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RESEARCHARTICLE

ANTI-BACTERIAL ACTIVITIES OF *LAWSONIAINERMIS* AND *CAMELLIASINENSIS* AGAINST SOME HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Background: Nowadays there are several bacteria show resistant to many antibiotics, therefore the discovery of new and more efficient antibacterial agents is essential.
Objective: to evaluate the antibacterial activities of *Lawsoniainermis* (henna) and *Camelliasinensis* (green tea) against some pathogenic bacteria.
Methodology: Fresh plants *Lawsoniainermis* and *Camellia*sinensis were collected with assistant by a plant taxonomist at the medicinal and aromatic plants research institute in the national center of research in Khartoum, Sudan. Then the plants were chapped into small slides and shade dried. By using Cup plates method; different concentrations of Ethanolic, Methanolic and boiling water extracts of plants were examined against different micro-organisms including clinical isolates and laboratory standard bacterial strain of *Staphylococcusaureus* ATCC 25923, *Escherichiacoli* ATCC 25922, *Proteusmirabilis* ATCC 49565, *Klebsiellapneumoniae* ATCC 35657, *Pseudomonasaeruginosa* ATCC 27853. Clinical isolates of these strain obtained from patients attending the Omdurman teaching hospital, Sudan.
Results: *Lawsoniainermis* show various antimicrobial activity against standard bacteria ranges from 9.9 mm to 22.9 mm, whilst against isolated organisms methanolic extract provide sensitivity zone ranging 4.6 mm to 12 mm for all isolates, whereby ethanolic and boiling water extract act only against *S. aureus*from 8 mm – 15 mm.
Camelliasinensis show various antimicrobial activity against standard bacteria ranging 7.2 mm – 24 mm except against *P.mirabilis* ATCC 49565 the boiling water extract show no activity, whereas against isolated organisms provide activity ranging from 3.6 mm to 19.8 mm with exception again no activity against *P.mirabilis.*
Also antibacterial activities of different antibiotics against both standard bacteria and isolated bacteria show various result from 0 to 38 mm.
Conclusion: The present studyclarify the antibacterial activities of used plants. Therefore these results supply the use of theseplant as antibacterial agent.

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INTRODUCTION

Medicinal plants have been a major source of therapeutic agents for alleviation and cure diseases. The antibiotic-resistant bacterial pathogens has been spreading worldwide (Reinthaler *et al*., 2013; Nazik *et al*., 2012). This statement illustrates the importance ofconducting scientific research to find a more effective antibacterial agents.

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Recent studies show the recognition of antibacterial activity of medicinal plants (Cheruiyot *et al*., 2009; Vieira *et al*., 2010; Cheruiyot *et al*., 2009; Peixoto *et al*., 2011). And identify different phytochemical agents:flavonoids, steroids, tannins, and glycine-rich peptide (Dhiman *et al*., 2011; Tavares *et al*., 2012). Furthermore, some plant extracts has antibacterial effect against strains of methicillin-resistant *Staphylococcus aureus* (Chomnawang *et al*., 2009). Thus, the chemical compounds with antimicrobial effect against human pathogens was essential. *Lawsoniainermis* leaves were exhibited antimicrobial against Gram-negative bacterial strains (Abulyazid*et al*., 2013). *Lawsoniainermis* and *Camellia sinensis* are widely consumed

as traditional beverages in Sudan and some regional countries. They are relatively cheap and the belief is that they improve health state and cure many diseases. The tea plants contain many phytochemical include alkaloids, saponins, tannins, catechin and polyphenols (Golden, 2009). As a result of increasing antibiotic resistant bacteria, many studies were conducted to regarding the antimicrobial effects and therapeutic properties of Green tea (Stoicov *et al*., 2009; Sharangi *et al*., 2009).

Objective

To evaluate the antibacterial effect of *Lawsoniainermis* (henna) and *Camelliasinensis* (green tea) against some pathogenic bacteria.

MATERIALS AND METHODS

Ethical approval was obtained from the ethical committee of the Omdurman Islamic University, Sudan.

Collection of plants samples

Fresh plants *Lawsoniainermis* and *Camellia*sinensis leaves collected and washed with water, then kept under shade until dried. This material was positively identified as henna and green tea by a plant taxonomist at the medicinal and aromatic plants research institute in the national center of research in Khartoum, Sudan. Then the plants were chapped into small slides and shade dried.

Plants extraction technique

A 100 g of each dry plant soaked in 500 mL of ethanol and methanol for three days, with adequate agitation. The mixture filtered by using Whatmann No. 1 (Whatmann International Ltd, Maidstone, UK), then harvest the crude extract and heated, at 48 °C in a water bath to get rid of the liquid. Aqueous extract for each dried ground plant (100g) was prepared by infusion using boiled distilled water. It was allowed to soak for 2 hours, then it was filtered, and taken 1 ml from the residue was then dried and weighted and the yield percent was obtained. Then various concentration of these extracts were used against different clinical isolates and a laboratory standard bacterial strain of *Staphylococcusaureus* ATCC 25923, *Escherichiacoli* ATCC 25922, *Proteusmirabilis* ATCC 49565, *Klebsiellapneumoniae* ATCC 35657, *Pseudomonasaeruginosa* ATCC 27853.

Invitro anti- microbial activity of crude plants and antibiotics techniques (Cup plates method)

Cup plates method was used for testing sensitivity of plants extract. Muller and Henton media was used as sensitivity test medium. The reconstituted medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes , allowed to cool at 48 ^{o}Cand 20 ml of the medium was inoculated with 0.1 ml of 24 hours broth culture of the tested organisms (about 1 x10^{8}/ml). The inoculated medium distributed aseptically in 20 ml volume into sterile Petri-dishes (90 mm internal diameter) and allowed to solidify on leveled surface. The agar plates were then stored at 4^{o}c surface till use. Three cups (15mm) were cut by using a10 mm sterile cork borer and the cut discs of agar were removed. A 0.2 ml of extract solutions were carefully added into these cups using measurable dropping pipettes and allowed to diffuse. Then inoculated plates were incubated at 37^{o}c for 24 hours. The used concentrations of extracts were 10%, 20% and 40% for ethanol and methanol extract whilst 40% only for Aqueous extract. Inhibition zone around the cup was measured in millimeters.

Samples

A numbers of 50 samples (urine, wound swab) were collected from hospitalized patients and transported in ice to the laboratory for immediate processing and culturing.

Identification of bacterial strain

The collected samples were inoculated onto Blood agar, incubated at 37°C for 24 hours for primary isolation. Then isolated bacteria were purified by several sub-culturing from single well-separated colony. The purity of the culture was checked by examining gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics and sensitivity of the isolates. This included staining reaction, organism morphology, growth condition, and the colony characteristics on different media, and biochemical characteristics include Catalase test, Coagulase test, Deoxyribonuclease (DNase) test, Oxidase test, Motility test, Indole test, Citrate utilization test, Urease test and Kligler iron agar.

RESULTS

In vitro antibacterial activity was examined for aqueous, ethanol and methanolic extracts from two different traditionally used medicinal plants *Lawsoniainermis* and *Camellia sinensis*. About 10^{8} CFU/ml of overnight broth cultures bacterial strains, were used to inoculated plate agar, which incubated at 37° for 24 h. antibacterial activity of extracts was evaluated by inhibition zones of bacterial growth. The results are represented as average zone of inhibition of all the isolates of individual species and standard strains.

Different strain of fresh isolate were used in the present study (Table 1)

Table 1. Ratio of bacterial isolates from different specimens

Isolates	Numbers	Percentages (%)
Staphylococcusaureus	12	33.33%
P.mirabilis	8	22.22%
Escherichiacoli	7	19.45%
*Klebsiellapneumonia*e	5	13.89%
Pseudomonas aeruginosa	4	11.11%
Total	36	100%

In the present study *Lawsoniainermis* and *Camelliasinensis* show various antimicrobial activity against both isolates strain and it's standard as shown in Table (2, 3, 4 and 5). Also in the present study antibiotic susceptibility test were done and appear various results against both isolates and standard bacteria (Table 6).

DISCUSSION

Recently there is a needing for develop and discover a new antimicrobial agents to challenge the increase of resistance to antibiotics.

(Fankam *et al*., 2011; Voukeng *et al*., 2012; Lacmata *et al*., 2012; Noumedem *et al*., 2013).

The present study was conducted to evaluate the in vitro antibacterial activity of *Lawsoniainermis* and *Camellia sinensis* against some pathogenic bacteria. The antibacterial activity of

Table 2. Anti-bacterial activities of *Lawsoniainermis* against standard organisms (inhibition zone per mm)

STD organisms	Methanol			Ethanol			Boiling water
	10%	20%	40%	10%	20%	40%	40%
*S. aureus*ATCC 25923	10	17	20	11.1	14.5	20	20
E.coli ATCC 25922	12.00	20.00	22.80	14.70	19.00	22.90	22.80
*P.mirabilis*ATCC 49565	11.00	18.90	21.00	11.00	19.00	21.50	19.00
*K.pnemoniae*ATCC 35657	10.00	17.50	20.50	10.60	18.80	20.50	19.90
*P.aeurginosa*ATCC 27853	9.90	17.670	19.85	10.00	13.54	15.50	16.00

Table 3. Anti-bacterial activities of *lawsoniainermis* against isolated organisms (inhibition zone per mm)

Isolated organism	Methanol			Ethanol			Boiling water40%
	10%	20%	40%	10%	20%	40%	
S. aureus	6	8	10	8	10	12	15
E.coli	8.00	9.90	12	0	0	0	0
P.mirabilis	7.70	8.00	9.10	0	0	0	0
K.pnemoniae	7.40	8.00	10.00	0	0	0	0
P.aeurginosa	4.60	5.35	6.25	0	0	0	0

Table 4. Anti-bacterial activities of *Camelliasinensis* against standard organisms (inhibition zone per mm)

STD organisms	Boiling water 40%	Methanol			Ethanol		
		10%	20%	40%	10%	20%	40%
S.aureus ATCC 25923	21.6	7.2	9.6	14.4	15.4	21.6	24
E.coli ATCC 25922	9	18	21	21	12	15	18
*P.mirabilis*ATCC 49565	0	7.2	8	10.8	13.7	15.9	18
*K.pneumoniae*ATCC 35657	19.8	8.8	11	11.6	9.9	11	19.8
*P.aeurginosa*ATCC 27853	12.8	9.6	10	12.8	6.4	12.8	16

Table 5. Anti-bacterial activities of *Camelliasinensis* against isolated organisms (inhibition zone per mm)

Isolated organisms	Boiling water 40%	Methanol			Ethanol		
		10%	20%	40%	10%	20%	40%
S.aureus	14.4	7.2	9.6	10	9.6	10	10.4
E.coli	9	6	9	9.6	6	9	12
P.mirabilis	0	3.6	7.2	8	10.8	11	11.4
K.pnemoniae	19.8	6.6	8.8	13.2	8.8	11	15.4
P.aeurginosa	12.8	9.6	12.8	16	6.4	12.8	16

Table 6. Anti-bacterial activities of different antibiotics against standard and isolated bacteria (inhibition zone per mm)

STD and Isolates	Ciprofloxacin (10mg)	Amikacin (30µg)	Cotrimexazol (25µg)	Trimethoprim
*S. aureus*ATCC 25923	35	31	38	34
S. aureus	26	24	0	28
E.coli ATCC 25922	0	35	0	0
E.coli	0	30	30	0
*P.mirabilis*ATCC 49565	21	36	30	25
P.mirabilis	38	36	16	0
*K.pnemoniae*ATCC 35657	0	22	20	28
K.pnemoniae	0	22	10	28
*P.aeurginosa*ATCC 27853	21	30	16	34
P.aeurginosa	38	32	20	34

The medicinal plants are the potential sources of new agents for their many bioactive compounds. Also plants have long traditionally used as antimicrobial agent for their low toxicity

different concentration of used plants was expressed at varying degrees. In this study used five different species of bacteria.Many Sudanese population were used Medicinal plants

as antimicrobial treatment and get true improvement of diseases conditions without Harmful side effects. Theresults of the present study were hopeful, as the *Lawsoniainermis* show antimicrobial activity against five different species of bacteria. Here I used various concentrations of the extract include (10%, 20% and 40%). It is estimated that if an inhibition is obtained by (10 -40%) of extract concentration, it considered as valuable plant.

The extract of *lawsoniainermis* was more active (Inhibition zone up to 22.9 mm) against isolates and standard bacteria. Methanolic extract of *lawsoniainermis* provide high activity against *E.coli* ATCC 25922 (Zone diameter of inhibition (ZDI) 22.8 mm) followed by *P.mirabilis* ATCC 49565 (ZDI = 21 mm), *K.pnemoniae* ATCC 35657 (ZDI = 20.5 mm), *S. aureus* ATCC 25923 (ZDI = 20 mm) and *P.aeurginosa* ATCC 27853 (ZDI = 19.85 mm). Also Ethanolic extract show high activity against *E.coli* ATCC 25922 (ZDI = 22.9 mm) followed by *P.mirabilis* ATCC 49565 (ZDI = 21.5 mm), *K.pnemoniae* ATCC 35657 (ZDI = 20.5 mm), *S. aureus*ATCC 25923 (ZDI = 20 mm) and *P.aeurginosa* ATCC 27853 (ZDI =15.5 mm). While boiling water extract provide activity against *E.coli* ATCC 25922 (ZDI = 22.8 mm) followed by *S. aureus* ATCC 25923 (ZDI = 20 mm), *K.pnemoniae* ATCC 35657 (ZDI = 19.9 mm), *P.mirabilis* ATCC 49565 (ZDI = 19 mm) and *P.aeurginosa* ATCC 27853 (ZDI = 16 mm).

Also findings of the present study have similarity to the study done by of Hussain *et al*. (2011) which show that Lawsoniainermis has antimicrobial activity against some gram positive and gram negative bacteria such as *S. aureus*, *E. coli*, *Klebsiellapneumoniae, Pseudomonas aeruginosa* (Hussain *et al*., 2011). In addition Kannahi and Vinotha (2013) stated that The activity of methanol extracts of Lawsoniainermis leaves against *Staphylococcus aureus* showed minimum activity (2.3±2.01mm) at 25 % concentration and maximum activity (9.3±8.9mm) at 100% level. The activity of methanol extracts of *Lawsoniainermis* against *pseudomonas aeruginosa*, showed maximum activity was obtained at 75% (4.6±3.16mm) followed by 100% (3.3±2.16mm), 50% (2.8±2.4mm) and 25% (2.6±2.1mm).

The ethanol extracts of *Lawsoniainermis* leaves against *Staphylococcus aureus* showed minimum activity (3.1±3.21mm) at 25% concentration and maximum activity (8.1±6.2mm) at 100% level. The ethanol extracts of *Lawsoniainermis* leaves against *pseudomonas aeruginosa* showed minimum activity (9.1±8.61mm) an 25% concentration and maximum activity (7.6±6.41mm) at 100% (Kannahi and Vinotha, 2013). On the other hand Ethanolic extract of *Camelliasinensis* appear high activity against *S.aureus* ATCC 25923 (ZDI = 24 mm) followed by *K.pneumoniae* ATCC 35657 (ZDI = 19.8 mm), *E.coli* ATCC 25922, *P.mirabilis* ATCC 49565 (ZDI = 18 mm each) and *P.aeurginosa* ATCC 27853 (ZDI = 16 mm). whereas Boiling water extract show high activity against *S.aureus* ATCC 25923 (ZDI = 21.6 mm) similar to finding of study done by Abdul Majid *et al.* 2013 which clarify in vitro antibacterial activity of *Camelliasinensis* leaf extracts to some pathogenic bacteria (Abdul Majid *et al*., 2013).

Followed by *K.pneumoniae* ATCC 35657 (ZDI = 19.8 mm), *P.aeurginosa* ATCC 27853 (ZDI = 12.8 mm) and *E.coli* ATCC 25922 (ZDI = 9 mm), whilst there is non-activity against *P.mirabilis* ATCC 49565 (ZDI = 0 mm). Whereby Methanolic extract provide high activity against *E.coli* ATCC 25922 (ZDI = 21 mm) followed by *S.aureus* ATCC 25923 (ZDI = 14.4 mm), *P.aeurginosa* ATCC 27853 (ZDI = 12.8 mm), *K.pneumoniae* ATCC 35657 (ZDI = 11.6 mm) and *P.mirabilis* ATCC 49565 (ZDI = 10.8 mm). In the peer side the antibiotics show various range of activity against different used bacteria. Amikacin (30µg) found to be the more active against all strain of both standard and isolates bacteria. It provided high activity against *P.mirabilis* ATCC 49565 (ZDI = 36 mm) followed by *E.coli* ATCC 25922 (ZDI = 35 mm), *S. aureus* ATCC 25923 (ZDI = 31 mm), *P.aeurginosa* ATCC 27853 (ZDI = 30 mm) and *K.pnemoniae* (ZDI = 22 mm). in contrast Ciprofloxacin (10mg) show the less activity as compared with other used antibiotics as it show high activity against *S. aureus* ATCC 25923 (ZDI = 35 mm), *P.mirabilis* ATCC 49565, *P.aeurginosa* ATCC 27853 (ZDI = 21 mm each) and no effect against *E.coli* ATCC 25922 and *K.pnemoniae*ATCC 35657 (ZDI = 0 mm).

Conclusion

The present study clarify antibacterial activities of *Lawsoniainermis* (henna) and *Camelliasinensis* (green tea) against some pathogenic bacteria. Therefore these results give hopeful baseline information for the potential use of the *Lawsoniainermis* and *Camelliasinensis* plants in the fight against pathogenic bacteria.

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