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Full Length Research Paper

Antistaphylococcal Activity of Henna extracts Lawsonia inermis L.(Lythraceae)

Ihsan Edan Abdulkareem Alsaimary

Department of Microbiolody, College of Medicine, University of Basrah, Iraq.

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Antibacterial activity of aqueous and alcoholic extracts of leaves of henna (Lawsonia inermis L.(Lythraceae)) against Staphylococcus aureus and Staphylococcus epidermidis isolated from clinical cases of acne vulgaris were carried out in this study. Alcoholic extracts were found to be more efficacious than aqueous extracts on staphylococci and Staph. Epidermidis was more susceptible to extract activity than Staph. aureus. The biggest diameter of inhibition zone (22) mm was recorded for 1000 µg /ml of aqueous extract against Staph. epidermidis. The range of minimal inhibitory concentration (MIC) for all concentrations was 200-700 µg/ml. In comparison with standard antibiotics, the vancomycin gave the biggest diameter of inhibition zone (28) mm and some antibiotics are not affected on staphylococci.

Keywords: *Staphylococcus aureus, Stap.epidermidis, henna, Lawsonia inermis*

INTRODUCTION

Plant materials are used throughout developed and developing countries as home remedies [1]. Many works have been done which aim at knowing the different antimicrobial and photochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant [2]. Moreover, antibacterial pharmaceuticals are not affordable to the majority of the communities in the developing countries [1].

An Increase in resistance calls for new antibacterial drugs, one source of which are traditional medicinal plants. Plants may provide a natural source of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally [3]. *Lawsonia Inermis* (*L. inermis*) is a scientific name of a tall shrub plant commonly known as Henna or Mehndi or mignonette tree belongs to the Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Myrtales, Family: Lythraceae, Genus: *Lawsonia*, Species: *L. inermis* [4]. Henna is a flowering plant, having a height of 5 meters, natal to subtropical and tropical regions of the world including South Asia, Africa, oases of Sahara Dessert and even in the northern regions of Australia. Leaves of the henna plant are entire, opposite, sub-sessile, oval-shaped and smooth [5]. The leaves have a length of 2–3 cm with 1–2 cm width. Henna shrub is highly branched and has greyish-brown barks. *Lawsonia inermis* (Henna) is used as a hair dye based on the staining properties of one of its constituents, e.g. Lawsone. Modified Henna products, such as Black Henna are also available to consumers.

The content of Lawsone among various modified Henna products may vary significantly, but these products contain some other substances for modifying the intensity of the color provided by Henna alone. *Lawsonia inermis* powder as a product of botanical origin is composed of various ingredients and cannot be examined per se for percutaneous absorption [6]. Consequently, it is necessary to identify and select a representative lead ingredient. Since Lawsone is an important ingredient and can be analyzed easily, it has been selected as

Corresponding Author: ihsanalsaimary@gmail.com

the lead ingredient. However, Lawsone is predominantly glycosidic bound and only a small amount is available in the plant powder. For practical and analytical reasons, Lawsone is therefore often added separately and mixed to the Lawsonia inermis powder, especially when the investigations were performed with a radiolabelled material. Main chemical constituents of henna are Lawsone (2-hydroxynaphthoquinone), mucilage, mannite, gallic acid and tannic acid [7]. Henna is known to be used as a cosmetic agent for dyeing hair, nails and skin [8]. In traditional medicine, henna plant is used to treat many diseases like edema, bronchitis, menstrual disorder, rheumatism, hemorrhoids and even in jaundice, leprosy, pain, spleen enlargement, dysentery and skin problems [1,2,9]. Henna can also be used as an astringent and antihemorragic agent and is also known for its hypertensive, cardio inhibitory and sedative effects [10]. In addition, henna is reported to show some other properties including hypoglycemic [11], immunostimulant , hepatoprotective, anti-inflammatory, tuberculostatic, anti-cancer and antioxidant properties [1,4,7,11,12].

The present research is designed to determine the Antistaphylococcal activity of leaves of *Lawsonia inermis* against certain skin staphylococci.

MATERIAL AND METHODS

L. inermis were collected from private gardens in Basra. The leaves were left to dry at room temperature for 24 hours. The dried leaves were ground to a powder and were kept in dry containers. Various concentrations (50 , 100 , 250 , 500 , 750 and 1000)mcg/ml (mcg mean µg(microgram)) were made from aqueous and alcoholic (ethanol 100%) extracts of leaves. All techniques of plant extraction were carried out according to (alsaimary , 1999,2002)[13,14]

The antibacterial effects of henna extracts *Staphylococcus aureus* (coagulase–positive staphylococci (CoPS), *Staphylococcus epidermidis* (coagulase–negative staphylococci (CoNS), were studied. These bacteria were isolated from patients with acne vulgaris of both sexes under 20 years who attended the Dermatology outpatient clinic in Basra teaching Hospital. Identification of bacterial types was carried depending on routine laboratory techniques. Determination of the inhibition zones (mm) and minimum inhibitory concentrations (MIC). The inhibition zones (mm) of L. inermis (henna) extracts were assessed using the Agar diffusion dilution method.

Muller Hinton agar was used with different diluted extract concentrations. 0.1 ml containing 10^5 CFU /ml (0.5 McFarland) was spread on the agar as described in NCCLS-2000[15] and minimum inhibitory concentrations (MIC) (mcg/ml) of plant extracts was carried by using brain heart infusion(Difco) by tube dilution method. Tetracycline, Ampicillin, Gentamicin, cephalexin, cloxacillin and vancomycin antibiotics were used in this study to evaluate the antibacterial efficacy of *L. inermis* (henna) extracts.

The statistical differences were tested by analysis of variance (ANOVA) for each treatment and using of chi square. The analysis was carried out using SPSS program ver. 17.

RESULTS

Staph.aureus and *Staph.epidermidis* were isolated from acne vulgaris cases as -predominant bacterial pathogens. All results are shown in tables 1, 2 and 3.

The results found that alcoholic extracts are more efficacious than the aqueous extract of henna leaves, and the highly effects associated with increase of extract concentrations with statistical differences between treatment. P< 0.05.

The biggest diameter of inhibition zone was 22 mm recorded for 1000 mcg/ml of alcoholic extract against *Staphylococcus epidermidis* followed by 20 mm against *Staphylococcus aureus*. Aqueous extract was less efficacious on *Staphylococcus aureus* and recorded 17 mm against *Staphylococcus epidermidis*.

The ranges of minimal inhibitory concentrations were between (400-700) **µ**g/ml and (200-300) **µ**g/ml for aqueous and alcoholic extracts, respectively with highly statistical differences between both MICs. P<0. 05 .

In comparison with standard antibiotics we can illustrate the results as follows:

Vancomycin (24, 28 mm), gentamicin (20 , 20 mm), cephalexin (16,20 mm), cloxacillin (14 , 18 mm), tetracycline (0,16) and ampicillin not affected, on *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively with statistical differences between each antibiotics. P<0.05.

DISCUSSION

Henna has many traditional and commercial uses, the most common being as a dye for hair, skin and fingernails, as a dye and preservative for leather and cloth, and as an anti-fungal. Henna body art is made by applying henna paste to the skin: the lawsone in the paste migrates into the outermost layer of the skin and makes a red-brown stain. Some pastes have been found to include: silver nitrate, carmine, pyrogallol, disperse orange dye, and chromium. These have been found to cause allergic reactions, chronic inflammatory reactions, or late-onset allergic reactions to hairdressing products and textile dyes.[16] Henna contains Lawsone in about 0.5 to 1.5% of its ingredients.

Lawsone (2-hydroxynapthoquinone) is the principal constituent responsible for the dyeing properties of the plant. However, henna also contains mannite, tannic acid, mucilage and gallic acid.[17] These substances are present in henna in the form of a mixture. Antimicrobial activity may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive. Water extracts did not show any antibacterial activity compared to alcoholic and oily extracts. This may be due to the lack of the solvent properties which plays an important role in antibacterial efficacy.[18,19,20]

The alcoholic extract showed the highest MICs compared to other types of extracts and this may be due to the large quantity of active substances that were precipitated during the extraction process due to the effect of the alcoholic extract itself. When compared with antibiotics, alcoholic henna extracts showed more antimicrobial activity and similar antibacterial activity compared to those of antibiotic. We concluded that henna has an in-vitro antibacterial activity against the tested bacterial strains. These findings have also been mentioned in literatures.[21-24]

Medicinal and herbal plants have been used as remedies for human diseases for centuries; this is because they contain components with therapeutic properties in their parts. The antibacterial effect of several plant extracts has been proven previously [11,12,16]. In this study, henna leaves and roselle calyxes extracted by either water or ethanol increased the inhibition zone on all of the tested bacteria; this result

Table.1: Antistaphylococcal activity of various concentrations and determination of minimal inhibitory concentrations (MICs) of aqueous extract of henna (*Lawsonia inermis*) on staphylococci species and determination of minimal inhibitory concentration (MIC).P<0.05

Staphylococci species	Diameter of inhibition zones (mm) for various aqueous extract (mcg/ml)						MIC µg/ml
	50	100	250	500	750	1000	
Staphylococcus aureus	NE	NE	NE	NE	NE	NE	600-700
Staphylococcus epidermidis	NE	NE	NE	NE	10	17	400-500

Table.2: Antistaphylococcal activity of various concentrations and determination of minimal inhibitory concentrations (MICs) of alcoholic extract of henna (Lawsonia inermis) on staphylococci species and determination of minimal inhibitory concentration (MIC). P<0.05

Staphylococci species	Diameter of inhibition zones (mm) for various alcoholic extract (mcg/ml)						MIC µg/ml
	50	100	250	500	750	1000	
Staphylococcus aureus	NE	NE	NE	NE	NE	NE	600-700
Staphylococcus epidermidis	NE	NE	NE	NE	10	17	400-500

Table.3: Diameters of inhibition zones of standard antibiotics against Staph.aureus and Staph.epidermidis. P<0.05

Staphylococci species	Diameter of inhibition zones (mm) for standard antibiotics					
	ampicillin	vancomycin	cloxacillin	tetracyclin	gentamicin	cephalexin
Staphylococcus aureus	NE	24	14	NE	20	16
Staphylococcus epidermidis	NE	28	18	16	20	20

confirmed their antibacterial activity. This is consistent with previous studies which concluded that the extracts of *L. alba* (henna) and *H. sabdariffa* (roselle) were shown to have promising antibacterial properties [4,5,6,9]. Some studies suggested that henna has a wide spectrum of antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activities. With the ever increasing resistant strains of microorganisms to the already available and synthesized antibiotics, the naturally available henna could be a potential alternative [25,26].

Antimicrobial activity may be due to numerous free hydroxyl ions that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive[27]. On the other hand, this study showed that ethanolic extract is more efficient than water extract for henna. This result is consistent with [28] who found slight higher inhibition of the roselle ethanol extract against *Bacillus subtilis* and *Staphylococcus aureus* than that of water extract. Also, antibacterial activity of henna extracted by alcohol or oil was more effective than that extracted by water[29]. This may be due to the lack of solvent the solvent properties which plays an important role in antibacterial efficacy [30,31].

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