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Extraction of betalains from Red Beetroot (*Beta vulgaris L.*) and to evaluate its antibacterial potential against Extended-spectrum betalactamases producing isolates

D.Vijaya [1], *Dr. N. Thangaraj[2]

[1] *Department of Botany, Kandaswami Kandar's College, Velur, Tamilnadu- 638182.*
[2] *Associate professor, Kandaswami Kandar's College, Velur, Tamilnadu- 638182.*

Abstract

Scientists and consumers favored concentrating on natural colorants due to the emergence of health consequences of synthetic colorants that are used in foods for many years. As a result of their antimicrobial effect, interest in natural colorants raises with each passing day. Beetroot (*Beta vulgaris*) is the main source for the natural red dye. The main component of this extract is Betalains. The betalains have potential as a natural colorant and functional food for pharmaceutical purposes in food applications. In the present study, betalanins was separated from *Beta vulgaris* with ethanol and determine the antibacterial activity against various genera of ESBL isolates. Among them, highest zone of clearance was observed against to *E.feacalis* and second, most was *K.pneumoniae*. This might be the first report of antimicrobial activity of Betalains against Extended-spectrum β-lactamases (ESBLs) producing isolates.

Key words: *Beta vulgaris*, Betalains, ESBL, Antibacterial activity

INTRODUCTION

Plant derivatives have been used for centuries for therapeutic and prophylactic purposes. At present, approximately 80% of the world's population is estimated to rely on botanical preparations as medicines to meet their health needs (1 and 2). There are many natural plant products with antifungal, antibacterial and antiprotozoal activity that can be used systemically or locally. Currently, from one- quarter to one- half of all pharmaceutical products of higher plant origin in the world. Countries such as India meet the needs of medicinal sources primarily from plants to cure infectious diseases (2). Among the various plant products, pigments are one of the alluring features on the Earth.

Currently, in the food industry, cosmetics, pharmaceuticals, pigments of various types and forms have been used as additives or supplements (3). The need for natural colorants has increased as consumers become more aware of the toxicity of synthetic colours. Natural colors often referred to as biocolors, usually derived from vegetables, fruit, roots and microorganisms due to their biological source. Pigments act as bioactive agents against skin diseases and act as antioxidants, anti- inflammatory, anti analgesic and chemopreventive agents against cancer (4).

Among pigments with natural origin, Betalains represent a group of valuable products for the pharmaceutical, chemical, food and feed industries not only because they can act as agents of antioxidant and possible tumor-inhibiting activity through the removal of oxygen radicals. Betalains are water-soluble nitrogen-containing pigments, found in high concentrations in red beet (*Beta vulgaris*). Betalains consist of two subclasses: betacyanins (red-violet pigments) and betaxanthins (5 and 6).

There are many reports published about medicinal properties of red beet containing Betalains, but no reports about the antibacterial activity of Betalains against ESBL producing isolates. Taking into account the biological activities of beetroot as well as a possible potential of plant by-products, in this study, Betalains was used to screen antimicrobial activity to ESBL producing clinical isolates.

MATERIALS AND METHODS

Test pathogens

The clinical isolates of 6 bacterial genera were procured from a clinical laboratory, Coimbatore, India. All isolates were confirmed with cultural characteristic by selective media and biochemical tests.

Determination of ESBL producing isolates by phenotypic method

Confirmation of the ESBL producing isolates was done by the phenotypic confirmatory test according to CLSI recommendation. All test pathogens were inoculated into the nutrient broth and incubated at 37ºC for 24hrs. After incubation, each bacterial isolates were swabbed in to each Muller Hinton agar plate. The first generation of betalactam antibiotics i.e. Amoxcillin disc (30µg) alone and in combination with clavulanic acid (10 µg) were placed on the surface on the plate. After overnight incubation at 37^{o}C, diameter of zone of inhibition was measured. A 5 mm or more increases in diameter of zone of inhibition for Amoxcillin tested in combination with clavulanic acid versus its zone when Amoxcillin tested alone confirms a ESBLs producing organism (7).

Collection of beetroot and preparation

The fresh red beet roots were purchased from the local vegetable market and were washed thoroughly to remove the soil. The vegetable material was cut into slices and weighed. About 200 g of red beet was mixed in a blender with 1 liter of ethanol (acidified with 2% citric acid) for 15 min at room temperature and left for 24 hours. The extract was filtered and concentrated under vacuum by a rotary vacuum evaporator at 40º C. as reported by Francis (8) and which was further analyzed for its characteristics.

Determination of total betalains

The concentrated red beet was diluted with distilled water and measured at a wavelength of 535 nm and measured as mg betalains/100 g using the following equation as determined by Castellar *et al.*, (9).

Total betalains content (mg / 100 g) = $A \times DF \times MW \times 1000 / €L$

Determination of the antibacterial activity of betalains

This test was carried out according to the method of Jahir (10). The Mueller - Hinton agar plates were inoculated with freshly prepared overnight inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml of bacteria. The 6mm diameter of the well was made with borer on the agar plates. Different concentrations of betalains were filled in well with the help of micropipette. The ampicillin 5microgram/20 micro liter was added in one well as a standard, 100 micro liter of ethanol was added in another well which was served as a control. Incubate the plate at 37^{o}C for 24hrs, then observed the zone of inhibition.

RESULTS AND DISCUSSION

Antimicrobial resistance is currently a major concern in hospitals, public health systems and veterinary practices throughout the world. In the clinical sector, the ESBL production of isolates is a major problem and frequently caused a nosocomial outbreak. Scientific efforts are pursuing alternative therapies to tackle these MDR strains to overcome this burgeoning problem. In the present study, 7 bacterial genera of 17 isolates were procured from clinical laboratory and subjected to determination of ESBL characterization. Among them, all bacterial genera were showed positive for ESBL production (Table1 and Plate 1).

Based on the results of the phenotypical ESBL tests, ESBL-producing isolates were responsible for 53% of the enterobacteriaceae isolates that occurred in patients with malignancy in this study. Among them, *E.coli* and *K.pneumoniae* were predominant isolates. In recent years there has been an increased incidence and prevalence of ESBL isolates enzymes that hydrolyze and cause resistance to some antibiotics, for example, oxyimino-cephalosporins and aztreonam (11). In this study, gram positive isolate of *E.faecalis* also produced the ESBL character.

The peril factor for the colonization or infection with ESBL isolates is due to an extended hospital stay, intensive care unit admission, urinary and arterial catheterization and exposure to antibiotics including extended spectrum cephalosporins (12). Against the backdrop of this problem, urgently we need alternative thereby with a natural source. A number of studies have reported beetroot containing betalains to have high antioxidant and anti-inflammatory capabilities in vitro and a variety of in vivo animal models (13 and 14).

In 2014 Jasna *et al* (15) demonstrates that red beet root extracts exhibited antibacterial activity against gram positive and negative isolates. Jacob *et al.*, (16) were suggested that red beet containing betalains play a significant role as an antioxidant, antiviral, antimicrobial, hepatoprotective and anti-cancerous agent. In this present study, this has sparked interest in a possible role for beetroot in antimicrobial activity against clinical pathogens.

TABLE1. ISOLATION OF ESBL PRODUCING ISOLATES

S. No	Bacterial genera	No. of isolates	No. of positive
1.	*E.coli*	3	2
2.	*K.pneumoniae*	3	2
3.	*E.faecalis*	3	1
4.	*Proteus spp*	2	1
5.	*Acinetobacter baumannii*	2	1
6.	*P. aeruginosa*	2	1
7.	*Salmonella spp*	2	1
		17	**9**

TABLE 2 ANTIMICROBIAL ACTIVITY OF BETALAINS AGAINST ESBL PRODUCING ISOLATES

S.No	Bacterial genera	Con. of Betalains (mg)				Ampicillin	Ethanol
		1	2	4	8		
1.	*E.coli*	-	8.3±0.47	9.3±0.47	11±0.81	-	-
2.	*K.pneumoniae*	-	10.3±0.47	12.6±0.47	14.6±0.47	-	-
3.	*E .faecalis*	8.3±0.47	10.3±0.47	12.6±0.47	14.6±0.47	-	-
4.	*Proteus spp*	-	-	-	-	-	-
5.	*Acinetobacter baumannii*	-	-	8.3±0.47	11±0.81	-	-
6.	*P. aeruginosa*	-	-	-	8.3±0.47	-	-
7.	*Salmonella spp*	-	-	11±0.81	12±0.81	-	-

Data are means of three replicates (n = 3) ± standard error.

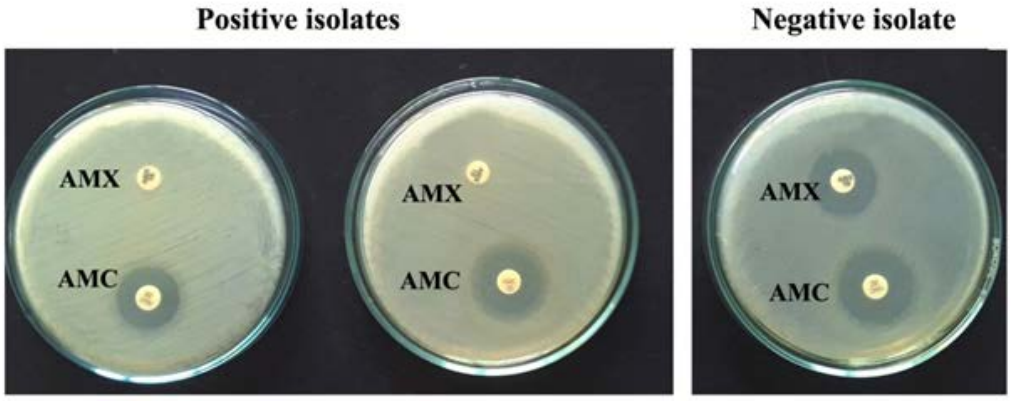

PLATE 1. ISOLATION OF EXTENDED -SPECTRUM BETALACTAMASE PRODUCING ISOLATES

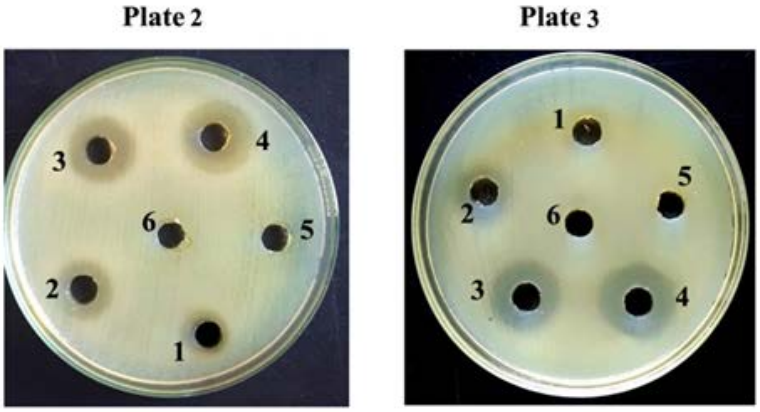

PLATE 2. ANTIBACTERIAL ACIIVITY OF BETALANINS AGAINST *Enterococcus faecalis*
PLATE 3. ANTIBACTERIAL ACIIVITY OF BETALANINS AGAINST *Klebsiella pneumoniae*
1-1milligram, 2-2-milligrams, 3-4 milligrams, 4-8milligrams, 5-Ampicillin, 6-Ethanol

Results of the extraction of betalains from red beet indicate that the content of total betalains was 156 mg / 100 g on fresh weights. The result obtained in general was in accordance with (17), who found that, 380 mg / 100 g of betalains on fresh weights. It was a low then previous study (18), who found that, the total betalains content of red beet was 250 to 850 mg/100g on fresh weight.

In vitro antibacterial activity of ethanol extract of betalain was evaluated by agar well diffusion method against 7 ESBL isolates (each genus), which were belongs to both Gram-positive and Gram-negative species. The results of the diameters of inhibition zones are shown in Table 2. While using ethanol extract, all isolates bacterial genera were suppressed except *Proteus spp*. The zone of clearance was ranged from 8.3±0.47mm to 14.6±0.47mm.

The highest zone of clearance was observed against to *E.feacalis* and second, most was *K.pneumoniae*. The zone of inhibition was started at even 1mg of betalain. On the other hand, a week antimicrobial activity was observed against *P. aeruginosa and Acinetobacter baumannii.* It is also important to note that these isolates were resistance to the ampicillin (Plate 2 and Plate 3). According to literature, no one demonstrate the antimicrobial activity of *Beta vulgaris* containing betalains, at same time Pawar *et al.*, (19) showed betalains extract of *Basella rubra* L fruits showed significant antimicrobial activity against *S.typhi.* In 2003 Strack and Vogt (20) suggested that Betalains are water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins and the yellow betaxanthins, they have antimicrobial and antiviral effects.

It can be clearly stated from the above experimental investigation that the beetroot containing betalains is a promising source of a natural antibacterial agent and certainly provides an alternative to synthetic colorant due to its beneficial properties and opens up a new aspect of the beetroot research trend as a natural antibacterial agents and viable food ingredient.

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