ejpmr, 2021,8(1), 416-423

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIMICROBIAL ACTIVITY OF LIBYAN *SALVIA FRUTICOSA MIL* AND MULTI-DRUG RESISTANT BACTERIA

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Article Received on 10/11/2020 **Article Revised on 30/11/2020** **Article Accepted on 20/12/2020**

ABSTRACT

This study was aimed to evaluate the antimicrobial activity and determine the minimum inhibition concentration (MIC) of the *Salvia fruticosa* extracts against standard microbe strains and clinical bacterial isolates. **Methods:** Soxhlet apparatus and rotary evaporator with maceration and Freeze drying were used for extraction and evaporation of the organic and aqueous extracts, respectively. Standard methods were used to evaluate the antimicrobial activity and minimum inhibition concentration. **Results:** The highest activity of extracts against standard strains was shown against the *B.subtilis* followed by *C.lbicans, S.aureus, Ps.aeruginosa* and *E.coli*, with range of inhibition zones of 21 ± 0.7 to 15 ± 0.01, respectively inhibition zones of 21 ± 0.7 to 15 ± 0.01. Among the tested clinical isolates, the methanol bark extract showed good growth inhibition zones ranged from 11.5mm ± 1.6 to17.5mm ± 4 against Gram negative bacteria; *Acinetobacter baumanii, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis* and *Pseudomonas aeruginosa* and 19mm ± 4.54 against MRSA compared with most of tested antibiotic references, except of Ciprofloxacin 30µg which showed weak activity against *A.baumannii*, but showed higher activity against other tested clinical isolates. The bark extract showed same activity of Gentamicin10µg against *Ps.aeruginosa* and MRSA and showed closed performance to that of Ceftazidime 30µg against *E.coli*. Compared with Augmentin 30µg and Ceftriaxone 30µg, the methanol bark extract *Salvia* extract was the only effective one against all tested clinical bacteria. **Conclusion:** This study concluded that *Salvia fruticosa* extracts have good antibacterial and antifungal activities and suggests that the methanol bark extract have different behaves by which can fights the bacteria. In addition this study gives the assumption that *Salvia fruticosa* could be considered as a key solution against multidrug resistant pathogenic bacteria.

INTRODUCTION

Medicinal plants are of great importance to the health of the individuals and communities, where considered as a rich resources of ingredients which can be used in drug development and synthesis (Sasidharan *et al*., 2011, Singh, 2015). The medicinal value of these plants lies in some bioactive constituents that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Lee *et al*., 2002). In addition to the use of *Salvia fruticosa* in a lot of cosmetic purposes such as skin and hair care (Barnes and Phillipson, 2007), it also has many different uses. Essentially, it has been used as herbal remedy for a wide range of disorders and illnesses by applying it either internally or externally. It is employed as diuretic, tonic, menstruation's promoter, antiseptic, anti-inflammatory, antifungal and spasmodic pain relief (Ioannides, 2002). It is also used as treatment for dysentery, coughing, indigestion, ulcer, varicose veins and insect bites (Dweck, 2000; Izzo, 2005). However, the plant should be used carefully since large doses can be toxic (Jellin *et al*., 2000). *Salvia officinalis* side effect range from depression to relieve aging symptoms (Scholey *et al*., 2008). The antimicrobial performance of aerial parts of *Salvia fruticosa* plant had been studied by many researchers. Results cleared that the plant oil showed inhibition performance on growth of tested Gram

positive and Gram negative bacteria with varied minimum inhibitory concentrations values. It has been concluded that *Salvia* plant has antimicrobial activity against the Gram positive *Streptococcus mutans*; the cause of dental cares and *Streptococcus* group D bacteria which completely inhibited after 10 minutes contact and also has antifungal activity against *Candida albicans* at lesser contact time and oil concentration. It has been proved that the plant essential oil showed a temporary bacteriostatic mode against *Salmonella typhi* (Khalil and Li, 2011, Giweli *et al.,* 2013, Beheshti-Rouy *et al*., 2015).

Pitarokili *et al*., (2003), reported that Camphor and 1,8-cinole has been proved as main constituents of *Salvia fruticosa* which showed an antifungal activity ranged from moderate to high growth inhibition capacity. Giweli *et al.,* (2013) and (Khali and Li, 2011) applied Gas chromatography-Mass spectrometry to analyze *Salvia fruticosa* extracts and identified approximately Forty five bioactive components including 1,8-cineole (49.34%), camphor (7.53%) and α –terpineol (3.25%). This study aimed to investigate the antimicrobial activity and determine the minimum inhibition concentration (MIC) of the Libyan *Salvia fruticosa* extracts against some standard and multi drug resistant (MDR) clinical bacterial isolates.

MATERIALS AND METHODS

Plant collection and preparation

The plants was collected with collaboration with Herbalists from around Al-Bayda city; located in Al-Jabal Al-Akhdar region, Northeast of Libya. The plant was cleaned, dried at room temperature, powdered using mixer and kept in clean closed container.

Plant extraction

One hundred grams of the powdered plant materials was successively extracted by Soxhlet apparatus with enough quantities (250-300 ml) of chloroform, methanol and ethanol 96%, respectively for (6 – 10 hours). Also one hundred grams of the plant powder extracted with maceration for 72 hour with water. All extracts were filtered. The organic solvent was evaporated under reduced pressure using Rotary-evaporator while the water extract was freeze dried. Each solvent extract yield was weighed; the yield percentage was calculated and kept in well labeled clean glass containers.

Tested microorganisms

Bacillus subtilis NCTC 8236, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 bacteria and the fungus *Candida albicans* ATCC 7596 are standard microbial strains used in this study and obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan. Hundred clinical isolates belonged to seven different genera; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii, Enterobacter cloacae, Klebsiella pneumonia* and *Proteus mirabilis*, were isolated from patients attending different departments in Medical Benghazi Centre, east of Libya.

Preparation of bacterial suspensions

Fresh suspension was prepared for each tested organism. A 24 hours bacterial growth was harvested and washed off with 100 ml sterile normal saline, the suspension was adjusted to McFarland 0.5 solution via dilution with sterile normal saline 0.9% to get a suspension containing about 10^8 C.F.U/ ml. Sabouraud dextrose agar media was used to prepare fungal cultures with incubation period of 2 days at 25°c.

Preparation of extract stock solution

In the day of the antimicrobial assay, 2gm of each of the pre-prepared extracts powder was dissolved in 20 ml sterile distilled water in order to prepare an extract solution of final concentration of 100 mg/ml. The chloroform extract (0.2g) was dissolved in 2 ml of a mixture of (2:1 V/V) of methanol and petroleum ether to get final concentration of 100 mg/ml and each of the methanolic extract (0.2g) and ethanolic extract (0.2g) was dissolved in 2 ml of methanol to get final concentration of 100 mg/ml.

Evaluation of the antimicrobial activity of extracts

Disc diffusion method was carried out according to Mukhtar and Ghori, (2012) with some minor modifications. One hundred microliters of freshly prepared bacterial suspension of each of the tested standard and clinical bacteria were separately swabbed uniformly on surface of Mueller Hinton agar (MHA) and the inoculums were allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of solution (100mg/ml) of each plant extracts. The inoculated plates were incubated at 37 °C for 16-18 hr. The diameters (mm) of the inhibition zones were measured. Antifungal activity of standard *Candida albicans* that cultured on Sabouraud dextrose agar was incubated for 2 days at 25 °C. The diameters (mm) of the inhibition zones were measured. Two replicates were carried out for each extract against each of the tested organisms.

Susceptibility of tested clinical bacteria to antibiotics references

Bacterial suspension of the clinical isolates were diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of Mueller Hinton agar (MHA) and the inoculums were allowed to dry for 5 minutes, antibiotic discs (Ciprofloxacin 30µg, Gentamicin 10 µg and Vancomycin 30µg, Ceftazidime 30µg, Ciprofloxacin 30µg and amoxicillin20µg +Clavulanic acid 10µg) were placed on the surface of the MHA. The inoculated bacterial plates were incubated at 37 °C for 16-18 hr. The

diameters (mm) of the inhibition zones were measured. Two replicates were carried out for each antibiotic disc against each of the tested organisms.

Determination of minimum inhibitory concentration (MIC)

According to Andrews (2006), agar dilution method was adapted with little modification. Serial dilutions were prepared for each extract in decreasing concentrations in the following order: 200,100, 50, 25, 12.5, 6.25, and 3.125. In sterile covered glass bottles, 5ml Melted double strength MHA cooled to 45 were mixed with 5ml of each dilution of the tested plant extract to get final serial dilutions of 100, 50, 25, 12.5, 6.25, 3.125 and 1.652 of each extract. The mix was poured to sterile small petri dishes, left to solidify and then the bottom of each plate was marked off into segments. A loop full of standard loop (0.01ml) of each of tested bacterial fresh suspension adjusted with McFarland 0.5 solution was spotted onto the surface of each segment of the marked MHA. The inoculums were allowed to be absorbed into the agar before incubated at 37°C for 18 hour. After the incubation period the least concentration mg/ml of the plant extract that completely inhibits growth of organism is considered as the MIC.

Statistical analysis

All determinations were conducted in duplicates and all inhibition zones results were calculated as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 20. Differences were considered significant at p value ≤ 0.05, highly significant at p value ≤ 0.01 and non-significant at P value ≥ 0.05.

RESULTS

Antimicrobial activity of *Salvia fruticosa Mil* extracts against standard microorganisms

Among tested standard microorganisms, this study showed that *Salvia fruticosa Mil* is more effective against Gram positive than Gram negative bacteria. Within Gram positive the plant appeared more active against *Bacillus subtilis* than *Staphylococcus aureus*. The highest inhibition zones shown against *Bacillus subtilis* and *Staphylococcus aureus* were 21mm and 16mm from Chloroform and Methanol extract of leaves and bark respectively (Table 1). Among tested Gram negative standard bacteria, the highest zones of inhibition revealed in this study against *E.coli* were 15mm and 12mm obtained from Methanol bark extract and Chloroform leaves extract, respectively. However, the highest inhibition zone shown against *Pseudomonase aeruginosa* was 16 mm from methanol bark extract followed with 13mm inhibition zone from ethanol extract of the plant leaves. Also 17 mm appeared as the highest inhibition zone revealed against the tested fungus; *Candida albicans* from bark methanol extract, while extracts of leaves ethanol, leaves Chloroform and bark Ethanol showed inhibition zones of 16 mm, 14 mm and 13 mm respectively. No activity was shown from the aqueous extracts of *Salvia fruticosa Mil* (Table 1). The statistical interpretation showed highly significant differences (P value ≤ 0.01) between the effects of different solvents extracts from different plants parts against tested standard organisms. Variable activities shown form ethanol extracts and no activities shown from the aqueous extracts of leaves and bark of *S.fruticosa* and (Table1).

Antimicrobial activity of methanol extract of bark of *Salvia fruticosa Mil* against clinical bacterial isolates

Since the methanol extract of bark of *Salvia fruticosa* showed good activity against all tested Gram positive and Gram negative and tested fungus, it has been investigated for its antibacterial activity against 100 clinical isolates belonged to 7 different genera. The results showed active growth inhibition performance from Methanol extract of bark of *Salvia fruticosa* against all tested clinical bacteria. The highest growth inhibition zone appeared was 19.5mm± 4.5 against *Staphylococcus aureus* followed by 17.5mm±4.0, 17.mm±4.0, 13.8mm ± 10, 12.50± 1.8, 12.4mm± 1.3 and 11.5±1.6 against *Pseudomonas aeruginosa*, *Eshcerichia coli, Klebsiella pneumonia, Acinetobacter baumanii, Proteus mirabilis* and *Enterobacter*, respectively. Compared with the antibiotic references used in this study, the plant extract appeared as the only effective agent against the tested clinical *Acinetobacter baumanii*. Moderate growth inhibition activity equal to what revealed from Ciprofloxacin 30µg has shown against tested *Enterobacter cloacae*. On the other hand active growth inhibition zones of 13.8mm± 1.0 and 19mm± 0.8 were seen against *Klebsiella pneumonia* from *Salvia fruticosa* extract and Ciprofloxacin 30µg, respectively. However no one of the other used references showed activity against these three tested bacteria (Table 2). For *Eshcerichia coli*, inhibition zones of 17mm± 4, 18.5mm± 1 and 31mm± 1.8 has been shown from the *Salvia* extract, Ceftazidime 30µg and Ciprofloxacin 30µg, respectively while other references were not active. Table (2) presented that inhibition zones of (12mm± 1.3, 17.5mm± 4), (14mm± 8, 10mm± 6), (37mm± 8, 39mm± 1) and (18mm± 1, 16mm± 9) have shown from *Salvia* extract, Ceftazidime 30µg, Ciprofloxacin 30µg and Gentamicin 10µg, respectively against tested *Proteus mirabilis* and *Pseudomonas aeruginosa*, respectively. Other tested antibiotic references showed no activity. Also this table proved that *Salvia fruticosa* methanol bark extract has the same active inhibition performance (19mm) shown by Vancomycin 30µg against tested clinical *Staphylococcus aureus*, where other tested references showed no activity.

Minimum inhibition concentration of *Salvia fruticosa* methanol bark extract against standard organisms

As shown in table 3. The minimum inhibition concentration of the methanol bark extract of *Salvia fruticosa* was 50 mg/ml for *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*. Also this table presented that *Salvia fruticosa* extract inhibited the

growth of *Pseudomonas aeruginosa* and *Candida albicans* with minimum inhibition concentrations of 25 mg/ml and 12.5 mg/ml respectively (Table 3).

DISCUSSION

In this study the methanol extract of *Salvia fruticosa* bark showed high antibacterial performance against tested standard Gram positive and Gram negative bacteria and fungi. The results claimed that the tested extract could be used in the treatment of infections caused by *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* which were inhibited with a MIC of 50mg/ml for each. On the other hand, this study pointed to that *Salvia fruticosa* bark will be more powerful in the treatment of diseases caused *by Candida albicans* and *Pseudomonas aeruginosa* as both were inhibited by lower MIC of 12.5 mg/ml and 25 mg/ml respectively.

Few studies have been found concerned with the antimicrobial activity of *Salvia fruticosa* in general and for the part bark in specific. *Salvia officinalis* is the most investigated species.

Delamare, *et al*., 2007, Pierozan, *et al*., 2009 and Khalil, *et al*., 2011, were screened the antimicrobial activity of the aerial part of the species *Officinalis*, and they reported that the essential oil of *Salvia officinalis* has moderate antibacterial activities against Gram positive and Gram negative bacteria; *Bacillus subtilis*, *Satphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, with an inhibition zone range of 8 mm to 10 mm. However, in a different way *Salvia fruticosa* in this assessment showed higher growth inhibitions zones with range of 15mm – 20mm and of 12.5mm – 19mm against standard and clinical bacterial isolates, respectively. This difference in activity might be contributed to the different plant species had investigated and also to the different plant constituents had been tested, where this study investigated an organic extracts while the above authors tested the antimicrobial activities of the essential oil of the plant. In addition, it is known that the composition of plant oils and extracts vary according to climatic and environmental conditions variation.

The issue which should be noticed from this assessment is that the bark of *Salvia fruticosa* presents a higher antibacterial activity than the essential oil of the aerial parts of *Salvia officinalis.* The point which makes *Salvia fruticosa* of a valuable worth in the treatment of infectious diseases.

This promising antimicrobial activity of the bark of *Salvia fruticosa* contributed to many bioactive constituents. Many researchers have reported that the antimicrobial activities of the essential oils extracted from the plant were due to the presence of some major and minor constituents. Dorman and Deans, 2000; Sur *et al*., 1991 and Tzakou *et al*., 2001, reported that the presence of Camphor and 1,8-cineole as constituents of *Salvia fruticosa* offers the plant an antifungal and antibacterial activities. Another study documeted that Flavonoids, Tannins and Triterpines are constituents of *Salvia fruticosa* responsible for the powerful antibacterial activity. In addition, thirty three bioactive constituents were revealed by gas chromatography mass spectrum analysis of the methanol extract of bark of *Salvia fruticosa*; among them there are six previously proved to have antimicrobial activities (Padmashree *et al*., 2018; Eltawaty, 2018).

This study makes the believe that the methanol extract of *Salvia fruticosa* bark has a broad spectrum antimicrobial activity as it showed active performance against all tested bacteria. This results figured out more than one probable idea about the antibacterial mechanism of the extract by which it fights the pathogenic bacteria. When the antibacterial activity of the methanol bark extract of *Salvia fruticosa* against clinical Methicillin resistant *Staphylococcu aureus*(MRSA) isolates had compared with that of a panel of most commonly used antibiotics, it showed similar activity compared with that shown by Gentamicin 10µg and Vancomycin30µg (The drug of choice against MRSA). By this results this study suggests that the tested extract probably has either a cell wall disruption activity as the Vancomycin do or in sense of that the multidrug-resistant clinical *S. aureus* have a greater likelihood of developing biofilms (Gwang, 2008), this study can suggests that the tested extract has the ability to inhibit the growth of MRSA by interfering of biofilm formation. In addition, it is known that Phosphatidylglycerol; the major bacterial lipid component in Gram-positive bacteria can be chemically modified by bacterial enzymes to convert the lipid from anionic to cationic or zwitterionic form, the process which leads to increased levels of resistance of bacteria against antimicrobial agents (Miller, 2016). According to this concept, this study suggests that the tested *Salvia fruticosa* methanol bark extract which actively inhibited the growth of MRSA might be have the ability to disable the bacterial enzymes responsible for phosphatidylglycerol modification. Furthermore, this assessment pointed out that the tested extract might be having a good affinity to bind with the small 30S sub unit of the bacterial ribosome by which it inhibits the protein synthesis, which then leads to the bacteria death, the mechanism by which the aminoglycoside Gentamicin follow to kill the bacteria. A fifth suggestion this assessment offers is that this extract has an anti beta-lactamases activity and can prevents the breakdown of Beta-lactam ring (the backbone of beta-lactam antibiotics). This assumption comes when the extract activity compared with the tested standard Beta-lactam antibiotics (Augmentin & Ceftriaxone), and showed a promising high antibacterial activity against all tested clinical isolates in time no activity was shown from both antibiotics. Among the Gram negative bacteria, all tested bacteria in this study appeared as multi-drug resistant bacteria (*A.baumanni, E.coli, K.pneumoniae, P.mirabilis, E.cloacae and Ps.aeruginosa*) where they resist three to

more of the used standard antibiotics nevertheless their growth actively inhibited by the tested extract. It has been documented that the resistance of Gram negatives might be due their ability to produce enzymes that can modify or destroy the antibiotics, such as aminoglycoside-modifying enzymes, extended spectrum -lactamases and carbapenemases or due to acquisition of mutations in bacterial targets such as topoisomerases, ribosomes, penicillin-binding proteins, and outer membrane porins that alter antibiotic efficacy or uptake (Blair *et al*., 2015, Epand *et al*., 2016). In addition, *Acinetobacter baumannii* in this assessment showed resistance to all different antibacterial mechanisms offered by all different used standard antibiotics nevertheless it was actively killed by *Salvia fruticosa* organic bark extract. This finding highlights the valuable, worth of this plant part extract on the management of infection caused by these worldwide problematic multi drug resistant bacteria, especially with the increasing rate of their pathogenic burden and medical importance in the last years. It is a very pretty finding that the bark of the Libyan *Salvia fruticosa* plant comprises a remarkable antibacterial activity against the multi-drug resistant Gram positive and Gram negative bacteria. In addition, according to the statistical analysis, variable significant differences (p value ≤ 0.05) had shown between the activity of the different tested solvent extracts and also between the activity of the Methanol bark extract and the antibiotics references used in this study. This finding with the variability had shown in the superscript letters, reinforces the idea of that the extract/s has different mechanisms of action can behave to fight the pathogens.

Table (1): Antimicrobial activity and Yields percentages of *Salvia fruticosa Mil* extracts against standard microorganisms.

Family Botanical name Local name	Plant part used	Solvent extract 100mg/ml	Yield %	Means of Diameters of Inhibition Zones (mm)				
				Gram positive		Gram negative		Fungus
				B.subtilis	*S.aureus*	*E.coli*	*Ps.aeruginosa*	*C.albicans*
Lamiaceae *Salvia fruticosa Mil* Toffah alshahi	leaves	Chloroform	15	$21^{a} \pm 0.7$	$15^{b} \pm .7$	$12.0^{c} \pm 0.7$	$0^{d} \pm 0.0$	$14.0^{b} \pm 0.7$
		Methanol	7.6	$14^{a} \pm 0.7$	$11^{b} \pm 0.7$	$0^{d} \pm 0.7$	$8^{c} \pm 0.71$	$0.0^{d} \pm 0.7$
		Ethanol	1.7	$12^{b} \pm 0.7$	$0^{c} \pm 0$	$11^{b} \pm 0.71$	$13^{b} \pm 0.7$	$16^{a} \pm 1.4$
		H_2O	6	$0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$
	bark	Chloroform	3	$20^{a} \pm 0.01$	$0^{c} \pm 0.0$	$10^{b} \pm 0.01$	$0^{c} \pm 0.0$	$0^{c} \pm 0.0$
		Methanol	6.5	$20^{a} \pm 0.01$	$16^{b} \pm 0.7$	$15^{b} \pm 0.01$	$16^{b} \pm 0.7$	$17^{b} \pm 2$
		Ethanol	1.6	$12^{ab} \pm 0.7$	$0^{d} \pm 0.0$	$10^{b} \pm 0.7$	$11^{b} \pm 0.7$	$13^{a} \pm 0.7$
		H_2O	2.5	$0^{a} \pm 0.00$	$0^{a} \pm 0.0$	$0^{a} \pm 0.0$	$2^{a} \pm 0.01$	$0^{a} \pm 0.0$

Means in same row with same superscript letter are not significantly different

Table (3): Minimum inhibitory concentration of methanol bark extract of *Salvia fruticosa* inhibit growth of standard tested micro-orgainsms.

Plant extract concentration mg/ml	Standard microorganisms				
	B.subtilis	*S.aureus*	*E.coli*	*Ps.aeruginosa*	*C.albicans*
100	-	-	-	-	-
50	-	-	-	-	-
25	+	+	+	-	-
12.5	+	+	+	+	-
6.25	+	+	+	+	+
3.125	+	+	+	+	+
1.56	+	+	+	+	+

(-) = No growth (+) = Growth

Table (2): The antimicrobial activities of *Salvia fruticosa* methanol bark extracts and some antibiotics against clinical isolates.

Antimicrobial agents	*A.baumanii*	*E.cloacae*	*E. coli*	*K. pneumonia*	*P.mirabilis*	*Ps. aeruginosa*	*S. aureus*
***Salvia fruticosa* bark extract**	12.5^{a} 10.8	11.50^{a} ± 1.6	17^{b} ± 4	13.8^{b} ± 10	12^{c} ± 13	17.5^{b} ± 4	19^{b} ± 4.54
Augmentine 30µg	0.0^{b} ± 0.	0.0^{b} ± 0.0	0.0^{d} ± 0.0	0.0^{c} ± 0.0	0.0^{d} ± 0.0	0.0^{d} ± 0.0	0.0^{c} ± 0.0
Ceftazidime 30µg	0.0^{b} ± 0.0	0.0^{b} ± 0.0	18.5^{b} ± 1	0.0^{c} ± 0.0	14^{c} ± 8	10^{c} ± 6	0.0^{c} ± 0.0
Ciprofloxacin 30µg	2^{b} ± 3	11^{a} ± 9.9	31^{a} ± 1.8	19^{a} ± 0.8	37.8^{a} ± 8	39^{a} ± 1	31.8^{a} ± 2.9
Gentamicin, 10µg	0.0^{b} ± 0.0	0.0^{b} ± 0.0	0.0^{d} ± 0.0	0.0^{c} ± 0.0	18.8^{b} ± 1	16.8^{b} ± 9	19.6^{b} ± 1.8
Ceftriaxone 30µg	0.0^{b} ± 0.0	0.0^{b} ± 0.0	0.0^{d} ± 0.0	0.0^{c} ± 0.0	0.0^{d} ± 0.0	0.0^{d} ± 0.0	0.0^{c} ± 0.0
Vancomycin 30µg	0.0^{b} ± 0.0	0.0^{b} ± 0.0	4.80^{c} ± 4	0.0^{c} ± 0.0	0.0^{d} ± 0.0	0.0^{d} ± 0.0	19.10^{b} ± 1.6

Means with superscript different letter are significantly differ.
Augmentin = Amoxicillin20µg + Clavulanic acid 10µg

CONCLUSION

This study concluded that the bark of the Libyan *Salvia fruticosa* have a good antibacterial and antifungal activities. This screening suggests that the Libyan *Salvia fruticosa* considered as promising alternative antibacterial agent to most common used antibiotics. Furthermore, both multi drug resistant Gram positive and Gram negative bacteria included in this assessment were actively inhibited, the result which gave the assumption that the methanol extracts of *Salvia fruticosa* bark may be have more than one inhibitory mechanism against growth of pathogenic bacteria. The highlighted result is that the plant appeared as the only effective agent against the worldwide problematic *Acinetobacter baumanii* bacteria compared with all tested antibiotics. In conclusion, this study introduces the methanol bark extract of the Libyan *Salvia fruticos* as a promising extract for developing new antimicrobial pharmaceutical products can help in the treatment of infectious diseases and promotes the health being.

Recommendation

This study recommends further investigation to examine if the extract has any synergistic activity to any of the common used antibiotics. Also further investigations are recommended to purify a pure bio-constituent/s which responsible for the antimicrobial activity and to determine the mechanism/s of action of the purified compound/s by which it can inhibits the bacterial growth.

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