



Phytochemical Investigations and Antibacterial Activity of Selected Medicinal Plants from Jordan

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Authors' contributions

This work was carried out in collaboration between all authors. Author SA designed the study, wrote the protocol and the first draft of the manuscript. Authors SA and SAO conducted the experimental works. Author RA performed the statistical analysis. Author PC managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the antibacterial effect of crude methanolic extracts of six selected medicinal plants grown in Jordan (*Paronychia argentea* Lam., *Inula viscosa* L., *Arbutus andrachne* L., *Asphodelus microcarpus* Salzm et Vivi, *Peganum harmala* L. and *Aloysia citriodora* Palau) against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

Study Design: *In vitro* assessment antibacterial study.

Place and Duration of Study: Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan, Amman, Jordan. Between (December 2012 and January 2013).

Methodology: *In-vitro* Laboratory experimental tests; preparation of plant extracts, phytochemical screening; susceptibility tests (zones of inhibition) and minimum inhibitory concentration (MIC) determination.

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Results: While the crude methanolic extract of *P. argentea*, *A. andrachne*, *A. microcarpus* had no antibacterial activity, crude extract of *P. harmala* showed good antibacterial activities against all the tested bacterial strains. MIC values for the seed and root extract of against *S. aureus* were 0.375 mg/ml and 1.5 mg/ml respectively while MIC values for seed and root extracts against *B. subtilis* were 0.375 and 6.25 mg/ml, respectively and also showed weak activity against Gram negative bacteria. The crude methanolic extract of *I. viscosa* and *A. citriodora* was also active against bacterial strains *S. aureus* and *B. subtilis* and inactive against *E. coli*. MIC value for *I. viscosa* extract against *S. aureus* were 6.25 mg/ml and against *B. subtilis* 0.375 mg/ml. Meanwhile, MIC value for *A. citriodora* against *S. aureus* were 12.5 mg/ml and against *B. subtilis* 1.5 mg/ml.

Conclusion: Results indicate the potential antibacterial activity of *I. viscosa* and *A. citriodora* towards Gram positive bacteria such as *B. subtilis* and *S. aureus*. The extracts phytochemical screening revealed the presence of terpenoids, flavonoids and phenolics. These preliminary results would be a guide in the selection of potential candidates for further pharmacological study and in search of new drug candidate for treatment of infections caused by Gram positive bacteria.

Keywords: Antibacterial activity; Jordanian medicinal plants; crude extract; phytochemical screening.

1. INTRODUCTION

During the last decades, there is increasing interest to unlock the secrets of ancient herbal remedies. For this purpose, various strategies have been developed e.g., biological screening, isolation as well as clinical trials for a variety of plants [1].

The bacterial organisms including gram positive and gram negative like different species of *Bacillus*, *Staphylococcus*, *Salmonella* and *Pseudomonas* are the main source to cause severe infections in humans [2]. Resistance to antimicrobials is a significant and growing problem, limiting treatment options, especially for serious Gram positive infections, among them *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis* and Gram negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* etc. Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Pneumococcus*, vancomycin-resistant *Enterococcus faecalis* (VRE), and multidrug-resistant *Mycobacterium tuberculosis* (MDRTB). These are the major cause of worldwide outbreaks of both hospitals and the community infections [3]. The spread of multidrug-resistant (MDR) strains of bacteria necessitates the discovery of new classes of antibacterials and compounds that inhibit these resistance mechanisms [4,5].

At present, there are no single chemical entity plant derived antibacterials used clinically, and this chemically diverse group deserves consideration as a source for two major reasons. First, plants have exceptional ability to produce cytotoxic agents and second there is an ecological rationale that antimicrobial natural products should be present or synthesised in plants following microbial attack to protect the producer from pathogenic microbes in its environment [6,7]. Plant derived antibacterials are always a source of novel therapeutics. Historically, plants have been placed at top among the sources of novel drugs with

antimicrobial activity, as traditional medicines based on plants and plant extracts have made considerable contributions to human health and well-being.

Plants are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, and polyphenols) represent different biological activities that depend on the diversity and quantity [8]. For example, Quinine (Cinchona) and berberine (Berberis) are alkaloids obtained from plants which are highly effective against microbes such as *S. aureus*, *E. coli* [1]. Therefore, the determination of the compounds responsible for any biological activity would facilitate the selection of the plants for future investigation.

Jordan is a land of biodiversity in terms of plant species. Various plants have been mentioned in traditional medicine literature, for their therapeutic advantages [9,10]. Many herbs used by herbalists show promising results in the treatment of various diseases and these herbs could be appropriate for large randomized trials. Many potent drugs have been purified from medicinal plants having antirheumatic, antithrombotic, antimalarial, anticancer, antidiabetic and antimicrobial properties [11].

The present study aimed to investigate the susceptibility of three clinically significant bacterial strains against six naturally growing plants methanolic crude extracts (*Paronychia argentea* Lam., *Inula viscosa* L., *Arbutus andrachne* L, *Asphodelus microcarpus* Salzm et Vivi. *Peganum harmala* L and *Aloysia citriodora* Palau). The minimum inhibitory concentration (MIC) values were also determined for the crude extracts showing significant activity against the bacterial strains selected for the susceptibility assay. The plant family name, herbarium code, part (s) used and traditional uses are tabulated in Table 1.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plants material *P.argentea*, *I. viscosa* , *A. andrachne* , *A. microcarpus* Salzm, *P.harmala* and *A.citriodora* were collected during spring from different geographical regions of Jordan (15 km of North West Amman). The collected plants were identified by Professor Suleiman Al-Olimat, Department of Pharmaceutical sciences; faculty of pharmacy, The University of Jordan, Amman, Jordan and a voucher for each plant material has been deposited in the herbarium of the university for future reference with suitable herbarium specimen code listed in Table1. Each plant material was thoroughly washed under running tap water and dried under shade. The dried plant materials were ground to powder form for extraction.

2.2 Extraction

The crude methanolic extracts were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 ml 80% methanol for 15 min and keeping the extract overnight for five days at room temperature with continuous shaking (Labtech, Korea). The crude methanolic extract was filtered using 125 mm filter paper (Albet, EEC). The volume of the filtered solution was increased to 100 ml with 80% methanol then the solvent was evaporated to dryness under reduced pressure using rotary evaporator (Heidolph laborota, Germany) to obtain 10% (equivalent to 100 mg/ml) crude methanolic extracts. The residues were further subjected to dryness by incubating them for 8 days at room temperature. The crude extracts were either used directly or stored in an air-tight container for further use.

2.3 Phytochemical Screening

Methanolic extracts (10%) of each of plants were subjected to TLC examination for group determination of the secondary metabolites. Modified Dragendorff's reagent for alkaloids, ferric chloride reagent for phenolics, Naturstoff reagent for flavonoids, ethanolic KOH for coumarins and vanillin/sulphuric acid reagent for terpenoids were used. Solvent systems for the development of ready coated analytical TLC plates were selected according to Harborne [24] and Evans [25].

2.4 Experimental Procedure

2.4.1 Antibacterial activity

2.4.1.1 Bacteria strains

In the present study, overnight cultures of three bacteria strains were used. These were two Gram positive strains (*Staphylococcus aureus*, ATCC25923 & *Bacillus subtilis*, ATCC441) and one Gram negative (*Escherichia coli*, ATCC8739). All bacteria were cultured on nutrient agar (Oxoid).

2.4.1.2 Preparation of inoculums

One single colony of each type of microorganism (from the nutrient agar stock culture) was taken with a sterile loop, and was transferred into 10 ml sterile nutrient broth (Oxoid). The broth cultures were incubated in a shaking incubator at 37°C for 16 - 20 hours.

2.4.1.3 Antibacterial susceptibility test: disc diffusion assay

The antimicrobial activity of crude methanolic extracts of plants were initially assessed against the three tested microorganisms using the agar diffusion method as recommended by the Clinical Laboratory Institute (CLSI) (NCCLS; 2003) [26].

Nutrient agar medium was prepared by suspending nutrient agar (Merck) 20 g/L in distilled water. The pH value of the media was adjusted to 7.0, autoclaved and allowed to cool up to 45°C. The media was seeded with 10⁶ CFU /ml prepared inocula. Subsequently, the seeded medium (75-80 ml) was poured into pre-labelled Petri plates (diameter = 14 cm) and allowed to solidify. Impregnated disks were prepared by the addition of 20 µl plant extract (10% w/v; 100 mg/ml) to "susceptibility blank disks" (Oxoid). These were subsequently applied to the inoculated agar plates and then incubated 24 hours at 37°C. Antibacterial activity was indicated when clear inhibition zones were noted around the discs.

The diameter of the inhibition zones was measured and the results were expressed as mean of three independent experiments. The test was repeated three times. Each extract was dissolved in 99.9% dimethyl sulfoxide (DMSO) (Sigma-Aldrich USA) to get 100 mg/ml concentration. Amoxicillin (30 µg) was prepared as positive control. Pure DMSO (99.9%) was used as negative control.

Table 1. Ethnobotanical data of the studied Jordanian medicinal plants

| Plant | Systematic name/ Herbarium Code | Common name | Family | Part(s) used | Traditional uses |
|-------|--|---|-----------------|--------------|---|
| | <i>Paronychia argentea</i> L. [2CAR PA] | Silver nail root, Silvery Whitlow Wort, Rejel el-hamama, Shoishet el-raei | Caryophyllaceae | Aerial part | Diuretic, dissolve kidney stones, urinary tract infections, gastric analgesic, bladder prostate and abdominal stomach ulcers ailments [10, 12, 13]. |
| | <i>Inula viscosa</i> L. [7 COMP IV] | Taioon, Tribe Inula | Asteraceae | Aerial part | Wound and ulcer treatment, bleeding joints pain, respiratory tract infections, Athlete's foot, hemorrhoids and intestinal worms. Bone repair, blood pressure, diabetes, Back ache, gum disorder and skin fungi treatment [14]. |
| | <i>Arbutus andrachne</i> L. [3 Erica AA] | Eastern Strawberry tree | Ericaceae | Leaves | Antiseptics, diuretics, laxatives, urinary tract infections, hypertension and diabetes. Antioxidants, potentially high value as a health promoting food [15]. |
| | <i>Asphodelus microcarpus</i> Salzm et Vivi. [4 LILIA AM] | Tall Asphodel | Liliaceae | Bulb & Root | Ectoderm parasites and jaundice [16]. |
| | <i>Peganum harmala</i> L. [5 ZYGO PH] | Syrian rue ,harmel | Nitrariaceae | Root & seeds | Analgesic, anti-inflammatory agent. harmaline, an active ingredient in <i>P. harmala</i> , is a central nervous system stimulant and a reversible inhibitor of MAO-A, a category of antidepressant. Antibacterial activity against drug-resistant bacteria. The root is applied to kill lice and insects. It is also used as an antihelmntic ,abortifacient and in large quantities, it can reduce spermatogenesis and male fertility in rats. Has antioxidant and antimutagenic properties [17, 18, 19, 20, 21]. |
| | <i>Aloysia citriodora</i> Palau. [AC-V1] | Lemon Verbana | Verbenaceae | Aerial part | Asthma, spasms, cold, fever, flatulence, colic, diarrhea, indigestion, insomnia and anxiety [22, 23]. |

2.4.1.4 Determination of minimum inhibitory concentration (MIC)

Minimal inhibitory Concentration (MIC) performed on extracts which showed positive activity in the preliminary screening using the microdilution method in 96-well plates (Cellstar®, Greiner Bio-One, Germany) (National Committee for Clinical Laboratory Standards NCCLS, 2003) [26].

Double-strength medium (100 µl) of the Mueller Hinton broth (Oxoid) bacterial culture were used to fill the first experimental well. The other wells were filled with single-strength medium (100 µl). A volume of 100 µl of the plant extract. Double-fold serial dilution was then carried out across the plate. The overnight batch culture of the microorganisms (10 µl) was used to inoculate each well to achieve an inoculum size of approximately 1×10^6 CFU/ml. The plates were incubated for 24 h at 37°C. The MIC (Minimal Inhibitory Concentration) was calculated. For each bacterial strain, controls were maintained where pure solvents were used. Amoxicillin (30 µg) positive controls were used. Each MIC determination was carried out in triplicate.

3. RESULTS AND DISCUSSION

Bacterial resistance to antibiotics has become a serious problem of public health that concerns almost all antibacterial agents and that manifests in all fields of their application. Novel antimicrobial compounds against new bacterial targets and drug resistance mechanisms are urgently needed. Plant derived antibacterials are always a source of novel therapeutics.

In such effort, we accessed the susceptibilities of some clinically significant bacterial species against various extracts made up from six selected Jordanian medicinal plants. It has been reported that biological activities in medicinal plants were exhibited by different class of phytochemicals [27] therefore, it is important to screen for phytochemical group in these plants. The qualitative estimation of the phytocompounds conducted on plant extracts revealed the presence of flavonoids, terpenoids, phenolics and coumarins. Alkaloids could be detected only in *P.harmala* Table 2.

In the initial screening using *E.coli*, a susceptible strain of *S .aureus* and *B.subtilis* crude methanolic extracts of *P. Argentea*, *A. andrachne*, *A. microcarpus* were inactive in all three tested bacteria strains. The extracts from *I.viscosa*, *A.citriodora* and *P. harmala* possessed good antibacterial activities against the two Gram positive tested strains. Data of antibacterial activity of various crude extracts are demonstrated in Table 3.

Data indicated that the most active extract was found to be *I.viscosa* of which inhibited two bacteria studied in the range of 11.16 mm to 14.4 mm size of inhibition zone against *S.aureus* and *B.subtilis* respectively. The crude extract of *A.citriodora* was also active in suppressing the growth of *S. aureus* and *B.subtilis* inhibition zone 11.0 mm and 12.4 mm. However, none of the extracts from these plants was active against the growth of the Gram negative bacteria *E.coli* except Harmal root and seed these were found to be slightly effective but, with comparatively lower activity than Gram positive bacteria.

The crude extract of *P.harmala* showed significant antibacterial activities against all the tested bacterial strains. Maximum activity was conferred against *S.aureus* 13.66, 12.83 mm for root and seed respectively; in addition both extracts showed good activity against *B.subtilis*, mean inhibition zone diameter 14.23, 12.85 mm, while lower activity observed against *E.coli* 8.16 and 7.7 mm, respectively. Results of MIC determination Table 4 showed that MIC values for the seed and root extract of against *S.aureus* were 0.375 mg/ml and 1.5 mg/ml, respectively while MIC values for seed and root extracts against *B.subtilis* were 0.375 and 6.25 mg/L, respectively. On the basis of the obtained results that are shown in Table 3, the seed and root extracts of *P.harmala* have a broad antibacterial activity. This is in agreement with previous reports by several workers about *P.harmala* with antibacterial activity of root and seed extracts against most of the tested gram positive bacteria [18, 19].

The MIC of the *I.viscosa* extract for *S.aureus* and *B.subtilis* organisms ranged between 6.25 and 0.375 mg/ml while that of crude extract of *A. citriodora* ranged between 12.5-1.5 mg/ml for *S.aureus* and *B.subtilis* respectively, Table 4.

The incidence of serious bacterial infection is increasing despite remarkable advances in antibiotic chemotherapy. Of great concern is the increasing incidence of infections caused by Gram positive bacteria with acquired multidrug resistance. New antibacterials with activity against multidrug resistant Gram positive pathogens are urgently needed for the treatment of severe multi-resistant hospital and community acquired infections

Gram positive bacteria selected for this study showed variable susceptibilities while Gram-negative *Ecoli* has shown to be completely resistance to all the tested extracts except harmal.

Our findings suggest that *I.viscosa* and *A.citriodora* have significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents of plant origin. Further phytochemical studies are required to determine and isolate compounds responsible for the antibacterial effects of these species.

Table 2. Classes of phytochemicals present in the plant extracts

| Plant botanical name | <i>Paronychia argentea</i> Lam. | <i>Inula viscosa</i> L. | <i>Arbutus andrachne</i> L. | <i>Asphodelus microcarpus</i> Salzm et Vivi. | <i>Peganum harmala</i> L. Root | <i>Peganum harmala</i> L. Seed | <i>Aloysia citriodora</i> Palau. |
|----------------------|---------------------------------|-------------------------|-----------------------------|--|--------------------------------|--------------------------------|----------------------------------|
| Alkaloids | - | - | - | - | +++ | +++ | - |
| Terpenoids | - | +++ | + | - | - | - | +++ |
| Flavonoids | ++ | ++ | + | - | - | - | ++ |
| Phenolics | ++ | ++ | ++ | + | - | - | + |
| Coumarins | - | + | - | ++ | + | + | - |

KEY: - = absence; + = presence; ++ = abundant; +++ = abundant in appreciable quantity

Table 3. Antibacterial activity of some medicinal plant methanol extracts (100mg/ml) and antibiotic (30 µg) against bacterial species tested by disc diffusion assay

| Bacterial Sp. | Zone of inhibition (mm) * | | | | | | | Amoxicillin | DMSO |
|--------------------|---------------------------|-------------------|---------------------|-----------------------|--------------------------|--------------------------|----------------------|-------------|------|
| | <i>P. argentea</i> | <i>I. viscosa</i> | <i>A. andrachne</i> | <i>A. microcarpus</i> | <i>P. harmala</i> (Root) | <i>P. harmala</i> (Seed) | <i>A. citriodora</i> | | |
| <i>S. aureus</i> | - | 11.16 ±0.66 | - | - | 13.66±0.33 | 12.83±0.3 | 11.00 ±0.24 | 7.2±0.11 | 2.8 |
| <i>B. subtilis</i> | - | 14.4 ±0.54 | - | - | 14.23±0.2 | 12.85±0.23 | 12.4±0.21 | 7.2±0.11 | 2.8 |
| <i>E. coli</i> | - | - | - | - | 8.16±0.33 | 7.7±0.22 | - | 7.2±0.20 | 2.8 |

*Values are mean inhibition zone (mm) ± Standard deviation of three replicate

Table 4. The Minimal inhibitory concentration (MIC) values of selected plant extracts against *S. aureus* and *B. subtilis*

| Plant name | MIC (mg/ml) against <i>S. aureus</i> | MIC (mg/ml) against <i>B. subtilis</i> |
|---------------------------------|---|---|
| <i>Inula viscosa</i> L. | 6.25 | 0.375 |
| <i>Peganum harmala</i> L. Root | 1.5 | 6.25 |
| <i>Peganum harmala</i> L. Seed | 0.375 | 0.375 |
| <i>Aloysia citriodora</i> Palau | 12.5 | 1.5 |

4. CONCLUSION

In conclusion, we have identified plant extracts with promising antibacterial activities towards Gram positive species. This study indicated that *I.viscosa*, *A.citriodora*, have significant antibacterial activity and it could be very useful in the discovery of novel antibacterial.

The antibacterial activity of *I.viscosa*, *A.citriodora*, *P.argentea*, *A.andrachne* and *A. microcarpus* crude extracts are reported for the first time. No previous reports about antibacterial activity of these species could be found in the literature.

These preliminary results would be a guide in the selection of potential candidates for further pharmacological study and in search of new drug candidate in treating Gram positive bacterial infections.

Further study can be made to isolate the pure compounds responsible for the activity from the extracts with the help of numerous advanced technologies such as GC-MS, LC-MS, IR and NMR.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bibi Y, Nisa S, Chaudhary FM, Zia M. Antibacterial activity of some selected medicinal plants of Pakistan. BMC. 2011;11(1):52.

2. Nathan C. Antibiotics at the crossroads. *Nature*. 2004;21(431):899-902.
3. Akins RL, Haase KK. Gram-positive resistance: pathogens, implications and treatment options: insights from the Society of Infectious Diseases Pharmacists. *Pharmacotherapy*. 2005;25(7):1001-1010.
4. Cornaglia G. Fighting infections due to multidrug-resistant Gram-positive pathogens. *Clin Microbiol Infect*. 2009;15(3):209-211.
5. Rice LB. Antimicrobial resistance in Gram positive bacteria. *Am J Infect Control*. 2006;34 (5 Suppl 1):S11-9
6. Perez C., Pauli, M, Bazerque, P. An antibiotic assay by the agar-well diffusion method. *Acta Biol Exp*.1990;15:113-115.
7. Nisbet J, Moore M. Will nature products remain an important source of drug research for the future?. *Curr Opin Biotechnol*.1997;8:708-712.
8. Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, et al. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *J Ethnopharmacol*. 2005;21(97):421-427.
9. Afifi FU, Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. *J Ethnopharmacol*. 2000;72(1-2):101-110.
10. Al-Khalil S. A survey of plants used in Jordanian traditional medicine. *Int. J. Pharmacogn*. 1995;33:317-323.
11. Abu-Irmaileh BE, Afifi FU. Herbal medicine in Jordan with special emphasis on commonly used herbs. *J Ethnopharmacol*. 2003;89 (2-3):193-197.
12. Hamdan II, Afifi FU. Studies on the in vitro and in vivo hypoglycaemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. *J Ethnopharmacol* 2004;93:117–121.
13. Ferreira A, Proença C, Serralheiro ML, Araújo ME. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *J Ethnopharmacol*. 2006;108:31-37.
14. Talib WH, Mahasneh AM. Antiproliferative Activity of Plant Extracts Used Against Cancer in Traditional Medicine. *Sci Pharm*. 2010;78(1):33-45.
15. Serce S, Ozgen M, Torun AA, Ercisli S. Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne* L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey. *J. Food Compos. Anal*. 2010;23(6):619–623.
16. Ljubuncic P, Azaizeh H, Portnaya I, Cogan U, Said O, Abu Saleh K, et al. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. *J Ethnopharmacol*. 2005;99:43–47.
17. Prashanth D, John S. Antibacterial activity of *Peganum harmala*. *Fitoterapia*. 1999;70(4):438-439.
18. Darabpour E, Bavi A , Motamedi H, Mansour S, Nejad S. Antibacterial activity of different parts of *Peganum harmala* L. growing in Iran against multi-drug resistant bacteria. *EXCLI Journal*. 2011;(10):252-263.
19. Shahverdi AR, Monsef-Esfahani HR, Nickavar B, Bitarafan L, Khodae S, Khoshakhlagh N. Antimicrobial activity and main chemical composition of two smoke condensates from *Peganum harmala* seeds. *Z Naturforsch C*. 2005;60(9-10):707-710.
20. Mirzaie M, Nosratabadi S, Derakhshanfar A, Sharif, I. Anti-leishmanial activity of *Peganum harmala* extract on the in vitro growth of *Leishmania major* promastigotes in comparison to a trivalent antimony drug. *Veterinarski arhiv*. 2007;77(4):365-375.
21. Astulla A, Zaima K, Matsuno Y, Hirasawa Y, Ekasari W, Widyawaruyanti A, et al. Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. *J Nat Med*. 2008;62(4):470-472.

22. Carnat A, Carnat AP, Fraise D, Lamaison JL. The aromatic and polyphenolic composition of lemon verbena tea. *Fitoterapia*. 1999;70(1):44-49.
23. Santos-Gomes PC, Fernandes-Ferreira M, Vicente AMS. Composition of the Essential Oils from Flowers and Leaves of Vervain [*Aloysia triphylla* (L'Herit.) Britton] Grown in Portugal. *J ESSENT OIL RES*. 2005;17(1):73-78.
24. Harborne JB. *Phytochemical Methods*. London: Chapman and Hall Publications; 1998.
25. [25] Evans WC, Trease and Evans *Pharmacognosy*, 14th ed, London: Bailliere Tindall W.B. Saunders company ltd; 2000.
26. *Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA: NCCLS. National Committee for Clinical Laboratory Standards; 2003.
27. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*; 2nd ed. Berlin: Springer Verlag; 1996.

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