DOI: https://doi.org/10.21323/2618-9771-2025-8-1-73-80

Received 22.07.2024
Accepted in revised 13.03.2025
Accepted for publication 17.03.2025

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

LAWSONIA INERMIS EXTRACT: ANTIBACTERIAL, ANTICANCER AND ANTIOXIDANT PROPERTIES

Seemaisamy Revathi[1], Elham K. Nasser [2], H. Khassaf Wafaa[3], Ammar B. Altemimi [2], Sutikno Sutikno[1], Titik Kartika[1], Ikhsan Guswenrivo[1], Farhang H. Awlqadr [4], Tarek Gamal Abedelmaksoud[5]*

[1] Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[2] Department of Food Science, College of Agriculture, University of Basrah, Basrah Iraq
[3] Date Palm Research Centre, University of Basrah, Iraq
[4] Food Science and Quality Control, Halabja Technical College, Sulaimani Polytechnic University, Sulaymaniyah, Iraq
[5] Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt

KEY WORDS:
herbal medicine, bioactive compounds, oxidative stress, cytotoxic effects, natural product research, phytochemical screening

ABSTRACT

Henna, scientifically known as Lawsonia inermis, is long used for its therapeutic benefits in several civilisations. This study examines the antibacterial, anticancer, and antioxidant properties of the leaf extract from L. inermis. The phytochemical study indicated the existence of alkaloids, flavonoids, glycosides, phenolic compounds, and tannins. GC–Mass Spectrometry detected notable bioactive chemicals, including tetracosamethyl-cyclododecasiloxane and cyclohexasiloxane. The extracts demonstrated strong antioxidant properties, as evidenced by their values of 254.32 and 121.25%, as well as their high FRAP values, demonstrating their effective ability to scavenge radicals. The antibacterial effectiveness, assessed by the disc-diffusion technique, demonstrated substantial inhibition zones against both Gram positive and Gram-negative microorganisms. The MTT experiment exhibited significant anticancer efficacy against MCF-7 breast cancer cell lines, with an IC_{50} value of 1.682 µg/mL. The results indicate that extracts from L. inermis contain significant bioactive substances that have the potential to be utilised in the development of innovative medications aimed at addressing oxidative stress, bacterial infections, and cancer.

Поступила 21.07.2024
Поступила после рецензирования 13.03.2025
Принята в печать 17.03.2025

https://www.fsjour.com/jour
Научная статья
Open access

ЭКСТРАКТ ЛАВСОНИИ ОБЫКНОВЕННОЙ: АНТИБАКТЕРИАЛЬНЫЕ, ПРОТИВОРАКОВЫЕ И АНТИОКСИДАНТНЫЕ СВОЙСТВА

Ревати С.[1], Нассер, Э. К.[2], Вафаа Х. Хассаф[3], Алтемими А. Б., Сутикно С.[1], Картика Т.[1], Гусвенриво И.[1], Авлкадр Ф. Х.[4], Абедельмаксуд Т. Г.[5]*

[1] Исследовательский центр прикладной зоологии, Научно-исследовательская организация в области наук о жизни и окружающей среде, Национальное агентство исследований и инноваций (BRIN), Богор, Индонезия
[2] Кафедра пищевых наук, Сельскохозяйственный колледж, Университет Басры, Басра, Ирак
[3] Исследовательский центр финиковой пальмы, Университет Басры, Ирак
[4] Технический колледж прикладных наук Халабджи, Исследовательский центр Халабджи, Сулейманский политехнический университет Сулеймания, Ирак
[5] Кафедра пищевых наук, Сельскохозяйственный факультет, Каирский университет, Гиза, Египет

КЛЮЧЕВЫЕ СЛОВА:
фитотерапия, биологически активные соединения, окислительный стресс, цитотоксические эффекты, исследование природного продукта, фитохимический скрининг

АННОТАЦИЯ

Хна (хенна), известная в науке как *Lawsonia inermis*, уже давно применяется во многих цивилизациях благодаря своим лечебным свойствам. В исследовании изучены антибактериальные, противораковые и антиоксидантные свойства экстракта листьев *L. inermis*. Фитохимическое исследование растения показало наличие алкалоидов, флавоноидов, гликозидов, фенольных соединений и танинов. С помощью ГХ-масс-спектрометрии были обнаружены биологически активные химические вещества, в том числе тетракозаметил-циклододекасилоксан и циклогексасилоксан. Экстракты продемонстрировали сильные антиоксидантные свойства, о чем свидетельствуют уровни их содержания — 254,32 и 121,25%, а также их высокие значения восстановления флуоресценции после фотообесцвечивания (FRAP), демонстрирующие их эффективную способность захватывать радикалы. Антибактериальная эффективность, оцененная с помощью дискодиффузионного метода, продемонстрировала весьма крупные зоны ингибирования роста как грамположительных, так и грамотрицательных микроорганизмов. Анализ с MTT показал значительную противораковую эффективность против линий клеток рака груди MCF-7 со значением IC50 1,682 мкг/мл. Результаты показывают, что экстракты *L. inermis* содержат значительные биоактивные вещества, которые могут быть использованы при разработке инновационных лекарств, направленных на борьбу с окислительным стрессом, бактериальными инфекциями и раком.

1. Introduction

Plants have long been utilized in traditional herbal medicine. Consequently, plants are currently acknowledged as an inherent source of potent novel antibacterial chemicals. Medicinal plants are used as ethnomedicine in several cultures worldwide due to their abundant and diverse medical effects. Additionally, they serve as a reservoir of natural chemicals that offer limitless potential for innovative pharmaceuticals [1]. Plants have been used in traditional herbal treatment for numerous years. Plants have been discovered to naturally produce powerful new antibacterial compounds. Medicinal plants are utilized as ethno-medicine

FOR CITATION: **Revathi, S., Nasser, E. K., Wafaa, H. H., Altemimi, A. B., Sutikno, S., Kartika, T. et al.** (2025). Lawsonia inermis extract: Antibacterial, anticancer and antioxidant properties. *Food Systems*, 8(1), 73–80. https://doi.org/10.21323/2618-9771-2025-8-1-73-80

ДЛЯ ЦИТИРОВАНИЯ: **Ревати, С., Нассер, Э. К. Вафаа, Х. Х., Алтемими, А. Б., Сутикно, С., Картика, Т. и др.** (2025). Экстракт лавсонии обыкновенной: антибактериальные, противораковые и антиоксидантные свойства. *Пищевые системы*, 8(1), 73–80. https://doi.org/10.21323/2618-9771-2025-8-1-73-80

in several countries worldwide due to their abundant and easily accessible therapeutic properties. Moreover, they offer an abundance of natural compounds that have the potential to be utilized in the development of numerous novel pharmaceuticals [1,2,3]. This well-known plant, sometimes known as "henna", is found in tropical regions of North Africa and South East Asia. Native to tropical and subtropical areas of the world, including as South Asia, Africa, the Sahara Desert, and even parts of northern Australia, the five-meter-tall blooming plant is known as henna. The leaves of the henna plant are sub sessile, smooth, oval-shaped, entire, and opposite. The leaves have proportions of 1–2 cm in width and 2–3 cm in length. The henna shrub has densely branched stalk, greyish-brown bark. People have used this plant for its herbal medicinal properties and cosmetic effects for years. Henna is the plant of medicinal importance, which provides significant biological effects both *in vitro* and *in vivo*. This plant has long been utilized in traditional oriental medical systems to treat a variety of ailments, including headaches, leprosy, and some skin issues. Henna was once used to treat hair to prevent dandruff and lice as well as to shield the hands and feet from fungal illnesses. Other traditional usage includes treating rheumatoid arthritis, headaches, ulcers, diarrhea, jaundice, leprosy, fever, enlarged spleen, diabetes, and other ailments. Again, it has astringent, antimicrobial, and antihemorrhagic qualities. It is also widely known for having cardiac inhibitory, hypertensive, and sedative properties [4]. Antioxidants are the compounds that have the ability to shield cells from the harmful effects of free radicals, which can cause oxidative damage. Natural products have exhibited high antioxidant action, potentially enhancing individuals' well-being. The pursuit of naturally occurring antioxidants with pharmacological importance has led to extensive research of numerous plant species and their active components. Researching medicinal plants entails more than just determining potential therapeutic applications. To optimize the use of medicinal plants with potential antioxidant activity and discover the qualities linked with them, many aspects of these plants require thorough investigation. Using phytochemical screening, a wide range of chemicals have been found from various parts of *L. inermis*, including coumarin, fatty acids, amino acids, phenolic compounds, terpenoids, sterols, tannins, xanthones, and derivatives of naphthoquinone. *L. inermis* leaf extracts show anti-proliferative effects against MCF-7 human breast cancer. Thus, in this work, we assessed the cytotoxic, antioxidant, and antibacterial qualities of the aqueous extract of *L. inermis* leaves.

2. Material and methods

2.1. Plant materials

Microorganisms, nutrient-rich substances, and chemical compounds were used for the research. The materials were obtained from Merck Co. (Darmstadt, Germany) include DPPH, ABTS, folin ciocalteu reagent, gallic acid, potassium persulfate $K_2S_2O_8$, and dimethyl sulfoxide (DMSO). The microbiological strains obtained from IMTECH, India, were used to isolate gram (negative and positive) bacteria, specifically *Pseudomonas putida* with strain number MTCC1194, *E. coli* MTCC1302, *K. pneumoniae* MTCC4727, and *S. marcescens* MTCC4822. The gram-positive bacterial strains comprised of *S. aureus* MTCC737, *B. subtilis* MTCC441, *B. megaterium* MTCC441, *B. cereus* MTCC6840, and *B. aryabhattai* MTCC14579. We purchased nutrient agar (NA), Mueller Hinton Broth (MHB) to use it as high-purity media.

2.2. Preparation of extract of L. inermis

To make the extract the plants of *L. inermis* were taken, 100 g of leaves were weighed separately and kept in a Soxhlet device for around 6 hours. The various chemical components were isolated using a variety of solvents, such as petroleum ether, chloroform, acetone, methanol, ethanol, and water, according to their polarity. The components were then selected for their antibacterial qualities. The extracts of *L. inermis* obtained with acetone and methanol proved them to be the most successful solvents among others. Bennour et al. [5] stated that the extract solvents were chilled for future using, after being evaporated with a Rota-evaporator 2198-H (Buchi T, India).

2.3. Initial phytochemical analysis

The initial phytochemical screening tests were performed using acknowledged methodologies to identify useful constituents, including alkaloids, terpenoids, glycosides, quinones, cardiac glycosides, coumarins, phlobatannins, anthraquinones, and phenols [6].

2.3.1. Inoculum development

Using both NA and MHB culture media, a stock culture of pathogenic bacteria was prepared. Strains of *Staphylococcus*, *Bacillus subtilis, Pseudomonas*, and *Escherichia coli* were cultured on agar slants for 24 hours at 37 °C using their original culture media. After incubation the cultures were maintained at 4 °C [7]. All of the analytical grade compounds were purchased from Sigma-Aldrich.

2.3.2. Preparation of plant extract

Making the leaf extract from *Lawsonia inermis* (henna). The henna plant, *Lawsonia inermis*, was taken from its natural habitat. We acquired fresh *Lawsonia inermis* (henna) leaves from the Science Campus of Alagappa University in Karaikudi, Tamil Nadu, India. The experimental field investigation was carried out in accordance with all applicable institutional policies and standards. The plant leaf was first prepared by washing, drying in the shade at the right temperature and humidity levels, and then were ground in a lab mill laboratory knife mill HM100 (STERICOX, India). The plant was then submerged at certain proportion (1:10 ratio; w/v) in water for one day at the temperature of the laboratory (approximately 30 °C), while being stirred as part of the maceration process. To remove the extract from the plant, filter paper was used and Rota-evaporator 2198-H (Buchi T, India). Benchtop High-Speed Centrifuge (TG24-WS, China) was used for the evaporation and centrifugation processes. The remaining sample was stored at 4 °C for subsequent analysis [8].

2.3.3. Purification of crude extract by column chromatography

For column chromatography, silica gel with particle sizes ranging from l00 to 200 µm was chosen. For packing the column, the silica gel was suspended in petroleum ether. A 40-centimeter corning glass tube with an internal diameter of 2.5 centimeters and a glass stopper at the bottom made up the column. To create a continuous column free of air bubbles, the washed silica gel suspension was progressively added to the tube's lower end, which held an attached disk. The column's ultimate dimensions were 25 by 2.5 cm. Methanol was used to equilibrate the column. Samples of volume up to 5 ml were run through the column at a flow rate of 0.2 ml/min using a gradient solvent solution made up of 9:1, 7:3, and 1:1 ratios of ethanol to chloroform. Ultimately, methanol was used to wash the column. Ten milliliters fractions from each solvent system were collected, and the homogeneity of each individual fraction was examined using thin layer chromatography (TLC). Iodine vapors were used to detect colorless compounds in the chromatograms, whereas colored spots were detected visually. Fractions with comparable Rf values were combined, and at 450 °C, they were evaporated to dryness under low pressure. Using the test pathogenic organisms, antimicrobial researches were conducted on all the pooled fractions.

2.4. Characterization with thin layer chromatography (TLC)

Following the collection of the extract, thin layer chromatography was used to ascertain its level of purity. The specimen was applied onto the plate coated with silica using capillary tubes, left to dry, and then the plates were subjected to development using the solvent mobile phase for duration of 30 minutes, applying a 1:1 ratio of ethanol to chloroform. With UV transilluminator, the obtained plates were inspected, and the retention factor was calculated using the subsequent formula:

$$\text{RF value} = \text{D.T. solution} / \text{D.T. solvent} \quad (1)$$

D.T. = Distance travelled.

2.5. Functional group analysis by FTIR

The sample was analysed using an FTIR spectrophotometer (FTIR8200 Affinity-1, Shimadzu Kyoto, Japan) to obtain the FTIR spectra. The analysis was conducted at the temperature in the laboratory (approximately 30 °C) and covered the range of 400–4000 cm^{-1} [8].

2.6. GC–MS analysis

The solvent extract underwent analysis using GC–MS spectrometry using a column that had the properties (film thickness = 0.25 µm, inner diameter (ID) = 0.25 mm. Length = 30 m). The analysis was performed using a Quadrapole spectrometer (Omni Star® GSD350 O, ultra-high vacuum Prisma Pro, Germany (. In order to perform the analysis, a volume of 1µl of the sample was introduced into helium gas of high purity at rate of 1 ml/min and the split ratio 20:1. The reaction was conducted with an ionization energy of 70 eV. The temperature of the injector column was initially maintained at 25 °C for 3 minutes, then increased at 10 °C/min gradually until it reached 280 °C, where it was held for three minutes. Finally, the temperature was maintained at 300 °C for 10 minutes. The obtained compounds were identified by matching their spectral configuration with the public NIST library database.

2.7. Antioxidant activity

2.7.1. DPPH test

The antioxidant activity of the extract was assessed using the approach outlined by Altemimi et al. [9] and Abedelmaksoud et al. [10]. Following the preparation of a methanolic solution with a concentration of

1 mg/ml, the extract was further diluted to a concentration range of 10–500 µg/mL. 1 ml of each concentration of the extract was combined with 1 mL of methanol and 2 mg of DPPH. The extract and control samples, which included all reaction components except the extract, were kept in darkness at a temperature of 24 °C for 30 minutes. The samples were observed UV-visible spectrophotometer Unico UV-2000 (Dayton, NJ, USA) at a wavelength of 517 nm [10]. The subsequent equation was used to ascertain the level of antioxidant activity:

$$I\% = A_{blank} - A_{sample}/A_{blank} \times 100. \quad (2)$$

"A_{sample}" and "A_{blank}" denote the extract and the blank sample absorption, respectively. The IC_{50} value was used to compare the extract's antioxidant activity to vitamin C and tertiary-butylhydroquinone (TBHQ) [9]. The IC_{50} was calculated using the radical scavenging activity (RSA) curve slope equation. The sample concentration that inhibits 50% of free radicals is the IC_{50}.

2.7.2. ABTS test

The extract was tested for ABTS radical inhibition using the Labiad et al. method [11]. Vitamin C and TBHQ were the reference standards. A solution of free radicals (7 mM ABTS, 2.4 mM potassium persulphate) was maintained at 24 °C without light for 14 hours. After adding 200 µL (10–500 µg/mL) of extract samples, the free radical solution (2 mL) was thoroughly mixed. Samples were analysed at 734 nm (UV-visible spectrophotometer Unico UV-2000, Dayton, NJ, USA) for 30 minutes. The IC_{50} test assessed ABTS free radical scavenging [11].

2.7.3. FRAP test

The FRAP assay measured extract's antioxidant capacity. A solution was made by mixing the extract with 0.2 M phosphate buffer (2.5 ml; pH 6.6) and potassium ferricyanide (2.5 ml; 1% w/v). The sample then was incubated at 50 °C for 20 minutes. 2.5 ml of 10% w/v trichloroacetic acid solution was added and centrifuged at 1000×g for 10 minutes to stop the reaction. The sample's 700 nm absorbance (UV-visible spectrophotometer Unico UV-2000, Dayton, NJ, USA) was measured 30 minutes after combining of 2.5 ml of supernatant, 2.5 ml of deionised water, and 0.5 ml of 0.1% chloride. FRAP was calculated using mg AAE/g extract. Vitamin C and TBHQ were the positive controls [11].

2.8. Cytotoxicity studies

Bahuguna et al. [12] conducted an experiment using the MTT assay (A. Type and C. Collection 2011) to determine the antiproliferative effect of tetracosamethyl-cyclododecasiloxane extracts from leaves of *L. inermis* on MCF-7 cell lines, which are human breast cancer cells. The MCF-7 cells, obtained from NCCS in Pune, India, were grown at a temperature of 37 °C in DMEM medium. The medium was supplemented with 10% FBS and 1% antibiotics (100 U·mL-1 of penicillin and 100 µg·mL^{-1} of streptomycin). The cells were cultured in a humid environment with 5% CO_2. The Trypsin-EDTA solution was used to isolate the individual cells, and a quantity of 5,000 cells per well was used for subculturing. Once the cell culture reached 50% confluence, it was treated with 20 µg of plant extract, which was diluted in 20 µL of DMSO. The cells were then incubated in a CO2 incubator at 37 °C for 24 hours. Subsequently, the medium was extracted. Finally, the samples were subjected to incubation for three hours with a concentration of 4 mg·mL-1. An ordinary microplate reader was employed to measure the absorbance at a wavelength of 540 nm (Reader, Plate Reader manufacturers & OEM manufacturers in India). Cytotoxicity was assessed by measuring cell viability as a percentage. The average value of three replicated experiments is shown to represent the findings. The following is the equation used to determine cell viability:

$$\text{Percent of cells viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100\%. \quad (3)$$

2.9. The minimum concentration of inhibitory or bactericidal activity

The minimum inhibitory concentration (MIC) of plant extract was determined through broth microdilution method technique following CLSI guidelines. The MHB was used to create a primary culture of pathogenic bacteria at 1.5 × 108 CFU/ml. The DMSO-containing extract was diluted to 1 mg/ml. Extract was poured into 96-well plates with capacity of 125 µl per each well. Into each well, bacteria were added. The plate was placed into a 37 °C incubator for 24 hours. To assess bacterial multiplication, 25 µl of triphenyltetrazolium chloride (5 mg/mL) was added to each well, that resulted in a dark red colour. At the lowest extractable concentration (MIC), no colour change was detected. Each well (100 µl) with no colour change was cultured on MHA at 37 °C for 24 hours. The minimum bactericidal concentration (MBC) was the dilution that fully stopped growth [13].

2.10. Statistical analysis

SPSS was used to perform a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) multiple comparison test to determine significant differences at $P \leq 0.05$.

3. Results and discussion

3.1. Initial phytochemical analysis

Lawsonia inermis leaf (Figure1) extracts prepared using petroleum ether, chloroform, ethyl acetate, and ethanol showed the presence of phytochemicals such as alkaloids, flavonoids, glycosides, saponins, steroils, tannins, proteins. According to the initial phytochemical analysis, Table 1 shows composition of the Lawsonia extract by GC–MS. The GC–MS analysis of *Lawsonia inermis* leaf extract identified a diverse range of bioactive compounds, primarily consisting of hydrocarbons, siloxanes, alcohols, and phenolic derivatives. Among the detected compounds, high molecular weight siloxanes, such as cyclo-decasiloxane eicosamethyl (740% area) and octasiloxane hexadecamethyl (578% area), were predominant, suggesting their significant contribution to the extract's chemical profile. Other notable siloxane derivatives, including cyclohexasiloxane dodecamethyl (444% area) and cyclononasiloxane octadecamethyl, also appeared in considerable proportions. In addition, hydrocarbons like eicosane (282.54% area), nonadecanol-1 (284.52% area), and 1-dodecanol (186.33% area) were present, potentially providing an effect on the extract's hydrophobic properties and biological activities. Phenolic compounds, such as phenol 2,4-bis(1,1-dimethyl ethyl), known for their antioxidant potential, were also detected.

Figure 1. ***Lawsonia inermis* leaves sample**
Рисунок 1. **Образец листьев растения *Lawsonia inermis***

Table 1. **Composition of the *Lawsonia inermis* leaves extract by GC–MS**
Таблица 1. **Состав экстракта листьев *Lawsonia inermis* согласно данным ГХ–МС**

Compound name	Formula	% area	Retention time
9-eicoscene	$C_{20}H_{40}$	1.01	26.694
Nonadecene	$C_{19}H_{38}$	0.68	28.88
Cyclohexasiloxanedodecamethyl	$C_{12}H_{36}O_6Si_6$	1.37	9.46
Cyclodecasiloxane, eicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	2.01	11.3
Hexacosene	$C_{26}H_{54}$	0.11	19.08
9-eicosene	$C_{20}H_{40}$	1.01	25.50
Dodecane	$C_{12}H_6$	170.34	0.339
Eicosane	$C_{20}H_{42}$	282.54	0.250
1-dodecanol	$C_{20}H_{42}O$	186.33	0.676
Phenol,2,4-bis (1,1-dimethyl ethyl)	$C_{14}H_{22}O$	206.32	1.115
Nonadecanol-1	$C_{19}H_{40}O$	284.52	7.956
Hexadene	$C_{16}H_{34}$	226.44	0.806
Octodecanoic acid, 9,10-dichloro-methyl ester	$C_{19}H_{36}Cl_2O_2$	367.40	4.166
Cyclo-decasiloxaneeicosamethyl	$C_{20}H_{60}O_{10}Si_{10}$	740	1.65
Octasiloxane1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15 hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	1.90
Cyclohexasiloxanedodecamethyl	$C_{12}H_{36}O_6Si_6$	444	2.61
Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$		1.75

The presence of octadecanoic acid, 9,10-dichloro-methyl ester, suggests possible antimicrobial or cytotoxic activity. Overall, the chemical composition of *L. inermis* extract, as revealed by GC–MS, highlights its complex phytochemical nature, which may contribute to its antibacterial, antioxidant, and anticancer properties.

3.2. GC–MS analysis

Using TLC analysis, the extract was partially purified. The extract that had been partially purified, underwent GC–MS analysis, and the NIST library was referred to in order to define a chemical profile. Based on the spectral values of the mass spectrometry data shown in Figure 2, the molecular weight was also determined. Fatty acids, esters, alkenes, and acidic chemicals were detected in the fractions after the chloroform, methanol extract was subjected to GC–MS analysis. Active chemicals were present as it was indicated by 23 peaks. The compounds that provided the greatest antibacterial and anticancer effect were dodecene (1.6%), eicoscene (0.2%), tetradecene (0.9%), cyclodecasiloxaneeicosamethyl (1.37%), and eptasiloxasanehexadecamethyl (0.351%). Additionally, 23 active metabolic peaks were detected in the GC–MS data at different retention times. The results showed that tetracosamethyl-cyclododecasiloxane is one of the physiologically active compounds with hepatoprotective activity, while cyclohexasiloxane, or dodecamethyl, is widely used as a lubricant, de-foaming agent, conditioning agent, and personal care product. This was verified by Ghribi et al. [14]. Using (GC–MS) to analyse the crude extract of the reported species, numerous significant organic volatile components were found. Therefore, the current study aligns with the findings of Plaza and Haarich [15]. Effective anti-arthritic, anti-acne, and anti-androgenic properties are present in the fatty acid ester group. When compared to phytochemicals derived from plants, Maheswari and Saraswathy found that ethyl acetate extract of alkene compounds, derived from bacillus, featured pharmacological activity. Additionally, different compounds derived from actinomycete and streptomycete demonstrated biological activity [16].

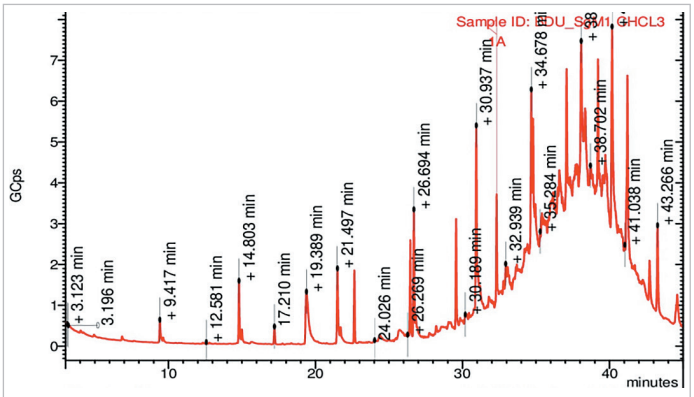

Figure 2. **Analysis of the fraction of the extract using GC–MS**
Рисунок 2. **Анализ фракций в экстракте по данным ГХ–МС**

3.3. FTIR

The wave range, that was recorded and shown in Figure 3, consisted of nine functional groups: 3361.00, 2928.84, 2161.79, 1637.12, 1557.12, 1405.07, 1271.01, 1069.07, 799.13, and 648.65 cm^{-1}. Furthermore, a functional group identified as fatty acids, alkenes, amino groups, volatile oils, hydrocarbons, and fatty acid esters was found within the unknown wave range of 2161.79. The active compound's functional groups of FTIR groups that include 648.85 and 799.13 fatty acids, respectively C-O stretch, ester, carboxylic acids, C-H-CH_2, at 1069.07C=N stretches of aliphatic amines, 1405.07 C=0 symmetric stretch of fatty acids and COO-amino acids, at 1557.12 aromatic C–C ring, at 1637.12 amide I of β-pleated sheet stretch, at 2161.79 there was unknown chemical, at 2928.84 there was C-H stretch of CH_2, at 3361.00 N-H, O-H vibration. The functional group was then established by FTIR analysis which was shown by active molecules like carboxyl, hydroxyl and amino acid, alkenes, hydrocarbons profile at 3361, 2928, 2161, 1637, 1557, 1405, 1271, 799, 648 (cm^{-1}) at various retention time the comparison. This was confirmed by several reports that shown the lipopeptide functional characterization [17].

3.3.1. Purification of crude extract by column chromatography

Using several solvent systems, we have shown for the first time that henna's colour is a combination of four components. Petroleum ether, ethanol, ethyl acetate, and chloroform were used as the solvents. After the fractions were collected and rinsed with color fractions, thin layer chromatography was used to further characterize them.

3.4. TLC analysis

The ethanol and chloroform (30:70) solvent solution was chosen based on the polarity of the active ingredient. The metabolites were observed on an analytical TLC plate, which was subjected to examination in an iodine and UV chamber. The findings revealed a single spot on the plate, with an Rf value of 0.96 and 0.68. The area displayed a blue hue when exposed to UV light and an iodine chamber. For additional examination, the active component was described.

3.5. Antioxidant potential

The ABST and DPPH radical scavenging activity and FRAP assay were used to assess the antioxidant capability of the *L. inermis* extract. The extract's antioxidant activity, natural antioxidant properties of the compounds such as vitamin C, and synthetic antioxidant properties TBHQ were all shown in the DPPH technique graph. Vitamin C, TBHQ, and extract samples had DPPH radical scavenging activity (% inhibition) values of 112.45, 75.12, and 254.32%, respectively. This suggests that antioxidant activity of the extract is greater than TBHQ and vitamin C, in that order. The extract's antioxidant activity dramatically increased ($p < 0.05$) as its concentration rose. The antioxidant activity of the control samples did not differ significantly ($p < 0.05$), although they did differ from the main sample. Table 2 displays the findings from the assessment of antioxidant activity using ABTS free radical scavenging effect. The sample's antioxidant activity grew significantly along with its concentration, suggesting a clear direct correlation between the extract's concentration and scavenging activity. Furthermore, compared to the plant extract, vitamin C and TBHQ had far less antioxidant activity. Vitamin C, extract, and TBHQ samples had values of 77.35, 54.21 and 121.25%, respectively. This suggests that the extract antioxidant activity is not greater than that of TBHQ and vitamin C, respectively. Additionally, the FRAP approach was applied, and Table 3 displays the results. The range of FRAP values for the extract, vitamin C, and TBHQ samples were 245.23, 65.23, and 454%, respectively. This antioxidant activity attributed to phenolic chemicals, which bind redoxactive metal ions and prevent hydroperoxides from becoming reactive oxyradicals, turning them to antioxidants. Interrupting

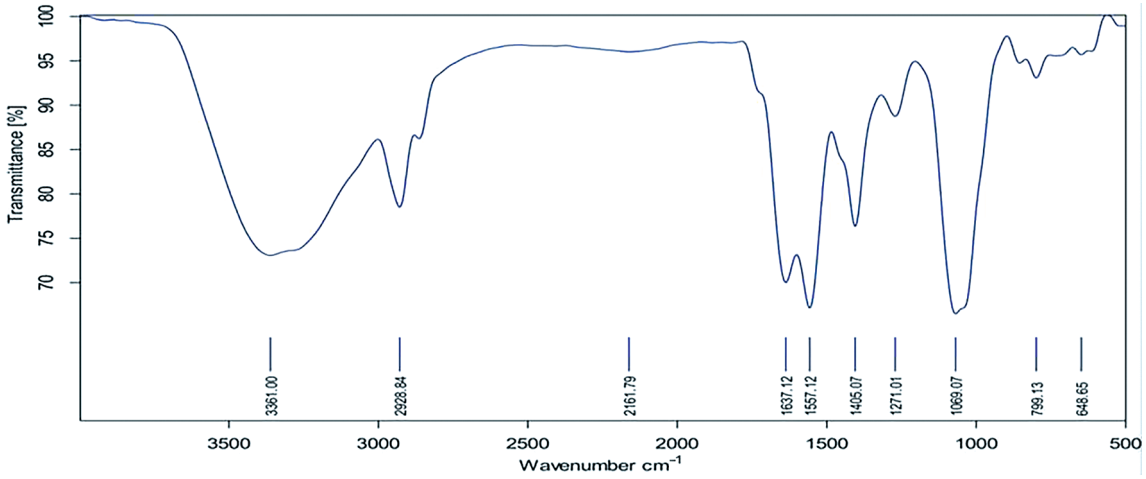

Figure 3. **The active molecules functional groups of isolated compounds**
Рисунок 3. **Функциональные группы активных молекул в выделенных соединениях**

chain reactions prevents free radical inactivation. Flavonoids form metal ion complexes to remove free radicals [18]. According to research, the leaf and skin extracts of *L. inermis,* prepared with solvent, had a total phenolic content (TPC) ranging from 20 to around 100 mg GAE/g of dry weight, and total flavonoid content (TFC) ranging from 18 to 92 mg of catechin equivalent/g of dry weight [19]. The total phenolic content (TPC) and total flavonoid content (TFC) of the *L. inermis* extract were determined to be moderately comparable to extracts from other plants, such as the *Ferulago angulata* used methanol extract method (TPC around 72 mg GAE/g, TFC around 30–35 mg QE/g) and *Tymus satureioides* used the hexane extract (TPC around 98 mg GAE/g, TFC around 92mg QE/g) [20]. In the other study by Gholamshahi amd Salehi Sardoei [21] documented varying total phenolic content (TPC) values for various components of the plant, with the leaf exhibiting the highest TPC value of around 9–10 mg GAE/g of dry weight. Several variables might influence the quantity of TPC and TFC (Total Flavonoid Content), such as the extraction technique, the kind of solvent used for extraction, meteorological conditions, the growing method, the type of plant, and the specific plant sections [22]. High concentrations of biological molecules, such as phenolic compounds and flavonoids, offer significant industrial and therapeutic advantages. They facilitate the mass production of these plants, thus enhancing the competitiveness of agricultural enterprises [23]. Al-Rowaily et al. conducted a study that revealed a significant concentration of carotenoids in the extract of *Lawsonia inermis* [24].

Table 2. **Free radical scavenging activity of vitamin C, TBHQ, and purified compound**

Табоица 2. **Активность захвата свободных радикалов витамином С, ТБГХ и очищенным соединением**

Free radical scavenging activity	Vitamin C	TBHQ	Purified compound
DPPH radical scavenging activity (% inhibition)	112.45 ± 2.23^{b}	75.12 ± 2.21^{a}	254.32 ± 1.31^{b}
ABTS radical scavenging activity (%)	77.35 ± 2.98^{c}	54.21 ± 4.23^{c}	121.25 ± 1.26^{c}
Ferric reducing antioxidant power (FRAP) assay	245.23 ± 3.21^{a}	65.23 ± 2.15^{b}	454 ± 3.56^{a}

Values followed by different superscripts between treatments are significantly different according to the ANOVA-Tukey's honestly significantly different (HSD) multiple comparison test (at $P \leqslant 0.05$).

3.6. Antibacterial activity

The antibacterial activity of purified substance of Tetracosamethyl-cyclododecasiloxane was assessed using the disc diffusion technique. The purified compound displayed the greatest antibacterial activity, as indicated in Table 3. The inhibition zones ranged from 25.06 ± 1.2, 26.03 ± 0.9, 21.03 ± 0.8, 20.05 ± 9.2, 25.09 ± 0.5, 28.12 ± 1.2, 30.12 ± 2.3, 25.04 ± 1.3, to 28.45 ± 25 mm, respectively. Gram-positive bacteria, specifically *Bacillus megaterium* MTCC441 and *Bacillus cereus* MTCC6840, exhibited greater general susceptibility. *Staphylococcus aureus* MTCC737, *Bacillus subtilis* MTCC441, *Bacillus aryabhattai* MCC14579, *Pseudomonas putida* MTCC1194, *Escherichia coli* MTCC1302, *Klebsiella pneumoniae* MTCC4727, and *Serratia marcescens* MTCC4822 all showed high susceptibility to gram-negative bacteria. Nevertheless, the refined chemical remained the most potent agents.

The present investigation provides evidence for the efficacy of purified compounds as powerful antimicrobials. The disc-diffusion approach revealed that the isolated component of *A. visnaga, L.* exhibits notable antibacterial action against both gram^{-} and gram^{+} bacteria, resulting in large zones of inhibition. The susceptibility of the examined microorganisms, including *Bacillus megaterium* MTCC441 and *Bacillus cereus* MTCC6840, was found to be higher with values ranging from 20.05 ± 9.2 to 30.12 ± 2.3 (21.03 ± 0.8, 20.05 ± 9.2, 25.09 ± 0.5, 28.12 ± 1.2, 30.12 ± 2.3, 25.04 ± 1.3, 28.45 ± 25) mm. Gram^{-} bacteria, including *Staphylococcus aureus* MTCC737, *B. subtilis* MTCC441, *B. aryabhattai* MTCC14579, *Pseudomonas putida* MTCC1194, and *Escherichia coli* MTCC1302, exhibited notable activity (Table 3). An inhibitory zone measuring 14 millimeters or greater, as determined by the disc diffusion test, is considered as exhibiting the significant antimicrobial activity [25]. The results of our study align with prior research that found that Tetracosamethyl-cyclododecasiloxane isolated from the fresh leaves of *Lawsonia inermis* had significant antibacterial effects against *Klebsiella pneumoniae, Escherichia coli and P. aeruginosa.*

The current study examined the antioxidant activity of a metabolite from a very effective strain, comparing it to ascorbic acid as a control sample (Table 2). The scavenging activity was observed to be greater in the extract compared to that of ascorbic acid. The findings indicated that

Table 3. **The diameters of inhibition zones treated with purified compound t Tetracosamethyl-cyclododecasiloxane**

Таблица 3. Диаметры участков ингибирования очищенным соединением тетракозаметил-циклододекасилоксана

Microorganism	Purified compound Tetracosamethyl-cyclododecasiloxane "Diameter of inhibition zone (mm)"
Staphylococcus aureus (MTCC737)	25.06 ± 1.2^{d}
Bacillus subtilis (MTCC441)	26.03 ± 0.9^{c}
Bacillus megaterium (MTCC441)	21.03 ± 0.8^{e}
Bacillus cereus (MTCC6840)	20.05 ± 9.2^{f}
Bacillus aryabhattai (MTCC14579)	25.09 ± 0.5^{d}
Pseudomonas putida (MTCC1194)	28.12 ± 1.2^{b}
Escherichia coli (MTCC1302)	30.12 ± 2.3^{a}
Klebsiella pneumoniae (MTCC4727)	25.04 ± 1.3^{d}
Serratia marcescens (MTCC4822)	28.45 ± 25^{b}

Values followed by different superscripts between treatments are significantly different according to the ANOVA-Tukey's honestly significantly different (HSD) multiple comparison test (at $P \leqslant 0.05$).

the metabolite exhibited strong antioxidant properties at a concentration of 2 µg/ml, which was lower than that of ascorbic acid. Moreover, the average range of DPPH radical scavenging activity of *L. inermis* was seen to be in close proximity to the control sample, thus suggesting a significant radical scavenging activity of the plant extract. The antioxidant assay (DPPH reduction assay) showed a gradual increase in the percentage of antioxidant activity, ranging from a minimum inhibition of 63.30% to a maximum of 89.95%. Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid, and tannic acid make up henna compounds. About 25% of market drugs are plant-based [26]. The chloroform and hexane extracts exhibited negligible antioxidant action, with an IC_{50} value greater than 100 mg/l. [27]. The alcoholic extracts derived from the leaves of *Lawsonia inermis* showed notable capability to remove DPPH free radicals. The petroleum ether fraction exhibited the highest scavenging rate of 79.16 ± 0.98%, which was similar to that of ascorbic acid around 78 ± 1.5%. It was followed by the ethyl acetate fraction with a scavenging rate of 73.77 ± 0.97% and the chloroform fraction with a scavenging rate of 72.61 ± 0.98%. The overall antioxidant capacity exhibited a positive correlation with the escalating concentration of the samples [28]. The antioxidant activity of the henna leaf extracts was assessed using the phosphor-molybdenum method, DPPH radical scavenging test, reducing power analysis, and lipid peroxidation inhibition assay. The ethanol extract exhibited superior antioxidant activity in all experiments compared to the petroleum ether, dichloromethane, and aqueous extracts. The efficacy of the aqueous extract was lower compared to the ethanol extract, but higher than that of the petroleum ether and dichloromethane extracts. The ethanol and aqueous extracts exhibited higher levels of phenolics (141.65 ± 0.29 and 51.46 ± 0.44 mg gallic acid equivalent/g extract, respectively) compared to the dichloromethane (4.60 ± 0.03 mg gallic acid equivalent/g extract) and petroleum ether extracts (3.72 ± 0.23 mg gallic acid equivalent/g extract) [29]. Predicting the mechanism that stands behind the increased anticancer activity of *L. inermis* is challenging due to its intricate pharmacological activities. Nevertheless, the inherent characteristics of *L. inermis* working together have a significant impact on improving the effectiveness of the desired biological outcome. Using the experimental results and existing literature, a hypothesis is formulated to explain the observed synergistic effect of *L. inermis*. Herbal medicines are widely regarded as being safe. Nevertheless, it is crucial to assess their biological safety prior to its using in order to prevent potentially lethal outcomes [30,31]. The pharmacological effects of *Lawsonia inermis* are unquestionable; nevertheless, it is also essential to conduct a toxicological assessment.

The present study aimed to analyze the properties of specific medicinal plants that have previously shown their antibacterial effects against drug-resistant gram^{+} and gram^{-} bacteria. The tests detailed in this report were specifically conducted to study the synthesis of *L. inermis* extract. It demonstrated high antibacterial efficacy against *E. coli, Staphylococcus* sp., *Pseudomona* ssp., *Bacillus* sp., it demonstrated the most prominent antibacterial efficacy, with a corresponding increase in the diameter of the inhibitory zone, as compared to the extracts derived from plants. The study measured the zone of inhibition (in centimeters) of *L. inermis* against *E. coli and Staphylococcus* sp. *E. Coli* exhibited the highest sensitivity to the ethanol extract at a concentration of 30 µl, followed by *Pseudomonas* sp. at a concentration of 90 µL, as observed in a 2 cm zone. However, none of the other solvents used for elution exhibited significant

antibacterial action, with the exception of ethanol. All extracts in the study showed antibacterial efficacy against all bacterial strains tested. Nevertheless, the bacterial strains exhibited varying levels of susceptibility to each extract. As per the research conducted by Gull et al. [32], The phytochemical elements demonstrate antibacterial activity specifically against gram-positive bacteria, however, they do not have any effect on gram-negative bacteria. Our analysis revealed that the substance had antibacterial action against both gram positive bacteria *(S. aureus* and *B. subtilis)* and gram negative bacteria *(E. coli* and *Pseudomonas aeruginosa)*. The research was conducted by Habbal and colleagues [33] in addition. The findings of Hussain et al. corroborate our own. The results of the *in vitro* antimicrobial assay indicated that the chloroform extract showed greater potential as an antibacterial agent for microorganisms mentioned below: *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, various species of Klebsiella,* and *Shigella*. On the other hand, the acetone extract demonstrated effectiveness against *E. coli and B. subtilis*. This study focuses on analyzing the antibacterial activities of extracts obtained from *L. inermis* leaves in order to inhibit the growth of specific gram-positive bacteria. The findings of this study demonstrate that varying doses of henna extract resulted in appearing of distinct inhibitory zones against the tested bacterium isolates [34]. On the other hand, Rathi et al. conducted a study in India where they found that the ethanolic extracts of henna had significant antibacterial activity against *Staphlococcus aureus* [35]. Moreover, in a similar study conducted by Gonelimali et al., it was reported that the highest level of inhibition was observed in ethanolic extracts against *Staphylococcus aureus* (26 mm) among the tested gram-positive bacteria. These findings demonstrate the vulnerability of this microorganism [36]. Furthermore, data from comparable studies have observed that *Staphylococcus aureus* exhibits greater vulnerability compared to other multi-drug-resistant bacteria when exposed to the applied plant extracts and antibiotics [37]. Ali and colleagues stated that. The rationale for this observation may be attributed to the diverse range of antibacterial chemicals that have been identified in the ethanolic extract [38]. The plant extract cleared a zone with diameter ranging from 18 mm to 30 mm, thus indicating its usability as an antibacterial agent against multidrug resistant organisms such as *Staphylococcus aureus*. These findings demonstrate the efficacy of henna extract against *Staphylococcus aureus* in a laboratory environment, and are consistent with the results obtained in previous investigations [39]. The antibacterial action of plants is primarily attributed to their secondary metabolites [40]. Phenolic chemicals and tannins are the main components of these secondary metabolites. Phytochemical investigations were conducted to identify the metabolites present in various extracts of *Lawsonia inermis*. Table 1 shows that methanol, acetone, and aqueous extracts contained cardioglycosides, terpenoids, carbohydrates, phenols, quinones and tannins. Likewise, according to Sharma et al. [41], the ethanolic extract of cinnamon did not show efficacy against gram-negative bacteria, however ginger shown the capability to inhibit the growth of these germs. Water soluble components found in most plant materials typically consist of starches, tannins, saponins, polypeptides, terpenoids, lectins, and other ions. On the other hand, the alcoholic extract of these materials contains flavonols, alkaloids, tannins, sterols, polyphenols, and so on [41].

3.7. Cytotoxic activity

The isolated compound Tetracosamethyl-cyclododecasiloxane obtained from *L. inermis,* was tested for its anticancer activity *in vitro* against the MCF-7 cell line using the MTT assay. The results indicated that at a concentration of 10µg/ml, it achieved 99.71% of cell death. The IC50 value, which represents the concentration required to inhibit 50% of cell growth, was found to be 1.682 µg/ml. Based on the MTT assay results, it can be inferred that the extent of cell death was directly correlated with the concentration (1.0, 5.0, and 10.0 µg/ml) of *L. inermis*. The MCF-7 breast cancer cell lines, which typically have a spherical shape, were treated with *L. inermis*. This therapy, known for its anticancer properties, resulted in the inhibition of cell development and finally led to cell death. The dead cells then aggregate together to form round structures. The phytochemical screening of *Abutilon indicum L.* revealed the existence of compounds that have proved to possess cytotoxic activity. In a previous study, Hussain et al. [42] It was also observed that the methanolic leaf extract and purified substance of *L. inermis* showed significant cytotoxic action at a concentration of 400 µg/ml.

Table 4. **The cytotoxic effect of the purified *S. ariculata* leaf component on cancer cell lines (MTT 72 h IC_{50}, µg/mL)**

Таблица 4. **Цитотоксическое воздействие очищенного вещества из листьев *S. ariculata* на линии раковых клеток (MTT 72 ч, $ИК_{50}$, мкг/мл)**

Samples	% of viability	IC_{50} µg/mL
Purified compound	99.71±0.19	< 20
Crude extract	54.64±0.14	20

4. Conclusion

The leaf extract of *L. inermis* contains bioactive chemicals that feature antioxidant, antibacterial, and anticancer properties. The extracts obtained from wild plants include a significant amount of essential fatty acids and numerous other bioactive chemicals, which have the potential for both industrial applications and biological effects. The most prevalent chemicals are phenol, tetracosamethyl-cyclododecasiloxane, and *L. inermis* leaf extract. These molecules can be used to create new pharmaceutical products for a range of non-infectious conditions, including cancer, hypertension, and diabetes. The identified chemicals should undergo additional investigations to determine their potential in the prevention and treatment of both infectious and non-infectious human disorders. In order to comprehend the mechanisms by which the active ingredients work, it is necessary to do thorough chemical analysis, as well as *in vivo* studies on the pharmacokinetics and pharmacodynamics of each component separately. Further research is required to elucidate the enduring consequences of combining pharmaceutical products with other medications.

REFERENCES

1. Gullo, V. P., Hughes, D. E. (2005). Exploiting new approaches for natural product drug discovery in the biotechnology industry. *Drug Discovery Today: Technologies*, 2(3), 281–286. https://doi.org/10.1016/j.ddtec.2005.08.002
2. Allaith, S. A., Abdel-aziz, M. E., Thabit, Z. A., Altemimi, A. B., Abd El-Ghany, K., Giuffrè, A. M. et al. (2022). Screening and molecular identification of lactic acid bacteria producing β-glucan in boza and cider. *Fermentation*, 8(8), Article 350. https://doi.org/10.3390/fermentation8080350
3. Thraeib, J. Z., Altemimi, A. B., Jabbar Abd Al-Manhel, A., Abedelmaksoud, T. G., El-Maksoud, A. A. A., Madankar, C. S. et al. (2022). Production and characterization of a bioemulsifier derived from microorganisms with potential application in the food industry. *Life*, 12(6), Article 924. https://doi.org/10.3390/life12060924
4. Al Bratty, M., Makeen, H. A., Alhazmi, H. A., Syame, S. M., Abdalla, A. N., Homeida, H. E. et al. (2020). Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of miswak plant, Salvadora persica L. *Journal of Chemistry*, 2020(1), Article 4521951. https://doi.org/10.1155/2020/4521951
5. Bennour, N., Mighri, H., Eljani, H., Zammouri, T., Akrout, A. (2020). Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of Moringa oleifera cultivated in Southern Tunisia. *South African Journal of Botany*, 129, 181–190. https://doi.org/10.1016/j.sajb.2019.05.005
6. Lawal, H. O., Etatuvie, S. O., Fawehinmi, A. B. (2012). Ethnomedicinal and pharmacological properties of Morinda lucida. *Journal of Natural Products*, 5, 93–99.
7. Survase, S. A., Annapure, U. S., Singhal, R. S. (2010). The effect of medium supplementation with second carbon source and amino acids for enhanced production of cyclosporin A. *Current Trends in Biotechnology and Pharmacy*, 4(3), 764–773.
8. Behbahani, B. A., Noshad, M., Falah, F. (2019). Study of chemical structure, antimicrobial, cytotoxic and mechanism of action of Syzygium aromaticum essential oil on foodborne pathogens. *Potravinarstvo Slovak Journal of Food Sciences,* 13(1), 875–883. https://doi.org/10.5219/1226
9. Altemimi, A.B., Al-Haliem, S.M., Alkanan, Z.T., Mohammed, M.J., Hesarinejad, M.A., Najm, M.A. et al. (2023). Exploring the phenolic profile, antibacterial, and antioxidant properties of walnut leaves (Juglans regia L.). *Food Science and Nutrition*, 11(11), 6845–6853. https://doi.org/10.1002/fsn3.3554
10. Abedelmaksoud, T. G., Hesarinejad, M. A., Shokrollahi Yancheshmeh, B. (2022). The effect of cold plasma on the enzymatic activity and quality characteristics of mango pulp. *Research and Innovation in Food Science and Technology*, 10(4), 341–350. https://doi.org/10.22101/JRIFST.2021.247462.1183
11. Labiad, M. H., Harhar, H., Ghanimi, A., Tabyaoui, M. (2017). Phytochemical screening and antioxidant activity of Moroccan Thymus satureioïdes extracts. *Journal of Materials and Environmental Sciences*, 8(6), 2132–2139.
12. Bahuguna, A., Khan, I., Bajpai, V. K., Kang, S. C. (2017). MTT assay to evaluate the cytotoxic potential of a drug. *Bangladesh Journal of Pharmacology*, 12(2), 115–118. https://doi.org/10.3329/bjp.v12i2.30892
13. Behbahani, B. A., Shahidi, F., Yazdi, F. T., Mortazavi, S. A., Mohebbi, M. (2017). Use of Plantago major seed mucilage as a novel edible coating incorporated with Anethum graveolens essential oil on shelf life extension of beef in refrigerated storage. *International Journal of Biological Macromolecules*, 94, 515–526. https://doi.org/10.1016/j.ijbiomac.2016.10.055
14. Ghribi, F., Bejaoui, S., Zupa, R., Trabelsi, W., Marengo, M., Chetoui, I. et al. (2023). New insight into the toxic effects of lithium in the ragworm Perinereis cultrifera as revealed by lipidomic biomarkers, redox status, and histopathological features. *Environmental Science and Pollution Research*, 30(26), 68821–68835. https://doi.org/10.1007/s11356-023-27223-7
15. Plaza, B., Haarich, S. N. (2015). The Guggenheim Museum Bilbao: Between regional embeddedness and global networking. *European Planning Studies*, 23(8), 1456–1475. https://doi.org/10.1080/09654313.2013.817543
16. Ibnouf, E. O., Aldawsari, M. F., Ali Waggiallah, H. (2022). Isolation and extrac-

tion of some compounds that act as antimicrobials from actinomycetes. *Saudi Journal of Biological Sciences*, 9(8), Article 103352. https://doi.org/10.1016/j.sjbs.2022.103352
17. Principe, P. P., Fisher, W. S. (2018). Spatial distribution of collections yielding marine natural products. *Journal of Natural Products*, 81(10), 2307–2320. https://doi.org/10.1021/acs.jnatprod.8b00288
18. Iqbal, K., Iqbal, J., Staerk, D., Kongstad, K. T. (2017). Characterization of antileishmanial compounds from Lawsonia inermis L. leaves using semi-high resolution antileishmanial profiling combined with HPLC-HRMS-SPE-NMR. *Frontiers in Pharmacology*, 8, Article 337. https://doi.org/10.3389/fphar.2017.00337
19. Mehmood, T., Arshad, H., Nawaz, S., Ullah, A., Hafeez, A., Anwar, F. et al. (2020). Pharmaceutical potential and phenolics profiling of leaves and bark of Calotropis procera in relation to extraction solvents. *Pharmaceutical Chemistry Journal*, 54(6), 631–641. https://doi.org/10.1007/s11094-020-02250-7
20. Ismaili, H., Milella, L., Fkih-Tetouani, S., Ilidrissi, A., Camporese, A., Sosa, S. et al. (2004). In vivo topical anti-inflammatory and in vitro antioxidant activities of two extracts of Thymus satureioides leaves. *Journal of Ethnopharmacology*, 91(1), 31–36. https://doi.org/10.1016/j.jep.2003.11.013
21. Gholamshahi, S., Salehi Sardoei, A. (2019). Phenolic compounds and antioxidant activity of plant milkweed (Calotropis Procera). *Eco-phytochemical Journal of Medicinal Plants*, 7(1), 77–86.
22. Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D. N., Karbancioglu-Guler, F., Capanoglu, E. (2020). Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules*, 25(20), Article 4711. https://doi.org/10.3390/molecules25204711
23. Ibrahim, N. I., Tadj, N. B.M.I., Sarker, Md. M.R., Mohamed, I.N. (2020). The potential mechanisms of the neuroprotective actions of oil palm phenolics: Implications for neurodegenerative diseases. *Molecules*, 25(21), Article 5159. https://doi.org/10.3390/molecules25215159
24. Al-Rowaily, S. L., Abd-ElGawad, A. M., Assaeed, A. M., Elgamal, A. M., Gendy, A. E.-N. G. E., Mohamed, T. A. et al. (2020). Essential oil of Calotropis procera: Comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. *Molecules*, 25(21), Article 5203. https://doi.org/10.3390/molecules25215203
25. Bouyahya, A., Et-Touys, A., Abrini, J., Talbaoui, A., Fellah, H., Bakri, Y. et al. (2017). Lavandula stoechas essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. *Biocatalysis and Agricultural Biotechnology*, 12, 179–184. https://doi.org/10.1016/j.bcab.2017.10.003
26. Dhouafli, Z., Ben Jannet, H., Mahjoub, B., Leri, M., Guillard, J., Saidani Tounsi, M. et al. (2019). 1, 2, 4-trihydroxynaphthalene-2-O-β-D-glucopyranoside: A new powerful antioxidant and inhibitor of Aβ42 aggregation isolated from the leaves of Lawsonia inermis. *Natural Product Research*, 33(10), 1406–1414. https://doi.org/10.1080/14786419.2017.1419229
27. Chaibi, R., Drine, S. Ferchichi, A. (2017). Chemical study and biological activities of various extracts from Lawsonia inermis (Henna) seeds. *Acta Medica Mediterranea*, 33, 981–986.
28. Hasan, K. M., Yesmin, S., Akhter, S. F., Paul, S., Sarker, S., Islam, A. et al. (2016). Hepatoprotective potentiality of various fractions of ethanolic extracts of Lawsonia inermis (henna) leaves against chemical-induced hepatitis in rats. *Biochemistry and Molecular Biology*, 1(2), 17–22. https://doi.org/10.11648/j.bmb.20160102.12
29. Philip, J. P., Madhumitha, G., Mary, S. A. (2011). Free radical scavenging and reducing power of Lawsonia inermis L. seeds. *Asian Pacific Journal of Tropical Medicine*, 4(6), 457–461. https://doi.org/10.1016/s1995-7645(11)60125-9
30. Yang, C.-S., Chen, J.-J., Huang, H.-C., Huang, G.-J., Wang, S.-Y., Chao, L.-K. et al. (2017). New flavone and eudesmane derivatives from Lawsonia inermis and their inhibitory activity against NO production. *Phytochemistry Letters*, 21, 123–127. https://doi.org/10.1016/j.phytol.2017.06.012
31. Altemimi, A. B., Alhelfi, N., Ali, A. A., Pasqualone, A., Fidan, H., Abedelmaksoud, T. G. et al. (2022). Evaluation of baseline cleanliness of food contact surfaces in Basrah Governorate restaurants using ATP-bioluminescence to assess the effectiveness of HACCP application in Iraq. *Italian Journal of Food Science*, 34(3), 66–90. https://doi.org/10.15586/ijfs.v34i3.2237
32. Gull, I., Sohail, M., Aslam, M., Athar, M. (2013). Phytochemical, toxicological and antimicrobial evaluation of Lawsonia inermis extracts against clinical isolates of pathogenic bacteria. *Annals of Clinical Microbiology and Antimicrobials*, 12(1), Article 36. https://doi.org/10.1186/1476-0711-12-36
33. Habbal, O. A., Al-Jabri, A. A., El-Hag, A. H., Al-Mahrooqi, Z. H., Al-Hashmi, N. A. (2005). In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna. *Saudi Medical Journal*, 26(1), 69–72.
34. Tariq Hussain, T. Arshad, M. Khan, S. Sattar, H, Qureshi, M. S. (2011). In vitro screening of methanol plant extracts for their antibacterial activity. *Pakistan Journal of Botany*, 43(1), 531–538.
35. Rathi, P. V., Ambhore, D., Jamode, P., Katkar, P., Kamble, P. (2017). Antimicrobial activity of Henna leaves against Staphylococcus aureus and Escherichia coli. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6(10), 981–990.
36. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M. et al. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9, Article 1639. https://doi.org/10.3389/fmicb.2018.01639
37. Ali, K. S., Al-Hood, F. A., Obad, K., Alshakka, M. (2016). Phytochemical screening and antibacterial activity of Yemeni Henna (Lawsonia inermis) against some bacterial pathogens. *Journal of Pharmaceutical and Biological Sciences*, 11(2), 24–27.
38. Boubaya, A., Marzougui, N., Yahia, L. B., Ferchichi, A. (2011). Chemical diversity analysis of Tunisian Lawsonia inermis L. populations. *African Journal of Biotechnology*, 10(25), 4980–4987.
39. Jain, V.C., Shah, D.P., Sonani, N.G., Dhakara, S., Patel, N. M. (2010). Pharmacognostic and preliminary phytochemical investigation of L leaf. *Romanian Journal of Biology — Plant Biology,* 55(2), 127–133.
40. Shastry, S., Kiran, U. P., Aswathanarayana, B. J. (2012). Effect of acute and chronic administration of the aqueous extract of Lawsonia inermis leaves on haloperidol induced catalepsy in albino mice. *Research Journal of Pharmaceutical Biological and Chemical Sciences,* 3(3), 1107–1116.
41. Sharma, R. K., Goel, A., Bhatia, A. K. (2016). Antityphoid activity and phytochemical screening of different extracts of L. inermis plant leaves. *International Journal of Current Research*, 8(08), 37539–37542.
42. Hussain, T., Bajpai, S., Saeed, M., Moin, A., Alafnan, A., Khan, M. et al. (2018). Potentiating effect of ethnomedicinal plants against proliferation on different cancer cell lines. *Current Drug Metabolism*, 19(7), 584–595. https://doi.org/10.2174/1389200219666180305144841

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Seemaisamy Revathi, Researcher, Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN) Cibinong city, Bogor, 1691, Indonesia Tel.: +91–908–053–11–24 E-mail: reva001@brin.go.id ORCID: https://orcid.org/0000-0003-2082-9425	**Ревати Симейсами** — научный сотрудник, Исследовательский центр прикладной зоологии, Научно-исследовательская организация в области наук о жизни и окружающей среде, Национальное агентство исследований и инноваций (BRIN) Чибинонг сити, Богор, 1691, Индонезия Тел.: +91–908–053–11–24 E-mail: reva001@brin.go.id ORCID: https://orcid.org/0000-0003-2082-9425
Elham K. Nasser, Associate Professor, Department of Food Science, College of Agriculture, University of Basra Basrah, 61004, Iraq Tel.: +964–773–491–31–98 E-mail: elham.nasser@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-8750	**Нассер Эльхам К.** — адъюнкт-профессор, кафедра пищевых наук, сельскохозяйственный колледж, Университет Басры Басра, 61004, Ирак Тел.: +964–773–564–00–90 E-mail: elham.nasser@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-8750
H. Khassaf Wafaa, Researcher, Date Palm Research Centre, University of Basrah Basrah, 61004, Iraq Tel.: +964–773–564–00–90 E-mail: wafaa.khassaf@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-8329-8436	**Вафаа Х. Хассаф** — научный сотрудник, Исследовательский центр финиковой пальмы, Университет Басры Басра, 61004, Ирак Тел.: +964–773–564–00–90 E-mail: wafaa.khassaf@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-8329-8436
Ammar B. Altemimi, Associate Professor, Department of Food Science, College of Agriculture, University of Basra Basrah, 61004, Iraq College of Medicine, University of Warith Al-Anbiyaa, Karbala, Iraq Tel.: +964–773–564–00–90 E-mail: ammar.ramddan@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-7750-5988	**Ал-Темими Аммар Б.** — адъюнкт-профессор, кафедра пищевых наук, сельскохозяйственный колледж, Университет Басры Басра, 61004, Ирак Медицинский колледж, Университет Варит Аль-Анбия, Кербела, Ирак Тел.: +964–770–567–37–84 E-mail: anfal.abdul_nabi@uobasrah.edu.iq ORCID: https://orcid.org/0009-0006-2658-2194

AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Sutikno Sutikno, Researcher, Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN) Cibinong city, Bogor, 1691, Indonesia Tel.: +62–898–657–63–00 E-mail: suti009@brin.go.id ORCID: https://orcid.org/0000-0002-2407-4747	**Сутикно Сутикно** — научный сотрудник, Исследовательский центр прикладной зоологии, Научно-исследовательская организация в области наук о жизни и окружающей среде, Национальное агентство исследований и инноваций (BRIN) Чибинонг сити, Богор, 1691, Индонезия Тел.: +62–898–657–63–00 E-mail: suti009@brin.go.id ORCID: https://orcid.org/0000-0002-2407-4747
Titik Kartika, Researcher, Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN) Cibinong city, Bogor, 1691, Indonesia Tel.: +62–812–7072–19–27 E-mail: titik.kartika@brin.go.id ORCID: https://orcid.org/0000-0002-9185-0400	**Картика Титик** — научный сотрудник, Исследовательский центр прикладной зоологии, Научно-исследовательская организация в области наук о жизни и окружающей среде, Национальное агентство исследований и инноваций (BRIN) Чибинонг сити, Богор, 1691, Индонезия Тел.: +62–812–7072–19–27 E-mail: titik.kartika@brin.go.id ORCID: https://orcid.org/0000-0002-9185-0400
Ikhsan Guswenrivo, Researcher, Research Center for Applied Zoology, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN) Cibinong city, Bogor, 1691, Indonesia Tel.: +62–813–1001–97–63 E-mail: ikhsan.guswenrivo@brin.go.id ORCID: https://orcid.org/0000-0002-3969-0067	**Гусвенриво Ихсан** — научный сотрудник, Исследовательский центр прикладной зоологии, Научно-исследовательская организация в области наук о жизни и окружающей среде, Национальное агентство исследований и инноваций (BRIN) Чибинонг сити, Богор, 1691, Индонезия Тел.: +62–813–1001–97–63 E-mail: ikhsan.guswenrivo@brin.go.id ORCID: https://orcid.org/0000-0002-3969-0067
Farhang H. Awlqadr, Researcher, Food Science and Quality Control, Halabja Technical College, Halabja Research Center, Sulaimani Polytechnic University P. O. Box: 70–236, Sulaymaniyah, Iraq Tel.: +96–477–014–209–62 E-mail: Farhang.hamid.a@spu.edu.iq ORCID: https://orcid.org/0000-0002-1737-5920	**Аулькадр Фарханг Х.** — научный сотрудник, Технический колледж прикладных наук Халабджи, Исследовательский центр Халабджи, Сулеймманский политехнический университет П/я 70–236, Сулеймания, Ирак Тел.: +96–477–014–209–62 E-mail: Farhang.hamid.a@spu.edu.iq ORCID: https://orcid.org/0000-0002-1737-5920
Tarek G. Abedelmaksoud, Associate Professor, Department of Food Science, Faculty of Agriculture, Cairo University. 1, Gamaa Street, Giza, 12613, Egypt Tel.: +2–0110–144–12–80 E-mail: tareekgamal_88@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-7012-6667 * corresponding author	**Абедельмаксуд Т. Г.** — адъюнкт-профессор, Кафедра науки о питании, Сельскохозяйственный факультет, Каирский Университет 12613, Египет, Гиза, ул. Гамаа, 1 Тел.: +2–0110–144–12–80 E mail: tareekgamal_88@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-7012-6667 * автор для контактов
Contribution	**Критерии авторства**
Authors are equally relevant to the writing of the manuscript, and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.