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Phytochemical Analysis and Antimicrobial Activity of *Lawsonia inermis* Leaf Extracts from Burkina Faso

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Abstract

Lawsonia inermis is a hairless plant growing in various regions of North Africa, the Indian subcontinent, and the Middle East. It possesses many medicinal attributes, including curative properties against infectious dermatoses. This study was carried out to evaluate the phytochemical profile of the crude ethanolic extract of the plant leaves and its fractions as well as their antimicrobial activities. The phytochemical profile was performed using high-performance thin-layer chromatography (HPTLC), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC). Additionally, the phenolic and flavonoid contents were determined using the Folin-Ciocalteu spectrophotometric and the aluminum trichloride methods. Antimicrobial activity was tested using disc diffusion and microdilution methods. The presence of flavonoids, tannins, sterols, and triterpenes was revealed. GC-MS detected twelve compounds main compounds consisting of saturated and unsaturated fatty acids and phenolic and terpenoid compounds among twenty-seven components. HPLC also detected high contents of phenolic acids and flavonoids. The most abundant triterpene and sterols were ursolic acid (around 43.14 g/100g DW, 13.9 g/100g dry weight (DW), and

0.68 g/100g DW) in the crude ethanolic extract of leaves (FeLi), hexane fraction (FHLi) and dichloromethane fraction (FDLi), respectively and, β-sitosterol in FeLi (56.7 mg/100g DW), FHLi (10.55 g/100g DW), FDLi (106.1 mg/100g DW) and butanol fraction (FBLi) (357.4 mg/100g DW). Among the flavonoids, rutin = 3.24 g/100g and quercetin = 0.63 g/100g in the ethanolic extract, rutin = 15.73 g/100g in the dichloromethane fraction, and rutin = 0.23 g/100g) in the aqueous fraction; and among phenolic compounds, caffeic acid (37.65 g/100g DW) and vanillic acid (22.70 g/100g DW) were the most important in the ethyl acetate fraction (FAeLi). All organic fractions exhibited interesting antibacterial and antifungal activities against the tested strains, with the best activity recorded with the dichloromethane and ethyl acetate fractions. The leaf extracts' phytochemical profile and antimicrobial activity support the use of *Lawsonia inermis* against infectious skin diseases.

Keywords

Lawsonia inermis, Phytochemical Profile, HPLC, GC-MS Analysis, Antimicrobial Activity

1. Introduction

After the tremendous deadly pandemics of past centuries, humanity is today faced with one of the most serious threats to health and the food industry: antibiotic resistance [1].

According to the World Health Organization (WHO), antimicrobial resistance will be the leading cause of death worldwide by 2050 unless preventive measures are taken [2]. Indeed, the enthusiasm generated by this historic discovery has given way to despair due to the overuse and misuse of these essential drugs in humans and animals [1]. Unfortunately, antimicrobial resistance has disastrous consequences such as treatment failures, increased healthcare costs, and prolonged hospital stays, and the situation is not likely to improve with the emergence and re-emergence of new infections, including COVID-19 and dengue [3] [4]. It is, therefore, of utmost urgency to develop new strategies to control antibiotic-resistant microorganisms. Due to their richness in bioactive compounds, medicinal plants constitute important raw materials for manufacturing new drugs [5]. Their preparations could play an important role as an emerging strategy to fight pathogenic microorganisms. Nowadays, interest in plants and their secondary metabolites is attracting more and more attention [4] [6].

Lawsonia inermis, commonly known as henna, is a woody, flowering plant 2 - 6 m tall found in North Africa and Southwest Asia [7]. It is the only species of the *Lawsonia* in the Lythraceae Champ family [5] [8]. In general, this glabrous, much-branched shrub or small tree with greyish-brown bark has opposite leaves, sub-sessile, elliptical or broadly lanceolate leaves, entire, acute or obtuse, 2 - 3 cm long and 1 - 2 cm wide [9].

Phytochemical studies on species from other regions have shown the richness of *L. inermis* in secondary metabolites such as flavonoids, tannins, coumarins, alkaloids, quinones, saponins, xanthones, sterols and terpenes [7]. The phenolic profile of *L. inermis* consists mainly of caffeic, ellagic, ferulic, gallic, coumaric acids, lawsoniaside (1,3,4-trihydroxynaphthalene 1,4-di-β-D-gluco-pyronoside) and flavonoid glycosides (quercetin, kaempferol, rutin, myricetin, luteolin) [9] [10]. The main coloring matter of the plant, lawsone (2-hydroxy-1,4-naphtoquinone) is present in dried leaves at the concentration of 0.4% - 1.5% [11] [12]. Benzenoid derivatives (lawsoinermone, inermidioic acid, inermic acid) from the aerial part, two alkaloids (harmine and harmaline), five triterpenes including rosamutin, euscaphic acid, ursolic and arjunic acids from the leaves, and coumarins, lacoumarin (5-allyoxy-7-hydroxycoumarin) were characterized and isolated from *Lawsonia inermis* [10]. Studies on henna and its components have revealed various pharmacological properties in the treatment and management of diseases such as leprosy, fever, leukorrhea, rheumatoid, arthritis, ulcers, heart diseases, wounds, blood infections, inflammations, diabetes, and headaches [8] [13]. The analgesic, hypoglycemic, hepatoprotective, immunostimulating, anti-inflammatory, antibacterial, antifungal, antivirus, anticancer, and anti-parasitic, etc. effects of the plant have been attributed to the presence of the phytochemicals mentioned above [10] [14]. *L. inermis* is also well known, particularly for its purposes of accelerating growth and dyeing hair and nails due to lawsone [12]. In Burkina Faso, women widely use henna in large cities like Ouagadougou for temporary eyebrow tattoos as a semi-permanent make-up [15].

In Burkina Faso, the botanical garden of the National Center for Scientific and Technological Research (CNRST), where the *L. inermis* material was collected, is an area for plant conservation and botanical experimentation. In a preliminary study [16], crude ethanol and aqueous extracts of *L. inermis* from the CNRST garden showed significant antimicrobial activity against Gram-negative bacilli (*P. aeruginosa* ATCC 27653), Gram-positive cocci (*S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 and *S. agalactiae* ATCC 13813) and fungal strains (*C. albicans* ATCC 90028 and *C. tropicalis* ATCC 750). This study complements the first, and aims to determine the qualitative and quantitative phytochemical profile and the antimicrobial potency of *L. inermis* fractions from Burkina Faso.

2. Materials and Methods

2.1. Plant Materials

The *Lawsonia inermis* leaves were harvested in January 2020 at the National Center for Scientific and Technological Research (CNRST) botanical garden. A CNRST botanist authenticated the samples and the specimens, and the specimens were deposited under the reference number 8734. The samples were washed thoroughly with water, dried in the shade, and ground to powder with a mechanical grinder. The powders were put in freezer bags and stored away from light at room temperature for later use.

2.2. Microbial Strains

Seven ATCC microbial strains including two Gram-negative bacilli (*Escherichia coli ATCC* 25922; *Pseudomonas aeruginosa ATCC* 27653), three Gram-positive cocci (*Staphylococcus aureus ATCC* 25923; *Streptococcus pyogenes ATCC* 19615; *Streptococcus agalactiae ATCC* 13813) and two fungal strains (*Candida albicans ATCC* 90028; *Candida tropicalis ATCC* 750*)* were used. These microbial strains were purchased from LGC standard distributor ATCC in South Africa.

2.3. Culture Media

Mueller Hinton (MH) agar (Liofilchem, lot: 032720504) was used for the isolation and susceptibility testing of *S. aureus* and Gram-negative bacilli (*E. coli* and *Pseudomonas aeruginosa*). Chocolate agar (GC Medium: Liofilchem, lot: 071320502) + Isovitalex (GC + IVx) were used for the isolation and antibiogram of Streptococci (*S. pyogenes* and *S. agalactiae*). Sabouraud agar was used to isolate and test the susceptibility of fungal strains (*C. albicans and C. tropicalis*). MH (Liofilchem, lot: 011822506), Sabouraud (Liofilchem, lot: 07280501), and Brain Heart Broth (BHI) (Liofilchem, lot: 110420503) were used for the determination of Minimum Inhibitory Concentration (MIC).

2.4. Chemicals and Standards

All solvents used were of analytical grade. Ethanol, n-hexane, and ethyl acetate were purchased from CARLO ERBA (Val de Reuil Cedex, France). One-butanol and dichloromethane were purchased from Honeywell (Riedel-de Haën Chemicals Seeize GmbH, Germany). Dimethyl sulfoxide (DMSO) (≥99.7%), vanillin (≥ 99%), sodium carbonate, ferric chloride, aluminum chloride (≥ 98%), 1,3,5-Triphenyltetrazolium Chloride (TTC) and Folin-Ciocalteu from Sigma-Aldrich (Laborchemikalien GmbH, Germany); MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) from EMD (Millipore Corp MW 414.3, Lot 3727633; USA). All the following standards were purchased from Sigma-Aldrich (St. Louis): Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Catechin, Quercetin, and Ascorbic acid. The water was of Milli-Q quality (Sartorius Arium® Pro).

2.5. Plant Extracts Preparation

2.5.1. Ethanolic Extracts

One hundred grams of sample powder was macerated at room temperature (25 ± 2˚C) for 24 h with 500 mL of 96˚ ethanol. During maceration, the mixture was shaken in closed flasks using a shaker (Modal T25 Digital Ultra Turrax) and filtered using a Buchner funnel and Whatman No.5 filter paper. The filtrate was concentrated under reduced pressure with an evaporator (Heidolph Rotacool, Allemagne Type: Laborota 4003) at 40˚C. The concentrated extract was dried at 40˚C in an oven, and the dry residue was weighed and stored at 4˚C until use.

2.5.2. Fractionation

The ethanolic extract was fractionated by liquid partitioning using a separatory funnel with organic solvents of increasing polarity: hexane, dichloromethane, ethyl acetate, and butanol. 5 g of each crude ethanol extract was initially dissolved in 25 mL of distilled water and extracted with 25 mL of each organic solvent (hexane, dichloromethane, ethyl acetate, and butanol successively). The experiment was carried out three times with each solvent. The organic phases were collected and concentrated at 40˚C using a rotary evaporator. Finally, the aqueous fraction was frozen and lyophilized. The different fractions were stored at 4˚C.

2.6. Phytochemical Screening

The phytochemical analysis of the ethanolic extract and its derived fractions was carried out using standard protocols and high-performance thin-layer chromatography (HPTLC).

2.6.1. Characterization Reactions in Tubes

Previous protocols [4] have been used to identify phytochemicals in plant extracts, including alkaloids, coumarins, flavonoids, tannins, triterpenes, steroids, saponosides, etc.

2.6.2. Screening by Using High-Performance Thin-Layer Chromatography (HPTLC)

Phytochemical screening of *L. inermis* leaf extracts was carried out on 200 mm × 100 mm HPTLC 60 F254 silica gel plates (Merck, Darmstadt, Germany) according to the method described by Koala *et al*., [17]. Briefly, 5 µL of each sample was applied to the plate reference line (8 mm from the down border) using a semi-automatic plate spotter Linomat V (CAMAG, Muttenz, Switzerland). The distance between the two extracts on the reference line was 3.4 mm. The distances between the left and right edges of the plate were 20 mm. A continuous flow rate of 100 nL/s was used for the application. The plates were placed in a glass chamber previously saturated with 10 mL of mobile phase vapor for 30 minutes. The mobile phase in the twin troughs of the chamber' consisted of:

Ethyl acetate-formic acid-acetic acid-water 100:11:11:26, v/v/v/v, for flavonoids, ethyl acetate-methanol-water-chloroform 18:2.4:2.1:6, v/v/v/v, for tannins, sterols, and triterpenes, n-hexane-ethyl acetate 20:4, v/v, for sterols and triterpenes and ethyl acetate-petroleum ether 10:20, v/v, for saponosides. The developed plates were then dried with a hairdryer and revealed by spraying Neu's reagent for flavonoids, 2% iron III trichloride for tannins, Lieberman Burchard reagent for sterols and triterpenes and anisaldehyde sulphuric acid for saponosides. The developed plates were heated at 110˚C for five minutes for flavonoids, sterols, and triterpenes), at 100˚C for two minutes for tannins, and at 100˚C for five to ten minutes for saponosides.

Interpreting of results

- *Flavonoids characterization*: flavonoids interact with various reagents (natural products and aluminum chloride) to generate complexes with brilliant colors

such as yellow and green, which glow under UV/366 nm or visible light. Flavonols were revealed as yellow and greenish-yellow spots, while the presence of flavones, ethylated flavone, isoflavones, flavanones, and chalcones was indicated by blue spots. Flavanols and aurones were characterized by green spots [17].

- *Sterols*, *triterpenes*, *and saponosides characterization*: according to the literature, triterpenes and steroids are revealed under ultraviolet light by the Liebermann-Burchard reagent in the form of blue, green, pink, brown, and yellow hues. At UV/366 nm, the Liebermann-Bürchard reagent reveals sterols in yellow and yellow-green. This reagent identifies triterpene genins if the spots fluoresce blue or purple. It classifies triterpenes as oleanane and ursane types if the patches have a red fluorescence and as lupine types if the patches have a yellow-orange fluorescence [17]. In visible light, the anisaldehyde-sulphuric acid reagent exhibited yellow and green fluorescence for saponosides.
- *Tannins characterization*: tannins appear as blue-black and brown-green luminescence with 2% $FeCl_3$ reagent [18]. After heating to 110˚C and spraying with 2% $FeCl_3$ reagent, tannins appear in brown and blackish-blue at visible light on the chromatogram. Condensed tannins acquire a brown-green hue [19], while hydrolysable tannins lead to blue-black spots [20].

2.6.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The phytochemical profile of the ethanolic extracts of *L. inermis* was carried out on a gas chromatograph, Agilent 6890N, equipped with an autosampler coupled to an Agilent capillary column mass spectrometer, HP5 MS (30 m long, 0.25 mm diameter and 0.25 µm layer thickness). The system is controlled by a computer equipped with Chemstation data acquisition and processing software. Helium N60, with a purity of 99.9999%, was used as the carrier gas at a constant flow rate of 1 ml/min. The oven temperature was set at 70˚C for 5 min with increases of 20˚C/min to 120˚C for 5 min, 10˚C/min to 200˚C and 5˚C/min to 310˚C for the 5 min. An aliquot of 1µl of the sample was injected into the column at 270˚C in splitless mode. The ionization energy of 69.9 eV was used, and electron ionization is involved. The mass range is 30 to 550 atomic mass units (amu), the inlet line temperature was 270˚C and the source temperature was 230˚C. The execution time of the GC program was 42.50 minutes.

Identification of compounds

Molecular identification was performed using an internal library (National Institute Standard and Technique (NIST Version-Year 2004). The relative percentage of each component was calculated by comparing its average peak area with the total area.

2.6.4. Determination of Total Phenolic: Folin-Ciocalteu Method

Each sample's total phenolic content (TPC) was determined using the Folin-Ciocalteu spectrophotometric method previously described by Koala *et al*. [17] with slight modifications. The reagent phosphomolybdate and sodium tungstate were reduced during the oxidation of phenols in alkaline media to a mixture of tungsten blue and molybdenum.

Briefly, 25 µL of diluted extract (1 mg/mL) was mixed with 125 µL of Folin-Ciocalteu reagent (FCR) solution (0.2 N). After 5 minutes of incubation at room temperature, 100 µL of a saturated sodium carbonate solution (7.5% in water) was added to the mixture. After 60 minutes of incubation in the dark at 37˚C, the absorbance of the resulting blue hue was measured using a SHIMADZU UV-Vis spectrophotometer at 760 nm. Using the calibration curve equation Y = 0.0516x + 0.0673, R^2 = 0.9936, the total phenolic content of the extract was calculated. Data are reported as milligrams of gallic acid equivalents (GAE) per gram of dry weight. Each measurement was performed in triplicate (n = 3).

2.6.5. Determination of Total Flavonoids Content

The total flavonoid content (TFC) was determined using the aluminum trichloride method and quercetin as a reference compound according to previous methods [4] [17]. Briefly, the crude ethanolic extract was dissolved in methanol at room temperature at 1 mg/ml and filtered. Quercetin was used as a standard and a calibration curve was produced using concentrations ranging from 0.001 to 0.5 mg/mL of a quercetin solution. Then, 100 µL of the extract solution was mixed with 100 µL of a 2% methanol aluminum trichloride ($AlCl_3$) solution. After one hour of incubation at room temperature, the absorbance of the supernatant was measured at 415 nm using a spectrophotometer. The TFC of the extract was obtained by relating the absorbance reading to the standard curve equation y = 0.0151x + 0.0593, R^2 = 0.9992. The total flavonoid content was expressed as mg quercetin equivalents per gram of dry weight. All measurements were performed in triplicate (n = 3).

2.6.6. Analysis by High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD)

The identification and quantification of triterpenes, sterols and polyphenolic compounds from *L. inermis* leaf extracts were carried out using the high-performance liquid chromatography method equipped with an HPLC-DAD diode array detector (Agilent, model 1100 series; serial number DE23917296) as described by [21]. The separation was carried out with an analytical Eclipse X08-C18 column (250 × 4.6 mm; 5 µm, Agilent, USA). The diode array detector was set at 200 - 400 nm acquisition range.

- ***Triterpenes and sterols***

The test was run in isocratic mode. The eluent consisting of 0.5% acetic acid (v/v) in methanol, acetonitrile, and water (70/25/5, v/v/v) was filtered through a membrane filter of 0.45 µm (Grosseron, ref: 0700817) and degassed by an ultrasonic bath before use. The sample extract and standard were dissolved in the mobile phase and also filtered as the eluent. Ursolic acid and three sterols were detected at 220 and 250 nm, respectively. The flow rate was 1 mL/minute, and the injection volume was 50 µL.

- ***Phenolic compounds***

The eluent consisting of 1% acetic acid (v/v) in methanol, acetonitrile and water (40/15/45, v/v/v) was filtered and degassed as before. The diluted ethanolic

extract, fractions and standard solutions were filtered through a 0.45 µm membrane. Flavonoids were detected at 365 nm, caffeic and sinapic acids at 327 nm and gallic, ellagic and vanillic acids at 271 nm. The flow rate was 0.5 mL/minute, and the injection volume was 10 µL.

➢ ***Identification and quantification of compounds***

Individual phenolic compounds were quantified using the calibration curves of the respective reference compounds. Twelves standards (Sigma-Aldrich, Berin, Germany), including a triterpene (ursolic acid, R^2 = 0.9987), three sterols (β-sitosterol, R^2 = 0.9998; stigmasterol, R^2 = 0.9914; campesterol, R^2 = 0.9998), three flavonoids (rutin, R^2 = 0.9928; quercetin, R^2 = 0.9994; and kaempferol, R^2 = 0.9978) and five phenolic acids (gallic acid, R^2 = 0.9842, vanillic acid, R^2 = 0.9982; ellagic acid, R^2 = 0.9959; cafeic acid, R^2 = 0.9961 and sinapic acid, R^2 = 0.9954) were used. For this purpose, each standard's stock solutions (1000 µg/mL) was diluted to concentrations of 6.25 to 500µg/mL. The chromatographic peaks of the extracts were confirmed by comparing their retention times and UV spectra to those of the standards. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the original calibration curve values (σ) and slope (S) using three independent analytical curves according to ICH guidelines. The following equations were used: LOD = 3.3 σ /S, LOQ = 10 σ /S [21].

2.7. Antimicrobial Activity

The antimicrobial activity of *Lawsonia inermis* leaf fractions was assessed using the disk diffusion method and the microdilution method described by Youl *et al.* [4].

2.7.1. Disc Diffusion Method in Agar Medium

Petri dishes containing 20 mL of appropriate agar medium were inoculated with an 18 - 24 h culture of bacterial strains from the nutrient broth. The inoculum was adjusted to approximately 10^8 UFC/mL with sterile saline solution. The fractions were solubilized in DMSO at 100 mg/mL. Twenty-five microliters of each sample were applied separately to sterile filter paper disks (Whatman No 1; 6 mm in diameter), and placed on the surface of the inoculated medium. Antibiotics (ciprofloxacin 5 µg, erythromycin 15 µg, and nystatin 100 IU) were used as positive control and diluted DMSO as negative control. A swab-inoculated Petri dish was used as a control for bacterial growth. The inoculated Petri dishes were placed in the oven according to the strain incubation conditions (**Table 1**). All tests were performed in triplicate.

Table 1. Incubation conditions of the microbial strains used.

Microbial group	Strains	Incubation in oven
Gram negative bacilli	*Escherichia coli* ATCC 25922	Agar plates inoculated and incubated for 24 hours at 37˚C
	Pseudomonas aeruginosa ATCC 27653	
Gram positive cocci	*Staphylococcus aureus* ATCC 25923	

Continued

Gram positive cocci	*Streptococcus pyogenes* ATCC 19615 *Streptococcus agalactiae* ATCC 13813	Inoculated agar plates were initially placed in moist, CO_2-rich jars and incubated for 24 - 48 hours at 37˚C
Fungi	*Candida albicans* ATCC 90028 *Candida tropicalis* ATCC 750	Incubation for 48 hours at 25˚C

2.7.2. Microdilution Method

The microdilution method (NCCLS, 2000) on a 96-well microplate was carried out for fractions presenting an inhibition zone ≥ 10 mm. Fresh colonies of each test strains were inoculated into sterile MH, Sabouraud and Brain Heart Infusion (BHI) broths and incubated according to the growth conditions of each strain. The overnight culture was appropriately diluted in broth to obtain a viable count of approximately 10^6 UFC/mL. Each fraction's dilution (0.025-25 mg/mL) was prepared from stock solutions. To each well of the microplate containing 100 µL of inoculum, 100 µL of diluted fraction was added.

DMSO (1/5) and Tween 80 (1/10) were used as negative controls; the growth of each strain (inoculum alone without fraction) was also monitored throughout the experiment. The microplates covered with their lids were packed in plastic bags and incubated (5% CO_2, 37˚C) for 24 to 48 hours. Then, 25 µL of triphenyltetrazolium chloride (TTC) or MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution at 2 mg/mL was added to each well and the plates were incubated at 37˚C. The indicator solution changes from clear to pink (TTC) or from yellow to purple or dark blue (MTT).

The indicator solution changing from clear to pink (TTC) or yellow to purple or dark blue (MTT) respectively attests to bacterial or fungal activity; however, it remains clear when microbial growth is inhibited.

The MIC was defined as the lowest fraction concentration which gave visible inhibition of the growth of the test strain. The MIC was defined as the lowest fraction concentration giving visible inhibition of the growth of the test strain. The minimum bactericidal concentration (MBC) was determined by spreading 10 µL of contents of transparent wells showing no visible growth onto appropriate agar plates and incubating according to the required conditions. The MBC was defined as the lowest extract concentration at which 99.99% of the bacteria present in the inoculum were killed. These concentrations correspond to less than 100 CFU per 10 µL of the contents of the well at concentrations below the MIC. The tests were performed in triplicate on the same microplate for each sample, and the experiment was repeated three times.

- ***Interpreting antimicrobial test results***

Bacterial sensitivity to fractions was interpreted based on the criteria established by Ponce *et al*. [22] and their and their efficacy was assessed based on the scale proposed by Kuete [23].

An inhibition diameter ≥10 mm on the microorganisms tested: fraction selected for the microdilution test.

The BMC/MIC ratio was used to classify the fractions [24]:

- BMC/MIC≤ 2: means the fraction has a bactericidal effect.
- BMC/MIC > 2: fraction having a bacteriostatic effect.
- Inhibition diameter ≥10 mm on the microorganisms tested: fraction selected for the microdilution test.

The strain is considered tolerant to the extract if the BMC/MIC ratio is ≥ 32.

3. Results

3.1. Phytochemical Analysis

3.1.1. Qualitative Phytochemical Screening by Tube Reactions and HPTLC Analysis

The results of the qualitative phytochemical screening of the crude ethanolic extract of *L. inermis* are summarized in **Table 2** and **Figure 1**. The presence of steroidal and triterpene glycosides (saponosides), tannins, reducing compounds, anthraquinones and flavonoids was noted in the ethanolic extract of the plant (**Table 2**). Anthocyanins, alkaloids and cardenolides were not detected.

Under UV/366 nm and white lights, the high-performance thin-layer chromatography profile (**Figure 1**) showed many different colored spots (orange, yellow, blue, green, pink, purple) which were revealed using specific indicators, as indicated in the methodology.

Sterols, Triterpenes and saponosides

L. inermis leaves contained sterols and triterpenes including oleanane, ursane and lupine types (**Figure 1(a)** and **Figure 1(b)**) and saponosides [17].

Flavonoids

Favonoids including flavonols flavones, ethylated flavone, isoflavones, flavanones chalcones flavanols and aurones (**Figure 1(c)**) were highly present in the ethanolic extract of the leaves of *L. inermis*.

Tannins

Figure 1(d) presents the chromatographic profile of the plant's tannins (condensed and hydrolysable tannins).

Table 2. Phytochemical groups identified in *Lawsonia inermis* ethanolic extract.

Compound group	Li
Steroidal and triterpenic glycosides (saponosides)	(+)
Anthraquinones	(+)
Anthocyanes	(-)
Alkaloids	(-)
Flavonoids	(+)
Coumarins and derivatives	(-)
Tannins	(+)
Reducing compounds	(+)
Cardenolides	(-)

Li: 96% ethanolic extract of *Lawsonia inermis* leaves. (-) not detected, (+) presence.

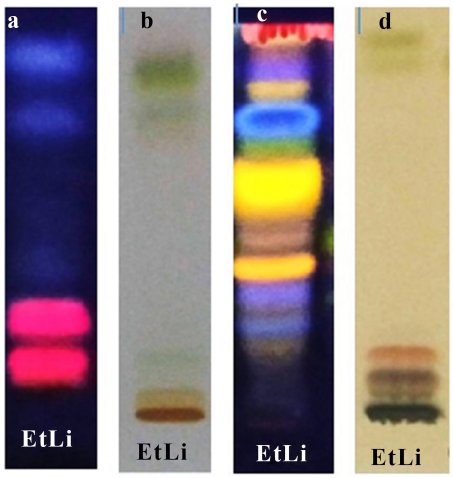

Figure 1. Chromatograms for detection of sterols and triterpenes (a), saponosides (b), flavonoids (c) and tannins (d). EtLi: 96% ethanolic extract of *Lawsonia inermis* leaves.

3.1.2. GC-MS Phytochemical Analysis

Table 3 and **Figure 2** show the phytochemical profile of the crude ethanolic extract of *L. inermis* by GC-MS. Twenty-seven peaks corresponding to various phytochemical compounds were identified. The proportion and retention time of compounds were also given.

Table 3. Phytochemical compounds identified from the chromatogram of the ethanolic extract of *Lawsonia inermis* leaves.

Peak N°	Compound name	Chemical structure	Mw	RT (min)	Area (%)
1	Benzofuran, 2,3-dihydro	C_8H_8O	120	9.141	1.61
2	1H-Isoindole-1,3(2H)-dione, 2-[hydroxymethyl]-	$C_9H_7NO_3$	177	11.714	2.68
3	D-Allose	$C_6H_{12}O_6$	180	12.470	1.04
4	1,4-Naphthalenedione, 2-hydroxy-(synonym **Lawson**)	$C_{10}H_6O_3$	174	13.144	2.35
5	α.-D-Glucopyranoside, α.-D-Glucopyranosyl	$C_{12}H_{22}O_{11}$	342	13.993	0.80
6	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	14.171	0.91
7	Coumarin-3-carboxylic acid, methyl ester	$C_{11}H_8O_4$	204	14.246	0.69
8	12-Oxododecanoic acid, ethyl ester	$C_{14}H_{26}O_3$	242	15.619	0.84
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	16.064	2.64
10	9-Eicosyne	$C_{20}H_{38}$	278	16.335	0.70
11	8-hexadecyne	$C_{16}H_{30}$	222	16.548	1.33
12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.621	5.86
13	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	17.938	7.53
14	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	19.231	0.79
15	Phytol	$C_{20}H_{40}O$	296	19.427	1.32

Continued

16	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	19.859	4.62
17	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	19.952	5.34
18	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	137	20.154	13.52
19	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_2$	320	20.252	14.85
20	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	326	20.581	1.30
21	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	23.425	0.75
22	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340	23.673	1.01
23	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	25.161	1.09
24	Tert-Heptadecanethiol	$C_{16}H_{34}S$	258	27.746	0.77
25	Squalene	$C_{30}H_{50}$	410	29.315	21.28
26	Vitamin E	$C_{29}H_{50}O_2$	430	33.411	2.89
27	γ -Sitosterol	$C_{29}H_{50}O$	414	35.626	2.92

Mw: Molecular weight, RT: Retention time.

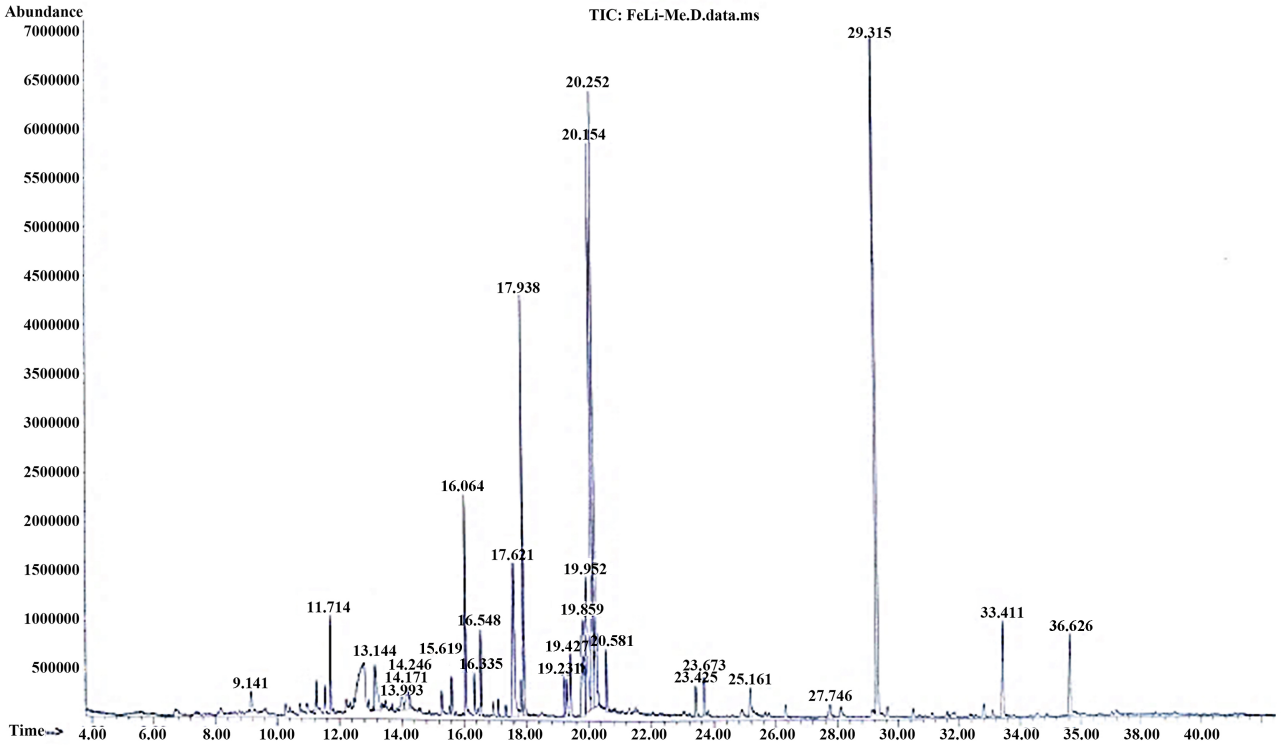

Figure 2. Chromatogram profile of the ethanolic extract of *L. inermis* leaves.

3.1.3. Quantitative Assessment

The total phenolic (TPC) and flavonoids (TFC) contents of *L. inermis* leaves are presented in **Figure 3**. TPC was expressed as gallic acid equivalent (mg of gallic acid equivalent per g of extract in dry weight (mg GA/g DW)) while TFC was expressed in mg of quercetin equivalents/g of dry weight of extract (QE/g DW).

The results were 84.53 ± 0.3 mg GAE/g DW and 40.47 ± 0.21 mg QE/g DW for TPC and TFC, respectively.

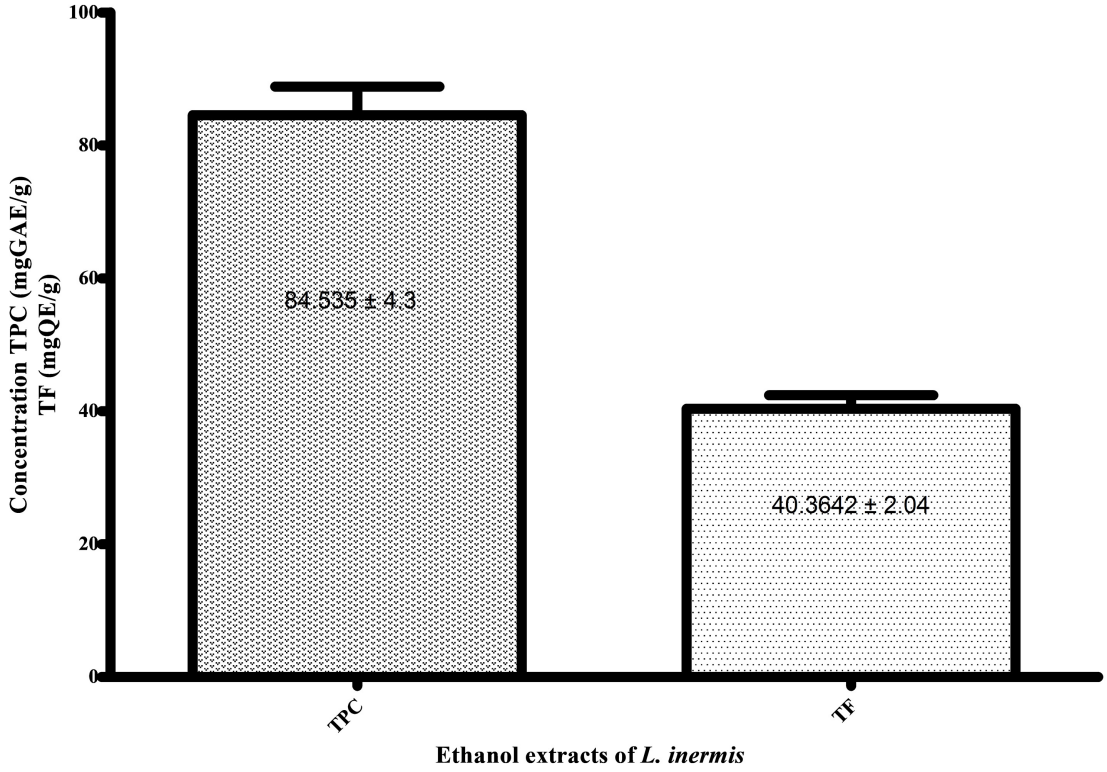

Figure 3. Content of total phenolic compounds and total flavonoids Values are presented in mean ± standard deviation (n = 3).

3.1.4. Compounds Identified and Quantified by HPLC-DAD

Triterpene and sterol compounds

The triterpene and sterol compounds identified and quantified by HPLC-DAD are presented in **Table 4**. Ursolic acid and *β*-sitosterol were the most detected. The crude ethanol extract of leaves (FeLi), hexane fraction (FHLi) and dichloromethane fraction (FDLi) of *L. inermis* contain ursolic acid (around 43.14 g/100g DW, 13.9 g/100g DW and 0.68 g/100g DW), respectively. In addition, *β*-sitosterol is detected in FeLi (56.7 mg/100g DW), FHLi (10.55 g/100g DW), FDLi (106.1 mg/100g DW) and butanolic fraction (FBLi) (357.4 mg/100g DW). Stigmasterol and campesterol were only detected in FDLi (0.95 g/100g DW) and FeLi (24.16 mg/100g DW) respectively.

Phenolic compounds

The results of the quantitative analysis of polyphenols compounds of ethanolic extracts of *L. inermis leaves* are presented in **Table 5**. The ethanolic leaf extracts and its derived fractions contained phenolic acids such as caffeic acid, gallic acid, vanillic acid and ellagic acid. Flavonoids were also detected in the ethanolic extract (Rutin = 3.24 g/100g and Quercetin = 0.63 g/100g); in the dichloromethane fraction (FDLi, (Rutin = 15.73 g/100g) and in the aqueous fraction (FAqLi (Rutin = 227.06 mg/100g). All extracts showed their richness in phenolic

acids and the highest amounts (caffeic acid = 37.65 g/100g DW, gallic acid = 8.62 g/100g DW, vanillic acid = 22.70 g/100g DW and ellagic acid = 8.04 g/100g DW) were recorded with the ethyl acetate fraction (FAeLi).

Table 4. Sterols detected in *L. inermis* leaf extracts.

Sample	Sterols (mg/100g dried weight extract)			
	Ursolic acid	Stigmasterol	Campesterol	-sitosterol
FeLi	43144.54 ± 24.78	Nd	24.16 ± 1.08	56.76 ± 0.7
FHLi	13940.66 ± 31.52	Nd	Nd	10548.84 ± 35.06
FDLi	683.83 ± 3.07	950.24 ± 18.01		106.1 ± 2.6
FAeLi	Nd	Nd	Nd	Nd
FAqLi	Nd	Nd	Nd	Nd
FBLi	Nd	Nd	Nd	357.44 ± 0.65

Values are presented in mean ± standard deviation (n = 3); Nd = no detected, FeLi: 96% ethanolic extract of *L. inermis* leaves, FHLi: hexane fraction, FDLi: dichloromethane fraction, FAeLi: ethyl acetate fraction, FAqLi: aqueous fraction, FBLi: butanolic fraction.

Table 5. Phenolic compounds detected in *L. inermis* leaf extracts.

Sample	Phenolic compounds (mg/100g dried weight extract)					
	Rutine (mg/100g DW)	Quercetin (mg/100g DW)	Caffeic acid (mg/100g DW)	Gallic acid (mg/100g DW)	Vanillic acid (mg/100g DW)	Ellagic acid (mg/100g DW)
FeLi	3241.02 ± 5.02	634.64 ± 0.02	12142.29 ± 0.18	2713.19 ± 0.47	5002.78 ± 1.09	2669.66 ± 0.4
FHLi	Nd	Nd	722.52 ± 0.09	350.80 ± 0.01	485.60 ± 0.17	136.06 ± 0.1
FDLi	15726.78 ± 22.04	Nd	21360.76 ± 0.06	4532.03 ± 0.19	9052.23 ± 0.36	4996.45 ± 01
FAeLi	Nd	Nd	37645.84 ± 0.58	8616.57 ± 0.08	22701.48 ± 11.01	8043.78 ± 0.2
FAqLi	227.06 ± 0.7	Nd	2285.18 ± 0.01	955.40 ± 003	1895.59 ± 2.36	652.64 ± 0.01
FBLi	Nd	Nd	Nd	Nd	Nd	Nd

Values are presented in mean ± standard deviation (n = 3); nd = no detected, FeLi: 96% ethanolic extract of *L. inermis* leaves, FHLi: hexane fraction, FDLi: dichloromethane fraction, FAeLi: ethyl acetate fraction, FAqLi: aqueous fraction, FBLi: butanolic fraction.

3.2. Antimicrobial Activity

Five fractions from the ethanolic extract of *L. inermis* were tested for their antimicrobial activity against a panel of Gram-positive cocci, Gram-negative bacilli and fungi. The antimicrobial activity expressed by the diameters of the inhibition zones and the minimum inhibitory concentrations are summarized in Table 6 and Table 7. The hexane (FHLi), dichloromethane (FDLi), ethyl acetate (FAeLi) and butanol (FBLi) fractions displayed significant antibacterial activity against microorganisms (Ø ranged from 8 mm to 24 mm) (Table 6). The most active ethyl acetate and dichloromethane fractions showed broad-spectrum antimicrobial activities against *P. aeruginosa*, Gram-positive cocci and fungi. FHLi inhibited only fungal strains at 100 mg/mL (Ø = 11 ± 0 mm and 20 ± 1 mm)

from a standardized inoculum of 10^8 CFU/mL of *C. albicans* and *C. tropicalis*, respectively. FBLi, on the other hand, only inhibited Gram-positive cocci with inhibition zone diameters of 11 ± 0.52 mm (*S. aureus*), 12 ± 0.52 mm (*S. agalactiae*) and 13 ± 0.67 mm (*S. pyogenes*).

By the microdilution method, FDLi was the most effective with a MIC value of 8.4 mg/mL, 0.52 mg/mL, 0.16 mg/mL and 0.23 mg/mL on standardized inoculum (10^6 CFU/mL) of *P. aeruginosa*, *S. aureus*, *S. agalactiae* and *S. pyogenes*, respectively. FAeLi showed moderate to low activity on the strains tested (MIC varying from 0.33 mg/mL to 4.1 mg/mL). Additionally, FHLi exhibited moderate to weak fungicidal activity against *C. tropicalis* and *C. albicans*, respectively; with no growth at concentrations of 0.66 mg/mL and 1.02 mg/mL for the two strains. Gram-positive cocci were the most sensitive (lowest MIC) to *L. inermis* extracts and fractions.

Table 6. Antimicrobial activity of *L. inermis* fractions determined by agar disc diffusion (Ø mm).

Souches / Extraits	*P. aeruginosa*	*E. coli*	*S. aureus*	*S. pyo-genes*	*S. aga-lactiae*	*C. albicans*	*C. tropicalis*
Negative control	0	0	0	0	0	0	0
Positive control	27.33 ± 0.57	35.67 ± 0.57	31.33 ± 1.15	37.67 ± 0.57	37.67 ± 0.57	20.67 ± 0.57	21 ± 1
L. inermis	8 ± 0	8 ± 0	9 ± 0.67	13.33 ± 1.1	12.33 ± 0.33	0	0
FHLi	0	8 ± 0	0	0	0	11 ± 0	20 ± 1
FDLi	12 ± 0	8 ± 0	15 ± 1	18 ± 1.67	20 ± 2.1	8 ± 0	12 ± 0.33
FAeLi	11 ± 0	8 ± 1	21 ± 0.67	24 ± 1.1	24 ± 1.2	12 ± 1.12	9 ± 0.67
FBLi	0	0	11 ± 0.52	13 ± 0.67	12 ± 0.52	0	0

Values are presented in mean ± standard deviation (n = 3); NC: negative control (DMSO 1/5); PC: positive control (Erythromycin 15 µg for gram-positive cocci; Ciprofloxacin 5µg for gram-negative bacilli and Nystatin 100UI for fungi); FHLi: Hexane fraction of leaves; FDLi: Dichloromethane fraction of leaves; FAeLI: Ethyl Acetate fraction of leaves; FBLi: Butanol fraction of leaves; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Sa: *Staphylococcus aureus*; Sp: *Streptococcus pyogenes*; Sag: *Streptococcus agalactiae*; Ca: *Candida albicans*; Ct: *Candida tropicalis*.

Table 7. Antimicrobial parameters (MIC; MBC) of active fractions (n = 3).

Extracts	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
		P. aeruginosa		
FAeLi	4.1 ± 0	16.3 ± 0	4	Bacteriostatic
FDLi	8.4 ± 0	67.2 ± 0	8	Bacteriostatic
		S. aureus		
FeLi	0.41 ± 0.29	1.56 ± 0.67	4	Bacteriostatic
FAeLi	1.02 ± 0	2.04 ± 0	2	Bactericidal
FDLi	0.52 ± 0	1.05 ± 0	2	Bactericidal
FBLi	2.44 ± 0	2.44 ± 0	1	Bactericidal

Continued

		S. agalactiae		
FeLi	0.41 ± 0	0.65 ± 0	1	Bactericidal
FAeLi	1.02 ± 0	2.6 ± 0	2	Bactericidal
FBLi	1.2 ± 0	2.6 ± 0	1	Bactericidal
FDLi	0.16 ± 0	0.33 ± 0	2	Bactericidal
		S. pyogenes		
FeLi	0.23 ± 0	0.47 ± 0	2	Bactericidal
FAeLi	0.33 ± 0	0.33 ± 0	1	Bactericidal
FBLi	0.65 ± 0	0.65 ± 0	1	Bactericidal
FDLi	0.23 ± 0	0.23 ± 0	1	Bactericidal
		C. albicans		
FHLi	1.2 ± 0.11	1.2 ± 0.33	1	Fungicidal
FAeLi	2.04 ± 0	2.04 ± 0	1	Fungicidal
		C. tropicalis		
FHLi	0.66 ± 0	0.66 ± 0	1	Fungicidal
FDLi	0.96 ± 0	0.96 ± 0	1	Fungicidal

FHLi: Hexane fraction of leaves; FDLi: Dichloromethane fraction of leaves; FAeLI : Ethyl Acetate fraction of leaves; FBLi : Butanol fraction of leaves; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Sa: *Staphylococcus aureus*; Sp: *Streptococcus pyogenes*; Sag: *Streptococcus agalactiae*; Ca: *Candida albicans*; Ct: *Candida tropicalis*.

4. Discussion

Lawsonia inermis (henna) is well-known as a plant with both industrial and medicinal properties [25]. It is a natural source of lawsone (2-hydroxy-1,4-naphthoquinone), a major bioactive and dying active compound [26]. Our study reported the phytochemical profile (polyphenols, sterols and triterpenes), and antimicrobial properties of extracts and fractions of *Lawsonia inermis* collected from the CNRST botanical garden.

Phytochemical analysis using standard protocols indicated the presence of sterols and triterpenes, flavonoids, tannins and reducing compounds in the crude ethanolic extract of *Lawsonia inermis* leaves. Our phytochemical results are consistent with those of previous works [27] [28].

Using the HPTLC method, triterpene compounds of the oleanane, ursane, and lupine types were identified, and among the flavonoids: flavonols, flavones, ethylated flavones, isoflavones, flavanones and chalcones, flavonols and aurones have been characterized. Flavonoids are a class of natural phenolic compounds of a 2-phenyl-benzo-c-pyrane nucleus comprising two benzene Rings, A and B, linked through a heterocyclic pyran or pyrone Ring C. Various subclasses, such as flavones, isoflavones, flavonols, flavanols (catechins), flavanones, flavanonols,

chalcones and dihydrochalcones, aurones and anthocyanidins have been distinguished according to the level of unsaturation and oxidation [29].

Previously, four ursane-type triterpenes (rosamutin, euscaphic acid, 1b,2b,3b,19a-tetrahydroxyurs-12-en-28-oic acid, ursolic acid) and one oleane-type triterpene (arjunic acid) were isolated from the methanolic extract of the leaves of *Lawsonia inermis* [10]. Condensed and hydrolyzed tannins were also characterized in our extracts. Tannins represent a high percentage (5% - 10%) of the constituents of henna extract and participate, with the lawsone molecule, in the reaction with skin proteins [30].

By GC-MS screening, twelve predominant compounds, including lawsone, the main marker of the plant, were identified. Most compounds identified in our study have been reported previously [31]-[33], but with a difference in content that can be explained by the experiment conditions, notably the nature of the solvent used for extraction. Elsewhere, variation in plant content between sites may be likely due to variation in local growing conditions as well as genetic and seasonal factors [32]. Previous studies have demonstrated the biological properties of some of the compounds revealed in the present study. Thus, squalene from soil fungi (*Talaromyces pinophilus* CJ15) has an antimicrobial inhibitory effect against pathogenic yeast strains such as *C. haemulonii* (d = 14 mm), *C. albicans* (d = 18 mm), *C. tropicalis* (d = 16 mm) and *C. glabrata* (d = 17 mm) at a concentration of 100 µL [34]. Several studies have revealed that squalene has anticancer, antioxidant, detoxifying, skin moisturizing, emollient and drug-carrying activities [35] [36]. The predominant fatty acids, such as hexadecanoic acid have antioxidant, hypocholesterolemic and nematicidal and pesticidal effects [37]. 9,12,15-octadecatrienoic acid and ethyl ester (Z, Z, Z) have anti-inflammatory, hepatoprotective, insect repellent, antihistamine, anticoronary and antimicrobial activities [38]. Vitamin E (α-tocopherol) plays an antioxidant role, reduces the risk of diabetes and protects the skin from UV rays [37]. Some of the minor compounds identified also possess remarkable biological properties. This is the case of benzofuran derivatives, which are used as antidepressants, anticancers, antivirals, antifungals, antioxidants and antipsychotics [39] and today represent the essential elements of new drugs against the hepatitis C virus and other cancers [40].

Quantitatively, the ethanolic extract of *L. inermis* leaves was also found to have a high content of total phenolics (84. 53 ± 0.3 mg GAE/g DWE) and total flavonoids (40.47 ± 0.21 mg QE/gDW) contents. According to literature data, our results are either similar [10] or different [41]. In their review, Al-Snafi *et al*. [10] highlighted that quercetin and isoquercitrin are regularly cited among the main phenolic compounds. On the other hand, Elansary *et al*. [26] showed a lower content of gallic acid (81.0 ± 13.2 mg/100 g DW) while a similar content of quercetin (1079.12 mg/100 g DW) was reported in the methanol extract of *L. inermis* leaves by Mustafa *et al*. [41].

Variation in phenol content among plant species is common and depends on

genetic, environmental and extraction conditions [42]. It has previously been shown that light intensity, temperature and altitude can influence flavonoid biosynthesis [41].

Phenolic acids (caffeic acid, gallic acid, vanillic acid and ellagic acid) and flavonoids (rutin and quercetin) were detected in high concentration in the ethanolic extract and fractions of *L. inermis*. Very few studies have evaluated the phenolic and flavonoid content of *L. inermis* leaves. The ethyl acetate fraction of *L. inermis* showed its richness in phenolic acids (caffeic, gallic, vanillic and ellagic acids); these results are consistent with those of a previous study which had characterized, in addition to other compounds, the molecules mentioned above [9] [13]. Elsewhere, Dhaouadi *et al*. [9] using HPLC-DAD and LC-ESI-MS confirmed the polyphenolic profile of *L. inermis* composed mainly of phenolic acids (caffeic acid, ellagic acid, ferulic acid, gallic acid, vanillic acid) and flavonoids (quercetin and others compounds).

Plant secondary metabolites play various roles, including defense and facilitating interactions with their living environment. These secondary metabolites have interesting therapeutic properties that could justify the traditional use of plants.

During our experiment, the best antimicrobial activity was recorded with the ethyl acetate and dichloromethane fractions, which showed a broad-spectrum antimicrobial effect against bacterial and fungal strains. The inhibitory effect of henna plant against both Gram-negative and Gram-positive bacteria and fungi was previously reported; indeed, a study demonstrated the inhibitory power of ethyl acetate and butanolic fractions of the hydromethanolic crude extract (70%) of *L. inermis* against *P. aeruginosa* ATCC 27853 and six clinical isolates of the same species from skin infections in diabetic patients [43].

Furthermore, the butanoic fractions showed a strong anti-biofilm effect on all *P. aeruginosa* isolates (MIC comprised between 3.12 and 6.25 mg/mL). The ethyl acetate fraction showed antibacterial activity against *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25923); *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) [43] and antityphoid activity against *Salmonella Typhi* [44]. Sharma et al. also reported antityphoid activity (11.66 ± 1.5 mm) at 20 mg/ml of the hexane fraction. In another study, dichloromethane and ethyl acetate fractions of leaves, fruits and flowers were found to have inhibitory activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *Bacillus subtilis*, with the highest activity obtained with the ethyl acetate fraction at 1 mg/100 µl. [10].

Elsewhere, Dahake *et al*., [7] also showed an antimicrobial effect of an ethanolic extract of *L. inermis* against *E. coli* (22.6 mm), *P. aeruginosa* (16.8 mm), *S. aureus* (24.4 mm), *S pyogenes* (20.7 mm) and many other germs. Furthermore, the antimicrobial activity of *L. inermis* leaf extract against eight pathogens of nosocomial infections, including *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*, was also reported by Kouadri *et al*., [28]. In Iraq, Ibrahim *et al*. [5] showed a dose-dependent inhibitory effect of the ethanolic extract of *L. inermis* leaves

against five Gram-positive bacteria isolated from wounds.

As mentioned in previous studies, the antimicrobial activity of *L. inermis* is due to the numerous free hydroxyls of the lawsone molecule which have the ability to combine with the proteins and carbohydrates of the bacterial cell wall, rendering the enzymes inactive by attachment to their site [5] [16]. These data highlighted the broad-spectrum antimicrobial activity of henna, including antimycotic, antiparasitic, antiviral, and antibacterial activities.

In addition to the lawsone molecule, some of the phytochemical compounds identified in our extracts and fractions are known to have antimicrobial activity. Indeed, phenolic acids like caffeic acid, ellagic acid, gallic acid and vanillic acid were found to possess direct or indirect antimicrobial activity as an adjuvant when combined with an antibiotic [45]-[49].

Flavonoids are well known to possess various biological properties, including direct antibacterial activity, synergy with antibiotics and suppression of bacterial virulence. In our study, we identified and quantified only quercetin and rutin (standards available); however, it should be noted that the *L. inermis* sample presented in the present study contains many other flavonoids evidenced by numerous fluorescent spots in **Figure 1(c)**.

The flavones, ethylflavones, isoflavones, flavanones, flavanols, chalcones and aurones of *L. inermis* have been shown to have direct antimicrobial activity against both Gram-negative and Gram-positive bacteria, including multidrug-resistant bacteria in previous studies [50] [51]. More specifically, quercetin and rutin isolated from herbal preparations showed broad-spectrum antibacterial activity against Gram-positive cocci (including *S. aureus* and MRSA) [52]-[54], Gram-negative bacilli (*P. aeruginosa* and *V. cholera*) [50] and an antifungal effect [55]. As is known for polyphenols, quercetin could exert its antibacterial activity by disrupting the integrity of the bacterial membrane [50]. The anti-MRSA activity would be expressed by the modulation of penicillin-binding protein 2a (PBP2a) [54], while the mechanism of antifungal activity suggests a possible alteration of the fatty acid pathway, which constitute specific targets for the development of new antifungal drugs [55].

Besides phenolic compounds, steroids/triterpenes and saponosides are also other groups of compounds with many biological properties. Thus, phytosterols obtained from medicinal plants are well known for their antidiabetic, anti-cardiovascular, anticancer and antimicrobial effects (notably on *S. aureus*, *E. coli* and *P. aeruginosa*) [56]. A previous study showed that stigmasterol at 100 µg/mL exerts a potent inhibition against various bacteria and fungi with average zones of inhibition ranging from 23 to 30 mm compared to ciprofloxacin (5 µg/mL) and fluconazole (5 µg/mL); the minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) of stigmasterol varied from 6.25 to 25 µg/mL and from 12.5 to 50 µg/mL, respectively [57]. In general, plant preparations containing β-sitosterol, stigmasterol and/or campesterol have demonstrated antibacterial [56] [58] [59] antiviral [60]

and antifungal [56] properties. According to the literature, the antimicrobial effect of sterols may be due to their similarity with sterols commonly used in cells, facilitating their incorporation into cell membranes [59]. Disruption of the membrane of microorganisms could be one of the possible mechanisms of action of sterols [56]; their mechanism of action is based on their ability to complex with saponins present in the membranes of fungi, leading to the formation of pores and the loss of membrane integrity [58].

Triterpenes of ursane and oleanane type were reported to possess antimicrobial, antiviral and antifungal properties [61] [62]. Experiments have shown that ursolic acid used alone is less active on bacteria and fungi. At the same time, its synthetically obtained ester derivatives significantly inhibit the growth of Gram-positive bacteria (*B. cereus* ATCC 11778 and *S. aureus* ATCC 29212), Gram-negative bacteria (E. coli ATCC 25922 and *S. typhimurium* ATCC 14028) and fungi (*C. albicans* ATCC 18804) at 100 µg/mL [63]. Similarly, plant extracts with high oleanane content showed pronounced antibacterial activity against *S. aureus* (MIC of 1.25%) and *B. subtilis* (MIC of 0.625%) [61]. In a word, fractions with a high number and content of detected compounds, including sterols and polyphenols, were the most active. Based on their polyphenolic profile, phenolic acid (caffeic, gallic, vanillic and ellagic acids) and flavonoid (rutin) are the best antimicrobial ingredients in the plant studied. It can be assumed that the antimicrobial activity observed above results from the individual and synergistic effects of the different compounds identified in this study.

5. Conclusions

Lawsonia inermis Linn. or Henna tree is a plant used for its coloring and medicinal properties, among which the antimicrobial effect. This study allowed the identification of several phytochemical compounds including phenolic acids (caffeic acid, gallic acid, vanillic acid and ellagic acid), flavonoids (quercetin, rutin), hydrolysed and condensed tannins and non-polar compounds like sterols (ursolic acid, stigmasterol, beta-sitosterol) and triterpenes (ursane and oleanane type). Extracts and fractions of the plant have been shown to elicit strong and broad-spectrum activity against bacteria and fungi responsible for infectious dermatoses. The observed strong antimicrobial activity could be attributed to the plant's varied and high content of phytochemicals. The primary outcome of this study is that the organic fractions (hexane, dichloromethane, ethyl acetate and butanol) were the most active and also concentrated the highest contents of phytochemicals compared to the aqueous fractions. The results of this study are complementary to previous studies, which identified the lawsone molecule, an organic compound from the naphthoquinone family, naturally present in henna leaves, as responsible for the antimicrobial activity.

Lawsonia inermis appears to be a rich cocktail of phytochemicals and a potential antimicrobial plant, particularly for therapeutic uses against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Candida tropicalis*

infections. Innovative approaches could be developed to valorize this plant material as an infectious agent.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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