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Original Article

# NATURAL ANTIMICROBIAL ACTIVITY OF *LAWSONIA INERMIS* AND *INDIGO TINCTORIA* AGAINST CLINICALLY ISOLATED MICROORGANISMS

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**ABSTRACT**

**Objective:** The present study aimed at evaluating the natural antimicrobial activity of *Lawsonia inermis* (*L. inermis*) and *Indigofera tinctoria* (*I. tinctoria*) against a reference and pathogenic clinically isolated strains compare with some antibiotics.

**Methods:** The antimicrobial activity of *Lawsonia inermis* (red henna) and *Indigo tinctoria* (black henna) was evaluated against clinically isolated strains from urinary tract and wounds infected patients. The six tested strains namely were *Staphylococcus aureus*, *Enterococcus fecalis*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Candida albicans.* The antimicrobial activity was evaluated by agar diffusion method and microplate dilution for measuring the minimum inhibitory concentration (MIC). An American Type Culture Collection (ATCC) of bacteria was used as a control for confirming the type of the isolated bacteria.

**Results:** Both red and black henna showed variable antimicrobial activity against tested bacteria and *C. albicans.* Alcoholic and oily extracts were more effective than water. *Staphylococcus epidermis* was significantly affected with water extract of black henna (20±1.1) and red henna (11±0.5). *Nigella sativa* oil extract with black henna had showed significant synergism effects against some microorganisms as *Enterococcus fecalis* (40±1.5), *P. aeruginosa* (15±0.9) and *Candida albicans* (18±0.6).

**Conclusion**: Black and red henna had antibacterial and antifungal activities. They exhibited synergistic effects when mixed with black seed oil.

**Keywords**: *Lawsonia inermis*, *Indigofera tinctoria*, Antimicrobial Activity


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## INTRODUCTION

Many plants have been used for many years as a traditional plant medicine. They have attained the status of the natural source of new and potent antimicrobial agents [1]. About 20% of the plants, which were found in the world had been submitted to pharmaceutical or biological tests and a sustainable number of new antibiotics introduced in the market were obtained from natural or semi-synthetic resources [2].

There are two types of natural henna, red henna and black henna. Red henna is a commercial name of *Lawsonia inermis* and the leaves powder gives red color for skin, hair and nails [3, 4]. Natural black henna is a commercial name of *Indigofera tinctoria* that gives black color for hair, skin and nail. *Lawsonia inermis* is a flowering plant which belongs to *lythraceae* family [5]. *Lawsonia inermis* contains a high amount of flavanol, phenolic acid and Quinones [5-7].

Many studies on *Lawsonia inermis* leaves extractions showed that it had antibacterial activity against Gram positive bacteria (*Bacillus spp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis* MRSA, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacteriodes fragilis*, *Clostridium perfringens*, *Streptococcus mutans*, *Micrococcus*, *Streptococcus salivarius*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Streptococcus gordonii*); and against Gram negative bacteria (*Escherichia coli*, *Salmonella spp*, *Klebsiella spp.*, *Shigella sonnei*, *Pseudomonas, aeruginosa*, *Citrobacter frewndii*, *Vibrio cholerae*, *Neisseria meningitides*, *Haemophilus influenzae*, *Aeromonas hydrophila*, *Micrococcus spp* and *Corynebacterium diphtheriae*) [8-10]; and antifungal activity aganist (*Candida albicans*, *Cryptococcus neoformans*, *A. niger* and *F. oxysporum*) [11].

*Indigofera tinctoria (Fabaceae)* is a species of plant; namely, the bean family which is an annual herb that is cultivated in India, China and other countries as a source of Indigo. [12] *Indigofera tinctoria* leaves contain high amount of alkaloids, flavonoids, saponins [13] and indigo. *In vitro,* the extracts of the indigo plant were found to be effective in suppressing the proliferation of cancer cells [14] and act as a strong antioxidant [15]. They had Antibacterial activity against (methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin sensitive *Enterococcus faecalis*, Vancomycin-resistant *Enterococcus* species, *Moraxella*, *Streptococcus* species, *Haemophilus influenzae*, *Bacillus subtilis*, *Escherichia coli* and *Pseudemonas aeruginosa*) [16, 17].

Both *Lawsonia inermis* and *Indigofera tinctoria* are Indian herbal plants which are used in the traditional systems of medicine to cure skin infections and burn wound [18, 19]. Neem and *Lawsonia inermis* mixed ethanolic extract have anti-inflammatory activity [20].

Seeds of *Nigella sativa* Linn commonly known as black seed or black cumin [21]. It contains nigellone and methyl isopropyl-pquinone [22]. The antimicrobial action could be attributed to the thymoquinone and melanin [23]. The purpose of this study was to evaluate the antimicrobial properties of *Lawsonia inermis* (*L. inermis*) and *Indigofera tinctoria* (*I. tinctoria*) against a reference and pathogenic clinically isolated strains and to compare them with some antibiotics.

## MATERIALS AND METHODS

### Chemicals and kits

The chemicals used were dimethyl sulfoxide (DMSO) (RCI Labscan, Thailand), tween 80 (AZ Chemicals, Canada), Antibiotic containing disks (Bioanalyse, Turkey), both ethanol 99.9 % and McFarland standard were purchased from (VWR, Germany).

### Culture media

Nutrient agar, Muller Hinton agar, Muller Hinton broth, Sabouraud dextrose broth and Sabouraud agar all were obtained from Oxoid laboratories, UK.

**Instruments**

Analytical balance (Phoenix Instrument, USA), autoclave (Rypa, Spain), incubator (Eurostar, EU), hot plate magnetic stirrer (Dragon, China), orbital shaker (Stuart, UK), filter paper (Wattman no 5, China), both sterile tubes and sterile swab (MWe, UK), micropipettes (Oxford, USA) and leofolyzer (Power Dry PL3000, Germany).

**Plant material**

*Dry* leaves powder of *L. inermis* (*Henna* 5 herbal blends-batch 13541-India) and I. *tinctoria* (Henna 5 herbal blends–batch RS 149-India) were obtained from a local market.

**Preparation of extracts**

**Water extracts**: 10 gm. of each henna powder were dissolved in 100 ml of distilled water then mixed with incubation at 60 °C for 24 h using orbital shaker at 200 rpm. The mixture was cooled and filtered by Buchner funnel and filter paper. Finally, the solvent was dried and concentrated using leofolyzer.

**Ethanolic extracts**: 10 gm. of each henna powder were dissolved in 100 ml of 99% ethyl alcohol and mixed for 24 h using orbital shaker at 200 rpm. Then, the mixture was cooled and filtered using 15 µm filter paper. The solvent was dried and concentrated using leofolyzer. Finally, the extracts dissolved in DMSO.

***Nigella sativa* (*N. sativa*) oil extracts**

The oil extraction method was from [24] 10 gm. of each henna powder was dissolved in 100 ml of black seed oil then mixed with incubation at60 °C for 24 h using orbital shaker at 200 rpm. Finally, the mixture was cooled and filtered with a negative pressure using Buchner funnel and filter paper.

**Tested organisms**

American type culture collection (ATCC) bacteria were used as a control for confirming the type of the isolated bacteria, which are *Escherichia coli* (*E. coli*) ATCC 10536, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027, *Staphylococcus epidermidis* (*S. epidermidis)* ATCC 12228, *Enterococcus fecalis* (*E. fecalis*) ATCC 51298, *Proteus mirabilis (P. mirabilis)* ATCC 29906. Also one yeast strain; namely, *Candida albicans* ATCC 10231. Thirty bacterial isolates of each organism were obtained from hospitals and labs. Bacteria and yeast were isolated from different UTI and infected wounds patients. The bacterial strains used throughout the present investigation were maintained on nutrient agar slants while the yeast was maintained on Sabouraud dextrose agar. The cultures were stored at 4 °C with a regular transfer at monthly intervals.

**Determination of antimicrobial activity**

The method of Rios [25] was used to determine the antimicrobial activity. An inoculum $1.5\times10^8$ CFU/ml equivalent to 0.5 McFarland was prepared, and 25 µl were swabbed over the surface of Müller-Hinton agar plate. Antimicrobial activity was carried out using a well-diffusion method after incubation for 24 h. At 37 °C, all plates were examined for zones of inhibition. The diameters of the inhibition zones were measured in millimeters (mm) [10]. The mean of five randomly selected isolates were calculated.

**Mesuring minimal inhibitory concentration (MIC)**

The most promising extract was tested for MIC by dilution method. This test was performed in sterile 96-well microtiter plates [26]. The cultures were diluted in Müller-Hinton broth at a density adjusted to a 0.5 McFarland turbidity. The final inoculum concentration was $1.5x10^8$CFU/ml of bacterial cultures. The wells were filled with 80 µl of sterile broth, 20 µl sterile tween 80 and 100 µl of the extract was added to the wells by serial two-fold dilution steps. Each well was inoculated with 100 µl of 0.5 McFarland standard bacterial suspensions so that each well got 1.5 $x10^8$ CFU/ml. The 96-well microtiter plates were covered, placed in plastic bags and incubated at 37 °C for 24 h. The MIC was the lowest concentration of the extract that resulted in a clear well.

**Antibiotic susceptibility testing for the tested microorganisms**

Five different antibiotics were selected for the present study and the test was done using disc-diffusion method [27]. The antibiotics were tetracycline (TE) 300 mcg, gentamicin (GN) 10 mcg, vancomycin (VA) 30 mcg, and penicillin G 10U (P G10), and nitrofurantoin (F) 300 mcg. Antibiotics were used in this study to evaluate the antibacterial efficacy of henna extracts. Antibiotic discs were spread on Muller Hinton agar as described in NCCLS-2000 [28].

All antimicrobial tests were done against the same tested isolates which were used for screening the efficacy of the different extract and the mean was calculated. The results of antimicrobial testing are compared with those of standard drugs. Isolates were reported as resistant (R), intermediate (I) and sensitive (S) according to the kit instructions.

**Statistical analysis**

Statistical analysis was carried out using statistical packages for social science software (SPSS) version 16, 2007, Chicago. Values expressed as mean±SD and values of $p<0.05$ were considered statistically significant according to LSD test.

**RESULTS**

Different extracts (water, ethanol and black seed oil) of *L. tinctoria* (black henna) were screened for their antimicrobial activity against Gram-positive, Gram-negative bacteria and yeast. Table (1) shows the results of antimicrobial activity of black henna extracts. The black seed oil control had no effects against all tested Gram positive and Gram negative isolates except for the yeast.

The black seed oil extracts had a significantly higher antimicrobial activity than water against *S. epidermids*. The diameter of the inhibition zone of oil, ethanol and water was 25, 24±1.1, and 20±1.1 mm respectively and their respective MICs were 0.0625, 0.125 and 0.5 mg/ml.

The other Gram-positive *E. fecalis* isolates were only sensitive to (*N. sativa*) oil with inhibition zone (40±1.5 mm) and MIC (0.0312 mg/ml). The antimicrobial activity of *N. sativa* oil against *E. feclais* was higher than of *S. epidermids*. Water had no antimicrobial activity against the Gram-negative *P. mirbalis*, *E. coli*, *E. fecalis* or *P. aerugonisa*.

Ethanol had the same effects as water against Gram-positive and the other Gram-negative bacteria expect for *P. mirbalis* where the zone of inhibition was (18±0.9 mm) and MIC (0.25 mg/ml). The black seed oil extracts had antimicrobial activity against the *E. fecalis* (40±1.5 mm at MIC 0.031 mg/ml) which was higher than *P. aurgonisa* (15±0.9 mm at MIC 0.5 mg/ml).

The highest effects against *C. albicans* were for alcoholic extracts (20±1 mm) and for black seed oil based extracts (18±0.6 mm) compared with significantly lower effects for black seed oil alone (14±0.5 mm) and no effects with water extracts. The MIC of black seed oil alone (0.5 mg/ml) was decreased (0.25 mg/ml) compared with black seed oil based extracts. Ethanol and black seed oil extracts had the same significant antimicrobial activity while the MIC of ethanol (0.125 mg/ml) was lower.

The results presented in table (2) indicated the antimicrobial action of red henna. The black seed oil alone had no antimicrobial activity against all the tested Gram negative and Gram positive isolates.

The antibacterial activity of oil-based extracts against *S. epidermids* was significantly more effective than ethanol extracts followed by aqueous extracts. The zone of inhibition diameters were 31±0.2, 20±0.9 and 11±0.5 mm respectively, and their MIC were 0.0625, 0.125 and 1 mg/ml respectively. *E. fecalis* was resistant to water, ethanol extracts and affected by black seed oil extracts. The zone of inhibition was (25±0.9 mm) and MIC was (0.125 mg/ml).

All red henna extracts were inactive against the Gram-negative *P. mirbalis* and *E. coli* except against *P. aeruginosa* where its zone of inhibition was (12±0.06 mm) and MIC 0.5 mg/ml. When measuring the antimicrobial activity against *C. albicans,* the MIC was the same (0.5 mg/ml) for black seed oil, black seed oil extracts and ethanol

extracts. The zone of inhibition was 14±0.5, 13±0.6 mm respectively. Ethanol extracts activity was significantly lower compared to all three isolates and water had no antimicrobial activity.

The antibiotics susceptibility test was done using five antibiotics (table 3) to compare the potency of black henna (table 1) and red henna (table2) with antibiotics.

*S. epidermids* was resistant to TE, GN compared to the largest inhibition zone (31±1.2 mm) at MIC (0.0625 mg/ml) with the black oil based extracts of red henna. *E. fecalis* showed promising results where it was resistant to all antibiotics with no inhibition zone compared to the largest zone of inhibition (40±1.5 mm) at MIC (0.0312 mg/ml) with black henna oil-based extracts.

The best zone of inhibition for *P. mirbalis* was obtained with black henna ethanolic extract (18±0.9 mm) at (MIC 0.25 mg/ml) when it was resistant to all tested antibiotics. *E. coli* was sensitive to GN and resistant to the different tested extracts. The maximum zone of inhibition, which was obtained against *P. aeruginosa,* was reached when black henna was mixed with black seed oil extracts (15±0.9 mm) and MIC (0.5 mg/ml).

**Table 1: Black henna extracts antimicrobial actions: zone of inhibition mean (mm) and MIC (mg/ml)**

| Microorganisms | Black seed oil control | | Water extracts | | Ethanol extracts | | Black seed oil extracts | |
|---|---|---|---|---|---|---|---|---|
| | Mean | MIC | Mean | MIC | Mean | MIC | Mean | MIC |
| *S. epidermidis* | 0* | 0 | 20±1.1a | 0.5 | 24±1.1b | 0.125 | 25b | 0.0625 |
| *E. fecalis* | 0 | 0 | 0 | 0 | 0 | 0 | 40±1.5 | 0.0312 |
| *P. mirabilis* | 0 | 0 | 0 | 0 | 18±0.9 | 0.25 | 0 | 0 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | 0 | 0 | 0 | 0 | 0 | 0 | 15±0.9 | 0.5 |
| *C. albicans* | 14±0.5a | 0.5 | 0 | 0 | 20±1b | 0.125 | 18±0.6b | 0.25 |

*The results were the mean±standard deviation (SD) of 5 isolates and means with the same letter within the same row do not differ significantly according to LSD test

**Table 2: Red henna extracts antimicrobial action: zone of inhibition mean (mm) and MIC (mg/ml)**

| Microorganisms | Black seed oil control | | Water extracts | | Ethanol extracts | | Black seed oil extracts | |
|---|---|---|---|---|---|---|---|---|
| | Mean | MIC | Mean | MIC | Mean | MIC | Mean | MIC |
| *S. epidermidis* | 0* | 0 | 11±0.5c | 1 | 20±0.9b | 0.125 | 31±1.2a | 0.0625 |
| *E. fecalis* | 0 | 0 | 0 | 0 | 0 | 0 | 25±0.9 | 0.125 |
| *P. mirabilis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | 0 | 0 | 0 | 0 | 12±0.6 | 0.5 | 0 | 0 |
| *Candida albicans* | 14±0.5a | 0.5 | 0 | 0 | 11±0.4 b | 0.5 | 13±0.6a | 0.5 |

*The results were the mean±standard deviation (SD) of 5 isolates and means with the same letter within the same row do not differ significantly according to LSD test.

**Table 3: The antibiotics susceptibility test against selected isolated strains (mm)**

| Microorganism | TE30 µg | GN10 µg | F300 µg | VA30 µg | P G1010 IU |
|---|---|---|---|---|---|
| *S. pidermidis* | 0* | 21±1.0[1] | 23±1.0 | 18±0.8 | 0 |
| *E. fecalis* | 0 | 0 | 0 | 0 | 0 |
| *P. mirabilis* | 12±0.5 | 23±1.1 | 24±1.2 | 14±0.5 | - |
| *E. coli* | 0 | 16±0.5 | 14±0.1 | 0 | - |
| *P. aeruginosa* | 13±0.5 | 20±1.0 | 0 | 0 | - |

*The results were the mean±standard deviation (SD) of the zone of inhibition diameter of 5 isolates, [1]Zone of inhibition for sensitivity discs (TE30 µg: ≥19 S, 15-18 I, ≤14 R), (GN 10 µg: ≥15 S, 13-14 I, ≤12 R), (F 300 µg: ≥17 S, 15-16 I, ≤14 R), (VA 30 µg: ≥12 S, 10-11 I, ≤ 9 R),(P G 10 IU: ≥29 S, 21-28 I, ≤ 20 R). (-): Not tested.

## DISCUSSION

In the present study, the antimicrobial efficacy of alcoholic extracts of black and red henna was greater than aqueous extracts. This result was in agreement with several reports as in [29, 30], where alcohols were generally the most superior solvent for extracting the active ingredients from *L. inermis* compared to water.

The black henna extracts generally showed high antimicrobial activity against Gram-positive, Gram-negative bacteria and *C. albicans* compared to red henna except for *P. aeruginosa*. *L. inermis* exhibited antimicrobial activity only against Gram-positive bacteria while was ineffective for Gram-negative bacteria [31]. Other studies had found that *L. inermis* had antimicrobial activity against both Gram-positive and Gram-negative bacteria [32].

Gram-positive bacteria had a susceptible cell wall since they had outer ineffective peptidogly can barrier [33] while the Gram-negative bacteria had outer phospholipids membrane impermeable to hydrophilic solutes [34]. Indigo was water-insoluble pigment [35]. The variation of antimicrobial activity of same plants could be explained by the different extracting methods, and the number of ingredients could be affected by the area and season of collection [36]. In addition, it could probably be related to the nature and combination of phytocompounds constituents [37], extraction methods and both dose and strain [38].

## CONCLUSION

According to the present study, it was concluded that black and red henna had proven to show significant antimicrobial activity. Our data also demonstrated pronounced antimicrobial effects against the pathogenic isolates which could be used as an effective natural antimicrobial product in pharmaceutical and industries against various microbial types through different inhibitory mechanisms. Further studies are required concerning dose, strain variation and phytoconstituents.

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**AUTHORS CONTRIBUTIONS**

- Maha AL Rimawi is the chief author, supervised the work and contributed in writing all parts.
- Mahmoud Al-Masri and Lena Sabrah were responsible for the laboratory work.
- Nedaa F. Husein and Ola Al Masim were responsible for writing and discussing the microbiology part.
- Arwa N T Al-Hinnawi was responsible for language reviewing and editing.

**CONFLICT OF INTERESTS**

The authors declare none

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