Study of antibacterial activity of *Lawsonia inermis* leaf extract

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Objectives The present study aims to determine antibacterial activity for *Lawsonia inermis* leaf extract against some pathogenic bacteria.
Methods Five solvents used are acetone, ethanol, methanol, ethyl acetate and distilled water to obtain of crude extract of *Lawsonia inermis* leaves, which tested the effectiveness on five types of bacteria are *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klebsila pneumonia* and *Staphylococcus aureus* to determine the most efficient solvent extraction of them, and then was use of a series of concentrations of the solvent is more efficient 0, 20, 40, 60, 80 and 100% to determine the most efficient concentration of solvent optimization, and then was determined the efficiency of minimum inhibitory concentration (MIC) of the extract.
Results The results of the current study showed that the most efficient in the extraction solvent is acetone and the diameters of inhibition zone are 18, 19.83, 17.16, 16.33, 16.5 mm for the types of bacteria above, respectively. The results showed that the concentration of acetone 100% is the best concentration in extraction, amounting to the diameters of inhibition zone at this concentration of 17.33, 20, 19.33, 17.66, 21.66 mm for each of the bacterial species above, respectively. The results also found that MIC is 6 mg/ml of *Pseudomonas eroginosa* and 7 mg/ml of *Pseudomonas oryzihabita* and 11 mg/ml of each *Proteus varaplis, Klebsila pneumonia* and *Staphylococcus aureus*.
Conclusion The most effective composites against pathogenic bacteria from *Lawsonia inermis* leaves are using acetone solvent with concentration of 100%.
Keywords acetone, *Lawsonia inermis,* antibacterial activity, MIC

Introduction

Bacteria are microorganisms that cause disease and some of which cause fatal diseases in humans. Every year millions of people die because of these microorganisms. The increasing of bacteria inside the human body takes advantage of any weakness found in body organs.[1]

In the past decade, interest on the topic of antimicrobial plant extract has been growing, and the use of herbal medicines in Asia represents a long history of human interaction with the environment. The plant used for traditional medicine contains a wide range of substance that can be used to treat chronic as well as infectious disease. A knowledge of how to use the plant against the different illness may be expected to have accumulated in areas where the use of plant is still of great importance the medicinal value of plant lies in some chemical substance that produce a definite physiological action on the human body. The most important of these bioactive compounds of plant are alkaloids, flavonoids, tannins and phenolic.[2]

The extract of leaves of *Lawsonia inermis* was applied on woollen yarn to investigate the dying characteristics and antimicrobial efficacy against common human pathogens such *E. coli*, *Staphylococcus aureus*, *Candida albicans.*[3] The active component of *Lawsonia inermis* is Lawsone (2-hydroxy1,4-naphthoquinone, CAS 83-72-7), which is also the principal dye ingredient. Current research suggests that lawsone is non-problematic for external use because of it slow toxicity and genotoxicity.[4]

The bioactive of *Lawsonia inermis* compound with ampicillin (antibacterial) and flucnecoul (antifungal) where found considerably active against test microorganism.[3]

Materials and Methods

Microorganisms

Five pathogenic bacteria due to disease caused by it are obtained from patient from AL-hussein hospital, and given numbers 1, 2, 3, 4, and 5. These bacteria are *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klebsila pneumonia,* and *Staphylococcus aureus*.

The Extraction Process

We take the dry leaves of *Lawsonia inermis* that obtained from Al-Fawo farms in Basra city, Iraq. They were blended into powder by electric blender. Then, we take 100 g of this powder and distributed to five beakers each beaker containing 20 g of powder, then it was put in 100 ml of solvent 70% (the ratio of extraction is 1 g: 5 ml) as was the use of solvents are five: acetone, ethanol, methanol, ethyl acetate and distilled water. Each beaker was labeled with its solvent name, and incubating it in shaking incubator overnight with 150 cycle/minute at 37°C. This method used for extraction were described by Al-Daamy et al.[5]

After 24 hr of shaking, the extract purified by cotton and gauze in other beaker (the filtration must be quietly with pressing the gauze to obtain all the substance) then pour the pre-collected substance of each beaker in tubes and centrifuge them in 3000 rpm for 5 minutes.

Then pour the substance in the tubes in glass petri dishes carefully without moving the precipitated material, and label each petri dish with the name of the solvent. Let the dishes to be dry (these take some time if the weather is cold and the temperature is less than 37°C). After drying of all dishes, we scrabbled each dish to obtain the extract powder. After that, we weighed each extract powder and recorded it to calculate the percentage of extraction and what is the best solvent used (we saved it in separated containers labeled with solvent name).

Determination of the Best Solvent for Extraction

From the pre-collected powder, we weighed 0.05 g from each container and put it in separate five tubes, and each tube contains extract of certain type of our five solvents. Add on each

tube 2 ml of ethanol 70% and shaking the tube until the powder melting, the final concentration of extract in each tube equal 25 mg/ml. In addition to our five tubes, we added a sixth tube containing 2 ml for control (70% ethanol without any extract).

We prepare 30 petri dishes of Muller Hinton agar and pour 20 ml in each dish and drill three wells in the media with a diameter of 5 mm to each well. After activation of bacteria in nutrient broth, we culture it in the prepared dishes. In each petri dish, 100 µl of each activation bacteria was added and spread it by sterilized spreader, then added 50 µl plant extract in each well. In the negative control, we added just the 50 µl ethanol 70%, also we used 50 µg/ml Gentamycin as a positive control. After culturing, we incubated all the dishes in an incubator for 24 hr in 37°C. Then, examine the inhibition zone of each well in each dish. The results were recorded to determine the best solvent that has a high activity against examined bacteria.[6]

Determine the Optimal Concentration of the Best Solvent

After determining the best solvent which is acetone, In five beakers with different concentrations of acetone 20, 40, 60, 80, 100%, 20 g of *Lawsonia inermis* extract powder was added, and repeated the same process of extraction method which described above and recorded the results.

In the next step, in five tubes put 0.05 g of dry extract in each tube, then add 2 ml of 70% ethanol to each tube to obtain on the final concentration 25 mg/ml. After that, we prepared 30 petri dishes with Muller Hinton agar and follow the three tests method to determine the best concentration of acetone that has a largest inhibition zone.[6]

Determine the Minimal Inhibitory Concentration (MIC)

Prepare two flasks:

- First flask has 50 ml nutrient broth media.
- Second flask have 25 ml nutrient broth and added to it 0.625 g from acetone 100% dry extract to obtain on 25 mg/ml concentration in this flask from extraction.

Then 26 glass tubes were labelled with numbers from 0 to 25. On each tube put the extract from *Lawsonia inermis* with acetone 100% concentration equivalent to the number of the label of the tube. For example: in tube number 25 put only 2 ml from flask 2 which represents 25 mg/ml concentration. In tube number 24 put 1.92 ml from flask 2 and 0.08 ml from flask 1 which represents 24 mg/ml concentration, so that with other tubes, in the tube number 0 put only 2 ml from flask 1 to obtain of 0 mg/ml in this tube. The distribution between flask 1 and 2 in these tubes according to the law C1 V1 = C2 V2.

After that, take a micro titter plate with 96 wells and numbered the wells from 0 to 25 µl, twice for each type of bacteria (each type of bacteria has 52 wells twice for each number), then added 150 µl from each tube in two wells of the same number on each type of bacteria, Then added 50 µl of each type of bacteria (after dilute it with both media and take from the 3rd dilution comparison with McFarland solution) in all the 52 wells from 0 to 25 µl concentrations. After that, cover the plates and incubate them in the incubator for 24 hr in 37°C.

Then examine the plates to see growth or no growth of bacteria and determine the minimal inhibitory concentration from extract for each type of bacteria.[7]

Statistical Analysis

Statistical analysis included random complete design (RCD) with three replicates. 0.05 is the level of probability that used to identify a significant difference. The significant differences between the averages were also tested by using the test less significant difference (LSD) at the level of probability of 0.05.[8]

Results

The results in Table 1 show that the percentage of substances using extraction are 2.35, 2.0, 1.7, 2.5, 1.75% by using each of solvents Acetone, ethanol, methanol, ethyl acetate, distilled water; respectively. In these results, unclear (show) that the maximum percentage of extraction is 2.35% by using ethyl acetate, and the minimal percentage of extraction is 1.7% using methanol.

After determining acetone as the best solvent in the extraction, the results in Table 2 shows the extract of, *Lowsonia inermis* leaves with acetone have a larger diameter inhibition zone on all types of bacteria 18, 19.83, 17.16, 16.33, 16.5 mm for each types of bacteria *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klepsila pneumonia, Staphylococcus aureus,* respectively, with significant differences ($P < 0.05$) against the control gentamycin and also against other solvents using.

Table 1. **The percentage of materials extracted from *Lawsonia inermis* leaves**

No.	Extraction solvent (100 ml)	Origin weight of plant powder (g)	Weigh of extract (g)	Percentage of extract materials (%)
1	Acetone	20	0.47	2.35
2	Ethanol	20	0.40	2.0
3	Methanol	20	0.34	1.7
4	Ethyl acetate	20	0.5	2.5
5	Distil water	20	0.35	1.75

Table 2. **Inhibition zone (mm) to extract *Lawsonia inermis* leaves against bacteria**

Bacteria	Extraction solvent (70%)						$LSD_{0.05}$
	Gentamycin 10 µg/ml	Acetone	Ethanol	Methanol	Ethyl acetate	Distil water	
Pseudomonas eroginosa	21.66 ± 0.88	18 ± 2.08	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**2.32**
Pseudomonas oryzihabita	20.33 ± 0.88	19.83 ± 1.36	10 ± 0.2	15.33 ± 1.45	0 ± 0	0 ± 0	**2.24**
Proteus varaplis	12.5 ± 0.28	17.16 ± 1.16	15.6 ± 2.42	0 ± 0	0 ± 0	0 ± 0	**2.78**
Klebsila pneumonia	17.66 ± 0.33	16.33 ± 0.44	10.5 ± 0.86	14.66 ± 0.6	15.3 ± 0.61	0 ± 0	**1.37**
Staphylococcus areus	17 ± 0.57	16.5 ± 1.8	14.5 ± 1.25	0 ± 0	13.83 ± 0.6	0 ± 0	**2.41**

Table 3. **Inhibition zone (mm) of extract *Lawsonia inermis* leaves against bacteria by using dilutions series of acetone**

Bacteria	Acetone Ratio (%)						$LSD_{0.05}$
	Gentamycin 10 µg/ml	20%	40%	60%	80%	100%	
Pseudomonas eroginosa	21.66 ± 0.88	0 ± 0	5.5 ± 0.28	7.33 ± 0.88	11.16 ± 0.72	17.33 ± 0.72	**1.68**
Pseudomonas oryzihabita	20.33 ± 0.88	0 ± 0	14.66 ± 0.6	17.5 ± 1.5	19 ± 0.57	20 ± 1.66	**1.98**
Proteus varaplis	12.5 ± 0.28	0 ± 0	12.5 ± 0.28	14.16 ± 0.6	15.33 ± 0.16	19.33 ± 0.62	**0.98**
Klebsila pneumonia	17.66 ± 0.33	9 ± 0.28	12.83 ± 0.72	14 ± 1.2	16.5 ± 1.32	17.66 ± 0.33	**1.94**
Staphylococcus aureus	17 ± 0.57	11.83 ± 0.16	13.33 ± 0.83	13.66 ± 0.16	14.33 ± 0.16	21.66 ± 0.88	**1.41**

Table 4. **Minimum inhibitory concentration (MIC) of acetonic extract *Lawsonia inermis* leaves**

Extract concentration (mg/ml)	Types of bacteria				
	Pseudomonas eroginosa	*Pseudomonas oryzihabita*	*Proteus varaplis*	*Klebsila pneumonia*	*Staphylococcus aureus*
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	–	+	+	+	+
7	–	–	+	+	+
8	–	–	+	+	+
9	–	–	+	+	+
10	–	–	+	+	+
11	–	–	–	–	–
12	–	–	–	–	–
13	–	–	–	–	–
14	–	–	–	–	–
15	–	–	–	–	–
16	–	–	–	–	–
17	–	–	–	–	–
18	–	–	–	–	–
19	–	–	–	–	–
20	–	–	–	–	–
21	–	–	–	–	–
22	–	–	–	–	–
23	–	–	–	–	–
24	–	–	–	–	–
25	–	–	–	–	–

+, means growth; –, means not growth.

The results in Table 3 shows the extract of plant with acetone 100% have a larger diameter inhibition zone on all types of bacteria 17.33, 20, 19.33, 17.66, 21.66 mm for bacteria *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klebsila pneumonia, Staphylococcus aureus,* respectively, with significant differences ($P < 0.05$) against gentamycin, also with significant differences ($P < 0.05$) against other solvents.

The results in Table 4 shows that the minimal concentration for inhibition (MIC) for bacteria *Pseudomonas eroginosa* is 6 mg/ml, and for *Pseudomonas oryzihabita* is 7 mg/ml, while for bacteria *Proteus varaplis, Klebsila pneumonia, Staphylococcus aureus* is 11 mg/ml.

Discussion

Lawsonia inermis leaves have antibacterial activity against many types of bacteria including *Staphylococcus aureus, Klebsila pneumonia,* where methanol was used as solvent.[4] In comparison with the present study, *Lawsonia inermis* leaves which consist of alkaloids, flavonoids, tannins, and phenolic,[2] using acetone 100%.

In our study, we found in the results that *Lawsonia inermis* leaves have minimum inhibitory concentration for *Staphylococcus aureus* and *klebsila pneumonia* is 11 mg/ml, but in other study the minimum inhibition concentration for these bacteria is 25 mg/ml,[4] that mean our extract has more activity than that test, and this contrasts may be due to the differences in the solvent and the difference in concentration in the solvent.

Also the other research showed that *Lawsonia inermis* leaves have antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*, using methanol as solvent.[9] Comparing this results with our results using *Lawsonia inermis* leaves with acetone 100% in our study and methanolic extract in the other research,[9] we see that our extract has antibacterial activity against *Pseudomonas aeruginosa* in Mueller Hintone agar well diffusion test and the diameter of inhibition zone is 17.33 mm, but in other test,[9] the diameter of inhibition zone of *lawsonia inermis* leaves with methanol against *pseudomonas aeruginosa* is 15 mm, that mean our extract is more active than methanolic extract, and this is either due to the less activity of their solvent than our solvent or due to the concentration of methanol that they have been used which is less activity.

Other research showed that *Lawsonia inermis* leaves have antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsila pneumonia* using methanolic extract.[10] When compare our research with this research, we find that in this research with considering the concentration of *Lawsonia inermis* leaves with methanol is 1000 µg/ml, where the diameter of inhibition zone of *Staphylococcus aureus* is 24 mm, and of *Pseudomonas aeruginosa* is 17 mm, and of *Klebsila pneumonia* is 18 mm. But in our test with considering we use *Lawsonia inermis* leaves with acetone 100%, where the diameter of inhibition zone of *Staphylococcus aureus* is 21.66 mm, and of *Pseudomonas aeruginosa* is 17.33 mm, and of *Klebsila pneumonia* is 17.66 mm, that mean in that test their extract more active than our extract against *Staphylococcus aureus*, and few more than our extract against *Klebsila pneumonia*, but our extract

has more activity than their extract against *Pseudomonas aeruginosa*. This contrast is due to type of solvent and concentration of solvent.

Conclusion

From the results of the present study, we concluded that the extract of *Lawsonia inermis* leaves by acetone 100% has high antibacterial activity against the pathogenic bacteria that isolated from patients.

Recommendations

Other study on *Lawsonia inermis* leaves is to purify the compound, which owns the effectiveness of antibacterial pathogenesis. ■

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