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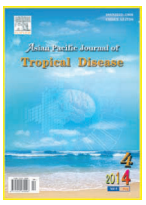

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Effect of henna and roselle extracts on pathogenic bacteria

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PEER REVIEW

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Comments

The study is good whereas the authors isolated the pathogens from wastewater and they tested the antimicrobial activity of water and ethanol extracts from henna and roselle against some pathogens. The results revealed that ethanolic extract had more antimicrobial activity than water extracts. Moreover, the ethanolic extracted from roselle showed highest antibacterial activity against all tested pathogens than the ethanolic extracted from henna.
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ABSTRACT

Objective: To investigate the antibacterial effects of water and ethanolic extracts of henna leaves and roselle calyxes against pathogenic bacteria isolated from domestic wastewater.
Methods: The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial activities of extracts (2.5%, 5.0% and 10.0% w/v) of both henna and roselle were tested against one Gram-positive *Bacillius subtilis*; two Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* human pathogenic bacteria.
Results: Ethanolic extracts had more antimicrobial activity than water extracts. Ethanolic extract of roselle had the highest antibacterial activity against all tested organisms, followed with ethanolic extract of henna. *Pseudomonas aeruginosa* was the most sensitive bacteria to plant extracts.
Conclusion: The results of this study suggested that roselle contains more phyto-chemicals with antimicrobial activity than henna on the bacteria strains under study, and these phyto-chemicals were more effective when extracted by ethanol rather than water.

KEYWORDS

Antimicrobial activity, Henna, Roselle, *Bacillius subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*

1. Introduction

Henna, *Lawsonia alba* Lam. (*L. alba*), is a medicinal plant belonging to the family Lythraceae. Powdered leaves of henna is commonly used as cosmetic for staining palm, hands, hairs and other body parts[1,2]. Lawsone, 2-hydroxy 1,4-naphthoquinone, is the chief coloring component of henna leaves which acts as a substantive dye for keratin and imparts orange color due to the presence of hydroxyl group in naphthoquinone structure[3,4]. Henna is found to have several pharmacological uses such as antitumor, anthelmintic, antioxidant, immunomodulatory, burn wound healing, UV protective, and antimicrobial properties[5-9]. Henna is a natural product with low health risk potential[10-12]. Leaves of henna contain major phyto-chemicals such as glycosides, phytosterol, steroids, saponins, tannins and flavonoids. Flavonoids and glycosides are commonly known to posse antimicrobial activity[13].

Roselle (*Hibiscus sabdariffa* L.) (*H. sabdariffa*) belongs to the Malvaceae family[14]. Roselle calyx is a famous source

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of anthocyanins which are the largest group of water-soluble pigments in plants. Anthocyanins are highly appreciated in the food industry for their coloring properties, which can give foods various hues of red and violet[15-17]. Also, there are several reports on the benefits of Roselle calyxes as an excellent source of natural antioxidants[18]. The extracts of calyx are also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever[19,20]. In addition to, roselle extracts are reported to have antimicrobial activity against different pathogenic microorganisms[21-23].

Bacteria are the most abundant group of organisms, and bacterial pathogens are the major source of disease and mortality to human populations, worldwide[24]. Treatment by antibiotics to face such pathogens is facing major challenges as bacteria are continued to evolve resistance to the synthesized antibiotics[25-27]. Nowadays, with taking into the account the side effects of chemical antibiotics, the use of plant extracts as pharmaceutical purposes is increased. In this study, we investigate the antimicrobial activity of henna and roselle extracted by two types of solvent (water and ethanol) against different pathogenic bacteria; *Bacillius subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*).

2. Materials and methods

2.1. Plant materials and extractions preparation

The plant materials used in this study were leaves of henna (*L. alba*) and calyxes of roselle (*H. sabdariffa*). The plant materials were collected from plants grown in the Agricultural Experimental Farm, Faculty of Agriculture, South Valley University, Qena, Egypt. The leaves and calyxes were left to dry at room temperature for 48 hours. The dried materials, henna leaves and roselle calyxes, were ground to a fine powder with an electric mill, then the materials were sterilized under UV-lighting for an hour. Two types of extract were prepared in this study; alcoholic and water-based extracts. The alcoholic extracts were prepared by mixing plant materials with 95% ethanol for four hours at concentrations of 2.5%, 5.0% and 10% (w/v). The extracts were filtered through filter paper (Whatmann No. 1) and the filtrates were collected for further using. Water based henna extract was prepared in the same way except that distilled water was used instead of alcohol. All tools used for the extraction were sterilized to avoid contamination.

2.2. Pathogenic bacteria

Microbial strains of *B. subtilis* (Gram positive), *E. coli*, and *P. aeruginosa* (Gram negative) were isolated from wastewater and used throughout the study. Wastewater samples were collected from South Valley University. The microorganism species were isolated and grown in nutrient broth medium containing (peptone 10 g/L and yeast extract 5 g/L at 37 ° C). The cells were grown in a 100 mL Erlenmeyer flask in a shaking incubator at 300 r/min at 37 °C.

2.3. Antimicrobial assay

The antimicrobial activity of investigated extracts was determined using Agar diffusion dilution method. Nutrient agar was used with different diluted extract concentrations (10-0.03 μg/mL). 0.1 mL containing 10^5 CFU/mL (0.5 McFarland) was spread on the agar as described in[28,29]. Discs of 5 mm in diameter were made using filter paper Whatmann No. 1. The discs were soaked in the prepared extractions for two hours, and then two discs were applied in Petri dish and incubated for 48 h at 37 °C. After the incubation period, the antimicrobial activity was evaluated by measuring the diameter of inhibition zone including the disc.

2.4. Statistical analysis

The statistical differences were tested by analysis of variance (ANOVA) for each treatment. The analysis was carried out using JMP (version 4; SAS Institute, Cary, NC, USA).

3. Results

3.1. Diagnosis of bacterial isolates

After isolating bacteria from wastewater, diagnosed bacteria was grown on nutrient agar for 24 h depending on the characteristics of the colony such as cell shape and biochemical characteristics (gram stain, oxidase, catalysis and dyes), as shown in Table 1.

Table 1
Characteristics of different bacterial isolates; *B. subtilis*, *E. coli* and *P. aeruginosa*.

Bacteria type	Cell shape	Biochemical characteristics			
		Gram stain	Oxidase	Catalysis	Dyes on media
B. subtilis	Rod-shaped	+	-	+	Gray/yellow
E. coli	Rod-shaped	-		+	
P. aeruginosa	Rod-shaped	-	+	+	Yellow/green