Antibacterial activity of medicinal plants extracts; *Rosmarinus officinalis* and *Nerium oleander*

Leila Ait Abderrahim [1*], Khaled Taibi [1*], Anas M. Alomery [2] and Nawel Ait Abderrahim [1]

1 Faculty of Life and Natural Sciences, Ibn Khaldoun University of Tiaret, Algeria.
2 Clinical Laboratory Department, Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia.

ID # (2898)
Received: 03/03/2019
In-revised: 25/04/2019
Correspondent Author:
Leila AIT ABDERRAHIM
Khaled TAIBI
E-mail:
aitleila-bio@hotmail.com
khaledtaibi@hotmail.com

KEYWORDS

Antibacterial activity, methanol extract, Rosmarinus officinalis, Nerium oleander.

ABSTRACT

Bacterial resistance to antibiotics has led scientists to search into nature for novel therapeutic molecules with less or no side effects. This study aims to determine the antibacterial activity of methanol extract of the leaves and stems of two plants species widely used in traditional medicine in the Mediterranean basin; *Rosmarinus officinalis* and *Nerium oleander*. The antibacterial activity was evaluated through determining the minimum inhibitory concentration (MIC) by the agar dilution method on four microbial strains; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus*. Results showed that extracts of the different parts of both species revealed inhibitory activity against all the tested strains with lower MICs exhibited by *R. officinalis*. *P. aeruginosa* was shown to be the most sensitive bacteria to *N. oleander* extracts and *S. aureus* the most resistant to them. However, *E. coli* was relatively the most resistant bacteria to *R. officinalis* extracts whereas *B. cereus* was the most sensitive to them. It should be noted that Gram-positive bacteria were the most sensitive to *R. officinalis* extracts while Gram-negative bacteria were more sensitive to *N. oleander* extracts. Taking all together, both plant species proved to be effective antibacterial agents and consequently, they may constitute a potential natural resource for new substances with antibacterial activity as alternative of the usual drugs to avoid microbial resistance.

النشاط المضاد للبكتيريا لمستخلصات النباتات الطبية؛ إكليل الجبل و الدفلى

ليلى آيت عبد الرحيم[1]، خالد طيبي[1]، أنس محمد العمري[2]، نوال آيت عبد الرحيم[1].

1 كلية علوم الطبيعة و الحياة ، جامعة ابن خلدون ، تيارت ، الجزائر.
2 قسم المختبرات السريرية ، كلية العلوم الطبية التطبيقية ، جامعة الطائف ، الطائف ، المملكة العربية السعودية.

رقم المسودة: (2898)
تاريخ استلام المسودة: 2019/03/03
تاريخ المسودة المُعَدّلة: 2019/04/25
الباحث المُرَاسِل:
ليلى آيت عبد الرحيم
خالد طيبي
بريد الكتروني :
aitleila-bio@hotmail.com
khaledtaibi@hotmail.com

الكلمات الدالة

النشاط المثبط للبكتيريا، مستخلص الميثانول، إكليل الجبل، الدفلى.

الملخص

لتفادي مقاومة البكتيريا للمضادات الحيوية قام العلماء بالبحث على جزيئات و مواد طبيعية جديدة مثبطة للبكتيريا بأقل آثار جانبية. تهدف هذه الدراسة إلى تحديد النشاط المضاد للبكتيريا لمستخلص ميثانول أوراق و سيقان نوعين من النباتات المستخدمة على نطاق واسع في الطب التقليدي في حوض البحر الأبيض المتوسط؛ الدفلى و إكليل الجبل. تم تقييم النشاط المثبط على أربعة سلالات بكتيرية؛ الاشريكية القولونية، الزائفة الزنجرية، المكورات العنقودية الذهبية و البكتيرية العصوية و ذلك من خلال قياس أقل تركيز مثبط عن طريق دمج تراكيز مختلفة من المستخلص النباتي في الوسط المغذي. و لقد بينت النتائج المحصل عليها أن مستخلص كلا النبتتين يحتوي على نشاط مثبط ضد كل السلالات البكتيرية المدروسة مع فعالية أكثر لمستخلص إكليل الجبل. كما لوحظ أن الزائفة الزنجرية هي البكتيريا الأكثر حساسية لمستخلص الدفلى بينما المكورات العنقودية الذهبية هي البكتيريا الأكثر مقاومة لهذا المستخلص. و كانت البكتيريا العصوية هي الأكثر حساسية لمستخلص إكليل الجبل بينما الاشريكية القولونية هي الأكثر مقاومة نسبيا لهذا المستخلص. و يجدر بالذكر أن البكتيريا الموجبة الغرام هي الأكثر حساسية لمستخلص إكليل الجبل بينما أظهرت البكتيريا السالبة الغرام حساسية أكبر لمستخلص نبات الدفلى. إعتمادا على ما سبق ذكره فقد اثبتت هذه الدراسة أن مستخلص الدفلى و إكليل الجبل يمتاز بفعالية تثبيط معتبرة ضد السلالات البكتيرية المدروسة و بالتالي فقد تُشكل النبتتان مورداً طبيعياً فعالاً للمواد و الجزيئات ذات النشاط المضاد للبكتيريا كبديل فعال للأدوية لتجنب المقاومة البكتيرية.

Introduction

The use of natural products in nutrition and medicine has a long history since ancient times. Medicinal plants represent an indispensable source of both preventive and curative traditional medicine preparations especially for poor populations (Jamal, et al. 2012). They have been used for years in daily life to treat diseases all over the world since they produce a diverse range of bioactive molecules and medicinal compounds (Hosseinzadeh, et al. 2015).

Nowadays, immense clinical problems have been raised in the treatment of infectious diseases. Infectious bacterial diseases are becoming serious threat mainly in developing countries where peoples are not aware of their primary healthcare (Ait Abderrahim, et al. 2017). This could be attributed mainly to the lack of appropriate treatment, the ignorance and indiscriminate use of antibiotics from a part (Jamal, et al. 2012), and to the development of bacterial resistance toward many commercial antibiotics as a common phenomenon from another part (Zhang, et al. 2015).

Algeria possesses rich and diversified flora of medicinal plants which are able to grow and develop in degraded environments where they constitute veritable nutritional and/or drug resources for the local populations. Nevertheless, the biological knowledge on native species in the arid regions constitutes a limit and a big handicap for the use and the valorisation of such plant genetic resources.

In this regard, species from the Lamiaceae family, such as rosemary *Rosmarinus officinalis* L., have been regarded with special interest because of their bioactive properties. This species originating from the Mediterranean region is a perennial aromatic plant forming a stiff shrub, much branched and densely bushy, with a characteristic aromatic smell (Pintore, 2002; Khorshid, 2009). It is used as a fragrance plant as well as herbal remedy with medical benefits as a tonic for the nerves, digester, anti-rheumatic, for wounds healing and a tonic for the circulation and the heart, treatment of migraines and as antiseptic and antimycotic agent (Antoine, 1999; Mark, 2003). Rosemary contains phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid and the antioxidants carnosic acid and carnosol (Mena, et al. 2016).

As well, oleander *Nerium oleander* Linn. belonging to the family Apocynaceae is also considered as a valuable species. It is a large glabrous evergreen shrub with milky latex widely distributed in temperate regions throughout the world. This species which is known to contain active cardiac glycosides has many therapeutic uses in different traditional medicine of the world. In ethnobotanical literature it is mentioned to be effective in the treatment of cardiac illnesses asthma, corns, prostate/breast cancer, epilepsy and also used as diuretic, anti-inflammatory agent, anti-parasitic and for neurological disorders (Siddiqui, et al. 1997; Al-Yahya, et al. 2000). Oleander is one of the most poisonous plants and contains numerous toxic compounds mainly oleandrin and neriine, which are cardiac glycosides. It is thought that Oleander may contain many other unknown compounds that may have dangerous effects (Yu, et al. 2007).

In general, the various parts of *R. officinalis* and *N. oleander* are reputed as therapeutic agents and have been used in traditional medicine since they possess valuable phytochemical compounds. Even though, both species hold several medicinal properties but they remain neglected.

In order to promote the use of such mistreated plants, this study aims to demonstrate the antibacterial activity of *R. officinalis* and *N. oleander* leaves and stems methanol extracts on some pathogenic bacterial species known to develop antibiotic resistance as well as resistant biofilms.

Material and methods

Plant material and extract preparation

The leaves and stems of *R. officinalis* and *N. oleander* were harvested in spring 2017 from the region of Tiaret; a steppic upland area in the Tell Atlas located about 160 kilometers inland from the Mediterranean seacoast of Algeria. Leaves and stems of each species were separated then kept for shade drying at room temperature. The

methanol extracts were prepared as described by Ait Abderrahim, et al. (2017). The dried samples were ground to fine powder using an electrical grinding machine. A 10 g of powder was macerated in 100 mL methanol in dark at room temperature for 48 h. The extract was filtered, and evaporated to dryness and kept in a dark bottle at 4 °C.

Extraction yield expressed as mass of extract/ mass of dry matter was also determined and used as an indicator of the effects of the extraction conditions.

Microbial strains and inoculums preparation

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 33862) and *Bacillus cereus* (isolate) tested in this study were kindly provided by the University Hospital Center (CHU) Mustapha Pacha, Algiers (Algeria). These microbial species are chosen since they are involved in many common infectious diseases. In addition, they are known to develop multidrug resistance. Prior to the experiment, the tested bacterial species were inoculated into nutrient agar media. The inoculum suspensions were prepared in sterile saline (0.85 % NaCl) from 24-hour-old cultures and the density was adjusted to the McFarland 0.5 turbidity standard (108 CFU/mL).

Antibacterial activity

The antibacterial activity was tested by the agar dilution method against the selected pathogenic microbial species (Ait Abderrahim, et al. 2017). Briefly, concentrations of the methanol extracts of leaves and stems of *R. officinalis* and *N. oleander* were incorporated separately into Mueller-Hinton agar media. The final volume of the mixture 'methanol extract-media' in each plate was 10 mL. The plates were inoculated and incubated at 37 °C for 24 h. The minimal inhibitory concentration (MIC) was established by determining the lowest concentration of the assayed antimicrobial agents that inhibits the visible growth of the species being investigated. Plates containing the media inoculated with each of the tested species were also prepared to serve as control for the viability of the tested microorganisms.

Results

The dilution methods are the most appropriate ones to estimate the minimal inhibitory concentration (MIC) of the tested extracts for this study. Nevertheless, the agar dilution method appears more suitable than the broth dilution method since the plant extract masks the detection of bacterial growth in the liquid medium (Balouiri, et al. 2016).

Extraction yields of methanol extracts were higher in leaves of both species in comparison to stems. In addition, leaves of *N. oleander* yielded higher than those of *R. officinalis* while the opposite is observed regarding stems. Extraction yields ranged from 7 % for stems to 17 % for leaves of *N. oleander* and from 10 % for stems to 16 % for leaves of *R. officinalis* (Fig. 1).

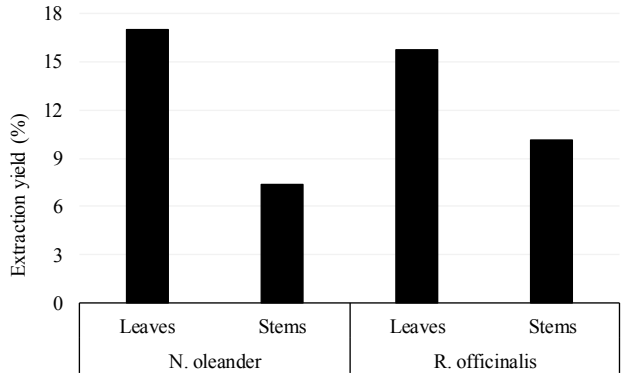

Figure 1. Extraction yields of leaves and stems methanol extracts of *N. oleander* and *R. officinalis*.

It is clearly noted that the methanol extracts of *N. oleander* and *R. officinalis* possesses significant antibacterial activity against all the tested Gram-positive and Gram-negative bacterial species. Nevertheless, the antibacterial effect was more evident and with lower MIC for *R. officinalis* extracts regarding all the tested strains.

Results demonstrated that the antibacterial activity of leaves extract was higher than that of stems extract in *R. officinalis*. In addition, the Gram-positive bacteria *S. aureus* and *B. cereus* were proven to be the most sensitive species to the leaves (with MIC values of 0.4 and 0.01 mg/ ml respectively) and stems extract (with MIC values of 0.6 and 0.3 mg/ ml respectively). However, the Gram-negative bacteria *P. aeruginosa* and

E. coli demonstrated higher MIC values of the stems extract (0.6 and 1 mg/ml respectively) and of leaves extract (0.6 and 1.1 mg/ml respectively) (Fig. 2).

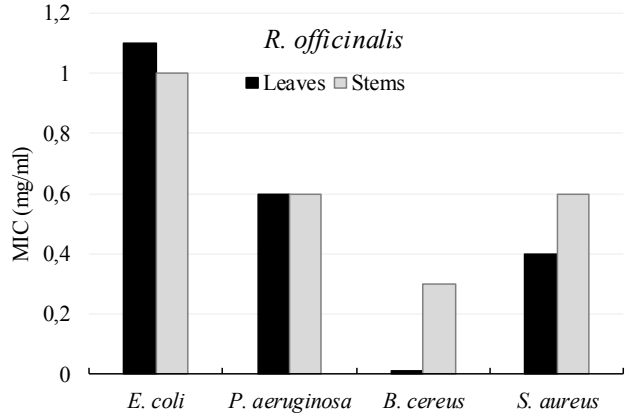

Figure 2. Minimal inhibitory concentration (MIC) of *R. officinalis* leaves and stems methanolic extracts on the tested microorganisms.

At the opposite, the Gram-negative bacteria were more sensitive than Gram-positive bacteria to leaves and stems extracts of *N. oleander*. *P. aeruginosa* was the most sensitive bacteria and was inhibited under MIC values of 0.6 and 0.8 mg/ml respectively for leaves and stems. However, *E. coli* and *B. cereus* were inhibited under 1 mg/ml of leaves extract and under 1.1 and 2 mg/ml of stems extract respectively. *S. aureus* was the most resistant bacteria and was inhibited under MIC values of 14 and 10 mg/ml of leaves and stems extracts respectively (Fig. 3).

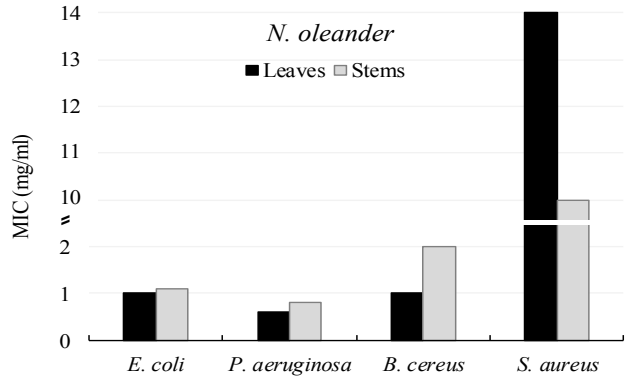

Figure 3. Minimal inhibitory concentration (MIC) of *N. oleander* leaves and stems methanol extracts on the tested microorganisms.

Discussion

Plants have been used since a long time for the treatment of several ailments and nowadays constitute a base of several drug compounds. This study is a contribution to the knowledge on plant genetic resources and medicinal plants along with their biological properties. It was conducted to investigate the antibacterial activity of methanol extracts of leaves and stems of two medicinal plants *R. officinalis* and *N. oleander* against selected pathogenic bacterial species.

In this work, extraction was performed using methanol as solvent. In fact, bioactive compounds usually occur in low concentration in plants and the extraction technique is very important to obtain extracts with high yield and with minimal changes to the functional properties of the isolated phytochemicals from plant materials (Quispe Candori, et al. 2008).

Extraction yields ranged from 7 % to 17 % for both species noting that the yield of leaves extracts of both species was higher than that of the stems. Actually, the main parameters affecting the yield of extract are the method of extraction, the nature of the tissue, the concentration of the phytochemicals, the particle size and the interfering substances (Do, et al. 2014). Several studies showed that methanol and ethanol are effective solvents for extraction. Alcoholic solvents can increase the permeability of cell walls by facilitating the extraction of numerous polar molecules as well as molecules of medium and low polarity (Franco, et al. 2008). Since almost all identified compounds from plants that are active against microorganisms are aromatic compounds or saturated organic compounds, they are most often obtained by ethanolic or methanolic extraction (Naili, et al. 2010).

Regarding the antibacterial activity, the results obtained demonstrate a difference in bacterial sensitivity to the plant extracts. This difference may lie in the permeability, composition and charge of the external structures of microorganisms (Bhuvaneshwari, 2007). It may also be due to variation in the rate of penetration of extracts across the cell wall and cell membrane structures (Alnamer, et al. 2013).

Studies demonstrated the presence of polyphenols, particularly flavonoids, phenolic

acids and phenolic terpenes in *R. officinalis* (Ibañez, et al. 2003). The mechanism of action of polyphenols on bacteria is very complex, they are thought to act either on nucleic acids, on cell envelopes, by substrate sequestration required for microbial growth or chelation of metals, by inhibiting microbial metabolism and/or non-specific interactions such as the establishment of hydrogen bridges with cell wall proteins or enzymes (Rsaissi, et al. 2013). Several studies have shown the antibacterial activity of *R. officinalis* essential oil against *E. coli, B. cereus* and *S. aureus* attributing its inhibitory effects to the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol (Burt, 2004). Studies of Tirumalasetty, et al. (2014) reported that the antimicrobial activity of rosemary is highly attributed to carnosol and carnosic acid (phenol diterpenes). Vegara, et al. (2011) also reported that the effectiveness of carnosic acid against pathogenic bacteria is superior to that of any other major extract component, including rosmarinic acid. However, Moreno, et al. (2006) and Ivanovic, et al. (2012) demonstrated that the effectiveness of rosemary is related to a possible synergy between the rosmarinic phenolic acid and the carnosic acid diterpene.

Regarding *N. oleander*, studies have shown that this plant contains numerous toxic compounds that are present in all parts of the plant; mainly oleandrin and nerine that are cardiac glycosides (Zibbu and Batra, 2010) as well cardenolides compounds that inhibit cellular membranes (Aslani, et al. 2004; Soto-Blanco, et al. 2006). A large amount of polyphenols is also present in the leaves of *N. oleander*. Cinnamic acid is the main component, the other components are epicatechin, catechin and chlorogenic acid (Sinha and Biswas, 2016). In addition to these compounds, the presence of tannins, alkaloids, saponins and terpenes which are known for their antioxidant and antimicrobial activity have been reported (Mohadjrani, 2012). Our results are in accordance with those of Hussain and Gorsi, (2004) that reported the antimicrobial activity of leaves and roots of *N. oleander* against *S. aureus, E. coli* and other strains belonging to the genus Bacillus namely *Bacillus pumilus* and *B. subtilis*. As well, our results are also consistent with those of El sawi, et al. (2010) and Tannu, et al. (2011) that demonstrated the antibacterial activity of the crude extract of *N. oleander* on *S. aureus, E. coli, B. cereus* and *P. aeruginosa* among others as well the antimicrobial activity of *N. oleander* stem extract on all the bacterial strains used in this study. Mohadjrani, (2012) also demonstrated the antibacterial activity of *N. oleander* extracts on *S. aureus* and *P. aeruginosa*. They also found that methanol is the most effective solvent in extracting the phenolic content of *N. oleander.*

Conclusion

Plant based antimicrobials represents a vast unexploited source for medicine. Their rare side effects compared to synthetic drugs confer them an enormous therapeutic potential. This study demonstrated the antibacterial activity of methanol extract of two medicinal plants species widely distributed in the steppe regions of the Mediterranean basin and commonly used in Algerian traditional medicine by the local population; *Rosmarinus officinalis* and *Nerium oleander.*

Leaves and stems extracts of both species revealed antibacterial activity against all the tested strains with lower MICs exhibited by *R. officinalis*. Furthermore, *P. aeruginosa* was shown to be the most sensitive bacteria to *N. oleander* extracts and *B. cereus* the most sensitive to *R. officinalis* extracts. However, *E. coli* was relatively the most resistant bacteria to *R. officinalis* extracts whereas *S. aureus* was the most resistant to *N. oleander* extracts. It should be noted also that Gram-positive bacteria were the most sensitive to *R. officinalis* extracts while Gram-negative bacteria were more sensitive to *N. oleander* extracts.

To conclude, both species proved to be effective antibacterial agents that is cost reduced, more accessible than the usual drugs and able to avoid microbial resistance. Advanced studies are recommended to determine the bioactive compounds of both medicinal plants species in order to obtain drug molecules with enriched properties.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

No funding sources are declared by the Authors.

References

Ait Abderrahim, L, Taibi, K, and Ait Abderrahim, C (2017) Assessment of the antimicrobial and antioxidant activities of *Ziziphus lotus* and *Peganum harmala*. Iran. J. Sci. Technol. Trans. Sci. https://doi.org/10.1007/s40995-017-0411-x

Alnamer, R, Alaoui, K, Doudach, L, Bouidida, EH, Chibani, F, AL-Sobarry, M, Benjouad, A, Cherrah, Y (2013) In vitro antibacterial activity of *Rosmarinus officinalis* methanolic and aqueous extracts. Int. J. Pharm. 3(1): 1-6.

Al-Yahya, MA, AL-Farhan, AH, Adam, SEA (2000) Preliminary toxicity study on the individual and combined effects of *Citrullus colocynthis* and *Nerium oleander* in rats. Fitoterapia 71: 385-391.

Antoine, BK (1998) Hungarian Musuotai of medicinal plants, Part I: Plants: Nature drug store. 1st edition. p: 63-67.

Aslani, MR, Movassaghi, AR, Mohri, M, Abbasian, A, Zarehpour, M (2004) Clinical and pathological aspects of experimental oleander *(Nerium oleander)* toxicosis in sheep. Vet. Res. Commun. 28: 609-616.

Balouiri, M, Sadiki, M, Koraichi Ibnsouda, S (2016) Methods for in vitro evaluating antimicrobial activity: A review. JPA 6: 71-79.

Bhuvaneshwari, L, Arthy, E, Anitha, C, Dhanabalan, K, Meena, M (2007) Phytochemical analysis and antibacterial activity of *Nerium oleander.* Anc. Sci. Life 26(4): 24–28.

Burt, S (2004) Essential oils: Their antibacterial properties and potential applications in foods—A review. Int. J. Food Microbiol. 94: 223–253.

Do, QD, Angkawijaya, AE, Tran-Nguyen, PL, Huynh, LH, Soetaredjo, FE, Ismadji, S, Ju, YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal. 22: 296-302.

El Sawi, NM, Geweely, NS, Qusti, S, Mohamed, M, Kamel, A (2010) Cytotoxicity and antimicrobial activity of *Nerium oleander* Extracts. J. Appl. Anim. Res. 37(1): 25-31.

Franco, D, Sineiroz, J, Rubilar, M, Sanchezz, M, Jerezz, M, Pinelo, M, Costoya, N, Nunez, MJ (2008) Polyphenols from Plant Materials: Extraction and Antioxidant Power. Electron J Environ. Agric. Food Chem. 7(8): 3210-3216.

Hosseinzadeh, S, Jafarikukhdan, A, Hosseini, A, Armand, R (2015) The application of medicinal plants in traditional and modern medicine: A review of *Thymus vulgaris.* Int. J. Clin. Med. 6: 635-642.

Hussain, MA, Gorsi, MS (2004) Antimicrobial activity of *Nerium oleander* Linn. Asian J. Plant Sci. 3(2): 177-180.

I**bañez, E, Kubátová, A, Señoráns, J, Cavero, S, Reglero, G, Hawthorne, SB (2003)** Subcritical water extraction of antioxidant compounds from rosemary plants. J. Agric. Food Chem. 51(2): 375–382

.

Ivanovic, J, Misic, D, Zizovic, I, Ristic, M (2012) In vitro control of multiplication of some food-associated bacteria by thyme, rosemary and sage isolates. Food Control 25: 110–116.

Jamal, AHM, Rahman, S, Islam, A, Karim, R, Alam, S, Rahman, Z (2012) Minimum inhibitory concentration analysis of *Nerium oleander* against bacterial pathogens. Asian Pac. J. Trop. Biomed. 2(3): S1664-S1666. https://doi.org/10.1016/S2221-1691(12)60473-0.

Khorshidi, J, Mohammadi, R, Fakhr, MT, Nourbakhsh, H (2009) Influence of drying methods, extraction time, and organ type on essential oil content of rosemary *(Rosmarinus officinalis L.).* Nature and Science 7(11): 42-44.

Mark, M, Jenny, C, Keith, W, Paul, D (2003) Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. Int. J. Neurosci. 113 (1): 15–38.

Mena, P, Cirlini, M, Tassotti, M, Herrlinger, KA, Dall'Asta, C, Del Rio, D (2016) Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis* L.) extract. Molecules 21(11): E1576. doi:10.3390/molecules21111576.

Mohadjerani, M (2012) Antioxidant activity and total phenolic content of *Nerium oleander* L. grown in North of Iran. Iran. J. Pharm. Res. 11(4): 1121-1126.

Moreno, S, Scheyer, T, Romano, CS, Vojnov, AA (2006) Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radic. Res. 40: 223–231.

Naili, MB, Alghazeer, RO, Saleh, NA, Al-Najjar, AY (2010) Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (Astraceae) and *Ziziphus lotus* (Rhamnacea). Arab. J. Chem. 3: 79-84.

Pintore, G, Usai, M, Bradesi, P, Juliano, C, Boatto, G, Tomi, F, Chessa, M, Cerri, R, Casanova, J (2002) Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oil from Sardinia and Corsica. Flav. Fragr. J. 17: 15-19.

Quispe Candori, S, Foglio, MA, Rosa, PTV, Meireles, MAA (2008) Obtaining β-caryophyllene from *Cordia verbenacea* de Candolle by super crtitical fluid extraction. J. Supercrit. Fluids 46: 27-32.

Rsaissi, N, Kamili, EL, Bencharki, B, Hillali, L, Bouhache, M (2013) Antimicrobial activity of fruits extracts of the wild Jujube *Ziziphus lotus* (L.) Desf. J. Sci. Eng. Res. 4(9): 1521-1528.

Siddiqui, BS, Sultana, R, Begum, S, Zia, A, Suria, A (1997) Cardenolides from the methanolic extract of *Nerium oleander* leaves possessing central nervous system depressant activity in mice. J. Nat. Prod. 60(6): 540-544.

Sinha, SN, Biswas, K (2016) A concise review on *Nerium oleander* L. An important medicinal plant. Trop. plant res. 3(2): 408-412.

Soto-Blanco, B, Fontenele-Neto, JD, Silva, DM, Reis, PF, Nóbrega, JE (2006) Acute cattle intoxication from *Nerium oleander* pods. Trop. Anim. Health Prod. 38(6): 451-454.

Tannu. G, Gupta, AK, Kumar, S, Singh, K (2011) Antimicrobial activity of *Nerium oleander* Steam extract. IJPPR 2:210-211.

Tirumalasetty, J, Basavaraju, A, Praveena (2014) Antimicrobial activity of methanolic extracts of *Azadirachta indica, Rosmarinus officinalis* and *Lagenaria siceraria* leaves on some important pathogenic organisms. J. Chem. Pharm. Res. 6(4): 766-770.

Vegara, S, Funes, L, Martí, N, Saura, D, Micol, V, Valero, M (2011) Bactericidal activities against pathogenic bacteria by selected constituents of plant extracts in carrot broth. Food Chem. 128: 872–877.

Yu, M, Wong, A, Soa, K, Fang, J, Yuen, W, Chang, R (2007) New polysaccharide from *Nerium indicum* protects neurons via stress kinase signaling pathway. Brain research 153: 221-230.

Zhang, L, Xu, SG, Liang, W, Mei, J, Di, YY, Lan, HH, Yang, Y, Wang, WW, Luo, YY, Wang, HZ (2015) Antibacterial activity and mode of action of *Mentha arvensis* ethanol extract against multidrugresistant *Acinetobacter baumannii*. Trop. J. Pharm. Res. 14: 2099–2106.

Zibbu, G, Batra, A (2010) A review on chemistry and pharmacological activity of *Nerium oleander* L. J. Chem. Phar