

Phytochemical analysis and Antibacterial Activities of Henna Leaf (*Lawsonia inermis*) against *Salmonella typhi* and *Escherichia coli*

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Abstract

The species Henna (*Lawsonia inermis*) L. is native to the African continent, being found mainly in the savannas of Sub-Saharan Africa at low altitudes, with 4 to 10 months of drought per year. All parts of the plant have a long history of ethnomedicinal use worldwide. This study was aimed at assessing the phytochemical and antibacterial activity of Henna (*Lawsonia inermis*) aqueous and ethanolic leaves extract on *Salmonella typhi* and *Escherichia coli*. The results of the phytochemical screening of Henna (*Lawsonia inermis*) leaves aqueous and ethanolic extracts shows the presence of flavonoids and saponins and the absence of alkaloids, tannins, and terpenoids. Ethanolic extracts of Henna (*Lawsonia inermis*) leaves were significantly more effective than aqueous extract against *Escherichia coli* with zone diameter of inhibition ranging from 16.0 ± 1.5 to 23.0 ± 0.7 and 11.0 ± 1.1 to 20.0 ± 0.2 respectively. Similarly, Ethanolic extracts of Henna (*Lawsonia inermis*) leaves were significantly more effective than aqueous extract against *Salmonella typhi* with zone diameter of inhibition ranging from 17.5 ± 1.0 to 22.0 ± 1.0 and 16.5 ± 1.0 to 20.0 ± 1.0 respectively. Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had a MIC of 250 mg/ml against *Escherichia coli* and MIC of 250 mg/ml and 125 mg/ml respectively against *Salmonella typhi*. Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had MBC of 500 mg/ml and 250 mg/ml respectively against *Escherichia coli* and MBC of 250 mg/ml against *Salmonella typhi*. This study affirms that aqueous and ethanolic leaf extracts Henna (*Lawsonia inermis*) has significant antibacterial activity on the test organisms, hence the therapeutic potential of the plant in the treatment and management of infectious and non-infectious diseases.

INTRODUCTION

The importance of herbs in the management of human ailments cannot be over-emphasized. According to Agatemor (2019), it is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Boham *et al.* (2022) defined medicinal plants as plants in which one or more of the organs contain substances that can be used for therapeutic purposes or which possess precursors for the manufacture of drugs and are useful for disease therapy. Medicinal plants since time immemorial have been used in virtually all cultures as a source of medicine.

In general, plants are a significant source of conventional medications, and most plants' medicinal properties have been related to the existence of plant secondary metabolites (Sofowora, 2018).

Over 5000 plants are known to be used for medicinal purposes in Africa but only a few have been described or studied. Natural product from plants is another potent source for the discovery of excellent biological activities (Janine de Aquino *et al.*, 2015). Almost 50 % of current pharmaceuticals are derived from the plant kingdom. Plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids, and flavonoids, which have been demonstrated to have in vitro antimicrobial properties (Habbal *et al.*, 2021).

Henna is a tropical and subtropical shrub, that grows naturally in North Africa, the Middle East, and the Indian subcontinent. Powder made from dry Henna leaves has been used for various medicinal and cosmetic purposes for millennia. Historically, Henna when applied in the form of paste onto the skin or hair can give a red coloration that may last for as long as twelve weeks (Wasim Raja *et al.*, 2013).

Besides its cosmetic applications, henna was also historically used in Persian, Arab, Turkish, and Jewish medicine to treat headaches, skin, and dental diseases, as well as animal bites (Wasim Raja *et al.*, 2013). Modern pharmacological research on henna leaves and their constituents has confirmed its anti-inflammatory, antipyretic, and analgesic effects, and provided evidence for its anti-carcinogenic potential. Henna leaves are used in the form of decoction or ointment in the treatment of burns, skin inflammations, wounds, and ulcers. The leaves also possess antifungal and antibacterial activities (Sakarkar *et al.*, 2017).

The antibacterial potential of Henna leaf has been attributed to its rich phytochemical composition, which includes flavonoids, tannins, and phenolic compounds. Several studies have shown that Henna extracts, when applied in adequate concentrations, can inhibit the growth of these bacterial strains, making it a promising natural alternative for treating bacterial infections (Habbal *et al.*, 2021; Raja *et al.*, 2013; Wasim *et al.*, 2013).

The mechanism of action of Henna leaf extract against *S. Typhi* and *E. coli* involves the disruption of the bacterial cell wall and membrane integrity. Flavonoids, one of the primary constituents of Henna, are known to bind with bacterial proteins, leading to the loss of cell membrane function and ultimately causing bacterial cell death. Moreover, tannins present in the Henna leaf have been reported to precipitate proteins and interfere with the cell wall synthesis of bacteria, thereby preventing their replication and spread.

MATERIALS AND METHODS

Study Area

The phytochemical analysis and antibacterial activity of Henna leaves (*Lawsonia inermis*) against *Salmonella typhi* and *Escherichia coli* was carried out at the Microbiology Laboratory, University of Abuja, Gwagwalada FCT-Abuja, Nigeria. Gwagwalada is one of the six Area Councils of the Federal Capital Territory of Nigeria, together with Abaji, Kuje, Bwari, Abuja Municipal Area Council (AMAC), and Kwali; the FCT also includes the City of Abuja. Gwagwalada is also the name of the main town in the Local Government Area, which has an area of 1,043 km² and a population of 157,770 at the 2006 census with an annual growth rate of 6.46% (Loop, 2021). The University of Abuja is located within Gwagwalada Area Council.

Materials

The materials to be used for the collection of samples include; sterile hand gloves, sterile sample bottles, and sterile spatula. Equipment to be used in the laboratory includes; the autoclave, incubator, refrigerator, weighing balance, and microscope. Other materials include; sterile Petri dishes, pipettes, inoculating wire loops, bunsen burner, sterile hand gloves, aluminium foil, spatula, cotton wool, and syringes. Glass wares to be used include; conical flask, measuring cylinder, glass spreader, pipette, beakers, test tubes, microscopic glass slides, and covers slips.

Preparation and Sterilization of Media

The media to be used include Nutrient agar, Mueller Hinton agar, Salmonella shigella agar, and, Eosin Methylene Blue Agar. The glass wares used were sterilized at 160 °C for 2 hrs in a hot air oven. All the media was prepared according to the manufacturers' specifications.

Collection and Identifications of Bacterial Isolates

Samples of clinical isolates of *Salmonella typhi* and *Escherichia coli* were collected from the University of Abuja Teaching Hospital Gwagwalada and brought to the Microbiology laboratory, University of Abuja FCT-Abuja for further identification. The isolates were characterized and identified based on their cultural, morphological, and biochemical characteristics.

Collection and Processing of Plant Materials (*Lawsonia inermis*)

Fresh Henna (*Lawsonia inermis*) leaves were collected from Giri town in Gwagwalada area council- Abuja, which was identified by the Botany Laboratory of the University of Abuja.

The Henna leaves obtained were cleaned with water and then air-dried indoors to a constant dry weight. The Henna leaves were pulverized into powder using a clean and disinfected laboratory blender and stored until further required.

Preparation of Henna (*Lawsonia inermis*) Leaves Extracts

One hundred grams (100 gm) of Henna (*Lawsonia inermis*) leaf powder was weighed into two 1000 ml Erlenmeyer flasks and 500 ml each of ethanol and distilled water (aqueous solvent) was added into each container and the contents allowed to stand for three days in a rotary shaker at regular interval under room temperature. After 72 hours, the content of each flask was filtered using Whatman no.1 filter paper and then the filtrate was concentrated using a water bath at 60°C. The concentrated extract was stored at 4°C in the refrigerator for further use (Obioma *et al.*, 2017; Kasolo *et al.*, 2020).

Qualitative Analysis of Phytochemicals

The Henna leaf extract was screened for the presence of the various plant metabolites using the method outlined by Trease and Evans, (2005) and Sofowora, (1993). Phytochemical screening was carried out to determine the presence of secondary metabolites or chemical constituents such as glycosides, saponins, tannins, alkaloids, anthraquinones, amino acids, and flavonoids.

Test for Alkaloids

About 1g of each of the extracts was separately boiled with water and 10 ml of Hydrochloric acid was added to each of the test tubes in a water bath and filtered. About 2ml of Mayer's reagents will then be added and observed for coloured precipitates or turbidity. Trease and Evans, (2005) and Sofowora, (1993).

Test for saponins

About 0.2g of each of the extracts was shaken with 5 ml of distilled water in each of the test tubes and then heated to a boil. Frothing (appearance of creamy-like bubbles) shows the presence of saponins. Trease and Evans, (2005) and Sofowora, (1993).

Test for tannins

About 1 g of each of the extracts was separately poured into a test tube and boiled with 20 ml of distilled water for five minutes in a water bath and then filtered while hot. 1ml of cool filtrate was distilled to 5ml of water and a drop (2-3) of 10% ferric chloride was added and observed for any colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins. Trease and Evans, (2005) and Sofowora, (1993).

Test for Flavonoids

About 1 g of each of the extracts was separately boiled with 10 ml of distilled water for 5 minutes and filtered while hot. A few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour shows the presence of flavonoids. Trease and Evans, (2005) and Sofowora, (1993).

Test for Terpenoids

1 ml of the extracts was dissolved in ethanol. To it, 1 ml of acetic anhydride was added followed by the addition of conc. H_2SO_4 . A change in colour from pink to violet will indicate the presence of terpenoids. Trease and Evans, (2005) and Sofowora, (1993).

Determination of Antimicrobial Activity

The antibacterial activity of both ethanolic and aqueous extracts of henna leaves was evaluated by the agar well diffusion method (Hudziki, 2019). The bacteria (*Salmonella typhi* and *Escherichia coli*) culture was adjusted to 0.5 Mc. Farland standard and poured onto freshly prepared Mueller Hinton agar plates. A sterile cork borer was used to make a well (6 mm in diameter) on the MHA plates. Aliquots of 100 μ L (i.e 0.1ml) of the extracts dilutions, reconstituted in distilled water at concentrations of 62.50, 125, 250 and 500 mg/ml, were applied in each of the wells in the culture plates previously seeded with the test organisms (Hudziki, 2019). The cultures were incubated at 37⁰C for 24 hours.

Similarly, a well was made in each of the culture plates and filled with 20 µl of 10 mg/ml of Chloramphenicol as control. Antimicrobial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). All the experiments were carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined against the test organism (*Salmonella typhi* and *Escherichia coli*) in test tubes. To 0.5 ml of varying concentrations of the extracts (62.50, 125, 250, and 500 mg/ml) in test tubes, Nutrient broth (2ml) was added and then a loopful of the test organism, previously diluted to 0.5 Mc Farland turbidity standard introduced. The procedure was repeated on the test organisms using the standard antibiotic (Chloramphenicol) (Kasolo *et al.*, 2020).

A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were incubated at 37⁰C for 24 h, after which the tubes were examined for microbial growth by observing for turbidity (Cheesebrough, 2016). All the experiments were carried out in triplicates.

Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any bacterial growth in the MIC and inoculated on sterile Nutrient agar by streaking. All the plates were incubated at 37 ⁰C for 24h. After the incubation period, the lowest concentration at which no visible growth occurs on the plate was noted as the Minimum Bactericidal Concentration (MBC) (Cheesebrough, 2016; Hudziki, 2019). All the experiments were carried out in triplicates.

RESULTS

Table 1: Phytochemical Content of Henna (*Lawsonia inermis*) leaves

Key: (-) absence, (+) present

Table 2: Morphological characteristics of the test organism

Table 3: Biochemical characteristics of the test organism

Key: GR = Gram Reaction, IN = Indole Test, CU = Citrate Utilization, CA = Catalase Test, MR = Methyl Red Test.

Table 4: Zone Diameter of Inhibition (ZDI) in millimeter of Aqueous and Ethanolic Henna (*Lawsonia inermis*) Leaves Extracts against *Escherichia coli* and *Salmonella typhi*

Each value represents mean ± standard deviation of duplicate values.

Table 5: Minimum Inhibitory Concentration of Aqueous and Ethanolic Extracts of Henna (*Lawsonia inermis*) against *Escherichia coli* and *Salmonella typhi*

Key: + =Present, - =Absent

Table 6: Minimum Bactericidal Concentration of Aqueous and Ethanolic Extracts of Henna (*Lawsonia inermis*) leaves against *Escherichia coli* and *Salmonella typhi*

Key: + =Present, - =Absent

DISCUSSION

The species Henna (*Lawsonia inermis*) L. is native to the African continent, being found mainly in the savannas of Sub-Saharan Africa at low altitudes, with 4 to 10 months of drought per year. All parts of the plant have a long history of ethnomedicinal use worldwide. Several phytochemical compounds have been identified in distinct parts of Henna (*Lawsonia inermis*) L. with demonstrated therapeutic effects in the management of different diseases. This plant exerts hypoglycemic, hypolipidemic, antimicrobial, analgesic, and antipyretic activities. This study was aimed at assessing the phytochemical and antibacterial activity of Henna (*Lawsonia inermis*) aqueous and ethanolic leaves extract on *Salmonella typhi* and *Escherichia coli*. The results of the phytochemical screening of Henna (*Lawsonia inermis*) leaves aqueous and ethanolic extracts shows the presence of flavonoids and saponins and the absence of alkaloids, tannins, and terpenoids. This finding is in consonance with Semwal *et al.* (2014) and Ali *et al.* (2017) but contrasts with Wagini *et al.* (2014) who reported the presence of alkaloids, tannins, and terpenoids. This could be due to the difference in extraction solvents.

Ethanolic extracts of Henna (*Lawsonia inermis*) leaves were significantly more effective than aqueous extract against *Escherichia coli* with zone diameter of inhibition ranging from 16.0±1.5 to 23.0±0.7 and 11.0±1.1 to 20.0±0.2 respectively. Similarly, Ethanolic extracts of Henna (*Lawsonia inermis*) leaves were significantly more effective than aqueous extract against *Salmonella typhi* with zone diameter of inhibition ranging from 17.5±1.0 to 22.0±1.0 and 16.5±1.0 to 20.0±1.0 respectively. This agrees with Alsamahi *et al.* (2023) and Nargish and Mannan (2024) who reported significant antibacterial activity of Henna (*Lawsonia inermis*) leaf and stem bark extracts against *Escherichia coli* and other pathogenic bacteria species.

Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had a MIC of 250 mg/ml against *Escherichia coli* and MIC of 250 mg/ml and 125 mg/ml respectively against *Salmonella typhi*. Ethanolic extract of Henna (*Lawsonia inermis*) leaves met the standard of the antibiotic used as positive control (Chloramphenicol) with MIC at 125 mg/ml. This report conforms with Alsamahi *et al.* (2023) who reported an MIC of 200 mg/ml against *Escherichia coli* and *Salmonella typhi*.

Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had MBC of 500 mg/ml and 250 mg/ml respectively against *Escherichia coli* and MBC of 250 mg/ml against *Salmonella typhi*. Ethanolic

extract of Henna (*Lawsonia inermis*) leaves met the standard of the antibiotic used as positive control (Chloramphenicol) against *Escherichia coli* and *Salmonella typhi* with MBC of 250 mg/ml. Aqueous extract of Henna (*Lawsonia inermis*) leaves also met the standard of the antibiotic used as positive control with MBC at 250 mg/ml. This conforms with Nagish and Mannan (2024) who reported similarly high MIC and MBC of Henna (*Lawsonia inermis*) extracts against *Escherichia coli*, *Salmonella typhi*, and *Salmonella typhi*. This study affirms that aqueous and ethanolic leaf extracts Henna (*Lawsonia inermis*) has significant antibacterial activity on the test organisms, hence the therapeutic potential of the plant in the treatment and management of infectious and non-infectious diseases.

CONCLUSION

This study assessed the phytochemical and antibacterial activity of aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves against *Escherichia coli* and *Salmonella typhi*. Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) possessed flavonoids and saponins.

Ethanolic extracts of Henna (*Lawsonia inermis*) had a significantly higher zone of inhibition against *Escherichia coli* and *Salmonella typhi* compared to the aqueous extract. Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had a MIC of 250 mg/ml against *Escherichia coli* and MIC of 250 mg/ml and 125 mg/ml respectively against *Salmonella typhi*. Ethanolic extract of Henna (*Lawsonia inermis*) leaves met the standard of the antibiotic used as positive control (Chloramphenicol) with MIC at 125 mg/ml.

Aqueous and ethanolic extracts of Henna (*Lawsonia inermis*) leaves had MBC of 500 mg/ml and 250 mg/ml respectively against *Escherichia coli* and MBC of 250 mg/ml against *Salmonella typhi*. Ethanolic extract of Henna (*Lawsonia inermis*) leaves met the standard of the antibiotic used as positive control (Chloramphenicol) against *Escherichia coli* and *Salmonella typhi* with MBC of 250 mg/ml. aqueous extract of Henna (*Lawsonia inermis*) leaves also met the standard of the antibiotic used as positive control with MBC at 250 mg/ml.

RECOMMENDATIONS

The significant antibacterial activity of Henna (*Lawsonia inermis*) leaves demonstrates its potential use in the treatment of infectious diseases.

The active ingredients should be extracted, characterized, and synthesized to make its adoption in orthodox medicine acceptable and widely available.

Further experiments on its in-vivo activity should be done to understand the full spectrum of its therapeutic applications and safety.

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Tables

Tables 1 to 6 are available in the Supplementary Files section.

Supplementary Files

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