



Original article

Antimicrobial effect of different herbal plant extracts against different microbial population

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ARTICLE INFO

Article history:

Received 26 June 2020

Revised 8 August 2020

Accepted 9 August 2020

Available online 13 August 2020

Keywords:

Antimicrobial effect

Herbal extract

Gram positive

Gram negative

Mycoplasma gallisepticum

ABSTRACT

This study evaluates the antimicrobial effects of ethanolic extract of five herbal plants; Guava (*Psidium guajava*), Sage (*Salvia officinalis*), Rhamnus (*Ziziphusspina Christi*), Mulberry (*Morusalba L.*), and Olive (*Oleaeuropaea L.*) leaves against several microbial population representing Gram positive, Gram negative and Mollicutes; *S. aureus*, *E. coli*, *Pasteurella multocida*, *B. cereus*, *Salmonella* Enteritidis and *M. gallisepticum* using standard agar disc diffusion technique and minimal inhibitory concentration (MIC). Different extracts reveal variable results against the microorganism under study. All extracts have no antibacterial potency for *Mycoplasma gallisepticum* except *Psidium guajava*. The results of minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extracts against the six bacteria ranged from 625 to 5000 µg/ml. The used herbal extract could inhibit the selected microorganism under study with variable minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

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1. Introduction

Infectious diseases are still a major health concern accounting for 41% of the global disease burden measured in terms of Disability – Adjusted Life Years (DALYS) (Noah and Fidas, 2000). One of the main causes of this problem is the widespread of acquired bacterial resistance to antibiotics in such a way that the world is facing today, a serious threat to global public health (Chopra, 2000) in the form of not only epidemics, but also pandemics of antibiotic resis-

tance (Chanda et al., 2010; Osman et al., 2012). Due to this problem of resistance against antibiotics, attention is now being shifted towards biologically active components isolated from plant species communing used as herbal medicine, as they may produce a new potent source of antibacterial and antifungal activities (Maiyo et al., 2010; Erfan and Marouf, 2019). The antimicrobial properties of plants related to their ability to give several secondary metabolites of relatively complex structures possessing antimicrobial activities (Matasyoh et al., 2009; Souza et al., 2005).

Guava (*Psidium guajava L.*) is a fruit plant belonging to the family *Myttaceae*, guava leaves, roots, and used for prevention and treatment of diarrhea (Lutterrodt, 1989; Alnieida et al., 1995), guava also showed a significant antibacterial activity against food borne diarrhea causing bacteria which are *Staphylococcus* species, *Shigella* species, and *Pseudomonas* species. (Alnieida et al., 1995; Jaiarj et al., 1999). Guava is also used as anti-inflammatory and antiseptic as well as treatment of diabetes, hypertension, pain fever, respiratory disorders, gastroenteritis, diarrhea and dysentery (Gutierrez et al., 2008).

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Peer review under responsibility of King Saud University.



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Sage (*Salvia officinalis* L.) from family *Lamiaceae* is aromatic plant used in traditional medicine for treating many ailments as inflammation of mouth and throat (Baricevic et al., 2001) and used as antimutagenic and anticarcinogenic agent (Craig, 1999; Simic et al., 2000). It is also employed as diuretic, tonic, local styptic, antiseptic, antifungal and spasmodic pain relief (Loannides, 2002).

Rhamnus (*Ziziphusspina Christi*) is a plant belongs to family *Rhamnaceae*, its extracts showed antiviral, antifungal and antibacterial activities and used in the Egyptian folk medicine for treatment of several diseases including gastrointestinal tract ailments, diabetes and diarrhea (Shahat et al., 2001).

Mulberry (*Morusalba* L) belongs to Family *Moraceae* which used to treat fever and headache, Mulberry can help treat chronic diseases of digestive tract and improve appetite. It is also used in treating chronic gastritis and hepatitis. It is helpful in Cough, dyspepsia, oedema and oligurea (Sunil and Ammani, 2009). Recent studies have shown *M. alba* has antioxidant, antibacterial, antiviral and anti-inflammatory properties (El-Beshbishy et al., 2006).

Olive tree leaves (*Olea europaea* L.) is widely tested for its pharmacological use, where the leaves are important for their secondary metabolites such as secoiridoid compounds which are responsible for hypotensive activity (Hansen et al., 1996) and hypoglycemic activity (Gonzalez et al., 1992). Several reports have shown that plant has capacity to lower blood pressure (Samuelsson, 1951), relieve arrhythmia and prevent intestinal muscle spasm (Garcia et al., 2000).

The present work designed to investigate the antimicrobial effects of ethanolic extract of five herbal plants, namely: Guava (*Psidium guajava*), Sage (*Salvia officinalis*), Rhamnus (*Ziziphusspina Christi*), Mulberry (*Morusalba* L.), and olive (*Olea europaea* L.) against several microbial strains (*S. aureus*, *E. coli*, *Pasteurella multocida*, *B. cereus*, *Salmonella* Enteritidis and *Mycoplasma gallisepticum*) which are incriminated in different diseases among livestock animals and birds.

2. Materials and method

2.1. Plant material

In the investigation, the whole plant of Guava (*Psidium guajava*), Sage (*Salvia officinalis*), Rhamnus (*Ziziphusspina Christi*), Mulberry (*Morusalba* L.), and olive (*Olea europaea* L) leaves were collected from field market of Egypt. The plants are air dried in an oven at 40 °C for 48 h. Two hundred and fifty grams of dried powdered plant sample was extracted by one liter of ethanol 70% at 30 °C for 48 h and filtering through Whatman No. 4 filter paper. The plants were extracted by rotatory evaporation at 50 °C till complete dryness occurs. The total extract was dissolved in water in a concentration of 500 mg/ml and stored at –20 °C for further use.

2.2. Test organisms

Six types of microorganisms were investigated; Gram positive (*S. aureus* and *B. cereus*), Gram negative (*E. coli*, *Pasteurella multocida*, *Salmonella* Enteritidis) and *Mollicutes* (*Mycoplasma gallisepticum*). All organisms obtained and well identified onto Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

2.3. Antimicrobial activity

The antimicrobial potentiality of the above mentioned plants ethanolic extracts were determined by standard agar disc diffusion technique and minimal inhibitory concentration (MIC) in accordance with the Clinical and Laboratory Standards Institute (CLSI,

2015) against the tested microorganisms. *Mycoplasma gallisepticum* was determined in accordance with (Hannan, 2000).

2.3.1. Disc diffusion method

Pure colonies of each microorganism were suspended in sterile saline until a turbidity match McFarland tube number 0.5 (1.5×10^8 CFU/ml). A loopful from each adjusted organisms were swabbed onto Muller Hinton agar (Difco Laboratories). Sterile paper discs (6 mm in diameter) were impregnated with 100 µl of each 10%, 25%, 50%, 75% and 100% diluted extracts then dried at 100 °C for two hours in hot air oven and were dispensed onto the surface of the inoculated agar plate. The plates were then incubated according to growth requirement of each organism. Each sample was tested in triplicates and antibacterial activity was evaluated by measuring and recorded the zones of inhibition in mm (including the 6 mm disk). A parallel analysis study with commercial antimicrobial agents included Amoxicillin-Clavulanic (10 µg), Ampicillin (10 µg), Vancomycin (30 µg), Lincomycin (30 µg), Gentamicin (10 µg), Streptomycin (10 µg) and Tetracycline (30 µg) was conducted in order to compare their antimicrobial potency with the plant extracts.

2.3.2. Minimal inhibitory concentration (MIC)

In dilution technique, two fold serial dilutions of the extracts were prepared in concentrations ranging from 625 to 10,000 µg/ml. From each dilution of each extract, one milliliter of was mixed with 9 ml of Muller Hinton Agar. Ten microliter of each standardized broth cultures (1.5×10^8 CFU/ml) was cultivated on the surface of the plates containing various concentrations of the extracts. The plates were then incubated according to growth requirement of each organism and observed for any visible bacterial growth. MIC was the lowest concentration of extract that resulted in no visible growth on the surface of the agar.

2.3.3. Minimum bactericidal concentration (MBC)

Blocks of agar plates showing no growth after MIC tests were inoculate to fresh nutrient broth (acting as the recovery medium) for the determination of the MBC. The broths were incubated according to growth requirement of each organism. The absence of turbidity in the recovery medium was evidence of bactericidal activity.

2.4. Statistical analysis

The inhibition zones were calculated as means \pm SD. The significance was evaluated by analysis of variance (ANOVA) using Microsoft Excel program. Significant differences in the data were established at the 5% level of significance.

3. Results

The ethanolic extracts of the used plants under study reveal that the extract can be a better antimicrobial agent in comparison with the result of commercial antimicrobial agents used in the work.

In Disk diffusion technique, the extracts of *Salvia officinalis* and *Psidium guajava* have the ability to inhibit *S. aureus* growth, while the extract of *Olea europaea* and *Morus alba* have antibacterial potency against *B. cereus*. *Salvia officinalis* and *Olea europaea* have the ability to stop *E. coli* growth. Moreover, *Olea europaea* and *Salvia officinalis* extracts can inhibit the growth of *Salmonella* Enteritidis. The plate of *Pasteurella multocida* show inhibition zone with *Psidium guajava* and *Morus alba*. All extracts have no antibacterial potency for *Mycoplasma gallisepticum* except *Psidium guajava*. The diameter of inhibition zone in correlation to concentration of

Table 1
Antimicrobial activity of examined plant extracts on six microorganism using disk diffusion method.

Organism	Inhibition zone of extract (mm)																								
	<i>Psidium guajava</i>					<i>Salvia officinalis</i>					<i>Ziziphusspinachristi</i>					<i>Morus alba</i>					<i>OleaEuropaea</i>				
	10%	25%	50%	100%	100%	10%	25%	50%	100%	100%	10%	25%	50%	100%	10%	25%	50%	100%	10%	25%	50%	100%			
<i>S. aureus</i>	-	8.42 ± 0.41	11.5 ± 0.31	15.62 ± 1.15	3.5 ± 0.32	9.45 ± 0.35	13.5 ± 0.25	17.05 ± 1.05	6.5 ± 0.4	9.5 ± 0.25	11.82 ± 2.5	8.5 ± 0.55	10.5 ± 1.15	5.5 ± 0.05	7.42 ± 0.4	10.5 ± 0.25	12.02 ± 2.05								
<i>B. cereus</i>	-	-	7.5 ± 0.25	10.05 ± 0.15	7.5 ± 0.32	10.05 ± 0.25	14.2 ± 0.05	16.45 ± 1.05	8.5 ± 0.25	11.5 ± 0.45	13.52 ± 2.1	12.5 ± 1.55	14.75 ± 0.15	9.1 ± 0.15	11.52 ± 0.07	13.5 ± 0.45	16.62 ± 1.05								
<i>E. coli</i>	-	-	8.5 ± 0.35	10.55 ± 0.15	9.5 ± 1.32	12.25 ± 1.05	16.5 ± 0.75	19.25 ± 0.65	-	8.5 ± 0.15	10.02 ± 0.05	-	7.5 ± 0.15	8.5 ± 1.05	10.72 ± 1.04	14.5 ± 0.75	16.72 ± 0.55								
<i>S. Enteritidis</i>	-	3.42 ± 1.4	7.5 ± 0.71	10.12 ± 0.55	7.75 ± 0.52	10.05 ± 0.35	14.5 ± 0.15	16.25 ± 0.75	7.5 ± 0.4	9.5 ± 0.25	12.82 ± 2.5	7.75 ± 0.25	12.02 ± 0.05	9.5 ± 0.55	11.72 ± 0.45	16.5 ± 0.35	18.02 ± 0.05								
<i>P. multocida</i>	9.57 ± 1.55	11.92 ± 1.45	16.75 ± 1.35	18.02 ± 0.95	-	-	7.5 ± 0.25	9.05 ± 1.05	-	6.5 ± 0.25	8.52 ± 2.5	11.55 ± 0.41	15.42 ± 0.15	3.52 ± 0.49	7.5 ± 0.25	9.12 ± 0.05									
<i>M. gallisepticum</i>	-	8.5 ± 0.98	11.54 ± 0.61	14.62 ± 0.55	-	-	-	4.82 ± 2.5	-	-	4.82 ± 2.5	8.5 ± 0.55	10.5 ± 1.15	-	-	5.02 ± 2.05									

extracts and types of organism is listed in Table 1 and demonstrated in Fig. 1.

All commercial antimicrobial agents admitted to the work reveal a verity of sensitivity and resistance against the used microorganism. Lincomycin is the only one that has killing action on all used organisms as described in Table 2.

The minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extracts against the six bacteria ranged from 625 to 5000 µg/ml. *S. aureus* reveals MIC value of 1250, 5000, 625 and 625 µg/ml for *Psidium guajava*, *Salvia officinalis*, *Ziziphusspina christi* and *Olea europaea L*, while MBC value of the extracts for *S. aureus* is 1250, 1250, 625 and 625 µg/ml in the same order. The MIC value for *B. cereus* at examination of *Salvia officinalis*, *Ziziphusspina christi*, *Morus alba* and *Olea europaea L* is 625, 625, 2500 and 5000 µg/ml, while the MBC value for the same organism and the same extracts is 625, 625, 1250 and 2500 µg/ml. The MIC and MBC of *Psidium guajava* at examination against *E. coli*, *Salmonella Enteritidis*, *Pasteurella multocida* and *Mycoplasma gallisepticum* are 625, 625; 625, 625; 5000, 2500 and 1250, 625 µg/ml respectively. The MIC and MBC of *Salvia officinalis* for *E. coli* and *Salmonella Enteritidis* are 2500, 2500; 2500, 1250 µg/ml, while The MIC and MBC of *Ziziphusspina christi* are 625 and 625 µg/ml for *Salmonella Enteritidis* in the same order, the MIC and MBC value for *Morus alba* against *Salmonella Enteritidis*, *P. multocida* and *M. gallisepticum* are 625; 1250 and 625 µg/ml. Finally the MIC and MBC of *Olea europaea* against *E. coli*, *Salmonella Enteritidis* and *Pasteurella multocida* are 250, 1250; 5000, 2500 and 625, 625 µg/ml (Table 3) and demonstrated in Fig. 2.

4. Discussion

Phytochemical screening revealed exist several classes of secondary metabolites such as alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, saponins, tannins, triterpenes and steroids. Several molecules are active on pathogenic microorganisms (Cowan, 1999; Awouafack et al., 2013; Tsopmo et al., 2013; Erfan and Marouf, 2019). Exist such metabolites in the tested plant extracts can give a preliminary explanation on their antimicrobial antibacterial activities. Differences were observed in the antibacterial activities of the extracts. These could be due to the differences in their chemical composition as well as in the mechanism of action of their bioactive constituents (Cowan, 1999). All the extracts are rich in secondary metabolites; however, activity does not depend only exist secondary metabolites in the plant extracts, but also on their concentration and the possible interaction with other components (Dzotam et al., 2016). The antibacterial effect for tannins attributed to its ability to react with proteins to form stable water-insoluble components since bacterial cell wall made from proteins (Dangoggo et al., 2012). In addition, it may bind to proline – rich protein and interfere with the protein synthesis (Shimada, 2006), also the antimicrobial activity of saponins rendered to cause leakage of proteins and certain enzymes from cell (detergent like properties). The antibacterial effect of alkaloids due to its ability to interchelate with DNA of both Gram positive and negative bacteria and interfere with cell division (Bukar et al., 2015), while flavonoids activity is due to their ability to bind with intracellular and soluble proteins and to bind with bacterial cell walls and the antibacterial properties of steroids are due to complex with membrane lipids and exerts its action by causing leakage (Marjorie, 1999). The problem of microbial resistance is growing by time and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to face this problem and to introduce the antibacterial activity of medicinal plants (Cowan, 1999; Nostro et al., 2000; Osman et al., 2013; Osman et al., 2014a, 2014b, 2014c; Wagdy et al., 2016;

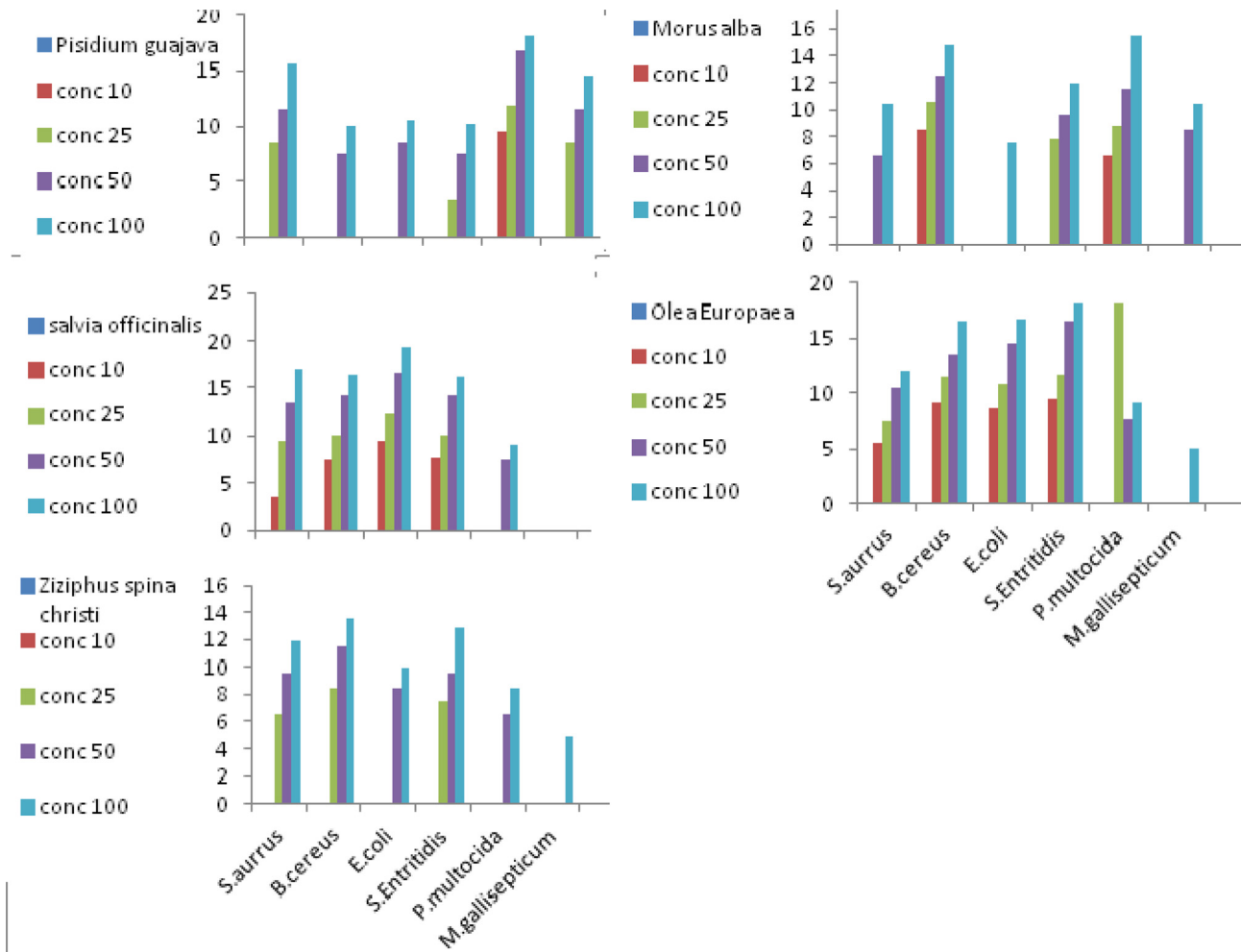


Fig. 1. Antimicrobial activity of examined plant extracts on six microorganism using disk diffusion method.

Table 2

Antimicrobial activity of commercial antimicrobial agentson six microorganism using disk diffusion method:

organism	Inhibition zone of extract (mm)						
	Amoxicillin-Clavulinic (10 µg)	Ampicillin (10 µg)	Vancomycin (30 µg)	Lincomycin (30 µg)	Gentamicin (10 µg)	Streptomycin (10 µg)	Tetracycline (30 µg)
<i>S. aureus</i>	25 ± 0.71 (S)	13.0 ± 0.4 (R)	17.0 ± 0.4(S)	27.0 ± 0.65 (S)	10.0 ± 0.23 (R)	12.0 ± 1.15 (R)	11.0 ± 0.71 (R)
<i>B. cereus</i>	23.0 ± 0.45 (S)	11.0 ± 1.25 (R)	18.0 ± 1.15 (S)	25.0 ± 0.02 (S)	10.0 ± 0.54 (R)	10.0 ± 0.71 (R)	17.0 ± 0.49 (I)
<i>E. coli</i>	17.0 ± 1.15 (I)	10.0 ± 0.71 (R)	19.0 ± 0.25 (S)	21.0 ± 0.25 (S)	21.0 ± 0.4 (S)	15.0 ± 0.75 (I)	10.0 ± 1.15 (R)
<i>S. Enteritidis</i>	16.0 ± 0.25 (I)	9.0 ± 1.15 (R)	19.0 ± 0.75 (S)	20.0 ± 1.15 (S)	22.0 ± 0.69 (S)	19.0 ± 0.4 (S)	13.0 ± 0.36 (S)
<i>P. multocida</i>	23.0 ± 1.05 (S)	8.0 ± 0.25 (R)	10.0 ± 0.55 (R)	17.0 ± 0.4 (I)	9.0 ± 0.71 (R)	11.0 ± 0.25 (R)	18.0 ± 0.25 (I)
<i>M. gallisepticum</i>	15 ± 1.15 (I)	10.0 ± 0.85 (R)	11.0 ± 1.25 (R)	21.0 ± 0.89 (S)	8.0 ± 0.46 (R)	11.0 ± 1.25 (R)	16.0 ± 0.4 (I)

S = Sensitive I = Intermediate R = Resistant.

Table 3

Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of examined plant extracts on six microorganism:

Organism	<i>Psidiumguajava</i>		<i>Salvia officinalis</i>		<i>Ziziphusspinachristi</i>		<i>Morus alba</i>		<i>OleaEuropaea</i>	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>S. aureus</i>	1250.00	1250.00	5000.00	1250.00	625.00	625.00	–	–	625.00	625.00
<i>B. cereus</i>	–	–	625.00	625.00	625.00	625.00	2500.00	1250.00	5000.00	2500.00
<i>E. coli</i>	625.00	625.00	2500.00	2500.00	–	–	–	–	2500.00	1250.00
<i>S. Enteritidis</i>	625.00	625.00	2500.00	1250.00	625.00	625.00	625.00	625.00	5000.00	2500.00
<i>P. multocida</i>	5000.00	2500.00	–	–	–	–	1250.00	1250.00	625.00	625.00
<i>M. gallisepticum</i>	1250.00	625.00	–	–	–	–	625.00	625.00	–	–

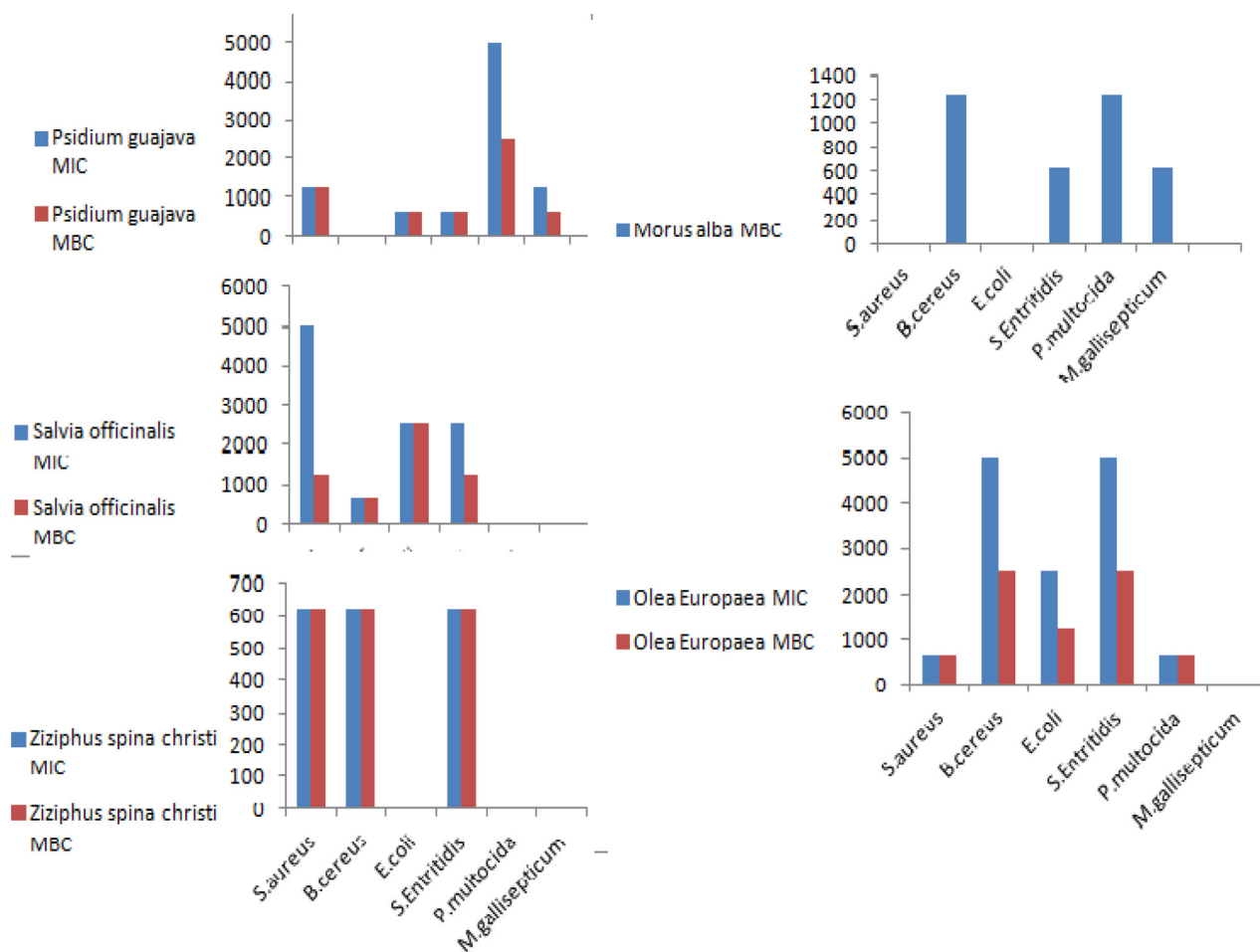


Fig. 2. Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of examined plant extracts on six microorganism.

Abdel Halium et al., 2019; Mohamed et al., 2019; Ibrahim et al., 2019). In this study the antibacterial findings of *Psidium guajava* extract at different concentration have that able to inhibit the growth of *S. aureus*, *Pasteurella multocida* and *Mycoplasma gallisepticum* that correlated with the findings of Concalves et al. (2008), who studied guava extracts against food born pathogen and spoilage bacteria that due to the phenolic components which make them effective against the tested microorganisms. This result confirmed by Malaviya and Mishra (2011) and these observations matched also with that of the findings of Hogue et al. (2007) who showed the antibacterial activity of guava leaf extracts based on how the phenolic components act particularly flavonoids. These observations also agreed with Ismail et al. (2012) who exhibited the antibacterial activity of guava against food borne pathogens. According to the study the extracts of *Ziziphusspina christi* of all parts act as antibacterial agent against the tested pathogenic bacterial strains. This activity attributed to exist tannins and leucocyanidin (Rizk et al., 1993), also due to exist other active components like saponins, flavonoids, steroids, glycosides (Lee, 2006; Lam, 2007). This result agreed with results recorded by Elkamali and Mahjoub 2009. Who recorded the seed extracts was effective only against three bacterial strains. The investigation also agreed with Al-Mutairi et al. (2016) who recorded that ethanol and methanol leaves extracts also were effective against most tested strains. The antibacterial activity of *S. officinalis* extract against *S. aureus*, *E. coli* and *Salmonella* Enteritidis, confirming results already reported by Velckovic et al. (2003) who found that sage ethanol extract possesses antibacterial activity against *S. aureus*, *B. subtilis*,

E. coli and *Salmonella* Enteritidis. Lai and Roy (2004) also reported that sage thanolic extract is effective against *B. cereus*, *S. aureus* and *Vibrio parahaemolyticus*. Availability of some compounds like α -pienene, camphor, 1,8-cineole, borneol in *salvia officinalis* shows that antibacterial effect attributed to these compounds (Knobloch et al., 1989). Investigation of *Morus alba* extract show antimicrobial potency against *B. cereus* and *Pasteurella multocida*, of the same with Sunil and Ammani (2009) who found that *Morus alba* ethanolic extract showed antibacterial activity against 15 bacterial and fungal spp. and also confirmed by Mohamed et al. (2013) who recorded that the most active extracts observed by *M. alba* against Gram positive and Gram negative bacteria. That activity may due to the plant is rich in phyto constituent slike; tannins, phytosterols, sitosterols, saponins, anthroquinones, glycosides and Oleanolic Compounds (Chen et al., 2005). The present results showed a good inhibitory effects olive extract on pathogenic bacteria. Many studies confirm the positive role of olive extracts in inhibitory pathogenic bacteria. Such as, Markinet al. (2003) who reported that water extract of olive leaf killed *E. coli*, *P. aeruginosa* *S. aureus* and *K. pnebdmonia* and *B. subtilis*. In another study, Korukluoglu et al. (2010) investigated the effect of the extraction solvent on the antimicrobial efficiency of *S. aureus*, *E. coli*, *Salmonella* Enteritidis, *Salmonella* Typhimurium, they also recorded that ethanol olive extracts showed the highest antimicrobial activity against *E. coli* and *Salmonella* Enteritidis. Papers about the antimicrobial properties of olive refer to compounds obtained from olive fruit particularly hydroxytyrosol and oleuropein (Bisignano et al., 1999).

5. Conclusion

The present investigation reveals a real solution for antimicrobial resistance by using a natural herbal extracts. The five above mentioned plant extracts have a powerful antimicrobial activity that inhibit or at less stop the growth of six microbial populations of different bacterial categories; so those extract may be used as food additives and food preservatives to control such microbial population and conserve human and animal health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Deanship of Scientific Research at King Saud University through the research group project No.: RG-162.

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