ANTIBACTERIA ACTION OF *LAWSONIA INERMIS* LEAVE EXTRACT ON WOUND ISOLATES

Enwa Felix Oghenemaro[1]*, Oyubu Levinson Obaro[2], Adjekuko Collins Ohwonigho[3], Eguvbe Peter Mamuro[4], Azeez Ayo Okiemute[5], Michael Oghenejobo[6]

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Abstract:
This study examines the Lawsonia inermis (Henna) plant's ability to combat isolates amid wound swabs. Lawsonia inermis leaves were bought, spotted, and extracted by applying the cold maceration process with seventy percent (70%) ethanol. The plant excerpt was subjected to phytochemical screening applying a standard analytical technique. Patients, who gave their consent, had their wound surfaces containing purulent material, swabbed. Lawsonia inermis crude, chloroform, and aqueous extract were used on the isolates from the wound surfaces. Five plant metabolites, namely saponins, tannins, alkaloids, steroids, as well as terpenoids, were found using ethanol as extracting solvent. However, flavonoids and cardiac glycosides were not present. This excerpt had a smaller inhibitory zone compared to the positive control results with zones of 10.5±0.5mm and 10±0.00mm respectively, against M9N (S. aureus) and M9C (P. aeruginosa). The crude excerpt's greater efficacy was observed at 100 mg/ml. With zones of restraining of 10.5±0.5 and 9±1, respectively, M16N (S. aureus) and F3Y (S. aureus) revealed maximum action for the chloroform excerpt. With areas of inhibition of 8.5±0.5 and 11.0±0, respectively, M1C (P. aeruginosa) and F4Y (S. aureus) revealed the maximum action for the aqueous excerpt. The results of this study have provided support for the traditional medical uses of Lawsonia inermis in the development of brand-new antibacterial medications for the effective treatment of bacterial illnesses.

Keywords: Lawsonia inermis, wounds, Antimicrobial, Plant extract

[1*,5, 6] Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.

[2]Department of Science Laboratory Technology, Faculty of Science, Delta State University, Abraka, Nigeria.

[3]Department of Biological Sciences, Faculty of Science, University of Delta, Agbor, Nigeria.

[4]Department of Chemical Sciences, Delta State University of Science & Technology, Ozoro, Nigeria

***Corresponding Author: -** Enwa Felix Oghenemaro

*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.

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Introduction

Natural substances derived from plants have been used to support human health ever since the advent of medicine. The biological activities of these chemicals have attracted a great deal of scientific attention due to the significance of plant active components in agriculture and medicine. The pharmaceutical industry has used plant-based active components (phytochemicals) as a key pipeline for drug discovery across time [1]. In addition to using plants for food, shelter, and clothing, man has also used them for a variety of other things, such as entertainment and medical treatment.

Due to the increasing failure of chemotherapy treatments and the antibiotic resistance exhibited by pathogenic organisms, researchers are concentrating more on employing medicinal plants in traditional medicine. To uncover new possibilities for creating more potent medications against microbial infections, many plants were evaluated for their possible antibacterial characteristics [2].

Lawsonia inermis, belongs to the Lythraceae family, which is commonly referred to as Henna. The *Lawsonia inermis* tree, a native of North Africa, is utilized all over the world. Despite the fact that the stem's bark, roots, blooms, andThis branching, glabrous shrub or small tree is grown for its leaves, while the seeds have also been utilized in traditional medicine. It has a maximum height of 25 feet. Henna was used for cosmetic and therapeutic purposes more than 9,000 years ago. It was frequently utilized there because of its cooling effects during the stifling Indian summers. With the use of its crude chloroform and aqueous extracts, the current study investigated *Lawsonia inermis's* antibacterial properties [3]. Henna paste or powder created from the leaves is widely used to create designs on people's hands, feet, and nails. Henna leaves have an orange-red color.

It can also be used to make hair dye. It is used to treat smallpox, *spermatorrhea*, *spermatorrhagia*, jaundice, and skin problems. The base for local scents is the perfume derived from aromatic flowers. The application of a flower infusion on bruises can be quite beneficial. The flower decoction is claimed to create an emmenagogue. Seeds can eliminate odors. A diet rich in pulverized seeds and clarified butter can help prevent dysentery. Applying a decoction made from the bark can be used to treat burns and scalds. Internal administration is used to treat a number of ailments, such as leprosy, calculus, jaundice, and obstinate skin problems. The root is thought to be effective treatment for gonorrhea and herpes. You can pulp the astringent root and apply it directly on hurting eyes. Pulped root can also be used to treat boils on children's heads [4]. A phytochemical analysis revealed that Lawsonia inermis included lipids, resin, tannins, phenolics, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, and xanthones. Additionally, it included 2-hydroxy-1,4 naphthoquinone (lawsone).

Numerous alkaloids, naphthoquinone derivatives, phenolics, and flavonoids were isolated from Lawsonia inermis using various parts of the plant. Lawsonia inermis was found to have a wide range of pharmacological effects, including analgesic, anti-inflammatory, antipyretic wound and burn healing, immunomodulatory, hypolipidemic, anti-ulcer, antidiarrheal, diuretic, anticancer, and many more [5].

Materials and Method

Materials

Nutritional agar, nutritional broth, cetrimide agar, Mueller Hinton agar, peptone water, Mannitol Salt Agar, and McConkey agar are examples of bacterial media.

Method

Collection and Extraction of Plant Material

We purchased Lawsonia inermis from a local supplier in Kaduna state in its already-pulverized form.

Using the cold maceration method, the powdered lawsonia inemis leaves were extracted with 70% ethanol over the course of 72 hours. After that, it was sieve-filtered. The final extract was weighed (101.2g) and refrigerated for later use.

Fraction A/ Crude Extract

Using the cold maceration method, the powdered lawsonia inemis leaves were extracted with 70% methanol over the course of 72 hours. After that, it was sieve-filtered. The final extract was weighed (101.2g) and refrigerated for later use.

Fraction B / Chloroform Extract

For the partitioning, 45.7g of the extract was weighed out and mixed with 100ml each of distilled water and methanol before being poured into a separating funnel. The separating funnel received 200ml of chloroform before being carefully covered and shaken. Before adding and shaking in another 100ml of chloroform, the funnel was allowed to stand for a while. After

that, the chloroform extract was taken out, weighed (1.55g), and kept in a freezer.

Fraction C/ Aqueous Extract
The leftover extract in the separating funnel received 200ml of water, which was then added and shook again before being left to stand. The resulting aqueous extract was removed, weighed (29.69g) and stored in a refrigerator.

Sterilization of Materials and Media
Test tubes, beakers, and measuring cylinders were wrapped in foil paper and sterilized for 15 minutes at 121 ^{o}C in an autoclave.

By using cotton wool cleaned with 99% methanol and flames over a Bunsen burner, cork borers were sterilized. Additionally, the work environment was sterilized by being cleaned with cotton wool dipped in disinfectant before to each task. Inoculating wire loops were sterilized by heating to redness with a Bunsen burner before each use, and research media were autoclaved at 121 ^{0}C for 15 minutes.

Collection of Bacteria Isolates
50 patients with diverse wounds or traumas were selected for samples from various clinics and hospitals, mainly the central hospital, Abraka. Using a sterile swab stick, the sample from the wound with aspirate (pus) was obtained. At Delta State University Abraka's Faculty of Pharmacy's Pharmaceutical Microbiology Laboratory, the swab sticks were collected, and bacteriology tests were conducted on them.

Isolation procedure
Before the three varieties of Agar—Mannitol salt agar, Cetrimide agar, and Nutrient agar—were produced in accordance with the manufacturer's instructions and placed in 50 sterile Petri dishes, one for each agar, and allowed to set, the surface was wiped with a disinfectant swab. The wire loop was then ignited by a spirit lamp and became sterile.

The results were then recorded after inoculating a bacterial colony from the indicated nutrients broth culture on the Agar plates according to their label.

Preservation of Bacterial colony
The bacterial colonies are shielded from the Cetrimide, Nutrient Agar, and Mannitol salt cultures by a slant that is made. A slant is made from nutrient agar, which was sterilized in an autoclave at 121°C for 15 minutes using aluminum foil-wrapped, clean bijou bottles. The 35 sterilized bijou bottles are filled with the Cetrimide, Mannitol salt, and Nutrient agar with growth agar once the agar has cooled to a temperature of 50 to 55 °C.In order to keep the slanted shape, the agar is then held in a slant posture and allowed to solidify. Then, using a sterile wire loop, each bacterial growth was inoculated into the different culture media (Mannitol salt, Cetrimide, and Nutrient agar), which were afterwards incubated for 24 hours at 37°C. The slants are then kept in a clean, covered, and sealed plastic bucket. This procedure was performed using an aseptic method [6]

Identification of Bacteria colony
I. Gram staining
Gram staining procedure was done following standard technique [6].

II. Biochemical Reactions
Based on their biochemical reactions with various biochemical chemicals, different isolates are subjected to biochemical testing in order to identify them. One of the more common approaches for identifying microorganisms is by biochemicals testing, which are frequently combined with phenotypic identification.

Determination of Antimicrobial activity
Antimicrobial Susceptibility Test
The Mueller Hinton agar was produced in accordance with the manufacturer's instructions, autoclaved for sterilization, and then allowed to harden. Upon solidification, various microbial strains were dispersed across the agar plate's surface. The plant's crude extract was serially diluted twice (200, 100, 50, 25, 12.5 and 6.25 mg/mL). A 6 mm cork borer was used to make six holes on the agar, and each one was labeled with the concentration it represented.

The various plant extracts were added to the culture plate in accordance with their labeled concentrations, allowed to diffuse for a while, and then incubated for 24 hours at 37 °C. Zones of inhibition for each concentration were seen during the incubation process, and the procedure was repeated for the chloroform and aqueous plant extract [6].

Minimum Inhibitory Concentration (MIC)
The test organisms that were active were subjected to the minimum inhibitory concentration. A two-fold serial dilution was used to generate the crude extract for about seven (7) concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL). According to the prepared

concentrations of the extract, seven Petri plates were given labels. The test was performed on each plate.

Each plate had labels for the various divisions of the organism. The varied concentrations were added to the corresponding plates that had been labeled. To ensure good mixing, the Mueller Hinton agar was put into the plate after being sterilized. It was permitted for the set plate to build up. Each organism had its varied sections of the specified plats vaccinated.

The infected plates were then incubated at 37 °C for 24 hours, and after that time, the concentration that had the least effect on the isolates' ability to develop was determined to be the minimal inhibitory concentration. The aqueous plant extract and chloroform underwent the same procedure [6]

Phytochemical Analysis

Lawsonia inermis leaf extract was subjected to a phytochemical study using techniques largely unchanged from those described by Taher et al. [7]. Flavonoids, tannins, saponins, steroids, terpenoids, alkaloids, and cardiac glycosides were among the secondary metabolites examined.

Statical Analysis

The Statistical Package for Social Sciences, Version 22 (SPSS 22) was used to analyze the data, which was then condensed using graphs, frequency tables, means, and standard deviations.

Ethics approval

Ethical approval was obtained from the Research and Ethical Committee, Delta State University Teaching Hospital (DELSUTH), Oghara

Results

Table 1 below lists the findings of the phytochemical screening of the ethanolic leaf extracts of Lawsonia inermis. 50 samples in total were taken; after inoculation, 35 of them produced growth and were identified. Of the 35 strains, 15 (42.9%) were gram-positive and 20 (57.1%) were gram-negative. The fifteen (15) isolated strains of Staphylococcus aureus were gram-positive. Two (2) Klebsiellaspp, three (3) Proteus spp, and fifteen (15) Pseudomonas aeruginosa were found among the twenty (20) gram-negative isolates. The results of the bacterial isolates' biochemical tests were provided in Table 2.

The test organism was labeled in numerous divisions on each of the plates. The varied concentrations were added to the corresponding plates that had been labeled. To ensure good mixing, the Mueller Hinton agar was put into the plate after being sterilized. It was permitted for the set plate to build up. Each organism had its varied sections of the specified plats vaccinated. The inoculated plates were then incubated. A ruler calibrated in millimeters was then used to measure the resulting zone of inhibition. The average reading was used to determine the bacterial isolate's zone of inhibition and is shown in Table 3.

Table 1: Results of the phytochemical Screening Test

TEST	OBSERVATION	INFERENCE
Tannins	Black colouration observed	Present
Saponin	Persistent foam formation	Present
Steroids	Brown ring at the interface	Present
Alkaloids	Brown colouration with dragendoffs reagent	Present
Terpenoids	Brown ring at the interface	Present
Flavonoids	Transparent solution observed	Absent
Cardiac glycosides	Brown ring at the interface	Absent

Table:2: Results of Biochemical Test of isolates from Wound Swabs

Isolates	Catalase	Citrate	Oxidase	Indole	H2S	Motility	MR	Urease	Coagulase	Gram Stain	Fermentation			Inference
											G	S	L	
M1C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
M2C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
M3C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
M4N	+	+	-	+	-	-	-	+	-	- rods	AG	AG	A	*Klebspp*
M5C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
M6C	+	+	+	-	-	+	-	-	-	- rods	A	-	-	*Pseudomonas aeruginosa*
M7C	+	+	+	-	-	+	-	-	-	- rods	A	-	-	*Pseudomonas aeruginosa*
M8N	+	+	-	+	+	+	+	+	-	- rods	AG	AG	A	*Proteus spp*
M9N	+	+	-	+	+	+	+	+	-	- rods	AG	AG	AG	*Proteuusspp*
M10Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	A	*Staphylococcus aureus*
M11N	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	A	*Staphylococcus aureus*
M12N	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	AG	*Staphylococcus aureus*
M13Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	A	*Staphylococcus aureus*
M14Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	A	*Staphylococcus aureus*
M15N	+	+	-	+	+	+	+	+	-	- rods	AG	AG	AG	*Proteus spp*
M16N	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	A	*Staphylococcus aureus*
M17Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	A	*Staphylococcus aureus*
M18Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG		*Staphylococcus aureus*
M13C	+	+	+	-	-	+	-	-	-	- rods	A	-	-	*Pseudomonas aeruginosa*
F1N	+	+	-	+	-	-	-	+	-	- rods	AG	A	AG	*Klebspp*
F2Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	AG	*Staphylococcus aureus*
F3Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	A	*Staphylococcus aureus*
F4Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	AG	*Staphylococcus aureus*
F5Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	AG	*Staphylococcus aureus*
F6Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	A	A	*Staphylococcus aureus*
F7C	+	+	+	-	-	+	-	-	-	- rods	A	-	-	*Pseudomonas aeruginosa*
F8C	+	+	+	-	-	+	-	-	-	- rods	A	-	-	*Pseudomonas aeruginosa*
F9C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
F10C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
F11N	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	AG	*Staphylococcus aureus*
F12Y	+	+	-	-	-	-	-	+	+	+ cocci	AG	AG	A	*Staphylococcus aureus*
F2C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
F3C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
F5C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*
F6C	+	+	+	-	-	+	-	-	-	- rods	AG	-	-	*Pseudomonas aeruginosa*

Key: + = Positive, - = Negative, spp = species, G = Glucose, S = Sucrose, L = Lactose, A = Acid, AG = Acid and Gas

Table 3: Diameter of Zone of Inhibition of the Crude Extract of *Lawsonia inermis* against the Test Organisms

	Concentration (mg/ml)/Zone Of Inhibition (mm)					
Isolates	100	50	5	12.5	6.25	Positive control
M8N	-	-	-	-	-	8±1
M9N	10.5±0.5	8±1	4.5±0.5	-	-	17±1
M4N	9±1	6.5±0.5	6±0	4.5±0.5	-	10.5±1.5
M15N	-	-	-	-	-	11.5±0.5
M16N	6±0	4±0	-	-	-	10.5±0.5
M5C	5±0	-	-	-	-	17±0
M2C	7.5±0.5	3±0	-	-	-	9±0
M13C	-	-	-	-	-	8±1
M3C	10±0	7±0	4.5±0.5	-	-	15.5±0.5
M1C	9.5±0.5	8±0	5.5±0.5	-	-	15±1
M14Y	9±0	7.5±0.5	6±0	-	-	23.5±0.5
F12Y	-	-	-	-	-	23±1
F4Y	-	-	-	-	-	17±0.5
F2Y	6.5±0.5	5±0	3.5±0.5	-	-	28.5±0.5
F3Y	8.5±0.5	5.5±0.5	4.5±0.5	-	-	26±1

KEY - = no inhibition

Table 4: Antibacterial Activity expressed at Minimum Inhibitory Concentration (MIC) 0f the Crude Extract of *Lawsonia inermis* against Test Organisms

	Concentration (mg/ml)				
Isolates	200	100	50	25	12.5
M9N	-	-	+	+	+
M4N	-	+	+	+	+
M16N	-	+	+	+	+
M5C	-	+	+	+	+
M2C	-	+	+	+	+
M3C	-	-	-	+	+
M1C	-	-	+	+	+
M14Y	-	-	+	+	+
F2Y	-	+	+	+	+
F3Y	-	+	+	+	+

Key: - = no growth, + = growth present

Table 5: Diameter Zone of Inhibition of Chloroform Extract of *Lawsonia inermis* against the Test Organisms

	Concentration (mg/ml)/Zone of Inhibition (mm)					
Isolates	100	50	25	12.5	6.25	Positive Control
M8N	-	-	-	-	-	15.5±0.5
M9N	-	-	-	-	-	14.5±0.5
M4N	-	-	-	-	-	9.5±0.5
M15N	-	-	-	-	-	11±1
M16N	10.5±0.5	6±1	3±1	-	-	17.5±1.5
M2C	6.5±0.5	4.5±1.5	-	-	-	8±1
M5C	-	-	-	-	-	16.5±0.5
M13C	-	-	-	-	-	10.5±1.5
M1C	-	-	-	-	-	15±1
M14Y	6±1	4.5±0.5	-	-	-	14.5±0.5
F12Y	-	-	-	-	-	10±0
F4Y	-	-	-	-	-	24±1
F2Y	6.5±1.5	4.5±1.5	2±1	-	-	10.5±0.5
F3Y	9±1	6±1	2.5±1.5	-	-	20±1
M3C	8.5±0.5	5±1	-	-	-	11.5±0.5

Key - = no inhibition

Table 6: Antibacterial Activity expressed at Minimum Inhibitory Concentration (MIC) of the Chloroform Extract of *Lawsonia inermis* against Test Organisms

	Concentration (mg/ml)				
Isolates	200	100	50	25	12.5
M2C	-	-	+	+	+
M16N	-	-	+	+	+
M14Y	-	-	+	+	+
F2Y	-	-	+	+	+
F3Y	-	-	+	+	+
M3C	-	-	+	+	+

Key: - = no growth, + = growth present

Table 7: Diameter Zone of Inhibition of Aqueous Extract of *Lawsonia inermis* against the Test Organisms

	Concentration (mg/ml)/Zone of Inhibition (mm)					
Isolates	**100**	**50**	**25**	**12.5**	**6.25**	**Positive control**
M8N	-	-	-	-	-	15±1
M9N	8±1	6±1	3±0	-	-	14.5±0.5
M4N	7.5±0.5	6.5+0.5	2.5±0.5	-	-	16±1
M15N	-	-	-	-	-	10±0
M16N	-	-	-	-	-	20±1
M2C	-	-	-	-	-	9.5±0.5
M5C	-	-	-	-	-	14.5±1.5
M13C	-	-	-	-	-	8±1
M3C	-	-	-	-	-	11+1
M1C	8.5±0.5	6.5±0.5	4±1	-	-	10.5±2.5
M14Y	-	-	-	-	-	27.5±1.5
F12Y	-	-	-	-	-	12±1
F4Y	11±0	9.5±0.5	7±0	4.5±0.5	3.5+1.5	14.5±1.5
F2Y	-	-	-	-	-	13±1
F3Y	-	-	-	-	-	14.5±0.5

Key: - = no inhibition

Table 8: Antibacterial Activity expressed at Minimum Inhibitory Concentration (MIC) of the Aqueous Extract of *Lawsonia inermis* against Test Organisms

	Concentration (mg/ml)				
Isolates	200	100	50	25	12.5
M9N	-	-	+	+	+
M4N	-	-	-	-	+
M1C	-	-	+	+	+
F4Y	-	-	+	+	+

Key: - = no growth, - = growth present

Discussion

The phytochemical examination of the ethanol extract of *Lawsonia inermis* revealed the presence of the bioactive substances saponins, tannins, alkaloids, steroids, and terpenoids, but not cardiac glycosides or flavonoids. These substances include tannins and flavonoids, which have biological effects like anticancer characteristics and are free radical scavengers with antioxidant qualities. Additionally, these phytochemicals aid in the antimicrobial capabilities of plants. Ethanol has been employed frequently to extract bioactive components from plant samples, and the results indicate that it is a successful solvent for doing so.

With their cooperation, 50 patients from various clinics and hospitals, especially the Central Hospital Abraka in Delta State, had wound swabs obtained from wounds containing purulent

material. After being identified and evaluated, only 35 of the isolates inoculated at the Pharmaceutical Microbiology Laboratory of Delta State University in Abraka demonstrated symptoms of growth. Fifteen (15) of the 35 strains (42.9%) were gram-positive, while 20 (57.1%) were gram-negative. The Lawsonia inermis plant's leaves were used in the antimicrobial susceptibility test, which revealed that the plant has strong antibacterial potency against isolates. With zones of inhibition of 10.5 0.5 and 10 0, respectively, M9N (Proteus spp.) and MC3 (*P. aeruginosa*) demonstrated the strongest activity using the crude extract (Table 3). With zones of inhibition of 10.5 0.5 and 9 1, respectively, M16N (S. aureus) and F3Y (S. aureus) exhibited the maximum activity for the chloroform extract (Table 5). With zones of inhibition of 8.5 0.5 and 11.0 0, respectively, M1C (P. aeruginosa) and F4Y (S. aureus) exhibited the maximum activity for the aqueous extract (Table 7).

While the standard shown higher antibacterial activity against the isolates, the activity of the extracts was lower. The organisms that shown activity at different doses with varied zones of inhibition were then subjected to additional antimicrobial testing, which is reported as the Minimum Inhibitory Concentration (MIC). The findings demonstrated that the isolates with the lowest levels of extract activity were between 200 and (Tables 4, 6, and 8) 50 mg/ml. The results of this study corroborate with the study of Liao et al. [8]. Because bioactive chemicals have been found in plant extracts to have medicinal and physiological effects, it is possible to attribute the antibacterial activity of Lawsonia inermis extract to their existence.

Conclusion

These findings were promising because *Lawsonia inermis* leaf samples showed potential antibacterial action. *Lawsonia inermis* leaves were mentioned as a potential source of natural antibacterial compounds for food supplementation and the pharmaceutical industry, maybe suited to replace synthetic preservatives. *Lawsonia inermis* leaves are a possible natural source of antimicrobial compounds due to their superior antibacterial testing protective characteristics. The Lawsonia inermis leaves and their active ingredient equivalents should be useful for the creation of environmentally friendly food supplementary agents and medications, according to recent and past studies in this field.

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