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ANTIBACTERIAL ACTIVITY OF *BRASSICA OLERACEA* L. *VAR. CAPITATA F. RUBRA* NATURAL DYE

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ABSTRACT
Objective: The present study has been designed to evaluate the *in-vitro* antibacterial activities of the natural dye of *Brassica oleracea* L. *var. capitata f. rubra*. **Methods:** Bacterial strains were subjected to antibiotic sensitivity testing by Kirby–Bauer's disc diffusion method. The antibacterial activity was calculated based on the zone of inhibition and activity index by using Muller–Hinton broth in a spread plate method. **Results:** The antibacterial analysis showed that the dye extract of *Brassica oleracea* L. *var. capitata f. rubra* inhibits growth of the bacteria. Natural dye of *Brassica oleracea* L. *var. capitata f. rubra* possessed highest antibacterial activity against *Staphylococcus aureus.* **Conclusions:** *Brassica oleracea*L. *var. capitata f. rubra*natural dye has the potential to be developed as antibacterial agents against some bacteria.

KEYWORDS: Antibacterial, activity index, *Brassica oleracea* L. *var.capitata f. rubra*, Kirby– Bauer's disc diffusion method and *Staphylococcus aureus.*

INTRODUCTION
Dyeing is an art of imparting colour to fabrics, food stuff, paper, leather, cosmetics, etc. Dyes can be obtained from both natural and synthetic sources. Synthetic dyes impart vibrant colours which are widely used. But in the recent decades, there is an increasing interest towards the natural dyes due to the toxic and allergic reaction associated with synthetic dyes. Many European countries have banned the use of numerous synthetic dyes. Germany was the first to take initiative to ban on numerous specific azo-dyes for their manufacturing and applications. Netherlands, India and some other countries also followed the ban (Patel, 2011).[1] A variety of harmonizing, gentle and soft natural colours can be obtained from different bio-sources. Plants are the major sources of these natural dyes. Natural dyes are considered as eco-friendly, non-toxic, medicinal properties and can recycled after use which is very important for maintaining environmental balance. Most of the natural dyes are safe and many of them have medicinal properties. There has been a growing interest in the investigation of the natural products from plants for the discovery of new antibacterial agent. It has been reported that the higher plants have shown as a potential source for the new antibacterial agents (Lalitha *et al.*, 2011).[2] Many of the dye yielding plants are classified as medicinal and some of them have antimicrobial effect.

Brassica oleracea L. *var. capitata f. Rubra* is a member of Brassicaceae family. It is a cool season cruciferous vegetable. *Brassica oleracea* L. *var. capitata f. rubra* is a type of cabbage, also well - known as red cabbage or purple cabbage and is widespread in the Mediterranean region. Red cabbage is a herbaceous, biennial, dicotyledonous flowering plant. The leaves of red cabbage are purple in colour and the pigment of Red cabbage has purple anthocyanin. These anthocyanins can be utilised as an excellent natural colorant and are dominant antioxidants that have anti-inflammatory properties which help to protect cells. So, the aim of this research work is to explore the antibacterial efficacy of natural dye of *Brassica oleracea* L. *var. capitata f. rubra.*

Materials and methods Preparation of Extraction
50g of fresh leaves of *Brassica oleracea* L. *var. capitata f. rubra* were weighed and cleaned with distilled water. They are cut into small pieces and grinded into paste with 100ml of distilled water. Then the mixture was stirred and filtered through Whatmann No.1 filter paper. The extract was concentrated with the help of rotary evaporator under few reduced pressure to yield semisolid mass which was dried in a desiccators and stored properly for further study.

Antibacterial Assay (Bauer and Kirby, 1966)[3]

The antibacterial potential of the *Brassica oleracea* L. *var. capitata f. rubra* dye extract is estimated by disc diffusion method. The disc diffusion is a simple and reliable test to find out the effect of plant extracts on pathogenic bacteria.

Source of Microbial Strains

The strains of common pathogenic microorganisms are used in this study such as *Proteus vulgaris*, *Bacillus megaterium, Pseudomonas aeruginosa* and *Staphylococcus aureus.* All the bacterial cultures are obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of Muller Agar Media

38g of Muller Hinton agar was dissolved in 1000ml of distilled water. The pH was adjusted to 7 and autoclaved for 30 minutes in 15lb pressure.

Preparation of Culture Plates

20ml of sterile Muller Hinton agar medium is poured into petriplates under sterile condition and kept in laminar air flow chamber for solidification. After solidification, the plates are dried for 30minutes in an oven to remove excess of moisture from the surface.

Preparation of Inoculums

Nutrient agar - 1gm
Bacteriological peptone - 0.5gm
Sodium chloride - 0.25gm
Distilled water -100ml

The above components are dissolved one by one in 100ml of distilled water and the pH was also adjusted to 7. 10ml of medium was poured into test tube and the mouth of the tube was covered with sterile cotton. The test tubes were autoclaved for 30minutes in 15lb pressure. After autoclaving, the test tubes were cooled in laminar air flow chamber and selected microorganisms were inoculated into the medium separately. The tubes were incubated overnight in 37ºC and used for inoculation.

Inoculation

The test microorganisms were inoculated in nutrient agar medium by spread plate method. About 10µl (106 cells/ml) of nutrient broth of overnight bacterial cultural was spread evenly on the solidification medium. Sterile cotton swabs were dipped separately into inoculums of organisms and swabbed inside the wall of the tubes. The agar surface of the plates was streaked in three directions by turning the plates to 60º angle between each streaking. The lid of the petriplates was on and kept at room temperature for 5-10 minutes to get confluent growth for accurate results.

Preparation and Application of Disc

Sterile discs of 6mm prepared by using Whatsmann No.1 filter paper. Various concentration of extract such as 30, 40, 50, 60 µg were dissolved in Dimethyl Sulfoxide (DMSO) and loaded in the discs. The standard antibiotic (Amoxicillin) was used as a control due to its broad spectrum of activity against various organisms. The impregnated discs were incubated at 37ºC for an hour. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the zones of inhibition. The discs were gently pressed on the surface of the medium and they were placed at least 25mm away from the edge.

Incubation

The plates were incubated at 37ºC for 16-18 hours in an incubator.

Measurement of Zone Inhibition

The diameter of the zone of inhibition was measured in mm at the end of incubation period of 18 hours and recorded. Each experiment was done in triplicate.

Determination of Activity Index (AI)

The activity index of the crude plant extract was calculated by comparing the mean value of the extracts with the mean value of zone of inhibition of standard antibiotic using the following formula,

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of extract}}{\text{Zone of inhibition of Standard antibiotic drug}}$$

RESULT AND DISCUSSION

The antibacterial activity of the *Brassica oleracea* L. *var. capitata f. rubra* natural dye was established by disc diffusion method and their potency was determined by measuring the diameter of growth inhibition zones. The dye of *Brassica oleracea* L. *var. capitata f. rubra* was active against five different bacteria. Four concentrations of the dye extract were used (20, 40, 80 and 100 µg) for the study. The natural dye showed a clear zone of inhibition against *Proteus vulgaris*, *Bacillus megaterium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Amoxicillin is used as the standard antibiotic. The natural dye extract of *Brassica oleracea* L. *var. capitata f. rubra* possess very significant antibacterial activity compared to the standard antibiotics. The zone of inhibition increased with the increase in the concentration of the dye. Among the five different bacteria, the natural dyes was more active against *Staphylococcus aureus* (28 mm) and less active against *Pseudomonas aeruginosa* (24mm) in concentration of 100µg. The plant dye extract showed maximum Activity index (AI) values against *Staphylococcus aureus* (0.96) and minimum Activity index (AI) values against *Pseudomonasaeruginosa* (0.85). The *Brassica oleracea* L. *var. capitata f. rubra* natural dye exhibits strong activity against *Staphylococcus aureus* and *Proteus vulgaris* > *Bacillus megaterium* > *Pseudomonas aeruginosa.* Similar results were also observed in previous studies various plant extracts have been shown

strong antibacterial activities (Ahmad *et al.*, 1998; Quarenghi *et al.*, 2000; Mann *et al.*, 2011).[4],[5],[6]

In the current study the variation in antibacterial potentiality of examined plants could be attributed to their disparate contents of biocidal agents and this is in accordance with the results of different studies which provided evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic resistant strains (Bin *et al.*, 2007).[7] The antibacterial property of natural dyes is due to the presence of phytochemicals such as terpenoids, steroids and saponins which may be responsible for the antibacterial activity (Akhilesh Bhat and Koteshwar AnandaraoRaveesh, 2014).[8] Tannins and flavonoids also have been reported to inhibit the growth of many fungi, yeast, bacteria and viruses (RekhaRavindran*et al.*, 2014).[9] Based on the results of present study, it is confirmed that the *Brassica oleracea* L. *var. capitata f. rubra* natural dye possess antibacterial property.

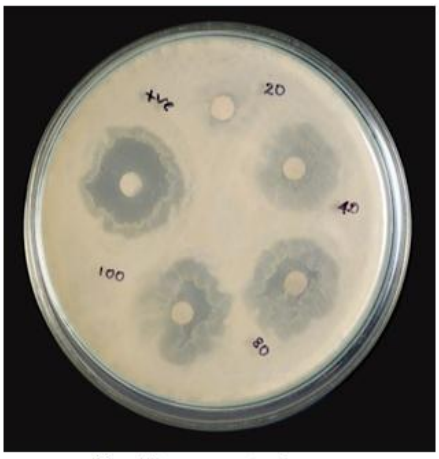
Table 1: Antibacterial activity of *Brassica oleracea* L. *var. capitata f. rubra* natural dye.

Sl.no	Name of the bacterium	Control (Amoxicillin)	Concentration(µg)			
			20	40	80	100
			Zone of Inhibition (mm)			
1.	*Proteus vulgaris* (MTCC No: 426)	27	9	10	19	26
2.	*Bacillus megaterium* (MTCC No: 428)	29	11	22	24	25
3.	*Pseudomonas aeruginosa* (MTCC No: 424)	28	15	16	22	24
4.	*Staphylococcus aureus* (MTCC No: 737)	29	13	18	24	28

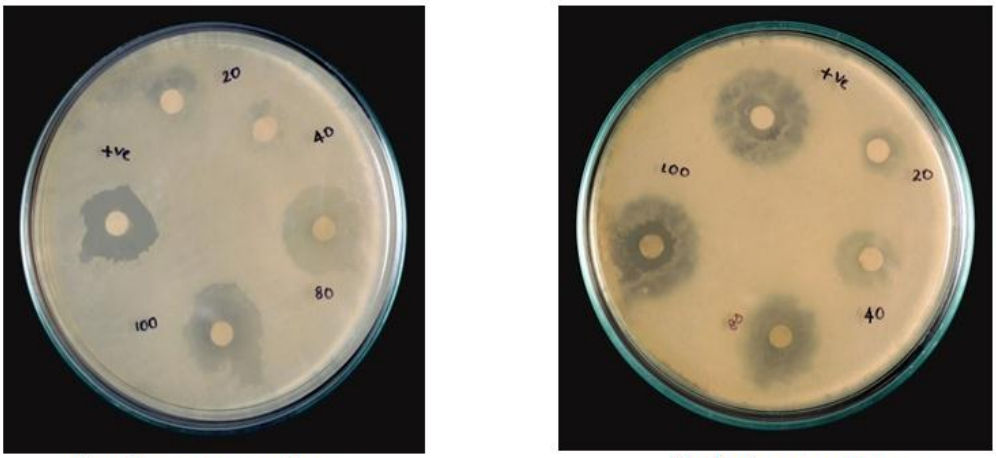
Table 2: Activity Index of *Brassica oleracea* L. *var. capitata f. rubra* natural dye.

Sl.no	Name of the bacterium	Concentration(µg)			
		20	40	80	100
		Activity Index			
1.	*Proteus vulgaris* (MTCC No: 426)	0.33	0.37	0.70	0.96
2.	*Bacillus megaterium* (MTCC No: 428)	0.37	0.75	0.82	0.86
3.	*Pseudomonas aeruginosa* (MTCC No: 424)	0.53	0.57	0.78	0.85
4.	*Staphylococcus aureus* (MTCC No: 737)	0.44	0.62	0.82	0.96

Proteus vulgaris



Bacillus megaterium



Pseudomonas aeruginosa *Staphylococcus aureus*

Figure 1: Antibacterial Activity of *Brassica oleracea* L. *var. capitata f. rubra* Dye.

CONCLUSION

The results of the study revealed that *Brassica oleracea* L. *var. capitata f. rubra* natural dye extract showed a clear zone of inhibition against *Proteus vulgaris*, *Bacillus megaterium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The highest activity was against *Staphylococcus aureus*. Therefore, the dye obtained from the *Brassica oleracea* L. *var. capitata f. rubra* can be used as a potential antibacterial agent to control some bacterial infections. Further, this natural dye can contribute antimicrobial property to the products they are used in.

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