

## ANTI-BACTERIAL ACTIVITIES OF SOME ACTIVE CONSTITUENTS ISOLATED FROM *PHLOMIS* *FLOCCOSA* D. DON.

Rehab A. Lotfy and Heba I. Abd El-Moaty\*

Department of Medicinal and Aromatic Plants, Desert Research  
Center El-Matareya, Cairo, Egypt

\*E-mail: torkeyheba@yahoo.com

The present study aimed to evaluate the anti-bacterial activities (*in vitro*) of the total extract of 70% methanol and successive extracts of different organic solvents (petroleum ether, chloroform, methanol, 50% methanol extracts), and their isolated compounds of the vegetative parts and flowers of *Phlomis floccosa* against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus* spp. and *Staphylococcus aureus*. Where the best antibacterial active extract for vegetative parts was chloroform, while it was petroleum ether extract for flowers. Isolation and purification of the major compounds of the active fractions concluded that, 7, 3', 4'-trimethoxy quercetin (flavonoid) and hahnfett (hydrocarbon) were detected in the vegetative parts, meanwhile, stewertiisin B (sesquiterpene) was detected in the flowers. The three isolated compounds showed active inhibition of the bacterial growth.

**Keywords:** *Phlomis floccosa*, flavonoid, hydrocarbons, sesquiterpene, antibacterial activities

Plant extracts constitute a natural source of antimicrobial mixtures or pure compounds for centuries. The purified components are used as natural antimicrobials in food systems, as well as to prevent the growth of food-borne bacteria and molds, resulting in extension of the shelf life of processed foods (Kalemba and Kunicka, 2003 and Burt, 2004). *Phlomis* species are explained by dioscorides as herbal medicines, and are in practice ethnopharmacological in herbal drugs for respiratory tract ailments and for local healing of injuries (Bucar et al., 1998). Some *Phlomis* species are used in folk medicine for their analgesic and antidiarrheal properties, and for the treatment of ulcers and hemorrhoids (Kirmizibekmez et al., 2005). There are few reports about the pharmacological and biological effects of *Phlomis*, some studies have shown various activities, such as anti-inflammatory, immunosuppressive, antimutagenic, anti-nociceptive, antifibriel, free radical scavenging, anti-malarial, and anti-microbial effects (Sarkhail et al., 2006).

Different classes of glycosides comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids have been identified from the genus *Phlomis*. Many of these phenylpropanoids showed significant biological activities, such as cytotoxic, cytostatic, antiinflammatory, immuno-suppressant and antimicrobial (Kamel et al., 2000 and Ben Amor et al., 2009), while Jabeen et al. (2013) isolated four new compounds (stewartiiside, stewartiisin A, stewartiisin B and stewartiisin C) and nine known compounds from *Phlomis stewartii*. The leaves of *Phlomis aurea* contained the 7-glucosides, 7-rutinosides and 7-*p*-coumaroylglucosides of naringenin, apigenin, luteolin and chrysoeriol, hispidulin 7-glucoside, luteolin 7-diglucoside, vicenin-2 and lucenin-2. The microscopic hairs on the leaves only contained the 7-monoglucosides and their acylated derivatives. *Phlomis floccosa* showed a similar flavonoid pattern, but with no flavanones (El-Negoumy et al., 1986).

## MATERIALS AND METHODS

### 1. Plant Material

The vegetative parts and flowers of *Phlomis floccosa* were collected at full flowering stage from Mersa Matruh during April 2011. The plant was identified in the Herbarium of the Desert Research Centre. They were air dried, then ground to fine powder and kept to be used for analyses.

### 2. Extraction of Total Extract

About 80 gram from each of the vegetative parts and the flowers were extracted separately with 70% methanol. The obtained residue from each part was dried and weighed.

### 3. Extraction Using Different Organic Solvents

#### Successive extraction technique

About 700 gram of the vegetative parts and 250 gram of the flowers were subjected to extraction with successive organic solvents using Soxhlet apparatus, in order to increase polarity, including petroleum ether (b.p. 40-60 °C), chloroform, methanol and 50% methanol. The obtained residue from each solvent was dried and weighed.

### 4. Antibacterial Activity of Different Extracts

Under aseptic conditions, the antibacterial activity of total extract, petroleum ether, chloroform, methanol and 50% methanol extracts (100 µg/ml), of the aerial parts and flowers of *Phlomis floccosa*, as well as the

three isolated compounds (25 µg/ml) were carried out by the cup diffusion method (Vivek et al., 2010). For preparation of bacterial inocula, the bacterial density was adjusted to approximately  $10^8$  colony forming units (CFU) per ml (optical density was adjusted to 0.5 McFarland turbidity). Bacterial suspension was spread over the plates containing Mueller-Hinton agar, using a sterile cotton swab, in three directions in order to get a uniform microbial growth (Kateryna and Mahendra, 2012). Three wells of 5 mm diameter each were made in agar Petri plate of the solidified agar medium using sterilized cork borer. All plates were then incubated at 37°C for 24 hours. After incubation, average diameters of inhibition zones around the discs were measured in mm (Bülent et al., 2011 and Hanene et al., 2013).

### 5. Tested Bacteria

Four clinical isolates of bacterial strains, were isolated from El-Demerdash hospital patients. They were identified and used for investigating the antibacterial activity of the plant extracts and isolated compounds, including *Escherichia coli*, *Klebsiella pneumonia*, *Proteus spp.* and *Staphylococcus aureus*.

### 6. Separation and Identification of Active Constituents

The most antibacterial active fractions of vegetative parts and flowers (chloroform and petroleum ether extracts, respectively) were applied separately on the top of a Sephadex LH- 20 column (A and B, respectively). Elution was started with pure ethanol, followed by a mixture of ethanol/water and finally distilled water was applied. The received fractions were evaporated and subjected to TLC using the solvent system chloroform: methanol (9: 1 v: v) where similar fractions were collected together.

The column (A) from the vegetative parts led to two main fractions (I and II). Fraction (I) contained one spot only represented compound (1). Fraction (II) reapplied to a Sephadex LH- 20 column using methanol as eluent, this led to isolation of compound (2). While, column (B) from the flowers led to isolation of one pure compound (3).

### 7. Identification of Isolated Compounds

The NMR measurements were carried out on Bruker High Performance Digital FT-NMR Spectrometer Advanced III 400 MHz (270 MHz for  $^1\text{H}$ -NMR and 67.5 MHz for  $^{13}\text{C}$ -NMR).

## RESULTS AND DISCUSSION

### 1. Extraction of Total Extract

The obtained data showed that, total extracts (70% methanol) of vegetative parts and flowers were 23.34 and 14.62%, respectively.

### 2. Successive Extraction Using Different Organic Solvents

Data presented at table (1) showed that, methanol extracts had the highest values (10.11 and 7.51%) obtained from vegetation parts and flowers, respectively.

**Table (1).** Successive extraction residues (%) of vegetative parts and flowers of *Phlomis floccosa* D. Don.

Solvent used	Residue percentage (%)	
	Vegetative parts	Flowers
Petroleum ether (b.p. 40-60 °C)	5.94	2.40
Chloroform	2.21	1.50
Methanol	10.11	7.51
Methanol 50%	5.84	4.95

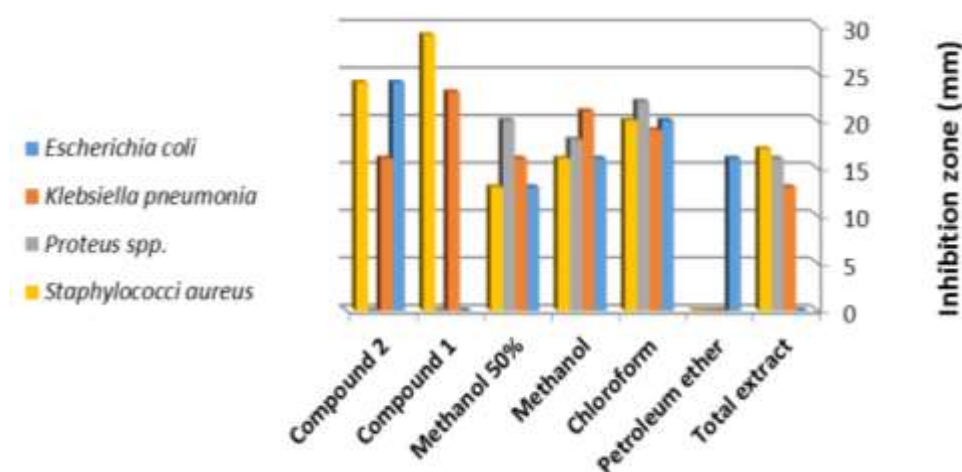
### 3. Anti-Bacterial Activity

Table (2) showed that, the most antibacterial active extracts were chloroform extract of the aerial parts and petroleum ether extract of the flowers. These revealed the isolation of flavonoid and hydrocarbon compounds from chloroform extract and one sesquiterpene from petroleum ether extract. The antibacterial activity of the isolated compounds as shown in fig. (1) indicated that, compounds (1) isolated from the vegetative parts inhibited the bacterial growth of both *Escherichia coli* and *Staphylococci aureus*, however it was inactive against the other two bacterial strains. It could also be concluded that, compound (2) posses antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococci aureus*. Fig. (2) showed that, compound (3) was active against *Escherichia coli* and *Staphylococci aureus*. All the isolated compounds exhibited no antibacterial activity against *Proteus spp.* This means that, the extractions from which the compounds were isolated are active against *Proteus spp.* due to the presence of mixture of compounds not to the isolated compounds alone. However, compounds (1, 2 and 3) showed better activity after isolation against the other three bacteria as shown in figs. (1 and 2).

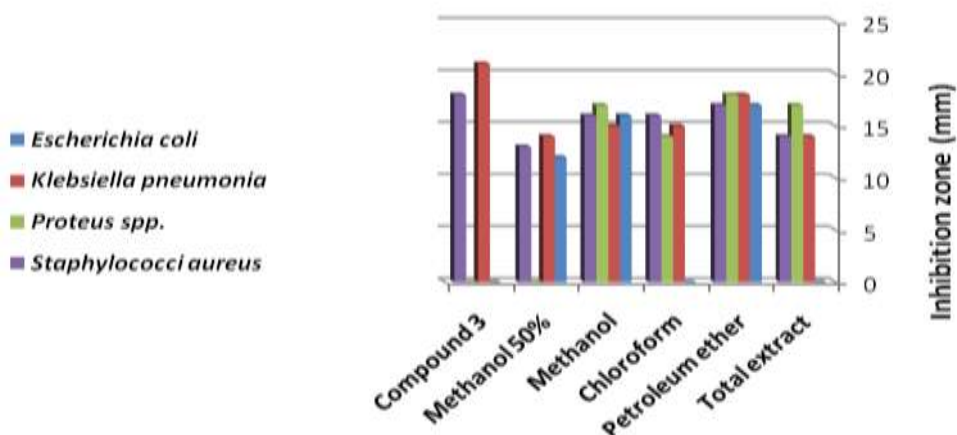
**Table (2).** Antibacterial activity of the total extracts, successive extractions and the isolated compounds of *Phlomis floccosa* D. Don. using disc diffusion assay.

Extracts	Inhibition zone (mm)*							
	<i>Escherichia coli</i>		<i>Klebsiella pneumonia</i>		<i>Proteus spp.</i>		<i>Staphylococci aureus</i>	
	Vegetative parts	Flowers	Vegetative parts	Flowers	Vegetative parts	Flowers	Vegetative parts	Flowers
Total extract (70% methanol)	0	0	13	14	16	17	17	14
Petroleum ether	16	17	0	18	0	18	0	17
Chloroform	20	0	19	15	22	14	20	16
Methanol	16	16	21	15	18	17	16	16
Methanol 50%	13	12	16	14	20	0	13	13
Compound 1	0		23		0		29	
Compound 2	24		16		0		24	
Compound 3		0		21		0		18

\*Values are the means of three replications



**Fig. (1).** Antibacterial activity of the total extract, successive extractions and the isolated compounds of *Phlomis floccosa* D. Don. vegetative parts.



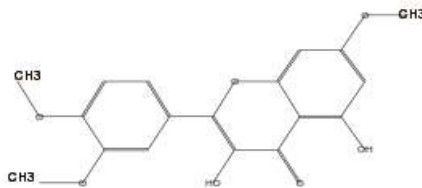
**Fig. (2).** Antibacterial activity of the total extract, successive extractions and the isolated compounds of *Phlomis floccosa* D. Don. flowers.

#### 4. Identification of Active Constituents

##### 4.1. Chloroform extract of vegetative parts

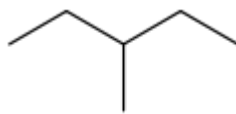
Two compounds were isolated from the chloroform extract of the vegetative parts. Compound (1) identified as 7, 3', 4' trimethoxy quercetin using  $^1\text{H-NMR}$  as follows:

$^1\text{H-NMR}$   $\delta$ : 3.90 (s, 9H,  $\text{OCH}_3$ ), 6.73 (d, 1H,  $J=2.44$ ,  $\text{C}_6\text{-H}$ ), 6.87 (d, 1H,  $J=8.24$ ,  $\text{C}_8\text{-H}$ ), 6.95 (d, 1H,  $J=8.36$ ,  $\text{C}_6'\text{-H}$ ), 7.80 (d, 1H,  $J=8.4$ ,  $\text{C}_5'\text{-H}$ ), 7.91 (d, 1H,  $J=4.84$ ,  $\text{C}_2'\text{-H}$ ).



The purified compound (2) was identified as hahnfett using NMR as follows:  
 $^1\text{H-NMR}$   $\delta$ : 0.80 (m, 6H,  $\text{C}_{1,6}\text{-H}$ ), 1.18 (m, 3H,  $\text{C}_4\text{-H}$ ), 1.54 (m, 4H,  $\text{C}_{2,5}\text{-H}$ ), 2.24 (m, 1H,  $\text{C}_3\text{-H}$ ).

$^{13}\text{C-NMR}$   $\delta$ : 14.12, 22.69, 29.70, 31.93.



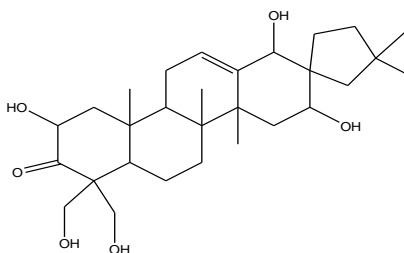
#### 4.2. Petroleum ether extract of the flowers

The purified compound (3) was identified as stewertiisin B [(17R)-19 (18-17)- abeo-2 $\alpha$ , 16 $\beta$ , 18 $\beta$ , 23, 24-pentahydroxy-28-norolean-12-ene-3-one] using NMR.

$^1\text{H}$ NMR  $\delta$ : 0.78 (s, 3H, C<sub>26</sub>-H), 0.80 (m, 2H, C<sub>7</sub>-H), 0.81 (s, 3H, C<sub>30</sub>-H), 1.06 (s, 3H, C<sub>29</sub>-H), 1.07 (s, 3H, C<sub>25</sub>-H), 1.09 (m, 2H, C<sub>19</sub>-H), 1.19 (m, 2H, C<sub>6</sub>-H), 1.22 (s, 3H, C<sub>27</sub>-H), 1.37 (m, 1H, C<sub>1</sub>-H), 1.51-2.23 (m, 11H, C<sub>9,21,22,5,15,1,11</sub>-H), 3.20 (s, 1H, C<sub>16</sub>-H), 3.21 (s, 1H, C<sub>18</sub>-H), 3.53 (dd, J= 21.2, 7.08, 1H, C<sub>2</sub>-H), 4.05 (s, 2H, C<sub>23</sub>-H), 4.24 (s, 2H, C<sub>24</sub>-H), 5.25 (s, 1H, C<sub>12</sub>-H).

$^{13}\text{C}$ NMR  $\delta$ : 13.11, 16.98, 22.36, 22.63, 24.62, 25.54, 26.76, 28.80, 29.06, 29.23, 29.36, 30.22, 31.69, 33.52, 33.69, 38.76, 46.99, 47.42, 47.63, 48.05, 48.27, 56.95, 61.95, 63.00, 69.26, 125.61, 129.41, 129.55, 173.44.

This compound was isolated as new compound from *Phlomis stewartii* (Jabeen et al., 2013).



#### CONCLUSION

It is the first time to study the antibacterial activity of *Phlomis floccosa* D. Don. vegetative parts and flowers. All the tested bacterial strains were clinical isolates. It could be concluded that, the most active extractions of vegetative parts and flowers were petroleum ether and chloroform extracts, respectively. The compounds of these two fractions were isolated, purified and identified. Two compounds were isolated from the vegetative parts namely, 7, 3', 4' trimethoxy quercetin and hahnfett and one compound from the flowers (stewertiisin B). The antimicrobial activity of the three isolated compounds were investigated and found that, 7, 3', 4' trimethoxy quercetin and stewertiisin B posses activity against *Klebsiella pneumonia* and *Staphylococci aureus*, while hahnfett was active against *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococci aureus*. It was found that, the three compounds were inactive against *Proteus* spp. It is recommended to further study the mechanism of action as antibacterial naturally acting agent of the isolated compounds, as well as studying the ability to produce them using tissue culture techniques to facilitate their industrialization.

## REFERENCES

- Ben Amor, I.L., J. Boubaker, M. Ben Sgaier, I. Skandrani, W. Bhourri, A. Neffati, S. Kilani, I. Bouhlel, K. Ghedira and L. Chekir-Ghedira (2009). Phytochemistry and biological activities of *Phlomis* species. *Journal of Ethnopharmacology*, 125 (2): 183–202.
- Bucar, F., S. Ninov, I. Ionkova, T. Kartnig, M. Schubert-Zsilavec, I. Asenov and B. Konuklugil (1998). Flavonoids from *Phlomis nissolii*. *Phytochemistry*, 48 (3): 573 -575.
- Bülent, C., C. Songul and C. Ramazan (2011). The investigation of antimicrobial activity of thyme and oregano essential oils. *Turk. J. Agric.*, 35: 145-154.
- Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. *International Journal of Food Microbiology*, 94: 223– 253.
- El-Negoumy, S.I., M.F. Abdalla and N.A.M. Saleh (1986). Flavonoids of *Phlomis aurea* and *P. floccosa*. *Phytochemistry*, 25 (3): 772-774.
- Hanene, M., R.B. Slama, D. Mili, S. Zouari, A. Bakhrouf and E. Ammar (2013). Essential oil of *Thymus vulgaris* L. and *Rosmarinus officinalis* L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. *Natural Science*, 5 (6): 729-739.
- Jabeen, B., N. Riaz, M. Saleem, M.A. Naveed, M. Ashraf, U. Alam, H.M. Rafiq, R.B. Tareen and A. Jabbar (2013). Isolation of natural compounds from *Phlomis stewartii* showing  $\alpha$ - glucosidase inhibitory activity. *Phytochemistry*, 96: 443-448.
- Kalemba, D. and A. Kunicka (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 10 (10): 813–829.
- Kamel, M.S., K.M. Mohamed, H.A. Hassanean, K. Ohtani, R. Kasai and K. Yamasaki (2000). Iridoid and megastigmane glycosides from *Phlomis aurea*. *Phytochem.*, 55: 353-357.
- Kateryna, K. and R. Mahendra (2012). Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with other essential oils. *Bioscience*, 4 (2): 50-56.
- Kirmizibekmez, H., P. Montoro, S. Piacente, C. Pizza, A. Doenmez and I. Calis (2005). Identification by HPLCPAD-MS and quantification by HPLC-PAD of phenylethanoid glycosides of five *Phlomis* species. *Phytochem. Anal.*, 16 (1): 1-6.



- Sarkhail, P., E.H.R. Monsef, G. Amin, M.H.S. Surmaghi and A. Shafiee (2006). Phytochemical study of *Phlomis olivieri* Benth. and *Phlomis persica* Boiss. DARU., 14: 115-121.
- Vivek, S., S. Nisha, P. Vijaylata, M.A. Reyaz, S. Bikramand and G.C. Raghbir (2010). GC-MS analysis and antimicrobial activity of essential oil of *Mentha piperita* L. from Kullu-a North Indian region of higher altitude Himalayas. Int. J. Drug Dev. Res., 2 (4): 40-46.

**Received: 21/8/2016**

**Accepted: 28/10/2016**

## الأنشطة المضادة للبكتيريا لبعض المكونات الفعالة لنبات ضرس الشايب

رحاب أحمد لطفى وهبة إبراهيم عبد المعطي\*  
قسم النباتات الطبية والعطرية، مركز بحوث الصحراء، المطرية، القاهرة، مصر

أوضحت النتائج (المعملية) لتأثير المستخلص الكلي (٧٠٪ ميثانول) والمستخلصات متتابعة القطبية لكل من الإيثر البترولي والكلوروفورم والميثانول وميثانول ٥٠٪ للأجزاء الخضرية والأزهار لنبات ضرس الشايب على بكتيريا (إيشيريشيا كولاي وكليبيلا بنومونيا وجنس البروتس وستافيلوكوكاس ايروس)، أن أفضل النتائج التي تم الحصول عليها والمثبطة للبكتيريا من المستخلصات كانت لمستخلص الكلوروفورم بالنسبة للأجزاء الخضرية، بينما كان مستخلص الإيثر البترولي هو الأفضل فاعلية بالنسبة للأزهار. وقد تم فصل وتنقية المركبات الرئيسية للمستخلصات الفعالة وهي ٣،٧،٤- تراى ميثوكسى كوارستين (فلافونيد) وهاهنفيت (هيدروكربون) وتم الحصول عليهما من الأجزاء الخضرية للنبات، بينما تم الحصول على ستويرتبيسين ب (سيسكوترابين) وتعريفه من الأزهار. وقد أوضحت النتائج أن المركبات الثلاثة التي تم فصلها لها تأثير مثبط لنمو سلالات البكتيريا المستخدمة.