International Journal of Scientific Research in Multidisciplinary Studies
Vol.6, Issue.10, pp.14-18, October (2020)

Research Paper
E-ISSN: 2454-9312
P-ISSN: 2454-6143

Antibacterial Activity of *Phlomis Floccosa* and *Marrubium Alysson* on Growth of Some Pathogenic Bacteria

H.I. IL Fergani[1], M.A. Alteerah[2*], Y.M. Adim[3], M.M. Alareefi[4], E.M. Elshikhi[5]

[1,2,3,4,5]Dept. of Botany, faculty of science, university of Benghazi, Benghazi, Libya

[*]*Corresponding Author: munay.abdulqadir@uob.edu.ly*

Available online at: www.isroset.org

Received: 27/Sept/2020, Accepted: 17/Oct/2020, Online: 31/Oct/2020

***Abstract*—** The inhibitory effect of an aqueous and a methanolic extract of *Phlomis floccosa* leaves and vegetative parts of *Marrubium alysson* were tested on growth of some pathogenic bacterial strains: *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus pyogenes* and *Staphylococcus aureus*. After incubation for 24 hours at 37 °C, using a well diffusion method and comparing their inhibitory effect with the capacity of many antibiotics: Ampicillin, Erythromycin, Amoikacin and Amikacin. The alcoholic extract of *Phlomis floccose* showed remarkable inhibition on 3 species of bacteria, and the efficacy increased by increasing of concentrations. At 100% concentration showed the highest inhibitory capacity on each *St. pyogenes*, *S. aureus*, and *Kl. Pneumonia* with no inhibiting effect on *E. coli*. The alcoholic extract of *Marrubium alysson* showed remarkable inhibition activity against both St. pyogenes & S. aureus with no inhibitory effect on *K. pneumonia* & *E. coli.* The both plants aqueous extract did not show any inhibition effect on bacteria under study. The minimum inhibition concentration of the methanol extract on *St. pyogenes* was at 25%, on S. aureus was at 50%, on *K. Pneumonia* was at 100%, while there was no inhibition effect on *E. coli*. The antimicrobial activity of *Ph. floccosa* exceeded the antibiotic effect of AK 30mcg, AM 10mcg and Ax 20 mcg on S. aureus, while E 30 mcg exceeded plant extracts activity for the same bacteria. AK 30mcg effected highly on *E. coli*, while the highest concentration of *M. alysson* extract exceeded E 30mcg activity on *St. pyogenes*. This study result indicated to the rich valuable medicine compound in *Marrubium alysson* and *Phlomis floccosa* which have inhibitory impact on pathogenic bacteria.

Keywords—Antibacterial activity, Plant extract, Medical plants, Phlomis floccosa, Marrubium alysson

I. INTRODUCTION

In our local community, dozens of different plants are traded as an alternative treatment for many diseases. The oldest Arab researchers have sought to extract the active compounds of medicinal plants. This has contributed to facilitate pharmaceutical studies by synthesis more effective drugs with low toxicity. Although there is a large number of innovative researches in this range, there are many plants in different areas in the Arab world, have not been studied in details, because hundreds of researches were carried out by scientists are scattered and lack to the integrated natural sequence of all the stages required by the pharmaceutical research. So, they can be used in the discovery of new and useful medicines [1].

Scientific researches in recent years have been interested in trying to discover safe substances for treatment of human and animal diseases away from various chemicals. Due to the importance of medicinal plants and aromatic herbs, about 80% of the developing countries population depends on the popular medical plants for their basic health care [2]. Combating the indiscriminate use of the commercial chemical drugs has been considered by successful treatments for many diseases using medicinal plants [3], [4]. Therefore, many scientists thought of trying to return to these vital sources of medicines, as they are more safe. This confirmed by the 14th Conference of Botanists held in Berlin in 1987, which recommended focusing on the need to increase attention to medicinal plants and preserve them from extinction. Their extracts are used as therapeutic substances for diseases that could not treated by chemical medicines [5] and now about 25% of the drugs prescribed in the world originate from plants. Libya possesses a wide variety of medicinal plants and herbs, which are known for their therapeutic ability and their effect on the growth of many pathogenic bacteria. Due to diseases and epidemics caused by microbes, these studies was started, which aimed to use Libyan medicinal plants to provide a number of active substances from natural sources to be used in the pharmaceutical industry as natural antibiotics and antioxidants. These natural products are concerned as a base for the modern drugs we use today.

In recent years, resistant to pathogenic microorganisms for both humans and plants has been studied by successful treatments for many diseases using medicinal plants [6]. The resistance of pathogenic bacteria to antibiotics is due to their ability to form a biosphere [7] which has increased their ability to resist antibiotics. The indiscriminate use of commercial antibiotics has increased microbial resistance to these antibiotics, encouraging scientists to look for new

antimicrobial substances from various sources, including medicinal plants [8].

Based on previuos studies, *Phlomis floccosa* contain on phenolic compound, and alkaloids [9]. *Marrubium alysson* contain on alkaloids such as Marrubiin, and essential oils. This study aimed on test the effect of two medicinal plants in the Libya that are known for their use in traditional medicine, *Phlomis floccosa* and *Marrubium alysson* and their inhibition of the growth of some types of pathogenic bacteria and comparing them with the inhibition ability of antibiotics.

II. METHODOLOGY

Plants sample collection and extract preparation

Plants samples were collected from two cities in Libya, *Phlomis floccosa* collected from Farzogha on January 2018, while *Marrubium alysson* collected from Benghazi on December 2017. The samples were cleaned and purified from all other plant residues, clay granules and suspended sand. The infected and damaged parts were removed. The samples dried and stored away from moisture until they were used. 50g of each dried plant sample was extracted by 250 ml of solvent in clean and sterile conical flasks. Water and methanol were used as solvents to obtain medicinal plant extracts. The flasks were put on shaker for 24 hours.

Plant extracts were filtered by filter paper. The aqueous extract was concentrated by fumigating the solvent in a water bath at 40°C for 6 hours and was kept in the refrigerator until used. While the methanol extract was concentrated in the fume hood until the evaporation of alcohol in order to obtain concentrated plant extracts. The aqueous extract was dissolved in distilled water, but the methanol extract was dissolved in Dimethyl sulfoxide (DMSO).

Bacterial strains

Four pure isolated strains of pathogenic bacteria were achieved from the Benghazi Children's hospital and identified by phoenix device. *Eschericia coli* & *Klebsiella pneumonia* are gram negative bacteria, while *Streptococcus pyogenes* & *Staphylococcus aureus* are gram positive bacteria. The bacteria were kept on nutrient agar and stored in the refrigerator at 4°C until use. The bacteria were cultured on nutrient agar and Muller Hinton agar, then testing of bacteria's sensitivity to plant extracts was performed.

Antibacterial assay

Effects of the plant extracts on bacterial growth was studied by a well diffusion method [10]. Activation was done for bacterial strains and preserved in the nutrient agar by replanting them to get spread individual colonies. 100µ of the plant extract was injected on the agar medium. Control was done by creating pits saturated with the original solvent (water, methanol) and DMSO as well, then incubated at 37°C for 24 hours. After the end of the incubation period, the results were recorded by measuring the diameter of the inhibition zone in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

To determine the lowest inhibitory concentration of plant extracts on pathogenic bacterial strains, alcohol extract dilutions were prepared 10% ,30%, 35%, 45%, 80%, 90% and 95% by DMSO. The minimum concentration of MIC inhibitor was then determined for the different concentrations of the plant extract.

Effects of the Antibiotics on bacterial growth

Sensitivity for antibiotic was tested using four types of antibiotics. Muller Hinton agar medium was injected by the bacterial suspension. The antibiotics were then fixed by sterile tongs. The petri dishes incubated at 37°C for 24 hours, the inhibition zone is measured in (mm).

Statistical analysis

One Way ANOVA was used to analyze the data at ($p \leq 0.05$) by Minitab® 16 software.

III. RESULTS AND DISCUSSION

Antimicrobial activity of Phlomis floccosa and Marrubium alysson extract

The inhibitory effect of plant extracts on bacterial growth using well diffusion method are shown in tables 1& 2. The results confirmed that the inhibitory effects on bacterial growth is due to the active chemicals extracted from plants and not from solvents used in the extraction process. The following is an explanation of the effect of various plant extracts on inhibiting the growth of bacterial strains.

Effect of Marrubium alysson leaves extract on bacterial growth

The results showed in Table 1, the effect of *Marrubium alysson* aqueous and methanol extracts in four concentrations 25%, 50%, 75%, and 100% on growth of the various bacterial strains. There is no a significant difference between different concentrations of *Marrubium alysson* for its effect on bacterial species ($p > 0.05$). The aqueous extract did not effect on the growth of all bacterial strains. While the methanol extract caused an inhibitory effect in average (10-15 mm) on *Staphylococcus aureus* at 50% and 75% concentrations and a strong effect (15-20 mm) at 100% concentration and no results at 25% concentration. These findings are agreed with study done by [11] on *Marrubium vulgare* and its effect on inhibition on *Staphyloccocus aureus*. The methanol extract exhibited inhibition on *Streptococcus pyogenes* in all concentrations, where it caused a weak effect (7-10 mm) in both concentrations 25% and 75% and a medium effect (10-15 mm) in both concentrations 50% and 100%.

Resistance of the gram-negative bacteria (*klebsiella pneumonia* and *Escherichia coli*) to the effect of methanol extract was observed as no results were shown. These results did not agree with a similar study carried out by [12] on the effect of *Marrubium alysson* methanol and other solvents extracts on *klebsiella pneumonia* and *E. coli* which showed inhibitory potency against them.

Table 1: The inhibition zone in mm of the aqueous and methanol extract of *Marrubium alysson* on the bacterial strains growth. The diameters of the inhibition zone measured in (mm), (-) no inhibition, (+) medium effect (10-15mm), (+++) strong effect (15-20mm), (++++) very strong effect (20-30mm).

Solvents	Aqueous extract concentration				Methanol extract concentrations			
Bacterial strains	25%	50%	75%	100%	25%	50%	75%	100%
Staphylococcus aureus	–	–	–	–	–	++	++	+++
Escherichia. Coli	–	–	–	–	–	–	–	–
Klebsiella pneumonia	–	–	–	–	–	–	–	–
Streptococcus pyogenes	–	–	–	–	+	++	+	++

The minimum inhibiting concentration of *Marrubium alysson* methanol extracts on growth of positive and negative bacterial strains was determined. MIC methanol extract on *Staphylococcus aureus* was 50% while on *Streptococcus pyogenes* was 25%.

Effect of Phlomis floccosa leaves extract on bacterial growth

The results showed in Table 2, the effect of *Phlomis floccosa* aqueous and methanol extracts in four concentrations 25%, 50%, 75%, and 100% on growth of the various bacterial strains. One Way ANOVA test showed significant differences in *Phlomis floccosa* effect on bacterial strains used in the experiment ($p< 0.05$). The aqueous extract did not effect on the growth of all bacterial strains as well. While the methanol extract caused a strong inhibitory effect in average (15-20 mm) on *Staphylococcus aureus* for concentration 25%, and very strong inhibitory effect in average (20 -30 mm) for concentrations 50%, 75%, and 100%.

The methanol extract did not exhibit any effects on *Streptococcus pyogenes* with concentration of 25%, while it exhibited a medium effect (10-15 mm) for concentration 50%, and a strong effect (15-20 mm) for concentration 75% and 100%. This extract exhibited a medium effect (10-15mm) on *Klebsiella pneumonia* at 100% concentration while it did not give any inhibitory effects at 25%, 50%, 75% concentrations.

Resistance of the gram-negative bacteria (*Escherichia coli*) was observed for the methanol extract, where no results were shown. In a similar study of the same plants carried out by [13]. *E. coli* as gram-negative bacteria, their cells are surrounded by a second membrane, the outer one functions as an effective barrier [14].

The minimum inhibiting concentration of *Phlomis floccosa* methanol extracts on growth of positive and negative bacterial strains was determined. MIC of the methanol extract on *Staphylococcus aureus* was 25%, while on Streptococcus pyogenes was 50%, and on the growth of *Klebsiella pneumonia* was 100%. *Klebsiella pneumonia* is protected by capsular polysaccharides that may explain its resistance to the low concentration of the methanol extract.

Table 2: The inhibition zone in mm of the aqueous and methanol extract of *Phlomis floccosa* on the bacterial strains growth. The diameters of the inhibition zone measured in (mm), (-) no inhibition, (+) medium effect (10-15mm), (+++) strong effect (15-20mm), (++++) very strong effect (20-30mm).

solvents	Aqueous extract concentration				Methanol extract concentration			
Bacterial strains	25%	50%	75%	100%	25%	50%	75%	100%
Staphylococcus aureus	_	_	_	_	+++	++++	++++	++++
Escherichia. Coli	_	_	_	_	_	_	_	_
Klebsiella pneumonia	_	_	_	_	_	_	_	++
streptococcus pyogenes	_	_	_	_	_	++	+++	+++

Effects of the antibiotics on bacterial growth

E. coli were mostly sensitive to AK 30 mcg, while were not any effects from others. *Staphylococcus aureus* were highly sensitive to all antibiotics. *Klebsiella pneumonia* was more sensitive to AK 30mcg. *Streptococcus pyogenes* was moderately affected by all antibiotics.

Table 3: Effects of different antibiotics on pathogenic bacterial growth. The diameters of the inhibition zone measured in (mm), (-) no inhibition, (+) medium effect (10-15mm), (+++) strong effect (15-20mm), (++++) very strong effect (20-30mm).

Antibiotic Bacterial strains	Amikacin 30mcg (AK)	Ampicillin 10mcg (AM)	Erythromycin 30mcg (E)	Amoxicillin 25mcg (AX)
Staphylococcus aureus	+++	+	+++	++
Escherichia. Coli	+++	-	-	-
Klebsiella pneumonia	+++	+	+	-
streptococcus pyogenes	+	+	+	+

Comparisons between the antibacterial activity of the both plants and the different antibiotics

From table 2 &3 can be observe clearly that the methanol extract of *Phlomis floccosa* was more inhibitory than the different antibiotics on *Streptococcus pyogenes*. All the methanol extract concentrations inhibit the growth of Staphylococcus aureus more than AM 10mcg & AX 25mcg. and the 100% concentrations of inhibit the growth of *Klebsiella pneumonia* more than AM 10mcg, E20 mcg &AX 25 mcg. *Phlomis floccosa* is used traditionally for treatment of boils and infections, which can be caused by *Staphylococcus aureus*.

From table 1 &3 can be observe also that the methanol extract of *Marrubium alysson* was more inhibitory than AM 10mcg antibiotics on the growth of *Staphylococcus aureus*. From these results, can be concluded that both *Phlomis floccosa* and *Marrubium alysson* are not much different in their antibacterial impact. These result is confirmed with previous studies that plant extracts are more active against gram-positive bacteria than gram-negative bacteria [15], [16].

IV. CONCLUSION AND FUTURE SCOPE

Using of natural herbal products for some diseases treatments, become developing alternative to antibiotics. The current study results indicated to the rich valuable medicine compounds in *Marrubium alysson* and *Phlomis floccosa* which have inhibitory impact on pathogenic bacteria, especially on the gram-positive bacteria. Because of resistance of bacteria to some of antibiotics, it has become very important to care about using of medicine plants. This study recommends to do more detailed research on the chemical composition of the both plants.

REFERENCES

[1] H. Shuhaireen & M. Hanafiah, study on acetylcholinesterase inhibitor derived from Datura metel L, Doctoral dissertation, University of Malaya, **2017**.

[2] S. J. Uddin, I. D. Grice and E. Tiralongo, "Cytotoxic Effect of Bangladeshi Medicinal Plant Extracts". Evidence -Based Complementary and Alternative Medicine, vol. **2011**, pp. **1-7, 2011**.

[3] B. Joshi, G. P. Sah, B. B. Basnet, M. R. Bhatt, D. Sharma, K. Subedi & R. Malla, "Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem)". J. Microbiol. Antimicrob, vol. **3**, no. **1**, pp. **1-7**, **2011.**

[4] N. C. C. Silva & A. Fernandes Júnior, "Biological properties of medicinal plants: a review of their antimicrobial activity", Journal of venomous Animals and Toxins including tropical diseases, vol. **16**, no. **3**, pp. **402-413, 2010.**

[5] J. Parekh, D. Jadeja & S. Chanda, "Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity", Turkish Journal of Biology, vol. **29**, no.**4**, pp. **203-210, 2006.**

[6] A. Sen & A. Batra, "Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L". Int J Curr Pharm Res, vol. **4**, no.**2**, pp. **67-73, 2012.**

[7] S.F. Leibirsh, M. A. Alteerah, M. M. Godeh, "Antibacterial Effects of Brown Algae Extracts of Cystoseira Sp. Collected from Tukra Beach, Mediterranean Sea Coast of Libya", International Journal of Scientific Research in Biological Sciences, vol.**7,** no.**4**, pp.**67-70**, **2020.**

[8] M. Ahanjan, J. Ghaffari & F. M. Hagi, "Antibacterial Activity and Chemical Composition of Medicinal Plant Satureja bakhtiarica Bung against Multi Drug-Resistant Acinetobacter baumannii [ESBL]". Iranian Journal of Public Health, vol.**43**, no.**2**. pp.**105, 2014.**

[9] Y. Zhang and Z.Z. Wang, "Comparative analysis of essential oil - components of three Phlomis species in Qinling Mountains of China", Journal of pharmaceutical and biomedical analysis, vol.**47**, no.**1**, pp. **213–217, 2008.**

[10] A. Daud, A. Gallo & A. S. Riera, "Antimicrobial properties of *Phrygilanthus acutifolius"*. Journal of ethnopharmacology, vol.**99**, no.**2**, pp.**193-197,2005**.

[11] M. Bokaeian, E. Saboori, S. Saeidi, A. A. Niazi, N. Amini-Borojeni, H. Khaje & S. Bazi, "Phytochemical Analysis, Antibacterial Activity of Marrubium vulgare L against Staphylococcus aureus in vitro". Zahedan J Res Med Sci, vol.**16**, no.**10**, pp. **60-64, 2014**.

[12] E. Hayet, A. Samia, G. Patrick, M. Mohamed, M. Maha, G. Lauann, Z. Mighri and A. Mahjoub, "Antimicrobial and cytotoxic Activity of Marrubium alysson and Retama raetam Grown in Tunisia", Pakistan Joumal of Biological Sciences, vol.**10** no.**10**,pp. **1759-1762, 2007.**

[13] Z. Ulukanlı, and A. Akkaya, "Antibacterial activities of Marrubium catariifolium and Phlomis pungens var. hirta grown wild in Eastern Anatolia", Turkey. Int. J. Agric. Biol., vol.**13**, no.**1,** pp. **105–109, 2011**.

[14] N. Atef, S. M. Shanab, S. I. Negm & Y. A. Abbas, "Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection". Bulletin of the National Research Centre, vol.**43**, no.**144**, pp. **1-11, 2019**.

[15] E. M. Tekwu, A. C. Pieme & V. P. Beng, "Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance", Journal of ethnopharmacology, vol.**142**, no.**1**, pp.**265-273, 2012.**

[16] A. Klančnik, S. Piskernik, B. Jeršek & S. S. Možina, "Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts", Journal of microbiological methods, vol.**81**, no.**2**, pp. **121-126, 2010**.

AUTHORS PROFILE

Dr. Hanan Ishtiwi IL Fergani
Bachelor from Botany department faculty of science, University of Benghazi in 2008, master of botany from Botany department, University of Benghazi in 2016. Member in staff teaching since 2017.

Dr. Munay A. Alteerah
Bachelor and master degree from Botany department, University of Benghazi in 2004, 2010, PhD from University Putra Malaysia in 2017. She is working as lecture in botany department, university of Benghazi since 2018.

Dr. Yusra Mohammed Adim
Bachelor from Botany department faculty of science, University of Benghazi in 2008, master of botany from Botany department, University of Benghazi in 2016. Member in staff teaching in botany epartment, university of Benghazi since 2017.

Ms. Malak Murad Alareefi
Bachelor from Botany department faculty of science, University of Benghazi in 2018

Ms. Embarka Muftah Elshikhi
Bachelor from Botany department faculty of science, University of Benghazi in 20018

