristics	Frequency	Percentage (%)
Location		
Annaba	12.0	57.1
El Tarf	9.0	42.9
Gender		
Men	20.0	95.2
Women	1.0	4.8
Age (years)		
[20-30[	2.0	9.5
[30-40[	8.0	38.1

[41-60[	10.0	47.6
≥60	1.0	4.8
Level Education		
Illiterate	8.0	38.1
Primary	5.0	23.8
Secondary	7.0	33.3
University	1.0	4.8
Information Source		
Professional experience	16.0	76.2
Internet documentation	5.0	23.8

3.2. Distribution of Medicinal Plants According To The Relative Frequency Index

Table 2 shows the descriptive analysis of ethnobotanical data collected from interviews with herbalists in the Annaba and El Tarf districts. Herbalists recommended 19 therapeutic herbs from 13 plant families, where the Lamiaceae was the most abundant species (five), followed by the Amaryllidaceae (two species) and the Asteraceae (two species).

The species were ranked according to their frequency of citations, and the top two species were *Teucrium polium* L. (57.14%), followed by *Allium sativum* L. (23.80%). Other significant species also cited were *Juniperus phoenicea* L. (14.28%) and *Rosmarinus officinalis* L. (9.52%). The results also showed that 14 medicinal plants [sp1: bulb, sp3: leaves, sp4: leaves, sp5: bark, sp6: fruit, sp7: resin, sp8: fruit, sp10: stem, sp12 seeds, sp13: aerial part, sp14: leaves, sp15: leaves, sp16: aerial part, and sp19: leaves] demonstrated a similar value in the frequency index (4.76%). The most represented parts were bark, flower, root, resin, and stem, with a similar frequency of occurrence estimated as 18.

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Table 2. Alphabetical list of antileishmanial species mentioned during the ethnobotanical survey.

N°_Species	Family	Local Name	Organ	Preparation Form	Application	Association	Frequency of use	Informants	RFC (%)
sp1_*Allium cepa* L.	Amaryllidaceae	*Bsal*	B_O	Cat_MP	Ta_F	No_AS	t2.d_FU	1	4.76
sp2_*Allium sativum* L.	Amaryllidaceae	*Thoum*	B_O	Cat_MP, Jce_MP	Ta_F	Oo_AS	t2.d_FU	5	23.80
sp3_*Aloe vera* L.	Aloeaceae	*Sebbae*	L_O	Ltx_MP	Ta_F	No_AS	t2.d_FU	1	4.76
sp4_*Artemisia herba alba* Asso.	Asteraceae	*Chih*	L_O	Oil_MP	Ta_F	No_AS	No_FU	1	4.76
sp5_*Cinnamomum zeylanicum* Blume	Lauraceae	*Qarfa*	Ba_O	Dec_MP	Oa_F	No_AS	t1.d_FU	1	4.76
sp6_*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	*Handel*	Fr_O	Cat_MP	Ta_F	Oo_AS Pl_AS	t1.d_FU	1	4.76
sp7_*Commiphora*	Burseraceae	*El Mour*	Rs_O	Ltx_MP	Ta_F	Oo_AS	t2.d_FU	1	4.76

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myrrha (T. Nees) Engl.									
sp8_*Ficus carica* L.	Moraceae	*Kartous*	Fr_O	Ltx_MP	Ta_F	No_AS	t1.d_FU	1	4.76
sp9_*Juniperus phoenicea* L.	Cupressaceae	*Aaraar*	L_O, Fr_O	Dec_MP	Ta_F	No_AS	t1.d_FU t2.d_FU t3.d_FU	3	14.28
sp10_*Launaea arborescens* (Batt.) Murb.	Asteraceae	*Oumlbina*	R_O	Ltx_MP	Ta_F	No_AS	t2.d_FU	1	4.76
sp11_*Lavandula angustifolia* Mill.	Lamiaceae	*Khzama*	Fl_O	Dec_MP	Ta_F	No_AS	t3.d_FU	3	14.28
sp12_*Lepidium sativum* L.	Brassicaceae	*Habrchad*	S_O	Pwd_MP	Ta_F	Cm_AS	t2.d_FU	1	4.76
sp13_*Marrubium vulgare* L.	Lamiaceae	*Merriouet*	Ap_O	Pwd_MP	Ta_F	No_AS	No_FU	1	4.76
sp14_*Nerium oleander* L.	Apocynaceae	*Defla*	L_O	Pwd_MP	Ta_F	No_AS	t3.d_FU	1	4.76

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sp15_*Olea europaea* L.	Oleaceae	*Zitoun*	L_O	Pwd_MP	Ta_F	Oo_AS	No_FU	1	4.76
sp16_*Retama raetam* (Forssk.) Webb	Fabaceae	*Rtem*	Ap_O	Dec_MP	Ta_F	Oo_AS Pl_AS	No_FU	1	4.76
sp17_*Rosmarinus officinalis* L.	Lamiaceae	*Iklil djabel*	L_O	Dec_MP	Ta_F	No_AS	t3.d_FU	2	9.52
sp18_*Teucrium polium* L.	Lamiaceae	*El-Khiyata*	Ap_O	Dec_MP, Inf_MP, Cat_MP	Ta_F	No_AS	t2.d_FU t3.d_FU	12	57.14
sp19_*Thymus vulgaris* L.	Lamiaceae	*Zaatar*	L_O	Dec_MP	Oa_F	H_AS	t2.d_FU	1	4.76

Analyzed of 25 qualitative variables: Ap_O: Aerial part, Ba_O: Bark, B_O: Bulb, Fl_O: Flower, Fr_O: Fruit, L_O: Leaves, R_O: Roots, Rs_O: Resin, S_O: Seeds, Dec_MP: Decoction, Inf_MP: Infusion, Cat_MP: Cataplasm, Jce_MP: Juice, Ltx_MP: Latex, Pwd_MP: Powder, Oil_MP: Oil, Ta_F: Topical administration, Oa_F: Oral administration, H_AS: Honey, Oo_AS: Olive Oil, Cm_AS: Cow's Milk, Pl_AS: Plant t1.d_FU: once a day, t2.d_FU: twice a day, t3.d_FU: thrice a day, No_AS: No association, No_FU: No frequency of use, **Modalities of variables:** Each variable was recoded with a binary code:"No"= "0": or "yes"="1", **Supplementary quantitative variable:** RFC: Relative Frequency Citation. The name of each species was checked with the online database «The Plant List».

3.3. Multiple Correspondence Analysis (MCA)

Regarding the descriptive results and the frequency of responses connected to CL traditional knowledge in Northeastern Algeria, all qualitative variables were chosen for MCA analysis. Three parameters were identified using this method: the eigenvalue *(p)*, the contribution *(ctr)*, and the square ratio correlation *(*η^2*)*.

3.3.1. Inertia Distribution and Eigenvalues

The variance for the first dimension (x-axis) was recorded to be 16.139% (p=0.155), while the second dimension (y-axis) was 12.926% (p=0.124). The inertia (sum of variances) for these two first dimensions was 29.07% of the whole dataset inertia; that means that 29.07% of the individuals (or variables) cloud total variability is explained by the plane (Figure 2).

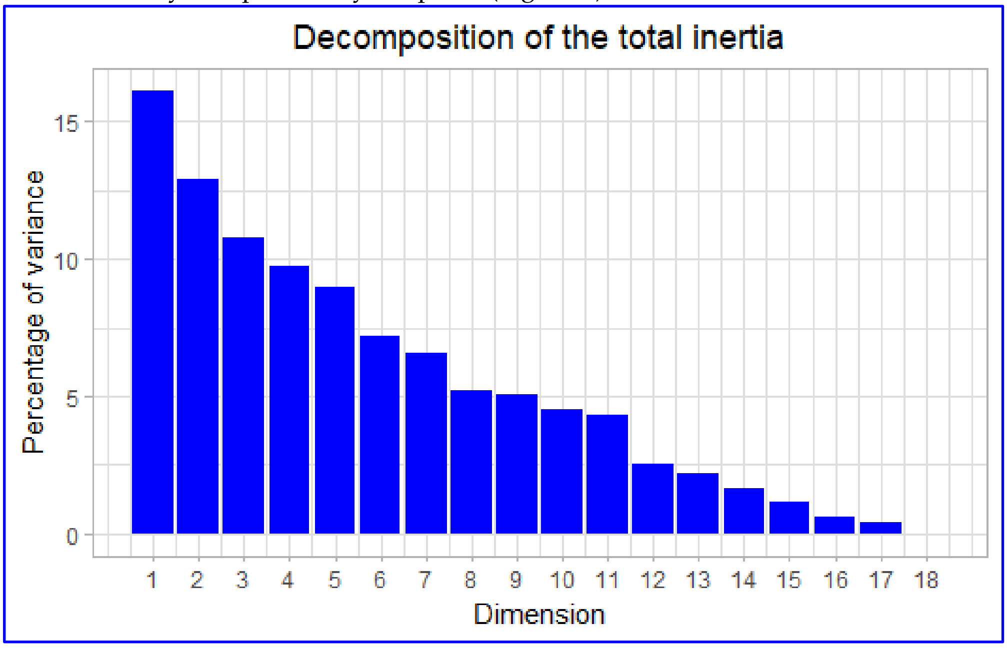

Figure 2. Percentage of explained variances of the overall dimensions.

3.3.2. Distribution of species within variables

The graphical representation of the contribution of the individual species on the first two axes is shown in the figure 3. This presentation analyzes the states of the first descriptor compared to the states of the second descriptor. The visualization of the results was carried out with a scatter diagram. The variability or similarity of species identified in the study was described according to the descriptors used.

The first dimension opposes species characterized by a higher contribution value with positive coordinates on the axis-x [**sp5**: ctr=17.738, x=0.723, **sp11**: ctr=11.128, x=0.573, **sp19**: ctr=25.292, x=0.864] to species with negative coordinate values on the axis-x [**sp 2**: ctr=11.869, x=-0.592, **sp6**: ctr= 9.425, x=-0.527, **sp16**: ctr= 10.633, x=-0.560). Concerning the first dimension, the second axis opposes similarities with a strongly positive coordinate on the axis-y [**sp16**: ctr=14.363, y=0.582, **sp6**: ctr= 11.221, y=0.515, and **sp18**: ctr=12.497, y= 0.543] to species [**sp12**, **sp10** and **sp7**] characterized by a negative coordinate value [ctr=28.727/y=-0.824, ctr=7.376/y=-0.417, ctr=4.635/ y=-0,331].

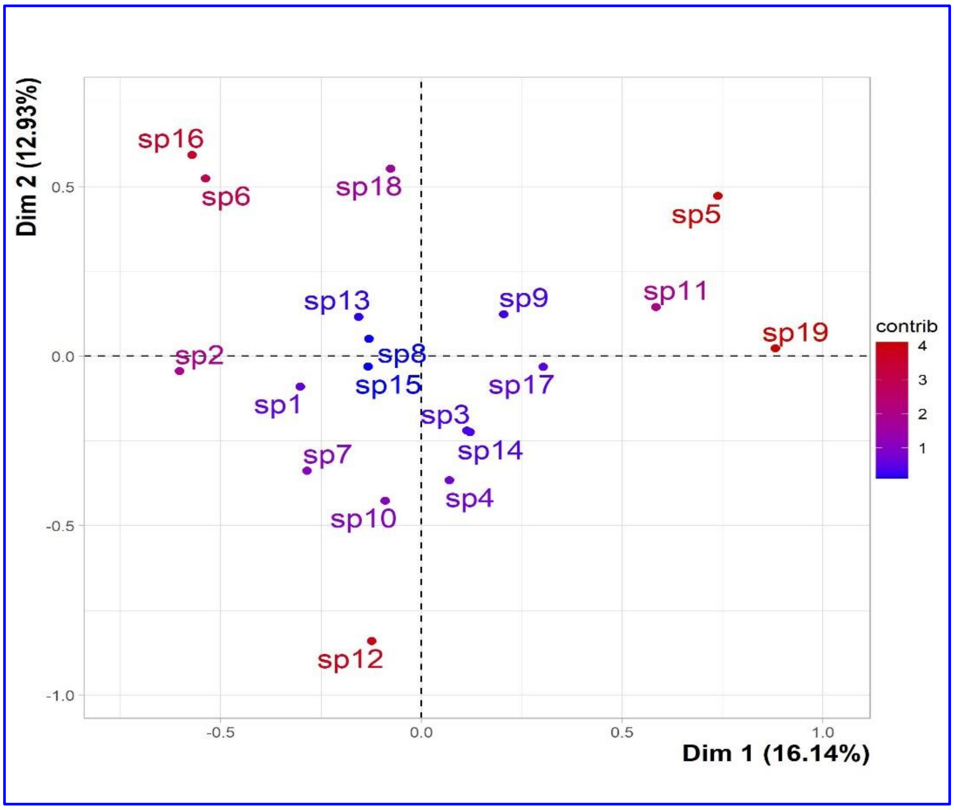

Figure 3. Scatter plot of plant species according to their contribution to the two first dimensions.

Legend of species (sp) factor map: The labeled individuals (species in red) are those with the higher contribution to the plane construction.

Species legends: **sp1**, Allium cepa L.; **sp2**, Allium sativum L.; **sp3**, Aloe vera L.; **sp4**, Artemisia herba alba Asso.; **sp5**, Cinnamomum zeylanicum Blume; **sp6**, Citrullus colocynthis (L.) Schrad.; **sp7**, Commiphora myrrha (T. Nees) Engl.; **sp8**, Ficus carica L.; **sp9**, Juniperus phoenicea L.; **sp10**, Launaea arborescens (Batt.) Murb.; **sp11**, Lavandula angustifolia Mill.; **sp12**, Lepidium sativum L.; **sp13**, Marrubium vulgare L.; **sp14**, Nerium oleander L.; **sp15**, Olea europaea L.; **sp16**, Retama raetam (Forssk.) Webb; **sp17**, Rosmarinus officinalis L.; **sp18**, Teucrium polium L.; **sp19**, Thymus vulgaris L.

To facilitate the interpretation of the cloud of species, a graphical depiction of variables was employed (Figure 4). In this presentation, each variable coordinates the square correlation ratios (denoted as η^2) between the variable and the coordinates of the individuals on the first and second axes, respectively.

In this way, the first dimension showed a higher correlation with the Ta_F variable, with the square correlation ratio (η^2) estimated as 0.626 ($p<0.05$). This correlation with the first dimension was followed by two variables, Oa_F and Dec_MP, with values of η^2: 0.550 and 0.477 ($p<0.05$). In parallel, the frequency of use (t1_FU) showed a higher correlation with the second dimension, with the η^2 value estimated as 0.509.

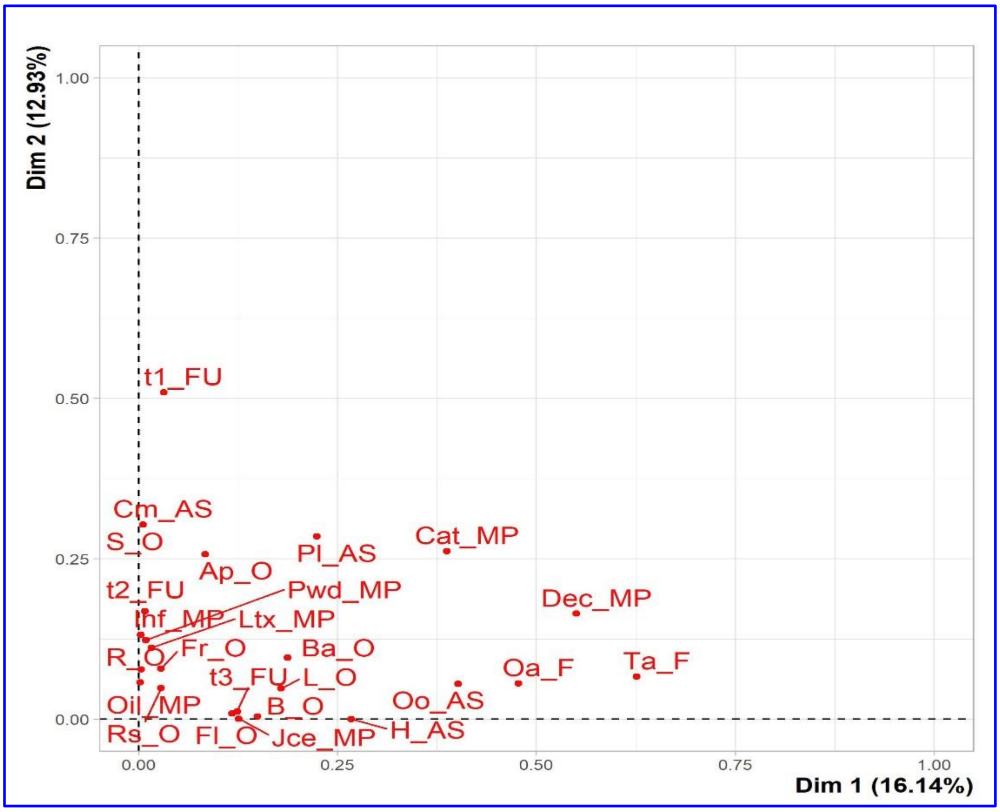

Figure 4. Square correlation ratios of the twenty-five qualitative variables on the two first dimensions.

Dim: Dimension

The data offered by the variables is broad and, hence, inadequate for interpreting the similarities and differences among the species. Consequently, it is necessary to conduct a comprehensive examination utilizing the classifications of those variables. A common method for visually depicting a category on a graph of persons is to place the category at the barycenter, which refers to the average location of the individuals who possess it in their response. Two categories exhibit proximity when the species that comprise them demonstrate a tight relationship, indicating that their general reactions to the variables are comparable [57]. The proximity between two categories is interpreted as proximity between two groups of species (Figure 5).

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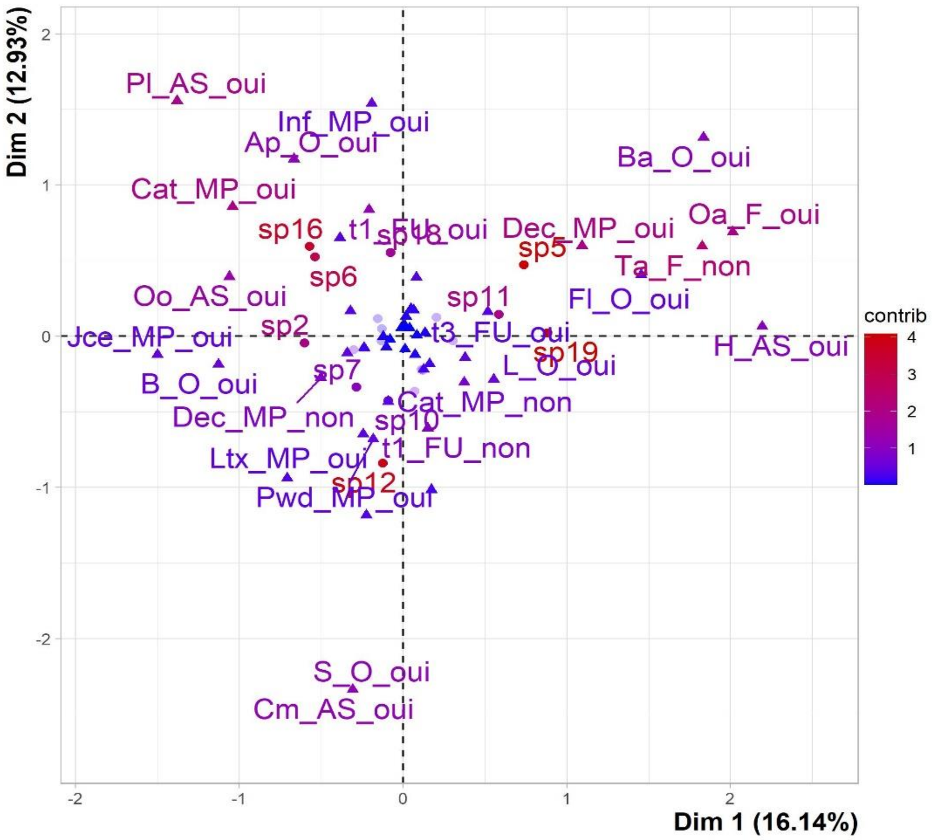

Figure 5. Two-dimensional MCA plot. Correlation between species and the category of each variable.

Here, for example, the categories Ta_F_no, Oa_F_yes, and Dec_MP_yes are superimposed as they are chosen by the same species [sp5, sp19, and sp11, sp9, sp17]. Moreover, the modalities Cat_MP_yes, Pl_AS_yes and Oo_AS_yes were closely associated with species [sp16, sp6, sp2, and sp18]. Table 3 shows the main features and their correlation ratios on the two first dimensions (1 and 2).

Table 3. Square correlation ratios of the main features with modalities.

Dimension 1			Dimension 2		
Modalities	**η^2**	***p*-value**	**Modalities**	**η^2**	***p*-value**
Ta_F_no	0.435	5.467E-05	t1_FU_yes	0.259	6.004E-04
Oa_F_yes	0.452	1.058E-03	Cm_AS_no	0.443	1.455E-02
Dec_MP_yes	0.320	2.780E-04	S_O_no	0.443	1.455E-02
Cat_MP_yes	0.283	4.442E-03	Pl_AS_yes	0.312	1.859E-02
Pl_AS_no	0.309	4.070E-02	Cat_MP_yes	0.209	2.506E-02
Oo_AS_no	0.289	3.573E-03	Ap_O_yes	0.250	2.660E-02

η^2: squared correlation ratio is an indicator used in the ANOVA model for testing the relationship between a qualitative variable and a quantitative variable (coordinates value of the qualitative variable on the axis).

Here, it represents the ratio of the between-categories variability over the total variability.

The quantitative variable of RFC is projected as a supplementary feature. Therefore, the graph is constructed as the graph of correlation in PCA. This method was detailed and reported by [57]. The correlation of the variable on dimension *k* corresponds to the correlation coefficient between the principal component *k* and the variable. Figure 6 demonstrates that the correlation coefficient between the second principal component and the frequency of the RFC index is 0.367.

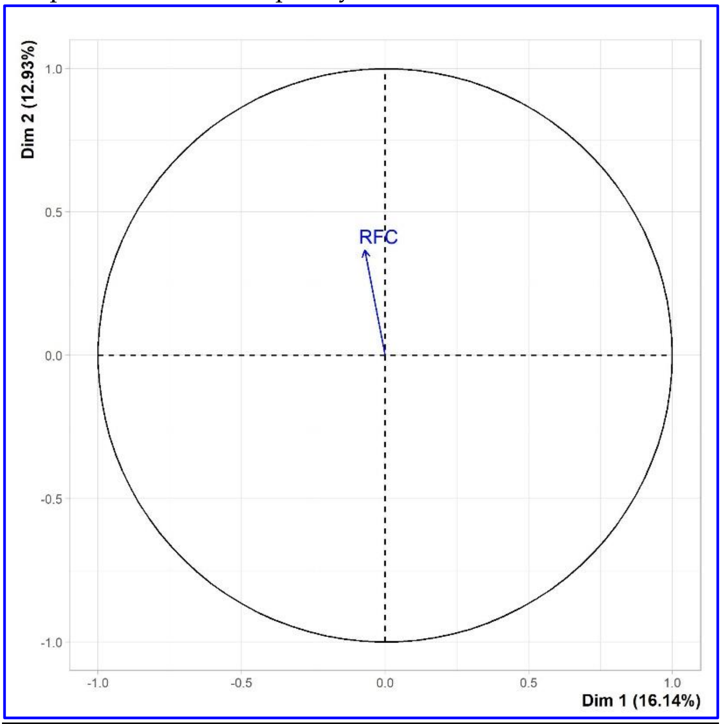

Figure 6. Correlation circle of the quantitative supplementary variable "RFC".

3.4. Cluster Analysis

3.4.1. Cluster Characterization

The chi-square test was performed between the categorical variable and the cluster variable, and the *p*-value was less than 0.05, showing that the categorical variable is linked to the cluster variable [58]. Indeed, the most characteristic variables affecting the partition of the clusters were **Oa_F**, **t3_FU**, **Dec_MP**, and **Ta_F** with respective *p*-values 7.48E-05, 5.44E-04, 3.95E-03, and 1.87E-03 (*p*-values<*0.05*).

The dendrogram suggests the existence of three clusters (Figure 7). Cluster 1 includes ten species, sp1, sp2, sp10, sp7, sp15, sp13, sp8, sp6, sp16, and sp12, while cluster 2 contains seven species, sp17, sp9, sp11, sp3, sp4, sp14, and sp18. In addition, cluster 3 includes the two species, sp5 and sp19.

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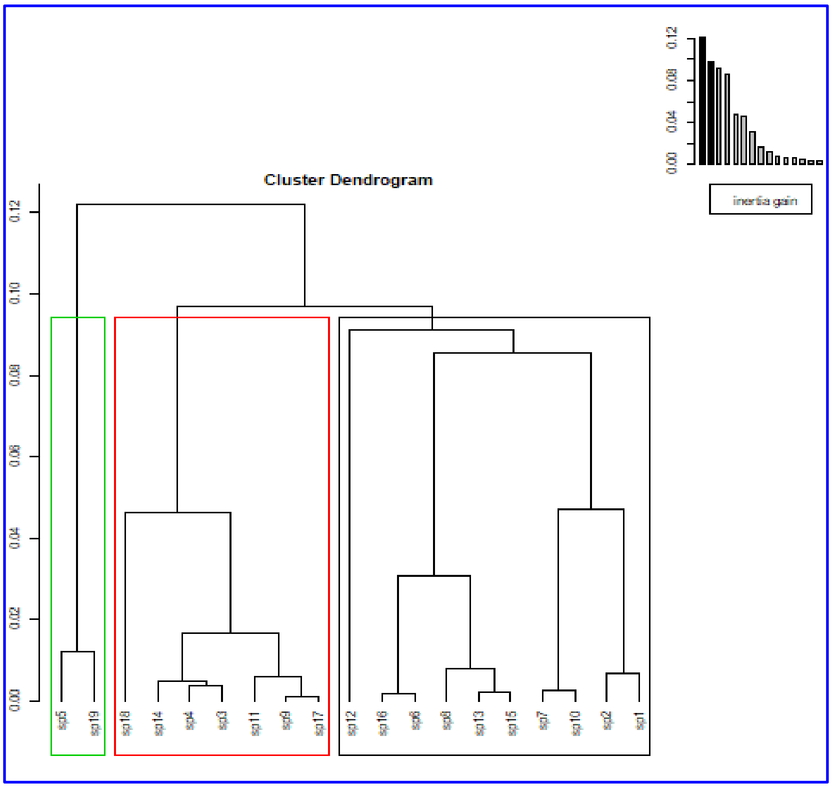

Figure 7. Dendrogram of plant species based on Elucidean distances of 25 variables.

3.4.2. Link between the cluster variable and the categorical variables

Determining the characteristic variables for the partition is measured by the difference between the values of the model of the nominal variable related to the cluster and the overall values. The modality is considered characteristic if its abundance in the cluster is significantly higher than expected, given its presence in the population. This is measured by a criterion known as the *test value* [59]. The test value is a criterion to assess a significant position of a modality or a category of the variable. The significant position is considered when the test value is greater than 2 in absolute value, corresponding to approximately the 5% threshold [60]. Table 4 defines the category of variables most related to the cluster variable with the test value.

Considering the partition of the categorical variable for each cluster, cluster 1 is characterized by two modalities, including the mode of preparation via decoction [Dec_MP_no] and the frequency of use [t3_FU_no]. More species are not used by decoction and a frequency of thrice per day in this cluster than in others. Indeed, 76.92% of species related to t3_FU_no/Dec_MP_no belong to cluster 1, and 100% of species in cluster 1 are cited with t3_FU_no/Dec_MP_no considered as significant modalities with similar test values estimated as 2.95. In contrast, 100% of species used with a frequency of use t3_FU_yes belong to cluster 2 (v.test=3.65). Then, the modality Oa_F_yes was significant and characterized 100% of the species for cluster 3 with the test value estimated as 2.75.

Table 4. The link between the cluster variable and the significant categorical variables (chi-square test).

Categorical variable	*p.* value	*v.* test
Cluster 1		
t3_FU=t3_FU_**no**	3.09E-03	+2.95
Dec_MP=Dec_MP_**no**	3.09E-03	+2.95
Cluster 2		

t3_FU=t3_FU_**yes**	2.57E-04	+3.65
t3_FU=t3_FU_**no**	2.57E-04	-3.65
Cluster 3		
Oa_F=Oa_F_**yes**	5.84E-03	+2.75
Ta_F=Ta_F_**no**	1.75-E02	+2.37

The sign of the v. test indicates if the mean of the cluster is lower **(-)** or greater **(+)** than the overall mean [58].

The analysis of the clustering partition and the phylogenetic relationship demonstrates the disorders of the distribution of the botanical family coupled with the traditional use of the antileishmanial species. This is justified by the occurrence of the Lamiaceae family in the three clusters: cluster 1 (sp_13_*Marrubium vulgare* L.), cluster 2 (sp_17_*Rosmarinus officinalis* L., sp_11_*Lavandula angustifolia* Mill., and sp_18_*Teucrium polium* L.), and for cluster 3 (sp_19_*Thymus vulgaris* L.).

However, the partition of the species belonging to the Asteraceae family is noticeable for the two clusters 1 (sp10_*Launaea arborescens* (Batt.) Murb) and 2 (sp4_*Artemisia herba alba* Asso). The applicability of the cluster analysis to the study of plant taxonomy in the context of ethnopharmacology was performed by Leduc et al., 2006, who reported the limitations of the classification within phylogenetic relationships [40].

4. Discussion

Considering the ethnobotanical research carried out in North Africa, it was found that 42% of species were not previously reported for the traditional treatment of cutaneous leishmaniasis. Among them, three species were reported for the first time: *Retama raetam* (Forssk.) Webb, *Ficus carica* L. and *Launaea arborescens* (Batt.) Murb.

Moreover, the ethnopharmacological investigation in the Ain Sekhouna region, located in the elevated regions of western Algeria, revealed the presence of three plant species that are utilized for treating cutaneous leishmaniasis (CL). These species include *Haloxylon scoparium* Pomel (Chenopodiaceae, 73%), *Artemisia herba-alba* Asso. (Asteraceae, 18%), and *Camellia sinensis* L. (Theaceae, 9%). These plants are administered primarily in the form of powdered extracts, either alone or in combination with substances such as butter, honey, olive oil, or cade oil, often applied as a poultice [30].

Sixty-one plants were cited by 272 herbalists and traditional healers for the treatment of CL in the Tafilalet oasis situated in the Errachidia district of Morocco. Thus, 96% of responses described that the external form is the most administered as the cataplasm, liniment, poultice, ointment, local rinsing, and powdered forms [61].

Subsequently, the diversity of leishmanicidal plants was investigated in the center of Morocco (Sefrou City). This ethnopharmacological survey included 16 herbalists and reported 16 species known for their potential to cure cutaneous lesions [62]. The most species cited by herbalists from Sefrou city and ranked by the percentage of the frequency index (FI %) were *Lavandula dentata* L. (FI = 93.75%), *Cistus salviifolius* L. (FI = 87.5%), *Berberis hispanica* Boiss. & Reut. (FI = 87.5%), *Crataegus oxyacantha* L. (FI = 81.25%), *Ephedra altissima* Desf. (FI = 75%), and *Rosmarinus officinalis* L. (FI = 62.5%).

The results of this bibliographic data analysis do not agree with our study, which highlighted the ranking of species cited by the herbalists from the Annaba and El Tarf districts based on the relative frequency index value. Thus, the most important species identified were *Teucrium polium* L., *Allium sativum* L., *Juniperus phoenicea* L., *Lavandula angustifolia* L. and *Rosmarinus officinalis* L.

The antileshmanial effect of these species has not been reported previously in Algeria. Table 5 reports their ethnopharmacological data and the scientific name of species, the origin of the plant, the part used, the form of parasitic strain tested, and the results of the concentration of drug that causes 50% growth inhibition of amastigotes and promastigotes forms of Leishmania.

Table 5. Ethnopharmacological and experimental data of antileishmanial plants.

Species	Family	Part used	origin	Extract	Parasitic Strain	IC_{50} value	Reference
Teucrium polium L.	Lamiaceae	Aerial Part	Tunisia	EO	*L.infantum*	0.09±0.02 µg/mL	[63]
					L. major	0.15±0.09 µg/mL	
Allium sativum L.	Amaryllidaceae	Bulb	Iran	AGE	*L. major*	37 µg/mL	[64]
							[65, 66]
				AGE		37 mg/mL (MTT assay)	
		Bulb	Sudan	MGE	*L. major*	4.94 µg/mL	
							[67]
		Bulb	Iran	AGE	*L. tropica*	19.2±2.51 µg/mL	[68]
				MGE		12.3±1.15 µg/mL	
		Bulb	India	MGE	*L. donovani* Promastigotes	89±7 µg/mL	[69]
					L. donovani Amastigotes	67±5 µg/mL	
		Bulb	Not specified	MGE	*L. major* Promastigotes	34.22 µg/mL	[70]
					L. donovani Promastigotes	37.41 µg/mL	
		Bulb	Not specified	DCE	*L. tarentolae*	2.89±0.4 µg/mL	[71]
		Bulb	Hadley, MA	Fresh bulb	*L. mexicana*	105 µg/mL	[72]
					L. chagasi	76 µg/m	

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		Bulb	Kenya	AGE	*L. major*	575.75 µg/mL	[73]
Juniperus phoenicea L.	Cupressaceae	Berries	Saudi Arabia	HXE	*L. donovani*	4.4 µg/mL	[74]
Rosmarinus officinalis L.	Lamiaceae	Not precise	Iran	EO	*L. infantum* *L. tropica* *L. major*	1.20± 0.36 µg/mL 3.50± 0.83 µg/mL 2.60 ±0.64 µg/mL	[35]
							[75]
				EO NE	*L. major*	0.26 µl/mL 0.08 µl/mL	
Lanvandula angustifolia L.	Lamiaceae	Not precisely		EO	*L. major*	0.11 µl/mL	[75]

AGE: Aqueous Garlic Extract, **MGE**: Methanolic Garlic Extract, **DCE**: Dichloromethane Extract, **HXE**: Hexane Extract, **EO:** Essential Oil, **NE:** Nanoemulsion formulated: the oil-in-water (o/w) nano-emulsions of Lavender and Rosemary essential oils were prepared using essential oil (1% w/w) and Span 60 (0.5% w/w) as the oil phase, and a mixture of Tween 80 (1% w/w) in deionized water as the aqueous phase, **IC_{50}:** concentration of drug that causes 50% growth inhibition of amastigotes and promastigotes forms of *Leishmania* spp.

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Moreover, Eddaikra et al. (2019) reported on the antileishmanial activity of seven Algerian species [*Erica arborea* L., *Ajuga iva* (L.) Schreb., *Ballota hirsuta* Benth., *Artemisia herba alba* Asso, *Marrubium vulgare* L., *Marrubium supinum* L., and *Marrubium deserti* (Noë) Coss.] against promastigotes and amastigotes of *Leishmania major* (MON 25) and *Leishmania infantum* (MON 1) [29]. *E. arborea*, *M. vulgare*, and *A. herba alba* were the most efficient species on the growth inhibition of *L. major* promastigotes form with respective values of IC_{50}: 43.98, 45.84, and 55.21 µg/mL. *M. vulgare* leaves were the most effective against *L. infantum* promastigotes, with an IC_{50} value of 35.63 µg/mL [29]. Three references were used as the positive control [Amphotericin B, Potassium antimony tartrate (SbIII), and Gluconate antimoniate of meglumine (Glucantime®)], the concentration of the inhibition of 50% growth (IC50) for parasitic strains *L. major* and *L. infantum* were evaluated. The highest activity was observed for Amphotericin B, with a similar value of IC50 for *L. infantum* and *L. major* estimated as 0.2 µg/mL. At the same time, SbIII was active on *L. major* and *L.infantum* promastigotes with IC50s of 3.59±0.56 µg/ml and 1.34±0.56 µg/ml, respectively. However, Glucantime® was less active against *L. major* and *L. infantum* amastigotes with IC50s of 20.44±1.02 µg/ml and 13.78±1.07 µg/ml, respectively.

A significant contribution was performed by Passero et al. (2021) who analyzed 294 articles and reported several criteria regarding the plants recommended for the treatment of leishmaniasis by traditional communities worldwide, namely species, vernacular name, botanical family, recipes, parts of the plants used, the method of preparation, and the route of administration [7].

From 20 articles, 378 citations referring to 292 plants indicated by several traditional communities around the world to treat leishmaniasis, the most frequent families used by traditional communities were Fabaceae (27 species), Araceae (23 species), and 22 each for the Asteraceae and Solanaceae. This classification was followed by the Euphorbiaceae (21 species) and Rubiaceae (20 species). Among the available data in the 378 citations, the most suitable route of administration for plants was the topical route at 74.6%, followed by the oral route and inhalation or nasal route (5%, *v.s* 1.3%). Notably, no specified route of administration was indicated at 20.6% [7].

Another review by Bahmani et al. (2015) highlighted the phytotherapy of cutaneous leishmaniasis in traditional Iranian medicine. Thirty medicinal plants were reported, with their effects supported *in vitro* by inhibiting the parasitic agent. The effective concentration of extracts against promastigotes of *L. major* was also detailed. According to these data, the most critical family with the best effect on leishmania is the Asteraceae, including the two genera *Artemisia* and *Tagetes* [76].

5. Conclusions

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

This is the first study to employ a quantitative ethnobotanical technique to investigate the traditional usage of antileshmanial medicinal plants from Northeastern Algeria. Even though this survey reported 19 species ranked according to their relative frequency index, practically all species cited had not been described in prior studies on Algerian antileishmanial plants. For the first time, three species are documented for their traditional use in treating CL.

The MCA multivariate tests and cluster analysis revealed that the traditional usage of antileshmanial species is distinguished by two factors: the mode of administration and the mode of preparation. In this case, *Cinnamomum zeylanicum* Blume and *Thymus vulgaris* L. are described for the first time for treating CL using *per os* as the mode of administration.

Once the active principles have been determined, a more diverse in vitro study on sustainable, standardized preparations of the most promising plants is required to identify a dosage schedule. Selected clinical research studies on standardized materials may be conducted to determine their safety and efficacy against certain strains of leishmaniasis.

Finally, the partition clustering analysis requires more phylogenetic links and ethnobotany classes. These constraints highlight the need to identify the exact metabolite profile and biological activity to establish a global matrix for a potential candidate plant.

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Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, A.B.; methodology, A.B.; software, A.B.; validation, A.B., M.L.A. and G.A.C.; formal analysis, A.B. and L.B.; investigation, A.G. and A.G.; resources, A.B. and L.B.; data curation, A.B., A.G. and A.G..; writing—original draft preparation, A.B., M.L.A. and G.A.C.; writing—review and editing, A.B., M.L.A. and G.A.C.; visualization, A.B.; supervision, A.B. and G.A.C.; project administration, A.B., A.G. and A.G.; funding acquisition, M.L.A. and E.R.E. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Abbreviations: MCA: Multiple Correspondence Analysis, HCA: Hierarchical Clustering Analysis, RFC: Relative Frequency Citation, CL: Cutaneous Leishmaniasis, VL: Visceral Leishmaniasis, MCL: Mucocutaneous Leishmaniasis, Csa: hot-summer Mediterranean climat, *p*: eigenvalue, *ctr*: contribution, η^2: square ratio correlation, ANOVA: Analysis of Variance, PCA: Principal Component Analysis, *v. test*: test value, FI: Frequency Index, IC_{50}: Half maximal inhibitory concentration.

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