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Antibacterial Potential of Broccoli Extracts against *Pseudomonas aeruginosa*

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A B S T R A C T

Broccoli is an edible green plant in the cabbage family and possesses antibacterial activity against wide range of bacterial pathogens. In the present study antibacterial activity of Broccoli extracts in Methanol and Distilled Water was evaluated against skin infection causing *Pseudomonas aeruginosa*. Antibiotic susceptibility test showed that 100% *Pseudomonas aeruginosa* strains were found to be sensitive to Meropenem followed by 40% to Cefixime. However, all the strains of *Pseudomonas aeruginosa* were resistant to Amoxyclav, Carbenicillin and Piperacillin each. Antibacterial activity of Broccoli extracts showed that all *Pseudomonas aeruginosa* (100%) were resistant to Distilled Water extracts. However, Broccoli flower extract was effective against 60% *Pseudomonas aeruginosa* followed by stem extract and leaves extract 20% each. Phytochemical analysis of Broccoli extracts showed the presence of Flavonoids, Glycosides, Saponins, Steroids and Terpenoids.

Introduction

Broccoli is an edible green plant in the cabbage family whose large flowering head is eaten as a vegetable. The word *broccoli* comes from the Italian plural of *broccolo*, which means "the flowering crest of a cabbage", and is the diminutive form of *brocco*, meaning "small nail" or "sprout". Broccoli is often boiled or steamed but may be eaten raw. Broccoli is a cool-weather crop that does poorly in hot summer weather. The ingestion of appropriate amounts of vegetables is known to reduce the risk of certain diseases due to the presence of beneficial biological molecules. For example, it has been shown that broccoli has anti- obesity, anticarcinogenic, antidiabetic, antimicrobial, hepatoprotective, cardioprotective, gastro protective, anti-inflamatory, anti-amnesic and immunomodulatory properties (Yang and Zhang, 2011; Mahn and Reyes, 2012; Owis, 2015; Vinha *et al.*, 2015; Park *et al.*, 2016). These activities are attributed to the presence of bioactive compounds such as vitamin C, carotenoids, polyphenols, glucosinolates, sulforaphane and enzymes (e.g. peroxidases, cystine lyases), among others (Fahey *et al.*, 2002). In particular, the concentration of these chemicals in broccoli depends on cultivation parameters, processing methods and variety of the vegetable (Fraire- Cordero *et al.*, 2010). However, *Pseudomonas aeruginosa* is an opportunistic pathogen. The bacterium takes

advantage of an individual's weakened immune system to create an infection and has the ability to produce tissue-damaging toxins. *Pseudomonas aeruginosa* causes urinary tract infections, respiratory infections, dermatitis, soft tissue infections, and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. *Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics (Wu *et al.,* 2011). Therefore the present study was undertaken to evaluate the antibacterial activity of Broccoli extracts against skin infection causing *Pseudomonas aeruginosa*.

Materials and Methods

Sample collection

Broccoli vegetables were purchased from local market and rinsed with sterile distilled water for several times to remove dirt from it.

Preparation of extract of Broccoli

Broccoli flower, Broccoli stem and Broccoli leaves were cut into small pieces and subjected to air drying under shade for one week. After drying completely it was grinded to make powder of it. A 5 gm of each samples were dissolved in 50 ml of solvents i.e. in Methanol and Distilled water and allowed to stand for one week with frequent shaking. After one week the solvents were filtered through Whatman Filter Paper No. 1 and concentrated to dryness under reduced pressure in a rotary evaporator and stored in sterile vials at 4°C until used (Sibi *et al.,* 2013).

Test organisms

Skin infection causing *Pseudomonas aeruginosa* were collected from pathology laboratory in Nagpur and were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

Antibiotic sensitivity test

Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer *et al.,* 1966). Five different types of antibiotics were used in the study (Table 1). *Pseudomonas aeruginosa* strains were grown on nutrient agar at 37^0C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The discs were placed on each inoculated Hi-sensitivity agar plate. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Antibacterial activity of Broccoli extracts against *Pseudomonas aeruginosa*

Antibacterial activity of Broccoli extracts was performed by well diffusion technique. *Pseudomonas aeruginosa* strains were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi-Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Bauer *et al.,* 1966).

Phytochemical analysis

Phytochemical analysis of the solvent extracts of broccoli was performed by following standard procedures (Sofowara, 1993). In brief, 0.5 ml of extract was added with a drop or two of Mayer's regent by the side of test tube and the formation of white or creamy precipitate indicates presence of alkaloids. Adding 1 ml of extract with ammonia and conc. sulphuric acid and disappearance of yellow colour on standing indicates flavonoids. Formation of brown ring at interface by the addition 2 ml of glacial acetic acid followed by few drops of ferric chloride solution and 1 ml of conc. sulphuric acid to the extracts revealed the presence of glycosides. Adding few drops of neutral ferric chloride to the extract and development of dark green color indicates the presence of the phenolic compounds. Existence of froth formation during warming and vigorous shaking indicates saponins. Change of colour from violet to blue or green after the addition of 2 ml of acetic anhydride and sulphuric acid gives positive result for steroids. Appearance of brownish green or blue black coloration after adding 0.1% ferric chloride to the cooled extract indicates tannins. Addition of 2 ml of chloroform and 3 ml of conc. sulphuric acid to the extract and formation of reddish brown layer at the junction of two solutions confirms terpenoids.

Results and Discussion

The present study was conducted to evaluate the effect of extracts of Broccoli extracts on *Pseudomonas aeruginosa*. It showed the comparative study of an extracts and antibiotic Ampicillin as a positive control and negative control (Methanol and Distilled water) against strains of *Pseudomonas aeruginosa*. Antibiotic susceptibility test showed that 100% *Pseudomonas aeruginosa* strains were found to be sensitive to Meropenem followed by 40% to Cefixime. However, all the strains of *Pseudomonas aeruginosa* were resistant to Amoxyclav, Carbenicillin and Piperacillin (Table 2). Antibacterial activity of Broccoli extracts showed that all *Pseudomonas aeruginosa* (100%) were resistant to Distilled Water extracts. However, Broccoli flower extract was effective against 60% *Pseudomonas aeruginosa* followed by stem extract and leaves extract 20% each (Table 3). Phytochemical analysis of Broccoli extracts showed the presence of Flavonoids, Glycosides, Saponins, Steroids and Terpenoids (Table 4).

Table.1 Antibiotics discs used in the study

Sr. No.	Antibiotics	Concentration
1	Amoxyclav	30 mcg
2	Carbenicillin	100 mcg
3	Cefixime	5 mcg
4	Meropenem	10 mcg
5	Piperacillin	100 mcg

Table.2 Antibiotics Susceptibility test of *Pseudomonas aeruginosa*

Antibiotics	R		S	
	No	%	No	%
Amoxyclav	5	100%	0	0%
Carbenicillin	5	100%	0	0%
Cefixime	3	60%	2	40%
Meropenem	0	0%	5	100%
Piperaeillin	5	100%	0	0%

Table.3 Antibacterial Activity of Broccoli Extracts against *Pseudomonas aeruginosa*

Extracts	Solvents	R		S	
		No.	%	No.	%
Broccoli Flower	Methanol	2	40%	3	60%
	D.W	5	100%	0	0%
Broccoli Stem	Methanol	4	80%	1	20%
	D.W	5	100%	0	0%
Broccoli Leaves	Methanol	4	80%	1	20%
	D.W.	5	100%	0	0%
Negative control	Methanol	5	100%0	0	0%
	D.W	5	100%	0	0%
Positive control (Ampicillin)	Methanol	2	40%	3	60%
	D.W	5	100%	0	0%

Table.4 Phytochemical analysis of broccoli extracts

Phytochemicals	Methanol	Distilled Water
Alkaloids	-	-
Flavonoids	+	-
Glycosides	+	-
Phenols	-	-
Saponins	+	-
Steroids	+	-
Tannins	-	-
Terpenoids	+	-

Where, + = Present; - = Absent

In the present study antibacterial activity of Broccoli extracts in Methanol and Distilled Water was evaluated against skin infection causing *Pseudomonas aeruginosa*. Antibiotic susceptibility test showed that 100% *Pseudomonas aeruginosa* strains were found to be sensitive to Meropenem followed by 40% to Cefixime. However, all the strains of *Pseudomonas aeruginosa* were resistant to Amoxyclav, Carbenicillin and Piperacillin. Antibacterial activity of Broccoli extracts showed that all *Pseudomonas aeruginosa* (100%) were resistant to Distilled Water extracts. However, Broccoli flower extract was effective against 60% *Pseudomonas aeruginosa* followed by stem extract and leaves extract 20% each. Farzinebrahimi *et al.*, (2012) has reported the antibacterial activity of leaf extracts of broccoli against *Pseudomonas aeroginosa*. Phytochemical analysis of Broccoli extracts showed the presence of Flavonoids, Glycosides, Saponins, Steroids and Terpenoids. In previous studies, it has been reported that broccoli crude extracts have activity against clinically significant bacteria (Hu *et al.*, 2004; Owis, 2015).

In conclusion, the present study demonstrates antibacterial activity of Broccoli extracts against skin infection causing *Pseudomonas aeruginosa*. From the results, it would seem logical to predict that broccoli could prevent the ailments caused by skin infection causing *Pseudomonas aeruginosa*. Further, owing to its strong antibacterial activity, bioactive compounds from broccoli have scope for the possible use in pharmaceutical industries to stay away from skin infection causing *Pseudomonas aeruginosa*.

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