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BACTERIAL, PLANT, AND ANIMAL TISSUE STAINING AND IN VITRO VISUALIZATION OF NUCLEIC ACID BY EGGPLANT PEEL EXTRACT

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ABSTRACT
The natural stain is always eco-friendly, and biodegradable, with less or no biohazard, compare to synthetic chemical stain. Anthocyanin a phenolic compound is the major pigment in eggplant and leads to coloration. At below pH 2, it gives red or orange color and possesses a positive charge. Eggplant samples were collected from panskura market, West Bengal, India (Latitude 22.3921654; Longitude 87.7428713); extracted with 3 % acetic acid and absolute ethyl alcohol as a solvent. After processing in a rotary evaporator and lyophilized; it was used for bacterial cells, plant tissue (onion peel), and animal tissue (squamous epithelium) staining at a concentration of 156.7 mg/ml diluted in 3% acetic acid. The pigment was also used as an ethidium bromide alternative in agarose electrophoresis. Antimicrobial activity of the processed extract was also performed against Proteus vulgaris, Salmonella typhi, Klebsiella pneumoniae, and staphylococcus aureus. We have got the positive result of simple staining using eggplant pigment for morphological study of the bacterial cell, plant tissue, and bit of animal tissue with little compromise in terms of resolution. Here the use of some fixative agents such as glycerol and sodium bicarbonate did not give any significant differences. The processed sample of eggplant pigment produced the green fluorescent bands of nucleic acid extracted from Lysinibacillus sp., grown as filamentous morphological conformation in agarose gel electrophoresis under the gel documentation system. We have found the mean value of zone of inhibition of antimicrobial activity as 4.20 and standard deviation (SD) .131 for 20 microliter samples at a concentration of 156.7 mg/ml. The eggplant peel may be used as a biological stain.

Keywords: Anthocyanin, Eggplant, staining, Ethidium bromide, Antimicrobial activity, Fluorescent

1. INTRODUCTION
Pigments produce colors with visual appeal. It is present in all organisms such as bacteria, fungi, algae, animals, and plants. Among them, the plant is the principal producer. Almost all the parts of the plant body produce pigment. It requires cellular growth and development, metabolism, and other vital processes like photosynthesis or even managing the oxidative stresses and so on. There are so many important natural pigments such as chlorophyll, carotenoids, anthocyanin, hemoglobin, melanin, flavonoid, quinone derivatives, etc. There are many pigments in therapeutic uses due to their pharmacological activities [1,2,3,4].

The pigment may be natural, synthetic, or inorganic based on origin. The synthetic pigment is produced in the laboratory. The natural and synthetic pigments are organic. Inorganic pigments can be existing as natural or can be produced by synthesis [5]. It is the chromophore that is responsible for color. Based on chromophores, pigments are classified as a conjugated system with chromophores such as anthocyanins, carotenoids, caramel, betalains, synthetic pigments, lakes, and metal-coordinated porphyrins such as chlorophyll, hemoglobin, myoglobin, and their derivatives [6].

The effectiveness of synthetic stains in the field of coloration, beautification, cell and molecular visualization, etc. is higher than natural stains. But it is reported synthetic stains led to biohazards and unbalancing the ecology [7]. They are often corrosive and damage the living tissues, are associated with oxidation, and dehydration, and are responsible for allergies and even led to cancer [8]. A common dye crystal violet routinely used in microbiology laboratories; has been reported as a carcinogen in mice and aquatic organisms [9]. Ethidium bromide commonly used in molecular biology laboratories acts as a carcinogen to the organisms as intercalating agent [10]. Again antimicrobial activity of some natural dyes has been reported. Pigments from Quercusinfectoria, Acacia catechu, Rumexmaritimus, Rubiacordifolia plants showed antimicrobial activity against pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumonia, and in Escherichia coli [11].

Eggplant is also known as brinjal is an economically important vegetable, especially in the tropical and subtropical zone. There are so many varieties in morphology as well as the nature and concentration of chemical constituents in eggplant. Anthocyanin a phenolic compound is the major pigment in eggplant and leads to coloration. Besides anthocyanin, there are so many phenolic acids in eggplant [11]. Anthocyanin contains eight conjugated double bonds with a positive charge. At acidic pH (below pH 2) it gives red or orange color. Upon

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increase of pH, it becomes colorless and again under the alkaline condition, it turns into blue color [12]. There is an ongoing trend to use natural pigment in various fields even in cellular morphology study, and biomolecule visualization by fluorescence nature of some natural pigments. The stain from the Kumkum plant along with some fixative agents named glycerol and sodium bicarbonate has been used as a microbial stain in Escherichia coli [13].

2. MATERIALS AND METHODS:

2.1 Collection of Samples and Preparation:

Eggplant sample is collected from panskura vegetable market, West Bengal, India (Latitude 22.3921654; Longitude 87.7428713). Eggplants are thoroughly washed in tap water and the outer pigmented part was carefully peeled out into small pieces. Then dried the peels at 40^0C for 72 hours. We added a 200 ml acid-alcohol mixture which contains 190ml absolute ethyl alcohol and 10 ml acetic acid (3%). Then we kept it in a shaker incubator for 24 hrs. at 32^0C aseptically. The reddish pigment was liberated and peels became white. The sample was then filtered out twice and put for centrifugation at 5000 rpm for 20 minutes at 4^0C and supernatant was collected. After that, for concentrating, we kept the sample in a rotary evaporator for 6hrs at 45^0C at 120 rpm. After that, we get the residual volume as 10 ml. This is used for microbial, plant, and animal tissue staining. At this point pH of the pigmented extract was 1.5. This is further concentrated in powdered form by lyophilized process and experiments carried out at a concentration of 156.7 mg/ml where the solvent was 3% acetic acid [14,15,16]. The eggplant sample and extract are represented in Fig.1.

2.2 Staining Process:

Simple staining is done using extracted sample against a Lysinibacillus sp. Then staining of squamous epithelial cells was also carried out as an animal tissue sample. For the plant tissue sample, we performed an onion peel staining process. In all cases, we used a phase-contrast microscope for visualization. We used the fixative agent to check any change in intensity for visualization under a microscope. For that purpose, we used 1ml glycerol and a pinch of sodium bicarbonate in a 10 ml prepared extract [13,17,18,19].

2.3 In-Vitro Visualization of Nucleic Acid:

We performed agarose electrophoresis using extracted nucleic acid of Lysinibacillus sp. in filamentous morphological conformation. For that purpose, we used 1.5ml extract in 50 ml 0.8% agarose at a concentration 156.7mg/ml. It is also noted we used 6X orange DNA loading dye as tracking. During the loading, we also loaded only tracking dye in some lanes along with samples in other lanes as control [20].

2.4 Antimicrobial Assay

We performed the antimicrobial activity test aseptically against some selected pathogens named Proteus vulgaris, Salmonella typhi, Klebsiella pneumoniae, and staphylococcus aureus in the Mueller-Hinton agar plate. We used 24 hours old culture at a volume of 0.1ml and followed the spread plate technique. Then the center of each plate was bored by a cork borer and 20 microliter extract was added to each bore at a concentration of 156.7 mg/ml [21].

3. RESULT AND DISCUSSION

3.1 Result Of The Bacterial Cell, Plant Tissue, and Animal Tissue Staining

We have got positive results from eggplant extract as the primary stain under the microscope for selected microbial cells, plant tissue, and animal tissue represented in Fig.2, Fig3, and Fig.4 respectively. But the resolution was not so high as chemical stains. But we can predict morphology with no problem. It is also noted that the use of fixative agents such as glycerol, and sodium bicarbonate did not increase the intensity of visualization in that case. For the bacterial cell visualization magnification was 1000X and magnification was 400X for plant and animal tissue.

3.2 Result of Nucleic Acid Visualization:

In the electrophoresis experiment, we got a green color fluorescent band of the nucleic acid of Lysinibacillus sp. under gel documentation systems as Fig.5. We did not get any band in the controlled lane of loading dye.

3.3 Result of Antimicrobial Sensitivity

In the antimicrobial sensitivity method, we have found the zone of inhibition against selected pathogens in Fig. 6 and Fig. 7. The mean value and standard deviation (SD) of the zone of inhibition are 4.20 and .131 respectively, and are represented in Table 1. The SD value clearly showed that there was an almost equal zone of inhibition against selected pathogens.

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4. CONCLUSION

As natural stain is eco-friendly, biodegradable, and less or not biohazardous compared to synthetic chemical stain; our main focus was the implementation of natural pigment as a primary stain for morphology study. As most of the cellular outer surface charge including nucleic acid is negative, so it was electrostatically demanded our stain should carry the positive charge to get positive results. As anthocyanin at acidic pH (below pH 2) possesses a positive charge; that's why it was our basic interest. As eggplant is a major source of anthocyanin; the major reason was selected as a sample. Again for ethidium bromide replacement, eggplant extract exhibited a positive result for in vitro visualization of nucleic acid and produces clear fluorescence. During staining, it should be ensuring the microbes especially the pathogen should not be in viable condition. Chemical stains can kill the microbes and limit the biohazard during the staining process. We have performed the antimicrobial activity test. The extract used as stain gives potential antimicrobial sensitivity; which is used in all the experiments. Finally, we got a positive result from eggplant pigment for morphological study and can be used as an ethidium bromide alternative during agarose electrophoresis. But we have to improve more in terms of resolution under instrumental visualization for morphology study and the effect on the mobility of DNA during electrophoresis in presence of eggplant pigment has to be checked. Anyhow it is one of the green methods in terms of biological stain with positive outcomes.

REFERENCES

1. Chattopadhyay P, Chatterjee S, Sen SK. Biotechnological potential of natural food grade biocolorants. African Journal of Biotechnology. 2008;7(17).
2. Hari RK, Patel TR, Martin AM. An overview of pigment production in biological systems: functions, biosynthesis, and applications in food industry. Food Reviews International. 1994 Feb 1;10(1):49-70.
3. Koes RE, Quattrocchio F, Mol JN. The flavonoid biosynthetic pathway in plants: function and evolution. BioEssays. 1994 Feb;16(2):123-32.
4. Mol J, Jenkins G, Schäfer E, Weiss D, Walbot V. Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Critical Reviews in Plant Sciences. 1996 Jan 1;15(5-6):525-57.
5. Bauernfeind JC. Natural food colors. Carotenoids as colorants and vitamin A precursor, Academic Press, New York. 1981 Jan 1:1-45.
6. Wong DW. Mechanism and theory in food chemistry. New York: Van Nostrand Reinhold; 1989 Sep 30.
7. Bordoloi B, Jaiswal R, Siddiqui S, Tandon A. Health hazards of special stains. Saudi Journal of Pathology and Microbiology. 2017;2(5):175-8.
8. Jones ML, Bancroft JD, Gamble M. Connective tissues and stains. Theory and practice of histological techniques. 2008; 6:135-60.
9. Littlefield NA, Blackwell BN, Hewitt CC, Gaylor DW. Chronic toxicity and carcinogenicity studies of gentian violet in mice. Toxicological Sciences. 1985 Oct 1;5(5):902-12.
10. Belyaev IY, Eriksson S, Nygren J, Torudd J, Harms-Ringdahl M. Effects of ethidium bromide on DNA loop organisation in human lymphocytes measured by anomalous viscosity time dependence and single cell gel electrophoresis. Biochimica et BiophysicaActa (BBA)-General Subjects. 1999 Aug 5;1428(2-3):348-56.
11. Singh R, Jain A, Panwar S, Gupta D, Khare SK. Antimicrobial activity of some natural dyes. Dyes and pigments. 2005 Aug 1;66(2):99-102.
12. Horbowicz M, Kosson R, Grzesiuk A, Dębski H. Anthocyanins of fruits and vegetables-their occurrence, analysis and role in human nutrition. Vegetable crops research bulletin. 2008 Jan 1;68(1):5-22.
13. Gupta A, Sengupta S, Nathan A, Agarwal A, Devadas M, Madhumati G, Suneetha V. Pharmaceutical Applications. Int. J. Drug Dev. & Res. 2014 Oct;6(4):0975-9344.
14. Garcıia-Viguera C, Zafrilla P, Tomás-Barberán FA. Determination of authenticity of fruit jams by HPLC analysis of anthocyanins. Journal of the Science of Food and Agriculture. 1997 Feb;73(2):207-13.
15. Todaro A, Cimino F, Rapisarda P, Catalano AE, Barbagallo RN, Spagna G. Recovery of anthocyanins from eggplant peel. Food Chemistry. 2009 May 15;114(2):434-9.

16. Salamon I, Mariychuk R, Grulova D. Optimal extraction of pure anthocyanins from fruits of Sambucusnigra. InI International Symposium on Elderberry 1061 2013 Jun 9 (pp. 73-78).
17. Jain A, Jain R, Jain S. Staining Methods–Simple Staining, Negative Staining, Gram's Staining and Acid-Fast Staining. InBasic Techniques in Biochemistry, Microbiology and Molecular Biology 2020 (pp. 111-116). Humana, New York, NY.
18. Prabhu S, Rajasekar M. Identification of colour pigments in root Vegetables-Carrot (Daucuscarota) and Beetroot (Beta vulgaris) by foldscope.
19. McMillan DB, Harris RJ. An atlas of comparative vertebrate histology. Academic Press; 2018 Jun 4.
20. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. JoVE (Journal of Visualized Experiments). 2012 Apr 20(62): e3923.
21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016 Apr 1;6(2):71-9.

Figures and Tables:

Fig.1 Eggplant Peel extract: A – Eggplant, B – Extract with solvent, C – processed lyophilized peel extract

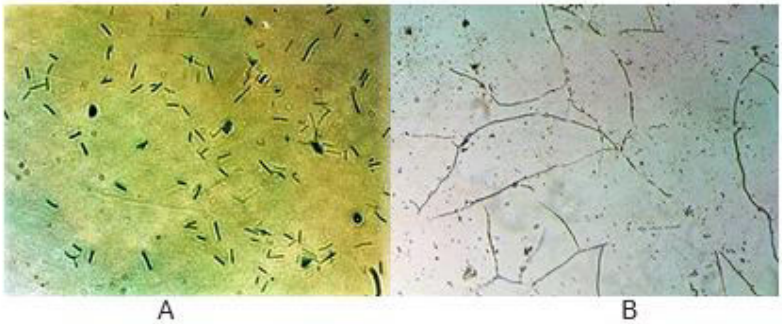

Fig.2 Simple staining of bacteria by eggplant peel extract: A – *Lysinibacillus sp.* 24 hrs old culture, B – *Lysinibacillus sp.* filamentous conformation upon long term incubation

Fig. 3 Staining of onion peel by eggplant peel extract

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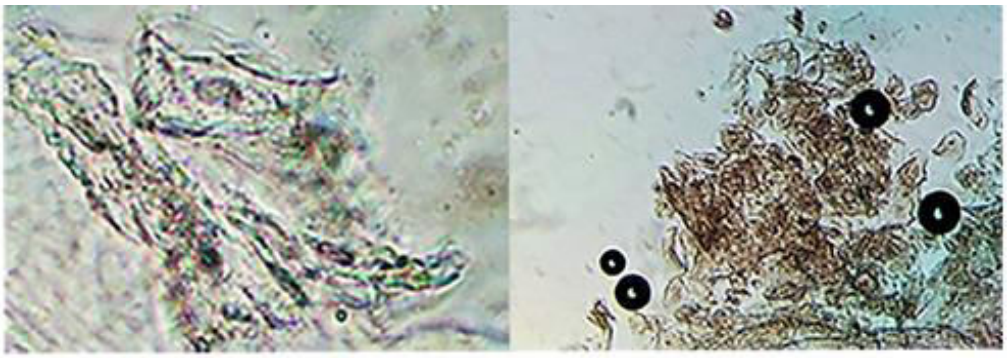
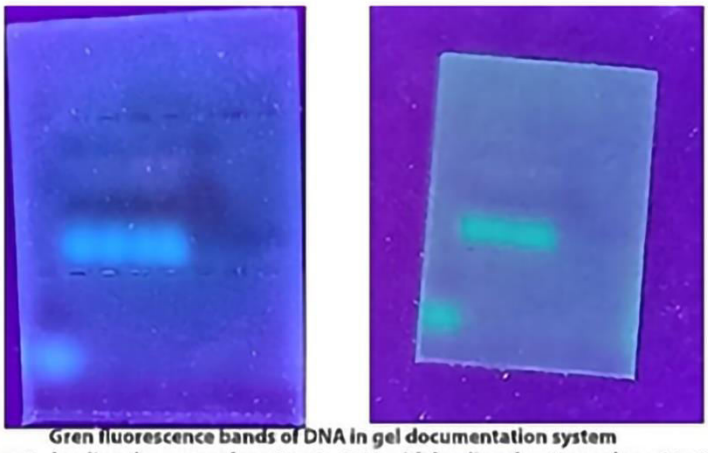


Fig. 4 Staining of squamous epithelium by eggplant peel extract



Gren fluorescence bands of DNA in gel documentation system
Upper lane1 - loading dye, upper lane 2 to 5 - DNA with loading dye, Upper lane 6 to 8 - blank
Lower lane 1 - DNA with loading dye, Lower lane - 2 to 8 - loading dye

Fig.5 Green fluorescent bands of nucleic acid by eggplant peel extract

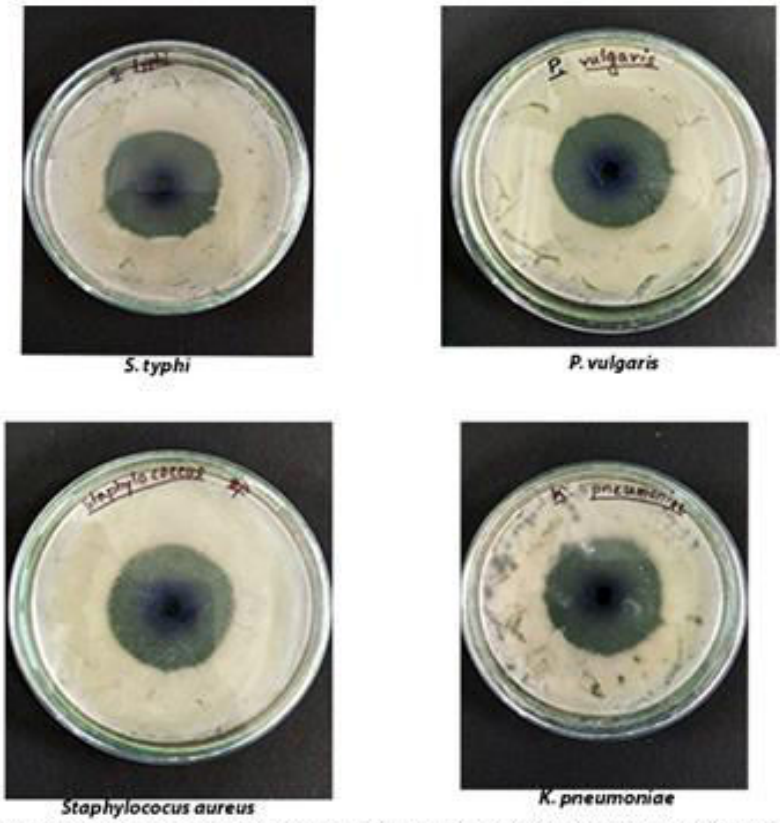


Fig.6 Antimicrobial activity of eggplant peel extract against selected pathogens

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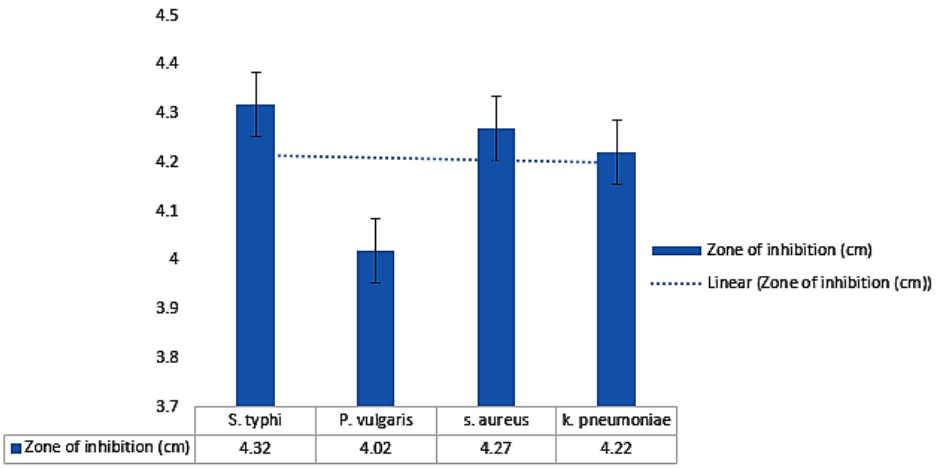

Fig. 7 Zone of inhibition against selected pathogens by eggplant peel extract

Table 1: Mean and SD value of zone of inhibition

Parameter	N	Mean	Std. Deviation	Std. Error Mean
Zone of inhibition in (cm)	4	4.2075	.13150	.06575