

## **In Vitro Antibacterial activity of Different Yemeni Leaves extracts of *Lawsoniainermis* against Some Bacterial Pathogens**

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**Abstract:** *The aim of this study was to evaluate antibacterial activity of the leaves of the Yemeni Lawsoniainermis L. The aqueous, methanol, ethanol, and acetone extract of Lawsoniainermis Leaves were tested against standard bacterial cultures by agar well diffusion method on Mueller Hinton agar for bacterial cultures. Acetone extract showed significantly higher inhibitory effect compared to aqueous, methanol, and ethanol extracts on tested organisms, with no activity for aqueous extract.*

*Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as therapeutic agent.*

**Keywords:** *Lawsoniainermis, Antibacterial activity, Solvent extracts*

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### **1. INTRODUCTION**

Infectious diseases are one of the major causes of health hazard in humans and animals. These infections are caused by pathogenic bacteria, virus and fungi. Severity of infectious disease is based on virulence factors produced by infectious agent. In recent years, various human pathogens have been reported to acquire resistance toward the common drugs. Drug resistant microbes are highly lethal and they increase the severity of infection especially in immune compromised patients (Sieradzki *et al.*, 1999).

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals/antibiotics. Species of higher plants are much less surveyed for antibacterial activity (Satish *et al.*, 2008). Where almost all parts of the plant viz., leaves, flowers and stem bark of this plant carry many medicinal properties and used in traditional medicinal system for the treatment of several diseases. (Kumar *et al.*, 2011).

Plants are rich in a wide variety of secondary metabolites, such as resins, tannins, terpenoids, alkaloids, flavonoids, pesticides and other pharmacological compounds it's have demonstrated *in-vitro* antimicrobial (Sanni *et al.*, 2010).

*Lawsoniainermis* (Lythraceae) commonly known as Henna is a shrub frequently cultivated in the Middle East, along the African coast of the Mediterranean Sea and

India. Besides its use in cosmetics for staining hands and as a hair dye, the leaves are used as a prophylactic against skin diseases. It was reported to be useful in jaundice, enlargement of the spleen, calculus affliction, and skin diseases (Chaudhary *et al.*, 2010). The extract of leaves of *L. inermis* has been shown to possess antimicrobial activity (Bhuvaneshwari *et al.*, 2002). Ethanolic extract of the whole plant was found to have antifungal activity (Rahmoun *et al.*, 2013).

Yemeni People especially in rural areas practice the use of plants in their daily life as medicines. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Aziza *et al.*, 2013).

Antimicrobial activity of plants can be dedicated by observing the growth response of various microorganisms to those plant tissues or extracts, which are placed in contact with them. Many methods for detecting such activity are available, but since they are not equally sensitive or even based on the same principle, the results obtained will also be influenced by the method selected and the microorganisms used for the test.

The present study was conducted to study the antibacterial activity of *Lawsoniainermis* used by Yemeni peoples to show that therapeutic properties against five bacterial strains.

## 2. MATERIAL AND METHODS

### 2.1. Plant Samples and Methods of Extraction

*L. inermis* were collected from local gardens in Sana'a City in Yemen. Leaves of the plant were washed thoroughly 2 - 3 times with running tap water followed by distilled water. Washed leaves were dried in hot air oven at a temperature of 40°C. Dried leaves were powdered by using an electrical grinder (Waring Blender, Tokyo, Japan). 30 grams of pulverized leave material was extracted with 300 ml of each methanol 70%, ethanol 70% and acetone 70%, also 30 grams of pulverized leave material was extracted with 300 ml distilled water separately at room temperature for 48 hours. Extracts were filtered by using filter paper (Whatman No. 1). Extracts were concentrated in rotary evaporator and dried by using Freeze-Dry Systems. The extract was transferred into glass sealed amber dark bottles and then stored in at 4 °C) for subsequent analyses.

### 2.2. Crude Extract

The methanol, ethanol, acetone, and distilled water powders were dissolved in 10 % dimethyl sulfoxide (DMSO) (10 % w/v) as 100 mg/ml stock solution. Another extract concentration was prepared from the first extract solution to make a concentration range from (250 -62.5 mg/ml). by three fold serial dilution from stock solution was prepared appropriate dilution with PBS. Aqueous extracts were dissolved directly in 5 ml PBS. Extract solutions were prepared just before use in DMSO.

### 2.3. Bacterial Cultures

Five bacterial strains were used for the study. Gram positive bacteria include (*Bacillus subtilis* (ATCC 6633), *Kocuriarhizophila* (ATCC 9341)(formerly *Micrococcus luteus*), *Listeria monocytogenes* (ATCC 7644), *Staphylococcus epidermidis* (ATCC 12228), and Gram negative bacteria (*Escherichia coli*(ATCC 11303).All the tested strains were reference strains and were obtained from Department of Biology, Division of Microbiology, Science College and Department of Food Sciences and Technology, Sana'a university. Strains were maintained in nutrient broth (Difco, 0003-01-6 - USA) at 37°C and maintained on Brain Heart Infusion Agar (OXOID, CM0375) slants at 4°C. These bacteria served as test pathogens for antibacterial activity assay.

### 2.4. Preliminary Phytochemical Screening

The preliminary phytochemical screening of *Lawsoniainermis* was carried out for the decotion of various Phyto-constituents using standard procedure (Evans, 1996). All the leaves extract were found to contain more flavonoids. The preliminary phytochemical screening of leaves extract reveals the presence of alkaloids, flavonoids, tannins and quinones.

### 2.5. Total Flavonoids Determination

Aluminum chloride colorimetric method was used for flavonoids determination in each extract.

### 2.6. Antibacterial Screening of Different Extracts of *L. Inermis* Leaf

Antibacterial activity of each extracts were determined by agar well diffusion method as described by (Satish, 2008) on Mueller-Hinton Agar(MHA) (Accumix-AM071, India) with some modifications. The concentration of bacterial suspension was determine by serial dilutions and pour plates technique. The bacterial suspensions were containing 10<sup>6</sup> CFU/ml of bacteria were spread on MHA plates by seeded agar technique. In each of these plates four wells were cut out using a standard cork borer (7 mm). Using a micropipette 100µl of each extract and negative control was added in to different wells. A positive control antibiotic disc was placed in the plate

## In Vitro Antibacterial activity of Different Yemeni Leaves extracts of *Lawsoniainermis* against Some Bacterial Pathogens

separated. All the plates were incubated for 24 h at 37°C. After incubation, bioactivity was evaluated by measuring the zone of inhibition. if any around the wells was measured in mm (millimeter).All the Experiment was performed in duplicates. Negative controls had solvent DMSO (10% in distilled water) without test compounds. Antibiotics standard Discs (Hi-Media, India) (5mcg) Enrofloxacin, (30mcg) Tetracycline, (10 mcg) Penicillin G, (10 mcg) Polymyxin-B, (10mcg) Gentamycin, (10 mcg) Amoxycillin were used as reference to determine the sensitivity of each bacterial species tested and used control positive.

### 3. RESULTS

Four different types of extracts were prepared including acetone extract, methanol extract, ethanol extract and aqueous extract. The results revealed that all extracts exhibited antimicrobial activity against all bacterial strains used in the present study, except for the aqueous extracts. However bacterial strains showed differential sensitivity for each extract (Table 1, 2, 3, and 4). Antibacterial activity was not observed with controls (DMSO and water),

But all the extracts showed inhibition effect against *Staphylococcus epidermidis* ( Table 1,2,3, and 4).The inhibition zones formed by standard antibiotics are showed in (Table 5).The Acetonic 70% extract showed more potent against *Bacillus subtilis*, *Kocuriarhizophila* and *Listeria monocytogenes* compare with another extract , Whereas methanolic 70% extract showed more potent against *E. coli* and *Staphylococcus epidermidis*. While ethanolic 70% extract showed moderately inhibition compare to remaining samples, But the aqueous extracts did not showed any inhibition effect against all the bacteria used in the study, except for the *Staphylococcus epidermidis*, which was similar to ethanolic 70% extract result at250 mg/ ml.

**Table 1.** Antibacterial activity of *Lawsoniainermis* acetonic extract against tested bacteria

Bacteria	Inhibition zones in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Bacillus subtilis</i> (ATCC 6633)	36	31	24
<i>Escherichia coli</i> (ATCC 11303)	17	15	12
<i>Kocuriarhizophila</i> *(ATCC 9341)	34	30	22
<i>Listeria monocytogenes</i> (ATCC 7644)	30	25	22
<i>Staphylococcus epidermidis</i> (ATCC 12228)	24	22	17

**Table 2.** Antibacterial activity of *Lawsoniainermis* methanolic extract against tested bacteria

Bacteria	Inhibition zones in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Bacillus subtilis</i> (ATCC 6633)	28	25	21
<i>Escherichia coli</i> (ATCC 11303)	22	19	16
<i>Kocuriarhizophila</i> *(ATCC 9341)	24	19	13
<i>Listeria monocytogenes</i> (ATCC 7644)	25	20	16
<i>Staphylococcus epidermidis</i> (ATCC 12228)	26	21	18

**Table 3.** Antibacterial activity of *Lawsoniainermis* athanolic extract against tested bacteria

Bacteria	Inhibition zones in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
<i>Bacillus subtilis</i> (ATCC 6633)	22	19	12
<i>Escherichia coli</i> (ATCC 11303)	12	10	-
<i>Kocuriarhizophila</i> *(ATCC 9341)	20	16	10
<i>Listeria monocytogenes</i> (ATCC 7644)	15	12	-
<i>Staphylococcus epidermidis</i> (ATCC 12228)	18	15	13

**Table 4.** Antibacterial activity of *Lawsoniainermis* aqueous extract against tested bacteria.

Bacteria	Inhibition zones in mm		
	250 mg/ml	125 mg/ml	62.5 mg/ml
Bacillus subtilis(ATCC 6633)	-	-	-
Escherichia coli(ATCC 11303)	-	-	-
Kocuriarhizophila *(ATCC 9341)	-	-	-
Listeria monocytogenes(ATCC 7644)	-	-	-
Staphylococcus epidermidis(ATCC 12228)	18	12	-

Three concentrations of each extract were used (250µg/ml, 125µg/ml and 62.5µg/ml).

Among the various extract, the acetonic extract of *lawsoniainermis* was more active against all the tested microorganisms. Each extracts showing significant activity except aqueous extract maybe due to the presence of alkaloids, flavonoids, tannins and quinones.

**Table 5.** The sensivity of the antibiotics on the bacteria used in the study

Antibiotics	Concentration (mcg)	<i>B.subtilis</i>	<i>E. coli</i>	<i>K. rhizophila</i>	<i>L. monocytogenes</i>	<i>S. epidermidis</i>
Enrofloxacin	10	28	29	24	30	26
Gentamycin	10	32	29	33	34	28
Tetracycline	10	30	28	29	33	32
Amoxycillin	10	30	28	23	30	29
Penicillin G	10	31	32	33	31	27
Polymyxin-B	10	29	29	31	35	33

### 3.1. Preliminary phytochemical screening

The preliminary phytochemical screening reveals the presence of Flavonoids Alkaloids, Tannins and quinones. The results were shown in table 6&7.

**Table 6.** Flavonoid contents in each extracts of *Lawsoniainermis*

Extracts	Flavonoids (mg/g)
Acetonic	29.12± 0.13
Methanolic	23.15±0.18
Athanolic	19.34±0.21
Aqueous	1.02±0.11

**Table 7.** Qualitative test

	Extracts of <i>Lawsoniainermis</i>			
	Acetonic	Methanolic	Athanolic	Aqueous
Alkaloids	+	+	+	+
Quinones	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+

## 4. DISCUSSION

The search for new antimicrobial agents is an important line of research because of the resistance acquired by several pathogenic microorganisms.

Dried leaves of *Lawsoniainermis* were used to prepare the extracts as it has been reported that dried preparation have more concentrated bioactive compounds than fresh plant material (Romero C *et, al* 2009).

In the previous studies has been found to be a best solvent for extraction of the active ingredient ( asarone ) from leaves. Though, solvents such as methnol, ethanol and aqueous used the most of the previous studies. It is well established that the β- asarones found in leaf, roots and rhizomes

## **In Vitro Antibacterial activity of Different Yemeni Leaves extracts of *Lawsoniainermis* against Some Bacterial Pathogens**

tissues are responsible for almost all of the antimicrobial activities of the *Lawsoniainermis* (MacGawet *et al.*, 2002). But our results demonstrated the first time report that use acetone extract as best solvent for high antibacterial activity.

According to the study of Iram *et al.*, (2013), phytochemical constituents of *Lawsoniainermis* exhibit antimicrobial activity against both gram positive and negative bacteria. In our study, it was interested to note that *Lawsoniainermis* had antibacterial activity similar to that found by (Iram *et al.*, 2013). The studies of Bhuwaneshwari *et al.* (2002); Habbal *et al.* (2005) and Hussain *et al.*, (2011) support our findings. These phytochemical constituents are good source of antimicrobial and antioxidant activity (Maurya and Akansha, 2010).

Acetone leaves extract of the Yemeni henna are strikingly most effective against the spectrum of bacteria tested as compared to other extracts. When comparing the antibiotics with those alcoholic, and acetone extracts showed pronounced antibacterial effects against the bacteria used in this study but aqueous extracts had no activity

Antimicrobial activity of alcoholic, and acetone extracts may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive (Harborne and Baxter., 1995). Water extracts did not show any antibacterial activity compared to alcoholic and acetone extracts. This may be due to the lack of the solvent properties which plays an important role in antibacterial efficacy (Kelmanson *et al.*, 2002).

We concluded that Yemeni leaves extract henna has an *in-vitro* antibacterial activity except water extract against the tested bacterial strains. These findings have also been mentioned in literatures (Hemem., 2002, Muhammad and Muhammad., 2005, Habbal *et al.* 2005).

### **5. CONCLUSION**

The present study provides the scientific rationale for medicinal use of *Lawsoniainermis*. The use of acetone extracts is of great significance as substitute antimicrobial agent in therapeutics. Acetone and alcoholic Henna extracts have significant effects to some of the antibiotics commonly used in clinical practice. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds

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