INTRODUCTION

Staphylococcus species are opportunistic bacteria and the most pathogenic species. Strains of Staphylococcus aureus are able to colonize all tissues [1], secrete various enzymes and toxins [2-4] and cause various diseases including skin infections [5,6]. Microbial infections are normally treated using antibiotics, but the development of resistant strains has become an increasingly common problem which constitutes a growing public health problem, especially since these multi-resistant strains are no longer confined to hospital environments but are also found in the community [7,8]. Moreover, the emergence of strains of S. aureus resistant to methicillin (MRSA) makes the bacterium very dangerous and put a therapeutic problem. This resistance has stepped up the use of vancomycin, main antibiotic drug and a large number of plants possess antimicrobial activity [11-13]. In this context, we target the region of Fez-Meknes located in the center of Morocco known by its particular geographical situation very rich in biodiversity to select anti skin infections medicinal plants and to evaluate theirs in vitro actions against Staphylococcus strains and especially MRSA.

METHODS

Study area

Located in the central North of Morocco (Fig. 1), partially integrating the plain of Saïss and along the mountain ranges of the Rif and the Middle Atlas, Fez-Meknes region covers an area of 40,075 km² corresponding to 5.7% of the national territory [14]. This region regroups the Prefectures of Fez and Meknes and the provinces of Boulemane, El Hajeb, Ifrane, Moulay Yaâcoub, Sefrou, Taounate and Taza [14].

The region of Fez-Meknes has 4,236,892 inhabitants [15], the density is 105.7 inhabitants per km², very high compared to the national average (47.6 hab/km²). Due to its particular strategic position, the region is characterized by three climatic types: (i) a continental climate in the northern part, very hot and very dry in summer and cold and wet in winter. The winds are dry and cold or cold and wet in winter and hot in summer (Chergui), (ii) a cold and humid climate in a mountainous area, very cold and very snowy in winter and temperate in summer, and (iii) semi-arid climate in the high hills of Boulemane. Winters are very cold and snowy [14]. The current study was undertaken in the prefectures of Fez and Meknes that are most populated (1,150,131 and 835,695 inhabitants, respectively) [15] and the province of Taounate that 90% of its communes are rural [16]. The local population of this province resorts commonly to herbal medicine.

ABSTRACT

Objective: The present study aims the investigation of the antimicrobial potential of medicinal plants selected in the central north of Morocco against methicillin-resistant Staphylococcus aureus and Staphylococcus epidermidis strain often involved in dermatitis.

Methods: Structured interviews were carried out among 91 herbalists and traditional healers through a specific information questionnaire, the in vitro susceptibility of Staphylococcus strains award ethanol extracts was evaluated using the well-diffusion assay, while the agar-microdilution method was used to determinate the minimal inhibitory concentrations (MIC). The total phenolic and flavonoids contents of all tested extracts were also determined.

Results: Based on the ethnomedical survey, a total of 55 plant species belonging to 30 families were mentioned. The Lamiaceae family was the most represented (18.80%) followed by the Apiaceae family (10.90%). Leaves (45.00%) were the favored used part. Decoction method (48.53%) was the most frequently used to prepare remedies that are taken externally (75.00%). Nine of the 17 most selected species have shown an effective antistaphylococcal activity; the most active extracts were Punica granatum and Rhamnus alaternus with MIC values ranging between 0.25 mg/ml and 2.00 mg/ml.

Conclusion: The current data confirm the good antistaphylococcal activity of P. granatum and R. alaternus and suggest that these species could constitute a promoter source for antistaphylococcal drugs with deeply studies.

Keywords: Ethnobotanical study, Morocco, Plant extracts, Antistaphylococcal activity, Phytochemical assay.
Ethnobotanical survey

Skin infections such as Impetigo or Furunculosis (Fig. 2) are specific infections caused by *S. aureus*, for this reason, they were the basis of a questionnaire among herbalists. The survey was carried out during the year 2017. In addition to personal sociodemographic data of the interviewers, the questionnaire has also included information about the recommended plants, vernacular names, used parts, preparation, and administration mode. In general, the most recommended plants were collected from the field; voucher specimens of each plant were identified by a specialist (from the Scientific Institute in Rabat-Morocco) and then deposited at a herbarium in Laboratory of Microbial Biotechnology in Faculty of Sciences and Techniques in Sidi Mohamed Ben Abdellah University of Fez-Morocco. The frequency index (FI) was calculated for each plant to evaluate the importance of each plant using the following formula:

\[ FI = \frac{n}{N} \times 100 \]

*n*: Total number of herbalists who listed a particular plant species.

*N*: Total number of interviewed herbalists.

Plants selection and preparation

Certainly, the local population usually uses the decoction mode to prepare remedies. However, our findings in a previous study show that aqueous extracts have not shown any antibacterial activity due to the influence of solvents nature and polarity. For this reason, ethanol extracts of the seventeen most common plants cited by the healers (FI≥8.88%) have been prepared in accordance to the methods described by Elaloui et al. and Yeo et al. [17,18]. The used part of each plant as mentioned by herbalists was grounded to powder, and then 10 g of the powder of each plant was macerated in 100 ml of ethanol under agitation (500 rpm), at room temperature for 6 h. The resulting mixture was filtered through Whatman filter n°1 then evaporated under vacuum. Dried extracts were stored in a refrigerator at 4°C until further use.

**ANTISTAPHYLOCOCCAL TESTING**

Target microorganisms

The prepared ethanolic extracts have been the objective for the antistaphylococcal activity using agar well diffusion method and the minimum inhibitory concentrations (MIC) determination against strains often involved in cutaneous infections including *S. aureus ATCC 29213*, *S. aureus* clinical isolate, and *Staphylococcus epidermidis ATCC 12228*. The antibiogram profile of strains’ bacteria was identified at the Laboratory of Bacteriology in Fez-Morocco, and it has shown that both *S. aureus* strains are methicillin-resistant (Table 1).

**Agar well diffusion method**

Re-vivification of bacteria has been performed by subculturing the agar plate surface Luria-Bertani (LB) pre-poured in Petri dishes and incubated at 37°C for 18 to 24 h. The microbial inoculums were obtained from fresh colonies through the direct colony suspension method. Hence, 1–2 colonies were suspended in sterile saline (NaCl 0.9%) and adjusted to 0.5 McFarland scale (10⁸ colony-forming unit/ml). Agar well diffusion method as described in Balouiri et al. [19] was used to evaluate the antimicrobial activity. The agar surface was inoculated

**Table 1: Antibiogram profile of the targets bacterial strains**

| Antibiotic family | Antibiotic | Dose per disk (µg) | *S. epidermidis* | *S. aureus ATCC 29213* | *S. aureus* clinical isolate |
|-------------------|------------|--------------------|-----------------|-------------------------|-----------------------------|
| Penicillins       | Penicillin | 10 units           | Susceptible     | Resistant               |
|                   | Ampicillin | 10                 | Susceptible     | Susceptible             |
|                   | Amoxicillin| 20                 | Susceptible     | Susceptible             |
|                   | Oxacillin  | 1                  | Susceptible     | Resistant               |
|                   | Methicillin| 5                  | Susceptible     | Susceptible             |
| Penicillin combinations | Amoxicillin/Clavulanate | 20/10 | Susceptible | Susceptible |
| Cephalosporins    | Ceftriaxone| 30                 | Susceptible     | Resistant               |
|                   | Ceftazidime| 30                 | Susceptible     | Resistant               |
| Glycopeptides     | Vancomycin | 30                 | Susceptible     | Susceptible             |
|                   | Teicoplanin| 30                 | Susceptible     | Susceptible             |
| Macrolides        | Erythromycin| 15                | Susceptible     | Resistant               |
|                   | Spiramycin | 15                 | Susceptible     | Resistant               |
| Tetracyclines     | Tetracycline| 30                | Resistant       | Susceptible             |
| Polypeptides      | Colistin   | 10                 | Resistant       | Resistant               |
| Others            | Fusidic acid| 10                | Susceptible     | Susceptible             |
|                   | Pristinamycin| 10              | Susceptible     | Susceptible             |

*S. aureus*: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*
| Botanical name | Family name | Local name  | Part used | Administration route | Preparation mode | FI (%) |
|---------------|-------------|-------------|-----------|----------------------|------------------|--------|
| Allium ampeloprasum (=Allium porrum) | Alliaceae | Krrat | Leave flower | Cutaneous | Decoction | 2.22 (FS) |
| Aloe vera | Aloeaceae | Alovera | Leaves | Cutaneous | Decoction | 5.88 (TN) |
| Daucus carota | Apiaceae | Khizzo/Jaada | Whole plant fruit | Cutaneous | Decoction | 2.22 (FS) |
| Petroselinum crispum | Apiaceae | Madnous | Leaves | Oral | Decoction | 2.22 (FS) |
| Ammi majus | Apiaceae | Trillan/Tillan | Leaves | Cutaneous | Decoction | 8.88 (FS) 11.76 (TN) |
| Ammi visnaga | Apiaceae | Elkhella el bariya/Bechnika; Kessiba Elwashch | Leaves | Cutaneous | Decoction | 2.22 (FS) |
| Ferula assa - foetida | Apiaceae | Chemmar/Besbas/Nafaâ/Amsi | Leaves | Cutaneous | Decoction | 2.22 (FS) |
| Aloe vera | Aloeaceae | Alovera | Leaves | Cutaneous | Decoction | 5.88 (TN) |
| Ferula assa - foetida | Apiaceae | Chemmar/Besbas/Nafaâ/Amsi | Leaves | Cutaneous | Decoction | 2.22 (FS) |
| Aloe vera | Aloeaceae | Alovera | Leaves | Cutaneous | Decoction | 5.88 (TN) |
| Carlinum officinale | Acanthaceae | Tafarkha | Roots | Cutaneous | Decoction | 2.22 (FS) |
| Arctium lappa (=Arctium lappa) | Asteraceae | Chih | Leaves | Cutaneous | Decoction | 4.44 (FS) 17.64 (MK) 5.88 (TN) |
| Dittrichia viscosa (=Inula viscosa) | Asteraceae | Magraman Bayraman | Leaves | Cutaneous | Decoction | 2.22 (FS) 6.25 (MK) 5.88 (TN) |
| Artemisia herba alba | Asteraceae | Chih | Leaves | Cutaneous | Decoction | 4.44 (FS) 17.64 (MK) 5.88 (TN) |
| Dittrichia viscosa (=Inula viscosa) | Asteraceae | Magraman Bayraman | Leaves | Cutaneous | Decoction | 2.22 (FS) 6.25 (MK) 5.88 (TN) |
| Arnica montana | Asteraceae | Addad | Roots | Cutaneous | Decoction | 2.22 (FS) |
Table 2: (Continued)

| Botanical name          | Family name  | Local name | Part used | Administration route | Preparation mode | FI (%) |
|-------------------------|--------------|------------|-----------|----------------------|------------------|--------|
| Laurus nobilis         | Lauraceae    | Elghar/Chajrat | Leaves   | Cutaneous            | Decoction        | 2.22 (FS) |
| Syzygium aromaticum    | Myrtaceae    | Sidna Moussa/Rand| Cloves   | Oral                 | Infusion         | 2.22 (FS) |
| Eucalyptus globulus    | Myrtaceae    | Krenfel/Oud | Leaves    | Cutaneous            | Decoction        | 2.22 (FS) |
| Olea europaea          | Oleaceae     | Emnawar    | Leaves    | Cutaneous            | Decoction        | 8.88 (FS) |
| Oenothera sp.          | Onagraceae   | Alkhdariya | Leaves    | Cutaneous            | Decoction        | 2.22 (FS) |
| Globularia alypium      | Plantaginaceae| Ain larneb/tasalgha/| Leaves | Cutaneous            | Decoction        | 6.66 (FS) |
| Plantago major         | Plantaginaceae| Leen Hamal/Aslouj | Leaves | Cutaneous            | Decoction        | 2.22 (FS) |
| Rhamnus alaternus      | Rhamnaceae   | Mililes    | Leaves    | Cutaneous            | Decoction        | 17.77 (FS) |
| Ziziphus lotus         | Rhamnaceae   | Nbeg       | Leaves    | Cutaneous            | Decoction        | 2.22 (FS) |
| Rubia tinctorum        | Rubiaceae    | Elfossa    | Roots     | Cutaneous            | Decoction        | 4.44 (FS) |
| Citrus aurantium       | Rutaceae     | Renj       | Fruit     | Oral                 | Cutaneous        | 2.22 (FS) |
| Crataegus monogyna (= Crataegus oxyacantha) | Rosaceae | Zeerour/adnam | Leaves arial part | Cutaneous     | Cutaneous        | 4.44 (FS) |
| Salvadoria persica     | Salvadoriaceae| Swak       | Roots     | Cutaneous            | Cataplasme       | 5.88 (MK) |
| Thymelaea tartonraira  | Thymelaceae  | El matan/ Talacaz | Leaves | Cutaneous            | Cataplasme       | 17.64 (TN) |
| Aquilaria malaccensis  | Thymelaceae  | Oud aghris | Roots     | Cutaneous            | Cataplasme       | 35.29 (MK) |
| Urtica dioica          | Urticaceae   | Herriga Herricha | Leaves flowers whole plant | Cutaneous | Decoction | 8.88 (FS) |
| Curcuma longa          | Zingiberaceae| Karkum/ Kharqoum | Roots | Cutaneous            | Decoction        | 2.22 (FS) |

% FI: Frequency index. Reported cities: FS: Fez, MK: Meknes, TN: Taounate

spreading bacterial inoculums. After 30 min of the drying process in ambient temperature and inoculums' diffusion, a hole with a diameter of 6 mm was punched aseptically using a tip, then 80 µl of each extract solution (50 µg/ml) were introduced into the wells. Finally, agar plates were incubated for 24 h at 37°C. Distilled water was used as negative control, while ampicillin (100 µg/ml) was used as positive control. After measuring the diameter of inhibition's zones around the well, means were calculated, and then the active extracts were subjected to the determination of the MIC.

Determination of the MIC

The MIC was determined following the agar dilution method described by Balouiri et al. with slight modifications [19]. It involves the incorporation of varying concentrations of extract as an antimicrobial agent into the agar medium before its solidification. Different concentrations of each extract ranging from 50 to 160 µg/ml per factor of (2) were prepared in dimethyl sulfoxide (20%), and 1 ml of each dilution was incorporated in 9 ml of medium culture (sterile and soft LB). The mixture was grounded carefully and distributed into Petri dishes. After medium’s solidification and from a suspension adjusted to 10⁵ UFC/ml, volumes of 5 µl were deposited on agar surface as spots. Finally, the dishes were incubated for 24 h at 37°C. A growth control agent into the agar medium before its solidification. Different concentrations of each extract ranging from 50 to 160 µg/ml per factor of (2) were prepared in dimethyl sulfoxide (20%), and 1 ml of each dilution was incorporated in 9 ml of medium culture (sterile and soft LB). The mixture was grounded carefully and distributed into Petri dishes. After medium’s solidification and from a suspension adjusted to 10⁵ UFC/ml, volumes of 5 µl were deposited on agar surface as spots. Finally, the dishes were incubated for 24 h at 37°C. A growth control

The flavonoid content has been determined as described in Bahorun et al. [20]. Technically, 0.5 ml of each extract was mixed with 0.1 ml of aluminum chloride (1%), 0.1 ml of potassium acetate (1 ml), and 4.3 ml of distilled water; after a vigorous agitation, the mixture was incubated for 30 min in ambient temperature. DO’s values have been read using spectrophotometer visible-UV at 415 nm. Flavonoid content was expressed as µg Quercetin equivalents per mg dry weight of the extract (µg GA eq mg E).

Total flavonoids quantification

The flavonoid content has been determined as described in Bahorun et al. [20]. Technically, 0.5 ml of each extract was mixed with 0.1 ml of aluminum chloride (1%), 0.1 ml of potassium acetate (1 ml), and 4.3 ml of distilled water; after a vigorous agitation, the mixture was incubated for 30 min in ambient temperature. DO’s values have been read using spectrophotometer visible-UV at 415 nm. Flavonoid content was expressed as µg Quercetin equivalents per mg dry weight of the extract (µg Quer eq mg E) using a calibration range from 25 to 300 µg/ml.

Data analysis

The data collected from the ethnobotanical surveys have been analyzed using Excel software. The other results have been presented as means ± standard deviation, and statistical analyses were performed using analysis of variance by IBM SPSS Statistics 21. Differences at p<0.05 were considered statistically significant.

RESULTS

Ethnobotanical survey

In the present survey, 91 traditional herbalists and healers (n=91) from provinces of Fez (FS), Meknes (MK) and Taounate (TN) were interviewed. 55 plant species distributed in 30 families were mentioned (Table 2). The most representative family was the Lamiaceae (18.18%) with 10 species, followed by the Apiaceae (10.91%) with 6 species and the Asteraceae (7.27%) with 4 species. Other families have been presented by one or two species.

On the one hand, leaves were the most frequently cited used part to prepare remedies with 45% followed by roots or whole plant with 12.5% each, then flowers (11.25%). The remedies were prepared

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using decoctions in water as solvent (48.53%), followed by cataplasm with 35.29%. Most of the anti-cutaneous infections preparations were administered externally (75%) against 25% orally.

On the other hand, the calculated FI has indicated that the most frequently cited (FI=8.88%) anti-dermatitis plant species in the three studied areas were Rhamnus alaternus (FI =17.77%; FI (MK)=29.41%; FI (TN)=29.41%). Punica granatum (FI =11.11%; and FI (MK)=23.52%); Dittrichia (Inula) viscosa (FI=0.2%; FI(MK/TN)=17.64%); Alkanna tinctoria (FI (MK)=23.52%). Aquilaria malaccensis (FI (MK)=35.29%); Urtica dioica (FI (FS)=8.88%); FI (MK)=11.76%); Crateagus monogyna (Fl (FS)=11.11%; FI (TN)=17.64%); Artemisia herba alba (FI (MK)=17.64%); Berberis hispanica (FI (FS)=8.88%; FI (MK)=11.76%); Rubia tinctoria (FI (MK)=17.64%); FI (TN)=11.76%); Thymelae a tartonraia (FI (MK)=17.64%); Globularia alypum (FI (TN)=11.76%); and Anmmi majus (FI (FS)=8.88%; FI (TN)=11.76%), then Nerium oleander, Eruca sativa, Olea europea with FI (FS)=8.88%. Aquilaria malaccensis has presented an important score of citation; however, it is not from Morocco and especially Fez-Meknes region, the species is reported from Asia so for that we didn’t test it.

**Antistaphylococcal activity**

The crude ethanolic extracts of the 17 most cited plants were tested against *S. epidermidis*, and two MRSA clinical isolate and *S. aureus* reference. The data pertaining to the in vitro assay are presented in Tables 3 and 4. The obtained data have revealed that among the 17 tested plants, nine were active against the target strains, and eight plant extracts have not shown antistaphylococcal activity. The quantitative determination of MIC values has shown that the antibacterial activity varied on the plant species and the target microorganism.

Both extracts from *P. granatum* peel and *R. alaternus* leaves have registered the highest effect against the three *Staphylococcus* strains with a MIC ranging between 0.25 and 2.00 mg/ml.

In the other side, *I. viscosa* leaves extract has exhibited the same effect with with MIC of 4.00 mg/ml against the three strains which were the same for *O. majorana* expect for *S. epidermidis* that its MIC was superior to 16.00 mg/ml. *O. europea* has also inhibited the growth of *S. aureus* clinical isolate at 4.00 mg/ml, but it was effective against *S. aureus* reference at MIC of 0.50 mg/ml and more than 16.00 mg/ml for *S. epidermidis*. In addition, the MIC values of the other active plant extracts were ranging from 0.25 mg/ml to upper than 16.00 mg/ml against the tested strains.

**Quantitative phytochemical assays**

The total phenolic and total flavonoids contents of the 17 extracts were presented in Table 5. As can be noted from this table, *O. majorana* and *O. europaea* have presented the highest content of total phenols (307.87±1.12 µg eq GA/mg E and 297.5±16.45 µg eq GA/mg E, respectively), followed by *P. granatum* extract which has noticed 153.4±14.36 µg eq GA/mg E, then *R. alaternus* with 119.38±3.71 µg eq GA/mg E. *L. viscosa* and *C. oxyacantha* extracts were in the same rang with 88.63 ±3.12 µg eq GA/mg E and 113.6±1.88 µg eq GA/mg E. The total phenols amount of the other plant extracts were ranging from 57.98±2.66 µg eq GA/mg E to 59.5±0.99 µg eq GA/mg E. *N. oleander*, *E. sativa*, and *A. tinctoria* extracts have indicated the lowest concentration of total phenols in comparison with the other extracts (p<0.05).

Regarding to flavonoids, the first range belonged to *R. alaternus* with a content of 321.03±0.63 µg eq Que/mg E, followed by *P. granatum* extract (125.07±3.90 µg eq Que/mg E) and *E. sativa* (152.53±4.85 µg eq Que/mg E), then *O. europaea* with 83.54±1.92 µg eq Que/mg E, followed by *A. majus*, *T. tartonraia*, *O. majorana*, *A. herba alba*, *J. oxycedrus*, *C. oxyacantha*, and *I. viscosa*, the flavonoid contents of these plants were between 73.64±1.47 and 49.90±2.80 µg eq Que/mg E. *B. hispanica* and *A. tinctoria* extracts have shown the lowest total flavonoids content (14.75±3.18 and 12.17±2.36 µg eq/mg E, respectively).

**DISCUSSION**

The current survey realized in three areas in the central north of Morocco aimed the identification of plants used in the treatment of skin infections, the in vitro evaluation of the most recommended plants against *Staphylococcus* strains often involved in dermatitis and the analysis of phytochemical compounds that could be responsible for skin care.

### Table 3: Antibacterial screening of the plant extracts using the agar well-diffusion method

| Plant extracts | Target microorganisms | S. epidermidis | S. aureus clinical isolate | S. aureus ATCC 29213 |
|----------------|-----------------------|---------------|---------------------------|----------------------|
| Punica granatum | 22.3±1.24             | 25.0±1.00     | 24.66±0.47                |
| Rhamnus         | 16.00±1.00            | 25.50±1.5     | 25.00±1.00                |
| Inula viscosa   | 11.66±1.24            | 13.66±1.88    | 13.00±0.81                |
| Crateagus       | 14.00±0.81            | 12.33±1.88    | 11.66±1.24                |
| Rubia tinctoria | 13.33±1.24            | 10.33±0.47    | 12.50±0.50                |
| Artemisia herba alba | 7.0±0.00          | 14.50±2.12    | 13.00±0.70                |
| Berberis hispanica | 24.0±0.00        | 26.0±1.00     | 24.00±1.00                |
| Olea europaea   | 25.33±0.94            | 14.50±0.5     | 14.00±0.81                |
| Origanum majorana | 8.00±0.00         | 10.00±0.81    | 11.00±0.81                |
| Nerium oleander | -                     | -             | -                         |
| Alkanna tinctoria | -                    | -             | -                         |
| Juniperas       | -                     | -             | -                         |
| J. oxycedrus    | -                     | -             | -                         |
| Eruca sativa    | -                     | -             | -                         |
| Urtica dioica   | -                     | -             | -                         |
| Thymelae a      | -                     | -             | -                         |
| Tartonraia      | -                     | -             | -                         |
| Globularia      | -                     | -             | -                         |
| A. albulm       | -                     | -             | -                         |
| Anmmi majus     | -                     | -             | -                         |
| Ampicillin      | 17.0                  | -             | 0.70                      |

**Table 4: MICs (mg/ml) of the most active studied extracts**

| Ethanolic extracts | CMI (mg/ml) | S. aureus clinical isolate | S. aureus ATCC 29213 | S. epidermidis |
|--------------------|-------------|----------------------------|----------------------|---------------|
| Artemisia herba alba | 8.00        | 8.00                       | 8.00                 |
| Berberis hispanica | 16.00       | 16.00                      | >16.00               |
| Crateagus oxyacantha | 16.00      | 16.00                      | 8.00                 |
| Inula viscosa      | 4.00        | 4.00                       | 4.00                 |
| Olea europaea      | 4.00        | 8.00                       | >16.00               |
| Origanum majorana  | 4.00        | 4.00                       | >16.00               |
| Punica granatum    | 1.00        | 0.25                       | 2.00                 |
| Rhamnus            | 0.50        | 0.50                       | 2.00                 |
| Rubia tinctoria    | 16.00       | 16.00                      | >16.00               |

*S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis*
In the present study, 55 medicinal plants belonging to 30 families have been prescribed by herbalists and traditional healers in Fez, Meknes, and Taounate to cure skin disorders that can be caused by the Staphylococcus genus. The Lamiaceae family was the most abundant (18.18%). This family has been demonstrated to have antibacterial and antifungal activities against skin infections [21]. Furthermore, several pharmacological properties have been attributed to this family due to its richness in active bio-molecules. Admittedly there has been a qualitative and quantitative difference in chemical composition of species belonging to the Lamiaceae family or else the same genus [22], but this family stills generally rich in polyphenols, saponins, irroisides, alkaloids, anthocyanins, and aldehydes [23]. Those compounds possess broad-spectrum antimicrobial [24].

The obtained results have also revealed that (i) the leaves were the most used part (45%) which accords other studies [24]. This extensive use could be explained by the abundance of phytochemical compounds in leaves which are the synthesis site of vegetal secondary metabolites [25]; (ii) decoction mode was the most recommended (48.53%). Many investigations concerning the plants’ uses in traditional medicine have highlighted the preponderance of decoction method to prepare remedies [26,27] which appears to have a number of advantages like the extraction of the maximum of herbal substances that are soluble in boiling water which makes them easily absorbed and perceived by human body [28]; (iii) external administration route was in the first ring (75.00%) including flushing and cataplasm depending on the patient preference. This is may be explained by the fact that both of methods could be fast and efficient. The internal use of medicinal plants consisted, for example, for digestive disease, stomachache, or rheumatic pain. However, skin infections need the use of external remedies which is in agreement with literature [29,30].

Nine of the most recommended plant species reported in the current survey were found to be efficient against the three studied Staphylococcus strains. These plants included P. granatum, R. alaternus, I. viscosa, O. europea, O. majorana, C. oxyacantha, B. hispanica, A. herba alba, and B. hispanica. In some cases, these findings support the traditional uses of the inventorying plant species through the ethnobotanical study.

Many previous investigations have confirmed the significant antibacterial activity of these plants against various bacterial species including S. aureus. Indeed, the anti-staphylococcal activity of B. hispanica extract is in agreement with a previous study that has reported the effect of ethanolic extract of B. hispanica roots against S. aureus [31]. Stelmarki et al. [32] reported that the aqueous extract of C. oxyacantha leaves has revealed antibacterial activity against S. aureus and S. epidermidis.

Table 5: Total phenolic and flavonoids contents of the ethanolic extracts

| Plants                  | Total phenolic contents (µg equivalent of gallic acid/mg of extract) | Total flavonoid contents (µg equivalent of quercetin/mg of extract) |
|-------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
| P. granatum             | 153.4±1.436ab                                                      | 125.0±3.90bc                                                       |
| R. alaternus            | 119.3±3.71c                                                        | 32.1±6.63a                                                         |
| I. viscosa              | 88.6±3.12de                                                        | 57.4±6.58d                                                        |
| C. oxyacantha           | 113.6±1.88cd                                                       | 57.01±5.38d                                                       |
| R. tinctorum            | 51.3±3.64f                                                         | 22.95±2.82e                                                       |
| A. herba alba           | 52.6±3.61f                                                         | 52.26±0.77f                                                       |
| O. majorana             | 307.8±7.12a                                                        | 73.64±1.47f                                                       |
| B. hispanica            | 50.7±8.34e                                                         | 14.75±3.18f                                                       |
| G. alypum               | 52.4±2.71f                                                         | 43.99±1.79f                                                       |
| O. europaea             | 297.5±1.64a                                                        | 83.54±1.92c                                                       |
| N. oleander             | 5.95±0.99f                                                         | 19.76±0.51f                                                       |
| A. tinctoria            | 8.50±3.40f                                                         | 12.17±2.36f                                                       |
| J. oxycedra             | 16.63±1.63h                                                        | 67.00±5.25f                                                       |
| E. sativa               | 9.25±0.88f                                                         | 15.23±4.85f                                                       |
| U. dioica               | 13.29±0.56f                                                        | 33.00±0.94f                                                       |
| T. tartaronaira         | 57.98±2.66d                                                        | 62.67±1.97f                                                       |
| A. majus                | 28.5±2.58h                                                         | 60.59±5.53f                                                       |

Means that not share the same letter are statistically different at p<0.05. Data represent mean±standard error of mean.
Numerous causes may justify the variations of total phenolic and flavonoids contents reported in this work. Indeed, the variation of the polyphenolic content of a plant could be influenced by many biotic factors (Plant species, used part, and physiological stage) and abiotic factors (Environment, solvent) which can affect the metabolism of the plant [50]. Based on all results in the present work, complementary studies are necessary to improve and strengthen these current preliminary findings. This is concern because bioassay-guided isolation, purification of the bioactive components, and in vivo and toxicity assays. Advanced and succeeding scientific research could lead to discover novel and cost-effective drugs against Staphylococcus genus and especially methicillin-resistant strains.

CONCLUSION

The ethnobotanical study conducted in Fez, Meknes and Taounat cities belonging to Fez-Meknes region in the central North of Morocco documented 55 plant species belonging to 30 families that were traditionally used by the local populations to cure dermatitis. The 17 most recommended plants were screened for their in vitro antistaphylococcal activity, nine of them found to demonstrate appreciable effects, namely P. granatum, R. alaternus, I. viscosa, O. europea, O. majorana, C. oxyacantha, R. tinctorium, A. herba alba, and B. hispanica with MIC values ranging between 0.25 mg/ml and 16 mg/ml against MRSA. These plants could be good candidates to overcome infectious diseases associated with Staphylococcus including MRSA infections. Hence, additional deep studies must be maintained such as the search for bioactive fractions and pure compounds that may serve as a potential source of new drugs to treat S. aureus, especially MRSA. Furthermore, despite the presence of rich herbal knowledge in the studied region, skin disorders are dermatologic conditions that have a similar clinical appearance, for this reason, much attention must be paid due to the correct diagnosis impacts both the prognosis and the treatment options.

ACKNOWLEDGMENTS

We are grateful to Professor IBN TATTOU Mohammed from the Scientific Institute in Rabat-Morocco for its help to identify the inventorying plants in this study. We thank also Doctor BALOUIRI Mounyr for his help and his bacterial strains.

AUTHORS’ CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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