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Assessment of Antibacterial Potentials of Lawsonia inermis L. (Henna) Leaf Aqueous Extracts

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Abstract

Antibiotic resistance has become a euphemism in the public health sector globally. The urge for alternative therapies led researchers to endorse the use of medicinal plants due to their cost-effectiveness and absence of side effects. This research was conducted to evaluate the antibacterial potential of henna leaf aqueous extract against clinical bacterial isolates. Different leaf aqueous concentrations of Henna (50, 25, 12.5, and 6.25 mg/ml) were tested against **Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,** ***and*** **Staphylococcus aureus.** ***The disc diffusion method was used to determine the antibacterial susceptibility of the isolates against the extracts. The phytochemical constituents of the extract were determined using standard procedures. The result revealed a significant difference (P≤0.05) in the effect of various concentrations of henna extracts on bacterial activities. The result showed that 50 mg/ml concentrations of henna leaf aqueous extract showed the highest activity on all the isolates. The activity of the 50 mg/ml extract is above 50% of that of the positive control. The MIC of the extract against the tested microorganisms ranged from 2.5-10.0 mg/ml, while the MBC obtained in the present study ranged between 20.0-40.0 mg/ml. The effect of the extract is concentration-dependent; it increases with an increase in concentration. The activity of the henna leaf aqueous extracts could be attributed to the presence of 7 active phytochemicals: Saponins, Steroids, Triterpenes, Flavonoids, Alkaloids, Glycosides, and Tannins. Thus 50mg/ml of henna aqueous extract is recommended against bacterial isolates.***

Keywords: Aqueous extract, Bacteria, Biosafety, Henna,

INTRODUCTION

Antibiotic resistance has become one of the major global problems in the public health sector, rapidly increasing in both developed and developing nations, leading to a high rate of morbidity and mortality (Ibrahim et al., 2021). Antimicrobial resistance was speculated to probably be the leading cause of death worldwide by 2050 unless preventive measures are taken (WHO, 2019; Youl et al., 2024). The increasing incidence of multiple resistances in human pathogenic bacteria in recent years could largely be attributed to the indiscriminate use of commercial antibiotics commonly employed in treating infectious diseases (Manso et al., 2021). The major problems of antibiotic resistance include treatment failures, increased healthcare costs, and prolonged hospital stays; the situation escalates with the emergence and re-emergence of new infections (Youl et al., 2023). It is, therefore, of utmost urgency, as Ibrahim et al. (2021) put it, to develop new strategies to control the further evolution of antibiotic-resistant bacterial species. Thus, scientists searched for new alternative antimicrobials that are safe to use, cost-effective, and with no side effects.

Medicinal plants were found to be the best options for the treatment of infectious diseases (Raja et al., 2013) due to their long history of usage by humans since the dawn of civilisation (Elaguel et al., 2019) and when compared to the numerous side effects of synthetic drugs in use, they have high safety margins and are cost-effective (Güler et al., 2023). Medicinal plants are very rich in phytochemicals, which can be structurally optimised and processed into new drugs. Nigeria is blessed with a diverse collection of medicinal plants, and previous research has

ascertained the efficacy of these plants (Ugboko et al., 2020). Various preparations of medicinal plants could play a significant role as an alternative strategy to fight pathogenic bacterial species (Youl et al., 2024). Nowadays, interest in plants and their secondary metabolites is attracting increasing attention (Álvarez-Martínez et al., 2020).

Lawsoniainermis (L.) commonly known as Henna or *'Lalle'* in Hausa, is a plant species belonging to the family Lythraceae (Bafghi *et al.*, 2022). The plant has been reported to contain a vast array of active constituents with both industrial and medicinal applications in its stem, bark, roots, leaves, flowers, and seeds (Batiha et al., 2024). It is used cosmetically in Hausa society by women for aesthetic purposes to dye palms, feet, and skin, and medicinally as an antioxidant and antimicrobial agent (El Massoudi *et al.*, 2023; Batiha *et al.*, 2024). It is mostly applied topically to affected areas to treat itching, eczema, scabies, wounds, and skin burns (Zaidi et al., 2023). It is also traditionally used to treat various ailments such as headaches, jaundice, amoebiasis, and spleen enlargement (Al-Snafi, 2019). Hafiz et al. (2012) reported the potential of henna leaf extracts as an alternative counterstain in Gram staining reactions. The objective of this study, therefore, is to determine the antibacterial potential of henna leaf aqueous extract against some clinical bacterial isolates.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh leaves of the henna plant were collected from the Botanical Garden(Latitude 11° 11' N; Longitude 7° 38'E), Ahmadu Bello University, Zaria.

Preparation of Plant Materials and Extraction Procedure

The fresh leaves were spread on sterile tiles to dry at a room temperature of 27°C at the Microbiology Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria, for 7 days. Dried leaves were ground into powder using a sterile pestle and mortar. The extraction method followed the method described by AOAC (2019). Five hundred grams (500g) of the sample was weighed and transferred into a conical flask and labeled accordingly. About 1.5 L of distilled water was added. The flask was shaken thoroughly and covered with a foil sheet to avoid contamination. The sample was periodically shaken every 24 hours for 3 days. Afterwards, the macerated product was filtered using sterile cotton wool, and retort stands were set up with a sterile glass funnel mounted on each. The content was emptied through the cotton wool into a clean and sterile conical flask beneath the glass funnel. The filtrate was decanted into a sterile evaporating dish and labeled accordingly, and placed into the laboratory oven to evaporate the solvents at a temperature of 45 °C. The filtrate dried up after 48 hours, and a sterile blade was used to scrape off the dried product from the evaporating dish and stored in a labeled air-tight container for further use.

Phytochemical Screening of the Extracts

The standard procedure as outlined by Adegoke et al. (2010) was used to qualitatively determine the presence of bioactive constituents present in the aqueous extract of Henna: Wagner's test for detection of Alkaloids, Lead sub-acetate test for detection of Tannins, Keller-Killiani's test for detection of Cardiac Glycosides, Frothing/Foaming test for detection of Saponins, Libermann-Burchard's test for detection of Steroids and Triterpenoids, Alkaline test for the detection of Flavonoids and Borntrager's test for the detection of Anthraquinones.

Antimicrobial Studies

The sample of the test organisms was collected from wound infections of patients attending the outpatient Department, Ahmadu Bello University Teaching Hospital, Zaria and the samples were identified according to standard procedure as described by Ibrahim *et al.* (2021). Nutrient Agar was used in the identification and isolation of the bacterial isolates. The obtained wound samples were inoculated into the agar plates and incubated for 24 hrs at 37°C. The isolates were confirmed to be: *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, and Pseudomonas aeruginosa.*

The antibacterial activity of Henna aqueous extract against the test organisms was evaluated using the agar well diffusion method of susceptibility test (Rad *et al.*, 2013). Mueller-Hinton agar plates were prepared according to the manufacturer's instructions and sterilised by autoclaving at 121°C for 15 minutes, and inoculated with 0.2 mL of standardised inoculum of each bacterium (in triplicate) using a 0.2 mL pipette and spread uniformly with sterile swab sticks. Six wells of 6 mm size were made with a sterile cork borer (6 mm) into the inoculated agar plates.

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The wells were properly labeled according to the different concentrations of the extract prepared. Using a micropipette, 0.2 mL volume of the various concentrations of the Henna extract, Ciprofloxacin (positive control), 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, each of the aqueous extracts was dispensed into wells of inoculated plates. The prepared plates were then left at room temperature (27 ºC) for 10 minutes, allowing the diffusion of the extracts, and incubated at 37 ºC for 24 hours. At the end of the incubation period, the plates were observed for any evidence of inhibition, which would appear as a clear zone completely devoid of growth around the wells (Zone of inhibition). The diameter of each zone was measured using a transparent ruler calibrated in millimeters, and the result was recorded. Distilled water was used as a negative control. Minimum inhibition concentrations (MIC) were determined according to procedures described by Sharifi-Rad et al. (2016). Distilled water was used as a negative control.

The minimum inhibitory concentration of the extract was determined using the tube dilution method with the Mueller-Hinton broth used as diluent (Rad *et al.*, 2013). The lowest concentration of the extract showing inhibition for each organism when the extract was tested during the sensitivity test was serially diluted in the test tubes containing Mueller-Hinton broth. The standardised organisms were inoculated into each tube containing the broth and extract. The inoculated tubes were then incubated at 37˚C for 24 hours. At the end of the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion; the lowest concentration in the series without a viable sign of growth (turbidity) was considered the minimum inhibitory concentration (MIC). The result was also recorded. A sterilised wire loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test, and a loop was taken and streaked on sterile nutrient agar plates. The plates were incubated at 37˚C for 18-24 hours. At the end of the incubation period, the plates were examined for the presence or absence of growth. This is to determine whether the antimicrobial effect of the extract is bacteriostatic or bacteriocidal.

Data Analyses

The data obtained for antimicrobial activity were analysed using analysis of variance with Duncan's New Multiple Range Test (DNMRT) used to separate significant means at a 5% level.

RESULT

The result of the phytochemical screening of Henna leaf aqueous extract is presented in Table 1. The result showed the presence of seven active constituents in the extracts, which include: Saponins, Flavonoids, Alkaloids, Anthraquinones, Tannins, Steroids, and Cardiac glycosides.

Table 1: Qualitative phytochemical screening of aqueous leaf extract of Henna

S/N	Constituents	Inference
1	Saponins	+
2	Flavonoids	+
3	Alkaloids	+
4	Anthraquinones	+
5	Tannins	+
6	Steroids	+
7	Triterpenes	+
8	Cardiac Glycosides	+

Key: + = Present, - = Absent

The result for the antibacterial activity of the henna aqueous leaf extracts on some clinical bacterial isolates is presented in Table 2. The result indicated a significant difference (P≤0.05) in the zone of inhibition of the bacterial isolates against the various concentrations of the extract. The highest activity was recorded among 50 mg/ml concentrations in all the isolates. The gradual decrease in the MIC with a decrease in the concentration of henna leaf aqueous extract. The activities of the 50 mg/ml extracts are above 50% of that of the positive control, which shows that they are effective.

Table 2:Zone of inhibition (mm) by Henna aqueous extracts (mg/ml) on bacterial isolates

Species	50	25	12.5	6.25	Distil water	Ciprofloxacin
Escherichia coli	22^{b}±1.02	18^{c}±0.01	0^{d}±0.00	0^{d}±0.00	0^{d}±0.00	45^{a}±1.37
Staphylococcus aureus	26^{b}±1.21	19^{c}±0.01	4^{d}±0.00	0^{e}±0.00	0^{e}±0.00	36^{a}±1.21
Pseudomonas aeruginosa	30^{b}±1.01	22^{c}±0.34	10^{d}±0.00	2^{e}±0.00	0^{f}±0.00	37^{a}±1.02
Salmonella typhi	33^{b}±1.08	24^{c}±1.01	16^{d}±0.87	0^{e}±0.00	0^{e}±0.00	42^{a}±1.25

N.B: Means with the same superscripts across each row are NOT significantly different at P≤0.05

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The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts of Henna against bacteria are shown in Table 3. The MIC of the extract against tested microorganisms ranged from 2.5-10.0 mg/ml, Escherichia coli and Salmonella typhi recorded the highest value for MIC. The MBC obtained in the present study ranged between 20.0-40.0 mg/ml.

Table 3: Minimum inhibitory and bactericidal concentrations of henna extract against bacterial species

Bacterial species	MIC(mg/ml)	MBC(mg/ml)
Escherichia coli	10.0^{a}±1.00	20.0^{b}±0.01
S. aureus	4.5^{c}±0.67	40.0^{a}±0.02
P. aeruginosa	2.5^{d}±0.45	0.0^{c}±0.00
Salmonella typhi	10.0^{b}±1.00	20.0^{b}±0.01

N.B: Means with the same superscripts down a column are NOT significantly different at P≤0.05

DISCUSSION

Lawsoniainermis has been used in herbal medicine since antiquity, as stressed by Batiha et al. (2024). The medicinal properties of this plant depend on the presence of some active constituents present in it. The presence of seven active phytochemicals in the aqueous extracts of henna leaves presented by this study agrees with the finding of Batiha et al. (2024), who reported similar phytochemical compounds among the constituents of henna extracts. Previous findings by Kouadri (2018), Meutia et al. (2021), and Youl et al. (2024) reported similar phytochemicals (Saponins, Flavonoids, Alkaloids, Anthraquinones, Tannins, Steroids and Cardiac glycosides) in Henna leaves. Similarly, El Massoudi et al. (2023) reported the presence of high amounts of phenolic compounds, flavonoids, saponins, and tannin in Moroccan Henna leaf extracts.

The present study reported the ability of henna extract to show the highest zones of inhibition of the highest concentrations of henna leaf aqueous extract against the test bacterial isolates. This finding agrees with that of the previous findings of Jothiprakasamet al. (2013), Ibrahim et al. (2021) and Zaidi et al. (2023), who individually reported the inhibitory activity of henna extracts against both Gram-negative and Gram-positive bacterial species. Al-Snafi (2019) ascribed the antimicrobial activity of Henna to the presence of phytochemicals similar to those reported by the present study.

With the ever-increasing strains of microbes to the already synthesised and accessible antibiotics, the naturally available Henna might be a potential alternative (Said et al., 2024). The antibacterial activity of henna leaves may probably be attributed to the presence of a vast array of free hydroxyls that can combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites, rendering them inactive as reported by Guler *et al.* (2023). According to the dose responses, the zone of inhibition increased with an increase in the concentration of the investigated extracts. The high concentration (50 mg/ml) inhibited the microbes the most and recorded obvious inhibition activity against all tested bacteria isolates.

CONCLUSION

The present study concluded that seven active phytochemical compounds were found in the aqueous leaf extracts of Henna. The highest dose (50 mg/ml) of henna leaf aqueous extract showed the highest activity against bacterial isolates. The antibacterial efficacy of the extract is concentration-dependent, increasing with an increase in concentration.

REFERENCES

Adegoke, A. A., Iberi, P. A., Akinpelu, D. A., Aiyegoro, O. A., & Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products, 3*(3), 6–12.

Al-Snafi, A. E. (2019). A review on *Lawsonia inermis*: A potential medicinal plant. *International Journal of Current Pharmaceutical Research, 11*(5), 1–13. [Crossref]

Álvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V. (2020). Antimicrobial capacity of plant polyphenols against gram-positive bacteria: A comprehensive review. *Current Medicinal Chemistry, 27*, 2576–2606. [Crossref]

AOAC. (2019). *Official methods of analysis* (18th ed.). Association of Official Analytical Chemists.

Bafghi, M. F., Salary, S., Mirzaei, F., Mahmoodian, H., Meftahizade, H., & Zareshahi, R. (2022). Antibacterial and anti-trichomonas characteristics of local landraces of *Lawsonia inermis* L. *BMC*

Complementary Medicine and Therapies, 22, 203. [Crossref]

Batiha, G. E., Teibo, J. O., Shaheen, H. M., Babalola, B. A., Teibo, T. K. A., Al-Kuraishy, H. M., Al-Garbeeb, A. I., Alexiou, A., & Papadakis, M. (2024). Therapeutic potential of *Lawsonia inermis* Linn: A comprehensive overview. *Naunyn-Schmiedeberg's Archives of Pharmacology, 397*, 3525-3540. [Crossref]

El Massoudi, S., Zinedine, A., Rocha, J. M., Benidir, M., Najjari, I., El Ghadraoui, L., Benjelloun, M., & Errachidi, F. (2023). Phenolic composition and wound healing potential assessment of Moroccan henna (*Lawsonia inermis*) aqueous extracts. *Cosmetics, 10*, 92. [Crossref]

Elaguel, A., Kallel, I., Gargouri, B., Amor, I. B., Hadrich, B., Mesaaoud, E. B., et al. (2019). *Lawsonia inermis* essential oil: Extraction optimisation by RSM, antioxidant activity, lipid peroxidation, and antiproliferative effects. *Lipids in Health and Disease, 18*(1), 196. [Crossref]

Güler, Ş., Torul, D., Kurt-Bayrakdar, S., Tayyarcan, E. K., Çamsarı, Ç., & Boyacı, İ. H. (2023). Evaluation of antibacterial efficacy of *Lawsonia inermis* Linn (Henna) on periodontal pathogens using agar well diffusion and broth microdilution methods: An in-vitro study. *BioMedicine, 13*(3), Article 3. [Crossref]

Hafiz, H., Chukwu, O. O. C., & Nura, S. (2012). The potentials of henna (*Lawsonia inermis* L.) leaves extracts as counter stain in gram staining reaction. *Bayero Journal of Pure and Applied Sciences, 5*(2), 56–60. [Crossref]

Ibrahim, M. S. S., Rasool, C. S., & Al-Asady, A. A. (2021). Antimicrobial activity of crude henna extract against Gram-positive bacteria. *MicroMedicine, 9*(1), 18–26. [Crossref]

Jothiprakasam, V., Ramesh, S., & Rajasekharan, S. (2013). Preliminary phytochemical screening and antibacterial activity of *Lawsonia inermis* Linn (Henna) leaf extracts against reference bacterial strains and clinically important AMPC beta-lactamases producing *Proteus mirabilis*. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(1), 219–222.

Kouadri, F. (2018). In vitro antibacterial and antifungal activities of the Saudi *Lawsonia inermis* extracts against some nosocomial infection pathogens. *Journal of Pure and Applied Microbiology, 12*, 281–286. [Crossref]

Manso, T., Lores, M., & de Miguel, T. (2021). Antimicrobial activity of polyphenols and natural polyphenolic extracts on clinical isolates. *Antibiotics, 11*, Article 46. [Crossref]

Meutia, N., Putra, B., & Jusuf, N. K. (2021). Antifungal activity of henna leaf extract (*Lawsonia inermis* Linn) against inhibition of *Trichophyton rubrum* fungal growth cause *Tinea unguium*. *Jurnal Buletin Farmatera, 6*, 72–79.

Rad, J.S., Alfatemi, S.M.H., Miri, A. and Rad, M.S. (2013). A study of antibacterial potentiality of some plants extracts against multi-drug resistant human pathogens. Ann. Biol. Res., 4:35-41.

Raja, W., Ovais, M., & Dubey, A. (2013). Phytochemical screening and antibacterial activity of *Lawsonia inermis* leaf extract. *International Journal of Microbiology Research, 4*(1), 33–36.

Said, A., Abu Elghait, M., Atta, H.M. and Salem, S.S. (2024). Antibacterial activity of green synthesized silver nanoparticles using Lawsonia inermis against common pathogens from urinary tract infection. Applied Biochemistry and Biotechnology, 196:85–98. [Crossref]

Sharifi-Rad, J., Mnayer, D., Roointan, A., Shahri, F., Ayatollahi, S. A. M., Sharifi-Rad, M., et al. (2016). Antibacterial activities of essential oils from Iranian medicinal plants on extended-spectrum ß-lactamase-producing *Escherichia coli*. *Cellular and Molecular Biology, 62*(9), 75–82.

Srinivasan, L., Sasaki, Y., Calado, D. P., Zhang, B., Paik, J. H., DePinho, R. A., Kutok, J. L., Kearney, J. F., Otipoby, K. L., & Rajewsky, K. (2009). PI3 kinase signals BCR-dependent mature B cell survival. *Cell, 139*, 573–586. [Crossref]

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H., & Omonhinmin, C. A. (2020). Antimicrobial importance of medicinal plants in Nigeria. *The Scientific World Journal, 2020*, Article ID 7059323, 1–10. [Crossref]

WHO. (2019). *New report calls for urgent action to avert antimicrobial resistance crisis*. who.int

Youl, O., Konaté, S., Sombié, E. N., Boly, R., Kaboré, B., Koala, M., Zoungrana, A.,

Savadogo, S., Tahita, C. M., Valea, I., Tinto, H., Hilou, A., & Traoré-Coulibaly, M. (2024). Phytochemical analysis and antimicrobial activity of *Lawsonia inermis* leaf extracts from Burkina Faso. *American Journal of Plant Sciences, 15*, 552–576. [Crossref]

Youl, O., Moné-Bassavé, B. R. H., Yougbaré, S., Yaro, B., Traoré, T. K., Boly, R., et al. (2023). Phytochemical screening, polyphenol and flavonoid contents, and antioxidant and antimicrobial activities of *Opilia amentacea* Roxb. (Opiliaceae) extracts. *Applied Biosciences, 2*, 493–512. [Crossref]

Zaidi, S., Zaidi, A., Amir, S., Ali, R. M., & Syabani, A. A. (2023). Palm art to therapeutic properties: Henna—A potential medicinal plant. *International Journal of Technology and Education Research, 1*(3), 1–8.