ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 3, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS IN THE CENTRAL NORTH OF MOROCCO: A POSSIBLE SOURCE OF ALTERNATIVE DRUGS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

IKRAME ZEOUK[1], ABDELHAKIM EL OUALI LALAMI[2,3], KHADIJA BEKHTI[1]*

[1]Department of Biology, Sidi Mohamed Ben Abdellah University, Faculty of Sciences and Techniques, Laboratory of Microbial Biotechnology, PB 2202, Fez, Morocco. [2]El Ghassani Hospital, Regional Health Directorate, Institute of Nursing Professions and Health Techniques Fez (Annex Meknès), 30000 Fez, Morocco. [3]El Ghassani Hospital, Regional Health Directorate, Regional Diagnostic Laboratory of Epidemiological and Environmental Health, 30000 Fez, Morocco. Email: bekhti.bki4@gmail.com

Received: 14 December 2018, Revised and Accepted: 20 November 2018

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 ethnobotanical 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.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i3.30395

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 award current antibiotics has become increasingly common 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 used to fight the MRSA infections; in 2002 a study showed that some strains of MRSA were also resistant to vancomycin (RVSA) [9]. The multi-resistance is serious; significant efforts must be invested to search new effective anti-MRSA bioactive molecules. Given that 75% of the drugs against infectious diseases are natural products or natural derivatives [10], herbal medications are more used, 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. The primary objective of the current study was to aid the progressive scientific works related to the antimicrobial activity of plants.

METHODS

Study area

Located in the central North of Morocco (Fig. 1), partially integrating the plain of Saïss and along siding the mountain ranges of the Rif and the Middle Atlas, Fez-Meknes region covers an area of 40.075 km^2 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 km^2, very high compared to the national average (47.6 hab/km^2). 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) a 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.

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:

$$FI=n/N*100$$

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

Fig. 1: Map of the studied areas

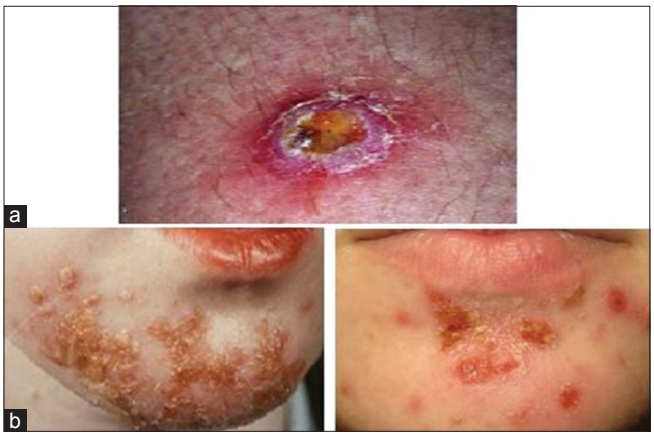

Fig. 2: Example of dermatitis photos showed to the herbalists in the studied region. (a) Furunculosis. (b) Impetigo

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*. with slight modifications [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

Revivification 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 (10^8 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	Resistant	
	Amoxicillin	20	Susceptible	Resistant	
	Oxacillin	1	Susceptible	Resistant	
	Methicillin	5	Susceptible	Resistant	
Penicillin combinations	Amoxicillin/Clavulanate	20/10	Susceptible	Resistant	
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	Resistant	
	Pristinamycin	10	Susceptible	Susceptible	

S. aureus: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*

Table 2: The inventorying ethno dermatological medicinal plants

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	Cataplasme decoction infusion	5.88 (TN)
Daucus carota	Apiaceae	Khizzo/Jaada	Whole plant fruit	Cutaneous	Decoction cataplasme	2.22 (FS)
Petroselinum crispum	Apiaceae	Maadnous	Leaves	Oral	Infusion decoction	2.22 (FS)
Ammi majus	Apiaceae	Trillan/Tlillan	Leaves	Cutaneous	Decoction	8.88 (FS) 11.76 (TN)
Ammi visnaga	Apiaceae	El khella el bariya/ Bechnikha; Kessiba	Leave Root	Cutaneous	Cataplasme decoction	2.22 (FS)
Ferula assa - foetida	Apiaceae	Elwachk	Leaves flowers	Cutaneous	Cataplasme decoction	2.22 (FS)
Foeniculum vulgare	Apiaceae	Chemmar/Besbas/ Nafaâ/Amsi	Leaves	Cutaneous	Decoction	2.22 (FS)
Nerium oleander	Apocynaceae	Defla	Leaves whole plant arial part	Cutaneous	Cataplasme	8.88 (FS)
Artemisia herba alba	Asteraceae	Chih	Leaves	Cutaneous	Cataplasme	4.44 (FS) 17.64 (MK) 5.88 (TN)
Dittrichia viscosa =(*Inula viscosa)*	Asteraceae	Magraman bayraman	Leaves whole plant	Cutaneous	Cataplasme	20 (FS) 17.64 (MK) 17.64 (TN)
Chamaemelum nobile	Asteraceae	Babounj	Leaves	Oral	Infusion	2.32 (FS) 6.25 (MK)
Carlina gummifera (*=Atractylis gummifera=*	Asteraceae	Addad	Roots	Cutaneous	Cataplasme	2.22 (FS)
Berberis hispanica	Berberidaceae	Azirki arghis	Roots	Cutaneous	Cataplasme	8.88 (FS) 11.76 (MK) 5.88 (TN)
Lepidium sativum	Brassicaceae	Heb errechad	Seeds	Oral	Decoction	5.88 (TN)
Eruca sativa	Brassicaceae	El Gergir/ Jerjir	Flowers seeds	Oral	Decoction	8.88 (FS)
Alkkanna tinctoria	Boraginaceae	Taymant	Roots	Cutaneous	Cataplasme	23.52 (MK)
Tetraclinis articulata	Cupressaceae	*Aarâar*	Leaves	Oral	Infusion	4.44 (FS) 5.88 (MK)
Juniperus oxycedrus	Cupressaceae	Taqqa, tiqqi	Seeds	Cutaneous	Decoction cataplasme	11.11 (FS) 17.64 (TN)
Ricinus communis	Euphorbiaceae	El kharwaae	Leaves	Cutaneous	Cataplasme decoction	5.88 (TN)
Glycyrrhiza glabra	Fabaceae	Erk sous	Roots	Cutaneous	Decoction	2.22 (FS)
Cicer arietinum	Fabaceae	Hemess	Fruit	Cutaneous	Cataplasme	2.22 (FS)
Pelargonium graveolens	Geraniaceae	Laatercha	Leaves flowers	Cutaneous	Decoction	2.22 (FS)
Illicium verum	Illiciaceae	Badiyan	Whole plant seed	Oral cutaneous	infusion cataplasme	2.22 (FS)
Crocus sativus	Iridaceae	Zaafrane	Stigma	Cutaneous	Decoction	2.22 (FS)
Juncus rigidus(= Juncus arabicus)	Juncaceae	Oud essemar/smar; your	Roots	Cutaneous	Cataplasme	5.88 (TN)
Thymus algeriensis	Lamiaceae	Zaater, marrad	Whole plant leaves	Oral	Infusion	6.66 (FS) 5.88 (MK) 5.88 (TN)
Lavandula dentata	Lamiaceae	Khiyata	Leaves flowers whole plant	Oral	Infusion	4.44 (FS) 5.88 (MK) 5.88 (TN)
Mentha piperita	Lamiaceae	Naânaâ Elfolfoli, Naânaâ El Âbdi	Leaves	Oral cutaneous	Infusion	2.22 (FS)
Thymus atlanticus	Lamiaceae	Zîitra	Leaves	Oral cutaneous	Decoction cataplasme	2.22 (FS)
Origanum majorana	Lamiaceae	Merdedouch/ Berkdouch	Leaves whole plant	Cutaneous	Decoction	11.11 (FS) 17.64 (TN)
Lavandula angustifolia	Lamiaceae	Khzama	Leaves flower whole plant	Oral	Infusion	5.88 (MK)
Marrubium vulgare	Lamiaceae	Meriwta, marriwa	Leaves flowers whole plant	Cutaneous	Decoction	5.88 (MK)
Rosmarinus officinalis	Lamiaceae	Azir	Leaves	Oral cutaneous	Infusion	2.22 (FS)
Ocimum canum	Lamiaceae	Errayhan el kafouri	Leaves	Oral	Decoction	2.22 (FS)
Salvia officinalis	Lamiaceae	Salmiya	Leaves	Cutaneous	Cataplasme	2.22 (FS)
Punica granatum	Lythraceae	Romman	Peel	Cutaneous	Decoction	11.11 (FS) 23.52 (MK)
Lawsonia inermis	Lythraceae	El Henna	Leaves	Cutaneous	Cataplasme	2.22 (FS)

(Contd...)

Table 2: ***(Continued)***

Botanical name	Family name	Local name	Part used	Administration route	Preparation mode	FI (%)
Laurus nobilis	Lauraceae	Elghar/Chajrat Sidna Moussa/Rand	Leaves	Cutaneous	Decoction	2.22 (FS)
Syzygium aromaticum	Myrtaceae	Krenfel/Oud Ennawar	Cloves	Oral	Infusion	2.22 (FS)
Eucalyptus globulus	Myrtaceae	Eucalyptus	Leaves	Cutaneous	Decoction	2.22 (FS)
Olea europaea	Oleaceae	Zitoun	Leaves fruit	Cutaneous	Decoction cataplasme	8.88 (FS)
Oenothera sp.	Onagraceae	Akhdariya	Leaves flowers	Cutaneous	Decoction	2.22 (FS)
Globularia alypum	Plantaginaceae	Ain larneb/ tasalgha; hallab rwa	Leaves	Cutaneous	Decoction	6.66 (FS) 11.76 (TN)
Plantago major	Plantaginaceae	Lsan lhamal/Aslouj	Leaves	Cutaneous	Decoction	2.22 (FS)
Rhamnus alaternus	Rhamnaceae	Mililess	Leaves	Cutaneous	Decoction cataplasme	17.77 (FS) 29.41 (MK)29.41 (TN)
Ziziphus lotus	Rhamnaceae	Nbeg	Leaves fruit	Cutaneous	Decoction cataplasme	2.22 (FS)
Rubia tinctorum	Rubiaceae	El fossa	Roots	Cutaneous	Decoction	4.44 (FS) 17.64 (MK) 11.76 (TN)
Citrus aurantium	Rutaceae	Renj	Fruit	Oral Cutaneous	Decoction	2.22 (FS)
Crataegus monogyna (= Crataegus oxyacantha)	Rosaceae	Zeerour/admam	Leaves arial part fruit	Cutaneous	Decoction	4.44 (FS) 23.52 (MK)
Salvadora persica	Salvadoraceae	Swak	Roots	Cutaneous	Cataplasme	5.88 (MK)
Thymelaea tartonraira	Thymelaeaceae	El matnan/ Talazazt	Leaves	Cutaneous	Cataplasme	17.64 (TN)
Aquilaria malaccensis	Thymelaeaceae	Oud aghris	Roots	Cutaneous	Cataplasme	35.29 (MK)
Urtica dioica	Urticaceae	Herriga Herricha	Leaves flowers whole plant	Cutaneous	Decoction	8.88 (FS) 11.76 (MK) 5.88 (TN)
Curcuma longa	Zingiberaceae	Karkum/ Kharqoum	Roots	Cutaneous	Decoction	2.22 (FS)

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

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 mg/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 mg/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 10^5 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 was prepared without plant extracts.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Total phenolic quantification

The quantification of total phenolic has been evaluated using the Folin–Ciocalteu reagent by introducing 1.5 ml of Folin–Ciocalteu reagent (10%) in 200 µl of extract (1 mg/ml Ethanol), the mixture was agitated carefully and allowed to react for 5 min in dark, then followed by adding 1.5 ml of sodium carbonate (5%). After 2 h of incubation in room temperature and in the dark again, values have been read using spectrophotometer visible-ultraviolet (UV) at 750 nm. Under the same conditions, a calibration range was made using gallic acid with different concentrations ranging between 300 µg/ml and 25 µg/ml. The total phenolic content was expressed as µg gallic acid 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 (10%), 0.1 ml of potassium acetate (1 m), 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 values±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

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 (FS) =17.77%; FI (MK)=29.41%; FI (TN)=29.41%), *Punica granatum* (FI (FS)=11.11%; and FI (MK)=23.52%); *Dittrichia* (*Inula*) *viscosa* (FI(FS)=20%; FI(MK/TN)=17.64%), *Alkkanna tinctoria* (FI (MK)=23.52%), *Aquilaria malaccensis* (FI (MK)=35.29%); *Urtica dioica* (FI (FS)=8.88%; FI (MK)=11,76%), *Crataegus monogyna* (FI (MK)=23.52%), *Origanum majorana* (FI (FS) =11.11%; FI (TN)= 17.64%), *Juniperus oxycedrus* (FI (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 tinctorum* (FI (MK)=17.64%; FI (TN)=11.76%), *Thymelaea tartonraira* (FI (TN)=17.64%), *Globularia alypum* (FI (TN)=11.76%), and *Ammi 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 08.00 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 08.00 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±0.12 µg eq GA/mg E and 297.51±6.45 µg eq GA/mg E, respectively), followed by *P. granatum* extract which has noticed 153.41±4.36 µg eq GA/mg E, then *R. alaternus* with 119.38±3.71 µg eq GA/mg E. *I. viscosa* and *C. oxyacantha* extracts were in the same rang with 88.63 ±3.12 µg eq GA/mg E and 113.60±1.88 µg eq GA/mg E, respectively. The total phenols amount of the other plant extracts were ranging from 57.98±2.66 µg eq GA/mg E to 5.95±0.99µg eq AG/mg d'E. *Nerium oleander, Eruca sativa,* and *Alkanna 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 d'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 *Ammi majus, Thymelaea tartonraira, Origanum majorana, Artemisia herba alba, Juniperus oxycedrus, Crataegus oxyacantha*, and *Inula viscosa*, the flavonoid contents of these plants were between 73.64±1.47 and 49.90±2.80µg eq Que/mg E. *Berberis hispanica* and *Alkanna tinctoria* extracts have shown the lowest total flavonoids content (14.75±3.18 and 12.17±2.36 µg eq/mg E, respectively).

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	25.00±1.00	24.66±0.47
Rhamnus alaternus	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
Crataegus oxyacantha	14.00±0. 81	12.33±1.88	11.66±1.24
Rubia tinctorium	13.33±1.24	10.33±0.47	12.50±0.50
Artemisia herba alba	7.00±0.00	14.50±2.12	13.00±0.70
Berberis hispanica	24.00±0.00	26.00±1.00	24.00±1.00
Olea europaea	25.33±0.94	14.50±0.5	14.00±0.81
Origanum majorana	08.00±0.00	10.00±0.81	11.00±0.81
Nerium oleander	-	-	-
Alkanna tinctoria	-	-	-
Juniperus oxycedrus	-	-	-
Eruca sativa	-	-	-
Urtica dioica	-	-	-
Thymelaea tartonraira	-	-	-
Globularia alypum	-	-	-
Ammi majus	-	-	-
Ampicillin	17.0	-	07.0

Non active: Data represents mean±standard error of mean.
S. aureus: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis*

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
Crataegus 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	1.00
Rhamnus alaternus	0.50	0.50	2.00
Rubia tinctorum	16.00	16.00	>16.00

S. aureus: *Staphylococcus aureus*, *S. epidermidis*: *Staphylococcus epidermidis,*
MICs: Minimum inhibitory concentrations

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 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)
Punica granatum	153.41±4.36^{b}	125.07±3.90^{b,c}
Rhamnus alaternus	119.38±3.71^{b,c}	321.03±0.63^{a}
Inula viscosa	88.63±3.12^{c,d}	57.74±6.58^{c,d}
Crataegus oxyacantha	113.60±1.88^{c,d}	57.01±5.38^{c,d}
Rubia tinctorum	51.37±3.64^{f,g}	22.95±2.82^{d}
Artemisia herba alba	52.68±3.61^{e,f}	52.26±0.77^{c,d}
Origanum majorana	307.87±0.12^{a}	73.64±1.47^{c,d}
Berberis hispanica	50.77±8.34^{f,g}	14.75±3.18^{d}
Globularia alypum	52.45±2.71^{e,f}	43.99±1.79^{d}
Olea europaea	297.51±6.45^{a}	83.54±1.92^{b,c,d}
Nerium oleander	5.95±0.99^{i}	19.76±0.51^{d}
Alkanna tinctoria	8.50±3.40^{i}	12.17±2.36^{d}
Juniperus oxycedrus	16.63±10.63^{h,i}	67.00±5.25^{c,d}
Eruca sativa	9.25±0.88^{i}	152.53±4.85^{b}
Urtica dioica	13.29±0.56^{i}	33.00±0.94^{d}
Thymelaea tartonraira	57.98±2.66^{e,f}	62.67±1.97^{c,d}
Ammi majus	28.20±5.88^{g,h}	60.59±5.53^{c,d}

Means that not share the same letter are statistically different at $p<0.05$. Data represent mean±standard error of mean

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, irroides, alkaloids, anthocyanins, and aldehydes [23]. Those compounds possess broad-spectrum antimicrobials [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, tract digestive disease, stomachache, or rheumatism 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*, *R. tinctorium, 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-*staphylococcus* 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]. Stelmakiene *et al.* [32] reported that the aqueous extract of *C. oxyacantha* leaves has revealed antistaphylococcal activity against *S. aureus* and *S. epidermidis.*

A number of studies have also been reported to confirm that *P. granatum* is effective in skin curing and protection. The extract of *P. granatum* has been mentioned as one of the ingredients in skin care formulations of an interesting invention [33]. This plant species has been also acclaimed to have a protective effect award Ultraviolet-Irradiated Human Skin Fibroblasts that could cause serious skin disorders [34,35]. The antistaphylococcal activity of *P. granatum* has been also demonstrated; a previous study has shown that the ethanolic extract prepared from *P. granatum* pericarp was effective against *S. aureus* and *S. epidermidis* [36].

The antibacterial activity of *R. alaternus* has been also evaluated against pathogenic bacteria; the leaves' extract was efficient against *S. aureus* [37]. Other *in vitro* investigations have also confirmed the antibacterial activity (especially against *staphylococcus* genus) of the extract prepared from the leaves of *I. viscosa* [38], *O. europea* [39], *O. majorana* [40], and the roots of *R. tinctorium* [41]. Among *A. herba alba*, it has proven its medicinal use for centuries as an antimicrobial agent [42].

However, our findings have noticed that eight of the most recommended plants have not shown any anti-staphylococcal potent, these extracts may have other biological activities against other parasitic or fungal pathogenic microorganisms that cause dermatitis, or else bacterial genus other than *Staphylococcus*. Another explanation may justify this result, it was reported in a recent study that not all the preparations could be useful to cure dermatitis. The skin defense is based on the efficacy of the chemical deactivation process through the enzymology mechanisms by broking down the inactive xenobiotics into a more polar inactive metabolite. This is may have place by forming functional groups such as -OH, -NH2, and –SH [43]. Moreover, in our study, the phytochemical analysis could also give an idea about the plant species effectiveness. It was reported that plants containing high contents of phytochemical compounds such as polyphenols and flavonoids have been considered as useful ingredients in skin cosmetic preparations [44]. Many research groups have explained the effectiveness of total phenols and flavonoids by direct action against germs or through the suppression of microbial virulence factors. For instance, it was reported that flavonoids can inhibit some of the bacterial virulence factors, including quorum-sensing signal receptors, enzymes, and toxins that are necessary for bacteria growth and metabolism [45]. Furthermore, it has been documented that the antibacterial activity of different groups of flavonoids can be attributable to numerous mechanisms such as the inhibition of energy metabolism of bacteria, the inhibition of nucleic acid synthesis, and the inhibition of cytoplasmic membrane function [46]. In the case of the *Staphylococcus* genus, it was demonstrated that flavonoids have an aggregatory effect on whole bacterial cells [47]. In our study, the active plants were rich in total phenols and total flavonoids which may explain their effectiveness against the target *Staphylococcus* strains. However, we have demonstrated the antistaphylococcal effect of *B. hispanica* despite the small phytochemical amounts that it contains. Based on an investigation conducted by Musumeci *et al.* [48], the biological effect of this species could be contributed to the presence of 5'-methoxyhydnocarpine-D and pheophorbide (chlorophyll decomposition products) synthesized by berberine containing in this genus, these substances have no antimicrobial activity, but they are responsible for inhibiting the expression of efflux pumps expression in *S. aureus* through the extruding of antimicrobial agents from bacterial cells [49].

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 bioassay-guided isolation, purification of the bioactive components, *in vivo* and toxicity assays. Advanced 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.

REFERENCES

1. Clarke SR, Foster SJ. Surface adhesins of *Staphylococcus aureus*. Adv Microb Physiol 2006;51:187-224.
2. Jarvis AW, Lawrence RC. Production of extracellular enzymes and enterotoxins A, B, and C by *Staphylococcus aureus*. Infect Immun 1971;4:110-5.
3. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, *et al.* Association between *Staphylococcus aureus* strains carrying gene for panton-valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 2002;359:753-9.
4. Valle J, Vadillo S, Piriz S, Gomez-Lucia E. Toxic shock syndrome toxin 1 (TSST-1) production by staphylococci isolated from goats and presence of specific antibodies to TSST-1 in serum and milk. Appl Environ Microbiol 1991;57:889-91.
5. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 2015;28:603-61.
6. Totté JEE, van Doorn MB, Pasmans SGMA. Successful treatment of chronic *Staphylococcus aureus*-Related dermatoses with the topical endolysin staphefekt SA.100: A Report of 3 cases. Case Rep Dermatol 2017;9:19-25.
7. Périchon B, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2009;53:4580-7.
8. Isha J, Ashwini H, Pooja R, Radhakrishna M. Nasal colonization of *Methicillin-resistant Staphylococcus aureus* among clinical postgraduates in a coastal Karnataka medical college prevalence and antibiogram pattern. Asian J Pharm Clin Res 2018;11:104-9.
9. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, *et al*. *Methicillin-resistant* S. Aureus infections among patients in the emergency department. N Engl J Med 2006;355:666-74.
10. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75:311-35.
11. Swarnamoni D, Mukundam B, Shagufa A. Antibacterial activity of the ethanolic extract of leaves of *Citrus maxima* (burm.) merr. On *Escherichia coli* and *Pseudomonas aeruginosa*. Asian J Pharm Clin Res 2013;6:136-9.
12. Badduri N, Gupta NV, Gowda DV, Manohar M. Formulation and development of topical anti acne formulation of spirulina extract. Int J App Pharm 2018;10:229-33.
13. Bikash G, Biswajit D, Sujata P, Aditya B. Preliminary phytochemical screening and *in vitro* anti-microbial activity of ethanolic extract of *Delonix regia* (hook) raf. bark (family-*Leguminosae*). Int J Curr Pharm Res 2018;10:43-6.
14. Moroccan Ministry of the Interior Wilaya of the Region Fez-Meknes, Monograph of the Region Fez-Menkes, Thèses et Mémoires, C28613/14, Thèse No. DL 1987/489; 2016.
15. Kingdom of Morocco: Ministry of Interior. Fez-Meknes Regional Direction, Regional Statistical Directory Fez Meknes, Regional Direction; 2016.
16. Agency for the Promotion and Development of the North (APDN). Morocco: The Province of Taounat; 2018.
17. Yeo YL, ChiaYY, Lee CH, Sow HS, Yap WS: Effectiveness of maceration periods with different extraction solvents on *in-vitro* antimicrobial activity from fruit of *Momordica charantia* L. J Appl Pharm Sci 2014;4:16-23.
18. Elaloui M, Laamouri A, Ennajah A, Cerny M, Mathieu C, Vilarem G, *et al*. Phytoconstituents of leaf extracts of *Ziziphus jujuba* Mill. plants harvested in Tunisia. Ind Crops Prod 2016;83:133-9.
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal 2016;6:71-9.
20. Bahorun T, Gressier B, Trotin F, Brunet C, Dine T, Luyckx M, *et al.* Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneimittelforschung 1996;46:1086-9.
21. Chaumont JP, Mandin D, Sanda K, Koba K, de Souza CA. Activités antimicrobiennes *in vitro* de cinq huiles essentielles de *Lamiacees* togolaises vis-à-vis de germes représentatifs de la microflore cutanée. Acta Bot Gall 2001;148:93-101.
22. Zgórka G, Głowniak K. Variation of free phenolic acids in medicinal plants belonging to the *Lamiaceae* family. J Pharm Biomed Anal 2001;26:79-87.
23. Btissam R, Fatima EIM, Mohamed N, Fatima EIM, Rubia L. *In vitro* Study of antibacterial activity of hydro-alcohol morrocan plants extracts. Pharmacogn J 2018;10:519-26.
24. Cocan I, Alexa E, Danciu C, Radulov I, Galuscan A, Obistioiu D, *et al.* Phytochemical screening and biological activity of *Lamiaceae* family plant extracts. Exp Ther Med 2018;15:1863-70.
25. Dulla O, Jahan FI. Ethnopharmacological survey on traditional medicinal plants at Kalaroa Upazila, Satkhira district, Khulna division, Bangladesh. J Intercult Ethnopharmacol 2017;6:316-25.
26. Azzi R, Rabah D, Farid L, Zohra SF, Houcine B, Nacéra B. Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. J Med Plants Res 2012;6:2041-50.
27. Rupani R, Chavez A. Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. Clin Dermatol 2018;36:306-9.
28. Bouasla A, Bouasla I. Ethnobotanical survey of medicinal plants in Northeastern of Algeria. Phytomedicine 2017;36:68-81.
29. Kolasani A, Xu H, Millikan M. Evaluation of mineral content of Chinese medicinal herbs used to improve kidney function with chemometrics. Food Chem 2011;127:1465-71.
30. Al-Qura'n S. Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. J Ethnopharmacol 2009;123:45-50.
31. Aribi I, Chemat S, Hamdi-Pacha Y, Luyten W. Isolation of berberine tannate using a chromatography activity-guided fractionation from root

bark of *Berberis hispanica* Boiss. and Reut. J Liq Chromatogr Relat Technol 2017;40:894-9.
32. Stelmakiene A, Ramanauskiene K, Petrikaite V, Jakstas V, Briedis V. Application of dry hawthorn (*Crataegus oxyacantha* l.) extract in natural topical formulations. Acta Pol Pharm 2016;73:955-65.
33. Burke-Colvin D, Hines M, Gan D. Skin Care Formulations. Justia Patents Number 9833642 United States; 2017.
34. Pacheco-Palencia LA, Noratto G, Hingorani L, Talcott ST, Mertens-Talcott SU. Protective effects of standardized pomegranate (*Punica granatum* L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts. J Agric Food Chem 2008;56:8434-41.
35. Moorthy K, Punitha T, Vinodhini R, Sureshkumar BT, Vijayalakshmi P, Thajuddin N: Antimicrobial activity and qualitative phytochemical analysis of *Punica granatum* Linn. (PERICARP). J Med Plants Res 2013;7:474-9.
36. Park HM, Moon E, Kim AJ, Kim MH, Lee S, Lee JB, *et al.* Extract of *Punica granatum* inhibits skin photoaging induced by UVB irradiation. Int J Dermatol 2010;49:276-82.
37. Ammar RB, Sghaier MB, Boubaker J, Bhouri W, Naffeti A, Skandrani I, *et al*. Antioxidant activity and inhibition of aflatoxin B1-, nifuroxazide-, and sodium azide-induced mutagenicity by extracts from *Rhamnus alaternus* L. Chem Biol Interact 2008;174:1-0.
38. Rhimi W, Salem IB, Immediato D, Saidi M, Boulila A, Cafarchia C, *et al.* Chemical composition, antibacterial and antifungal activities of crude *Dittrichia viscosa* (L.) greuter leaf extracts. Molecules 2017;22:E942.
39. Sudjana AN, D'Orazio C, Ryan V, Rasool N, Ng J, Islam N, *et al.* Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int J Antimicrob Agents 2009;33:461-3.
40. Leeja L, Thoppil JE. Antimicrobial activity of methanol extract of *Origanum majorana* L. (Sweet marjoram). J Environ Biol 2007;28:145-6.
41. Abachi S, Khademi F, Fatemi H, Malekzadeh F. Study of antimicrobial activity of selected Iranian plant extracts on vancomycin resistant *Staphylococcus epidermidis*. J Dent Med Sci 2013;4:59-63.
42. Yashphe J, Segal R, Breuer A, Erdreich-Naftali G. Antibacterial activity of artemisia herba-alba. J Pharm Sci 1979;68:924-5.
43. Dlova NC, Ollengo MA. Traditional and ethnobotanic dermatology practices in Africa. Clin Dermatol 2018;36:353-63.
44. Draelos ZD. Updates in medical skin care. Adv Cosmet Surg 2018;1:211-7.
45. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 2011;38:99-107.
46. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26:343-56.
47. Cushnie TP, Hamilton VE, Chapman DG, Taylor PW, Lamb AJ. Aggregation of *Staphylococcus aureus* following treatment with the antibacterial flavonol galangin. J Appl Microbiol 2007;103:1562-7.
48. Musumeci R, Speciale A, Costanzo R, Annino A, Ragusa S, Rapisarda A, *et al. Berberis aetnensis* C. Presl. Extracts: Antimicrobial properties and interaction with ciprofloxacin. Int J Antimicrob Agents 2003;22:48-53.
49. Stapleton PD, Shah S, Ehlert K, Hara Y, Taylor PW. The beta-lactam-resistance modifier (-)-epicatechin gallate alters the architecture of the cell wall of *Staphylococcus aureus*. Microbiology 2007;153:2093-103.
50. Orhan DD, Hartevioğlu A, Küpeli E, Yesilada E. *In vivo* anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. Fruits. J Ethnopharmacol 2007;112:394-400.