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Antimicrobial Activity of Methanol, n-Hexane and Dichloromethane Extract of *Portulaca oleracea*

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Abstract

Portulaca oleracea (Family: Portulacaceae). The antimicrobial activity was performed by disc diffusion method by determining the zone of inhibition of living microorganisms. The methanolic extract showed significant to mild antimicrobial activity than n-hexane and dichloromethane in compared with the standard drug Amoxicillin. The present study we used gram-positive (*Klebsiella pneumoniae* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*; *Shigella boydii* and *Pseudomonas aeruginosa*). The present results support the use of this plant in the treatment of diseases like abscesses, bilious conditions, and coughs in traditional medicine and also suggest the possibility of isolating antibacterial agents from *P. oleracea*.

Keywords: *Portulaca oleracea;* Portulacaceae; Microorganisms; Antimicrobial activity; Amoxicillin

Introduction

Portulaca oleracea L. (Portulacaceae) is a summer annual which is grown in several parts of Bangladesh. Annual succulent, glabrous, prostrate or ascending plant, 10-40 cm high; very much branched from the base. Leaves alternate, fleshy, obovate or spathulate with a cuneate base and obtuse apex, smooth and waxy on an upper surface, margins sometimes purple; sessile or indistinctly petiolate, 1-2(4)cm long,0.5-1(1.5) wide. Flowers solitary or clustered, axillary or terminal, surrounded by 2 glabrous bracts; 2 unequal sepals, 5 glabrous yellow petals, stamens 6-15. Fruit brown rounded capsule, 6-8(10) mm long, opening at top with lid. Seeds numerous, small, 0.8 mm broad, reniform and black in color.

It is used in Iranian folk medicine as a diuretic, vermifuge, antiscorbutic, antitussive, analgesic and gastro esophageal reflex [1]. In traditional medicine, this plant is utilized as anti-vomiting, antibleeding, anti-hepatitis and in the treatment of gastric mucosal diseases [2]. Recent pharmacological activity showed that it possesses anticonvulsive, analgesic and anti inflammatory, hepatoprotective, antifungal and muscle relaxant effects [3-7]. Our recent study is to evaluate the cytotoxicity and antimicrobial activity of *P. oleracea.*

Materials and Methods

Plant Collection and Extraction

P. oleracea collected from a personal garden in Bogra, Bangladesh. The specific identification and authentication of *P. oleracea* plant for the present study identified by the Scientific Officer of Bangladesh National Herbarium which situated at Mirpur in Dhaka, Bangladesh. The verified voucher specimen Code (DACB: 37936) deposited at

Herbarium center for further references. The dried *P. oleracea* leaves (250g) soaked into 900mL n-hexane, Dichloromethane, and methanol. During extraction process that soaked plant stirring with glass rod for every 18 h interval. The crude extract analyzed for the antimicrobial activity.

Preparation of Medium

The calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25°C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. The pressure at 121°C for 20 minutes. The slants were used for making the fresh culture of microorganisms that were in turn used for sensitivity study.

Test Organisms

The microbial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram-positive and gram-negative organisms were taken for the test and they are listed in the (Table 1).

Gram Positive Bacteria	Gram Negative Bacteria
Klebsiella pneumoniae	*Escherichia coli*
Staphylococcus aureus	*Shigella boydii*
-- -- -- --	*Pseudomonas aeruginosa*

Table 1: Gram positive and gram-negative organisms were taken for the test.

Preparation of the Test Samples

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The microbial suspension was immediately transferred to the sterilized petri dishes. The petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

Preparation of Disc

The measured amount of each test sample was dissolved in the specific volume of solvent (chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried (Table 2).

Plants	Sample	Dose (µg/disc)
Portulaca oleracea	n-hexane extract of the Plants	500
	Dicholoro methane extract of the Plants	500
	Methanolic extract of the Plants	500

Table 2: Preparation of sample discs.

Standard Ciprofloxacin (30 µg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for the comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

Antimicrobial Screening

The anti-bacterial screening of an agent is essential to ascertain its spectrum against various types of pathogenic organisms. The antimicrobial potency of the plant can be visualized by antimicrobial screening which measures the ability of a test sample to inhibit the *in vitro* microbial growth by Disc diffusion method. Dried and sterilized filter paper discs (6mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (kanamycin) discs and blank discs are used as the positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry, 1976). The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as the zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of the zone of inhibition expressed in millimeter [8].

Determination of the Zone of Inhibition

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test microorganisms [9]. The plates were then kept in a

refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours. After incubation, the antimicrobial activity of the test materials was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Results and Discussion

Medicinal plants have used from the ancestor as the antimicrobial agent in several parts of Bangladesh [10]. The antimicrobial activities of n-hexane, dichloromethane and methanolic extracts of the Plants of *P. oleracea* were examined in the present study (Table 3).

	MEL	DCMEL	NHEL	Amoxicillin
Gram positive bact.				
Klebsiella pneumoniae	8	7	6	17
Staphylococcus aureus	7	6	6	35
Gram negative bact.				
Escherichia coli	8	6	6	8
Shigella boydii	9	6	7	11
Pseudomonas aeruginosa	9	7	7	14

Table 3: Antimicrobial activity of test samples of *portulaca oleracea.*

NHEL: n-hexane extract of the Plants. (500 µg/disc)
DCMEL: Dichloromethane extract of the Plants.(500 µg/disc).
DCMEL: Dichloromethane extract of the Plants.(500 µg/disc).

Antibacterial effects of methanol plant extract against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in man and skin diseases. The effects of methanol extract more than n-hexane and Dichloromethane plant extract. The n-hexane and dichloromethane soluble extract of the Plants showed resistant to the tested microorganisms. In case of both gram positive and gram negative bacteria, methanol fraction increased the zone of inhibition compared with Amoxicillin. The crude methanolic extract of the plants showed mild antimicrobial activity against the growth of *E. coli* (8mm), *Shigella boydii* (9 mm), *Pseudomonas aeruginosa* (9 mm), *Klebsiella pneumoniae* (8 mm) when compared with the standard drug Amoxicillin (8-35mm).

Conclusion

This work has revealed further potentials of this plant in the area of pharmacology as an antimicrobial agent. As a result of the high antimicrobial activity, the extract of *P. oleracea* would be considered a safe antimicrobial agent.

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Competing Interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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