Screening of Selected Medicinal Wild Plant Extracts Antibacterial Effect as Natural Alternatives

Article · August 2013

CITATIONS
3

READS
470

4 authors:

Ghadeer Omar
An-Najah National University
7 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Lubna Abdallah
An-Najah National University
16 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Shurooq Ismail
An-Najah National University
5 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Motasem Almasri
An-Najah National University
35 PUBLICATIONS **75** CITATIONS
SEE PROFILE

Screening of Selected Medicinal Wild Plant Extracts Antibacterial Effect as Natural Alternatives

Ghadeer Omar
Plant Taxonomy, Department of Biology & Biotechnology, Faculty of Science, An-Najah National University, Nablus, P.O.Box 7, Palestine

Lubna A. Abdallah
Biotechnology, Department of Biology & Biotechnology, Faculty of Science, An-Najah National University, Nablus, P.O.Box 7, Palestine

Shurooq Ismail
Microbial Botany, Department of Biology & Biotechnology, Faculty of Science, An-Najah National University, Nablus, P.O.Box 7, Palestine

Motasem Y. Almasri
Medical Microbiology, Department of Biology & Biotechnology, Faculty of Science, An-Najah National University, Nablus, P.O.Box 7, Palestine

Corresponding author Email: ghader.omar@gmail.com

ABSTRACT

The aim of the present study was to evaluate the antimicrobial potential of aqueous and ethanol extracts of *Thymbra spicata* L. (Lamiaceae), *Nepeta curviflora* Boiss. and *Paronychia argentea* Lam. (Caryophyllaceae) against six Gram negative bacteria and one Gram positive bacterium. Agar well diffusion method was adopted to examine the antimicrobial activity of all plant extracts being studied. Out of the seven bacterial isolates, a clinical isolate of *Proteus mirabilis* (II) was the most susceptible one for all the examined plant extracts except for *N. curviflora* ethanol extract. Moreover, the ethanol extract of *P. argentea* exhibited the highest antimicrobial potential against most of the tested bacteria except for *Klebsiella pneumoniae* and *Escherichia coli*. On the other hand, all investigated ethanol plant extracts displayed antibacterial effect against the other clinical isolate of *Proteus mirabilis* (I), which showed resistance against the broad spectrum antibiotic Gentamycin. Further more, micro-broth dilution method was used to measure the minimum inhibitory concentration (MIC) of the effective plant extracts. The examined ethanol plant extracts demonstrated higher MIC values than the aqueous extracts ranging from 1.56 to 50 mg/ml. Accordingly, the obtained results form the platform for further phytochemical and pharmacological studies which are invited to purify and characterize the active ingredient (s) of the studied plant species by the future focus on their extracts fractionation in hope of identifying the active components.

Keywords- Antibacterial effect, plant extract, *Thymbra spicata*, *Nepeta curviflora*, *Paronychia argentea*, West Bank.

1. INTRODUCTION

Plants defend themselves chemically against grazing or infection via the production of a host of bioactive molecules such as tannins, terpenoids, alkaloids and flavonoids ([1], [2]). Some plants are known as medicinal because they contain active substances that cause certain reactions from relenting to the cure of human diseases [3]. Therefore, different countries used plants medicinally as a source of many potent and powerful drugs [4] from different plant parts including root, stem, flower, fruit, twigs and modified plant organs [5]. Medicinal plants play a key role in health care with about 80% of the world's populations relying on the use of traditional medicine, which is predominantly based on plants [6]. Bacterial genetic ability to transmit and acquire resistance to drug and therapeutic agents created a global problem of antimicrobial resistance [7]. This obstacle can be resolved through the new and innovative antimicrobials from plants [8].

Medicinal plants are important element of the indigenous medical system of developing countries as well as Palestine [9]. The utilization of complementary and alternative medicine in Palestine is very common [10]. Due to the rapid increase of antibiotic resistance in our region, plants which have been used as medicines over hundreds of years, constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore [11]. Several studies in Palestine have been published concerning many plant extracts biological active properties such as antibacterial, antitumor, antifungal and antioxidant of wild plants ([5], [12]-[17]). Nevertheless, Documentation of scientific information about the efficacy and safety of wild plants in West Bank, Palestine is one of our research goals based on their accurate taxonomical identification. Systematic screening of folk medicine plants may result in the discovery of novel effective compounds and natural alternatives for disease treatment. As well, some medicinal herbs for some reasons have not found wider application. Taking into account the increasing demand for the natural ingredients that might be used as food additives, preventing plant diseases and nutraceuticals as well as for other applications, it is reasonable to

revise the wild plants by assessing their applicability and benefits using modern scientific analysis methods [18]. Therefore, this study was conducted to screen in vitro the antibacterial potential of the aqueous and ethanol extract of *Thymbra spicata* L. (Lamiaceae), *Nepeta curviflora* Boiss. and *Paronychia argentea* Lam. (Caryophyllaceae) against Gram negative bacteria which are *Pseudomonas aeruginosa* (ATCC 27853 and a clinical isolate), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (two clinical isolates), *Klebsiella pneumoniae* (a clinical isolate) and a Gram positive bacterium which is *Staphylococcus aureus* (ATCC 25923). The plant species, *Thymbra spicata* was a target plant for antibacterial assay in this research, as the genus *Thymbra* from the family Lamiaceae is widely used in Middle East folk medicine. Besides, it is used in the food industry for flavor, aroma and preservation [19]. Several studies were conducted on the antimicrobial activity of *T. spicata* essential oils against several bacteria ([20], [21], [22]). Moreover, the antibacterial activity of a species of the genus *Nepeta* was explored in our study. This genus is one of perennial or annual herbs from Lamiaceae family. *Nepeta* species are widely used in folk medicine in different countries because of their medicinal properties. They are used as a traditional medicine in many countries and have large ethnobotanical effects: diuretic, diaphoretic, vulnerary, antitussive, antispasmodic, antiasthmatic, tonic, febri-fuge and carminative ([23], [24], [25]). Therefore, it was subjected to several antimicrobial activities assay. Different species such as: *N. nuda*, *N. cataria* and many other species were examined against a variety of microbial species ([26], [27], [28]). While, the Silvery whittle-wart, *Paronychia argentea* Lam. (Caryophyllaceae) is commonly used in many different indigenous countries communities for medicinal purposes causing this plant species to be subjected to several antimicrobial activity detections. Abou Elkhair et al. [11] investigated the antimicrobial effect of the aqueous, chloroform and ethanol extracts of several wild plants in Gaza, Palestine; one of which was *P. argentea*. In addition, a research team in Jordan, has tested the antimicrobial effect of some plant leaves crude extracts. *Paronychia argentea* was one of their target plants detecting its antibacterial effect against five bacterial species [2].

Therefore, the present study aimed to evaluate the antibacterial potential of water and ethanol extract of *T. spicata* as the previous studies concentrated on the essential oils biological activity assay. In addition, to study the antibacterial effect of water and ethanol extract of one *Nepeta* species existing in our region, which is *N. curviflora*, providing the possibility of considering it as natural treatment for infectious diseases caused by different pathogens. Furthermore, this study will re-examine the anitibacterial effect of *P. argentea* including a bacterium, which has not been investigated for its sensitivity to *P. argentea*.

2. MATERIALS AND METHODS

2.1. Plant Materials

The three investigated plants (*Thymbra spicata, Nepeta curviflora* and *Paronychia argentea*) were collected from different locations in West Bank, Palestine. The plants were identified by Ghadeer Omar, Department of Biology & Biotechnology, An-Najah National University, Palestine. Representative plant specimens of the studied plant species were collected, pressed till drying, treated chemically, mounted on herbarium sheets and provided with voucher numbers, after then are deposited at An-Najah National University herbarium. The plant materials for the antibacterial assay were washed, air-dried, ground into powder using grinder and stored at room temperature until they were used.

2.2. Aqueous Extraction of Plants

Ten grams of each plant powder were soaked in 100 ml boiled distilled water for one week with interval shaking. Then the mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were evaporated by freeze-drying. The extracted powder of each of the plant species under investigation was dissolved in distilled water to a final concentration equal to 100 mg/ml.

2.3. Ethanol Extraction of Plants

Ten grams of each plant powder were soaked in 100 ml of 70 % ethanol for one week with interval shaking. Then the mixtures were centrifuged for 5 min at 5000 rpm. The supernatants were evaporated at room temperature under aseptic conditions. The extracted powder of each of the plant species under investigation was dissolved in 5 % dimethyl sulfoxide (DMSO) to a final concentration equal to 100 mg/ml.

2.4. Test Microorganisms

The in vitro antibacterial activities of the plant extracts were evaluated against a total of seven bacterial isolates, which includes six Gram negative bacteria; *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas aeruginosa* (clinical isolate), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (two clinical isolates *P. mirabilis* I obtained from wound infected sample and *P. mirabilis* II obtained from urine sample), and *Klebsiella pneumoniae* (clinical isolate). In addition to one Gram positive bacterium *Staphylococcus aureus* (ATCC 25923). The clinical isolates were obtained from Rafidia hospital and their identification was confirmed by API20 (bioM'erieux, France). American type culture collection (ATCC) numbers represent the standard strain numbers assigned to these microorganisms.

2.5. Antibacterial Activity Assay

The antibacterial activities of the plant extracts under study were determined by well diffusion method [29]. The tested bacteria were grown over night on nutrient agar plates. Broth turbidity was adjusted to 0.5 McFarland (1.5×10^8 CFU). Then each bacterium was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. After 10 min, 6 mm wells were bored in the agar. Each plant extract under study was

checked for the antibacterial activity by introducing 25 µl of a 100 mg/ml concentration of the plant extract into each well. The plates were allowed to stand at room temperature for 30 min for extract to diffuse into the agar and then they were incubated at 37°C for 18 h. Then the plates were examined for bacterial growth inhibition by measuring the inhibition zone diameter (IZD) to the nearest mm. The test was performed in duplicates. Antibiotic Gentamycin was used as positive control and sterilized distilled water and 5% DMSO were used as negative controls.

Minimum inhibitory concentration (MIC) for active plant extracts was determined by micro-broth dilution method [30]. The prepared extract was serially diluted two fold in nutrient broth medium. Duplicates of each dilution (50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781 0.391, 0.195 and 0.098 mg/ml) were inoculated with 1 µl of 5×10^7 (CFU). The last two duplicate wells were not inoculated. After then, the inoculated microtiter plates were incubated at 37°C for 18 h. The lowest extract concentration (highest dilution) that inhibited the growth of tested microorganisms was considered as MIC.

3. RESULTS

Agar well diffusion assay of the aqueous and ethanol plant extracts under study posses potential antibacterial activity on at least one of the tested bacteria (Table 1).

Figures 1 and 2 illustrate that the Gram positive bacterium *S. areus* was susceptible to the aqueous and ethanol extracts of *T. spicata* and *P. argentea*, while *N. curviflora* extracts had no antibacterial effect on this Gram positive bacterium. From our results it was clearly noticed that *T. spicata* extracts had no antibacterial effect on the two strains of *P. aeruginosa*. On the other hand, *T. spicata* aqueous extract was the only plant extract in the current study that inhibited the growth of *K. pneumoniae*. Interestingly, the clinical isolate of *P. mirabilis* I was sensitive to all plant ethanol extracts (Figure 2), in spite of its resistance to the wide spectrum antibiotic Gentamycin. However all plant aqueous and ethanol extracts except for *N. curviflora* ethanol extract had antibacterial activity against the clinical isolate *P. mirabilis* II. The recorded antibacterial activity of these plant extracts against this clinical isolate of *P. mirabilis* II was higher than the wide spectrum antibiotic Gentamycin. Unfortunately, *E. coli* ATCC 25922 was resistant to all plant extracts being tested. Significant antibacterial effects expressed as minimum inhibitory concentration (MIC) of promising aqueous and ethanol crude extracts against test bacteria were shown in Figures 3 and 4. Ethanol extracts of the three plants inhibited bacterial growth at concentrations ranged from 1.56 to 50 mg/ml. While aqueous extracts of these plants were only effective at concentrations higher than 25 mg/ml. Ethanol extract of *P. argentea* has had the lowest MIC among the tested effective plant extracts equaling to 1.56 mg/ml, which was against *S. aureus*.

Table 1: Antibacterial activity of aqueous and ethanol extracts of *T. spicata*, *N. curviflora* and *P. argentea* with their minimum inhibitory concentrations.

* (IZD) diameter of inhibition zones (mm) including the diameter of the well (6 mm); values are the mean of two duplicates; (0) not active
** (MIC) minimum inhibitory concentration (mg/ml)
*** Positive control Gentamycin (10 µg/disc).

Plant / Bacteria	*T. spicata*				*N. curviflora*				*P. argentea*				+ve control
	Water		Ethanol		Water		Ethanol		Water		Ethanol		
	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD
P. aeruginosa (ATCC27853)	0	0	0	0	12	50	0	0	0	0	13	50	16
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	12	3.125	15
K. pneumonia	10	50	0	0	0	0	0	0	0	0	0	0	19
P. mirabilis I	0	0	13	50	0	0	9	50	0	0	12	50	0
P. mirabilis II	18	50	35	12.5	20	50	0	0	8	50	23	25	14
E. coli (ATCC25922)	0	0	0	0	0	0	0	0	0	0	0	0	17
S. aureus (ATCC25923)	13	50	14	12.5	0	0	0	0	8	25	15	1.56	20

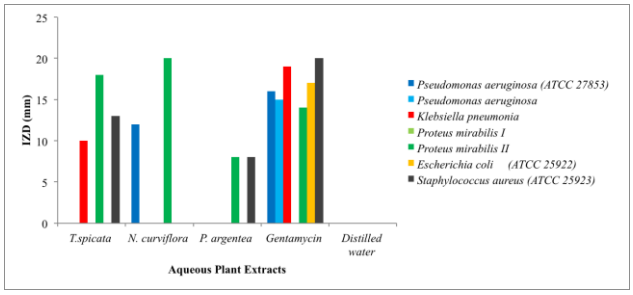

Figure 1: Antibacterial activity of aqueous extracts using agar well diffusion method; (IZD) inhibition zone diameters including well diameter (6 mm).

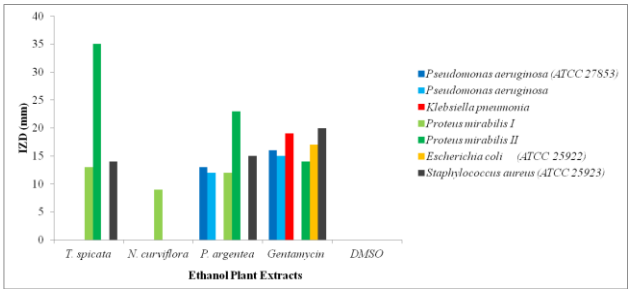

Figure 2: Antibacterial activity of ethanol extracts using agar well diffusion method; (IZD) inhibition zone diameters including well diameter (6 mm).

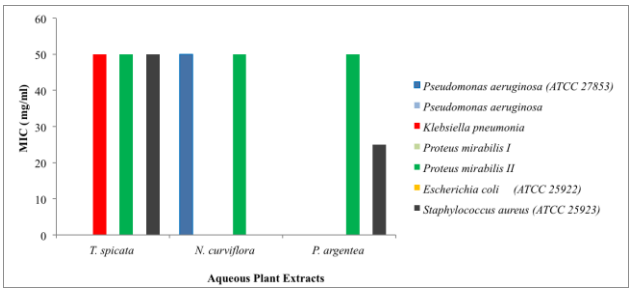

Figure 3: Antibacterial activity of aqueous extracts using micro-broth dilution method; (MIC) minimum inhibitory concentration (mg/ml).

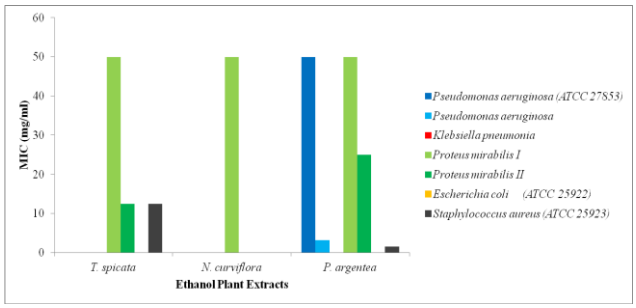

Figure 4: Antibacterial activity of ethanol extracts using micro-broth dilution method; (MIC) minimum inhibitory concentration (mg/ml).

4. DISCUSSION

Therefore, the results of this study provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic resistant strains. In this study it was observed that the extracts of the plant species have had antibacterial activity against tested Gram negative and Gram positive bacteria. The obtained results showed that, the ethanol and water extracts of *T. spicata* revealed a wide antibacterial spectrum against the most tested bacterial strains except of *P. aeruginosa* and *E. coli*. These findings are consistent with those obtained in some previous studies to a certain degree considering the antibacterial effect of the essential oils of *T. spicata* ([20], [21], [22]). Nevertheless, for the first time, the antibacterial effect of the water and ethanol extracts of *T. spicata* was tested against two clinical isolates of *P. mirabilis* I and II which were sensitive to at least one extract type. In addition to that, all previous studies on the antibacterial activity of *T. spicata* were on its essential oils rather than the water and ethanol extracts.

In spite of that the previous studies on the antimicrobial activity of the genus *Nepeta* have considered different species ([26], [27], [28]) other than the one under investigation in this study, which is *N. curviflora*, different bacterial species showed sensitivity to the studied *Nepeta* species. This study is the first comprehensive report that assesses antibacterial activity against *P. mirabilis.* Our findings provided an evidence of the efficacy of the ethanol extract on the clinical isolate of *P. mirabilis* I, which showed resistance to the wide spectrum antibiotic Gentamycin. In addition to that, the water extract showed antibacterial effect against another clinical isolate of *P. mirabilis* II more than Gentamycin. In this context, this study can be useful as a starting point for further applications of the ethanol and water extracts and their constituents in pharmaceutical preparations for drugs against clinical resistant strains of *P. mirabilis*.

Furthermore, the data in this study indicated that *P. argentea* water and ethanol extracts were very effective against all tested bacterial species except for *E. coli* and *K. pneumoniae*. The recorded data agreed with some previous studies ([11], [2]). However, the water and ethanol extract of *P. argentea* showed antibacterial effect against *S. aureus* as indicated by Abou Elkhair et al. [11]. On the other hand, the recorded result is in contrast to what have been indicated by Obeidat et al. [2]. Moreover, it was recorded for the first time the sensitivity of *P. mirabilis* clinical isolates (I and II) to both the water and ethanol extracts of *P. argentea*.

5. CONCLUSIONS

In general, all the tested microorganisms were inhibited by several plants extracts of different solvents used in this study. Thus, the efficacy of the plant extracts evaluation as antibacterial agents were related to the solvent of extraction. In classifying the antibacterial activity as Gram positive or Gram negative, it would be generally expected that a much greater number would be active against Gram-positive than Gram negative bacteria. In this research, the water and ethanol extracts of two plant species out of the three studied plant species showed activity against Gram positive bacteria (*S. aureus*) supporting the aforementioned view. Nevertheless, the growth of different Gram negative bacteria was controlled by the water and/or ethanol extract of one or more of the studied plant species. However, all solvent extracts of all studied plant species showed no antibacterial effect on *E. coli*. Different extract

types of the examined plant species are recommended to be investigated which may reveal an antibacterial activity against this resistant bacterium.

The results of the current investigation clearly indicate that the antibacterial activity varies with the species of the plants and the extract type. This antibacterial variation of the different screened plant extracts could be explained by the fact that different solvents have various degrees of solubility for different phyto constituents. The antibacterial activities significantly differed depending on the taxonomic characteristics of the plant species as well as biological characteristics of the tested bacteria. Therefore, the antibacterial activity of the plant extracts relies on the species of the plant, the type of solvent and the type of the tested microorganism.

Accordingly, the obtained results form the platform for further phytochemical and pharmacological studies which are invited to purify and characterize the active ingredient (s) of the studied plant species by the future focus on their extracts fractionation in hopes of identifying the active components.

In conclusion, this research confirms the folkloric anticipation of the antibacterial effectiveness and the therapeutic applications of the examined plants, proving that the traditional medicinal plants are an important source of natural products in treating common infectious bacteria.

ACKNOWLEDGEMENT

The authors are grateful to the Hospital of Rafidia for supplying this study with the antibiotic resistant pathogenic bacterial strains

REFERENCES

[1] P. A. Cox, and M. J. Balick, "The ethnobotanical approach to drug discovery", Scientific American., vol. 6, pp. 82-87, June. 1994.

[2] M. Obeidat, M. Shtnawi, M. Al-alawi, E. AlZub'i, H.Al-Dmoor, M.Al-Qudah, J.Al-Qudah, and I. Otri, "Antimicrobial Activity of Crude Extracts of Some Plant Leaves", Research Journal of Microbiology., vol. 7, pp. 59-67, Jan. 2012.

[3] A.A. Silva Junior, V. J. Vizotto, E. Giorgi,S. G. Macedo, and L. F. Marques, "Plantas medicinais, caracterizacoa e cultivo, EPAGRI". Bol Tecnico Florianopolis., vol. 68, pp.1-71, Sept. 1994.

[4] J.Srivastava, J.Lambert, and N.Vietmeyer, "Medicinal plants: An expanding role in development", World Bank Technical Paper.1996. Paper 320.

[5] A.B.Suhaib, N. K. Azra, A.G. Bashir. A. L., Bashir, and S. Saleem, "Evaluation of antimicrobial activity of aqueous extracts of *Nepeta cataria*", Journal of Pharmacy Research., vol. 4,pp. 3141-3142, July. 2011.

[6] O.J.Owolabi, E. K. I. Omogbai, and O. Obasuyi, "Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella Africana* (Bignoniaceae) stem bark", African Journal of Biotechnology., vol. 6, pp. 1677-1680, July. 2007.

[7] G. G. F. Nascimento, J. Locatelli, P. C. Freitas, and G. L. Silva, "Antibacterial activity of plant extracts and phytochemicals on antibiotic- resistant bacteria", Brazilian Journal of Microbiology., vol. 31, pp. 247-256, Jan. 2000.

[8] A. Lino, and O. Deogracious, "The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea* Ugandan medicinal plants", African Health Science. vol. 6, pp. 31-50, Mar. 2006.

[9] G. Adwan, B. Abu-Shanab,K. Adwan, and F. Abu-Sanab, "Antibacterial effect of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*", Turkish Journal of Biology.,vol. 30, pp. 239-242, Mar. 2006.

[10] A. F. Sawalha, "Complementary and alternative medicine (CAM) in Palestine: use and safety implications", The Journal of Alternative and Complementary Medicine., vol. 13, pp. 263-270, Mar. 2007.

[11] E.Abou-Elkhair, H.Fadda, and U. Abu-Mohsen, "Antibacterial activity and Phytochemical analysis of some medicinal plants from Gaza Strip-Palestine", Journal of Al-Azhar University-Gaza., vol. 12, pp. 45-54, Oct. 2010.

[12] M.S. Ali-Shtayeh, M. A. Al-Nuri,R. M. Yaghmour, and Y. R. Faidi, "Antimicrobial activity of *Micromeria nervosa* from the Palestinian area", Journal of Ethnopharmacology., vol.58, pp. 143-7, Dec. 1997

[13] M. S. Ali-Shtayeh, R. M. Yaghmour, Y. R. Faidi,K.Salem, and M.A. Al-Nuri, "Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area", Journal of Ethnopharmacology., vol. 60, pp. 265-271, Apr. 1998.

[14] M.S.Ali-Shtayeh, and S.I.Abu-Ghdeib,"Antifungal activity of plant extracts against dermatophytes", Mycoses., vol. 42, pp. 665-672, July. 1998.

[15] B.Abu-Shanab, G. Adwan, and D. Abou-Safiya, "Antibacterial activities of some plant extracts utilized in popular medicine in Palestine", Turkish Journal of Biology., vol. 28, pp. 99-102, Sept. 2004.

[16] B.Abu-Shanab, G. Adwan, and N. Jarrar, "Antibacterial activity of four plant extract used in Palestine in Folkloric medicine against Methicillin-Resistant *Staphylococcus aureus*", Turkish Journal of Biology., vol. 30, pp. 195-198, June. 2006.

[17] A. I. Husein, M. S. Ali-Shtayeh MS, N. Zater, and W. Jondi, "Modification of biologically active compounds from selected medicinal plants in Pales-

tine", PhD thesis. An-Najah National University pp.4-10, 2010.

[18] S. M. Abdel Rahman, S. A. Abd-Ellatif, S. F. Deraz, and A. A. Khalil, "Antibacterial activity of some medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious diseases treatment", African Journal of Biotechnology., vol. 10, pp. 10733-10743, July. 2011.

[19] S. Daneshaver – Royandezagh, K. M. Khawar, and S. Ozcan, "In vitro micropropagation of garden thyme (*Thymbra spicata* L. var *spicata* L.) collected from southern Turkey using cotyledon node". Biotechnology & Biotechnological Equipment., vol. 3, pp. 1319-1321, Aug. 2009.

[20] T. Kilic, "Analysis of essential oil composition of *Thymbra spicata* var. *spicata*: Antifungal, Antibacterial and Anti-microbacterial activities", Z. Naturforsch., vol. 61, pp. 324-328, May. 2006.

[21] M. Akin, D. Oguz, and H. T, Saracoglu, "Antibacterial effect of essential oil from *Thymbra spicata* var. *spicata* L. and *Teucrium polium* (Stapf Brig)", International Journal of Pharmaceutical and applied sciences., vol. 1, pp. 55-58, Jan. 2010.

[22] T. Markovic, P. Chatopoulou, and J. Siljegovic, "Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components", Archives of Biological Sciences., vol. 63, pp. 457-464, 2011.

[23] A. Ghannadi, F. Aghazari, M. Mehrabani, A. Mohagheghzadeh, and I. Mehregan, "Quantity and composition of the SDE prepared essential oil of *Nepeta macrosiphon* Boiss", Iranian Journal of Pharmacology Research., vol. 2, pp. 103-105, May. 2003.

[24] A.Nasto, M. A. Cannatelli, C. Giuseppe, and V. Alonzo,"The effect of *Nepeta cataria* extract on adherence an enzyme production of *Staphylococcus aureus*", International Journal of Antimicrobial Agents., vol. 18, pp. 583-585, Dec. 2001.

[25] K..C.Baser, N. Kirimer, M. Kurchcuoglu, and B. Demirci, "Essential oil of *Nepeta* species growing in Turkey", Chemistry of Natural Compounds., vol. 36, pp. 356-359, July. 2000.

[26] A.Alim, I. Goze , A. Cetin, A. D. Atas, S. A. Cetinus, and N. Vural, "Chemical composition and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp. *albiflora* (Boiss.) gams", African Journal of Microbiology Research., vol. 3, pp. 463-467, Aug. 2009.

[27] A.Adiguzel, H. Ozer, M. Sokmen, M. Gulluce, A. Sokmen, H. Kilic, F. Sahin, and O. Baris, "Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*", Polish Journal of Microbiology., vol. 58, pp. 69-76, Dec. 2009.

[28] D. S. Bisht, R. C. Padalia, L. Singh L, P. Veena, L. Priyanka, and M. Chandra, "Constituents and antimicrobial activity of the essential oils of six Himalayan *Nepeta* species", Journal of the Serbian Chemical Society., vol. 75, pp. 739-747, 2010.

[29] National Committee for Clinical Laboratory Standards NCCLS. Performance standards for antimicrobial susceptibility tests, Approved standards NCCLS Publication M2-A5. Villanova, PA, USA 1999.

[30] National Committee for Clinical Laboratory Standards NCCLS. Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards- fifth edition. NCCLS document M7-A5. NCCLS: Wayne, PA, USA 2000.