

Antimicrobial and Antiquorum Sensing Activity of Different Parts of *Laurus nobilis* L. Extracts

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Abstract

Since quorum sensing controls the density and pathogenesis of a wide variety of bacteria, it became inviting to test some plant extracts for their anti-quorum sensing (AQS) and antimicrobial activity. *Laurus nobilis* L. commonly known as bay leaf belongs to the Lauracea family. It has been a potential culinary and medicinal plant in east Mediterranean including Jordan. In this study, 24 aqueous, ethanol, butanol, hexane, chloroform and methanol extracts of *L.nobilis* L. leaves, bark, fruit and flowers were tested for their antiquorum sensing, antibacterial and antifungal activities. Test microorganisms included *Chromobacterium violaceum* for anti-quorum sensing activity. Two gram positive, two gram negative bacteria and four fungi species. Of these extracts, significant antiquorum sensing activity was associated mainly with the hexane (25mm of AQS halo) and ethanol (21 mm) flowers extracts of *L.nobilis* L.

Other extracts showed low (10-14 mm halo) to moderate ¹⁵⁻²⁰ varying degrees of antiquorum sensing activity. As for antimicrobial testing, the magnitude of activity varied in terms of the type and number of bacteria and fungi tested as well as to the degree of inhibition. Superior antifungal activity as compared with nystatin was recorded for ethanol and hexane leaf and bark extracts especially against *Aspergillus fumigatus* (25,23 mm inhibition zone), *Aspergillus niger* ^{21,20} and *Candida albicans*. ^{11,13} The antibacterial activity also varied among different extracts and different gram positive and negative bacterial species. To be highlighted is the superior activity of aqueous bark extract against methicillin resistant *Staphylococcus aureus* (25 mm) compared with penicillin G (33 mm).

A moderate antibacterial activity of flowers, fruits and leaves extracts against gram negative *Klebsiella pneumoniae* and *Salmonella typhimurium* was also recorded. MICs values for both bacteria and fungi were relatively high (525-3000 µg), this is understandable since we are dealing with crude extracts. These results stress for the first time the importance of antiquorum sensing activity of *L.nobilis* L. extracts as an antipathogenic agents and being a possible new alternative for bacterial disease therapy. It also substantiates and validates medicinal plants use in folk medicine.

Keywords: *Laurus nobilis* L. plant extracts; antimicrobial activity; antiquorum sensing.

(J Med J 2009; Vol. 43 (4):286-298)

Received

October 21, 2008

Accepted

January 18, 2009

Introduction

In drug discovery, most studies have looked on the antimicrobial potential of medicinal plants

and other natural products ^{1, 2} measured as either killing or inhibiting the microbial growth without giving proper attention to the antiquorum properties of such materials.

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However, natural products including medicinal plants are still major sources of innovative therapeutic agents for various conditions of human diseases.³

The increased role of antibiotic resistant pathogenic microorganisms is greatly mediated by the increased frequency of mutations, misuse of antibiotics and other factors.⁴ Combating such situation so far is dependent upon the traditional treatment of such microbial infections based on substances that kill or inhibit growth of causative pathogens.⁵

Quorum sensing (QS) or cell to cell communication which is a generic regulatory mechanism in bacteria perceiving and responding to bacterial population density and certain gene expression by a small molecule called autoinducers is thought to have a role in bacterial pathogenesis.^{6,7}

This quorum sensing mechanism is also believed to lend itself to pathogenic bacteria to minimize or stop host immune responses through arresting the production of tissue-damaging virulence factors until sufficient bacterial density is attained.^{8,9} The notion that quorum sensing is somehow linked to pathogenicity and virulence may suggest controlling pathogens by inhibiting their quorum sensing systems.¹⁰ However, quorum sensing does not control virulence only but biofilm formation in certain bacteria as well.¹¹

The traditional chemotherapeutic agents exhibit a broad range efficacy through toxicity or growth inhibition to target microorganisms. Due to misuse of such agents in addition to selective pressure upon pathogens, an increased level of antibiotic resistance is on the rise.^{4,5} An alternative to 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 antipathogenic drugs. Quorum sensing inhibitors would undoubtedly help in providing means of controlling and treating some

microbial infections without selecting for increased resistant strains.¹² Given the large number of organisms including some plants that would use quorum sensing inhibition to control the activity of microbial pathogenic colonizers,^{13,14} we embarked on studying the antimicrobial as well as the antiquorum sensing activity of some medicinal and culinary plants in Jordan. Among these is the laurel tree (*Laurus nobilis* L.) commonly known as el-Ghar and a member of the Lauraceae family. It is traditionally used in food flavoring, herbal teas and in cosmetics industry for its oils and pleasant scent.¹⁵ Although it has been widely used in the eastern Mediterranean, *L.nobilis* L. has scarcely been comprehensively assayed for quorum sensing and antimicrobial antagonistic activities.¹⁶ However, the antinociceptive, analgesic, anti-inflammatory activity and antioxidant activity of the leaf extracts of *L.nobilis* L. were investigated.¹⁶⁻¹⁸

In this report, leaves, flowers, fruits and stem bark extract of *L.nobilis* L. are studied for their anti-quorum sensing, antibacterial and antifungal activities. This work is a part of an ongoing effort of our laboratory¹⁹⁻²² to screen and test as much as possible of medicinal and culinary plants indigenous and introduced into Jordan for bioactivity. It is a modest contribution to the quest of promoting human health by preventing microbial pathogenesis.

Methods

Plant Material

Laurus nobilis L. leaves, flowers, fruits and stem bark were collected during March-November 2007, from Jubaiha gardens, 10 Km north of Amman, Jordan.

The plant was authenticated by Prof. A. EL-Oqlah, a plant taxonomist at the Yarmouk University, Irbid, Jordan. A voucher specimen (MAHAS 1) is deposited in the Department of Biological Sciences, University of Jordan, Amman.

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

Extractions of leaves, flowers, fruits and stem bark

The air-dried leaves (350 g), flowers(260 g), fruits (263 g)and bark (96g) of *L.nobilis* L. were separately extracted twice at room temperature with ethanol 95% (500 ml/100 g of plant material each run). The final ethanol extract was filtered using (Whatman) filter paper and was evaporated under vacuum using rotary vacuum evaporator (Buchi R-215, Switezland) at 40 °C. The resultant residues from leaves (55 g), flowers (21.3 g), fruits (35g), and bark (17 g) were further fractionated according to (Mahasneh, 2002; Mahasneh and EL-Qqlah, 1999) as in (Fig.1) 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: Methacillin resistant *Staphylococcus aureus* (MRSA Clinical isolate), *Bacillus cereus* (Toxigenic strain). Gram negative bacteria: *Salmonella typhimurium* (ATCC 14028), *Klebsilla pneumoniae* (ATCC 10031) and both filamentous fungi; *Aspergillus fumigatus* (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 on to nutrient agar slants at 4 °C. Subcultures were freshly prepared before the assay. 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. 1×10^8 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.

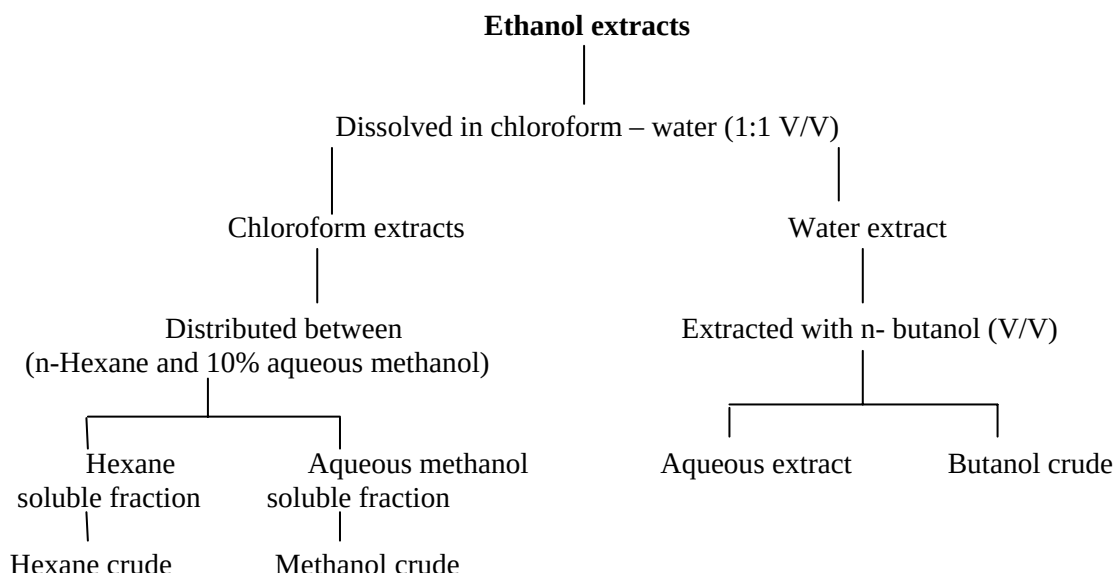


Figure (1): Further fractionation of ethanol extract.

Antimicrobial activity testing

Extracts of the leaves, flowers, fruits and bark of *L.nobilis* L. were dissolved in 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 525- 3000 µg of the sterile appropriate extract and were placed in duplicates onto Muller–Hinton agar (Oxoid, England) plates for bacteria and malt extract agar for yeast and filamentous fungi.

These plates were earlier surface inoculated with 100 µl of freshly prepared bacteria, fungal spores or yeast cells suspension (Ca.10⁸ CFU/ml). The plates were kept for 2 h at 4 °C to facilitate diffusion of the substances into the agar and were then incubated for 24h at 37 °C for bacteria and for 48 h at 28 °C for fungi. Inhibition zone diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for two replicates.

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

Minimum inhibitory concentrations (MIC_s) for the tested samples were determined by the agar diffusion assay (NCCLS, 2003) using media and incubation temperature as above for both bacteria and fungi. Negative controls of DMSO alone were included as well as positive controls of the standard antibiotic ampicillin and tetracycline. MIC was defined as the lowest concentration of the extract that totally inhibited the growth of the tested microorganisms.

Antiquorum sensing assay

The bacterial biomonitor strain *Chromobacterium*

violaceum ATCC 12427 was a kind gift from Professor Robert Mclean of the Department of Biology, Texas State University–San Marcos, USA. This strain produces a purple pigment, violacein which is under quorum sensing control. The inhibition of violacein production by antiquorum sensing material makes this bacterium an excellent model for the isolation of antiquorum sensing substances from natural products. The disc diffusion assay was employed to test the antiquorum sensing activity of the different extracts of *L.nobilis* L. In this test the bacterial growth inhibition would exhibit a clear halo around the disc while quorum sensing inhibition is exhibited by a turbid halo harboring pigmentless bacterial cells of the monitor strain. Each extract (525-3000 µg/disc) was impregnated onto sterile (6 mm diameter) filter paper discs placed on to Luria-Bertani agar plates (Merck, Germany) surface inoculated with 100 µl of the (16-18 h) culture of *Chromobacterium violaceum* monitor strain adjusted to 0.5 McFarland standard (Ca.10⁸ CFU/ml). Plates were kept for 2 h in the refrigerator to facilitate diffusion and were then incubated at 30 °C for 24h.

Quorum sensing inhibition activity was recorded by observing a ring of colorless but viable bacterial cells around the disc. These were positively controlled by observing bacterial growth inhibition zones by standard tetracycline discs (30µg/disc). To validate this result for quorum sensing inhibition, the flask incubation assay test of Choo²³ for the quantification of quorum sensing inhibitory activity of some plant extracts was carried out using *C.violaceum* monitor strain.

Results

Plant extracts

Table (1) shows the ethanol extract starting material and percentage yields of different plant forms. The highest yield recorded from *L.nobilis* L. bark 18%, followed by leaves 16%, fruit 13% and flowers 8% w/w. These ethanol fraction yields when further fractionated as in methods gave varying yields with other solvents including chloroform, hexane, aqueous methanol, butanol

and aqueous extracts. Lowest yields were observed with methanol extract where only yields of 2.5 % (leaf), 13 % (flowers), 10% (fruits) and 7% (bark) were recorded.

Other solvents yields varied with highest for flowers aqueous and bark butanol 47 and 35% w/w respectively followed by leaves 28% and fruits 26% hexane extracts.

Table (1): *Laurus nobilis* L. plant forms extracted and yields from different solvents.

Plant form Starting weight(g)	Extracts yields (%) w/w					
	E	C	H	M	B	A
Leaves (350)	16	23	28	2.5	4.5	1.8
Flowers (260)	8.0	8.5	3.0	13	19.5	47
Fruits (263)	13	23	26	10	7.0	11
Bark (96)	18	8.0	5.0	7.0	35	22

(E) Ethanol; (C) Chloroform; (H) Hexane; (M) Methanol ; (B) Butanol; (A) Aqueous.

An antiquorum sensing activity of different plant form extracts using *Chromobacterium violaceum* 12427 monitor strain

Disappearance of the violet colored pigment in *Chromobacterium violaceum* is a strong evidence of quorum-sensing inhibition by the different *L.nobilis* L. extracts. Table (2) presents results of the antiquorum sensing (AQS) activity of plant extracts regardless of the type of solvent used. Antiquorum sensing activity was clear as indicated by the size of the quorum sensing halo surrounding the extract discs compared to the antibiotic tetracycline (30µg/disc) zone of growth inhibition of the *C. violaceum*. The best antiquorum sensing activity as measured by the diameter of the zone of the colorless (halo) *C. violaceum* growth was exhibited by *L.nobilis* L.

flowers irrespective of the extraction solvent. Diameters of the halo of quorum sensing inhibition were (16 mm) lowest for the aqueous extract at (3mg /disc) to highest (25 mm) for hexane extract at the same concentration (Table 2).

However, strongest antiquorum sensing activity at (3 mg/disc) was also prevalent for hexane extracts of flowers (25 mm), fruits (21 mm), bark (19 mm) and leaves (18 mm). Never the less, comparable butanol leaf extracts anti-quorum sensing activities (19.5mm), chloroform fruits (18mm), ethanol bark (19 mm) and butanol leaves extracts (19.5mm) were recorded at the 3 mg/disc concentrations. According to results obtained, low AQS activity (10-14 mm halo) and moderate (15-20 mm) were recorded for the different plants extracts (Table 2).

Table (2): Antiquorum sensing activity of different plant forms of *L.nobilis* L. extracted with varying solvents. Diameters (mm) of anti-quorum sensing halo at a concentration of 3 mg of the extract (average of 3 measurements).

Solvent	Extraction		Plant form extracts	
	Leaves	Bark	Fruit	Flower
Aqueous (A)	10	14	10	16
Butanol (B)	19.5	17	17	18
Methanol (M)	11	15	9.5	17
Hexane (H)	18	19	21	25
Chloroform (C)	17.5	17	18	19
Ethanol (E)	17.5	19	15	21

To substantiate these results, the quantitative flask assay of Choo²³ for quantification of quorum sensing inhibitory activity was carried out. Interestingly, the different *L.nobilis* L. flower extracts (Fig.2) showed concentration-dependent quorum sensing inhibitory activity where a significant drop in violacein production by *C. violaceum* as measured as O.D (585 nm) was observed.

At a concentration of 3 mg per ml of the culture assay, flowers hexane extract reduced violacein production by about 95% and ethanol flowers extract reduced it by about 83% (Fig.2). Other extracts, namely *L.nobilis* L. hexane bark extract showed a similar trend (unpublished data).

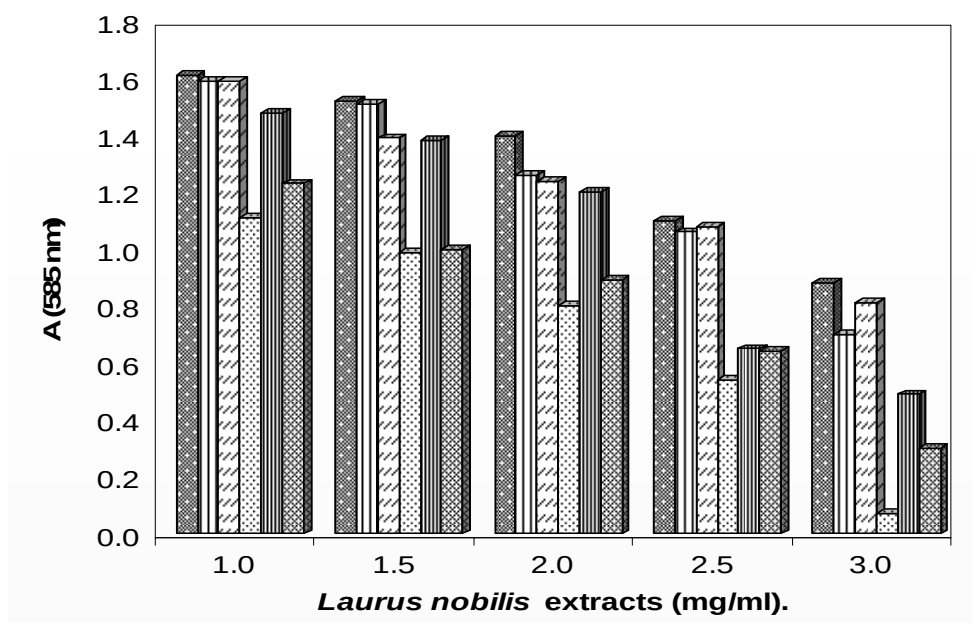


Figure (2): Anti-quorum sensing activity of *L.nobilis* L. flower extracts at different concentrations.
Aqueous ■ Butanol □ Methanol ▨ Hexane ▩ Chloroform ▤ Ethanol ▦

Antimicrobial activity

Microbial growth inhibition by *L.nobilis* L. different extracts would produce a clear zone of no growth compared with the antiquorum sensing halo of colorless cells of *C. violaceum*. We confirmed this by studying the effect of the different extracts upon the growth of several selected bacterial and fungal strains (Table 3).

The *L.nobilis* L. leaves extracts showed varying degrees of activities against microorganisms

tested. Depending upon values recoded, these activities fall in either of a moderate (8-10 mm inhibition zone), good (10-15mm) to superior (>15 mm) for all extracts of different solvents used. However, butanol and hexane leaf extracts were superior (> 15 mm inhibition zones) to others especially against gram positives *S.aureus* (MRSA), and *B.cereus* and the gram negative *K.pneumoniae* and *S.typhimurium*.

These results (Table 3) at 3 mg/disc are comparable to the reference standard antibiotics used i.e. penicillin G and tetracycline considering that these are crude extracts. As for antifungal activity, ethanol, hexane and chloroform leaf extracts were superior on the whole, especially against the filamentous fungi i.e. *A.niger* and *A.fumigatus* where inhibition zones (20-25 mm)

were in fact at 3 mg /disc of the extract greater than the inhibition zones for the standard reference nystatin (22-23 mm) at 100 µg/disc. (Table 3). Other extracts were of the good order of inhibition for both filamentous fungi *A.niger*, *A.fumigatus*, and non filamentous (yeasts) *C.glabrata* and *C. albicans* respectively (Table 3).

Table (3): Antimicrobial activity of *L.nobilis* L. leaves extracts at (3 mg /disc).

Microorganism	Plant leaves extract								
	A	B	M	H	C	E	P	T	N
<i>S.aureus</i> (MRSA)	15	19	14	16.5	15.5	16	33	28	N.T.
<i>B. cereus</i> (TS)	10.5	15.5	13	15	11	14	30	15	N.T.
<i>C.violaceum</i>	6.5	17	8	15	13	14	N.T.	40	N.T.
<i>K. pneumoniae</i>	12	13	13	16	16	18	N.T.	28	N.T.
<i>S.typhimurium</i>	14	12	11	14	8.5	10	N.T.	26	N.T.
<i>C.glabrata</i>	15	12	10	15	10.5	12.5	N.T.	N.T.	27
<i>C.albicans</i>	9	15	14	13	13	11	N.T.	N.T.	26
<i>A.fumigatus</i>	10	11	13	23	17.5	25	N.T.	N.T.	23
<i>A. niger</i>	11	14	8.5	20	21	21	N.T.	N.T.	22

(Diameter of inhibition zone mm, 8-10 moderate, 10-15 good, >15 superior) Standard penicillin (P) 10 unit/disc, tetracycline (T) 30 µg/disc and nystatin (N) 100 µg/disc were included. Values are average of 2 replicates. (N.T.) not tested. MRSA Methicillin Resistant *Staphylococcus aureus* (*S.aureus* MRSA), *Bacillus cereus* Toxigenic strain (*B.cereus* TS), *Chromobacterium violaceum* (*C.violaceum*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella typhimurium* (*S. typhimurium*), *Candida glabrata* (*C.glabrata*), *Candida albicans* (*C.albicans*), *Aspergillus fumigatus* (*A.fumigatus*), *Aspergillus niger* (*A. niger*).

MICs of these extracts for bacteria and fungi tested ranged from 525 µg of the butanol, hexane and ethanol extracts for gram positive *S.aureus* (MRSA) and *B.cereus*, to 525, 1000 and 3000 µg for butanol, aqueous and ethanol for gram negative *C.violaceum*, *K. pneumonia* and *S. typhimurium* (Table 7).

MICs for fungi were of somehow close to values for bacteria where butanol, hexane and ethanol extracts showed an MIC values of 525, 2000 and 1200 µg when tested against *C. albicans*, *C.glabrata* or *A.fumigatus*. Of all these, leaf extracts of butanol, hexane and ethanol appeared to be the most active against both gram positive and gram negative bacteria. The plant bark extracts showed variable degrees of antibacterial and antifungal activities (Table 4).

Interestingly the bark aqueous extract at 3 mg/disc exhibited a prominent antibacterial activity (25 mm inhibition zone) against methicillin resistant *S.aureus* which was comparable to tetracycline (28 mm) and penicillin G (33mm). This aqueous extract activity was not as noticeable when tested against other bacteria and fungi where it fell in the moderate to good range of activity (Table 4).

Other bark extracts such as butanol, hexane and ethanol were again of higher potential compared with the other extracts and the same trend as above was observed for leaf extracts with the exception of the hexane bark extracts being superior again against the non filamentous fungi *C. albicans* (20 mm inhibition zone) and *C.glabrata* (18 mm inhibition zone).

MICs for bark extracts were 525 µg for gram positive *S.aureus* and *B.cereus* for all solvents extracts and 525-3000 µg for both gram negative and fungi tested (Table 7). The highest MICs recorded were for the hexane (3000 µg) and ethanol (2400 µg) bark extracts for *K. pneumonia* (Table 7). The *L.nobilis* L. fruits extracts showed variable degrees of antibacterial and antifungal activities of the moderate to good order of inhibition against test organisms.

Surprisingly, the hexane and chloroform fruits extracts showed an impressive antibacterial activity against the methicillin resistant *S.aureus* where inhibition zone diameters recorded were 21 and 22 mm versus 28 mm for standard tetracycline and 33 mm for penicillin G (Table 5).

MICs for the fruits extracts against bacteria and fungi were generally higher than these recorded for other plant forms (Table 7). MIC values were 2-3 folds higher than these for leaves and bark extracts.

The antimicrobial activity of the *L.nobilis* L. flower extracts were equally of the moderate range against gram positive and gram negative bacteria and again the hexane flower extract exhibited a noticeable superior activity against bacteria and fungi tested (Table 6).

The aqueous, butanol and chloroform flowers extract exhibited a rather striking activity against filamentous fungi (*A.niger* and *A.fumigatus*) an activity exceeding that of the standard anti fungal nystatin (Table 6). It is worthy to say that hexane flowers extract was vary active against the non filamentous fungi (*C. albicans* and *C. glabrata*).

MICs as determined for the flowers extracts were closely related to values recorded for fruit extracts tending to be higher compared with MICs for the leaf and bark extracts (Table 7).

However, it could be easily recognized (Table 7) that MICs for the aqueous, butanol and ethanol extracts of the flowers are relatively higher compared with the other plant parts extracts.

Table (4): Antimicrobial activity (diameter of inhibition zone mm) of *L.nobilis* L. bark extracts at (3 mg /disc). Details as in Table (3).

Microorganism	Plant bark extract								
	A	B	M	H	C	E	P	T	N
<i>S.aureus</i> (MRSA)	25	18.5	15	10	19	18	33	28	N.T.
<i>B. cereus</i> (TS)	13	12	14	22	12	18	30	15	N.T.
<i>C.violaceum</i>	11	13	11	16	14	16	N.T.	40	N.T.
<i>K. pneumoniae</i>	7.5	6.5	10.5	9	9	7	N.T.	28	N.T.
<i>S.typhimurium</i>	10.5	12	8	10.5	11	9	N.T.	26	N.T.
<i>C.glabrata</i>	8.5	11	12	18	17	12	N.T.	N.T.	27
<i>C.albicans</i>	9.0	10	12	20	15	11	N.T.	N.T.	26
<i>A.fumigatus</i>	17	15	12	12	10	15	N.T.	N.T.	23
<i>A. niger</i>	15	13	10	13	18	18	N.T.	N.T.	22

Table (5): Antimicrobial activity (diameter of inhibition zone mm) of *L.nobilis* L. fruits extracts at (3 mg /disc). Details as in Table 3.

Microorganism	Plant fruits extract								
	A	B	M	H	C	E	P	T	N
<i>S.aureus</i> (MRSA)	11	12	14	21	20	12	33	28	N.T.
<i>B. cereus</i> (TS)	10.5	11	8.5	14.5	12	14.5	30	15	N.T.
<i>C.violaceum</i>	8.0	13	7.0	8.0	10	9.0	N.T.	40	N.T.
<i>K. pneumoniae</i>	11	10.5	7.5	10	10.5	10	N.T.	28	N.T.
<i>S.typhimurium</i>	9.0	9.0	10.5	10.5	9.5	9.0	N.T.	26	N.T.
<i>C.glabrata</i>	9.0	12	12	15	15	9.0	N.T.	N.T.	27
<i>C.albicans</i>	9.5	14	11.5	15	13	9.5	N.T.	N.T.	26
<i>A.fumigatus</i>	9.0	11	10	25	13	13	N.T.	N.T.	23
<i>A. niger</i>	11	15	13	10	14	11	N.T.	N.T.	22

Table (6): Antimicrobial activity (diameter of inhibition zone mm) of *L.nobilis* L. flowers extracts at (3 mg/disc). Details as in Table (3).

Microorganism	Plant flowers extract								
	A	B	M	H	C	E	P	T	N
<i>S.aureus</i> (MRSA)	12.5	13	14.5	17	11	11	33	28	N.T.
<i>B. cereus</i> (TS)	7.0	10	11	21	13.5	11	30	15	N.T.
<i>C.violaceum</i>	12	13	9.0	22	14	15	N.T.	40	N.T.
<i>K. pneumoniae</i>	11.5	12.5	11	15	12	11	N.T.	28	N.T.
<i>S.typhimurium</i>	11	9.0	13	8.5	8.0	10	N.T.	26	N.T.
<i>C.glabrata</i>	11	9.0	9.0	17	13.5	11.5	N.T.	N.T.	27
<i>C.albicans</i>	9.0	10.5	13	20	16	12.5	N.T.	N.T.	26
<i>A.fumigatus</i>	17	22	13	17	15.5	16.5	N.T.	N.T.	23
<i>A. niger</i>	22	24	17	16	20	19	N.T.	N.T.	22

Table (7): Minimum inhibitory concentration (MICs) of some different extracts of different plant form of *L.nobilis* L. Solvents initials as in Table (1).

Plant form	Extracts	MICs (µg/disc)								
		S.a.	B.c.	C.v.	K.p.	S.t.	C.g.	C.a.	A.f.	A.n.
Leaf	A	525	525	3000	525	525	525	1200	525	525
	B	525	525	525	525	525	525	525	525	525
	H	525	525	525	525	525	525	2000	525	525
	E	525	525	525	525	1000	750	1200	525	525
Bark	A	525	525	525	2000	525	1500	2000	525	525
	B	525	525	525	3000	525	525	525	525	525
	H	525	525	525	1000	525	525	525	525	750
	E	525	525	525	2400	1000	525	525	525	525
Fruit	A	1000	1500	1500	525	1000	1200	1000	1500	1000
	B	525	525	1200	1000	1200	525	750	525	525
	H	525	525	1500	1200	525	525	525	525	525
	E	525	525	1000	1500	1500	1000	750	525	525
Flower	A	525	2400	525	525	750	1500	2000	525	525
	B	750	1000	525	750	1500	1200	1000	525	525
	H	525	525	525	525	1200	525	525	525	525
	E	1000	525	525	1000	1200	1000	525	750	525
Tetracycline	-	N.T.	N.T.	0.62	0.62	1.11	N.T.	N.T.	N.T.	N.T.
Ampicillin	-	0.06	0.93	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.

S.aureus (MRSA); *B.c.*: *B. cereus* (TS); *C.v.*: *C.violaceum*; *K.p.*: *K. pneumoniae*; *S.t.*: *S.typhimurium*; *C. glabrata*; *C.a.*: *C.albicans*; *A.f.*: *A.fumigatus*; *A.n.*: *A. niger*; N.T.: not tested.

Discussion

In the east Mediterranean materia medica, *L.nobilis* L. known as bay laurel leaves, flowers, fruits and bark are described for the treatment of different afflictions.¹⁶ It is also one of the widely used spices in the western world.¹⁵ The main aim of this study was to validate some aspects of the traditional uses of *L.nobilis* L. as antibacterial and antifungal agents² as well as the antipathogenic potential of different solvents extracts by looking at the anti-quorum sensing activity of such extracts. The interruption of bacterial quorum sensing is just an example of the antipathogenic activity of these extracts which is very poorly researched from natural products compared to the direct antimicrobial activities.¹³

Our results indicated the anti-quorum sensing activity to be associated mainly with the flowers extracts of *L.nobilis* L. irrespective of the solvent used (Table 2). However, hexane and ethanol flower extracts were prominent compared with other solvents as well as other plant form extracts i.e. leaves, fruits and bark (Table 2).

Fang¹⁵ found that the hexane extract of the *L.nobilis* L. leaves exhibited the strongest apoptotic biological activity but did not test flowers, fruits or bark. The least antiquorum sensing activity in our study was recorded for the aqueous extracts irrespective of the plant form, though the leaf and fruit extracts were lowest (Table 2).

Fray²⁴ indicated the incidence of antiquorum sensing among higher plants. Our results with *L.nobilis* L. do agree with this notion since the leaf, flowers, fruit and bark extracts of this plant showed varying degrees of antiquorum sensing activity. These results confirm the claim that antiquorum sensing activity of plants against bacterial systems is diverse and more complicated than previously thought.

From this, it could be said that different signaling molecules are present in these extracts of the different organic solvents we used. This explains the varying degrees and spectrum of the anti-

quorum sensing of *L.nobilis* L. different plant forms (Table 2). It is also important to highlight here the effect of the solvents used in the screening process and their preparation impact upon the bioactivity, a factor which is not greatly regarded in medicinal plants studies.² The different *L.nobilis* L. extracts were also tested for their antibacterial and antifungal activity against an array of gram positive and gram negative bacteria and fungi (Table 3).

The biological activities of leaves, flowers, fruits and bark of *L.nobilis* L. has not previously been studied comprehensively. However, leaves and flowers which are traditionally used orally in our part of the world to treat gastrointestinal problems among other afflictions, have showed some trypanocidal activity.²⁵ In this study, different extracts of the leaves, flowers, fruits, and bark showed varying degrees of antibacterial as well as antifungal activities (Tables 3, 4, 5, 6).

The magnitude of activity varied in terms of the type and number of bacteria and fungal species inhibited and the degree of inhibition as related to the plant form, solvent used for extraction and the standard antibiotics tested. Matsuda²⁶ reported different pharmacological properties of *L.nobilis* L. leaf extracts. Fang¹⁵ also isolated cytotoxic compounds from bay leaf.

Plant leaves hexane and butanol extracts showed a broad range of antibacterial and antifungal activities. Superior antifungal activity was recorded for the ethanol and hexane leaf extracts. This activity was prominent against the filamentous fungi *A.fumigatus* and *A.niger* where an inhibition zones diameters of 25 and 21 mm respectively were recorded for the ethanol leaf extract at 3 mg/disc compared to the antifungal standard nystatin (23 mm inhibition zone) (Table 3).

Barla¹⁶ found the fruit extract of *L.nobilis* L. to be marginally active against some yeasts, while leaf and flower extracts were cytotoxic against ovarian cancer cells. The antifungal activity against the non filamentous fungi (yeasts) *C.glabrata* and *C.albicans* was of the moderate

type in the case of hexane and ethanol leaf extracts. While in the case of the bark extracts, the aqueous extract showed the highest activity at 3 mg/disc (25 mm inhibition zone) against the *S.aureus* (MRSA) compared with standard penicillin G (33 mm inhibition zone).

As for fungi, hexane bark extract at 3 mg/disc (20 mm inhibition zone) was very comparable with nystatin (26 mm inhibition zone) when tested against the yeast *C. albicans* (Table 4). The fruit extracts of *L. nobilis* L. which are usually used for its essential oils did not show any unique antibacterial activity against both gram negative and gram positive bacterial strains tested (Table 5). However, hexane fruit extract exhibited a superior antifungal activity against *A. fumigatus* (25 mm inhibition zone) as compared to the standard nystatin (23 mm). Baytop²⁷ reported the use of *L.nobilis* L. leaf and fruit extracts as antiseptics and antimicrobial in Turkish folk medicine.

The antibacterial and antifungal flower extracts of *L.nobilis* L. (Table 6) were closely related in the pattern of inhibition to that of fruit extracts. A moderate antibacterial activity against both gram positive and gram negative strains was observed. Unlike the antibacterial activity, butanol, hexane and ethanol flowers extracts at 3mg/disc exhibited a very strong antifungal activity 24, 17, 19 (mm inhibition zone). This activity against the filamentous *A.fumigatus* and *A.niger* was very comparable with standard nystatin (23-22 mm inhibition zone). Uchiyama²⁵ isolated trypanocidal components from different parts of *L.nobilis* L. and they reported the leaf methanol extracts to be of such unique activity.

As for the effect of the solvent used for extraction, it was clear that hexane, ethanol and butanol yielded compounds with superior biological activities both in the case of antiquorum sensing activity and antimicrobial testing. The variations in the different extracts activities may suggest that these active compounds are either more or less polar than water.

However, it is unclear for us whether the antiquorum and antimicrobial activities in these plant extracts are a result of the same or different ingredients.

Mahasneh;²⁰ Mahasneh and EL-Oqleh²¹ and Mahasneh, 2002 reported the same trend of the effect of different extraction solvents when extracting other medicinal plants for antimicrobial activity testing.

In conclusion, this study provides encouraging results about the possible use of antipathogenic (antiquorum sensing) activity of certain plants extracts to control pathogenesis. It also presents data indicating superior antifungal activity of *L.nobilis* L. extracts against both filamentous and non filamentous pathogenic fungi. Realizing that the majority of studies on natural products test only the antimicrobial activity of such products leaving unmined area of medicinally useful plant to go unobserved^{28, 29} necessates more studies on anti-quorum sensing activities of natural products in general.

Finally, we share the notion of Adonizio² which states that studying bioactivity of natural products should follow additional tools including antiquorum sensing activity testing, which definitely is as important as studying antimicrobial activity. This would partially contribute to the understanding of natural products and their potential use in folk medicine.

Acknowledgments

The authors would like to thank the University of Jordan for the financial support provided.

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الفعالية المثبطة لنمو وإحساس النصاب في الأحياء الدقيقة لمستخلصات أجزاء مختلفة من

نبات الغار *L. nobilis*

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الملخص

بما أن إحساس النصاب يلعب دوراً في امراضية العديد من أنواع البكتيريا فقد رأينا أن من المناسب دراسة مستخلصات أجزاء مختلفة من نبات الغار في تثبيط النمو وإحساس النصاب كذلك، علماً بأن هذا النبات ينتمي لعائلة Lauraceae ويستعمل كثيراً في مناطق المتوسط بما فيها الأردن ، وقد تم هنا دراسة 24 مستخلصاً مائياً والايتانول والبيتانول والهكستين والكلورفوروم والميثانول لكل من الأوراق واللحاء والثمار والأزهار لهذه النبتة لمعرفة تأثيرها في منع نمو مجموعة من البكتيريا الموجبة والسالبة لصبغة جرام وكذلك دراسة تعطيل إحساس النصاب باستعمال بكتيريا *Chromobacterium violaceum*.

بينت النتائج أن أعظم فعالية في تعطيل إحساس النصاب كانت مع مستخلصي زهور النبات للهكسين والايتانول حيث كان قطر منع إحساس النصاب 25 و 21 ملم على التوالي، في حين أن المستخلصات الأخرى كانت فعالة بدرجات منخفضة (10-14 ملم) إلى معتدلة (15-20 ملم)، وتجدر الإشارة هنا إلى أن هذه هي المرة الأولى التي تدرس فيها فعالية إحساس النصاب لهذه المستخلصات. وفيما يتعلق بفعالية هذه المستخلصات تجاه تثبيط نمو البكتيريا والفطريات فقد تبينت فعالية المستخلصات اعتماداً على نوع الكائن الدقيق وكذلك المستخلص ولوحظ فعالية متميزة تجاه فطريات *Aspergillus flavus* و *Aspergillus niger* وكذلك *Candida albicans* من مستخلص الايتانول والهكسين لأوراق ولحاء النبتة حيث كان قطر منطقة منع النمو يتراوح ما بين (11-25 ملم) وهي قيمة عالية إذا ما قورنت بالمضاد الفطري المعياري Nystatin، ومن حيث الفعالية تجاه البكتيريا فقد لوحظ أن فعالية عالية (25 ملم) للمستخلص المائي للحاء النبتة تجاه بكتيريا *Staphylococcus aureus* المقاومة لمضاد الميثيسيلين عند مقارنتها بفعالية البنسلين G (33 ملم).

ولوحظ أيضاً فعالية معتدلة لمستخلصات الزهور والثمار والأوراق تجاه البكتيريا السالبة لصبغة جرام مثل *Klebsiella pneumoniae* و *Salmonella typhimurium* وعند دراسة تراكيز الحد الأدنى المثبطة لنمو البكتيريا (MIC) لوحظ أنها مرتفعة نسبياً حيث تراوحت ما بين 525-3000 مايكرو جرام. ويمكن فهم ذلك الارتفاع لأننا نتعامل مع مستخلصات خام (Crude) وليس مع مواد نقية. يتضح مما تقدم أن هذه أول دراسة على نبات الغار تعنى بفعالية تثبيط إحساس النصاب كمدخل للسيطرة على بعض أنواع البكتيريا كتوجه جديد يساعد في تقليل ظهور بعض العزلات المقاومة للمضادات الحيوية التقليدية.

الكلمات الدالة: نبات الغار *L. nobilis*، مستخلصات أجزاء النبات، فعالية النبات المثبطة لنمو الميكروبات، إحساس النصاب.