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SCREENING OF CRUDE ETHANOLIC EXTRACTS OF NINE LIBYAN MEDICINAL PLANTS FOR ANTIBACTERIAL ACTIVITY AGAINST*Staphylococcus aureus*AND*Escherichia coli*

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ABSTRACT

The purpose of this study was to determine the effects of different concentrations of crude ethanolic extracts on two pathogenic bacterial strains. The various concentration of 10%, 5%, and 1% of crude ethanolic extracts of 9 plants belonging to diverse families was estimated for antibacterial activity against Staphylococcus aureus and Escherichia coli. The dried and ground plant materials were extracted in absolute ethanol 99.9% by cold-soaking for 48 h. The in vitro antibacterial activity was done by disc diffusion Assay (DDA). The zone of inhibition for the tested microbial strains was measured using the disc diffusion method. Imipenem (Ipm 10) was used as the positive control, while DMSO was the negative control. Among 9 medicinal plants used in this study, only (7) ethanolic extracts {H. stoechas L., M.parviflora (L.) Demonstr, S. arenariaForskal, P. rupestre L. M. longipetalaL., E. microcarpa and R. alba L.} Showed the range of inhibition zone between 7.66±0.57 to14.67±0.56 mm. However, the crude ethanolic extract of L.resedifolia (L.) O. Kuntze, and B. tournefortiiGouan presented no activity at all against pathogenic bacteria. Antibacterial susceptibility exposed that the most active antibiotics were ciprofloxacin, chloramphenicol, imipenem, and ticarcillin against S. aureus; ciprofloxacin, chloramphenicol, imipenem, and polymyxin against E. coli.

Keywords: Antibacterial activity, ethanol extracts, Staphylococcus aureus, Escherichia coli.

الملخص

الهدف من هذه الدراسة هو دراسة تأثير تراكيز مختلفة من مستخلصات 9 نباتات طبية علي نوعين من البكتيريا الممرضة للإنسان، هذه التراكيز هي 1% ، 5%، 10%. وتم تجفيف وطحن النباتات واستخلاصها بالايثانول بطريقة النقع عالبارد لمدة 48 ساعة. وقد تم اختبار الفاعلية التثبيطية بواسطة الانتشار بالقرص . هذا وقد تم قياس منطقة التثبيط بالمسطرة. وتم استخدام المضاد الحيوي اميبينيم كاشاهد موجب و بينما استخدم داي كلوروميثيل سلفوكسويد كاشاهد سالب في التجربة . وقد أظهرت النتائج ان 7 من 9 نباتات طبية أظهرت تاثير كبير عند معدل تثبيط يتراوح بين **7.66±0.57**الي **14.67±0.56**مم .بينما لم يلاحظ أي تاثير لكلا من نباتي **L. resedifolia (L.) O. Kuntze, and B. tournefortiiGouan**علي بكتيريا قيد الدراسة.

اما بالنسبة لاختبارالمضادات الحيوية علي العزلات البكتيرية فانه لوحظ ان اكثر المضادات فعالية علي **S. aureus** كانت كلورامفينيكول ، اميبينيم ، سيبروفلكساسين. كما وجد ان كلا من سيبروفلوكساسين و كلورامفينيكول وبولي ميكسين اكثر تاثيرا علي **E. coli**.

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الكلمات المفتاحية:نشاط ضد بكتيري، مستخلص الايثانول، *E. coli*، *S. aureus*.

1. INTRODUCTION

Introduction Antibacterial agents extracted from medicinal plants are, easily available, cheap, most essentially, and very rarely have side effects, and the extracts are active on many pathogenic bacteria (Durairaj S., 2009). Although several traditional medicinal plants are used for the treatment of many infectious diseases, injuries, and inflammations in several parts of Libya (Al-Kathe & Al-Ramah, 1997), there are only limited reports on their antibacterial activities (Hussein Ayoub, 1989, 1990; Hussain &Tobji, 1997). Therefore, it is important to carry out an antibacterial screening of these plants to confirm their ethnopharmacological use and to identify their active components.

Staphylococcus aureus causes respiratory and skin infections along with Escherichia coli causing gastrointestinal, urogenital diseases and wound contamination are resistant to almost all of the older antibiotics (Elisha, 2017). For a long time, the problems that occurred to the human body because of antibiotic resistance are increasing. Therefore, the development of alternative drugs to treat such infectious diseases is urgently needed.

In this study, we report the results of in vitro antibacterial assays performed on ethanolic extracts obtained from different parts of nine plant species (*Reseda alba, Scabiosa arenaria, Erucariamicrocarpa, Phagnalonrupestre, Malva parviflora, Matthiolalongipetala, Helichrysum stoechas, Launaearesedifolia, and Brassica tourneffortii*) that are generally used in Libyan folk medicine for treating illnesses likely to be caused by two Gram-positive, and two Gram-negative bacteria strain isolated.

2.METHODOLOGY

2.1 Materials

Analytical grade solvents ethanol absolute 99.9 % was purchased from Nur Al Elmia Company, Tripoli, Libya. The solvent was used in the extraction processes. Deionized water was supplied by the Alqamoudi factory, Tajora, Tripoli, Libya. Media used for the antibacterial activities included: Mueller–Hinton broth (MHB), Mueller–Hinton agar (MHA), and nutrient agar (NA), which were provided by Oxoid Ltd. (Basingstoke, UK). Hydrogen peroxide (H_2O_2) supplied by Alfarapy company for drugs, Tripoli, Libya. Dimethyl sulfoxide (DMSO) 100%; Fisher Scientific, Leicestershire, United Kingdom).

2.2 Plant Material

The plant species were chosen based on their traditional uses described in available literature (Boulos, 198311; Al-Yahya et al., 198410; Al-Kathe, 19952; Al-Kathe & Al-Ramah, 19974; Al-Kathe & Al-Maghrebi, 19993; Hamed, 200723 E.A. Sobhy and S.S. El-Feky, 2007 17; Akrout Ahmed et al 2010 26); (Ibrahim. et al 201627); Góngora L, 2002 21); Louhaichi, 201122); Malek BesbesHlila, 201318); Ashraf A, El-Bassuony, 200620); Omara Naser 202016); (Abella, S. R, 201319); (Shabana Marwan, 201325)) Al YaforLubna, 20071);. Table 1 summarizes the ethnobotanical data of the plants selected for the study.

Table 1. Ethnobotanical data on medicinal plants

Botanical name	Family	Local names	Traditional uses	Active constituents	Ref.
H. stoechas (L.) Moench	Asteraceae	Ashbatel -arnab	Urinary infections	Caffeoylquinic acid, dicaffeoylquinic acids, a pigenin glucosides, quercetin and kaempferol.	23, 1
R. alba L.	Resedaceae	Fattolet El-Holi.	Colic, diarrheas and poisonings Stomach disorders	-	(17,26,27)
E. microcarpa. Boiss.	Brassicaceae	-	Anti diabetic, anti bacterial, anti fungal, anti cancer, and anti rheumatic	Chromone, lanosterol, coumarins, quercetin,	28,10, 27,17
*M. longipetala (*L.) Maire.	Brassicaceae	Chgara	Anti-inflammatory,	-	21,22
P. rupestre (L.) Dc.	Asteraceae	Rock phagnalon, Qadeeh	used in the past to make deliberate, burns, to treat asthma, an anesthetic for toothache to treat headache	Hydroquinones, luteolin 7-O-beta-glucoside	22,21
*S. arenaria*Forssk,	Dipsacaceae	-	diaphoretic, stomachic, asthma, pneumonia, ulcers influenza,herpes ringworm, scabies	gallic acid, quercetin, catechin	1,18,26
M. parviflora (L.) Demonstr,	Malvaceae	Khobbeiza	Relief teeth pain, laxative, cough, antitussive, colitis, tonsillitis, abscess gastroenteritis,	Malvin, tannin, and mucilage	1,26
L. resedifolia (L.) O. kuntze	Asteraceae	Adeeda	Liver aches. liver, lungs, and stomach, as well as to heal infected wounds. Jaundice, leucorrhoea	Coumarins, luteolin, oleanolic acid. cichoriinI , esculetin II , scopoletin III and isoscopoletin IV	20,1
B. tournefortii Gouan	Brassicaceae	Shultam	Lichen, vitiligo, and gastric ulcer. antioxidant, anti-inflammatory, anti-microbial, anti-allergic,	glucosinolates, polyphenols, carotenoïds, and vitamins.	19,25, 11, 16

The plant materials were collected in March 2021 from different regions of Sabratha city, Libya, during the flowering period. The taxonomic identification of plants was confirmed at the Department of Botany, Faculty of Science, University of Tripoli, Tripoli, Libya. The procured material was air-dried and put in storage at the Laboratory of Microbiology, National Cancer Institute Sabratha, Sabratha, Libya. A powerful blender (Waring, model 32 BL 80, New Hartford, USA) was used to pulverize the dried samples into fine power. The powdered samples were stored in an airtight polyethylene plastic bag and put in storage in a - 80°C fridge**.**

2.3 Plant extraction

The extraction of each sample was done utilizing the soaked method illustrated by Alqadeeri*et al.* (2019) with some modifications. The organic solvent used in the extraction was absolute ethanol (R & M Chemicals, 99.9%). The dried sample was ground to obtain as a powder. Samples extraction have been done once. It was performed using 400 ml room-temperature solvent and 48 hours of conventional shaking. Filtration of the plant's extracts was done using Whatman filter paper size No. 2 (Whatman International Ltd., Middlesex, England). Following this, the extracts were concentrated using a rotary vacuum evaporator at 40°C for 3-4 hours to obtain an ethanol extract of the dried for all samples. The temperature of the rotary evaporator was increased to 85°C for 2 × 30 sec at the end of the extraction process to ensure that the extract is ethanol-free (Madiha*et al*., 2017).

2.4Antibacterial Activity Assay

2.4.1 Sample Preparation

The stock ethanolic extract of nine plants samples was primed by dissolving a crude extract of each sample in 100% dimethyl sulfoxide (DMSO) to obtain a 100 mg/mL concentration (10%). Further dilution of the solution was done using sterile deionized distilled water, ddH2O to produce two different concentrations of 5% (50 mg/mL) and 1% (10 mg/mL) plants samples extract. All the extracts concentration were put in storage at 4 °C up to the time it was ready for use. The final concentration of 10% DMSO used in the present study was not effective in killing the tested microorganisms. (Alqadeeri et al. (2020)).

2.4.2 Bacterial strains and inoculums preparation

Two bacterial species, gram-positive *Staphylococcus aureus*, and Gram-negative *Escherichia coli* (*E. coli*) were used. These microorganisms were acquired and identified using the morphological and biochemical diagnostic tests at the Microbiology Department, Faculty of science, Tripoli University, Tripoli. The bacteria were sub-cultured overnight at 37 °C in nutrient agar.

The stock culture of the bacteria was grown on MHA at 37°C for 12-24 hours (Rukayadi*et al*., 2013). A sterile cotton swab was used to transfer 2-3 colonies of strains to 1 mL of MHB and mixed using a vortex for 15 minutes. The bacteria suspension was then grown at 37°C for 12-24 hours. Ten microliters of the bacteria suspension were transferred into 10 mL of MHB. The turbidity of inoculums was standardized between 10^5 - 10^8 CFU/mL before testing by using the standard broth microdilution method (Rukayadi*et al*., 2013) and inoculum quantification (Indira, 2014), which was carried out by plating 20 µL bacteria suspension on MHA and a count of the visible colonies was made after incubation at 37°C for 12-24 hours (CLSI, 2012).

2.4.3Disc Diffusion Assay (DDA)

The method suggested by the Clinical and Laboratory Standard Institute (CLSI) (2012) was employed to carry out the disc diffusion assay against 2 *E. coli* strains and 2 *S. aureus* strains. The inoculum was prepared and immediately spread on an MHA plate as a single uniform colony with a sterile cotton swab. A sterile self-punched disc paper with a diameter of 6 mm was attached to the inoculated MHA agar. Each paper disc was imbued with 10 mg/mL (1%), 50 mg/mL (5%) and 100 mg/mL (10%) of each extract in

the amount of 20 µL extract. The negative controls were prepared using 10% for DMSO. After a 24- hour incubation of the plates at 37°C, the diameter of the inhibition zone was measured (in millimeters (mm)) and recorded. Analysis was carried out three times in triplicate data (n = 3 × 3). Antibiotics; Imipenem were also used as a positive control.

2.5 Antibiotic Susceptibility assay

A sterile forceps were used to place the antibiotic disks on the surface of the inoculated MHA plate. Each of the disks was carefully placed and slightly pressed on the MHA plates using the forceps. The placement of the disks close to the edges of the plate was avoided to ensure zone measurement errors. The MHA plates were incubated at 37°C for 24 hours. The antibiotics and their potency were presented in Table2.

Table 2: List of the used antibiotics and their potency

Antibiotic	Antibiotic potency (µg)
Aztreonam	**30**
Oxacillin	**1**
Cephalexin	**30**
Ampicillin	**10**
Ticarcillin	**75**
Polymyxin B	**300**
Ceftriaxone	**30**
Imipenem	**10**
Chloramphenicol	**5**
Ciprofloxacin	**5**

2.5.1 Measurement of the zone of inhibition

The zone of inhibition for each of the antibiotics was observed on the incubated MHA plate. The size of zones for each antibiotic was measured carefully in millimeters (mm) using a ruler by observing the back of the petri dish. Each zone size was measured from three angles of the zone to ensure accuracy and was then recorded.

2.6 Statistical Analysis

Excel (v. 2010), and Graph Pad Prism version 6.00 for Windows (v. 6.00, Graph Pad Software, San Diego, CA, USA) were employed to perform the statistical analysis. Results were given as a mean of three replicates ± SD. The significant difference at $p < 0.05$ was established by performing ANOVA.

3. RESULTS

3.1Extraction yield

Dried of nine Libyan samples were extracted using absolute ethanol 96.9% (v/v) and the yields of extracts were presented in Table 2. The yield of herbal extracts is influenced by the types of soaking solvent, the ratio of soaking solvent, type of extraction technique, and soaking period (Sultana et al., 2009; Abdullah et al., 2015). The extracts yield percentages were calculated using the following formula:

Extract yield% = R/S * 100 (where R; the weight of extracted Plants residues and S; the weight of plant raw sample).

Table 3. The total yield of medicinal plants ethanol extracts.

Plant Species	Dried samples (g)	Plant part[a]	Yield (g)	Yield extract (%)
R. alba	50.00	AP	6.56	13.11
S. arenaria	50.00	AP	4.21	8.43
E. microcarpa	50.00	AP	3.71	7.42
P. rupestre	50.00	AP	5.72	11.44
M. parviflora	25.00	S	2.30	9.21

M. longipetala	25.00	AP	4.00	16.00
H. stoechas	25.00	AP	10.85	43.38
L. resedifolia	25.00	AP	6.82	27.23
B. tournefortii	25.00	S	3.79	15.16

[a]AP, aerial parts; S, seeds.

3.2 Disc diffusion assay (DDA)

Disc diffusion assay (DDA) is a preliminary screening for determining the antibacterial activity of selected plants against selected bacteria species. The principle of DDA is that a larger inhibition zone indicates greater antibacterial activity. In this study, DDA was carried out to screen the antibacterial activity of ethanolic extracts of nine Libyan plants against *S. aureus* and *E. coli*. The results for the DDA of the ethanolic extracts are presented in Table 4.

Table 4. Disc diffusion Assay (DDA) of extracts against *S. aureus* and *E. coli.*

Plants species	**E.E.C[a] (mg/mL)**	**Zone of inhibition [d](in mm)**			
		Gram-negative bacteria		**Gram-positive bacteria**	
		***E. coli*[c](iso.1)**	***E. coli* (iso.2)**	***S. aureus* (iso.1)**	***S. aureus* (iso.2)**
R. alba	10	[b]n.a	n.a	n.a	n.a
	50	n.a	n.a	n.a	n.a
	100	11.67±0.53	9.00±0.00	11.00±0.00	9.66±0.67
S. arenaria	10	n.a	n.a	n.a	n.a
	50	8.67±0.57	8.00± 0.00	11.00± 0.00	7.66±0.57
	100	12.00±0.00	9.00±0.00	11.00±0.00	9.67±0.57
E. microcarpa	10	n.a	n.a	9.00±0.00	n.a
	50	n.a	8.00±0.00	10.00±0.00	n.a
	100	10.00±0.00	9.00±0.00	13.00±0.00	n.a
P. rupestre	10	n.a	n.a	n.a	n.a
	50	n.a	8.00±0.00	9.00±0.00	n.a
	100	n.a	10.00±0.00	11.00±0.00	n,a
M. parviflora	10	n.a	n.a	9.66±0.57	n.a
	50	n.a	n.a	11.33±0.56	n.a
	100	n.a	n.a	12.33±0.67	n.a
M. longipetala,	10	9.77±0.67	n.a	10.00±0.00	n.a
	50	10.66±0.57	n.a	10.33±0.67	n.a
	100	14.60±0.56	9.67±0.68	11.77±0.67	n.a
H. stoechas	10	10.00±0.00	n.a	10.00±0.00	n.a
	50	10.00±0.00	n.a	11.33±0.67	n.a
	100	12.00±0.00	9.00±0.00	12.33±0.55	n.a
L. resedifolia	10	n.a	n.a	n.a	n.a
	50	n.a	n.a	n.a	n.a
	100	n.a	n.a	n.a	n.a
B. tournefortii	10	n.a	n.a	n.a	n.a
	50	n.a	n.a	n.a	n.a
	100	n.a	n.a	n.a	n.a
[f]DMSO(10%V/V)		n.a	n.a	n.a	n.a
[h]IMP,(mg/mL)		22	21	30	23

[a]E.E.C mg/mL; Ethanolic extract concentration in mg/mL.[b]n.a; not active. [c]Iso; isolate. [d]Zone of inhibition (in mm) excluding the diameter of the disc. [f]10% (v/v) solution of dimethylsulfoxide (DMSO) was assayed as negative control. [h]Imipenem(Ipm) was used as antibiotic positive reference standards for bacteria. *E. coli*; *Escherichia coli. S. aureus; Staphylococcus aureus*

3.3 Antibiotic Susceptibility Test

For antibiotic susceptibility examination, Mueller-Hinton agar is mainly used in which the culture of tested bacteria is completed. Commercially available antibiotic disks were used for this test. The technique is called the Kirby-Bauer method. In case the bacterium is resistant to any particular antibiotic it will grow over the antibiotic disks and if the bacterium is sensitive to the antibiotic, a clear circle, or zone of inhibition will be observed. Occasionally bacteria may gain resistance after a certain period and thus creating secondary zones of growth (Bogdadi, 2007), Read AF, 2014). The list of commercial antibiotics used are given below:

Table 5. Effect of some antibiotics on *E.coli* 1, 2, and *S. aureus* 1,2 (all values in mm)

Zone of inhibition of antibiotics	**Bacterial strains**				Total	%
	E. coli iso.1	*E. coli* iso.2	*S. aureus* iso.1	*S. aureus* iso.2		
ATM 30	-	-	-	-	0	0
OX 1	-	-	-	-	0	0
CL30	-	-	14	-	1	5
AMP10	-	-	20	-	1	5
TIC75	10	-	26	9	3	15
PB300	11	9	-	-	2	10
CRO30	16	-	11	-	2	10
IPM10	22	21	30	23	4	20
CIP5	20	-	20	8	3	15
C5	16	16	12	15	4	20
%	6(30%)	3(15%)	7(35%)	4(20%)	20	

4.DISCUSSION

4.1 Extraction yield

Ethanol extraction gave different extraction yields, *H. stoechas*had the highest yield (43.38%), followed by *L. resedifolia*(27.23%). The lowest extraction yield was obtained with *E. microcarpa* 7.42%. Extraction yield from a plant has a great effect on the overall efficacy and selection for bioprospecting and in the calculation of total activity (Elisha et al, 2017).

4.2 Disc diffusion assay (DDA)

The results of DDA show that the ethanolic extract with concentrate (100 mg/mL) 10% of *Reseda alba* and (100 mg/mL) 10%, and (50 mg/mL) 5% of *Scabiosa arenaria* exhibited antibacterial activity against all gram-positive and gram-negative bacterial.However, the crude ethanolic extract of *L.resedifolia* (L.) O. Kuntze, and *B. tournefortii*Gouan with different concentrations 1%, 5%, 10% presented no activity at all bacterial strains under the study. On the other hand, from the results, we have observed significant inhibitory effect of areal parts ethanol extracts of *H.stoechas, M. longipetala, P. rupestre* , *E. microcarpa,S. arenaria*, and *R. alba* together with seeds ethanol extract of *M. parviflora* against *S. aureus* (iso.1) indicating their potential antistaphylococcal properties.

Although crude chloroform extract of *H. stoechas*aerial part (Rios et al., 1987) possessed significant antimicrobial activity in the previous study, we also noted the significant antimicrobial effect of ethanolic extract against the strains current study. Moreover, methanol extract of this species showed a significant inhibitory effect on all microorganisms tested in the previous study. Except for phloroglucinol acetophenone and a-pyrone derivatives found in chloroform and dichloromethane extracts (Tomas-Barberan et al., 1990; Rios et al., 1991), there are no reports of isolation of other antimicrobial active components of *H. stoechas*. (Louhaichi, 2011)

The alteration in the sensitivity between Gram-positive and Gram-negative bacteria may be due to the difference in their cell wall construction. The Gram-positive bacterial cell wall consists of 70–100 layers of peptidoglycans. Peptidoglycan is included of two polysaccharides, N-acetyl-glucosamine and N-acetyl-

muramic acid cross-linked by peptide side chains and cross bridges. This is certainly an oversimplification as an explanation and other mechanisms probably play a role in resistance from Gram-negative bacteria against antibiotics like penicillin originates from the secretion of the lactamase enzyme in the periplasmic space between the thin outer membrane and the cytoplasmic membrane. (Louhaichi,2011).

Also, earlier studies have shown the antibacterial activities of aqueous garlic extracts against *Staphylococcus aureus*, *Staphylococcus epidermids*, *Staphylococcus pneumoniae*, *Staphylococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella spp*., and *Proteus spp*. whose widths of the zone of inhibition were calculated and characterized to range from 19.8-24.5 for gram-negative and 20.2-23.7 mm for gram-positive (Durairaj et al., 2009). The difference in the zone of inhibition between researchers could be due to variation in soil type, climate, pH, plant category or varieties, the random concentration used, and extraction procedures. Additional literature has shown that antibacterial extracted from the aqueous solution are less effective than those extracted from ethanol.

The results for some disc diffusion assays are inaccurate due to the limited ability of the extracts to pass through the hole discs and the hydrophobic compounds were then unable to diffuse into the agar media. Gangoué-Piéboji et *al*. (2009) specified that, when using discs, some active compounds might be stuck in the disc pores. These compounds are not able to pass through to the inoculated media and as a consequence are not be able to fulfill their functions. The disc diffusion assays are generally used as a first screening procedure to determine that the active compounds in plant extracts can pass through; this is done before performing a further determination.

Differences in the antibacterial activity of the examination plants are related to changes in the contents of active compounds. The most active species studied in this work seem to have similar antibacterial active compounds including essential oils, flavonoids and triterpenoids, and other compounds of phenolic nature or with free hydroxyl groups, which are classified as active antibacterial compounds. On the other hand, some of the moderately active and least active plants were also stated to have similar and/or other active compounds but possibly in smaller quantities. (Shtayeh Ali, 1998).

4.3 Antibiotic Susceptibility Test

4.3.1*E. coli* isolates 1 and 2

The antibiotic susceptibility examination was carried out on 2 isolates using 10 antibiotics and the result showed that the isolate (1) was fully resistant to Oxacillin, Aztreonam, Ampicillin, and Cephalexin and for the isolate (2) was resistant to Oxacillin, Aztreonam, Ampicillin, and Cephalexin, Ticarcillin, Ciprofloxacin, and Ceftriaxone, these antibiotics, when tested against all the isolates, produced no zone of inhibition. However, all the isolates were fully sensitive to Chloramphenicol, Polymyxin B, and Imipenem. The total percentage (%) sensitivity of *E. coli* isolate (1) and (2) to the commercial antibiotic were 30% and 15%, respectively.

4.3.2 *S. aureus* isolates 1 and 2

The antibiotic susceptibility analysis was carried out on 2 isolates using 10 antibiotics and the result showed that the isolate (1) was fully resistant to Oxacillin, Aztreonam, and Polymyxin B and the isolate (2) were resistant to Oxacillin, Aztreonam, Ampicillin, Cephalexin Ceftriaxone, and Polymyxin B. The total percentage (%) sensitivity of *S. aureus* isolate (1) and (2) to the commercial antibiotic were 35% and 20%, respectively.

5.CONCLUSIONS

Ethanol extracts of 9 Libyan plants belonging to 5 families were studied for their antibacterial activity. The results obtained from the current study suggest that the extracts of *R. alba*, *S. arenaria*, *E. microcarpa*, *P. rupestre*, *M. parviflora*, *M..longipetala*, *H. stoechas*on *Staphylococcus aureus*, and *R. alba*, *S. arenaria*, *E. microcarpa*, *M. longipetala.*, *H. stoechas*, on *E.coli*. possess significant antibacterial properties. However, the mechanisms of killing require further investigation. Out of the 9 plant ethanol

extracts tested, two plant extracts, of *L. resedifolia* (L.) O. Kuntze, and *B. tournefortii*Gouan with different concentrations of 1%, 5%, 10% showed no activity against most of the organisms. Moreover, the combination of some of these extracts and the antibiotics could be tested to determine any synergistic effects that might increase the antibacterial activity. Several studies have been reported on the potential of this synergistic effect to improve the biological activity of some low-activity plant extracts by combining them with higher activity plants or combining lower activity plants with higher activity antibiotics.

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