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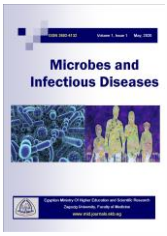

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Original article

Phytotherapy to prevent and treat COVID-19 in the province of Azilal, Morocco

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ABSTRACT

Background: COVID-19 is one of the most widely spread viruses worldwide and different conventional and traditional strategies have been tried to prevent and treat the infection. The current study was designed to document traditional knowledge on medicinal plants used to prevent and treat COVID-19 among herbalists of Azilal region, Morocco. **Methods**: Semi-structured direct interviews were conducted with 20 herbalists were undertaken to archive different medicinal plants and traditional remedies widely used to treat COVID-19 in the Azilal region, Morocco. **Results:** The results revealed that 20 plant species from 20 genera and 13 families used by herbalists in the prefecture of Azilal for the prevention and treatment of COVID-19. The most mentioned plants were *Eucalyptus globulus Labill*, *Eugenia* caryophyllata *Thunb*, *Artemisia annua* L, *Olea europaea* L, *Allium cepa* L, *A. sativum* L, *Citrus limon* (L.) *Eugenia caryophyllata* Thunb, *Zingiber officinale* Roscoe, and *Salvia officinalis* L. The most commonly used plant parts for herbal preparations were leaves (30%) followed by seeds (20%), and the majority of remedies were prepared by infusion. **Conclusion:** Ethnopharmacological research is extremely important for identifying plants used for the treatment of COVID-19 and creating a database of medicinal plants well-known for their biological properties. They may lead to the discovery of medicinal plants as prospects for drug discovery.

Introduction

The coronavirus (CoV), a class of single-stranded RNA viruses, can affect animals and humans, causing respiratory, gastrointestinal, liver and neurological diseases [1]. The first case of the novel coronavirus was reported on December 30, 2019 in Wuhan City, Hubei Province, People's Republic of China [2]. This virus was temporarily named severe acute respiratory syndrome coronavirus 2 and the corresponding infected disease was named coronavirus disease 2019 (COVID-19) by the World Health Organization [3]. On March 11, the WHO officially announced that COVID-19 was a pandemic. The latter was confirmed to have reached Morocco on March 2, 2020, when the first case of COVID-19 was confirmed in Casablanca, after being contracted by a Moroccan immigrant who came from Italy on February 27, 2020.

Since the very beginning of human civilization, plants have been an essential supply for prevention and healing for humans and livestock, especially in developing countries. This is illustrated by the following quote from Hippocrates, which dates from the 4th century BC: "Nature is the medicine of the sick". Consequently, research on

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plant species and their use is one of the most fundamental human interests and has spread worldwide [4,5].

Developing countries have a bias towards traditional medicine, due to limited medical personnel and provisions such as repair equipment and access to basic medicine. The ratio of doctors to patients in Morocco is not reasonable. For example, according to the latest available information, it revealed the following figures for doctors in Morocco (0.5% per 1000 inhabitants) and hospital beds (% 1.0 beds per 1000 inhabitants) [6].

Therefore, human has managed various medicinal plants known for a long time in ancient folk medicine as alternatives. However, in the health system, whether in cities or rural communities, medicinal plants have contributed permanently in the field, due to the low cost and easy access to plant products in all markets of the country [7]. Morocco is one of the nations of the Mediterranean area with diverse favorable climatic conditions to the development of rich and varied vegetation, and rich in plant species. In addition, Morocco has excellent medical knowledge and traditional experience of basic medicinal plants [8]. Mounting evidence has found that numerous medicinal plants contain effective natural agents against COVID-19 [9–11]. COVID-19 treatments can be divided into two groups according to their objectives targeting the immune system or CoV [12]. So far, ethnopharmacological studies have reported that several plants have antiviral activities against herpes virus, influenza virus, human immunodeficiency virus (HIV), hepatitis B and C, MERS, and SARS [12–14]. The pleiotropic effect of different phytocompounds found in medicinal plants makes them an excellent candidate to fight viruses affecting their lifecycle, replication, viral macromolecule assembly, viral release, and host-specific interactions [12]. Medicinal plants are well-known for their protective effects to prevent viral infections.

The present study aimed to investigate the traditional use of plants by herbalists for therapeutic purposes in response to the coronavirus pandemic in Azilal province, Morocco, in 2021.

Material and methods

Study area

The city of Azilal is located in the High Atlas, at an altitude of 1,351 m in central Morocco, 86 km from the city of Beni Mellal, and 242 km from Marrakech **(Figure 1)**.

Azilal has 30,000 inhabitants and enjoys a mild climate in relation to the topography of the region. Azilal is bordered by the Atlas mountain range, which separates it from the region of Kelâat Mgouna where the most important mountain of the region is located: The N'goun which culminates at more than 4071 meters.

Epidemiological situation in Azilal province

At the time of the study (February 1, 2021 to May 29, 2021), the Moroccan Ministry of Health had reported that 63 confirmed cases of COVID-19 had been reported in the province of Azilal. Laboratory confirmation of COVID-19 was carried out at the laboratory of the high Atlas hospital, in Azilal. RT-PCR tests were performed according to the protocol established by the WHO. As for the treatment protocol, Moroccan health authorities have authorized their hospitals to use antimalarial drugs to treat the new coronavirus. The Minister of Health, Mr. Khaled Ait Taleb, has invited, through a note addressed to the directors of hospitals, which receive patients with COVID-19, to use the drugs "Hydroxychloroquine", "Azithromycin" "Paracetamol", "Zinc Sulfate" and "Vitamin C" to treat cases with symptoms of the virus.

Herbalists in Morocco

Herbalists in Morocco are generally males, and over 20 years old. Training to become an herbalist is usually either traditional knowledge inherited from parents or acquired through formal training. Among the herbalists, some followed an ancient traditional lifestyle, while others, especially the young ones, were more associated with a modern Moroccan herbalist (UMH)" Unfortunately, there are no official statistics on their total number.

Methodology

Ethnopharmacological data collection

A semi-structured questionnaire was prepared to document an indigenous anti-coronavirus plant, traditional knowledge and determine the level of use of traditional medicinal plants for the prevention and treatment of COVID-19 by the 20 herbalists from different communes of the Azilal prefecture. Data were collected through face-to-face interviews over the period of February 1, 2021 to May 31, 2021, ranging from 30 to 40 min. The interviews were mainly conducted in the local language (Arabic or Berber) spoken by the herbalists in the study area. The inclusion criterion

was that the herbalists had to be qualified health professionals and had always lived in the area. The exclusion criterion was that they did not live in the study area.

Information collected in this study included sociodemographic characteristics of the herbalists interviewed (age, gender, academic level, and experience in herbal medicine) and ethnopharmacological information, including local and scientific name of the species, plant parts used, modes of use, method of preservation, mode of administration, and toxicity **(Appendix A)**.

Taxonomic identification of the species

Medicinal plants reported by herbalists were recorded with local names and photographed. Each medicinal plant species reported was collected, compressed, dehydrated and identified. The identification and nomenclature of the collected plant material was first done in the field. These plant species mentioned by the sources were identified taxonomically using the following references, "The Medicinal Plants of Morocco" [15], "The Practical Flora of Morocco", volumes I, II and III [16–18], and "Catalogues of Vascular Plants of Northern Morocco", including identification keys, volumes I and II [19]. All reference specimens were preserved during documentation and deposited in the Herbarium of the University Sidi Mohamed Ben Abdellah, Faculty of Sciences Dhar el Mahraz, Morocco for future reference.

Ethical approval

The study was authorized by the ethics committee of Sidi Mohamed Ben Abdellah University. Before the beginning of data collection, we obtained informed oral permission in each case at the site level and then individually before each interview. We also informed the herbalists that this was a student university project and that the survey was for our research purposes only, and not for financial or other purposes. Herbalists provided verbal informed consent to engage in this study; they were free to withdraw their information at any time. These informants freely accepted the idea and consented to the publication of their names and personal data.

Data analysis

The data obtained in the field and collected from the herbalists were classified and examined with the statistical program IBM SPSS Statistics 21 Premium (SPSS 2019), in order to determine the proportions of the different sociodemographic variables of the herbalists and the ethnopharmacological data.

Results

Informant demographics

A total of 20 men herbalists were interviewed using semi-structured questionnaires. The results showed that the use of medicinal species is widespread in all age groups with different percentages. Half of the herbalists interviewed were between 30 and 50 years old. Concerning the academic level, our results showed that more than half of the herbalists (55%) have secondary education, 25% have primary education, 15% are illiterate and only 5% of the herbalists have higher education **(Table 1)**.

Botanical families of plants used

The present research showed that a total of 20 plant species belonging to 13 families were frequently used by herbalists in the Azilal prefecture in the prevention and treatment of COVID-19.

The most used plants were *Eucalyptus globulus* Labill (20 citations), *Eugenia caryophyllata* Thunb (19 citations), *Artemisia annua* L (18 citations), *Olea europaea* L (16 citations), *Allium cepa* L (15 citations), *Allium sativum* L (13 citations), *Citrus limon* (L.) (12 citations) *Eugenia caryophyllata* Thunb (10 citations), *Zingiber officinale* Roscoe (7 citations), *Salvia officinalis* L (6 citations), their families, mode of preparation, parts used, are illustrated in **Table 2**.

Plant parts used

Different parts of the plant were harvested for the preparation of herbal remedies (e.g., seeds, roots, flowers and leaves). The results of the interviews revealed that leaves are the most frequently used part of medicinal plants (30%) of the total, followed by seeds (20%), whole plant (15%), bulb (10%), rhizome (10%), flower (5%) fruit (5%) and other part (5%) **(Table 2)**.

Methods and conditions of preparation of medicines

In the prefecture of Azilal, most of the herbal remedies for COVID-19 (70.8%) were prepared from fresh material followed by their dried form (29.2%). In most cases, the preparations were obtained from individual plants, but some plant compounds were also reported. The preparation of ancestral medicine requires components and solutions. The main solvent was water, but milk,

butter, tea and honey, cereal oils and vinegar were also widely used ingredients. The main method of preparation of traditional medicine reported was infusion (35%), followed by decoction (30%), powder (15%) and maceration (10%). The percentage of other preparation methods grouped together (poultice, fumigation, cream, bath and plaster) did not exceed 10% **(Table 2)**.

Symptoms and treatment of COVID-19

Herbalists perceived common COVID-19 as a combination of several symptoms. The most common were fever, fatigue, sore throat, loss of taste and smell, headache, and dry cough. Herbalists believed that treatment with herbal remedies for 7 days resulted in a marked improvement in the rate of symptom recovery, and herbalists reported that herbal capsules reduced the duration of fever by three days and fatigue symptom.

Table 1. Demographic characteristics of informants (n = 20).

Variables	Categories	Number of informants (percentage)
Age	Between 30 and 50	10 (50%)
	More than 50	05 (25%)
Sex	Female	00 (0 %)
	Male	20 (100 %)
Level of study	Illiterate	03 (15 %)
	Primary	05 (25 %)
	Secondary	11 (55 %)
	University	01 (05 %)
Years of experience as an herbalist	Between 01 - 10	03 (15 %)
	Between 10 and 20	04 (20 %)
	More than 20	13 (65%)

Table 2. Factors affecting COVID-19 severity among the studied population.

Family	**Common name**	**Scientific name**	**Vernacular name**	**Used part**	**Preparation method**	Bioactive compounds	Biological activities	References
Amaryllidaceae	Oignon	*Allium cepa* L.	البصل:Basla	Bulb	Other	Γ-Glutamyl Peptides, Diallyl Thiosulphinate, 2,2-Diphenyl-1-Picrylhydrazyl, quercetin, kaempferol	Antiinflammatory, Antioxidant, antiviral, antibacterial, anticancer activities, antiproliferative	[20]
	Garlic	*Allium sativum* L.	الثوم: Touma	Bulb	Other	Flavonoids, organosulfur compound and saponins, essential oils, diallyl-Trisulfide, diallyl-Disulfide	Antioxidant, dysentery, anti-inflammatory,antiviral, anticancer activities	[21,22]
Fabaceae	Batons de réglisse	*Glycyrrhiza glabra* L.	عرق: Sous Arq ضوش	Rhizome	Decoction	glycyrrhizin, 18β glycyrrhetinic acid, glabrin A and B, isoflavones, Licochalcone A	anti-inflammatory, antimicrobial, Antioxidant, Antiviral, Antitussic, expectorant	[23–25]
	Fenugrec	*Trigonella foenum-graecum* L.	الحلبت: Lhalba	Seed	Decoction	Magnesium, Calcium, Vitamins A, B1, C, Nicotinic Acid, Alkaloids, Flavonoids	Fungicidal, antiviral, Analgesic, anti-inflammatory, antiradical, antioxidant	[26–28]
	Romarin	*Rosmarinus officinalis* L.	االزير: Azir	Leaf	Infusion	Bornyl Acetate (11.3–17.0%) And Verbenone (4.4–24.9%), While 1,8-Cineole (3.4–11.3%),Borneol (6.2–7.3%), and Camphor	Antiviral, Anti-proliferative, antioxidant, antinociceptive	[29–31]

Family	Common name	Scientific name	Vernacular name	Used part	Preparation method	Bioactive compounds	Biological activities	References
Lamiaceae	Sauge officinale	*Salvia officinalis* L.	Salmya : الطالويت	Leaf	Infusion	Thyme;12-O-Methyl Carnosol;Apianane, Terpenoid; Abietane Diterpenoid; Flavonoid	anti-inflammatory, Anticancer, Antioxidant, antimutagenic, antinociceptive	[32]
	Thym	*Thymus vulgaris* L.	Zaatar :السعتر	Whole plant	Infusion	Γ-Terpinene, Camphenilone, Tricyclene, A-Thujene,1,8-Cineole	Antimicrobialactivity, antioxidant, fungicidal, antimicrobial	[33,34]
Lauraceae	Cannelle	*Cinnamomum zeylanicum* Blume	Lqarfa القرفة	Seed	Powder	Hydrocinnamaldehyde, Benzaldehyde, 3-Phenylpropyl Acetate, N-Heptadecane, 2-Hexadecanone	Antimicrobial, fungicidal, antibiotic, SARS-CoV	[35,36]
Meliaceae	Margousier	*Azadirachtai ndica A.Juss.*	Neem :النين	Leaf	Infusion	Ethanol, Methanol, Acetone, Alkaloids, Phenolic	Antimicrobial activities against human pathogenic bacteria (Salmonella typhi)	[37,38]
Myrtaceae	Eucalyptus	*Eucalyptus globulus Labill.*	Lqalitous :الكاليبتوش	Leaf	Infusion	Myricetin, linalool, urosolic acid, apigenin, quercetin, narasin	SARS-CoV, antiviral, coxsackievirus, hepatitis B and C virus	[39,40]
	Clou de girofle	*Eugenia caryophyllat a Thunb.*	Lqronfel : القرنفل	Flower	Maceration	2-Phenyl Ethanol, BenzylAlcohol, Eugenol, Chavicol, 4-Hydroxy, 3-Methoxy- Benzeneacetic Acid, Hexadecanoic Acid	Anti-inflammatory, Antimicrobial, Antioxidant	[41,42]
Oleaceae	Olivier	*Olea europaea L.*	Zitoun :الميتوى	Leaf	Decoction	Flavonoids, Flavone Glycosides, Flavanones, Iridoids, Triterpenes, Biophenols	Antioxidant, antiviral, antimicrobial, anti-diabetic, influenza, cardioprotective effects	[43]
Ranunculaceae	Nigelle	*Nigella sativa L.*	Hbatlbarka :حبت االبركت	Seed	Powder	Nigellidine, Nigellicine, Carvacrol, Thymol, A-Hederin	Anti-inflammatory, analgesic, antipyretic	[44]
Rhamnaceae	Jujubier	*Ziziphus lotus (L.) Lam.*	Sedr :الطدر	Other	Decoction	Alkaloids, Flavonoids, Terpenoids, Saponin, Pectin, Triterpenoic Acids, Lipids	Anti-inflammatory, analgesic, antioxidant, antimicrobial	[45,46]
Rutaceae	Citron	*Citrus limon (L.) Osbeck*	Lhamed : الحاهض	Fruit	Maceration	Hesperetin, eriodictyol and naringenin,3-Cyclohexene- 1-Methanol (11%), 2-Isoprpenyl-5-Metyhlhex-4-Enal (10%), Limonene (9%), B-Citral (8%)	Antibacterial, fungicidal, influenza, Haemolytic	[47,48]
Zingiberaceae	Gingembre	*Zingiber officinale Roscoe*	Sqinjbir : السنجبيل	Rhizome	Powder	Zingiberenol, Zingiberene, Hexanol, Γ- Terpinene, Menthol, B-Caryophyllene, Trans-Linalool Oxide	Antimicrobial, Antiinflammatory, antimicrobial properties, antioxidant, Hepatoprotective	[49]

Figure 1. Geographical location of the study area, Azilal – Morocco.

Discussion

During our interview, it became clear that men have a monopoly on the herbal market, while there are no women in this field. These results are consistent with the results of other national and international ethnobotanical studies [50,51], that confirmed that men are mainly familiar with traditional herbs. Regarding educational level, our results revealed that more than half of the informants (55%) have a high school diploma. Among the 20 herbalists participating in our survey, only one herbalist has a diploma in herbal medicine. While the Dahir of 19 February 1960, with its article 17, stipulates that in order to hold and sell medicinal plants or parts of plants, whether fresh or dried, with the exception of plants classified in the various tables of poisonous substances, the interested party must be in possession of a certificate in herbal medicine and approved under the conditions provided for in article 2 of the same Dahir. This indicates that with a higher level of education, experience in traditional herbal medicine decreases. Consequently, advanced education reduces the ancestral therapeutic knowledge of the younger generation [52]. Previous ethnobotanical studies [53–57], report similar results. All reported acquiring their traditional knowledge and experience in medicinal plants from relatives, friends, and the elderly.

The present study revealed that a total of 20 medicinal species belonging to 13 botanical families were commonly used by herbalists in the prevention and treatment of COVID-19. The Lamiaceae family was present in a large number of medicinal plants (3 species, 15%). The results showed that *Eucalyptus globulus* Labill. ranked first. These results are similar to international work, especially in Pakistan.

The results of the interviews revealed that leaves are the most frequently used part of the medicinal plants (30%), followed by seeds (20%) and the whole plant (15%). The choice of leaves was due to their availability, collection and simplicity in preparing the medicine. Comparable findings showed that leaves are the main part of the plant used in the preparation of medicinal plants in Morocco [58–61] and in Africa [62–64].

The main method of preparation of herbal medicine described was infusion (35%) followed by decoction (30%). The frequent use of infusion can be justified by the fact that it allows the accumulation of the multiple effective components and attenuates or eliminates some of the remaining toxic ingredients. Ethnobotanical studies conducted in other regions of Morocco have shown that the

majority of informants prepared herbal remedies by infusion and decoction [65–67].

A large number of chemicals and bioactive compounds extracted from different higher plants are responsible for many pharmacological effects [68–70] such as antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant, antipyretic, antiseptic, antibiotic, analgesic, antitumor, antiallergic, antiatherogenic, antimutagenic and antinociceptive.

A variable number of alkaloids such as emetine, tylophorine and mycophenolate mofetil have been reported as very important antiviral compounds [71,72]. Chloroquine is a good candidate, asynthetic derivative of quinine for the development of an effective drug to treat COVID-19 due to its DNA intercalation properties [73]. The isoquinoline alkaloids tetrandrine, fangchinoline and cepharanthine could inhibit the expression of spike and nucleocapsid proteins in SARS-CoV-OC43 in human lung cells [74].

Essential oils and other aromatic plants with antiviral activities have been well documented by several researchers [69,75–77]. Eucalyptol, a vital essential oil of *E. globulus* Labill. has been identified as an effective antiviral compound against coronavirus, especially COVID-19 because this major component of eucalyptus oil consists of ether (-O), ketone (=O), and hydroxyl (-OH) groups that play the main inhibitory role against SARS -CoV-2 [78]. Essential oils can insert themselves non-specifically into the lipid bilayer of the viral envelope, altering the fluidity of the membrane [76].

The antimicrobial activity of alkaloids has been widely reviewed [79,80]. Recently, Özçelik et al, published a comprehensive review on this topic. The review examined many alkaloids, flavonoids and phenolic acids with antimicrobial activity and reported that bioactive alkaloids could be found in acridone-, aporphine-, benzophenanthridine-, bisbenzylisoquinoline-, indole-, isoquinoline-, piperidine-, protoberberine-, quinoline-, terpenoid-, and steroid-type alkaloids [81]. It has also been reported that flavonoid derivatives possess antiviral activity against a wide range of viruses such as HSV, HIV, Coxsackie B virus, coronavirus, cytomegalovirus, poliomyelitis virus, rhinovirus, rotavirus, poliovirus, sindbis virus and rabies virus [82,83]. Phenolic compounds, polyphenols, steroids, terpenoids, other active phytochemicals and their derivatives are common plant secondary metabolites that contain aromatic rings with 1 or more hydroxyl groups. For a wide range of viruses, several studies have confirmed the aforementioned activity of several phenolic antiviral compounds such as curcumin, luteolin-7glucoside, epicatechin gallate, catechin, demethoxycurcumin, bavachinin, apigenin-7 glucoside, silvestrol, hypericin, psoralidin, mycophenolate mofetil, corylifol, and tomentine [72,84,85]. According to Wink [77], polyphenols are able to bind readily to lipoproteins of the viral envelope, which may prevent viral invasion into host cells.

We can see that some herbs are effective in preventing and treating COVID-19 for some people, however, there is currently not enough data regarding the use of herbal remedies for the new coronavirus.

Limitations

No information is provided on the pharmacological efficacy of the herbal species in this study. These are herbs that are used for the symptoms of COVID-19, the effects of which remain unknown.

Conclusion

Medicinal plants are a promising source of natural antiviral agents acting their effects synergistically. The present study revealed that Azilal population treated their viral infections, especially COVID-19 by multiple natural remedies. In this vision, the present study highlights and provides the traditional remedies used to treat COVID-19 by the population of Azilal region, Morocco, which could enrich the Moroccan database about traditional remedies of viral infections. Furthermore, there is a need for more experiments to provide scientific evidence on the antiviral activities of the plants used to treat COVID-19.

Ethics statement: Prior to the survey, we obtained oral informed consent from each participant.

Consent for publications: Not applicable.

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Conflicts of interest: The authors declare that there are no conflicts of interest in this article.

Availability of data and materials: data are available from the first author upon reasonable request.

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