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Antibacterial effect of the methanol extract of *Salix babylonica* against important bacteria in public Health

Efecto antibacteriano del extracto metanólico de *Salix babylonica* sobre bacterias de importancia en salud pública

González-Alamilla Eddy[1], Rivas-Jacobo Marco[1], Sosa-Gutiérrez Carolina[2], Delgadillo-Ruiz Lucía[3], Valladares-Carranza Benjamín[4], Rosenfeld-Miranda Carla[5], Zaragoza-Bastida Adrián[2*], Rivero-Pérez Nallely[2*]

[1] Autonomous University of San Luis Potosí, Faculty of Agronomy and Veterinary Medicine. [2]Autonomous University of Hidalgo State, Institute of Agricultural Sciences, Academic Area of Veterinary Medicine and Zootechnics. [3]Academic Unit of Biological Sciences, Autonomous University of Zacatecas. Zacatecas, Mexico [4] Autonomous University of Mexico State, Faculty of Veterinary Medicine and Zootechnics, [5] Faculty of Veterinary Sciences, Universidad Austral de Chile, Isla Teja s/n, Casilla 567, Valdivia, Chile * Responsible and correspondence author: Adrián Zaragoza-Bastida, Nallely Rivero -Peace. University ranch Av. Universidad km. 1, Ex Hacienda de Aquetzalpa, PO Box No. 32, Tulancingo de Bravo, Hidalgo, Mexico. eddynglez24@gmail.com, marco.rivas@uaslp.mx, carolina_sosa@uaeh.edu.mx, delgadillolucia@gmail.com, crosenfe@uach.cl, adrian_zaragoza@uaeh.edu.mx, nallely_rivero@uaeh.edu.mx.

RESUMEN

El uso excesivo de antimicrobianos ha generado resistencia de los microorganismos a estos, se han buscado alternativas que sean eficaces para el tratamiento de enfermedades producidas por microorganismos resistentes o multirresistentes a antibióticos, dentro de estas alternativas están las plantas, las cuales por su contenido de compuesto secundarios presentan actividad antibacteriana. El objetivo del presente estudio fue caracterizar y determinar la actividad antibacteriana del extracto metanólico de *Salix babylonica* (SB) sobre bacterias de importancia en salud pública. Para la obtención del extracto se utilizó la técnica de maceración, se realizó una caracterización química cualitativa y cuantitativa por cromatografía de gases. Para determinar la actividad antibacteriana, se determinó la Concentración Mínima Inhibitoria (CMI) y la Concentración Mínima Bactericida (CMB) y la caracterización del extracto permitió identificar compuestos fenólicos, cumarinas, lactonas, flavonoles, quinonas, saponinas, triterpenos y compuestos esteroidales, además de Timol (0.5319 mg/mL) y Carvacrol (0.4158 mg/ml). Con respecto a la actividad antibacteriana la mejor actividad se presentó contra *Bacillus. subtillis (*CMI: 12.5 mg/mL y CMB: 25 mg/mL*), Listeria. monocytogenes* y *Staphylococcus. aureus (*CMI: 25 mg/mL y CMB: 50 mg/mL*).* Se concluye que el extracto metanólico de SB puede ser una alternativa para el tratamiento de enfermedades producidas por bacterias resistentes o multirresistentes a antibióticos.

Palabras clave: *Salix babylonica*, caracterización, efecto antibacteriano.

ABSTRACT

The excessive use of antibiotics, has generated resistance of microorganisms to these, have been searched effectives alternatives for treating diseases caused by resistant or multiresistant microorganism, and within of these alternatives are plants, which by its content of secondary compounds have antibacterial activity. The aim on the present experiment was characterize and determine the antibacterial activity of methanolic extract of *Salix babylonica* (SB) against important bacteria in public health. To obtain extract, the maceration technique was used, qualitative and quantitative (gas chromatography) chemical characterization was carried. For antibacterial activity, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was determined, the characterization of the extract allowed the identification of phenolic compounds, coumarins, lactones, flavonols, quinones, saponins, triterpenes and steroidal

compounds, also Thymol (0.5319 mg/mL) and Carvacrol (0.4158 mg/mL). The extract showed the best activity against *Bacillus. subtilis* (MIC: 12.5 mg/mL and WBC: 25 mg/mL), *Listeria. monocytogenes* and *Staphylococcus. aureus* (MIC: 25 mg/mL and MBC: 50 mg/mL). It is concluded that the methanolic extract of SB can be an alternative for the treatment of diseases produced by resistant or multiresistant bacteria to antibiotics.
Keywords: *Salix babylonica***,** characterization, antibacterial effect.

INTRODUCTION

Infectious diseases caused by microorganisms have been one of the most important causes of death in humanity (Lozano *et al*., 2012). Bacterial agents including *Staphylococcus aureus, Escherichia coli, Pseudonomas aeruginosa, Bacillus subtilis and Proteus vulgaris* have caused important infectious diseases within public health (Khan *et al.*, 2013).

The introduction of antimicrobial agents in medicine has been one of the most important interventions to control and reduce the prevalence of infectious diseases (Alós, 2015); However, a growing threat in recent years that has diminished the effectiveness of these drugs is bacterial resistance to antibiotics; generated because the microorganisms have acquired the ability to prevent an antimicrobial from acting against it. As a result, the treatments of choice become ineffective, infections persist and can spread to other individuals (WHO, 2017).

In most populations of different developing countries, humanity has used plants to treat common infectious diseases, which could be a potential alternative to produce new drugs of great health benefit (Renisheya *et al.*, 2011; Khan *et al.*, 2013).

One of the plants considered important for the study of its photochemical properties is *Salix babylonica*, commonly known as weeping willow. This species belongs to the Salix genus of the Salicaceae family, *Salix babylonica* is one of the best known species in willows, distributed in some areas of Asia, Europe and America; commonly used as an ornamental and medicinal plant (Wahab *et al*., 2018).

There are reports in which the pharmacological properties associated with the evaluation of leaf, bark and stem extracts are evidenced; obtained from *Salix babylonica*. Among the phytochemical properties attributable to *Salix babylonica*, there are: anthelmintic, antiseptic, antiarthritic, astringent, analgesic, anticancer, antipyretic, antimalarial, antioxidant, antifungal, anthelmintic and antibacterial activity; these properties are associated with its content of secondary compounds such as total phenolics, flavonoids, terpenes and lignans (Sulaiman *et al*., 2013; Wahab *et al*., 2018).

Based on the aforementioned approaches, the objective of the present research work was to characterize and determine the antibacterial activity of the methanolic extract of *Salix babylonica*, on bacteria of importance in public health.

MATERIAL AND METHODS

Obtaining the extract

To obtain the extract, approximately 1 kg of *Salix babylonica* plant material was collected in different phenological stages, these were collected in the municipality of Tulancingo, Hidalgo. The aerial part collected from *Salix babylonica* was dried in shade at room temperature, after drying it was crushed and the maceration technique was performed. 250 g of the dry material was macerated in 1000 ml of methanol for 48 hours at room temperature and in the absence of light. The liquid extract from the maceration was obtained by filtration with filter paper (Whatman® 42) and cotton. The liquid extract obtained was concentrated under reduced pressure in a rotary evaporator, in order to remove the solvents and concentrate the secondary metabolites, according to the methodology described by Rivero *et al*., 2016.

Chemical characterization of *Salix babylonica* methanolic extract

Qualitative chemical profile: The chemical profile was carried out according to the procedure described by Bañuelos-Valenzuela *et al*., 2018, for the determination of instalations, phenolics, sterols, triterpenes, coumarins, sesquiterpenlactones, flavonoids, alkaloids, tanids, fluorataninos, steroids and saponins.

Gas chromatography: The chemical composition was determined by gas chromatography (CG; Agilent Technologies series 6890N manufactured in U.S.A), with a DB_WAXetr polar column, at 250 °C and 12.13 psi with a flow of He 36.5 ml min^{-1} after injection. The conditions for the column were: initial temperature 50 °C from zero to two min, increasing from 10 in 10 °C until reaching 250 °C, keeping the temperature constant for 5 min and then descending to 50 °C for two min with a flow of He of 1.6 ml min^{-1} at a pressure of 12.13 psi and an average speed of 25 cm s^{-1}, using an ionizing flame detector (FID), at a temperature of 210 °C with a flow of H2 of 40 ml min^{-1} and an air flow of 450 ml min^{-1}. The standards (Sigma-Aldrich), were used in different concentrations (Table 1).

Antibacterial activity

To determine the antibacterial activity of the methanol extract of *Salíx babylonica*, the following methods were used: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), following the CLSI specifications (CLSI, 2012).

Table 1. Concentrations of standards used for the chemical determination of *Salix babylonica* methanolic extract by gas chromatography.

Standard	Compound mg/mL^{-1}				
	Timol	Carvacrol	Linalool	Terpinene	Limonene
1	10.373	8.284	7.744	7.154	8.496
2	5.186	4.142	3.872	3.577	4.248
3	2.593	2.071	1.936	1.789	2.124
4	1.297	1.035	0.968	0.894	1.062
5	0.648	0.518	0.484	0.447	0.531
6	0.324	0.259	0.242	0.224	0.265

The antimicrobial activity test was carried out with the ATCC 6538 strains of *Staphylococcus aureus*, 6633 of *Bacillus subtilis*, 35218 of *Escherichia coli*, 9027 of *Pseudomona aeruginosa*, 14028 of Salmonella typhi, 10708 of *Salmonella cholerasuis* and 19113 of *Listeria monocyto*. A colony of each bacterium was inoculated in nutrient broth (BD Bioxon), which was incubated under constant agitation (70 rpm) for 24 hours at 37 °C. After the incubation time, the inoculum was adjusted with nutrient broth to 0.5 of the Mc Farland turbidity pattern, which corresponds to 150 x 106 cell/ml.

For the determination of the MIC, the plate microdilution method was used, using concentrations of 400, 200, 100, 50, 25, 12.50, 6.25, 3.12 mg/ml, of the methanolic extract of *Salix babylonica*. Each concentration was prepared with nutrient broth (BD Bioxon). The procedure was performed in triplicate in 96-well plates, placing 100 µl of each of the dilutions of the extract plus 10 µl of the bacterial suspension, previously adjusted to 0.5 McFarland. Once the inoculation was performed, the plate was incubated at 37 °C for 24 hours at 70 rpm under constant agitation, the positive control was Kanamycin (AppliChem 4K10421) at concentrations of 64, 32, 16, 8.0, 4.0, 2.0, 1.0 and 0.5 µg/ml and the negative control was nutritious broth.

To determine the MIC, a colorimetric method was used, based on the use of tetrazolium salts (Balouiri *et al*., 2016). After the incubation time elapsed, 20 µl of a 0.04% (w / v) solution of p-iodonitrotetrazolium was added to each well; it was incubated for 30 minutes at 37 ° C and the reading was carried out, determining as the minimum inhibitory concentration, the concentration at which the solution turns pink (Kaewpiboon *et al*., 2012; Mothana, *et al*., 2009).) .

To determine the MBC, after adding p-iodonitrotetrazolium, 5 µl of each well was inoculated into Mueller Hinton agar, then incubated at 37 ° C for 24 hours. After the incubation time, the reading was carried out to determine the minimum bactericidal concentration of the extract, that is, the concentration at which no bacterial growth was observed in the plate.

RESULTS

The qualitative characterization carried out on the methanolic extract of *Salix babylonica*, indicates the presence of unsaturations, phenolic oxidriles, coumarins, lactones, flavonols, quinones, saponins, aromaticity and polyphenols; In addition to being positive to the Lieberman-Buchard test, which indicates the presence of triterpenes and steroidal compounds. (Table 2).

Table 2. Qualitative tests of the chemical profile of the methanolic extract of *Salix babylonica*.

Test	Result
Unsaturation	+
Phenolic Oxidriles	+
Coumarins	+
Lactones	+
Salkowski	-
Flavonols	+
Flavones	-
chalcones	-
Quinones	+
Shinoda	-
Sesquiterpenlactones	-
Agitation	+
Bicarbonate	-
Saponins	+
Aromaticity	+
Triterpenes	+
Tannins	-
Floratannins	+
Steroids	+

Chemical composition

The analysis on the gas chromatograph was 20 min with a retention time for terpinen of 6.40 min, limonene 6.66 min, linalool 11.28 min, thymol 18.04 min and carvacrol 18.37 min. To calculate the concentration of the samples, we worked with five standards with six concentrations each (table 1).

Once the calibration curves were carried out and the equations were taken, it was determined that *Salix babylonica* methanolic extract contains Timol and Carvacrol in concentrations of 0.5319 mg / ml, 0.4158 mg/ml respectively. Table 3

Table 3. Chemical composition of *Salix babylonica* methanolic extract

Standard/ Extract	Compuesto mg/mL				
	Terpinene	Limonene	Linalool	Timol	Carvacrol
1	10.373	8.284	7.744	7.154	8.496
2	5.186	4.142	3.872	3.577	4.248
3	2.593	2.071	1.936	1.789	2.124
4	1.297	1.035	0.968	0.894	1.062
5	0.648	0.518	0.484	0.447	0.531
6	0.324	0.259	0.242	0.224	0.265
Salix babylonica	**0**	**0**	**0**	**0.5319**	**0.4158**

Antibacterial activity

Minimum inhibitory concentration

The minimum inhibitory concentration of *Sálix babylonica* methanolic extract was 100 mg/ml for *Escherichia coli*, *Salmonella typhi*, *Salmonella cholerasuis* and *Pseudomonas aeruginosa*; 25 mg/ml for *Listeria monocytogenes* and *Staphylococcus aureus*. The lowest concentration at which the extract had activity was 12.5 mg/ml, compared to *Bacillus subtillis* (Table 4).

Table 4. Minimum Inhibitory Concentration of *Salix Babylonica* Methanolic Extract

Bacterium	**Concentrations (mg/mL)**							
	400	**200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.12**
Escherichia coli	-	-	MIC	+	+	+	+	+
Salmonella typhi	-	-	MIC	+	+	+	+	+
Salmonella cholerasuis	-	-	MIC	+	+	+	+	+
Pseudomonas aeruginosa	-	-	MIC	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	MIC	+	+	+
Listeria monocytogenes	-	-	-	-	MIC	+	+	+
Bacillus subtillis	-	-	-	-	-	MIC	+	+

(-)No color change, (+) Change of color

Minimum bactericidal concentration

The minimum bactericidal concentration of the methanolic extract of *Sálix babylonica* was determined to be 200 mg/ml; for *Escherichia coli, Salmonella typhi*, *Salmonella cholerasuis* and *Pseudomonas aeruginosa*, 50 mg/ml; and for *Listeria monocytogenes* and *Staphylococcus aureus* and 25 mg/ml for *Bacillus subtillis* (Table 5).

Table 5. Minimum Bactericidal Concentration of *Salix Babylonica* Methanolic Extract

Bacterium	Concentrations (mg/mL)							
	400	200	100	50	25	12.5	6.25	3.12
Escherichia coli	-	MBC	+	+	+	+	+	+
Salmonella typhi	-	MBC	+	+	+	+	+	+
Salmonella cholerasuis	-	MBC	+	+	+	+	+	+
Pseudomonas aeruginosa	-	MBC	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	MBC	+	+	+	+
Listeria monocytogenes	-	-	-	MBC	+	+	+	+
Bacillus subtillis	-	-	-	-	MBC	-	-	-

(-) No growth , (+) Growth

DISCUSSION

The presence of unsaturations, phenolic oxydriles, coumarins, lactones, flavonols, quinones, fluoratanинos, triterpene steroids and saponins in the metonolic extract of Salix babylonica was determined by means of qualitative tests. In previous studies, compounds such as tritetracontane, 1,2,3-propanetriol ester, octadecanoic acid, methyl ester of hexadecanoic acid and 1,3-dioxane-4- (hexadecyl oxy) -2-pentadecyl have been identified; most of them classified as phenolic compounds, in addition to 7-O-β-D-glucopyranoside of luteolin, luteolin and crisoeriol; compounds classified as flavonoids (Salem *et al*., 2011).

Biological activities such as anticancer, antiulcer, antimalarial, antidiarrheal, antifungal, antitussive, anti-inflammatory, anthelmintic and antibacterial have been reported; in studies conducted with phenolic compounds, alkaloids, glycosides and terpenes (Hernández-Alvarado *et al*., 2018).

On the other hand, gas chromatography allowed to identify Timol and Carvacrol at concentrations of 0.5319 mg/ml, 0.4158 mg/ml respectively. These compounds are classified as essential oils of volatile nature, with some biological activities reported as: expectorant, antifungal, anti-inflammatory, analgesic, antiseptic, antioxidant, anti-rheumatic, antispasmodic, anti-hepatotoxic and antibacterial; both against Gram positive and Gram negative bacteria (*Magi et al.*, 2015).

When carrying out the antibacterial evaluation of the methanolic extract of *Salix babylonica*, it was determined that the extract exhibits better activity against Gram positive bacteria (*Staphylococcus aureus, Listeria monocytogenes and Bacillus subtilis*); which against Gram negative bacteria (*Escherichia coli, Salmonella typhi, Salmonella cholerasuis, Pseudomonas aeruginosa*). This effect is based on the structure of Gram negative bacteria, which by having a phospholipid membrane that prevents the cell wall from being penetrated by lipophilic solutes; while the porins constitute a selective barrier

for hydrophilic solutes, so the bacteria are protected from being penetrated by compounds such as antibiotics or some secondary metabolites derived from plants (Kaye *et al*., 2004; Ndhlala *et al*., 2015).

For the determination of antibacterial activity, it is important to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC); defined as MIC at the lowest concentration of antimicrobial agent that inhibits the growth of the microorganism, detected visually (CLSI, 2012). In the present experiment to determine the end point of the WCC, a colorimetric method was used, based on the use of tetrazolium salts (Balouiri *et al*., 2016); which allows to observe a color change from yellow to pink, caused by the entry of this salt into the cell, which is reduced by the NAD (P)H-dependent oxidoreductases and the dehydrogenases of metabolically active cells, producing the change from color to pink (Berridge *et al.*, 2005).

Sulaiman *et al*., in 2013, carried out a study in which they evaluated the antimicrobial activity of the ethanol extract of *Sálix alba* bark, belonging to the genus sálix and family salicaeae; same as *Sálix babylonica*. In this study they determined that *Sálix alba* has better antibacterial activity against *Staphylococcus aureus*; medium activity against *Pseudomonas aeruginosa* and had no effect against *Escherichia coli* and *Klebsiella pneumoniae*. The concentrations evaluated were 10, 20, 40, 60 and 80 mg/ml, using the agar diffusion technique; observing the greatest halos of inhibition at 80 mg/ml. The results of this study correspond to those observed in the present experiment, since the extract had a better effect against Gram positive bacteria (*Staphylococcus aureus*, 25 mg/ml; *Listeria monocytogenes*, 25 mg/ml and *Bacillus subtilis*, 12.5 mg/ml), which against Gram negative bacteria (*Escherichia coli, Salmonella typhi, Salmonella cholerasuis* and *Pseudomonas aeruginosa*, 100mg/ml, for each one); however, the extract was obtained from the bark of *Sálix alba*. The concentrations change and the technique also, so the results are not 100 % comparable, although the trees belong to the same genus and family.

On the other hand, in a study conducted by Wahab and colaboradores en 2018, who evaluated the methanolic extracts of the leaves and bark of *Sálix babylonica*; in addition to its fractions of petroleum ether, methylene chloride and ethyl acetate (diluted in dimethylsulfoxide), to determine its antimicrobial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (E*scherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria, using the agar diffusion technique and the extract at a concentration of 100 µg. The results showed that both the methanolic extract of the leaves and the bark have moderate or weak antimicrobial activity against the challenged microorganisms; observing the greatest halos of inhibition (10 mm) with *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae* (9 mm), finally *Escherichia coli, Staphylococcus aureus* (8 mm).

Because in the mentioned studies only techniques are applied to determine the sensitivity of the microorganism to a certain compound by the agar diffusion method; It is not possible to compare the results with those obtained in the present experiment, since in this case the minimum inhibitory concentration was determined by the plate microdilution method; In addition to the study by Wahab *et al*., dimethyl sulfoxide was used to dilute extracts and fractions; compound that is used to increase the permeability of the bacterial membrane, increasing the activity of the compounds and reducing the concentrations of use (Borges *et al*., 2013; Sulaiman *et al*., 2013; Wahab *et al*., 2018).

The Minimum Inhibitory Concentration is defined as the lowest concentration of antimicrobial agent necessary to kill 99.9% of the final inoculum, after incubation for 24 hours under a standardized set of conditions described by the CLSI (Balouiri *et al*., 2016). The determination of the MIC is not a viable option to know 100 % the efficacy of a drug or compound, since within each well there may still be viable cells if the drug evaluated only had a bacteriostatic effect on the bacterial species under study (Wiegand *et al*., 2008).

In the present experiment, the MBCs of the methanolic extract were determined against *Escherichia coli, Salmonella typhi, Salmonella cholerasuis* and *Pseudomonas aeruginosa* (200 mg/ml); *Listeria monocytogenes* and *Staphylococcus aureus* (50 mg/ml) and *Bacillus subtillis* (25 mg/ml); however, there are no studies reported with *Sálix babylonica* or another species of the genus *Sálix*, with which this activity has been reported.

CONCLUSION

In the present study it was demonstrated that the methanolic extract of *Sálix babylonica* has potential antibacterial activity on some bacterial pathogens of importance in public health; being an alternative for the treatment of diseases caused by bacteria resistant or multi-resistant to antibiotics.

CITED LITERATURE

ALÓS JI. 2014. Resistencia bacteriana a los antibóticos: una crisis global. *Enfermedades Infecciosas y Microbiologias Clinica*. 33(10):692–699. http://dx.doi.org/10.1016/j.eimc.2014.10.004

BALOUIRI M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis.* 6(2):71-79. https://doi.org/10.1016/j.jpha.2015.11.005.

BAÑUELOS-VALENZUELA R, Delgadillo L, Chairez F, Delgadillo O, Meza-López C. 2018. Composición química y FTIR de extractos etanólicos de *Larrea tridentata, Origanum vulgare, Artemisa ludoviciana y Ruta graveolens* Agrociencia. 52(3): 309-321. https://dialnet.unirioja.es/servlet/articulo?codigo=6423180.

BERRIDGE MV, Herst PM, Tan AS. 2005. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnology Annual Review.* 11:127-152. https://doi.org/10.1016/s1387-2656(05)11004-7.

BORGES A, Ferreira C, Saavedra MJ, Simoes, M. 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance*. 19(4): 256-265. https://doi.org/10.1089/mdr.2012.0244.

CLSI (Clinical and Laboratory Standards Institute). 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition. Pp. 88. USA.

HERNÁNDEZ-ALVARADO J, Zaragoza-Bastida A, López-Rodríguez G, Peláez-Acero A, Olmedo-Juárez A, Rivero-Perez N. 2018. Actividad antibacteriana y sobre nematodos gastrointestinales de metabolitos secundarios vegetales: enfoque en Medicina Veterinaria. *Abanico Veterinario*. 8(1):14-27. http://dx.doi.org/10.21929/abavet2018.81.1.

KAEWPIBOON C, Lirdprapamongkol K, Srisomsap C, Winayanuwattikun P, Yongvanich T, Puwaprisirisan P, Svasti J, Assavalapsakul W. 2012. Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. *BMC Complementary and Alternative Medicine*. 12(1):217. https://doi.org/10.1186/1472-6882-12-217.

KAYE KS, Engemann JJ, Fraimow HS, Abrutyn E. 2004. Pathogens resistant to antimicrobial agents: epidemiology, molecular mechanisms, and clinical management. *Infectious disease clinics of North America.* 18(3):467-511. https://doi.org/10.1016/j.idc.2004.04.003.

KHAN UA, Rahman H, Niaz Z, Qasim M, Khan J, Tayyaba, Rehman B. 2013. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology and Immunology.* 3(4): 272–274. . https://doi.org/10.1556/EuJMI.3.2013.4.6

LOZANO R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al*., 2012.Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. 2012. *The Lancet.* 380(9859):2095-2128. https://doi.org/10.1016/S0140-6736(12)61728-0

MAGI G, Marini E, Facinelli B. 2015. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. *Frontiers in Microbiology*. 6:165. https://doi.org/10.3389/fmicb.2015.00165.

MOTHANA RA, Lindequist U, Gruenert R, Bednarski PJ. 2009. Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants

from the island Soqotra. *BMC Complementary and Alternative Medicine*. 9: 7. https://doi.org/10.1186/1472-6882-9-7.

NDHLALA AR, Ghebrehiwot HM, Ncube B, Aremu AOJ, Gruz M, Subrtova J, Van Staden A. 2015. Antimicrobial, anthelmintic activities and characterization of functional phenolic acids of Achyranthes aspera linn, a medicinal plant used for the treatment of wounds and ringworm in east Africa. *Frontiers in Pharmacology*. 6:274. https://doi.org/10.3389/fphar.2015.00274.

RENISHEYA JJMT, Johnson M, Mary UM, Arthy A. 2011. Antibacterial activity of ethanolic extracts of selected medicinal plants against human pathogens. *Asian Pacific Journal of Tropical Biomedicine*.1(1):S76-S78. https://doi.org/10.1016/S2221-1691(11)60128-7.

RIVERO-PEREZ N, Ayala-Martínez M, Zepeda-Bastida A, Meneses-Mayo M, Ojeda-Ramírez D. 2016. Anti-inflammatory effect of aqueous extracts of spent Pleurotus ostreatus substrates in mouse ears treated with 12-O-tetradecanoylphorbol-13-acetate. *Indian Journal of Pharmacology*. 48(2):141-144. https://dx.doi.org/10.4103%2F0253-7613.178826.

SALEM AFZ, Salem MZ, González-Ronquillo M, Camacho LM, Cipriano M. 2011. Major chemical constituents of *Leucaena leucocephala* and *Salix babylonica* leaf extracts. *Journal of Tropical Agriculture*. 49: 95-98. http://jtropag.kau.in/index.php/ojs2/article/view/244

SULAIMAN GM, Hussien NN, Marzoog TR, Awad, HA. 2013. Phenolic content, antioxidant, antimicrobial and cytotoxic activities of ethanolic extract of Salix alba. *American Journal of Biochemistry and Biotechnology*. 9(1): 41-46. https://thescipub.com/PDF/ajbbsp.2013.41.46.pdf.

WAHAB GA, Sallam A, Elgaml A, Lahhloub M, Afifi MS. 2018. Antioxidant and antimicrobial activities of Salix babylonica extracts. *World Journal of Pharmaceutical Sciences*. 6(4): 1-6. http://www.wjpsonline.org/.

WHO (World Health Organization). 2017. *Antimicrobial resistance*. https://www.who.int/antimicrobial-resistance/en/

WIEGAND I, Hilpert K, Hancock REW. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*. 3(2):163-175. https://doi.org/10.1038/nprot.2007.521.