Journal of Education for Pure Science- University of Thi-Qar
Vol.9, No.2 (June, 2019)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.09.02.09

Evaluation of Biological Activity and Chemical Content of Some Medicinal Plants Using Different Methods

Muntadher Hafiz Ali[1], Yass Khudair Abbass[2], Zuhair Radhi Adai[3]

[1,2,3] College of Education for Pure Sciences, University of Thi-Qar, Thi-Qar, Iraq.

Received 14/11/2018, Accepted 16/01/2019, Published 02/06/2019

Abstract:

This study was conducted to evaluate the biological and chemical effectiveness of some medicinal plants using different methods. Five species of medicinal plants(*Origanum majorana*, *Lawsonia inermis*, *Salvia officinolis*, *Conocarpus erectuus* and *Zizphus spina-christi*)were used as antimicrobial for three types of bacteria (*Staphylococcus aureus, Proteus mirabili*s and *Pseudomonas aeruginosa*) and antioxidants agents. The study included the use of four organic solvents (ethanol, methanol, acetone) and water for the purpose of determining the efficiency of each solvent in the separation of active compounds. Secondary metabolism compounds, were detected by qualitative detection. Antioxidants were tested by the 2,2-diphenyl-1-picrylhydrazyl(DPPH) test and total phenolic content(TPC) was calculated. Antibiotics (Amikacin, Gentamicin, Imipenem, Norflaxacin, Tetracycline, Tobramycin, Trimethoprim, Vancomycin) were used. Imipenem antibiotic was better than all plant extracts.

Key words: *Conocarpus erectuus, Lawsonia inermis*, Antioxidants, Antibiotics.

1.Introduction:

Medicinal plant is defined as a plant that contains one or more of its chemical substances, in low or high concentration, which has the ability to treat a particular disease or at least it reduces the symptoms of infection (Hamza, 2006).Today, the biggest problem facing the world is the so-called undeclared war that takes place between bacteria and antibiotics. Most germs are characterized by their great resistance to antibiotics because they have many factors of ferocity (Hansen *et al*., 2004). humans have used herbs to treat some of the diseases that afflict them or their pets since about 6000 years(Farid and Rachid ,2013). The inhabitants of Iraq and for thousands of years BC had used plants in the treatment of diseases and can be considered the oldest tablets of medicine in the world (Hadi, 2007).The use of medicinal plants in the pharmaceutical field has increased significantly in the present time. Therefore, many researchers in this field focused on the study of medicinal plants spread around the world, which are used for therapeutic purposes, including the treatment of colds, fever, diarrhea and others where the use of medicinal plants is common in The population of the regions where these plants grow is known as folk medicine (Khafagi and Dewedar, 2000). Medicinal plants contain various compounds that play a key role in protecting the plant

itself from disease by its activity against the regeneration of pathogenic bacteria (Abu-Shanab *et al*., 2005). The components of medicinal plants are divided according to their effectiveness to active ingredients and non-effective ingredients, as the active ingredients are the substances that have therapeutic values such as phenols, alkaloids, volatile oils, turbines and tannins. The ineffective ingredients are substances that have no therapeutic value such as cellulose (Aburjai *et al*., 2001).The plant kingdom contains many plant varieties, which are an essential source of natural products with inhibitory effect of various types of microscopic organisms (Nascimento *et al*., 2000). The Prophet's Hadiths mentioned many plants and their reasons for use and their nutritional benefits. These include henna, figs, dates, garlic, etc. (Barham, 2007). The reason for the use of natural herbs in treatment is to overcome the side effects of manufactured drugs (Poole, 2001). The aim of this study to evaluate the inhibitory effectiveness of some plant extracts on some types pathogenic bacteria.

2. Materials and Methods

a)Plants collection and preparation

The leaves collected from each of *Origanum majorana*, *Lawsonia inermis* and *Salvia officinolis* from the local markets of AL-Nasiriyah city (south of Iraq) these plants were classified by the professor of Plant taxonomist at the College of Education for Pure Sciences, Dr. Yass Khudair, than, leaves were cleaned from impurities and grinded by the electric grinder and placed in the bottles of opaque and kept in the refrigerator until the preparation of extracts. The bark of both *Zizphus spina-christi* and *Conocarpus erectuus* were collected from one of the orchards located west of AL- Nasiriyah city, It was cleaned from impurities and dried by the sun with continuous stirring. After that it was grinded and then placed in darkened bottles and kept in the refrigerator until preparation of the extracts, alcoholic ,acetone and water.

b)Extraction process of antioxidants

Preparation of plant extracts plant materials were extracted using the methods described previously (Musa *et al*, 2011). Briefly, 0.1 g dried plant powder and 10 ml 50% aqueous acetone were stirred for 1 h in a 25-mL universal bottle at 1,000 rpm using a magnetic stirrer (IKA, Staufen, Germany). Samples were then centrifuged at 4,750 g for 10 min using a mini centrifuge (Thermo-line, China) and the supernatants were used for further analyses.

c)Total Phenol Content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of (Musa *et al*. 2011). About 100 µL plant extract was added with 0.5 mL diluted Folin Ciocalteu reagent. The samples extracts with Folin Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dry sample (mg GA/100 g of DW).

d)DPPH Radical Scavenging Activity

The determination of antioxidant activity through 2,2- diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of (Musa et al. 2011). Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 µL sample extract with 1 ml methanolic

DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow:

DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100. Where A is the absorbance.

e)Antibacterial Assay

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Proteus mirabilis* were used in experiment. The bacterial isolates were obtained from the Microbiology Laboratory, College of Education for Pure Sciences, Thi-Qar University. Mueller Hinton agar was used in antibacterial assay. Plant extracts were dissolved in (acetone, ethanol, methanol and water) to obtain a concentration of (75,100,200 and 300)µg/10µL. Antibacterial assays were conducted using the disc diffusion method as previously described by (Kusuma *et al*. 2010). Negative controls were prepared using the same solvent employed to dissolve the plant extract. Gentamicin discs (10 µg/ disc, Oxoid, UK) were used as control and positive controls. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

f)Phytochemical Analysis

One gram of the plant methanol extracts was dissolved in 100 mL methanol and subjected to preliminary phytochemical screening following standard methods(Harborne , 1998 ،Kokate 2001).

3.Results and Discussion

3. 1-Effect of organic solvents on the effectiveness of *Conocarpus erectus* extract against *Pseudomonas aeruginosa*

Natural products may be a particularly rich source of anti-infective agents. The antimicrobial activity on pathogenic strains of Gram-positive *Pseudomonas aeruginosa* bacteria of *Conocarpus erectus* bark extracts was evaluated in the present study (Table 1).The antimicrobial activity of *Conocarpus erectus* bark and As it can be observed from this Table, all extracts exhibited antibacterial action against *Pseudomonas aeruginosa*. The diameter of the zone of inhibition varied ranging from (11mm) to (17mm) for bark extract. The results of the present study disagree with the study by Hajar and Gumgumjee (2013) where they found that the ethanol extract of *Conocarpus erectus* inhibits *Pseudomonas aeruginosa* at 24.00mm at 100mg/mL at 33.33mm at 200mg/mL concentration, the reason may be variation in the extraction method used or because of the agricultural location of the plant and different weather conditions in terms of temperature. The results of this study are similar to the study carried out by Abdel-Hammed *et al*. (2012), where they found during their studies that the average rate of inhibition of the methanol extract of the conocarps stalks against *Pseudomonas aeruginosa* is (15.00 ± 0.11) mm. Figure (1).

Table 1: The diameter of the inhibition zones (mm) of *Conocarpus erectus* extract against *Pseudomonas aeruginosa*

Solvent	Concentrations mg/mL			
	100	200	300	Mean
Ethanol	7.33±3.78	12.00±2.64	16.00±2.64	11.77
Methanol	11.00±1.00	13.00±1.00	14.66±0.57	12.88
Acetone	13.66±1.52	16.66±0.57	17.66±0.57	16.00
Water	11.33±2.51	17.00±1.00	17.66±0.57	15.33
Mean	10.83	14.66	16.50	

L.S.D=3.12

3-2-Antibiotics against the pathogenic bacterial isolates used

From Table(2) *P. aeruginosa* and *S. aureus* were sensitive to the antibiotic (GN and IPE) while *Proteus mirabilis* was not sensitive to the antibiotic (AK).Results agree with Jaloob and Gafile (2012),they found that the antibiotic(AK) resistance ratio of bacteria(*Proteus mirabilis*) was 100%.but different from the study conducted by Dulaimi *et al* (2016),they found that *Proteus mirabilis* was sensitive to the antibiotic (AK). The antibiotic(AK) inhibits the synthesis of the bacterial cell wall and the antibody (GN) interferes with protein synthesis in bacteria by altering the ribosome portion (Susan *et al*., 2003).The antibiotic(IPE) inhibits the formation of peptidoglycan as it has the ability to penetrate the protein channels and thus destroys the Gram-negative bacteria (Bagge *et al*., 2004). It also disrupts the permeability barrier to the membrane of the Gram-positive bacteria and thus destroys the cell (Carson *et al*.,2002).All bacterial isolates were sensitive to antagonist(NOR), this was consistent with Hussein *et al*. (2017). Figure (2).

Table 2: The diameter of the inhibition zones (mm) of antibiotics against the bacterial isolates.

Antibiotic	Bacteria		
	P. aeruginosa	*S. aureus*	*P. mirabilis*
Amikacin (AK)	8	18	0.00
Gentamicin (GN)	4	20	2
Imipenem (IPE)	26	28	32
Norflaxacin (NOR)	30	22	4
Tetracycline (TE)	22	14	9
Tobramycin (TOB)	18	10	4

3-3- Specific data on active compounds in medicinal plants used

Table(3)shows that the water extract of *Conocarpus erectuus* contains phenols, carotenoids, saponins, alkaloids, flavonoids and does not contain tannins, carotenoids and terpenoids. These results are consistent with the study of Abdel-Hameed *et al*. (2012), found that *Conocarpus erectuus* bark contains phenols and saponins. The current study disagrees with Shohayeb *et al*. (2013), where it confirmed that antibacterial activity against microorganisms is due to the presence of tannins. This may be due to different methods of detection of materials or because of different plant source and country of plant cultivation. The results showed that the water extract of *Lawsonia inermis* leaves contains phenols, glycosides, saponins, turbines, carotenoids and flavonoids and did not contain alkaloids and tannins. The results of the present study agree with the study carried out by AL-Hamdani, and Al-Mahna(2009), they found that *Lawsonia inermis* leaves contain phenols, glycoside, soap, dyes, comarins, and resins. However, the results of this study did not agree with Raja *et al*. (2013). The results showed that the water extract of *Origanum majorana* leaves contains both the following compounds phenols, flavonoids, alkaloids, carotenoids and tannins, and

did not contain glycosides, saponins and terpenoids, these results disagree with the study conducted by Farid and Rashid (2013), they found that the leaves of *Origanum majorana* did not contain alkaloids. The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Flavonoids have been reported to possess antibacterial and antioxidant, saponins showed hypocholesterolemic and ant diabetic properties(Miller ,1996).

Table 3: Phytochemicals analysis of plant extracts

Compounds	Plants				
	Conocarpus erectuus	***Lawsonia inermis***	***Origanum majorana***	***Salvia officinolis***	***Zizphus spina-christi***
Phenols	+	+	+	+	+
Glycosides	+	+	-	-	-
Saponins	+	+	-	+	-
Terpenoids	-	+	-	-	+
Alkaloids	+	-	+	-	-
Carotenoids	-	+	+	+	-
Taninns	-	-	+	+	-
Flavonoids	+	+	+	+	+

* Where(+) represents the positive result(-) represents the negative result

3-4-Total phenol content determination (TPC)

The TPC varied markedly among the tested plant extracts ranging from 132.00 to 62.40 mg/GAE. Among the plants in this study, *Conocarpus erectus* showed the highest value (132.00 mg/ GAE).also note significant differences between *Conocarpus erectus* and *Lawsonia inermis* and the results of the current study consistent with Al-Fikki and Rikabi (2013),which confirmed that the methanol extract of *Lawsonia inermis* leaves contained phenolic compounds (25 mg / GAE),this does not agree with Hosein and Zinab (2007),they found that the amount of phenols in *Lawsonia inermis* leaves is (145mg / GAE). The cause of the difference is the difference of plant source. Abdel-Hameed *et al*. (2012),found that *Conocarpus erectus* contain large quantities of phenols, they also found that the ethyl acetate extract of *Conocarpus erectus* contains amount of phenols (181.61 ± 3.98 mg / GAE),While the butanol extract contains amount of phenols (416.09 ± 14.35 mg / GAE). Olajuyigbe and Afolayan (2011) found that the ethanol extract of *Zizphus spina-christi* contains a large amount of phenol compared with the water extract of *Zizphus spina-christi*. Figure (3).

3-5- DPPH radical scavenging activity

DPPH radical scavenging activity varied from 78 to 37 % representing an approximately 41%-fold variation between the lowest and highest activities. *Origanum majorana* showed the highest antioxidant activity, followed by *Salvia officinolis, Zizphus spina-christi, Lawsonia inermis* and *Conocarpus erectus*(76,55,51 and 37 % respectively).*Conocarpus erectus* showed the lowest antioxidant activity (46 %) in the DPPH assay. Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location extraction methods used (Uma and Wan Aida .,2010). Figure (4).

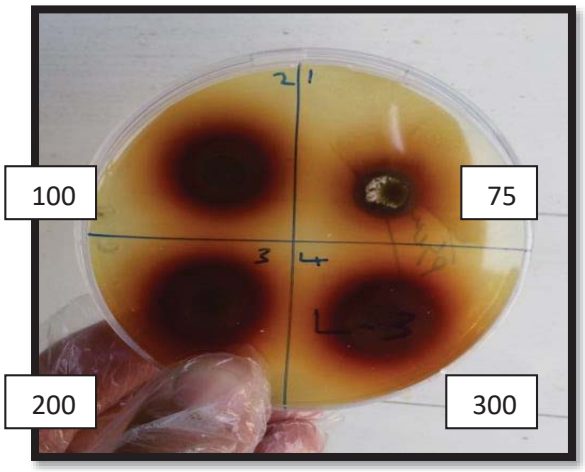


Figure 1: The areas of inhibition of the acetone extract of *Conocarpus erectuus* against *P. aeruginosa*

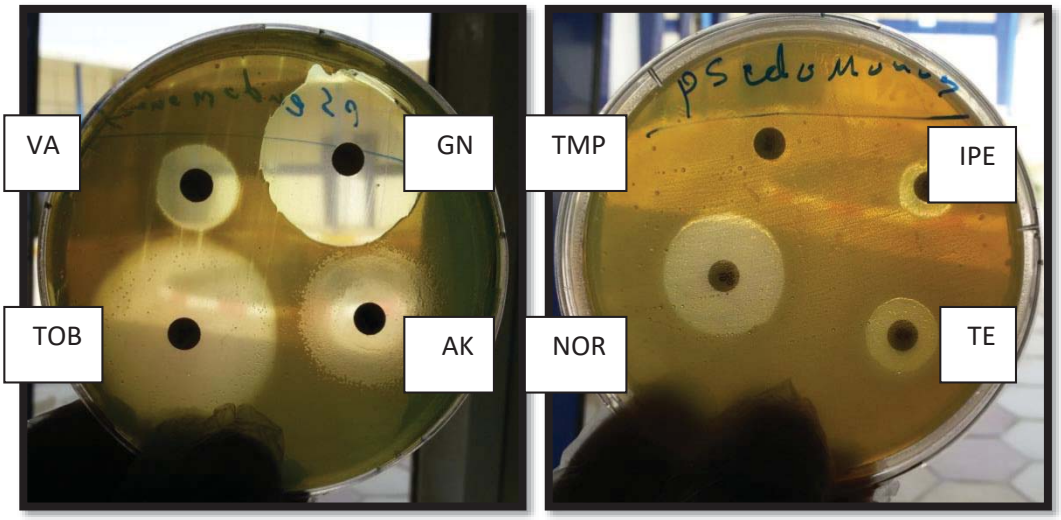
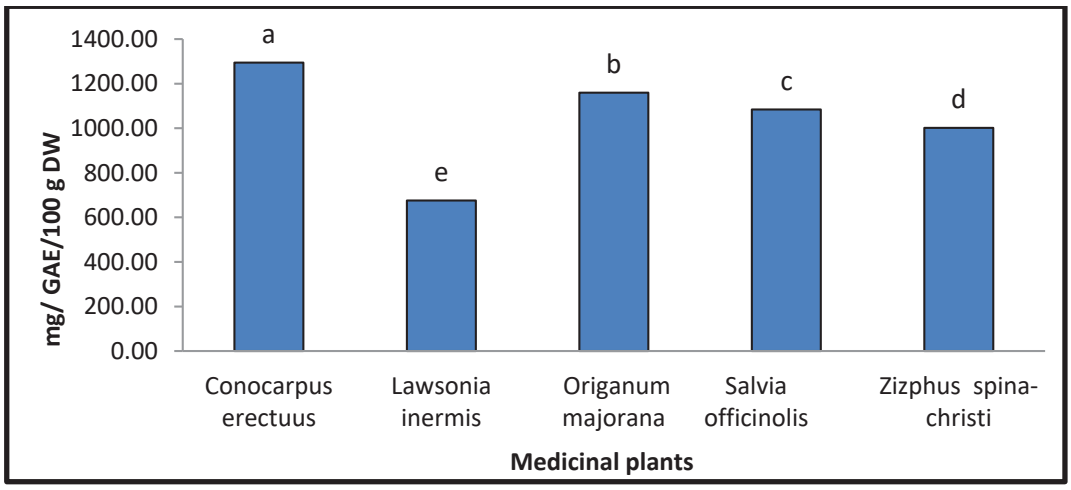
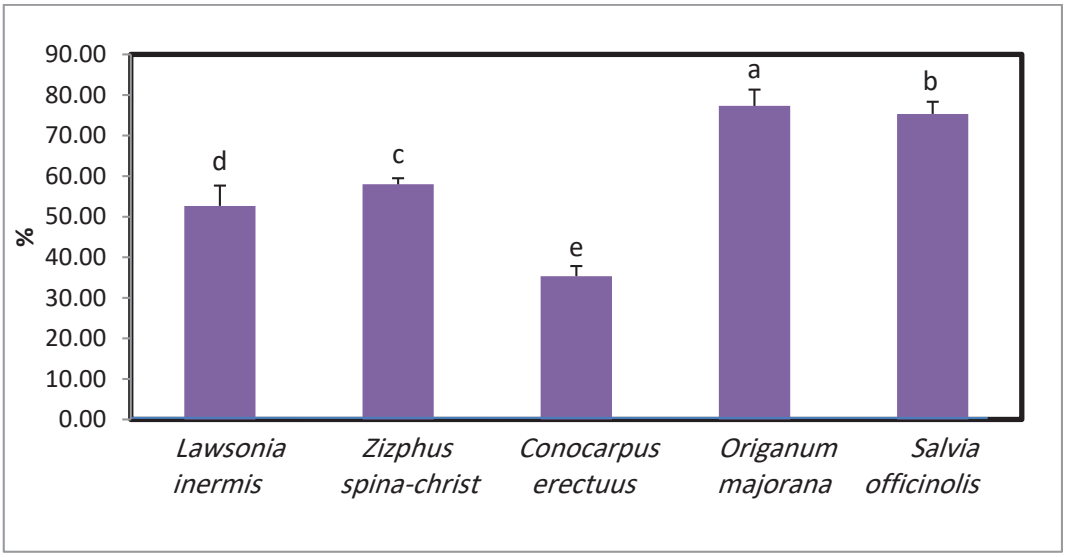


Figure 2: The effect of antibiotics against bacteria (*P.aeruginsoa*).



[a-d]Mean with different letters are significantly different (P<0.05)

Figure 3: The total phenol content of plants used in the study



[a-e]Mean with different letters are significantly different ($p<0.05$)

Figure 4: Radical-scavenging activity (DPPH) of medicinal plants

Conclusion

These results showed that the different medicinal plants contained significantly different amount of antioxidant capacity and antibacterial activity. According to this results, *Origanum majorana*, *Lawsonia inermis*, *Salvia officinolis*, *Conocarpus erectuus* and *Zizphus spina-christi* extracts with higher phenolic content, antioxidant capacity and antibacterial activity. Further study using bioassay guided of crude extracts of *Origanum majorana*, *Lawsonia inermis*, *Salvia officinolis*, *Conocarpus erectuus* and *Zizphus spina-christi* extracts are needed to isolate and identify the active compounds. Their active constituents may be potential candidates with therapeutic value in the treatment of bacterial and oxidation.

References:

- **Abdel-Hameed, E. S. S., Bazaid, S. A., Shohayeb, M. M., El-Sayed, M. M., and El-Wakil, E. A. (2012).** Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. *European J. of Medicinal Plants, 2(2),* 93-١١٢ .
- **Aburjai, T., Darwish, R. M., Al-Khalil, S., Mahafzah, A., and Al-Abbadi, A. (2001).** Screening of antibiotic resistant inhibitors from local plant materials against two different strains of Pseudomonas aeruginosa. *J. of ethnopharmacology*,*76*(1), 39-44.
- **Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Adwan, K., and Abu-Shanab, M. (2005).** Antibacterial activity of *Rhus coriaria* L. extracts growing in Palestine. *J. Islam. Univ. Gaza*, *13*(2), 147-153.
- **Aghashami, S. (1982).** Study of some pharmacological and toxicological properties of cesium flower. Master Thesis submitted to the Faculty of Veterinary Medicine - University of Baghdad.
- **AL-Fikkia, F. A. and Alrkabi, A. K. J. (2013).** Extraction and diagnosis of phenolic compounds from *Lawsonia inermis* and its effectiveness as antioxidants. *Thi-Qar University J. of Agricultural Research, 2 (2):* 140-157.

- **AL-Hamdani, A. H. O. and Al-Mahna, B. M. M. (2009).** Study of the effect of *Lawsonia inermis* in the growth of *Trichophyton mentagrophytes* in vitro and in vivo. *Qadisiyah J. of Veterinary Medicine, 8 (1):* 108-115.
- **Al-Shaihat, A. N. (1986).** Medicinal herbs and plants. First edition, Dar al-Behar Beirut Lebanon.
- **Bagge, N., Hentzer, M., Andersen, J. B., Ciofu, O., Givskov, M., and Høiby, N. (2004).** Dynamics and spatial distribution of β-lactamase expression in *Pseudomonas aeruginosa* biofilms. Antimicrobial agents and chemotherapy , 48(4), 1168-1174.
- **Barham, Z. S. (2007)**. Treat Yourself With Herbs. Umm Al - Qura Foundation for Translation, Publishing and Distribution.
- **Carson, C. F., Mee, B. J., and Riley, T. V. (2002).** Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrobial agents and chemotherapy, 46(6), 1914-1920.
- **Dulaimi, F. Y., Abbas A. F. and Al-Zubaidi N. A. (2016).** Biological efficacy of the aquatic and alcoholic extracts of chamomile plants and the ring against bacteria isolated from different clinical sources. Master Thesis, College of Education for Pure Sciences, Diyala University, Iraq.
- **Farid, A. K. and Rachid, B. K. (2013).** Separation and Determination of Secondary Metabolic Products and Study of Antibiotic Biological Effect of Ethan Acetate Extract of *Origanum vulgare* L. glandulosum (Desf) Ietswaart. Master of Science - Faculty of Fine Sciences- Monitory University of Constantine - Algeria.
- **Hadi, S. M. (2007)**. the inhibitory effect of Rosemarinus officinalis in some types of microbiology, Master Thesis, Faculty of Science, University of Baghdad.
- **Hajar, A. S., and Gumgumjee, N. M. (2013)**. Antibacterial efficiency and DNA impairmentunveilin some bacteria strains treated with *Conocarpus erectus* L. extract.
- **Hamdani, A. H., and Al-Mahna B. M. (2009).** Study of the Effect of Henson Plant Extract in the Growth of Trichophyton Mentagrophytes in Vitro and in vivo. *Qadisiyah J. of Veterinary Sciences*, 8 (1): 108-115.
- **Hammer, K. A., Carson, C. F., and Riley, T. V. (2002).** In vitro activity of *Melaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. *J. of Antimicrobial Chemotherapy, 50(2)*, 195-199.
- **Hamza, A. M. (2006).** International Medical Plants - Labeling - Specifications, Al-Jalal Printing Company - Alexandria - Egypt, pp. 371.
- **Hansen, D. S. ; Aucken, H. M. ; Abiola, T. and Podschun (2004).** Recommended Test Panel For Differentiation Of *Klebsiella* Species on The Basis Of Trilateral Interlaboratory Evaluation Of 18 Biochemical Tests . *J . Clin . Microbiol . 42(8)*:3665-3669.
- **Harborne J.B (1998).** Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, 3rd ed. New York: Chapman and Hall Int Ed. 14(2);234−45.
- **Harborne, J. B. (1984).** Methods of plant analysis. In Phytochemical methods (pp. 1-36). Springer, Dordrecht.
- **Hosein, H. K. M., and D. Zinab. (2007).** Phenolic compounds and antioxidant activity of henna leaves extracts (*Lawsonia inermis*).*World J. of Dairy and Food Sciences, 2(1),* 38-41.

- **Hussein, S. S., Khatem, A. O. and Nihad, K. T. (2017).** Study of the effect of some antibiotics on *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria isolated from burns and wounds. *J. of Bab University, 25 (1)*: 69-80.
- **Jaloob, A. A., and Gafil, F. A. A. (2012).** Effect of some antibiotics on aerobic pathogenic bacteria causing obits media and urinary tract infection in Al-Manathera city in Iraq: A comparative in vitro study. AL-*QADISIYAH MEDICAL JOURNAL, 8(13)*, 156-168.
- **Khafagi, I. K., and Dewedar, A. (2000).** The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. *J. of ethnopharmacology*, 71(3):365-367.
- **Kokate C.K. (2001).** Pharmacognosy, 16th ed. Mumbai, India: Nirali Prakashan.
- **Kusuma, I. W., Arung, E. T., Rosamah, E., Purwatiningsih, S., Kuspradini, H., Astuti, J., ... and Shimizu, K. (2010).** Antidermatophyte and antimelanogenesis compound from Eleutherine americana grown in Indonesia. *J. of natural medicines*, *64*(2), 223-226.
- **Miller, A. L. (1996).** Antioxidant flavonoids: structure, function and clinical usage. *Alt Med Rev*, *1*(2), 103-111.
- **Musa, K. H., Abdullah, A., Jusoh, K., and Subramaniam, V. (2011).** Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. Food Analytical Methods, 4(1), 100-107.
- **Nascimento, G. G., Locatelli, J., Freitas, P. C., and Silva, G. L. (2000).** Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian J. of microbiology*,*31*(4), 247-256.
- **Olajuyigbe, O. O., and Afolayan, A. J. (2011).** Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* Willd. Subsp. mucronata Willd. BMC complementary and alternative medicine , 11(1), 130.
- **Phillipson, J. D. (1995).** A matter of some sensitivity. *Phytochemistry*, *38*(6), 1319-1343.
- **Poole, K. (2001).** Overcoming antimicrobial resistance by targeting resistance mechanisms. *J. of pharmacy and pharmacology*, 53(3):283-294.
- **Raja, W., Ovais, M., and Dubey, A. (2013).** Phytochemical screening and antibacterial activity of *Lawsonia inermis* leaf extract. medicine, 6, 8.
- **Shohayeb, M., Hameed, E. A., and Bazaid, S. (2013).** Antimicrobial activity of tannins and extracts of different parts of *Conocarpus erectus* L. Int. *J. Pharm. Bio. Sci, 3*, 544-553.
- **Singh, K. (2012).** Phytochemical determination and antibacterial activity of Trichosanthes dioica Roxb (Patal), Cucurbita Maxima (pumpkin) and Abelmoschus esculentus Moench (Okra) plant seeds.Thesis -NationaInstitute of Technology-Rourkela .pp41.
- **Susan, K., Micota , D.V.M., Donald, C.P. and D. Pharm. (2003)** .Amikacin Sulfate. Elephant care International. http:// www.elephantcare.org/.
- **Uma, D. B., Ho, C. W., and Wan Aida, W. M. (2010).** Optimization of extraction parameters of total phenolic compounds from henna (Lawsonia inermis) leaves. *Sains Malaysiana*, *39*(1), 119-128.