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ANTIMICROBIAL ACTIVITY OF *Lawsonia inermis (henna)* EXTRACTS

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ABSTRACT
Lawsonia inermis (henna) has been widely used over centuries for medication and cosmetics in some regions of the world. The leaves and seeds of henna are known for alleviating a number of skin diseases including fungal infections and cracks on feet. The plant parts were extracted using maceration method. The antimicrobial activities as well as phytochemical screening of aqueous, ethanolic and petroleum ether extracts of leaves of Lawsonia inermis against clinical isolates of Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Epidermophyton floccosum were investigated using the agar well diffusion method. The phytochemical screening showed the presence of saponins, flavonoids and steroids in all the extracts. All tested isolates were susceptible to all the extracts at 1000µg/ml. The highest activities were observed in the aqueous extract of the plant against Staphylococcus aureus and Epidermophyton floccosum (19mm). While the standard drugs active against the same isolates at 20 µg/ml with zones of 28mm and 17mm respectively. The findings of this study suggest that Lawsonia inermis could serve as a potential antimicrobial agent and provide the basis for isolation and identification of biologically active constituents in these extracts.
Key words: Lawsonia inermis, Henna, antimicrobial activity, clinical isolates, bacteria, fungi, Phytochemicals.

INTRODUCTION

The efficacies of many indigenous plants for several disorders have been described by practioners of traditional herbal medicines (Muhammad and Muhammad, 2005). These plants provide on interesting and still largely unexplored source in the creation and the development of potential new drugs for chemotherapy which might help to overcome the growing problem of resistance and the toxicity of the currently available synthetic antibiotics (Rahmoun *et al.,* 2013).

Lawsonia inermis Linn. (*Lythraceae*) or Henna is a biennial dicotyledonous herbaceous shrub. Being native of North Africa and South – West Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant (Gagandeep *et al.,* 2010). Henna has been used cosmetically and medicinally for other 9,000 years. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a wide variety of ailments which include rheumatoid arthritis, headache, ulcers, diarrhea, leprosy etc. The leaves are used in alleviating skin diseases (Gagandeep *et al.,* 2010). The traditional medicinal method, especially the use of medicinal plants, still plays a vital role to cover the basic health needs in the developing countries. Therefore, it is of great interest to carry out screening of these plants to validate their use in folk medicine. Hence, this research was aimed at evaluating the Antimicrobial activity of *Lawsonia inermis* extracts against some pathogens.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Lawsonia inermis* were obtained from Duzau village of Babura local government area of Jigawa State, Nigeria. The leaves were authenticated at the herbarium unit of the department of plant Biology, Bayero University, Kano with batch number BUKHAN 0379. The leaves were air dried and pulverized to fine powder using Mortar and pestle as demonstrated by Muhammad and Muhammad, (2005).

Extraction

The plant materials were extracted using percolation method as described by (Fatope *et al.,* 1993) with slight modifications. Briefly, 30g of *L inermis* powder were soaked in 150ml of water, ethanol, and petroleum ether respectively for 7days at ambient temperature, followed by filtration using Whatman No. 1 filter paper and evaporated using rotary evaporator. The extracts were further dried in hot air oven at 50^{o}C for 24h and finally kept at 4^{o}C until further use.

Test Organisms

Stock cultures of *S. aureus, P. aeruginosa, E. floccosum* and *C. albicans* were obtained from Bela Dermatology hospital Kano Nigeria. Nutrient broth was used for growing and diluting the bacterial suspension at 37^{o}C for 24h. While the fungal suspension were cultured on Sabouraud's dextrose agar medium and then incubated at 25^{o}C for 7 days to obtain inocula for testing.

PREPARATION OF STOCK SOLUTION

One gram of each of the recovered extracts was weighed using electronic weighing balance and dissolved in 1ml of DMSO in which four different concentrations of different solvent extracts were prepared for sensitivity testing (i.e. 1000ug/ml, 500ug/ml, 250ug/ml and 125ug/ml) using serial dilution in a sterile bijou bottles.

Phytochemical analysis of plant extracts

The extracts of *Lawsonia inermis* prepared in the present study were screened for phytochemicals including Alkaloids, Flavonids, Saponins, Steroids and Tannins, by phytochemical analysis as below (Sofowora, 1993).

i- Alkaloids: To 3ml of the extract lml of 1% Hcl was added. This resulting mixture was then treated with few drops of mayers reagent the appearance of a white precipitate confirmed the presence of alkaloids (Sofowora, 1993)

ii- Flavonoids: To lml of the extract 3 drops of ammonia solution (NH_3^+) was added followed by 0.5ml of concentrated Hcl. The resultant pale brown colouration of the entire mixture indicated the presence of Flavonoids (Sofowara, 1993).

iii- Saponins : Five drops of olive oil was added to 2ml of the plant extract and the mixture shaken vigorously. The formation of stable emulsion indicated the presence of Saponins (Sofowora, 1993).

iv- Tannins
Two drops of 5% $fecl_3$ was to lml of the plant extract. The appearance of a dirty green precipitate indicated the presence of Tannins (Sofowora, 1993).

v- Steroids: 1ml of concentrated H_2S0_4 was added to lml of the test extract. A red colour indicated positive test (Sofowora, 1993).

Bioassay

The sensitivity test was done using agar well diffusion method as described by (Al-Mahmood, 2009)/. Six 6mm wells were made on nutrient agar (Bacteria) and Sabouraud Dextrose gar (fungi) using sterile cork borer. A loopful of test bacterial and fungal inoculums were streaked on the surface of the agar plates as appropriate. Ciprofloxacin (0.1ml of 20µg/ml) and Ketoconazole (0.1ml of 20µg/ml) were included for the bacterial and fungal plates respectively as controls. Thereafter the wells were filled with 0.1ml of *L. inermis* extracts (1000µg/ml, 500µg/ml and 125µg/ml). The plates were incubated at 37^{o}C for 24h for bacteria. While the fungal plates were incubated at 25^{o}C for 10 days. The diameter of zone of inhibition was measured in millimeter using ruler.

Determination of Minimum inhibitory concentration (MIC)

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution using dimethyl sulfoxide (DMSO) and incorporated into test tube containing 2ml nutrient broth. Specifically 0.1ml of standardized inocula was added to each test tube and incubated at 37^{o}C for 24hrs. The lowest concentration of extracts that produced no visible growth (turbidity) was recorded as MIC. Tubes containing broth and extracts without inocula save as positive control while tubes containing broth and inocula without extracts served as negative control (Fatope *et al.*, 1993).

RESULTS

The phytochemical constituents of all the 3 extracts of *Lawsonia inermis* are summarized in Table 1. The result reveals the presence of medicinally active constituents. Flavonoids,Saponins and Steroids were present in the extracts while Alkaloids and Tannins were absent.

The antimicrobial activity of the leaves extracts of *Lawsonia inermis* against bacterial and fungal isolates tested are shown in Table 2. At a concentration of 1000 µg/ml the aqueous extract exhibited the highest antimicrobial activity against *S aureus* (19mm) and *Epidermophyton floccosum* (19mm) followed by ethanolic and petroleum ether extracts at different concentrations.

The minimum inhibitory concentrations of *Lawsonia inermis* extracts were moderate (125 µg /ml) for all tested isolates (aqueous and ethanolic extracts).Whereas it was maximum (500 µg/ml) for all the test isolates with the petroleum ether extract except for *Epidermophyton floccosum* (125 µg /ml).

Table 1: Phytochemical constituents of different extracts of *Lawsonia inermis*

Phytochemicals	Aqueous	Ethanol	Pet ether
Alkaloids	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	-	-	-

Key:
(+) Indicate presence
(-) indicate absence

Table 2: Antimicrobial activity of *Lawsonia inermis* extracts against clinical isolates

Test isolates	Extract	Concentration	Zone of inhibition(diameter mm)	control 20µg/ml	CPX/KET
S. aureus	Aqueous	125 µg/ml	8		
		250 µg/ml	11	28	
		500µg/ml	15		
		1000 µg/ml	19		
	Ethanol	125 µg/ml	8		
		250 µg/ml	10	28	
		500µg/ml	12		
		1000 µg/ml	13		
	Pet ether	125 µg/ml	7		
		250 µg/ml	7	28	
		500µg/ml	8		
		1000 µg/ml	9		
P. aeruginosa	Aqueous	125 µg/ml	7		
		250 µg/ml	10	18	
		500µg/ml	11		
		1000 µg/ml	15		
	Ethanol	125 µg/ml	7		
		250 µg/ml	9	18	
		500µg/ml	11		
		1000 µg/ml	13		
	Pet ether	125 µg/ml	7		
		250 µg/ml	9	18	
		500µg/ml	13		
		1000 µg/ml	15		
C. albicans	Aqueous	125 µg/ml	8		
		250 µg/ml	12	20	
		500µg/ml	14		
		1000 µg/ml	16		
	Ethanol	125 µg/ml	7		
		250 µg/ml	12	20	
		500µg/ml	14		
		1000 µg/ml	16		
	Pet ether	125 µg/ml	7		
		250 µg/ml	9		
		500µg/ml	14	20	
		1000 µg/ml	16		
E. floccosum	Aqueous	125 µg/ml	8		
		250 µg/ml	15	17	
		500µg/ml	17		
		1000 µg/ml	19		
	Ethanol	125 µg/ml	10		
		250 µg/ml	12	17	
		500µg/ml	14		
		1000 µg/ml	15		
	Pet ether	125 µg/ml	9		
		250 µg/ml	10	17	
		500µg/ml	15		
		1000 µg/ml	18		

Table 3: Minimum inhibitory concentration (MIC) of *Lawsonia inermis* Extracts

MIC (µg/ml) Test isolates	Aqueous	Ethanol	Pet ether
S. aureus	125µg/ml	125µg/ml	500µg/ml
P. aeruginosa	125µg/ml	125µg/ml	500µg/ml
C. albicans	125µg/ml	125µg/ml	500µg/ml
E. floccosum	125µg/ml	125µg/ml	125µg/ml

DISCUSSION

Three different extracts of *L. inermis* (aqueous, ethanol and pet ether) were phytochemically screened. The results confirm the presence of phytoconstituents (saponins, flavonoids and steroids) which are known to exhibit medicinal as well as physiological activity (Sofowora, 1993). While alkaloids and tannins were absent. In similar studies, Basiran *et al.*, 2012 detected the presence of Steroids,Flavanoids and Tannins in ethanolic extract. Also in 2012, Jeyaseelan *et al.*, demonstrated the presence of Tannins, Terpenoids, Flavanoids and Glycosides in ethanolic extract of *L. inermis*. Plants are generally regarded as active against both fungi and bacteria when zone of inhibition is greater than 6mm (Muhammad and Muhammad, 2005).

The results of the antimicrobial activity of *L. inermis* revealed that all the extracts exhibited antimicrobial activity against all the isolates used in the study. However, isolates showed differential sensitivity to each extract. The aqueous extracts showed higher bioactivity against the test isolates. Maximum inhibition was observed at 1000µg/ml against *S aureus* (19mm) and *Epidermophyton floccosum* (19mm) as compared to that of ethanol and petroleum ether extracts. These finding agrees with the result of the study conducted by Saadabi, (2007), where he evaluated the antimicrobial activity of Sudanese Henna extracts against bacterial and fungal isolates. It has been widely observed and accepted that phytochemical constituents mainly attribute to the antimicrobial activity of plants (Gull *et al.*, 2013).

It was observed that all the isolates showed minimum value (MIC) for aqueous ethanolic extracts which ranges from 125 to 1000µg/ml. The MIC values of this study were less than the MIC values reported by Gull *et al.*, (2013). The MIC values where in the range of 9.27mg/ml and 2.3mg/ml for *E. coli* and *B. subtilis* using acetone extract. Pandey and Kumar in 2011 also observed MIC values of 0.02mg/ml for ethanolic extract against *S aureus*, 0.38mg/ml for ethyl acetate extract against *S aureus*.

CONCLUSION

The present study revealed that *L. inermis* has antimicrobial activity against the bacterial and fungal isolates used in the study, especially on *S aureus* and *Epidermophyton floccosum*.This therefore indicates that *L. inermis* could serve as a potential source of biological antimicrobial agent.

Recommendations

The plant should be investigated further to evaluate the significance of these extracts, clinical role and the medical system of indigenous people. In addition, intense research is necessary to isolate and characterize their active components for pharmacological testing.

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