A study of antimicrobial and phytochemical properties of leaf and root of Henna (*Lausonia Inermis L.*) against common microorganism

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Abstract
Medicinal plants are globally being used for one or more drugs combination in health care, almost all modern drugs today are originating from traditional medicine. Lawsonia inermis L. commonly known as Henna was reported to have antibacterial, antifungal, antiparasitic and anti-inflammatory properties.

The antibacterial and antifungal activities of the extracts with different solvents were tested, the results showed Staphylococcus aureus with highest zone of inhibition of 19.5±2mm while Aspergillus flavus extract showed no activities. A minimum inhibitory concentration was conducted for the best five extracts and the highest activity at 250m/g of root extract methanol was found against S. aureus. Phytochemical test was also conducted to identify the presence or absence of secondary metabolites such as flavonoid, tannin, saponin, phenolic compound and terpenoids. This result may open important perspectives for alternative bacterial therapies.

Keywords: Henna, Antimicrobial, Phytochemical and Common Microorganisms.

Introduction

Plants are important source of medicine and play a key role around the globe, the medicinal plants have been known to be an important potential source of curative aids. The use of medicinal plants has attained a commanding role in health system all over the world[3]. Many countries in the world and around two-third of the world's population depends on medicinal plant for primary health care. The reason for this is because of their better cultural acceptability, better compatibility and adaptability with the human body and poses lesser side effects.

Most of the used drugs contain plant extracts. Some contain active ingredients (bioactive components or substances)[1]. Henna (*Lawsonia inermis)* is cultivated by many farmers for cosmetic and pharmaceutical purposes, it belongs to the group of plants that are popular in nature and all parts of the plant (root, stem, leaf, flower pod and seed) are of great antimicrobial properties[7].

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Material and Methods

Sample Collection: Fresh leaves and roots of Henna (*Lawsonia inermis L)* were obtained from Sabon Layin Galadima Village, Faskari Local Government, Katsina State.

Maintenance of Test Organisms: The cultures were inoculated in nutrient broth, nutrient agar and the slants were incubated at 37^0C for 24hours. The slants and broth were stored at 4^0C and maintained in active stage by regular sub-culturing throughout the research.

Drying of Sample: The fresh leaves and roots of Henna (*Lawsonia inermis L.)* were shed dried at normal temperature for two weeks, then grinded into fine powder using mortar and pestle in the laboratory and the sieve powder using mesh, then stored in an air tight container until used.

Preparation of Extracts

Aqueous (Hot Water): 15g air dried powder of the dried leaves and roots of Henna (*Lawsonia inermis L)* was dissolved in 150ml of distilled water respectively, boil in water bath till one fourth of the extract is left after evaporation. The solution was filtered using muslin cloth and centrifuged at 5000rpm for 15minutes, re-filtered using Whatmann filter no. 1 under aseptic condition and then solution was collected in fresh sterilized bottles and stored at 4^0C until used.

Ethanol and Methanol: 36g air-dried powder of leaves and roots were introduced into five hundred millilitre flasks separately and 150ml of 95% ethanol/methanol was added to each. The mixtures were agitated overnight in a multifunctional oscillator at 120rpm after which the supernatants were separated from the residue by decanting and filtered through muslin cloth and then re-filtered by passing through Whatmann filter paper no.1. The samples were then evaporated to removes traces of the extraction solvent with the help of rotary evaporator at 4^0C and evaporated to dryness in 50ml sampling bottles using an electric thermostatic drying oven at 45^0C. The weight of the dry mass was determined and used to calculate the concentration of the extracts in each solution in mg/ml and stored at 4^0C in sterilized bottles until used.

Preparation of Inoculums: Active cultures for experiments were prepared from the stock cultures maintained at 4^0C on slopes of nutrient agar and potato dextrose agar by

transferring a loopful of cells to 50ml prepared nutrient broth (NB) for bacteria and potato dextrose broth (PDB) for fungi[8].

Antimicrobial Sensitivity Assay

Well Diffusion Techniques for Bacteria: The bacterial and fungal cultures were used separately (by spreading it on prepared solidified media) by well diffusion method. One well of 3 mm size was made with sterile jell cutter under aseptic condition in laminar air flow chamber. The wells were loaded with plant extracts and negative control while antibiotic was used as positive control respectively. The plates were incubated at 37^0C for 24 hours (Bacteria), 28^0C for 72 hours (Fungi). The plates were observed for clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well. The readings were taken in all the replicates of the average values[2].

Minimum Inhibitory Concentration (MIC) Assay: For bacterial assay, take different concentration of the plant extract ranging from 250,200,150,100 and 50µg/ml. 2ml of each concentration was mixed in different tubes that contains 2ml of nutrient broth, each tube was inoculated with 2ml of bacterial stain and two control tubes ware included, one with NB and bacterial stain (Positive control) while the other one with only NB (Negative control). All tubes ware incubated at 37 ^{0}C for 24 hours. Transmission of different concentration in each tube was measured using Spectrophotometer at wave length of 620nm and all values recorded respectively[4].

Phytochemical Screening: Chemical test for the screening and identification of bioactive chemical constituents in the plants under study (Leaf and root of henna) was carried out in extracts using a combined modified standard procedures as described by Predesh.[7] The presence of bioactive component such as Flavonoids, Phenolic Compounds, Tannins, Saponins and Terpenoids was tested for both leaves and roots of henna prepared with aqueous, ethanol and methanol[8].

Results and Discussion

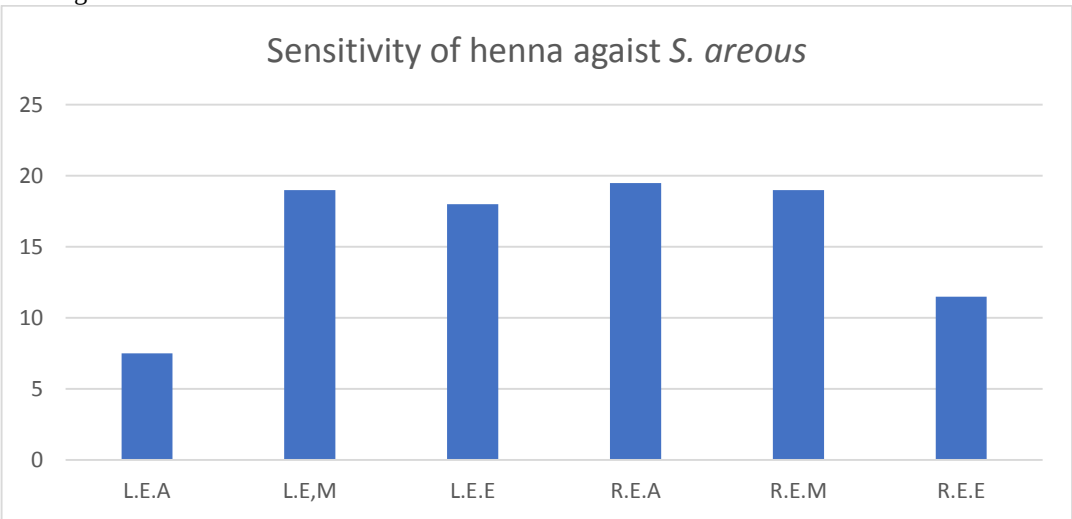
Antimicrobial Sensitivity Assay - Primary screening of *Staphylococcus aureus*: It was observe that, the crude extracts of henna (Aqueous, Methanol and Ethanol) were all active against tested bacteria (*Staphylococcus aureus)* in which zone of inhibition was shown (fig. 1) and measurements were taken with plastic ruler whereas the leaf extract aqueous 7.5.5±3, root extract aqueous 19.5±7, root extract ethanol 11.5.5±3, leaf extract methanol 19±4, leaf extract ethanol 18.5±1, root extract methanol 19±2, are presented in table 1. While in comparison, the root extract aqueous has the highest zone of inhibition of 19.5±7 as indicated in graph 1.

Table 1

Sensitivity test of root and leaf henna plant against *S. aureus*

S.N.	Sample	+tive control	Zone of Inhibition	-tive Control
1.	L.E.A	20 ±1	7.5±3	0
2.	L.E.M	18±2	19±4	6.5±2
3.	L.E.E	19±2	18.5±1	6±2
4.	R.E.A	23 ±2	19.5±7	0
5.	R.E.M	22 ±4	19±2	8 ±4
6.	R.E.E	16±3	11.5±3	5.5±1

Key: S/N= Serial number, *S. aurous* = *Staphylococcus aurous*, L.E.A= Leaf Extract Aqueous, L.E.M= Leaf Extract Methanol, L.E.E = Leave Extract Ethanol, R.E.A= Root Extract Aqueous, R.E.M = Root Extract, Methanol, R.E.E= Root Extract Ethanol, +tive= Positive and -tive= Negative.



Graph 1: Sensitivity test against *S. aureus*

This result is in agreement with the result reported by Pandey et al[6] who tested the antimicrobial activities of Henna extract of methanol and ethanol on three Bacterial pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and was found to be active against all the three bacteria.

Primary screening of *Aspergillus flavus*: All extracts of leaf extract aqueous (L.E.A), leaf extract methanol (L.E.M), leave extract ethanol (L.E.E), root extract aqueous, (R.E.A) root extract, methanol (R.E.M) and root extract ethanol (R.E.E) were found to be inactive ageist *Aspergillus flavus*, with zero zone of inhibition as presented in table 2 and this could be explained as the resistance of the stain used in this study. This result is in agreement with the result reported by Saddik and Mohmed[9] who demonstrated that most of the medicinal plants have weak antifungal activity compered to bacteria.

Minimum inhibitory concentration (MIC) - Secondary Screening of *S. aureus*: The best five extract (leaf extract methanol (L.E.M), root extract methanol (R.E.M), leaf extract aqueous (L.E. A), root extract aqueous (R.E.A) and leaf extract ethanol (L.E.E) were tested for bactericidal action (*S.aureus,)* and the result obtained revealed that almost all the extract have a bactericidal action depending on the test organisms and concentration used. L.E.M was more active against at 150mg, R.E.M was also very active at 200mg, L.E.A shows the highest activity at 50mg, R.E.A at 250mg and L.E.E showed less cloudy and bacterial growth at 150mg.

The most active extracts for MIC showed higher transmission and less absorbance at 620 wave length using spectrophotometer and the result is presented in table 3 while in comparison at 250mg, all tested bacteria were found to be more active as shown in graph 2.

Phytochemical Screening: The presence (positive) of five tested bioactive component such as Phenolic acid, Terpenoid, Flavonoid, Tannin ,Saponin in L.E.A and L.E.M, Terpenoid absence (Negative) in L.E.E and all the reaming four presence (positive), in the R.E.A Tannin absence (Negative) while all other tested bioactive component presence (positive) was confirmed. All five tested bioactive component showed presence in R.E.M and three showed presence in R.E.E (table 4). According to the Papageorgious[5], phytochemical constituents of *Lawsonia inermis* exhibit antimicrobial activity only against gram positive bacteria. Other studies have shown that *Lawsonia inermis* had antimicrobial activity against both gram positive and gram negative[2].

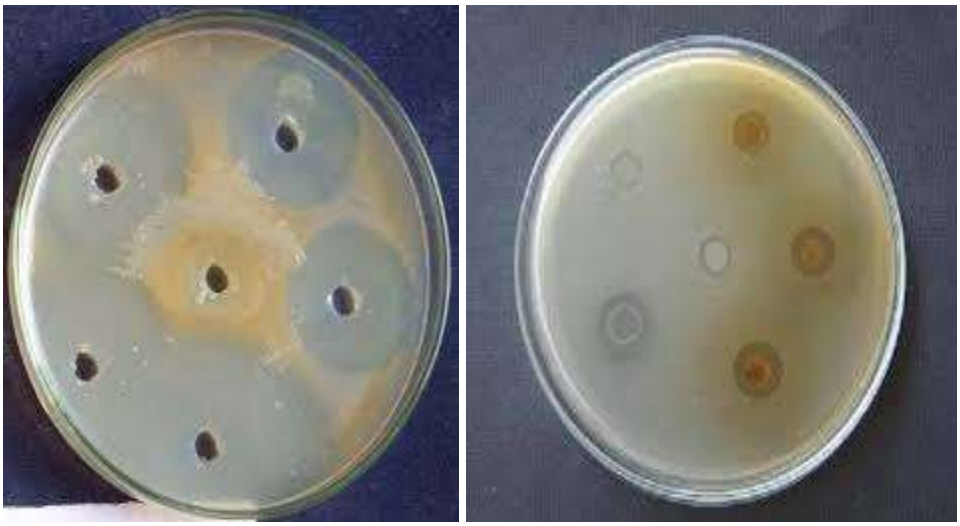


Fig. 1: Antibacterial activity of root and leave of henna extract against *S. aureus*

Table 2
Sensitivity test of root and leaf henna plant against *A. flavus*

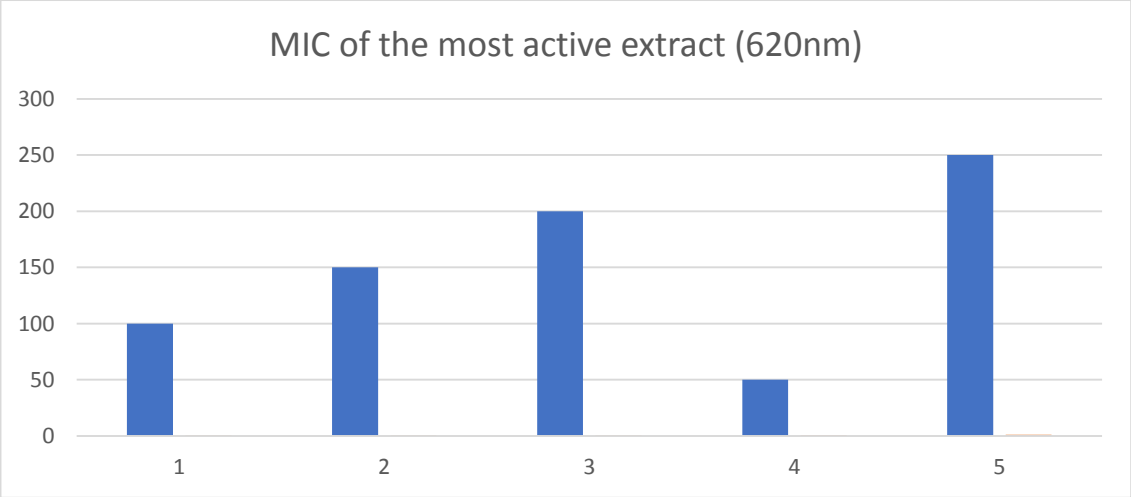
S.N.	Sample	+tive control	Zone of Inhibition	-tive Control
1.	L.E.A	25.5 ±1	0	0
2.	L.E.M	16±2	0	7±2
3.	L.E.E	22.5±2	0	6±2
4.	R.E.A	24±2	0	0
5.	R.E.M	20.5±3	0	6.5±1
6.	R.E.E	28±4	0	4.5±1

Key: S/N= Serial number, L.E.A= Leave Extract Aqueous, L.E.M= Leave Extract Methanol, L.E.E = Leave Extract Ethanol, R.E.A= Root Extract Aqueous, R.E.M = Root Extract, Methanol, R.E.E= Root Extract Ethanol, +tive= Positive and -tive= Negative.

Table 3
Minimum Inhibitory concentration (MIC) of the Most Active Extract (620nm)

S.N.	SAMPLE	TEST ORG.	250 µg/ml	200 µg/ml	150 µg/ml	100 µg/ml	50 µg/ml	+ve C
1.	L.E.A	*S. aureus*	0.98	0.137	0.382	**0.571**	0.901	0.997
2.	L.E.M	*S. aureus*	0.96	0.294	**0.458**	0.699	0.761	0.801
3.	L.E.E	*S.aureus*	0.108	**0.323**	0.625	0.857	0.929	0.989
4.	R.E.A	*S. aureus*	0.63	0.211	0.359	0.472	**0.616**	0.963
5.	R.E.M	*S. aureus*	**0.998**	0.344	0.566	0.763	0.991	0.991

Key: S/N= Serial number, MIC= minimum inhibitory concentration, Org= organisms, mg= milligram L.E.A= Leave Extract Aqueous, L.E.M= Leave Extract Methanol, L.E.E = Leave Extract Ethanol, R.E.A= Root Extract Aqueous, R.E.M = Root Extract, Methanol, R.E.E= Root Extract Ethanol, *S. aureus* = *Staphylococcus aureus*



Graph 2: Minimum Inhibitory Concentration

Table 4
Phytochemical properties of root and leaf of Henna Plant

Crude Extract	Phenolic	Terpeniod	Flavonoid	Tannin	Saponin
Leaf Extract Aqueous	+tive	+tive	+tive	+tive	+tive
Leaf Extract Methanol	+tive	-tive	+tive	+tive	+tive
Leaf Extract Ethanol	+tive	-tive	+tive	+tive	+tive
Root Extract Aqueous	+tive	+tive	+tive	-tive	+tive
Root Extract Methanol	+tive	+tive	+tive	+tive	+tive
Root Extract Ethanol	-tive	+tive	-tive	+tive	+tive

Key: S/N= Serial number, +tive= Positive and -tive= Negative

Table 5
Overall Comparison between the Activity Root and Leaf of Henna (T-Test)

	Variable 1	Variable 2
Mean	22.66667	19.54167
Variance	95.15152	40.11174
Observations	12	12
Hypothesized Mean Difference	0	
Df	19	
t Stat	0.930788	
P(T<=t) two-tail	0.36364	
t Critical two-tail	2.093024	

Key: df= difference, p= probability, t stat = t statistic

Conclusion

Medicinal plants are better alternatives of chemically synthesized drugs for its abundance, cheap and eco-friendliness. Sample root and leaf extracts of Henna plant (*Lawsonia inermis L.*) were prepared with aqueous, methanol and ethanol and tested against *Staphylococcus aureus* and found to be effective. The extract with highest activity was selected for minimum inhibitory concentration despite its ability of coursing several infections to human while *Aspergillus flavus* shows no activity against all extracts. The activity is reported to be associated with the presence of bioactive components such as phenol.

Acknowledgement

The authors are thankful to Noida International University, Department of Biotechnology and Microbiology especially Dr. Shiphy Singh and to Al-Qalam University, Katsina for proving infrastructure and technical support during experiments.

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(Received 02nd August 2022, accepted 01st October 2022)