# Antibacterial and Antifungal Activity of Ethanol Extract of Different Parts of Medicinal Plants in Jordan

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## ABSTRACT

Agar diffusion assay and Minimum Inhibitory Concentration (MIC) determinations, *in vitro* were used to evaluate antimicrobial activity of plant extracts against nine bacteria and four fungi. Fourteen ethanolic extracts of different plant parts from *Tecoma capensis* Thunb. Lindl (Bignoniaceae), *Lavandula angustifolia* Mill. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Jasminum sambac* Ait. (Oleaceae), *Populus alba* L. (Salicaceae), *Populus nigra* L. (Salicaceae), *Sonchus oleraceus* L. (Asteraceae), and *Laurus nobilis* L. (Lauraceae) were assayed. Ethanolic extract yields varied among plants and parts of the same plant. The highest yields (18% w/w) were from *L. nobilis* bark, the lowest (4%) from *P. nigra* leaves. Other yields ranged from 7-17%. Antimicrobial activity at 3 mg per disc varied between 8-25 mm inhibition zones. *L. nobilis* leaf extract exhibited highest antibacterial activity (22 mm and 0.5 mg per disc MIC) against *Bacillus subtilis* and the highest antifungal towards *Aspergillus niger* (25 mm and 0.5 mg MIC). *S. oleraceus* aerial parts, *P. nigra*, *P. alba* leaves and *L. nobilis* fruit extracts were the least active against bacteria and fungi (8-10 mm and 2.0-3.0 MICs). Interestingly, *T. capensis* flower extract exhibited strong antifungal activity (17 mm and 0.5 mg MIC) and inhibited moderately methicillin resistant *Staphylococcus aureus* which is reported for the first time. These plant extracts showed interesting antimicrobial activity against bacteria and fungi, thus validating their use and further investigations are needed.

**Keywords**: Antimicrobial, Bacteria, Fungi, Jordan, Plant extracts.

## INTRODUCTION

In drug discovery, most studies have examined on the antimicrobial potential of medicinal plants and other natural products[1,2] measured as either killing or inhibiting the microbial growth. Natural products including medicinal plants are still major sources of innovative therapeutic agents for the various conditions of human diseases[3,4]. The populations in rural developing countries rely heavily on traditional healers and medicinal plants as a basis to treat various maladies[5] inspite of the availability of modern medicine[6]. The world health organization reported that 80% of the world populations rely mainly on traditional medicine[7]. Herbal medicine of natives in every country forms a major part of the world heritage of the plant materia medica[1] and Jordan is not an exception[8].

Although active ingredients may occur in lower concentrations, plant extracts may be a better source of antimicrobials than synthetic drugs[9]. The increased role of antibiotic resistant pathogenic microorganisms is greatly mediated by the increased frequency of mutations, misuse of antibiotics and other factors[10]. Evolving resistant microbial strains has compromised the use of newer generations of antibiotics[11]. Combating such situation has been so far dependent upon the traditional treatment of such microbial infections based on substances that kill or inhibit growth of causative

Received on 7/1/2010 and Accepted for Publication on 28/6/2010.

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pathogens. Synergistic effects are often crucial to bioactivity in plant extract and some activity is usually lost during purification[12]. It is also believed that bacterial resistance to synergistic drug combinations present in plants may be slower than that for single drug therapies.

The traditional chemotherapeutic agents exhibit a broad range efficacy through toxicity or growth inhibition to target microorganisms[6,13]. Due to the misuse of such agents in addition to selective pressure upon pathogens, an increased level of antibiotic resistance is on the rise [10,14,15]. An alternative to the inhibition of bacterial growth would lie in an approach to prevent the pathogens from establishing a successful infection. This approach may be realized through developing new antipathogenic drugs. Given the large number of organisms, including some plants that harbor or produce inhibitory metabolites, to control the activity of microbial pathogenic colonizers offers a continued challenge to search for new and novel antimicrobial substances[16,17,18]. In this study, some medicinal and culinary plants in Jordan were tested for their antibacterial and antifungal activity against an array of microorganisms, keeping in mind that under different environmental conditions, plants synthesize variable substances of different biological activities[19]. Among these is the laurel tree *Laurus nobilis* L. (Lauraceae) commonly known as El-Ghar which is traditionally used in food flavoring, herbal teas and in cosmetics industry for its oils and pleasant scent[20]. Although it has been widely used in the eastern Mediterranean, *L. nobilis* L. has scarcely been comprehensively assayed for microbial growth antagonistic activities[21].

*Rosmarinus officinalis* L. (Lamiaceae) and *Lavandula angustifolia* Mill. (Lamiaceae) which are used as a tonic and are widely used as food spices in Jordan have been studied for their essential oils[22,23] and more information is needed due to their wide use in folk medicine recepies[24]. *Jasminum sambac* Ait. (Oleaceae), *Sonchus oleraceus* L. (Asteraceae), *Populus alba* L. (Salicaceae), *Populus nigra* L. (Salicaceae), and *Tecoma capensis* Thunb. Lindl (Bignoniaceae) are poorly studied for their antimicrobial and antipathogenic characteristics specially their different parts[23,25]. Although *Tecoma capensis* is reported to be used to relieve pain, yet, nothing has been published about its antimicrobial activity[26].

Jordan, though small in area, is a rich source of folk medicinal plants due to climatic variations where plant communities exist between 400 meters below sea level and up to 2000 meters above sea level. In this report, and whenever possible leaves, flowers, fruits and stem bark extracts of *Laurus nobilis*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Jasminum sambac*, *Populus alba*, *Populus nigra*, *Tecoma capensis* and *Sonchus oleraceus* were tested for their antibacterial and antifungal activities.

This work is a part of an ongoing effort of our laboratory to screen and test as much as possible of folk medicinal and culinary plants indigenous and introduced into Jordan for bioactivity[8,27,28]. It is a modest contribution to the quest of promoting human health and the fight against microbial diseases.

## MATERIALS AND METHODS:

### Plant Material

Leaves, flowers, fruits and stem bark of the plants (Table 1) were collected whenever available during March-November 2007, from Jubaiha, 10 Km north of Amman, Jordan. The plants were authenticated by Prof. A. El-Oqlah, a plant taxonomist at Yarmouk University, Irbid, Jordan and Prof D. Al-Esawi of the Department of Biological Sciences at the University of Jordan, Amman, Jordan. Voucher specimens (Table 1) were deposited in the Department of Biological Sciences, University of Jordan, Amman.

The collected plant materials were air-dried under shade at room temperature, milled into a fine powder using an electric mill (Breams, UK) and were stored in an airtight plastic sampling bags for later analysis.

### Plant Extraction

The air-dried plant materials were separately extracted twice at room temperature with ethanol 95% (500 ml/100 g of plant material each run). The final ethanol extract of each plant part was filtered using filter paper (Whatman) and was evaporated under vacuum at 40 °C using rotary vacuum evaporator (Buchi R-215, Switezland). The

resultant residues from the different plants parts were further fractionated according to Mahasneh[8], Mahasneh and El-Oqlah[27] and were stored at -20 °C for further analysis.

**Antimicrobial Assays**

**Microbial Cultures**

Microorganisms used for the determination of antimicrobial activities of the different extracts included Gram positive bacteria: *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* (MRSA Clinical isolate), *Bacillus cereus* (Toxigenic strain) and *Bacillus subtilis*. Gram negative bacteria: *Salmonella typhimurium* (ATCC 14028), *Klebsiella pneumonia*e (ATCC 10031), *Escherichia coli*, *Pseudomonas aeruginosa*, *Chromobacterium violaceum* and both filamentous fungi; *Aspergillus fumigates* (Clinical isolate), *Aspergillus niger* (ATCC 16404) and yeasts: *Candida glabrata* (Clinical isolate), *Candida albicans* (ATCC 10231). All microbial strains were obtained from our stock culture in the Department of Biological Sciences, University of Jordan, Amman.

The different bacterial strains were maintained onto nutrient agar slants at 4 °C. For antibacterial activity testing, bacterial cultures were prepared into a nutrient broth (Idg, England) tube containing (5 ml) and incubated at 37 °C for an overnight. The optical densities of the cultures were adjusted to match 0.5 McFarland standard i.e. $1\times10^8$ colony forming units per ml. Cultures of filamentous fungi and yeasts were grown on malt extract agar (Merck, Germany) at 28 °C and maintained at 4 °C onto malt extract agar slants.

**Antimicrobial Activity Testing**

Different extracts of the respective plants parts were dissolved in Dimethylsulfoxid (DMSO), membrane filter (pore size 0.45 µm) sterilized and tested for antimicrobial activity using the agar diffusion method. Sterile 6 mm diameter filter paper discs were impregnated with 3 mg of the sterile appropriate extract and were placed in duplicates at least onto Muller-Hinton agar (Oxoid, England) plates for bacteria and malt extract agar for yeast and filamentous fungi.

These plates were earlier surface inoculated separately with 100 µl of either freshly prepared bacteria, fungal spores or yeast cells suspension (Ca.$10^8$ CFU/ml). The plates were kept for 2 h at 4 °C to facilitate diffusion of the extracts into the agar and were then incubated for 24 h at 37 °C for bacteria and for 48-72 h at 28 °C for fungi. Inhibition zone diameters around each of the discs were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for at least two replicates.

Separate negative control discs contained either sterile DMSO or ethanol (the solvents were allowed to evaporate from control discs to eliminate toxicity). For comparative purposes, standard antibacterial penicillin G (10 U/disc), tetracycline (30 µg/disc) and antifungal nystatin (100 µg/disc) (Oxoid, Basingstoke, UK) were included in the assay.

Minimum Inhibitory Concentrations (MICs) for the tested samples were determined by the agar diffusion assay[29] using media and incubation temperature as recommended for both bacteria and fungi. Negative controls of DMSO alone were included as well as positive controls of the standard antibiotics ampicillin and tetracycline. MIC was defined as the lowest concentration of the extract that totally inhibited the growth of the tested microorganisms.

**Statistical Analysis**

Antimicrobial activity values are expressed as mean ± S.D. The software Graph Pad Prism was employed.

## RESULTS

**Plant Extract**

Ethanolic plants extracts yields varied with the plant part used where values ranged from 4% w/w in the case of *Populus nigra* leaves up to 18 and 17% in *Laurus nobilis* bark and *Jasminum sambac* leaves extracts, respectively (Table 2). Among the plants leaves tested, *Jasminum sambac* leaves yielded 17%, followed by *Laurus nobilis* 16% and *Tecoma capensis* 15%. Flowers yielded lower percentages ranging from 7% for *Lavandula angustifolia* up to 14% for *Tecoma capensis*. As it appears in table (2), the highest yield recorded was from *L. nobilis* bark 18%, followed by leaves 16%, fruits

16% and flowers 8% w/w. Other plants parts gave ethanolic yields ranging between 4 as in *Populus nigra* and 13 for *Rosmarinus officinalis* leaves.

**Antimicrobial Activity**

Table (3) presents results of the ethanol crude extracts of the different parts of the eight plant species tested at the concentration of 3.0 mg. These results showed antimicrobial activity that can be described as moderate (8-10 mm inhibition zone), good (11-15 mm) and superior (>16 mm) for both bacteria and fungi tested. *L. nobilis* ethanol crude extracts, specially leaves extract, showed the best potential activity against Gram positive *Bacillus subtilis* (22 mm inhibition zone), also recorded superior activity against filamentous fungi *Aspergillus niger*, and *Aspergillus fumigatus* (25 and 21 mm inhibition zones), respectively (Table 3). Likewise, a superior activity from leaves against *Pseudomonas aeruginosa* (17 mm) compared to standard tetracycline (15 mm) was recorded. It has been noticed that the superior (>16 mm) antifilamentous fungi (*A. niger* and *A. fumigatus*) activity of the leaves, flowers, and bark ethanolic extracts of *L. nobilis* as compared to the standard antifungal nystatin.

Flowers and leaves extracts of *Tecoma capensis*, *Lavandula angustifolia*, *Rosmarinus officinalis*, and *Jasminum sambac* exhibited almost good activity (10-15 mm inhibition zone) against Gram positive bacteria including the Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* while a moderate activity was recorded against Gram negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae*. On the other hand, *Tecoma capensis* leaves and flowers and *Lavandula angustifolia* (flowers) extracts showed good activity against the yeasts *Candida albicans* and *Candida glabrata* as well as against the filamentous fungi *Aspergillus niger* and *Aspergillus fumigatus*.

*Rosmarinus officinalis* leaves extract exhibited superior (>16 mm) antifungal activity against *Candida albicans* (16.5 mm), *A. niger* (17.5 mm) and *A. fumigatus* (16 mm). Less prominent antimicrobial activities were recorded for ethanolic extracts of aerial parts of *Populus alba*, *Populus nigra* and *Sonchus oleraceus*. These plants showed moderate (8-10 mm) variable degrees (Table 3) of antimicrobial activity against Gram positive, Gram negative bacteria and both filamentous and nonfilamentous pathogenic fungi tested.

**Minimum Inhibitory Concentration (MIC) Determination**

Results of MIC values for the 14 different plant extracts against the 4 Gram positive, five Gram negative bacterial species and four fungal species are presented in (Table 4). MIC values of 0.5-3.0 mg were recorded for the ethanolic extracts of *L. nobilis* fruits, leaves, flowers and bark against microorganisms tested being the highest (3.0 mg) for *L. nobilis* leaves extract against *Chromobacterium violaceum* to the lowest (0.5 mg) against an array of tested Gram positive bacteria such as MRSA, *Bacillus cereus* and Gram negative bacteria including *Klebsiella pneumoniae* and *Salmonella typhimurium*. However, MIC values of *L. nobilis* different extracts varied between 2.0 mg as in the case of *Candida albicans* to 0.5 mg for both *A. niger* and *A. fumigatus*.

MIC values for *Populus alba*, *Sonchus oleraceus*, *Tecoma capensis*, *Lavandula angustifolia*, *Jasminum sambac* and *Rosmarinus officinalis* ethanol extracts also fall within the range 0.5-3.0 mg (Table 4) for bacteria and fungi tested. A noteworthy observation was the highest MIC values (3.0 mg) for *Pseudomonas aeruginosa* among several plant extracts tested. It is also of interest to point out that *K. pneumoniae* was the most extract sensitive among Gram negative bacteria tested (Table 4).

**Table 1. Ethnobotanical data about the studied plants**

| Plant | | Ethnobotanical information | | |
|---|---|---|---|---|
| **Scientific name,family, Voucher numbers** | **Local name** | **Parts used** | **Traditional and/or medicinal use** | **Route of administration** |
| *L. nobilis* L. Lauraceae, MAHAS 1 | El- ghar | Leaves | Condiment, flavoring | Cooked meats |
| | | | Carminative, digestive problems | Infusion/oral |
| | | | Cold, bronchitis | Vapor bath, decoction |
| | | Fruits | Menstruation, earache, furunculosis | Row berries, essential oils/ ointment |
| | | Flowers | | |
| | Al-Hoor al-abyad | Bark | Diuretic, anti- rheumatic | Infusion/liniment |
| | | | As in leaves | Food flavoring |
| | Al-Hoor al-aswad | Leaves | As in leaves | Food flavoring |
| *Populus alba* L. Salicaceae, MAHAS 2 | khuzama, lavender | Leaves | Depurative, tooth decay | Decoction, Infusion/oral |
| | | Flowers | | |
| *Populus nigra* L. Salicaceae, MAHAS 3 | Hasa-alban | Leaves | Tonic, antiseptic | Decoction/ external use |
| *Lavandula angustifolia* Mill. Lamiaceae, MAHAS 4 | | Flowers | Bronchitis, cough | Infusion/oral |
| | | | Antiseptic | Essential oils/ liniment |
| *Rosmarinus officinalis* L. Lamiaceae, MAHAS 5 | Juwaidihia, juadheedh | Aerial parts | Antiseptic, antispasmodic, and food flavoring. | Essential oils/ liniment |
| | | Leaves | Tonic, stimulant | Decoction |
| *Sonchus oleraceus* L. Asteraceae, | Sareemat aljeddy | Flowers | As in leaves | Intact |
| | | | Bronchitis, pertussis, | Infusion/oral, liniment |

| Plant Scientific name,family, Voucher numbers | Local name | Parts used | Ethnobotanical information Traditional and/or medicinal use | Route of administration |
|---|---|---|---|---|
| MAHAS 6 | | | ophthalmia | |
| | Yasmeen | | | |
| *Tecoma capensis* Thunb. | | Leaves | | |
| Lindl. | | | Pneumonia, enteritis, diarrhea | Infusion/oral |
| Bignoniaceae, | | | Fragrance, tonic | |
| MAHAS 7 | | | | External use |
| *Jasminum sambac* Ait. | | | Ulceration, dermatosis, fever | |
| Oleaceae, | | | | Infusion |
| MAHAS 8 | | | Eyewash | |
| | | | | Infusion |

**Table 2. Plants starting weights (g), ethanolic crude extracts weight (g) and percentage yields**

| Plant name | Part used | Dry weight (g) | Ethanolic crude weight (g) | % yield w/w |
|---|---|---|---|---|
| *Tecoma capensis* | Flowers | 1006 | 141 | 14 |
| | | | 38.2 | 15 |
| *Lavandula angustifolia* | Leaves | 250 | 39.7 | 07 |
| | Flowers | | | |
| *Rosmarinus officinalis* | | 538 | 58 | 12 |
| | Flowers | | 22.8 | 13 |
| *Jasminum sambac* | Leaves | | 73.3 | 17 |
| | Leaves | | 32.3 | 13 |
| *Populus nigra* | Flowers | 500 | 21.3 | 04 |
| | Leaves | | | |
| *Populus alba* | | 170 | 26.8 | 11 |
| | Leaves | | | |
| *Sonchus oleraceus* | | 428 | 20.5 | 08 |
| | Aerial | | | |
| *Laurus nobilis* | | 250 | 55 | 16 |
| | Leaves | | 21.3 | 08 |
| | Flowers | 500 | 42 | 16 |
| | Fruits | | 17 | 18 |
| | Bark | | | |

**Table 3. Antimicrobial activity (mm inhibition zones diameter) of the ethanol plants extracts at 3 mg / disc**

| Plant | | Microbial growth inhibition[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **Part used** | **S.a** | **MRSA** | **B.s** | **B.c** | **K.p** | **S.t** | **E.c** | **P.a** | **C.v** | **C.a** | **C.g** |
| T | Leaves | 10±1.4 | 8.0±1.4 | 19±0.7 | 9.0±1.4 | 10±1.4 | 9.0±1.4 | 9.5±0.7 | 7.5±0.7 | 8.5±0.7 | ND | ND |
| | Flowers | 8.0±1.4 | 12.5±0.7 | 10±1.4 | 7.5±0.7 | 8.0±1.4 | 10±1.4 | 8.0±1.4 | 6.5±0.7 | ND | 15±0.7 | 14±0.7 |
| La | Flowers | 9.5±0.7 | 15±0.7 | 12.5±0.7 | 10±1.4 | 10.5±0. | 7.5±0.7 | 10±1.4 | ND | 7.0±0.7 | 11±0.0 | 9.0±1.4 |
| R | Leaves | 10±1.4 | 12±2.1 | 12±2.1 | 13±0.7 | ND | 13±0.7 | 10±1.4 | ND | 7.5±0.7 | 16.5±0.7 | 12±2.1 |
| | Flowers | 14.4±0.7 | 13±0.7 | 14±0.7 | 14±0.7 | 10±1.4 | 11±0.0 | 10±1.4 | 9.0±1.4 | 8.01.4 | 11±0.0 | 12±2.1 |
| J | Leaves | 13±0.7 | 13±0.7 | 10±1.4 | 11±0.0 | 13±0.7 | 15±0.7 | 11±0.0 | 7.5±0.7 | 8.0±1.4 | 11±0.0 | 10±1.4 |
| | Flowers | 10±1.4 | 16±0.7 | 11±0.0 | 11±0.7 | 13±0.7 | 9.0±1.4 | 11.5±0.7 | ND | 7.5±0.7 | 13±0.7 | 12±2.1 |
| P.a | Leaves | 9.0±1.4 | 8.0±1.4 | 7.0±0.7 | 10±0.7 | 10±1.4 | ND | 7.5±0.7 | ND | 8.0±1.4 | 9.0±1.4 | 11±0.0 |
| P.n | Leaves | 8.0±1.4 | 10±1.4 | 8.0±1.4 | 10±0.7 | 7.5±0.7 | ND | 10±1.4 | ND | 6.5±0.7 | 10±1.4 | 10.5±0.7 |
| S | Aerial | 11±0.0 | 10±1.4 | 15±0.7 | ND | 12±2.1 | 6.5±0.7 | 9.0±1.4 | 10±1.4 | 10±1.4 | 9.5±0.7 | 8.0±1.4 |
| L.n | Fruits | 9.5±0.7 | 12±2.1 | 9.5±0.7 | 14.5±0.7 | 10±1.4 | 9.0±1.4 | 9.5±0.7 | 8.0±1.4 | 9.0±1.4 | 9.0±1.4 | 9.5±0.7 |
| | Leaves | 15±0.7 | 16±0.7 | 22±0.0 | 14±0.7 | 18±0.7 | 10±1.4 | 14.5±0.7 | 17±0.7 | 14±0.7 | 12.5±0.7 | 11±0.0 |
| | Flowers | 13±0.7 | 11±0.0 | 11±0.0 | 11±0.0 | 11±0.0 | 10±1.4 | 8.0±1.4 | 11±0.0 | 15±0.7 | 11.5±0.7 | 12.5±0.7 |
| | Bark | 16±0.7 | 18±0.7 | 13.5±0.7 | 18±0.7 | 7.0±0.7 | 9.0±1.4 | 6.5±0.7 | 6.5±0.7 | 12±2.1 | 11±0.0 | 11±0.0 |
| P | - | 39±1.4 | 33±1.4 | 26±1.4 | 30±2.1 | - | - | - | - | - | - | - |
| T | - | - | - | - | - | 28±1.4 | 26±1.4 | 22±0.0 | 15±0.7 | 40±2.8 | - | - |
| N | - | - | - | - | - | - | - | - | - | - | 26±1.4 | 27±1.4 |

[a] Expressed as the x ± S.D. mean diameter ( mm) of growth inhibition zone and S.D. plant species: *Tecoma capensis* (T); *Lavandula angustifolia* (La); *Rosmarinus officinalis* (R); *Jasminum sambac* (J); *Populus alba* (Pa); *populus nigra* (Pn ); *Sonchus oleraceus* (S); *Laurus nobilis* (Ln). Microbial species: *Staphylococcus aureus* (S.a); Methicillin resistant S. *aureus* (MRSA); *Bacillus subtilis* (B.s); *Bacillus cereus* (B.c); *Escherichia coli* (E.c); *Klebsiella pneumoniae* (K.p); *Salmonella typhimurium* (S.t); *Pseudomonas aeruginosa* (P.a); *Chromobacterium violaceum* (C.v); *Candida albicans* (C.a); *Candida glabrata* (C.g); *Aspergillus niger* (A.n); *Aspergillus fumigatus* (

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A.n** | 14±0.7 | 13±0.7 | 15±0.7 | 12±2.1 | 16±0.7 | 11±0.0 | 9.0±1.4 | 11±0.0 | 13±0.7 | 10.5±0.7 | 13±0.7 | 25±0.7 | 17±0.0 | 17±0.0 | - | - | 22±0.0 | | | |
| **A.f** | 15±0.7 | 15±0.7 | 15±0.7 | 10±1.4 | 17.5±0.7 | 15±0.7 | 9.5±0.7 | 11±0.0 | 11±0.0 | 11±0.0 | 11±0.0 | 21±0.7 | 19±0.7 | 18±0.7 | - | - | 23±0.7 | | | |

**Table 4. Minimum Inhibitory Concentration (MIC) in mg/disk for different ethanol plants extracts**

| **Plants** | **MIC (mg/disc)** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **S.a** | **MRSA** | **B.s** | **B.c** | **E.c** | **K.p** | **S.t** | **P.a** | **C.v** | **C.a** | **C.g** | **A.n** | **A.f** |
| *Laurus nobilis* | | | | | | | | | | | | | |
| Fruit | 0.75 | 0.5 | 0.75 | 0.5 | 1.5 | 1.5 | 1.5 | 2.0 | 1.0 | 0.75 | 1.0 | 0.5 | 0.5 |
| Leaves | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1.0 | 0.5 | 0.5 | 1.2 | 0.75 | 0.5 | 0.5 |
| Bark | 0.5 | 0.5 | 0.5 | 0.5 | 3.0 | 2.4 | 1.0 | 3.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Flower | 0.5 | 1.0 | 1.0 | 0.5 | 2.0 | 1.0 | 1.2 | 1.0 | 0.5 | 0.5 | 1.0 | 0.5 | 0.75 |
| *Jasminum sambac* | | | | | | | | | | | | | |
| Flower | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1.0 | ND | 2.4 | 0.5 | 0.5 | 0.5 | 0.5 |
| Leaves | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 2.0 | 1.5 | 0.75 | 0.5 | 1.2 | 2.0 |
| *Rosmarinus officinalis* | | | | | | | | | | | | | |
| Flower | 1.2 | 1.2 | 0.75 | 0.5 | 1.5 | ND | 1.0 | ND | 3.0 | 0.75 | 0.5 | 0.5 | 1.2 |
| Leaves | 0.5 | 0.5 | 0.5 | 0.5 | 1.0 | 1.0 | 1.0 | 2.0 | 2.4 | 0.5 | 1.0 | 1.0 | 0.75 |
| *Tecoma capensis* | | | | | | | | | | | | | |
| Flower | 2.4 | 0.5 | 0.5 | 2.0 | 2.0 | 1.5 | 1.2 | 3.0 | 3.0 | 0.5 | 0.5 | 0.5 | 0.5 |
| Leaves | 1.0 | 1.5 | 0.5 | 1.0 | 1.0 | 1.0 | 2.0 | 2.4 | 2.0 | ND | 3.0 | 0.75 | 0.5 |
| *Sonchus oleraceus* | | | | | | | | | | | | | |
| Aerial | 1.2 | 0.75 | 0.5 | ND | 1.2 | 1.2 | 3.0 | 1.5 | 2.0 | 2.0 | 1.5 | 2.0 | 2.0 |
| *Lavandula angustifolia* | | | | | | | | | | | | | |
| Flower | 1.2 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 2.4 | ND | 3.0 | 1.2 | 0.5 | 0.5 | 0.75 |
| *Populus nigra* | | | | | | | | | | | | | |
| Leaves | 1.5 | 0.5 | 1.5 | 0.5 | 0.5 | 2.0 | ND | ND | 3.0 | 0.5 | 0.5 | 0.5 | 0.75 |
| *Populus alba* | | | | | | | | | | | | | |
| Leaves | 1.0 | 1.5 | 2.4 | 0.5 | 2.0 | 0.5 | ND | ND | 2.0 | 0.5 | 1.0 | 0.5 | 0.5 |
| Ampicillin | $1.3^{-4}$ | $6.0^{-5}$ | $3.0^{-4}$ $9.0^{-4}$ | | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| Tetracycline | NT | NT | NT | NT | $6.0^{-4}$ | $6.0^{-4}$ | $6.0^{-4}$ | $9.0^{-4}$ | $6.0^{-4}$ | | NT | NT | NT | NT |

Microbial species: *Staphylococcus aureus* (S.a); Methicillin resistant *S. aureus* (MRSA); *Bacillus subtilis* (B.s); *Bacillus cereus* (B.c); *Escherichia coli* (E.c); *Klebsiella pneumonia* (K.p); *Salmonella typhimurium* (S.t); *Pseudomonas aeruginosa* (P.a); *Chromobacterium*

*violaceum* (C.v); *Candida albicans* (C.a); *Candida glabrata* (C.g); *Aspergillus niger* (A.n); *Aspergillus fumigatus* (A.f).
30% DMSO as a negative control did not show any inhibitory activity.
ND: no detected activity at this concentration.
NT: not tested.

## DISCUSSION:

Ethanol plant extracts varied with the plant part used (leaves, flowers, stems or bark of different plants). While this study reports yields between 4-18% w/w of the different plant parts, Abdillahi *et al*[30] reported yields of 8-26% for ethanol extracts of different parts of 4 *Podocarpus* species. These values substantiate the effect of the solvent used in screening processes and their preparation effect upon bioactivity, a factor which is not greatly regarded in medicinal plants studies[2].

The antibacterial and antifungal activities of the different parts of most of the plants studied have not been previously studied comprehensively. Detailed studies of parts such as leaves, flowers, bark and fruits of *Tecoma* species[31], *Lavandula angustifolia*[24], *Populus alba*[32], *Laurus nobilis*[20], *Sonchus* spp.[33] and *Jasminum sambac* [34] are lacking, however, some have been individually sporadically tested or in combinations to treat gastrointestinal problems among other afflictions [(21)]. Despite the wide use of folk medicinal potential of plants in Jordan, detailed knowledge and studies are scarce except for some preliminary reports [8,27,28]

According to the present findings, all 14 ethanol extracts of the different plants parts studied were effective against the four Gram positive, 5 Gram negative bacteria and 4 fungi. These extracts varied in their inhibitory activity but in some cases they harboured higher activity or similar to penicillin, tetracycline and nystatin, which were used for comparison (Table 3). Leaves extract of *L. nobilis* was more active against *B. subtilis* and *P. aeruginosa* and the filamentous fungi *A. fumigatus* and *A. niger* was in fact more active than the standard nystatin against *A. niger* and almost similar against *A. fumigatus*. Other extracts have shown striking activity against *Candida albicans* and *Candida glabrata* and other filamentous fungi namely *Tecoma capensis* flower extracts (Table 3). Guiso *et al*.[35] isolated some fractions from *Tecoma capensis* leaves but did not discuss its bioactivity. *Populus alba* and *Populus nigra* extracts unexpectedly showed very weak activity against bacteria as well as fungi although *Populus* leaves extracts are used in Jordan folk medicine in skin disinfection. Adam *et al*.[32] mentioned the plant in the European folk medicine but our study is among the very few that talk about its bioactivity. Activities of plant extracts vary according to the antimicrobial properties of the active ingredients which in turn vary with extractant and the plant part[36]. Although almost all ethanol extracts exhibited varying degrees of antibacterial and antifungal activities but as table (4) shows, Minimum Inhibitory Concentrations (MIC's) varied between the different plants ethanol extracts as well as among extracts of different plant parts. MIC values ranged between 0.5-3.0 mg per disc. Gram negative bacteria including *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Chromobacterium violaceum* were more tolerant to *L. nobilis* leaves, flowers, bark and fruits extracts expressing this through high MIC values ranging from 0.5 mg/disc (leaves) to 2.0 mg (flowers) and 3.0 mg (bark). MIC values of the same plant extracts against Gram positive bacteria such as *B. subtilis*, MRSA were 0.5 mg for both (leaves and bark) and 0.75 mg (fruits), and 1.0 mg (flowers for *B. subtilis* and MRSA). Al-Bakri and Afifi[37] reported similar trends of antibacterial activity of some selected Jordanian plant extracts.

As for yeasts, MIC values for *Candida albicans* and *Candida glabrata* varied according to plant parts extracts but were within 0.5-1.0 mg per disk while filamentous fungi MIC values were of a lower diameter (0.5-0.75 mg). The same trend was observed with ethanol extracts of *Jasminum sambac*, *Rosmarinus officinalis*, *Tecoma capensis* and *Populus* species plant parts extracts. Dadalioglu and Evrendilek[38] indicated the antibacterial

activity of essential oils of some *Lavandula* sp. and *L. nobilis* but did not study their antifungal activity. Del Campo *et al*.[39] studied also the antibacterial activity of rosemary extracts with no mention of antifungal activity. The high MIC values of *Tecoma capensis* has been noticed against both Gram negative and Gram positive bacteria (*Pseudomonas aeruginosa* 3 and 2.4 mg) flower and leaf extract and *Bacillus cereus* 2.0 and 1.0 mg, respectively. This trend was also observed with the aerial parts extract of *Sonchus oleraceus* which is tested for the first time specially with yeasts and filamentous fungi. The positive control penicillin, tetracycline and nystatin had much lower MIC values than our extracts. From all the extracts tested *Tecoma capensis*, *Laurus nobilis* and *Lavandula angustifolia* appeared to be the most active against most bacteria and fungi tested with potential MIC values. Joy and Raja[34] reported the antibacterial activity of *Jasminum sambac* and their data cannot be compared with our results where the concentrations they used ranged between 250-500 mg per ml which is very high in such studies. Results obtained for *Populus* spp. and *Sonchus oleraceus* did not rise conclusively to substantiate their use in the materia medica of Jordan and no published data are available for comparison. In conclusion, the data presented in this study indicated that the *in vitro* activity of the ethanolic extracts of these plants against fungi and bacteria varied with the type of microorganism and plant parts used. However, MIC values though relatively high compared with positive control pure antibiotics may substantiate and validate the medicinal properties of such plants used by traditional healers.

## CONCLUSIONS

Ethanolic extracts of the tested medicinal plants exhibited varying degrees of antibacterial and antifungal activity against an array of Gram-positive, Gram-negative bacteria and fungi. Minimum inhibitory concentration and diameter of inhibition zones of some extract showed superior activities although the MIC values tended to be relatively high for some microorganisms. *Tecoma capensis* and *Sonchus oleraceus* antimicrobial activity is reported for the first time. Results validate the use of these plants in the folk medicine of Jordan, however further studies are needed to identify the active ingredients.

## ACKNOWLEDGEMENTS

The authors are grateful to the University of Jordan for their financial support.

## REFERENCES

(1) Wallace J., Antimicrobial properties of plant secondary metabolites. *Proceedings of the nutrition society*. (2004); 63: 621-629.

(2) Adonizio Al., Donnum K., Bennett BC., Matheek., Antiqourum sensing activity of medicinal plants in Southern Florida. *J Ethnopharmacol*. (2006); 105: 427-435.

(3) Erturk O., Ozbucak TB., Bayrak A., Antimicrobial activities of some medicinal essential oils. *Herba Polonica*. (2006); 52: 58-66.

(4) Rangasamy O., Rakotoniriana G., Cheuk FE., Urvergo-Ratismamanga K., Quentin-Leclercq S., Gurib-Fakim J., Subratty AH., Screening for antiinfective properties of several medicinal plants of the Mauritians flora. *J Ethnopharmacol*. (2007); 109: 331-337.

(5) Mantle D., Gok MA., Adverse and beneficial effects of plant extracts on skin and skin disorders. *Adve Drug React and Toxcol Rev*. (2001); 20: 89-103.

(6) Kumar VP., Chauhan NS., Rajani HPM., Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol*. (2006); 107: 182-188.

(7) Tadeg H., Mohammad E., Asres K., Gebre-Mariam T., Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *J Ethnopharmacol*. (2005); 100: 168-175.

(8) Mahasneh AM., Screening of some indigenous Qatari medicinal plants for antimicrobial activity. *Phytother Res*. (2002); 16: 751-753.

(9) Quave CL., Pieroni A., Bennett BC., Dermatological

remedies in the traditional pharmacopoeia of Vulture-Alto Bardano, inland Southern Italy. *J Ethnobiol Ethnomed*. (2008); 4(5): 1-10.

(10) Hentzer M., Givskov M., Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J Clini Invest*. (2003); 112: 1300-1307.

(11) Kultur S., Medicinal plants used in kirklareli province (Turkey). *J Ethnopharmacol*. (2007); 111: 341-364.

(12) Cos P., Vlietink AJ., Berghe DV., Maes L., Antiinfective potential of natural products: How to develop a stronger *in vitro* "proof of concept". *J Ethnopharmacol*. (2006); 106: 290-302.

(13) Hiller K., Melzig FM., (2006): *Lexikon der Arzneipflanzen und Drogen, Spektrum Academischer Verlag*, Heidelberg, pp. 72-76.

(14) Lewis K., Riddle of biofilm resistance. *Antimicro Agents Chemother*. (2001); 45: 1007-1012.

(15) Chomnawang MT., Surassomo S., Wongsariya K., Bunyapraphastara N., Antibacterial activity of Thai medicinal plants against methicillin-resistant *S. aureus*. *Fitoterapia*. (2009); 80:102-104.

(16) Wax R., Lewis K., Salyers A., Taber H., (2008). *Bacterial resistance to antimicrobials*, 2nd ed. Florida: CRC press, pp. 28-36.

(17) Gao M., Teplitski M., Robinson JB., Bauer WD., Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. *The American Phytopatho Soc*. (2003); 16: 827-834.

(18) Poonguzhali S., Madhaiyan M., Sa T., Quorum sensing signals produced by plant-growth promoting *Burkholderia* strain under *in vitro* and in plant conditions. *Res Microbiol*. (2007); 158: 287-294.

(19) Rios JL., Recio MC., Medicinal plants and antimicrobial activity. *J Ethnopharmacol*. (2005); 100: 80-84.

(20) Fang F., Sang S., Chen KY., Gosslau C., Rosen RT., Isolation and identification of cytotoxic compounds from bay leaf *Laurus nobilis*. *Food Chemistry*. (2005); 93: 496-501.

(21) Barla A., Topcu G., Oksuz S., Tumen G., Kingston DGI., Identification of Cytotoxic Sesquiterpenes from *Laurus nobilis* L. *Phytochemistry*. (2007); 31(7): 2537-2538.

(22) Braun L., Cohen M. *Herbs and Natural Supplements* 2nd ed. Australia: Churchill Living Stone, (2007). pp. 31-62.

(23) Duke J. *CRC Handbook of Medicinal Herbs*, 2nd ed. Florida: CRC press, (2002). pp. 42-58.

(24) Hajhashemi V., Ghannadi A., Sharif B., antiinflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. *J Ethnopharmacol*. (2003); 89:67-71.

(25) Duke J. *Dukes Handbook of Medicinal Plants of the Bible*, 1st ed. Florida: CRC press, (2008). pp. 122-141.

(26) Duke J. *CRC Handbook of Edible Weeds*, 1st ed. Florida: CRC press, (1992). pp. 22-70.

(27) Mahasneh AM., El-Oqlah AA., Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. *J Ethnopharmacol*. (1999); 64: 271-276.

(28) Al-Hussaini R., Mahasneh AM., Antimicrobial and antiquorum sensing activity of different parts of *Laurus nobilis* L. extracts. *Jordan Med Journal.* (2009), 43(4):286-289

(29) National Committee for Clinical Laboratory Standards. Methods for Disk Susceptibility Tests for Bacteria that Grow Aerobically. NCCLS M2-A7: Wayne, PA, USA, 2003; p. 7.

(30) Abdillahi HS., Stafford GI., Finnie JF., Van Staden J., Antimicrobial activity of South Africa *Podocarpus* species. *J Ethnopharmacol*. (2008); 119(2): 191-194.

(31) Hashem FA., Free radical scavenging activity of the flavonoids isolated from *Tecoma radicans*. *J Basic Appl Sci*. (2007); 3(1): 59-73.

(32) Adam M., Berset C., Kessler M., Hamburger M., (2009): Medicinal herbs for the treatment of rheumatic disorders-A survey of European herbals from the 16th and 17th century. *J Ethnopharmacol* 121(3): 343-359.

(33) Guil-Guerrero JL., Gimenez-Gimenez A., Rodriguez-Garcia I., Torija-Isasa ME., Nutritional composition of *Sonchus* spp (*S. asper* L., *Soleraceus* L. and *Stenerrimus* L.). *J Sci Food Agri*. (1998): 76: 628-632.

(34) Joy P., Raja P., Antibacterial activity studies of *Jasminum grandiflorum* and *Jasminum sambac*. *Ethnobotanical Leaflets*. (2008); 12: 481-483.

(35) Guiso M., Marra C., Piccioni F., Nicolettim., Iridoid and phenylpropanoid glucosides from *Tecoma capensis*. *Phytochemistry*. (1997); 45(1): 193-194.

(36) Kalyoncu F., Cetin B., Saglam H., Antimicrobial activity of common mader (*Rubia tinctorum* L.). *Phytother Res*. (2006); 20: 490-492.

(37) Al-Bakri, AG., Afifi FU., Evaluation of antimicrobial activity of selected plant extracts by rapid XTT colorimetry and bacterial enumeration. *J Microbiol*.

*Methods*. (2007); 68: 19-25.

(38) Dadalioglu I., Evrendilek GA., Chemical compositions and antibacterial effects of essential oils of Turkish oregano *O. minutiflorum*, *L. nobilis*, *Lavandula stoechas* and *F. vulgare* on common food pathogens. *J Agricult Food Chem*. (2004); 52(2): 8255-8260.

(39) Del Campo J., Amiot MJ., Nguyen-The C., Antimicrobial effect of rosemary extracts. *J Food Protec*. (2000); 63(100): 1359-1368.

# تأثير مستخلص الإيثانول لأجزاء مختلفة من نباتات طبية في الأردن في تثبيط نمو البكتيريا والفطريات

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## ملخـص

لقد تم تحديد فعالية مستخلص الإيثانول النباتي بتحديد قيمة الحد الأدنى المثبط لنمو تسع عزلات بكتيرية وأربع عزلات فطرية.

واشتملت الدراسة على أربعة عشر مستخلصاً لأجزاء مختلفة من نباتات: *Tecoma capensis* Thunb. Lindl (Bignoniaceae) ، *Lavandula angustifolia* Mill. (Lamiaceae)، *Rosmarinus officinalis* L. (Lamiaceae)، *Jasminum sambac* Ait. (Oleaceae)، *Populus alba* L. (Salicaceae)، *Populus nigra* (Salicaceae)، *Sonchus oleraceus* L. (Asteraceae)، و *Laurus nobilis* L. (Lauraceae). وقد لوحظ تباين كميات المواد المستخلصة من النبتة الواحدة، وكذلك تباين بين أجزاء النبتة الواحدة؛ حيث كانت أعلى النسب (18%) تلك المستخلصة من قلف نباتات الغار، بينما كانت أقل نسبة (4%) المستخلصة من أوراق نبات *P. nigra* وتراوحت النسب الأخرى ما بين 7-17%. كما تراوحت الفعالية المثبطة لنمو الكائنات الدقيقة عند استعمال تركيز 3 ملغم ما بين 8-25 ملمتر (قطر منطقة منع النمو)، في حين أن مستخلص أوراق نبات الغار أظهر أعلى فعالية تجاه البكتيريا (22 ملمتراً) والحد الأدنى للجرعة المانعة للنمو (500 مايكروجرام) في حالة بكتيريا *Bacillus subtilis*. وكذلك الحال تجاه فطر *Aspergillus niger* حيث كانت الفعالية 25 مليمتر قطراً و 500 مايكروجرام الحد الأدنى للجرعة المانعة للنمو، وكانت مستخلصات *S. oleraceus* وأوراق *P. nigra* و *P. alba* هي الأقل فعالية تجاه البكتيريا والفطريات تحت الدراسة. وقد لوحظ أن مستخلص أزهار نبات *T. capensis* قد تمتع بفعالية عالية تجاه الفطريات (17 ملمتراً والحد الأدنى للجرعة 500 مايكروجرام)، وكذلك بفعالية معتدلة تجاه بكتيريا *Staphylococcus aureus* المقاومة للميثيسيلين، وهذه أول إشارة لفعالية هذا المستخلص. وقد بينت دراسة هذه المستخلصات فعالية مثيرة للاهتمام، مما يدلل على صحة استعمالها في الطب الشعبي، ويدعو إلى إجراء دراسات أكثر تفصيلاً.

**الكلمات الدالة**: تثبيط الكائنات الدقيقة، بكتيريا، فطريات، الأردن، نباتات طبية.

تاريخ استلام البحث 2010/1/7 وتاريخ قبوله للنشر 2010/6/28.