International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 741-746
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF NATURAL DYE FROM *BETA VULGARIS* L.

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ABSTRACT

Betalain pigment of Beta vulgaris (L.) comprises an excellent natural dye. Beta vulgaris (L.) is good source of much health promoting and protective phytochemicals. The present study was conducted to assess the qualitative phytochemical analysis and in-vitro antibacterial activities of the Beta vulgaris natural dye. ***Methods:*** *The Beta vulgaris (L.) natural dye was screened to determine the presence of alkaloids, carbohydrates, glycosides, phytosterols, tannins, phenolic compounds, flavonoids, terpenoids, saponins and quinones through preliminary phytochemical screening. And 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 using Muller–Hinton broth in a spread plate method.* ***Results:*** *The dye extract of Beta vulgaris (L.) revealed the presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannins, flavonoids, terpenoids, saponins and quinones. The antibacterial analysis showed that Beta vulgaris dye extract does not allow the growth of bacteria due to its antibacterial activity. Natural dye of Beta vulgaris (L.) possessed highest antibacterial activity against Staphylococcus aureus.* ***Conclusions****: Beta vulgaris natural dye has the potential to be developed as antibacterial agents against some bacteria due to the presence of active phtyochemicals.*

KEY WORDS

Activity index, Antibacterial, Beta vulgaris, Kirby–Bauer's disc diffusion method and Phytochemical screening.

INTRODUCTION

In recent years, there has been an increasing tendency towards the natural dyes due to the increasing awareness of toxicity and serious health hazards of synthetic dyes. Unlike synthetic dyes, natural dyes are non- toxic, non- allergic, non- carcinogenic, non-poisonous, less polluting, easily available and produce soothing and soft shades as compared to synthetic dyes. Practically not or mild chemical reactions are involved in the production of natural dyes. Above all, they are environment- friendly and can recycle after use. Natural dyes are obtained from renewable and sustainable bio resource products. Plants are the major source of natural dyes. Almost all parts of plants produce natural dyes.

Besides of its colouring property, several natural dyes possess bioactive properties and have been used as therapeutic agents and as diagnostic tools. Some natural dyes have been reported for analgesics, antibacterial, antifungal, antileprotic, antiviral and anti-inflammatory effects. Medicinal potential of the plants lies in bioactive phytochemical constituents such as alkaloids, flavonoids, essential oils, tannins, terpenoids, saponins, phenolic compounds, etc. and that produce definite physiological action on the human body. Some phytochemicals produced by the plants have antimicrobial activity and used for the development of

new antimicrobial drugs [1]. Many of the plants used for dye extraction are classified as medicinal and some of these have remarkable antimicrobial activity. Many of human illnesses are caused by infection with bacteria. Bacterial infections are of particular concern mainly due to the development of antibiotic resistance.

Betalain pigment of *Beta vulgaris* (L.) comprises an excellent natural dye. *Beta vulgaris* is good source of much health promoting and protective phytochemicals including glycosides, flavonoids, phenolic acids like chlorogenic acid, caffeic acid, ferulic acid, cinnamic acid and p-coumaric acid in addition to small amount of vitamin A, vitamin C, vitamin B12, iron, potassium, sodium, zinc and calcium. Flavonoids displayed remarkable anti-inflammatory, antibacterial, antioxidant, antiallergic, hepatoprotective, antiviral and anticarcinogenic activities. Phenolic compounds like phenolic acids, phenols, flavonoids, phenyl proponoids, phenolic quinines acted as antiseptic and anti-inflammatory [2]. The aim of this study was to extract the natural water-soluble dye from *Beta vulgaris* (L.) and to evaluate the prepared dye regarding the Phytochemical and Microbiological analysis.

MATERIALS AND METHODS

Preparation of extraction

50g of fresh tap roots of *Beta vulgaris* (L.) was 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.

Phytochemical screening

Phytochemical examinations of the natural dye extract were carried out as per the standard methods.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

A. Mayer's test - Test solutions were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of yellow coloured precipitate indicates the presence of alkaloids.

B. Wagner's Test - Test solutions were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of carbohydrates

A. Benedict's test - The extracts were treated with Benedict's reagent and heated gently, the orange red precipitate indicates the presence of reducing sugars.

B. Fehling's Test - The extracts were hydrolysed with dil.HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the formation of reducing sugars.

Detection of glycosides

A. Keller – Killiani Test - A small portion of the extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of ferric chloride solution. The mixture was then poured into another test tube containing 2 ml of conc.H_2SO_4. The appearance of brown ring indicates the presence of glycosides.

B. Modified Borntrager's Test - Extract were treated with Ferric chloride solution and immersed in boiling water for about 5minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of glycosides.

Detection of phytosterols

A. Salkowaski Test - To the plant extracts add few drops of chloroform and 2ml conc. H_2SO_4. Shake well for few minutes. Red colour developed in the $CHCl_3$ layer indicates the presence of phytosterols.

B. Libermann Burchard`s test - Extracts were treated with chloroform and filtered. The filtrate were treated with few drop of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterol.

Detection of tannins and phenols

A. Ferric chloride test - The filtrate was treated with 5% $FeCl_3$ solution. A violet precipitate was formed which indicates the presence of tannins and phenols.

B. Test with lead acetate solution - Few ml of filtrate was treated with lead acetate solution, a white precipitate was formed which indicates the presence of tannins and phenolic compounds.

Detection of flavonoids

A. Alkaline reagent test - Extracts were treated with few drops of sodium hydroxide solution formation of intense yellow colour, which becomes colourless on addition of dilute acid indicates the presence of Flavonoids.

B. Lead acetate test - Extracts were treated with few drops lead acetate solution. Formation of yellow colour precipitate indicates the presence of Flavonoids.

Detection of terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added to and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

Detection of saponins

A. Froth test - Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

B. Foam test - 0.5gm of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of quinones:

A small amount of extract was treated with concentrated HCl and formation of yellow precipitate (or coloration) indicates the presence of quinones.

Antibacterial assay [3]

The antibacterial potential of the *Beta vulgaris* (L.) dye extract was 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 were used in this study such as *Proteus vulgaris*, *Bacillus megaterium, Pseudomonas aeruginosa* and *Staphylococcus aureus.* All the bacterial cultures were 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

Preliminary phytochemical screening

The preliminary phytochemical screening of natural dye of *Beta vulgaris* (L.) revealed the presence of carbohydrates, glycosides, phenolic compounds, tannins, saponins, flavonoids, terpenoids, phytosterols and quinones. This result may provide a basic idea about the phytochemical constituent of the dye extract. The detailed results of the analysis are given in Table 1.

Table:1 Preliminary phytochemical analysis of *Beta vulgaris* (L.) natural dye

Sl. NO	CONSTITUENTS	TEST	REACTIONS
1	Alkaloids	Mayer's test	-
		Wagner's test	-
2	Carbohydrates	Benedict's test	+
		Fehling's test	+
3	Glycosides	Keller-Killiani test	+
		Borntrager's test	+
4	Tannins and Phenolic compounds	Ferric chloride test	+
		Lead acetate test	+
5	Saponins	Froth test	+
		Foam test	+
6	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
7	Terpenoids	Salkowaski test	+
8	Phytosterols	Salkowaski test	+
		Libermann Burchard's test	+
9	Quinones	Conc. HCl test	+

The medicinal value of the plant depends on the phytochemicals such as alkaloids, flavonoids, phenolic compounds and other nutrients like amino acids and protein [4]. Plants containing carbohydrates and glycosides are known to exert a beneficial action on immune system by improving body strength and hence are valuable as dietary supplement, while the saponin has the property of precipitating and coagulating red blood cells. The tannin containing plants are used to tract non-specific diarrhoea, inflammations of mouth and throat and slightly injured skins [5]. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidant cell damage have strong anticancer activity [6 and 7]. Thus, the study revealed that the *Beta vulgaris* (L.) natural dye has the potential to cure many diseases.

Antibacterial assay

The antibacterial activity of the natural dye of *Beta vulgaris* (L.) was established by disc diffusion method and their potency was determined by measuring the diameter of growth inhibition zones. The *Beta vulgaris* (L.) dye was active against five different bacteria. Four concentrations of the dye extract were used (20, 40, 80 and 100 µg) for the study. The extracted dye showed a clear zone of inhibition against *Proteus vulgaris*, *Bacillus megaterium, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The standard antibiotic used for the study is Amoxicillin. The zone of inhibition increased with the increase in the concentration of the dye. Among the studied bacteria, the natural dye was more active against *Staphylococcus aureus* (28 mm) and less active against *Proteus vulgaris* (24 mm) in concentration 100µg (Table-2). The dye extract showed maximum Activity index (AI) values (Table-3) against *Staphylococcus aureus* (0.96) and minimum Activity index (AI) values against *Bacillus megaterium* (0.86).

The antimicrobial properties of the plants are due to the presence of phytochemicals. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable. Flavonoids inhibit microbes which are resistant to antibiotics. The antimicrobial activity of Beta vulgaris (L.) dye might be due to the presence of these phytochemicals. From the

results of the study, it can be clearly stated that the natural dye of Beta vulgaris (L.) is a potent source of antibacterial agent.

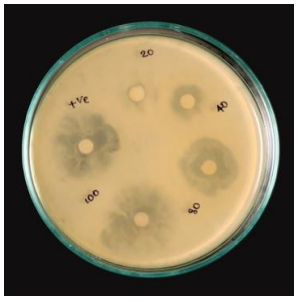
Table: 2 Antibacterial activities of *Beta vulgaris* (L.) natural dye

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	15	21	24
2.	*Bacillus megaterium* (MTCC No: 428)	29	14	19	22	25
3.	*Pseudomonas aeruginosa* (MTCC No: 424)	28	17	18	24	26
4.	*Staphylococcus aureus* (MTCC No: 737)	29	10	19	26	28

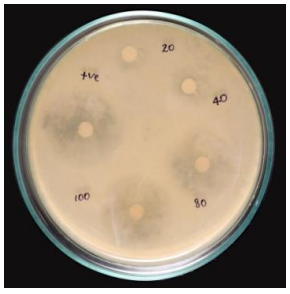
Table:3 Activity Index of *Beta vulgaris* (L.) natural dye

Sl.no	Name of the bacterium	Concentration(µg)			
		20	40	80	100
		Activity Index			
1.	*Proteus vulgaris* (MTCC No: 426)	0.33	0.55	0.77	0.88
2.	*Bacillus megaterium* (MTCC No: 428)	0.48	0.65	0.75	0.86
3.	*Pseudomonas aeruginosa* (MTCC No: 424)	0.60	0.64	0.85	0.92
4.	*Staphylococcus aureus* (MTCCNo: 737)	0.34	0.65	0.89	0.96

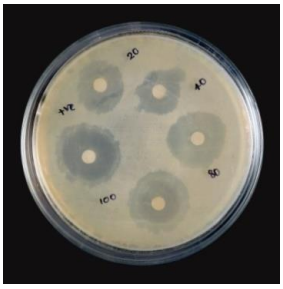
Figure-1. Antibacterial activity of *Beta vulgaris* (L.) natural dye



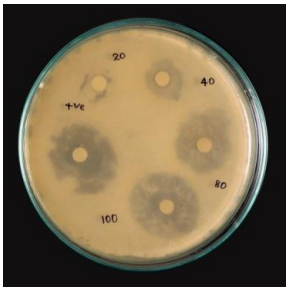
Proteus vulgaris



Bacillus megaterium



Pseudomonas aeruginosa



Staphylococcus aureus

CONCLUSION

The preliminary phytochemical screening of *Beta vulgaris* (L.) natural dye revealed the presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannins, flavonoids, terpenoids, saponins and quinones. The 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* (28 mm). Therefore, the dye obtained from the *Beta vulgaris* (L.) might be an alternative source to synthetic dyes with medicinal and antibacterial properties.

ACKNOWLEDGEMENT

The authors are thankful to management of Vivekanandha College of Arts and Sciences for Women (Autonomous) for providing laboratory facilities to carry out the research experiments.

Received:06.05.18, Accepted: 07.06.18, Published:01.07.2018

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