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Cystitis treatment with phytotherapy within the Rif, Northern Morocco



Noureddine Chaachouay^{1*}, Hicham Orch² and Lahcen Zidane²

Abstract

Background: Cystitis is often caused by a bacterial infection, which is the most widespread type among other urinary infections. This investigation was aimed to document detailed ethnobotanical information of medicinal plants used to heal cystitis problems because of their effective therapeutic properties. The study was carried in the Rif, from March 1, 2015, to April 15, 2017. Semi-structured direct interviews were carried with 657 interviewees to collect the indigenous therapeutic knowledge. Surveys included interviewed demographic profile and ethnomedicinal information. UR and MUV were applied in data analysis.

Results: A total of 60 plant species distributed in 51 genera and 31 families were commonly used by our interviewees in the therapy of cystitis. Apiaceae was designed by the highest number of species (7 species); *Capparis spinosa* L. was the medicinal plant most frequently prescribed by regional people. Leaves were the most commonly used plant part (41.5%), and the majority of herbal remedies were prepared from decoction (55 %).

Conclusions: This research is the first contribution to the ethnobotanical study of this region. It is suggested that the ingredients of natural plant species documented are being investigated to discover the therapeutic effects and mechanisms of action. Primary consideration must be paid to the preservation of medicinal species, comprehensive documentation of popular medicinal data, and biological validation of listed species.

Keywords: Rif, Morocco, Phytotherapy, Cystitis, Ethnomedicinal, Knowledge

Background

Urinary infections are a real public health problem around the world. Infections are classified as urethritis, simple acute cystitis, recurrent acute cystitis, simple acute pyelonephritis, and urinary infection of pregnant and postmenopausal women [1].

Cystitis (Nboula in the Moroccan dialect) is a medical term that describes inflammation of the bladder. Inflammation is most usually produced by a bacterial infection (*Escherichia coli, Proteus,* and *Enterobacter*), called a urinary tract infection. A bladder infection can be painful and irritating and can display a dangerous health difficulty if the infection reaches the kidneys.

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The specificity of the management of urinary infections in Morocco lies in the difficulties of accessing care, whether in private or public health facilities. Besides, the evaluation of the management and follow-up of the patient is unknown, as well as the evolution of resistance and the misuse of antibiotics.

Today, despite the development of chemical drugs to combat urinary tract diseases, there is often a return to plants as a source of active ingredients. Besides, an important part of the population, especially in rural areas, prefers medicinal plants, for economic reasons and sometimes because of difficulty in accessing medical care. It is also added that between 8 and 65% of *Escherichia coli* associated with urinary infections are resistant to ciprofloxacin, an antibiotic commonly used against these infections [2].

This research aims to establish, from an ethnobotanical survey of local people, the list of plant drugs available in

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the Rif and that are used in the treatment of cystitis. We suggest, subsequently, a classification of these medicinal plant species reported, according to the use, and we present them in the form of a catalog.

Methods

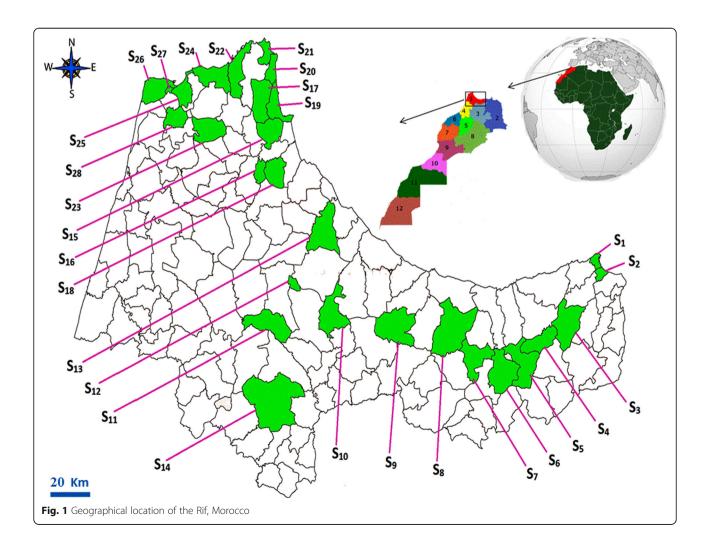
Study area

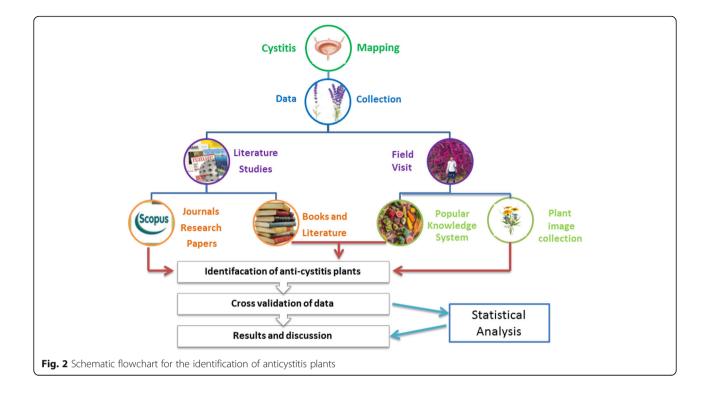
The current study was conducted out in the Rif region. It extends between 34 and 36° of latitude in the North and 4 to 6° of longitude in the East. It is bounded in the North by the Strait of Gibraltar and the Mediterranean Sea, in the South by the Rabat-Sale-Kenitra region and Fez-Meknes region, in the East by the Eastern Region, and in the West by the Atlantic Ocean (Fig. 1). The total geographical area of the Rif is 11,570 km², and the population of the city is about 3,549,512 inhabitants with an average population density of 222.2/km² [3]. The Rif is marked by Mediterranean weather with the highest temperature up to 45 °C during summer (July–August) and below 0 °C during winter (December–January), and the average annual rainfall ranges from 700 to 1300 mm which falls mainly between October and February [4]. The area is dominated by species such as *Abies marocana* Trab., *Pinus halepensis* Mill., *Cannabis sativa* L., and *Cedrus atlantica* (Endl.). The population is mixed between Arabic and Amazigh ethnicity. Principally, families of this region are very much dependent on subsistence farming, livestock, and, to a more secondary space, forest resources for their livelihood.

Methodology

Ethnobotanical data collection

To document an indigenous anticystitis plant ancestral knowledge and discover the level of utilization of traditional medicinal plants for prevention and therapy of cystitis by the local people (Fig. 2) (412 males and 245 females) from different rural and urban communes of the Rif region, namely, S_1 : Al Hoceim (30), S_2 : Ajdir (20), S_3 : Izefzafen (15), S_4 : Bni Hadifa (15), S_5 : Targuist





(25), S₆: Tizi n Tchin (22), S₇: Issaguen (30), S₈: Bab Berred (30), S₉: Cherrafate (30), S₁₀: Bab Taza (20), S₁₁: Derdara (10), S₁₂: Chefchaouen (30), S₁₃: Akchour (30), S₁₄: Fifi (30), S₁₅: Tétouan (30), S₁₆: Bni Karrich (30), S₁₇: Mallalyène (15), S₁₈: Zinat (30), S₁₉: Martil (20), S₂₀: Md'q (20), S₂₁: Fnideq (20), S₂₂: Belyounich (30), S₂₃: Melloussa (30), S₂₄: Ksar Esghir (25), S₂₅: Bni Ouassin (15), S₂₆: Tanger (30), S₂₇: Al Bahraouiyne (15), and S₂₈: Jouamaa (10), a semi-structured questionnaire was prepared, and data were collected through face-to-face interviews over a period between March 1, 2015, and April 15, 2017.

The information collected during this study includes the sociodemographic characteristics of the surveyed (age, gender, level academic) and ethnobotanical data, including the local and scientific name of the species, local names, plant parts used, methods of use, preservation technique, administration mode, and toxicity (Appendix). Interviews were generally carried in the local language (Arab dialect and Tamazight); all documented data were then translated to English.

Taxonomic nomenclature of the plant species

Medicinal species being mentioned by the informants were registered with local names and photographed. For each reported plant species, the plant species were accumulated and classified, and voucher specimens were archived. The identification and nomenclature of the collected vegetal material were done first in the field and completed at the Plant, Animal Productions and Agroindustry Laboratory by one of the authors using some floristic literatures as well as The medicinal plants of Morocco [5]; Practical Flora of Morocco; tomes I, II, and III [6–8]; and Catalogs of vascular plants of northern Morocco, including identification keys and tomes I and II [9]. Taxonomy and denominations of species were validated using "The Plant List 2020" database (http:// www.theplantlist.org). Voucher specimens have been kept at our university, for future reference.

Statistical analysis

The data collected from the field and obtained from the local people were organized and studied with the statistical program IBM SPSS Statistics 21 Premium (SPSS 2019), to determine the proportions of various variable sociodemographics of the interviewees and ethnobotanical data. Quantitative value records were also determined for the general practices of these medicinal plants using the use reports (UR) and medicinal use value (MUV).

Use reports (UR)

The use reports (UR) of a plant or its significance in the practice of a community is recognized by its mentioning degree or its mention frequency by informants. It was determined by the formula [10]: UR = $\frac{N_i}{n}$ where N_i is the

sum of the informant who cited the species and n is the entire of respondents interviewed.

Medicinal use value (MUV)

The MUV of documented therapeutic plants was defined by using this formula [11]: $MUV = \frac{\sum UR_i}{N}$ where $\sum UR_i$ is the total sum of use reports per plants and N is the total of interviewees interrogated for given medicinal species. The MUV rate will be more significant if there are several useful reports for a species, indicating that the plant is significant, whereas they will be near 0 if there are few reports compared to its use [12].

Results

Demographic information of informants

A total of 657 study informants, including 213 herbalists, 178 herbal sellers, 46 pharmacists, 40 midwives, and 180 other healers (bonesetters, Fougha, cautery installer, farmers, elder people, and nobles), were interrogated using semistructured surveys and group interviews. In the study area, both sexes are interested in phytotherapy. However, the numbers of male participants were more important (412 informants) than those of females (245 informants). In this study, results showed that the utilization of medicinal species is widespread in all age groups with different percentages. The bulk of informants surveyed were over 50 years old (350), and between 30 and 50 years old (304), while 3 of the informants were aged less than 30 years old. Concerning the educational level, our results revealed that the majority of the informants (43%) were uneducated, 29.4% have secondary education, 26.8% have primary education, and only 0.8% of the informants had high education (Table 1).

Botanical families of plants used

This investigation revealed that a total of 60 plant species belonging to 51 genera and 31 families were commonly used by local people from the Rif region in the treatment of cystitis. The family Apiaceae was designed by the largest number of plant species (7 species), followed by Ericaceae with 6 species; Lamiaceae, Poaceae, and Rutaceae (4 species each); and Asteraceae and Caryophyllaceae (3 species each), whereas the rest of botanical families were represented by one or two species in each. The vernacular names, scientific names of documented species, their families, mode of preparations, used parts, MUV, and UR are illustrated in Table 2.

Medicinal use plants (MUV)

MUV is used to find the most frequently used plant species in the study area. Its value ranged from 0.200 to 0.277 (Table 2). The calculated results of MUV showed

Variables	Categories	Number of informants	%
Sex	Male	412	62.7
	Female	245	37.3
Age groups	Less 30 years	3	0.5
	Between 30 and 50	304	46.3
	Above 50 years	350	53.2
Educational level	Illiterate	283	43
	Primary	176	26.8
	Secondary	193	29.4
	University	5	0.8
Profession	Herbalist	213	32.4
	Herb seller	178	27.1
	Pharmacist	46	7
	Midwives	40	6.1
	Other healers	180	27.4

that *Capparis spinosa* L. was ranked first (MUV=0.277) followed by *Apium graveolens* L. (MUV=0.267), *Zizyphus vulgaris* Lam. (MUV=0.247), *Herniaria glabra* L. (MUV=0.233), *Anethum graveolens* L. (MUV=0.224), *Spergularia rubra* (L.) J.Presl & C.Presl (MUV=0.216), and *Nigella sativa* L. (MUV=0.210), while the lowest value was found for *Citrus reticulata* Blanco., *Convolvulus althaeoides* L., and *Eleusine indica* (L.) Gaertn. (MUV=0.200 each).

Parts of the plant used

In the Rif region, indigenous people collect diverse plant parts for the preparation of phytotherapy (e.g., seed, root, flower, and leaf). The discussion result revealed that leaves are the most frequently used part of the medicinal plants 41.5% of the sum, followed by the whole plant (26.5%), fruit (10%), seed (9%), flower (7%), and rhizome (6%).

Modes and routes of medicine preparation

The preparation of herbal remedies needs liquids. The main solution was water, but milk, butter, tea, and honey are also used by the Rif's population. The principal method of traditional medicine preparation reported was decoction (55%), followed by infusion (33.1%), cataplasm (1.4%), and cooking (1.1%). The percentage of the other modes of preparation grouped (fumigation, bath, maceration, powder, and plaster) does not exceed 9.4%. Concerning the route of administration, the majority of informants' prepared remedies were applied mostly by oral (73.2%) followed by topical (18.7%) and dermal (8.1%).

Family and scientific name Vernacular name Used parts Preparation mode Route of administration UR MUV Amaranthaceae Atriplex halimus L. Infusion Oral 03 0.005 Legtef Leaf Anacardiaceae Pistacia lentiscus L. Drou Leaf Infusion Topical 97 0.145 Apiaceae Decoction Oral Khella Fruit 0.177 Ammi visnaga (L.) Lam. 116 Anethum graveolens L. Karwiya Amia Whole plant Infusion Oral 147 0.224 Apium graveolens L. Lkrafes Whole plant Infusion Oral 175 0.267 Conium maculatum L. Choukran Oral 13 0.020 Leaf Cataplasm Lkalkha Ferula communis L. Leaf Decoction Topical 93 0.142 Petroselinum sativum Hoffm. Maâdnous Oral 103 Leaf Decoction 0.157 Pimpinella anisum L. Habbat Hlawa Other 35 0.053 Seed Oral Apocynaceae Caralluma europaea (Guss.) N.E.Br. Infusion Oral 89 0.135 Daghmous Leaf Arecaceae Serenoa repens (W.Bartram) Small Nakhil Florida Fruit Infusion Topical 05 0.008 Aristolochiaceae 0.065 Aristolochia baetica L. Berztem Leaf Cataplasm Dermal 43 Asparagaceae Asparagus officinalis L. Sekkoum Rhizome Decoction Topical 117 0.178 Dermal 0.043 Urginea maritima L. Bsel Edib, Ansel Whole plant Other 28 Asteraceae Hieracium pilosella L. Wden Lfar Whole plant Decoction Oral 61 0.093 Silybum marianum (L.) Gaertn. Tawra, Seed Decoction Dermal 66 0.100 Lhindbae, Ûdjem 0.012 Taraxacum vulgare Lam. Leaf Decoction Oral 08 Betulaceae Betula pendula Roth Al Kodban Whole plant Infusion Oral 46 0.070 Boraginaceae Borago officinalis L. Harricha, Lsan Tor Flower Infusion Oral 02 0.003 Brassicaceae Nasturtium officinale R.Br. El Jerjir Leaf Decoction Oral 72 0.110 Cactaceae Opuntia vulgaris Mill. Lhendya Flower Decoction Topical 40 0.061 Capparaceae Lkabbar Capparis spinosa L. Leaf Decoction Oral 182 0.277 Caryophyllaceae 0.108 Corrigiola telephiifolia Pourr. Sarghina Whole plant Decoction Oral 71 Herniaria glabra L. Herras Lehjar Whole plant Decoction Oral 153 0.233 Spergularia rubra (L.) J.Presl & C.Presl Ftat Lhjer Leaf Infusion Oral 142 0.216 Cucurbitaceae Dermal 0.038 Cucurbita pepo L. Seed Decoction 25 Lgraa Lhamra Cupressaceae 0.068 Juniperus communis L. Taga Leaf Decoction Topical 45 Tetraclinis articulata (Vahl) Mast. Dermal Leaf Decoction 87 0.132

Table 2 Catalog of medicinal plants used to treat cystitis problems in the Rif, Morocco

Araar

Table 2 Catalog of medicinal plants used to treat cystitis problems in the Rif, Morocco (Continued)

Family and scientific name	Vernacular name	Used parts	Preparation mode	Route of administration	UR	MUV
Ericaceae						
Erica australis L.	Bouhadad	Flower	Infusion	Oral	36	0.055
Erica ciliaris L.	El Khlenj	Leaf	Infusion	Oral	53	0.081
Erica multiflora L.	El Khlenj	Flower	Decoction	Oral	5	0.008
Erica scoparia L.	El Khlenj	Leaf	Infusion	Oral	14	0.021
Erica terminalis Salisb.	El Khlenj	Flower	Decoction	Oral	10	0.015
Erica umbellata L.	El Khlenj	Flower	Infusion	Oral	8	0.012
Euphorbiaceae						
Mercurialis annua L.	Hrriyga Lmelsa	Whole plant	Decoction	Topical	05	0.008
Fabaceae						
Cicer arietinum L.	Hommes	Seed	Decoction	Oral	18	0.027
Gentianaceae						
Centaurium erythraea Rafn	Merarat El hnech	Leaf	Decoction	Topical	17	0.026
Grossulariaceae						
Ribes nigrum L.	Kashmesh khel	Leaf	Decoction	Oral	56	0.085
.amiaceae						
Convolvulus althaeoides L.	Louwaya	Leaf	Decoction	Oral	01	0.002
Lavandula officinalis Chaix.	Lkhzama	Flower	Infusion	Oral	112	0.170
Marrubium vulgare L.	Merriwt	Leaf	Other	Topical	69	0.105
Origanum vulgare L.	Merdkkouch	Leaf	Infusion	Oral	83	0.126
Myrtaceae						
Myrtus communis L.	Rayhan	Leaf	Decoction	Topical	56	0.0185
Pimenta dioica (L.) Merr.	Nwiwira	Fruit	Infusion	Dermal	08	0.012
Pedaliaceae						
Sesamum indicum L.	Jenjlane	Seed	Infusion	Oral	07	0.011
Poaceae						
Agropyrum repens (L.) P.B.	Njem	Rhizome	Decoction	Oral	76	0.116
Festuca glauca Vill.	Aguzmir	Seed	Infusion	Oral	05	0.008
Eleusine indica (L.) Gaertn.	Njem	Whole plant	Decoction	Oral	01	0.002
Zea mays L.	Dra	Whole plant	Decoction	Oral	79	0.120
Ranunculaceae						
Nigella sativa L.	Lhabba Ssawda	Seed	Decoction	Oral	138	0.210
Rhamnaceae						
Zizyphus vulgaris Lam.	Zefzouf	Fruit	Other	Oral	162	0.247
Rosaceae						
Eriobotrya japonica (Thunb.) Lindl.	Lemzah	Leaf	Infusion	Oral	02	0.003
Prunus cerasus L.	Habb Lmlouk	Fruit	Infusion	Oral	19	0.029
Rutaceae						
Citrus limon (L.) Osbeck.	Lhamed	Fruit	Other	Oral	06	0.009
Citrus limetta Risso.	Lhamed Beldi	Fruit	Cooked	Oral	06	0.009
Citrus × aurantium L.	Larnej	Flower	Cooked	Oral	25	0.038
Citrus reticulata Blanco.	Lmandarine	Fruit	Cooked	Oral	01	0.002
Urticaceae						
Urtica dioica L.	Lhurriga	Whole plant	Decoction	Topical	38	0.058

	,	1				
Family and scientific name	Vernacular name	Used parts	Preparation mode	Route of administration	UR	MUV
Urtica urens L.	Lhurriga	Whole plant	Decoction	Topical	14	0.021
Zygophylaceae						
Tribulus terrestris L.	Ders Elajouz	Whole plant	Cooked	Dermal	02	0.003

Table 2 Catalog of medicinal plants used to treat cystitis problems in the Rif, Morocco (Continued)

Discussion

Our analysis of the results shows that both sexes are involved in phytotherapy. But, the numbers of male informants were more important than those of females. Men were predominantly represented in the sampling because of working. Because of the customs and traditions of the inhabitants of the Rif region, women must remain at home. They tend to abide by religious laws and close down the community. This explains the absence of females in the field during our discussions. This result reinforces the conclusions of other ethnobotanical national and international investigations [13-17] which have besides verified that males are more popular for vegetative information. For age groups, results revealed that interviewees above 50 years old had higher knowledge of medicinal species, while interviewees age less than 30 years old were less informed about it; this is due to the high secrecy of ancestral knowledge by older peoples. Concerning the academic level, our results revealed that the bulk of the informants (43 %) were illiterate. Preceding ethnobotanical investigation studies have comparable conclusions [14, 18]. This means that with a greater level of education, the experience of traditional phytotherapy decreases. Consequently, advanced instruction diminishes the ancestral therapeutic experience of the young generation [19, 20].

The floristic analysis showed that a total of 60 plant species belonging to 51 genera and 31 botanical families were commonly utilized by local people in the therapy of cystitis. The botanical family Apiaceae was described by the most important number of medicinal plants (7 species) followed by Ericaceae with 6 species. The determined results of MUV noted that Capparis spinosa L. was ranked first (MUV=0.277) followed by Apium graveolens L. (MUV=0.267) and Zizyphus vulgaris Lam. (MUV=0.247). These results are compared to other studies especially in Morocco and Fars Province of Iran [20, 21]. These plant species producing leading MUV must be further evaluated for toxicological, phytochemical, and pharmacological investigations to know their active components for an efficient and non-toxic medicine extraction.

The interview result revealed that leaf is the most frequently used part of the plants 41.5% of the total, followed by the whole plant (26.5%) and fruit (10%).

The selection of leaves was due to its natural availability, easy gathering, and simplicity in herbal remedy preparation. Besides, the leaves are the site of photosynthesis and sometimes the storage of the secondary metabolites active for the pharmaceutical properties of the medicinal plant. Similar results indicated that the leaf is used as a principal part of plants in phytotherapy in different studies [22-26]. The principal method of remedy preparation described by our interviewees was decoction (55%). The frequent employment of the decoction can be justified by the evidence that this method makes it possible to conserve the effective multiple ingredients and attenuates or eliminates out the poisonous result of some plant constituents. Ethnobotanical researches carried in many regions of the globe discovered that the majority of informants prepared herbal remedies by decoction and infusion [13, 17, 27-29]. This result confirms that there is a continual transfer of information on the effectiveness of medicinal plants between the people of Morocco. Concerning the route of administration, the main route of application for herbal medicines was oral (82.4%). Moreover, the oral form of treatment is a preferred route all over the planet [18, 30-37]. The predominance of oral treatment can be explained by the fact that cystitis is a common internal disease that is decimating the health of the Rif's population.

Conclusion

In light of the results obtained in this study, medical plants appear to continue to play a pivotal role in covering the basic needs of the health coverage system of the local population living in the Rif region notwithstanding technological progress in modern health. The sum of plant species used by informants to treat cystitis is also evidence that this region is full of medical knowledge inherited from generations ago.

In light of these encouraging results, additional research is suggested on the sustainable and reasonable use and conservation of medicinal plant species. Finally, it is advisable to conduct pharmacological, phytochemical, and toxicological investigations on these species that have been inventoried for laboratory validation of ancestral uses of these plants and to obtain the traditional medicine Bios.

Appendix

Appendix A					
Qustionnaire sheets: Medicinal plants and herbal medicine					
Date					
Region					
Commune					
Commune					
Survey number					
Informant:					
Profession:					
Sex: Male	Female				
	$0-60\} \square \{ \ge 60 \square$				
Family situation: Single Divorced					
	Secondary 🗌 University 🗌				
Locality: Nomadic 🗌 Town 🗌	Village City				
Income / month (MAD): Unemployed 🗌 {250 - 1500} 🔲 {15	$\{00 - 5000\}$ \square $\{ \ge 5000\}$ \square				
TI					
Therapeutic practices : When you feel sick, you address:					
To traditional medicine, why?					
Effective Cheapest Acquisit	tion 🔲 Ineffective medication 🗌				
To modern medicine, why? Effective More precise					
If it is two that it is the first: Traditional medicine	Modern medicine				
Scientific Name: Plant Type: Spontaneous Cultivate	ed 🗌 Introduced 🗌				
Use of the plant: Therapeutic Cost	metic D Other				
	Mechanical				
Harvest Time: Summer Fall Winter Drug preparation: Plant alone	Spring Any year Possible association (of plants)				
If association of plants, quote the recipe:					
Use of the plant: Fresh Desiccated					
If desiccated, drying method: Sun exposure	In the Shade				
Used part: Stem Flower Fruit S	Seed 🗌 🛛 Bark 🗌 Bulb 🗌				
Root ☐ Rhizome ☐ Leaf ☐ Who Form of employment: Tisane ☐ Powder ☐ Essential oil	ole plant Other combination				
Form of employment: Tisane — Powder 🖵 Essential oil	□ Oily oil □ Tincture □				
Method of preparation: Infusion Decoction Cataplasm					
Dose used: Pinch Handle					
Precise Dose: Quantity in g / glass:Quantity in g Administration mode: Oral Massage Rinse					
Administration mode: Oral Massage Rinse Dosage: number of doses per day:	Swapping Other				
	3time / day D Other D				
For adults: 1time / day 🗌 2time / day 📋	3time / day D Other				
For older people: 1 time / day 🗌 2 time / day 🔲	3time / day D Other				
Length of Use: One Day 🗌 A Week 🗌 One m	onth 🗌 Until healing 🗌				
Conservation method: Sheltered from the light	Exposed to light D Other				
Expiration date:					
U.s.					
UNC :					
Use : Diagnosis By: Himself Doctor D	Herbalist 🗌 Other [

Abbreviations

MUV: Medicinal use plants; UR: Use report; MPs: Medicinal plants

Side effect: Toxicity:

Acknowledgements

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Caution of use:

Plant identification

Botanical identification of the plant parts was carried out at Plant, Animal Productions and Agro-industry Laboratory, Department of Biology.

Authors' contributions

NC carried out field research in the Rif; compiled the literature sources, data analysis, and interpretation; and wrote the manuscript, and performed data

analysis. HO helped in data and made a substantial contribution to data analysis. LZ designed the research and identification of plant species. All authors read and approved the final manuscript.

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Availability of data and materials

All data collected and analyzed in this paper are included in the article and attached in the form of "Appendices" as additional files. Medicinal plant specimens are deposited in our university.

Declarations

Ethics approval and consent to participate

The study was carried out following the recommendations of the Code of Ethics of the International Society of Ethnobiology. Ethics approval was authorized by the Ethics Committee of Plant, Animal Productions and Agroindustry Laboratory, Department of Biology, Faculty of Sciences, Ibn Tofail University, through *No. NHEL/06/06/2016*. During this discussion, the research objectives and interview procedure were explained to each informant, and confidentiality was assured. Consent for audio recording was also obtained. Interviewees were informed that the intentions of the research were not for commercial purposes or other interests but for academic reasons. Informants provided verbal informed consent to share in this study; they were free to withdraw their knowledge at any point in time. After informing the committee that we had only obtained the oral consent of the informants, the committee immediately accepted the completion of this study.

Consent for publication

Consent for publication was obtained from participants.

Competing interests

The authors declare that they have no competing interest.

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