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Antimicrobial Agents from Selected Medicinal Plants in Libya

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ABSTRACT **Objective**: To test the *in vitro* antimicrobial efficacy of water and methanol extracts of 23 plant species that are commonly used in Libyan folk medicine. **Methods**: The antimicrobial activity was determined using the well-diffusion method. Four test microorganisms were used namely, *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus* and *Bacillus subtilis*. The minimum inhibitory concentration (MIC) was determined for the high biologically active crude plant extracts. **Results**: Among 23 medicinal plants used in the study, only 5 methanolic extracts [*Rosmarinus offcinalis* L., *Carduus marianium* L., *Lantana camara* L., *Rhus tripartite* (ueria) *Grande*, and *Thymus capitatus* (L.) *Hoffm* (link)] showed the highest antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella* species, while 22 methanolic and aqueous extracts showed moderate to weak antimicrobial activity on all tested organisms. However 19 of the extracts showed no activity at all against Gram –ve and Gram +ve microorganisms. MIC was found to be 1.25 mg/mL (*Thymus capitatus*), 3 mg/mL (*Rhus tripartite*), 4 mg/mL (*Carduus marianium*), 5 mg/mL (*Rosamarinus officinalis*) and 5 mg/mL (*Lantana camara*), respectively. **Conclusions**: The present results revealed that, crude methanolic extracts of the investigated Libyan folk medicinal plants exhibited mild to high *in vitro* antibacterial activities against Gram-positive and Gram-negative microorganisms.

KEYWORDS medicinal plant extracts, antimicrobial activity, minimum inhibitory concentration, Libyan folk medicine

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Today it is estimated that more than two thirds of the world's population relies on plant derived drugs, some 7,000 medicinal compounds used in the Western pharmacopoeia are derived from plants.[1] In USA approximately 25% of all prescription drugs used contain one or more bioactive compounds derived from vascular plants.[2,3] Yet fewer than 10% of the world's plant species have been examined for the presence of bioactive compounds.[4,5] Hence screenings of antimicrobial plants for new agents poses an enormous challenge and are important especially with the emergence of drug resistant disease strains. It is valuable to screen ethno-medicinal plants commonly used by Libyans for their antimicrobial potentials to disclose which of them might be useful for curing infectious diseases based on analytical basis.

Recently much attention has been paid to extracts and biologically active compounds isolated from plant species and analyze their pharmacological activities.[6,7] Antimicrobials of plant origin have enormous therapeutic potential; they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.[8]

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine.[9] Extracts from *Citrus aurantifolia* (*Rutaceae*), *Punica granatum* (*Punicaceae*), *Phyllanthus acidus* (*Euphorbiaceae*) and *Tamarindus indica* (*Caesalpiniaceae*) possess strong *in vitro* antibacterial activity against many microorganisms.[10] The recent appearance of bacterial strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies.[11]

In the present investigation, the antimicrobial potential of 23 medicinally important plants *Artemisa cmpestris* (L.) *Compositae*, *Artemisia herba* (alba)

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assa Compositae, *Asphodelus microcarpus* (*Solzm* viv) *Liliaceae*, *Brassica tounefortii* (Gouan) *Cruciferae*, *Ceratonia siliqua* (L.) *Leguminosae*, *Cynomorium coccineum* (L.) *Cynomoriaceae*, *Eriobotrya japonica* (Thunb.) *Lindl Rosaceae*, *Marrubium vulgare* (L.) *Labiatae*, *Matricaria chamomill*a (L.) *Compositae*, *Olea europaea* (L.) *Oleaceae*, *Peganum harmala* (L.) *Zygoophllaceae*, *Retama raetem* (Forsk) Webb *Leguminosae*, *Rosmarinus officinalis* (L.) *Labiatae*, *Carduus marianum* (L.) *Compositae*, *Sonchus oleraceus* (L.) *Compositae*, *Thymus capitatus* (L.) Hoffm & Link Labiatae, *Urtica urens* (L.) *Urticaceae*, *Haloxylon salicornicum* (Moq.) *Bunge ex Boiss*. *Chenopoddiaceae*, *Citrus medica* (L.) Var. *Amara Rutaceae*, *Lantana camara* (L.) *Verbenaceae*, *Rhus tripartite* (ueria) *Grande*, *Launaea rescdifolia* (L.) *O*. *Kuutze* and *Thymelæa hirsuta* (L.) Eudl from various locations of (Tripoli, Jefara, El-Zawya and other locations of Western North of Libya),[12] were tested against selected pathogenic bacteria S*taphylococcus aureus*, *Bacillus subtilis*, *Salmonella* species and *Escherichia coli*. The minimum inhibitory concentration (MIC) was determined for the five plants which gave remarkable inhibition in the pilot study. Moreover preliminary purified of the most biologically active crude plant extracts were also carried out using thin layer chromatography technique.

METHODS

Chemicals and Instruments

Methanol was supplied by S.O.C. methanol Factory (Libya), sterile distilled water was from Al-Zahra Kidney Hospital (Libya), the other chemicals and instruments included dimethylsulphoxide (DMSO), acetone, NH_4OH (Aldrich Chemical Co. USA), liquid nitrogen, Mueller Hinton agar, grinder machine, shaker KL-2 (Edmund Buhler, Germany), rotary evaporator (Heidolph, Germany), freeze dryer (Qingdao Borui, China), hot plate magnetic stirrer (Heidolph, Germany), incubator and centrifuge (Shanghai Hengyue Medical Instruments Co., Ltd., China), TLC plates F254 Silica gel (Merck, Germany), nutrient broth, sterile cork borers, Petri dishes (42 mm Ø, disposable), pipettes (0.1 mL and 1 mL), standard antibiotic disks (Streptomycin, Ampicillin) and crude plant extracts (test sample).

Plant Material

Twenty three medicinal plants were collected from Western North of Libya between June 2005 and April 2006, classified and authenticated by Department of Botany Faculty of Science, University of Al-Fateh. The leaves, flowers and stems were plucked (Table 1), and washed under running tap water and shade dried. The dried samples were cut into small pieces and ground in a grinding machine into fine powder. The powder samples were sieved to get uniform particle size (2 mm), and then preserved in an air-tight colored glass container in dark place. Exposure to sunlight was avoided to prevent the loss of light-sensitive active components until further use.

Test Organisms

The test organisms used were *Escherichia coli* species, *Bacillus subtilis*, *Staphylococcus aureous* and *Salmonella* species (Table 2).

Preparation of Plant Extracts by Maceration Method

Methanolic extraction: 50 g of powdered plant material was mixed with 250 mL of methanol. The mixture was kept for 24 hrs in tightly sealed closed vessels with continuous shaking at room temperature (25 ℃) and was protected from sunlight. The extracts were filtered under vacuum through Whatman No.1 filter paper. The methanol was removed by the rotator evaporation to get semisolid material. The semisolid extract so obtained, was kept in the beakers covered with holed aluminum-foil for several days to get dry powder. Extract from this method was stored in small tightly closed container in the freezer until further use.

Aqueous extraction: 50 g of powdered plant material was treated with 300 mL of distilled water. The mixture was kept for 24 h in tightly sealed vessels with continually shaking in automatic shaking machine at room temperature. It was then filtered off through cotton wool, water was removed through freeze dryer. After 24 h the solid powdered material was obtained, then it was washed and stored in a freezer for further use. Extract from this method was then weighed and stored in small container in the freezer, until further use.

Agar Diffusion Assay

Aliquots 10 mL of nutrient broth was inoculated with the test organisms and incubated at 37 ℃ for 24 h. Using a sterile pipette 0.6 mL of the broth culture of the test organism was added to 60 mL of molten agar which had been cooled to 45 ℃, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 mL of the culture is added to

Table 1. *In vitro* Antibacterial Activity of Plant Methanolic Extracts

No.	Scientific name of the plant	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella* species	*Escherichia coli*
1	*Artemisa compestris* L.	++ve	++ve	–ve	–ve
2	*Artemisa herbas alba Assa*	–ve	–ve	–ve	–ve
3	*Asphodelus microcarpus Salzm viv*	+ve	+ve	–ve	–ve
4	*Brassica tounefortii Gouan*	–ve	–ve	+ve	+ve
5	*Ceratonia siliqua* L.	–ve	–ve	+ve	+ve
6	*Cynomorium coccineum* L.	++ve	++ve	–ve	–ve
7	*Eriobotrya japonica* (Thunb.) *Lindl*	–ve	–ve	–ve	–ve
8	*Marrubium vulgare* L.	+ve	+ve	–ve	–ve
9	*Matricaria chamonilla* L.	+ve	+ve	+ve	+ve
10	*Olea europaea* L.	–ve	–ve	–ve	–ve
11	*Peganum harmala* L.	–ve	–ve	+ve	+ve
12	*Retama raetem* (forsk) *webb*	–ve	+ve	+ve	+ve
13	*Rosmarinus officinalis* L.	+++ve	+++ve	+ve	+ve
14	*Carduus marianium* L.	+++ve	+++ve	+ve	+ve
15	*Sonchus oleraceus L.*	+ve	+ve	–ve	–ve
16	*Thymus capitatus* (L.) *Hoffm* (link)	+++ve	+++ve	++ve	++ve
17	*Urtica urens* L.	–ve	–ve	–ve	–ve
18	*Haloxylon salicornicum* (Moq) *Bunge ex boiss*	–ve	+ve	–ve	–ve
19	*Citrus medica* L.	–ve	–ve	+ve	+ve
20	*Lantana camara* L.	+ve	+++ve	–ve	–ve
21	*Rhus tripartite (ueria) Grande*	++ve	+++ve	–ve	–ve
22	*Launaea resedifolia* (L.) *O.kuutze*	–ve	–ve	–ve	–ve
23	*D. Gnidium* L. *(Thymelæa hirsute* (L) *Eudl*	+ve	+ve	–ve	–ve
A	*Ampicillin*	+++ve	+++ve	–ve	–ve
S	*Streptomycine*	+++ve	+++ve	+++ve	++ve

Notes: –ve=6.0 mm, +ve=8–10 mm, ++ve=10–12 mm, +++ve=13–18 mm, A=Ampicillin (1 μg/mL), S=streptomycin (1 μg/mL)

20 mL of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and required numbers of holes were cut using a sterile cork borer ensuring proper distribution of holes (cups) in the periphery and one in the center. Agar plugs are removed. Different cork borers were used for different test organisms. Using a 0.1-mL pipette, 100 μL of the test sample dissolved in an appropriate solvent was poured into appropriately labeled cups (marked at the bark of the cup before filling). The plates were left at room temperature for 2 h to allow diffusion of the sample and incubated face upwards at 37 ℃ for 24 h. The diameter of the zones of inhibition was measured to the nearest mm (the cup size also being noted).[13]

Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of test samples that resulted in a complete inhibition of visible growth.[14] The dilution method as recommended by the National Committee for Clinical Laboratory Standards in 1997 was used in the study.[15] To obtain stable dispersion, stock solutions of the highest inhibition of five plants obtained were prepared according to the literature.[16] Stock solutions were prepared using the same solvent (Methanol) yielding concentrations from 0.0125 to 0.060 g/mL. Methanol was used as control. Experiments were carried out in triplicate. Inhibition of microbial growth in the plates containing tested solutions was judged by comparison with growth in blank control plates. The methanol had no inhibitory effect.

Thin Layer Chromatography Purification

Suitable amount of 0.06 g/mL of the most active plant extracts was applied to 5 × 20 cm TLC-F254 Silica

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Table 2. *In vitro* Antibacterial Activity of Plant Aqueous Extracts

No.	Scientific name of the plant	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella* species	*Escherichia coli*
1	*Artemisa compestris* L.	+++ve	+++ve	++ve	–ve
2	*Artemisa herbas alba Assa*	–ve	–ve	–ve	–ve
3	*Asphodelus microcarpus Salzm viv*	–ve	–ve	–ve	–ve
4	*Brassica tounefortii Gouan*	+ve	+ve	–ve	–ve
5	*Ceratonia siliqua* L.	–ve	–ve	–ve	–ve
6	*Cynomorium coccineum* L.	+ve	+ve	–ve	+ve
7	*Eriobotrya japonica* (Thunb.) *Lindl*	–ve	–ve	–ve	–ve
8	*Marrubium vulgare* L.	–ve	–ve	–ve	–ve
9	*Matricaria chamonilla* L.	–ve	–ve	–ve	–ve
10	*Olea europaea* L.	++ve	++ve	+ve	+ve
11	*Peganum harmala* L.	–ve	–ve	–ve	–ve
12	*Retama raetem* (forsk) *webb*	–ve	–ve	–ve	–ve
13	*Rosmarinus officinalis* L.	+ve	+ve	–ve	–ve
14	*Carduus marianium* L.	–ve	–ve	–ve	–ve
15	*Sonchus oleraceus* L.	–ve	–ve	–ve	–ve
16	*Thymus capitatus* (L.) *Hoffm* (link)	–ve	–ve	–ve	–ve
17	*Urtica urens* L.	+ve	+ve	–ve	–ve
18	*Haloxylon salicornicum* (Moq) *Bunge ex boiss*	–ve	–ve	–ve	–ve
19	*Citrus medica* L.	–ve	–ve	–ve	–ve
20	*Lantana camara* L.	–ve	–ve	–ve	–ve
21	*Rhus tripartite* (ueria) *Grande*	–ve	+ve	–ve	–ve
22	*Launaea resedifolia* (L.) *O.kuutze*	++ve	++ve	–ve	–ve
23	*D. Gnidium* L. *(Thymelæa hirsute* (L) *Eudl*	–ve	–ve	–ve	–ve
A	*Ampicillin*	+++ve	+++ve	–ve	–ve
S	*Streptomycine*	+++ve	+++ve	+++ve	++ve

Notes : –ve=6.0 mm, +ve =8–10 mm, ++ve=10–12 mm, +++ve=13–18 mm, A=Ampicillin (1 μg/mL), S=streptomycin (1 μg/mL)

gel (Merck) glass plate. Best separations were achieved with mixture of methanol: acetone: NH_4OH (8:1:1; v/v).[17] The developed TLC plates were dried. Rf values were calculated and every single zone was scratched into watch glass dishes then re-dissolved in methanol, filtered and assayed by the paper disc assay method on the bacterial species to identify the active spots.

Microbiological Assay

Tables 1 and 2 represent the average diameters of the clear zones of inhibition (mm) induced by the crude extracts of the selected folklore medicinal plants; the well diameter is 6.0 mm. The methanolic extract of 5 plants (*Rosmarinus officinalis* (L.), *Carduus marianium* (L.), *Thymus capitatus* (L.) *Hoffm* (link), *Lantana camara* (L.) and *Rhus tripartite* (ueria) *Grande*, showed substantially high antimicrobial activities against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus* species), whereas 6 methanolic and aqueous extracts of three plants (*Artemisa compestris* L., *Olea europaea* L., *Launaea resedifolia* (L.) *O. kuutze*) showed moderate antibacterial activities especially against Gram-positive bacteria, 6 methanolic and 4 aqueous extracts showed weak antibacterial activity on *Staphylococcus aureus*, whereas 7 methanolic and 5 aqueous extracts showed weak antibacterial activities against *Bacillus subtilis*. Eight methanolic and 2 aqueous extracts also had weak antibacterial activities on *Salmonella* species and *Escherichia coli*. Meanwhile other plant extracts showed no such activity.

Assay of Combined Plant Extracts on Bacteria

The 5 methanolic plant extracts which induced the highest inhibitional effect were combined each two plant extracts together and all the 5 plant extracts also combined and applied on the same bacteria. This showed high antibacterial activity and no change from

Table 3. *In vitro* Antibacterial Activity of Combined Plant Methanolic Extracts

No.	The mixture of the extracts	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella* species	*Escherichia coli*
1	A&B	+++ve	+++ve	++ve	+ve
2	A&C	+++ve	+++ve	+++ve	+++ve
3	A&D	+++ve	+++ve	+ve	–ve
4	A&E	+++ve	+++ve	+++ve	++ve
5	B&C	+++ve	+++ve	+++ve	+++ve
6	B&D	+++ve	+++ve	++ve	++ve
7	B&E	+++ve	+++ve	+ve	+ve
8	C&D	+++ve	+++ve	+++ve	+++ve
9	C&E	+++ve	+++ve	+++ve	+++ve
10	D&E	+++ve	+++ve	–ve	–ve
11	[A,B,C,D&E]*	>20	>20	>20	>20

Notes : *diameter of inhibition zone is > 20 mm, tests was performed as 2 extracts per Petri dish; +++ve, diameter of inhibition zone: 13–18 mm; Where the extract (A) = *Rosmarinus officinalis* L., (B) = *Carduus maricinum* L., (C) = *Lantana camara* L., (D) = *Rhus tripartite* (Ueria) *Grande*, (E) = *Thymus capitatus* (L) ftoffm & Link

single plants extract as shown in Table 3.

MIC

MIC of the most biologically active methanolic crude plant extracts were measured and found to be 1.25 mg/mL for *Thymus capitatus* (L.) *Hoffm* (link), 3 mg/mL for *Rhus tripartite* (ueria) *Grande*, 4 mg/mL for *Carduus marianium* (L.), 5 mg/mL for *Rosmarinus officinalis* (L.) and *Lantana camara* (L.) when applied on Bacillus species, 2 mg/mL for *Cardus marianium* (L.), 4 mg/mL for *Rosmarinus officinalis* (L.) and *Thymus capitatus* (L.) *Hoffm* (link), when applied on *Staphylococcus aureus* whereas showed 6 mg/mL for *Thymus capitatus* (L.) *Hoffm* (link) with *Salmonella* species as shown in Table 4.

Table 4. MIC of Five Most Potent Methanolic Extracts of Plants (mg/mL)

No.	Scientific name of the plant	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella* species
1	*Rosmarinus officinalis* L.	4.00	5.00	ND
2	*Carduus marianium* L.	2.00	4.00	ND
3	*Lantana camara* L.	ND	5.00	ND
4	*Rhus tripartite* (ueria) *Grande*	ND	3.00	ND
5	*Thymus capitatus* (L.) *Hoffm* (link)	5.00	1.25	6.00

Note: ND = not determined

RESULTS

The crude methanolic and aqueous extracts were investigated for their possible *in vitro* antibacterial activities using two Gram-positive (*Staph. aureus* and *Bacillus subtilis*) and two Gram-negative (*Salmonella sp*. and *E. coli*) bacteria. The mostly recommended hole-plate diffusion method was adopted herein, since it was primarily found to be better than the disc diffusion method, especially for aqueous extracts.

The present results revealed that, crude methanolic extracts of the investigated Libyan folk medicinal plants exhibited mild to high *in vitro* antibacterial activities against Gram-positive (14 extracts, 61%), Gram-negative (9 extracts, –39%), while 5 (21.7%) out of the 23 extracts were lacking this property.

Effects of Methanolic Extracts

Extracts 13, 14 and 16 were most potent (+++) while extracts 1, 6 and 21 were moderately effective (++) with mild (+) efficacy of extracts 3, 8, 9, 15, 20 and 23 against *Staphylococcus aureus*. In case of activity against *Bacillus subtilis* 13, 14, 16, 20 and 21 had very potent activity, while extracts 1 and 6 had moderate and extracts 3, 8, 9, 12, 15, 18 and 23 had weak antimicrobial activity.

In general antimicrobial activity against Gram –ve organisms-both *Salmonella* species and *Escherrichia coli* was only moderate (++) with extract 16 while 8 extracts (No. 4, 5, 9, 11, 12, 13, 14 and 19) had very mild activity. Out of these methanolic extracts, only extract 16 showed high-moderate activity against Gram +ve and Gram –ve microbes.

Concerning the crude aqueous extracts of the same 23 plants, (8 extracts –34.8%) were anti-Gram-positive, with 3 anti-Gram-negative activity (–13%). Meanwhile, greater percentiles (15 extracts –65%) were completely lacking antibacterial activity against both species. Two plants *viz Retama raetem*[12] and *Haloxylon salicornicum*[18] extracts (methanolic or aqueous extracts of *Artemisa compestris*[1] and *Olea europaea*[10] showed mild to weak antimicrobial activity against both Gram-positive and Gram-negative species.

Effects of Aqueous Extracts

Three extracts (1, 10 and 22) had moderate activity against both Gram +ve microorganisms *Staphylococcus aureus* and *Bacillus subtilis* while 5 extracts (4, 6, 13, 17 and 21) had mild activity. Only 2 extracts (1 and 10) had mild activity against *Slmonella* while extracts (6 and 10) had this activity against *Escherichia coli*. However extracts (1 and 6) were also effective with lesser potency in this regard.

DISCUSSION

Out of all the extracts (both methanolic and aqueous) extract 1 stands very prominent for its high activity against both types of bacteria (Gram +ve and Gram –ve) and should be a promising candidate to be promoted for the clinical trials.

The aforementioned results revealed methanol extraction to be beneficial solvent of choice than water, since higher ratio of bioactive materials is extractable in methanol. This property was found and confirmed by many research laboratories.[18,19]

One of the unexpected results of this work is that aqueous extracts of all tested plants except extracts 1, 6 and 10 do not possess any anti-Gram-negative activity. This is somewhat unexplainable; however, it could be partially attributed to the low ambient temperature during preparing decoction. Some reports, therefore, prefer slight, short period heating or even boiling for efficient extraction with water,[20] nevertheless, aqueous extracts of most of the tested plant members which seemed ineffective, it may by useful to be tested against other microbial species like fungi or viruses.

The tested plant extracts, *Artemisia herbas* (No. 2), *Marrubium vulgar* (No. 8), *Citrus medica* (No. 19), *Lantana camara* (No. 20) and *Launaca resedidolia* (No. 22) showed high inhibitory activity especially against *Staphlococcus aureus*, than Gram-negative bacteria, as reported.[21] This indeed, could be attributed to the less complicated architecture of the Gram-positive cell wall and lack of natural sieve effect against large molecules. *E. coli* showed weak to no response, especially in case of testing the whole crude aqueous extracts of the studied plants. This is also strengthened by other report.[22] This observed resistance of *E. coli* probably could be due to more resistant cell membrane, with less permeability or due to other bacterial genetic factors. Concerning this point, similar results were reported[22] with other indigenous plants.

The five different plant methanolic extracts *Rosmarinus officinalis* (No. 13), *Carduus marianium* (No. 14), *Thymus capitatus* (No. 16), *Lantana camara* (No. 20) and *Rhus tripartite* (No. 21) exhibited extraordinary inhibitory effects on *Staphylococcus aureus* and *Bacillus subtilis* while aqueous extracts of *Carduus marianium* (No. 14) and *Urtica urens* (No. 17) lack this activity. On the other hand, water extracts of *Brassica tounefortii* (No. 4), *Rosmarinus officinalis* (No. 13) and *Urtica urens* (No. 17), although of detectable antibacterial effect against Gram +ve bacteria, yet their methanolic counterparts were completely inactive. This might throw some light on the differential extractability of these two solvents and reflects the different nature of some plants if active or inactive ingredients were extracted.

In this concern the high ratio (–65%) of the inactive aqueous plant extracts ascertains its insufficiency to extract the majority of most polar components like flavanoids, alkaloids, volatile oils, phenols etc. That is usually extracted in ethanol.

Furthermore the combination of two extracts with high activity mostly against both Gram +ve and Gram –ve microbes were also studied for their antimicrobial activity (Table 5), Surprisingly, almost all these combinations showed excellent antimicrobial activity against both types of microorganisms showing mutual potentiating effect — a very favorable finding which suggests further toxicity testing as safety index determination.

The preparative TLC purification of the crude methanolic extracts of the 5 selected most biologically

Table 5. The *in vitro* Antibacterial Proof of the 23 Plant Crude Extracts as Being Tested on Two Gram-Positive and Two Gram-Negative Species

Plant crude extracts	*In vitro* antibacterial activity pattern[a]				
	Gram-positive	Gram-negative[d]	Both G+ve/G–ve	Inactive	Remarks
Methanol	1[b], 3, 6[b], 8, 9, 14, 15, 16, 18, 20[b], 21[b], 23 {14%–34.8%}[e]	4, 5, 9, 11, 12, 13, 14, 16, 19 {9%–39%}	9, 13[b], 14[b], 16[b] {5%–21.7%)	2, 7, 10, 17 and 22 {5%–21.7%}	12 and 18 are active against 2 bacteria and one of the other sp. {2%– 8.5%}
Aqueous	1[b], 4, 6,10, 13, 17, 21[b], 22[b] {8%–34.8%}[e]	1, 6, 10 {3%– 8.7}	1 and {2%–8.7%}	2, 3, 5, 7, 9, 11, 12, 14, 15, 16, 18, 19, 20 and 23 {15%–65%}	

Notes: [a]Tested use the agar well diffusion method; [b]Numbers express the plant extract that show *in vitro* antibacterial activity. [c]Gram-positive bacteria are: *St. aureus* and *Bacillus subtilis*; [d]Gram-negative bacteria are: *E. coli* and *salmonella*; [e]values in brackets represent total number of active extracts and their %. 1. *A. compestris*, 2. *A. herba-alba*, 3. *A. microcarpus Salzm viv*, 4. *B. tounefortii*, 5. *C. siliqua*, 6. *C. coccineum*, 7. *E. japonica*, 8. *M. vulgare* L., 9. *M. chamonilla*, 10. *O. Europaea*, 11. *P. harmala*, 12. *R. raetem*, 13. *R. officinalis*, 14. *C. marianium*, 15. *S. oleraceus*, 16. *T. capitatus*, 17. *U. urens*, 18. *H. Salicornicum*, 19. *C. medica*, 20. *L. camara*, 21. *R. Tripartite*, 22. *L. resedifolia*, 23. *D. Gnidium*

active plants (Table 6), revealed the multi-composition nature of these extracts and the antibacterial activity restricted to specific separated zones. This may give preliminary information about the common TLC developing solvents and the relative Rf values of the antimicrobial spots. Further, chemical analysis is needed to elucidate the chemical nature of the TLC-separated spots.

Table 6. Thin Layer Chromatography of the Most Biologically Active Crude Methanolic Plant Extracts (Semi Purification Result)

Sampnale name	Spot No.	HRf@	Biologicalaction against	
			B. sublilis	*E. coli*
Rosmarinus officinalis L.	1	00	–ve	–ve
	2	75	–ve	–ve
	3	84	+ve	+ve
	4	91	–ve	–ve
	Background	Nil	Nil	Nil
Carduus marianium L.	1	68	+ve	+ve
	2	74	++ve	+ve
	3	96	+ve	+ve
	Background	Nil	Nil	Nil
Rhus tripartite (ueria) *Grande*	1	00	+ve	+ve
	2	65	–ve	–ve
	3	78	++ve	+ve
	4	83	–ve	–ve
	Background	Nil	Nil	Nil
Thymus capitatus (L.) *Hoffm* (link)	1	00	–ve	–ve
	2	83	–ve	–ve
	3	86	++ve	+ve
	Background	Nil	Nil	Nil

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

All the authors contributed equally for the present manuscript. Hasan MHM, Miftah MAM, Fadel AD, Aboclaid AR, and Omer MT collected the medicinal plants, carried out their extraction and screened their crude extracts for antimicrobial activities. Mehtab P interpreted the results and wrote the article.

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