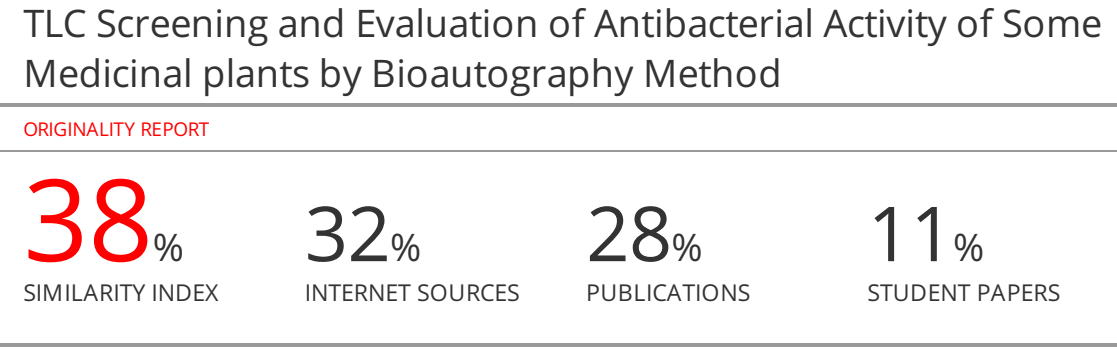
**Reviewer’s Comments**

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**Screening and Evaluation of Antibacterial Activity of Some Medicinal plants by BioautographyMethod**

**ABSTRACT**

This study aimed to conduct a qualitative detection of the active compounds in *Acacia cyanophylla*, *Phlomissyriaca* and *Scolymushispanicus* plants by thin layer chromatography (TLC) method. The antibacterial activity for of the aqueous and ethanolic extracts of the aerial parts of the three plants was evaluated using minimum inhibitory concentration (MIC) test. The active compounds that may be responsible for the antibacterial effect was isolated by direct bioautograph method. The results showed that the three plants contained flavonoids, saponin, bitter principles and essential oils by TLC method, and all extracts showed antibacterial activity, but the ethanolic extract of *Acacia cyanophylla* was the most effective as the MIC values ​​ranged from 0.097to 3.125mg/ml. Bioautography showed that *Escherichia coli* was inhibited by most of the separated flavonoids on the TLC plates. The study concluded that the *Acacia cyanophylla*has strong antibacterial properties among the selected plants due to the presence of flavonoids.

***Key words:***Bioautography, Antibacterial, Flavonoids,*Acacia cyanophylla, Phlomissyriaca, Scolymushispanicus*.

**INTRODUCTION**

Herbal medicine in Asia has a long history of human interactions with the environment. Natural products, such as plant extracts, are available either as pure compounds or as standard extracts provide great opportunities for new drug discoveries due to the unparalleled availability of chemicals diversity 1. At present, the world is facinggreat challenges in modern healthcare services with antimicrobial agents that have lost their effectiveness in treating infectious diseases due to the development of microbial resistance strains2. Epidemiological studies revealed high morbidity and mortality rates from infectious diseases due to antibiotics resistance. A wide variety of phytochemicals have been proven to be potential antibacterial agents, including terpenoids, essential oils, alkalis, lectins, polypeptides, phenols and polyphenols 3. Many plant extracts are rich in phenolic compounds that may exhibit antibacterial activity in addition to their antioxidant activity. The mechanisms of the antibacterial effect of phenolic compounds have not been fully explored. Modification of the permeability of cell membranes, loss of functions within cells due to hydrogen binding of phenolic compounds to enzymes and modifying of cell wall stiffness with loss of integration have been documented 3. The elevated lipophilic character of phenolic compounds enhances their antimicrobial activity. Flavonoids which are the base class of polypehols with C6-C3-C6skeleton may bind to soluble proteins found outside of cells and with the bacterial cell walls thus promoting the formation of complexes. in addition, flavonoids may also act by inhibiting both energy metabolism and nucleic acids synthesis 3,4. Thin layer chromatography (TLC) has been widely used in analysis of plant extracts for active components because many samples can be analyzed in one development. If required, the confirmation can be done by means of automated techniques such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometer (LC-MS). However, TLC continues the first-choice method due to its simplicity and low cost. The unique feature of TLC is that it can provide information about biological properties of the sample, for example, antioxidants and antimicrobial when combined with direct bioautography5. Bioautography is planar chromatographic analysis hyphenated with the biological detection method 6, it is simple, economical, time-saving, do not require advanced equipment and more sensitive than any other methods 7.

The World Health Organization (WHO) has listed more than 21,000 plants which are used for many medicinal purposesworldwide8 and a large number of medicinal plants have been recognized as valuable sources of natural antimicrobial compounds 9. The genus *Acacia* belongs to the Fabaceae family and contains a large number of species (about 1500), making it the largest genus within the previous family. It is widespread in Australia, Asia, Africa and the Americas 10. *Acacia* species have been reported to contain secondary metabolites including amines, alkaloids, fatty acids, amino acids, terpenes (including essential oils, diterpene and triterpene), hydrolyzed tannins, flavonoids, and condensed tannins 11. Several studies have reported that *Acacia cyanophylla* has strong potential antimicrobial effects 12. It also has important antioxidant and anti-acetylcholinesterase activity 13. The genus *Phlomis* L. is one of the largest genera of the *Lamiaceae*family with more than 100 species including herbs, shrubs. This genus is distributed in the northern temperate regions, especially in Europe and Asia 14 andpossess anti-inflammatory, immunosuppressive, antioxidant and antimicrobial activities. Various classes of glycosides, consisting mainly of diterpenoids, iridiodes, phenylpropanoid, phenylthanoid, flavonoids essential oils have been identified 15. *Scolymushispanicus* L. is a thistle-like plant in the family *Asteraceae*, a prickly perennial herb with a circum-Mediterranean distribution which grows all over Spain but it is scarce in the north of the country 16.  Although its leaf and root is usually consumed as a vegetable, it is also used in alternative medicine. *Scolymushispanicus* L. leaves, stems and flowers are traditionally used as a “bitter” tonic to stimulate appetite17.  It has many medicinal properties such as diuretic, depurative, digestive, choleretic and lithiuretic16. The aerial parts contain bioactive compounds such as flavonoids, flavonoid glucosides and flavonolrutinosides18 and this plant is rich in dietary fiber, total phenolic compounds, and showed high antioxidant capacity 17. Among which *Acacia cyanophylla, Phlomis syriaca* and *Scolymushispanicus* are known to be native in the flora of Syria.In particular, despite widespread of these plants, the literature contains few reports of antibacterial activity and chemical composition of these plants. This study aimed to identify the various phyto-constituents components in the crude extracts of *Acacia cyanophylla, Phlomissyriaca* and *Scolymushispanicus* that are responsible for antibacterial activity.

**METHODS**

**Plant Materials**

Fresh aerial parts (leaves, flowers and stems) of *Acacia cyanophylla*, *Phlomis syriaca* and *Scolymushispanicus* were collected between March to April 2020 from different regions of northern Syria. The plant materials were authenticated by Dr Ahmad Jaddouh, an expert at Faculty of Agriculture,University of Aleppo, Syria. The plant samples werecleaned with distilled water and dried at normal room temperature 19,20 for 15 days. The dried samples wereground to powder with the domestic blender and kept in airtight glass container until use. 21

**Bacterial Strains**

Fifteen *Escherichia coli*isolates include was obtained from urinary tract infections from at Aleppo University Hospital laboratory.  Isolates were defined by Gram stain, their microscopically appearance and their growth on the differential media. Isolates are kept in the liquid nutrient medium with 30% glycerol, at -20 oCuntil use 8.

**Plant Extraction**

Fine powder of three plants (30g) wereextracted using distilled waterandethanol 95%, respectivelyfor one hour in an ultrasonic bath (POWERSONIC 405 (Hwashin Technology Co, Korea). The temperature was maintained at 50°C. The plantandsolvent ratio was 1:10 (w/v). The extract solutions were filtered through Whatman No. 1 filter papers, and the residual material was re-extracted twice using the same procedure. The combined extracts were evaporated to dryness in a rotary evaporator (Rotary evaporator, Heidolph Instruments, Germany) at 50°C and under reduced pressure to remove the solvent 20. The obtained crude extracts were stored in dark glass bottles and refrigerated at -4°C until use 21.

**AntibacterialSusceptibility**

The antibiotic susceptibility of isolates was tested using the Disk diffusion method (Kirby Bauer) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 22 to eight antibiotics belonging to four main groups: Ampicillin&Sulbactum, Cefepime, Nalidixic acid, Ceftazidime, Cefpodoxime, Cifuroxim, Ceftriaxone and Nitrofurantoin.

**Minimum Inhibitory Concentration (MIC) Test**

The antibacterial activity of the extracts were determined by establishing the minimum inhibitory concentration (MIC) using microdilution method. For this purpose, stock solutions of examined extracts and Mueller-Hinton broth were prepared. The stock solution of each extract was prepared by dissolving 200 mg/mL of extract in dimethyl sulfoxide (DMSO). First, adding 50 𝜇L of Mueller Hinton broth (MHB) and 50 𝜇L of bacterial suspension at a concentration of 0.5 McFarland to the first well as a negative control. Then, adding 50 𝜇L of the selected plant extract at the highest concentration to the second well and 50 𝜇L of bacterial suspension as a positive control. Second, 50 𝜇L of the selected plant extract at the highest concentration to the third well and it is diluted by adding 50 𝜇L of MHB, then 50 µL are taken from the mixture for the fourth well and so on, the concentrations of tested extracts ranged between 0.097to 100 mg\mL.  then 50 𝜇L of bacterial suspension are added for each well. The microtiter plate was sealed with parafilm and incubated at 37°C for 24 h. The MIC of each tested isolate was detected by adding 20 𝜇L of 0.2 mg/mL indicator dye of 2, 3, 5-triphenyltetrazolium chloride (TTC) into the microtiter plate wells and incubated for 30 min at 37°C and observed any color change 2, 23. TTC is colorless in the oxidized form and red when reduced. The dehydrogenase of living bacteria reduces tetrazolium salt into intensely pinkish-red formazan. When solutions of microtiter plate wells remain without color change, infer that the bacterial were inhibited. 2,7

**Thin Layer Chromatography (TLC)**

Three types of extracts were prepared:

Extract1: Powdered plant (1 g) is extracted by heating on a water bath for 15 min with 5 mLmethanol then filtrated (for analysis of bitter principles and flavonoids).

Extract2: Powdered plant (1 g) is moistened with 1 ml of 10% ammonia solution, and then extracted by shaking for 15 min at 600C with 5 ml methanol (for analysis of alkaloids).

Extract3: A methanol extract wasprepared as in extract 1. is the eluate was evaporated and then 1 mLof the filtrate was mixed with 0.5 mLwater, followed by3 mLbutanol (for analysis of saponins).

Extract4: Powdered plant (1 g) wasextracted by heating under reflux for 15 min with 10 mLDichloromethane. The filtrate wasevaporated to dryness, and the residue was dissolved in 1 mLtoluene(for analysis of essential oils).

From each extract, 20 uLof extract was applied to a TLC silica gel plate (60F254, 10 cm x 10 cm). Chromatography wasperformed in one of the following solvent systems:

1) Ethyl acetate-methanol-water (100: 13.5: 10): For the analysis of bitter principles, flavonoids, alkaloids and saponins.

2) Toluene-ethyl acetate (93: 7): For the analysis of essential oils.

 Both solvents are allowed to run for a distance of 8 cm. After inspection in UV- 254 nm and UV-365 nm, each chromatogram is analyzed for the presence of one of the groups of plant constituents by spraying with an appropriate reagent such as:

1-Bornträger reagent (10% ethanolic KOH): for detection anthraquinones.

2-Dragendorff reagent: for detection alkaloids.

3-Natural products-polyethyleneglycol reagent (NP/PEG): for detection flavonoids.

4-Vanillin-sulphuric acid reagent: for detection Bitter principles and saponins. 24

**Direct BioautographyMethod**

Flavonoids were selected for microbiological tests in the presented study using bioautography method. Directbioautography was performed with a culture of *Escherichia coli* (isolate 10) which showed a good sensitivity to the ethanolic extract of the three plants. TLC plates of ethanolic extract of three plant were prepared in ethyl acetate-methanol-water (100: 13.5: 10) as mobile phase to separate flavonoids. TLC plates were carefully dried for complete removal of the solvents 25 and sprayed with or dipped into bacterial suspension at a concentration of 0.5 McFarland. Incorporated a new medium for direct TLC bioautography which is fluid enough to disperse microorganisms and viscous enough to adhere to the TLC plates; according to them a mixture of MullerHinton (MH) broth and MH agar in the ratio of 90:10 fulfills this requirement. The TLC plates were thenincubated at 37°C for 24h under humid condition. After 24 h of incubation, for visualization of microbial growth, tetrazolium salts are used. These salts are sprayed onto the plates and are re-incubated at 37°C for 1 h. Clear white zones against a purple background on the TLC plate indicate antimicrobial activity of the sample. Then, this zones of inhibition were compared with the RF of the related spots on the reference TLC plate 6.

**RESULTS AND DISCUSSION**

**Antimicrobial Susceptibility**

The results of antibacterialsusceptibility of *Escherichia coli* isolates against selective antimicrobial agents are listed in Table (1). The results showed complete sensitivity to nitrofurantoin and prevalence of resistance with different percentage    to other different antibiotics ranged from 20% to 86.67%. The emergence of antimicrobial resistance among uropathogenic*E. coli* is well documented in our country 26.

**Table (1): Susceptibility of *Escherichia coli* isolates against studied antibiotics**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolates**  **number** | **SAM**  **10/10 µg** | **FEP**  **30 µg** | **NAL**  **30 µg** | **CAZ**  **30 µg** | **CPD**  **10 μg** | **CXM**  **30 µg** | **CRO**  **30 µg** | NIF300 µg |
| 1 | S | S | R | R | R | R | R | S |
| 2 | S | S | R | S | S | S | S | S |
| 3 | S | S | R | S | R | R | R | S |
| 4 | S | S | R | S | S | S | S | S |
| 5 | I | R | R | R | R | R | R | S |
| 6 | S | S | R | S | R | R | R | S |
| 7 | S | S | R | R | R | R | R | S |
| 8 | R | R | S | R | R | R | R | S |
| 9 | R | S | R | R | R | R | R | S |
| 10 | S | S | R | S | R | R | R | S |
| 11 | S | R | R | R | R | R | R | S |
| 12 | S | S | S | R | R | R | R | S |
| 13 | R | I | R | R | R | R | R | S |
| 14 | R | S | S | R | R | R | R | S |
| 15 | R | S | R | R | S | S | R | S |
| Resistance | 40% | 26% | 80% | 60% | 80% | 80% | %86.7 | %0 |

# SAM:ampicillin-​sulbactam, FEP: Cefepime, NAL: nalidixic acid,CAZ: Ceftazidime, CPD:  Cefpodoxime, CXM :Cefuroxim, CRO: Ceftriaxone, NIF: Nitrofurantoin , R: Resistant, S: Sensitive, I: Intermediate

**MIC Assay**

Finding healing powers in plants is an ancient idea. With the increasing prevalence of antibiotic resistance, the search for natural alternatives was an imperative to eradicate resistant strains. 27

Table (2) shows the MIC values ​​for the six extracts from the three plants against *Escherichia coli* isolates. The MIC value was taken as the lowest concentration of the extract that inhibiting any visible bacterial growth and this is detected after adding tetrazolium salts and observed color change.

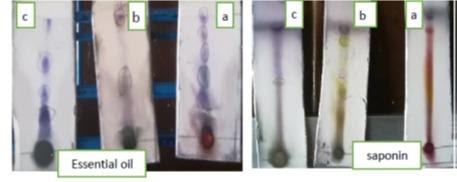
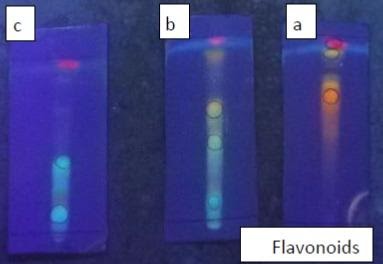
In general, the ethanolic extracts were more effective than the aqueous extracts for the same plant. However, the ethanolic*Acacia cyanophylla* extract was the most effective against *E. coli* isolates as its MIC values ​​ranged from 0.097to 3.125mg\ml. The aqueous extract also showed good efficacy on *E. coli*isolatesas its MIC ranged between3.125to 12.5 mg\ml. The ethanolicextracts of *Phlomis syriaca*showed good efficacy on *E. coli* isolates, as the activity started at a concentration of 6.25 mg/mL for some isolates, while the highest activity was reached at a concentration of 0.78 mg/mL.  The aqueous extracts MICs ranged between 12.5to 3.125 mg/mLfor the studied isolate.

Both the ethanolic and aqueous extracts of *Scolymushispanicus* showed similar efficacy against *E. coli* isolates, where the efficacy ranged between 25to 6.25mg/mL. The highest antibacterial efficacy of *Acacia cyanophylla*ethanolic extracts maybe be attributed to the richness in phenols and flavonoids, as documented in previous study where thetotal phenol content (TPC) recorded 98.39 ±4.755 mg GAE g/g of dry extract and total flavonoids content (TFC) recorded 121.64 ±6.469 mg RE/g of dry extract 28. This is in agreement with a ([SADIQ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sadiq%20MB%5BAuthor%5D&cauthor=true&cauthor_uid=28098806) et al., ~~2017~~) study on *Acacia nilotica*plant extracts where MIC values ​​for *acacia* leaf extracts were 1.56to 3.12 mg/mL, while fruit and bark extracts showed somewhat higher values ​​of 3.12to 6.25 mg/mL on *E. coli* and *Salmonella*. 29 It also converges with Marmouzi et al., study, where *Scolymushispanicus* extract was tested against five types of bacteria, and the results showed that *Scolymushispanicus* roots had the strongest antibacterial activity against *E. coli* bacteria at MIC value of 1.56 mg/mL30. This variation in values can be explained by the fact that the flavonoids and phenols content are influenced by different parameters such as time and place of harvest, climate, geographical conditions, method and time of extraction, solubility and degree maturation of the plan 31.

**Table (2): The MIC values for the aqueous and ethanolic extracts from *Acacia cyanophylla, Phlomis syriaca* and *Scolymushispanicus* against *Escherichia coli* isolates.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Isolates number | *Acacia cyanophylla* | | *Phlomissyriaca* | | *Scolymushispanicus* | |
| Aqueous  mg/mL | Ethanolic  mg/mL | Aqueous  mg/mL | Ethanolic  mg/mL | Aqueous  mg/mL | Ethanolic  mg/mL |
| 1 | 3.125 | 0.19 | 3.125 | 3.125 | 6.25 | 6.25 |
| 2 | 12.5 | 0.19 | 6.25 | 3.125 | 12.5 | 12.5 |
| 3 | 6.25 | 0.39 | 3.125 | 3.125 | 12.5 | 12.5 |
| 4 | 3.125 | 0.39 | 12.5 | 3.125 | 25.0 | 25.0 |
| 5 | 6.25 | 0.78 | 6.25 | 6.25 | 25.0 | 25.0 |
| 6 | 12.5 | 0.39 | 12.5 | 0.78 | 12.5 | 6.25 |
| 7 | 6.25 | 0.78 | 6.25 | 1.56 | 12.5 | 12.5 |
| 8 | 12.5 | 3.125 | 12.5 | 6.25 | 25.0 | 25.0 |
| 9 | 6.25 | 0.39 | 3.125 | 1.56 | 12.5 | 12.5 |
| 10 | 6.25 | 0.78 | 6.25 | 0.78 | 12.5 | 12.5 |
| 11 | 6.25 | 3.125 | 12.5 | 1.56 | 25.0 | 25.0 |
| 12 | 12.5 | 0.39 | 6.25 | 3.125 | 12.5 | 12.5 |
| 13 | 6.25 | 0.78 | 6.25 | 3.125 | 12.5 | 12.5 |
| 14 | 3.125 | 0.097 | 6.25 | 6.25 | 12.5 | 12.5 |
| 15 | 3.125 | 0.19 | 1.56 | 1.56 | 12.5 | 12.5 |

**Thin Layer Chromatography (TLC)**Qualitative phytochemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of a particular secondary metabolite in the plant extract of clinical significance. The presence of any significant bioactive natural product indicates the necessity of separation of the compounds through suitable chromatographic techniques 32. In the present study, forth extracts i.e. methanolic, butanolic, methanolic with ammonia and dichloromethane were checked by thin layer chromatography. Greenish and orange florescent spots were observed after NP-PEG indicates the presence of flavonoids, whereas violet bluish spots after spraying withvanillin-sulphuric acid and heating indicates presence ofsaponins and essential oils. Red-brown spots were observed using dragendorff reagent indicates the presence of alkaloids 24. Hence, it has been proven that the three plants contain diverse classes of bioactive compounds such as flavonoids, saponin, bitter principles and essential oils. Alkaloids were present in *Acacia cyanophylla* while it was absent in *Phlomissyriaca* and *Scolymushispanicus* (Figure 1). These results are in agreement with the literature review on the studied plants 11, 15-18. These constituents are responsible for the various pharmacological properties of these plants, as many reports are available on the antibacterial and antioxidant properties.12,15-17.



**Figure (1): Separated compounds of ethanolic extract of *Acacia cyanophylla, Phlomissyriaca* and *Scolymushispanicus* aerial part on TLC**

**Detection of antibacterial activity by Bioautography**

The chromatograms of ethanolic extract of three plantswere developed in order to calculate Rf values of the spots Table (3) andFigure (1). Table (2) showed that the three plants showed several flavonoids spots of different colors after spraying with the NP / PEG reagent where it was observed with *Acacia cyanophylla* six spots of different colors with Rf values 0.42, 0.55, 0.6, 0.83, 0.88, 0.92, respectively. While it appeared with *Phlomissyriaca*, four color spots with Rf values of 0.48, 0.85, 0.90, 0.96 respectively. Also featured with *Scolymushispanicus* are seven spots in different colors with RF values of 0.16, 0.30, 0.48, 0.58, 0.78, 0.86, 0.92, respectively. Previous studies indicate that when the fluorescence appears orange- yellow colors, the compound may be flavonols while yellow-green fluorescence indicates possible presence Kaempferol, isorhamnetin and their glycosides and Apigenin and their glycosides. Also the appearance of fluorescence orange indicates the possibility of presence flavones such as Luteolin and their glycosides 33.

After spraying with tetrazolium salts some separated flavonoids compounds showed antibacterial activity by revealing yellow inhibition zones on *Escherichia coli* Figure (2).

Figure (2) shows that the antibacterial activity with *Acacia cyanophylla* was shown in spots (1, 2, 3, 4) with Rfvaluesof0.42, 0.55, 0.6, 0.83 While in *Phlomissyriaca* it appeared in only one spot (1) with Rf values (0.48) and in *Scolymushispanicus*, the antibacterial activity appeared in the spots (1, 2, 3, 4, 5) with Rf values of 0.16, 0.3, 0.48, 0.58, 0.87 .This is consistent with previous studies where it was found *in vitro* that the antibacterial activity of flavonoids it can be practiced in three ways: direct and synergistic killing of bacteria the antibiotics activate and weaken the bacteria pathogenicity. Also, the flavonoids showed inhibitory activity against the flow pump bacterium, and it also restricted peptidoglycan synthesis and ribosome in amoxicillin-resistant *Escherichiacoli* (AREC). They also showed inhibition activity against different types of lactamases produced by bacteria; they are the main enzymes that inactivate the common antibiotics 3. The reports also documented that *Acacia cyanophylla, Phlomissyriaca*and*Scolymushispanicus* have a total phenolic content in ethanolic extract of aerial part reached to 98.39, 46.73, 14.48 mg GAE g/ g extract respectively and total flavonoids contents reached to 121.64, 52.49, 35.59 mg of RE/g extract respectively 28.



**Figure (2) Antibacterial activity of separated flavonoids from ethanolic extracts of *(a) Acacia cyanophylla (b) Phlomissyriaca* and (c) *Scolymushispanicus* aerial part by bioautography on *Escherichia coli***

**Table (3) Separated compounds of ethanolic extract of *Acacia cyanophylla, Phlomissyriaca* and *Scolymushispanicus* aerial part on TLC and their RFvalues.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ethanolic plant extract** | **Spots** | UV /NP/PEG | **Rf values** |
| *Acacia cyanophylla* | 1 | Red | 0.42 |
| 2 | Orange | 0.55 |
| 3 | Green | 0.6 |
| 4 | Yellow | 0.83 |
| 5 | Green | 0.88 |
| 6 | Red | 0.92 |
| *Phlomissyriaca* | 1 | Yellow | 0.48 |
| 2 | Green | 0.85 |
| 3 | Blue | 0.9 |
| 4 | Red | 0.96 |
| *Scolymushispanicus* | 1 | Blue | 0.16 |
| 2 | Blue | 0.3 |
| 3 | Orange | 0.48 |
| 4 | Green | 0.58 |
| 5 | Blue | 0.78 |
| 6 | Blue | 0.86 |
| 7 | Red | 0.92 |

**CONCLUSION**

In this study, TLC results showed that the three plants contain flavonoids, saponins, bitter principles and essential oils. The ethanolic extract of *A. cyanophylla* have strong antibacterial activity compared to the other two plants studied.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**Author’s Contribution**

**REFERENCES**

[1]Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2011; 8(1): 1–10.

[2]Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Determination of Antimicrobial Activity of Extracts of Indigenous Wild Mushrooms against Pathogenic Organisms. Evidence-based Complementary and Alternative Medicine, 2019; (2019). ***https://doi.org/10.1155/2019/6212673***

[3]Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Current Medicinal Chemistry. 2015;(22): 132-149

[4]Chibane LB, Forquet V, Lantéri P, et al. Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models. Frontier in Microbiology*,* 2019;(10): 829. ***doi: 10.3389/fmicb.2019.00829***

[5]Jesionek W, Grzelak EM, Dziedzic BM, Choma IM. Thin-Layer Chromatography – Direct Bioautography for the Screening of Antimicrobial Properties of Plant Extracts. Journal of Planar Chromatography - Modern TLC*.* 2013; 2(26): 109-113.

[6]Dewanjee S, Gangopadhyay M, Bhattacharya N, Khanra R, Dua TK. Bioautography and its Scope in the Field of Natural Product Chemistry. Journal of Pharmaceutical Analysis. 2015; 5(2):75–84. ***http://dx.doi.org/10.1016/j.jpha.2014.07.001***

[7]Choma IM, Grzelak EM.  BioautographyDetection in Thin-Layer Chromatography. Journal of Chromatography A, 2011; (1218): 2684–2691. ***doi:10.1016/j.chroma.2010.12.069***

[8] Sufi Da, Sunday E, Mustapha T.Antibacterial Effect of *Acacia nilotica*and *Acacia senegalensis*Fruit Extracts on *Escherichia coli*and *Salmonella Typhi*. FUTY Journal of the Environment, 2020; (2)14.

[9]  Kayali R, Kitaz A, Haroun M. Antibacterial Activity of *Asphodelinlutea* and *Asphodelusmicrocarpus* Against Methicillin Resistant *Staphylococcus aureus* Isolates.  International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(12): 1964-1968.

[10]Maroyi A. *Acacia karroo* Hayne: EthnomedicinalUses, Phytochemistryand Pharmacology of an Important Medicinal Plant in Southern Africa. Asian Pacific Journal of Tropical Medicine. 2017; 10(4): 351–360.

[11]Abbasian K, Asgarpanah J, Ziarati P.  Chemical Composition Profile of *Acacia nilotica*Seed Growing Wild in South of Iran. Oriental Journal of Chemistry. 2015; 31(2):1027-1033.

[12] Noreen I, Iqbal A, Rabbi F, Muhammad A, Shah Z, Rahman ZU. Antimicrobial Activity of Different Solvent Extract of *Acacia cyanophylla*. [Pakistan Journal of Weed Science Research](https://www.citefactor.org/impact-factor/impact-factor-of-journal-Pakistan-Journal-of-Weed-Science-Research.php). 2017; 23(1): 79-90,

[13]Ghribia L, Ghouilaa H, Omrib A, Besbesb M, Janneta HB. Antioxidant and Anti-acetylcholinesterase Activities of Extracts and Secondary Metabolites from *Acacia cyanophylla*. Asian Pacific Journal of Tropical Biomedicine. 2014; 4(1): 417-423 ***doi:10.12980/APJTB.4.2014C1038***

[14]Firdous S, Ahmed H, Hussain M, Shah M. Pollen Morphology of *Ajugal L., Lamium L.* and *Phlomis L*. (Lamiaceae) from District Abbott Abad Pakistan. *Pakistan* Journal of Botany*,* 2015; 47(1): 269-274.

[15] Al-Qudah MA, Obeidat SM, Saleh AM, El-Oqlah AA, Al-Masaeed E, Al-Jaber HI. Volatile Components Analysis, Total Phenolic, Flavonoid Contents, and Antioxidant Activity of *Phlomis* species Collected from Jordan. Journal of Essential Oil Bearing Plants. 2018; 21(3): 583-599. ***https://doi.org/10.1080/0972060X.2018.1489739***

[16] Polo S, Tardío J, Burgo AV, Molina M, Santayana MP. Knowledge, Use and Ecology of Golden Thistle (*Scolymushispanicus* L.) in Central Spain. Journal of Ethnobiology and Ethnomedicine. 2009; 5:42. ***doi:10.1186/1746-4269-5-42***

[17]Altiner DD, Sahan Y, A Functional Food Additive: *ScolymusHispanicus L.* Flour. International Journal of Food Engineering. 2016; 2(2): 124-127. ***doi: 10.18178/ijfe.2.2.124-127***

[18] Ahmad B. Extraction of Phytochemicals from *Scolymushispanicus* and Determination of Potential Health Effects. Thesis, Master of science in Biotechnology, Izmir Institute, Turkey. 2017; 76 P.

[19]Meena R K, Ansari K, Kishor N, Chouhan N. Green Synthesis of Silver Nanoparticles using *Acacia concinna*Plant Extract and Their Antibacterial Activity. Research Journal of Recent Sciences. 2018; 7(3): 1-6.

[20]Sahraoui R, Djellali S, Chaker AN. Morphological, Anatomical, Secondary Metabolites Investigation and Physicochemical Analysis of *Cistus creticus*. Pharmacognosy Communications. 2013; 4(3): 58-63. ***DOI: 10.5530/pc.2013.4.8***

[21]Stanković MS. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubiumperegrinum L.* extracts. Kragujevac Journal of Science. 2011; 33: 63-72.

[22] Wayne Pa. CLSI, Performance Standards for Antimicrobial Susceptibility Testing. 27thed CLSI supplement M100. Clinical and Laboratory Standards Institute, 2017. p. 240.

[23][Adamczak](https://www.ncbi.nlm.nih.gov/pubmed/?term=Adamczak%20A%5BAuthor%5D&cauthor=true&cauthor_uid=31906141) A, [Ożarowski](https://www.ncbi.nlm.nih.gov/pubmed/?term=O%26%23x0017c%3Barowski%20M%5BAuthor%5D&cauthor=true&cauthor_uid=31906141) M, [Karpiński](https://www.ncbi.nlm.nih.gov/pubmed/?term=Karpi%26%23x00144%3Bski%20TM%5BAuthor%5D&cauthor=true&cauthor_uid=31906141) TM. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. Journal of Clinical Medicine. 2020; 9 (1): 109.

[24] Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis, A Thin Layer Chromatography Atlas.1sted., Springer-verlag berlin heidelberg, Germany; 1984. p. 314.

[25]Masoko P, Gololo SS, Mokgotho MP, Eloff JN, Howard RL, Mampuru LJ. Evaluation of the Antioxidant, Antibacterial, and Antiproliferative Activities of the Acetone Extract of the Roots of *Senna italica*(Fabaceae).[African Journal of Traditional, Complementary and Alternative Medicines](https://www.ajol.info/index.php/ajtcam/index). 2010; 7(2): 138–148. ***doi: 10.4314/ajtcam.v7i2.50873***

[26]Al-kayali, R. Study of biofilm formation in clinical bacterial isolates of rinary tract infection. Tishreen universityJournal, Medical Sciences Series, 2017;39 (3):220-227.

[27]Ciocan ID, Băra II.Plant products as antimicrobial agents. Clinical Microbiology Reviews Journal**.** 1999; 12(4): 564-582.

[28]  Jalab J, Kitaz A, Abdelwahed W, Kayali R.Green synthesis of silver nanoparticles using some medicinal plants.International Research Journal of Pure & Applied Chemistry. 2020; 21(24): 13-26.***DOI:*** [***10.9734/irjpac/2020/v21i2430330***](https://doi.org/10.9734/irjpac/2020/v21i2430330)

[29][Sadiq](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sadiq%20MB%5BAuthor%5D&cauthor=true&cauthor_uid=28098806) MB, [Tarning](https://www.ncbi.nlm.nih.gov/pubmed/?term=Tarning%20J%5BAuthor%5D&cauthor=true&cauthor_uid=28098806) J, [Cho](https://www.ncbi.nlm.nih.gov/pubmed/?term=Aye%20Cho%20TZ%5BAuthor%5D&cauthor=true&cauthor_uid=28098806) TZA, [Anal](https://www.ncbi.nlm.nih.gov/pubmed/?term=Anal%20AK%5BAuthor%5D&cauthor=true&cauthor_uid=28098806) AK. Antibacterial Activities and Possible Modes of Action of Acacia nilotica (L.) Del. against Multidrug-Resistant *Escherichia coli* and *Salmonella*. Molecules*.* 2017; *22*(1): 47.

[30]Marmouzi I, Karbane M, Hamdani M, et al. Phytochemical and pharmacological variability in Golden Thistle functional parts: comparative study of roots, stems, leaves and flowers. Natural Product Research Formerly Natural Product Letters. 2017; 31(22): 2669-2674.

[31]Rajhi I, Ben Dhia MT, Abderrabba M, Ouzari-Hadda I, Ayadi S. Phytochemical screening, in vitro antioxidant and antibacterial activities of methanolic extracts of *Capparisspionsa*L. different parts from Tunisia. Journal of Materials and Environmental Sciences. 2019; 10(3): 234-243.

[32] Sharif S, Kitaz A, Al-Kayali R. TLC Screening and Evaluation of Antioxidant, Antibacterial Activity of *Onopordonmacrocephalum*by Bioautography Method.  Iranian Journal of Pharmaceutical Sciences*.* 2016;12(2): 1-8.

[33]Debenedetti SL. TLC and PC in Isolation, Identification and Characterization of Allelochemicals. In:  Vattuone MA, Catalán CA, Sampietro DA, editors. Textbook of Isolation, Identification and Characterization of Allelochemicals, CRC Press;2009. p. 103-133.