"EXTRACTION OF PLANT AND MICROBIAL PIGMENT, COMPARISION AND TO CHECK ITS STABILITY"

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ABSTRACT

Pigments are the secondary metabolites produced by plants and microorganisms for their various applications in their life. Pigments are coloring compound which are used by humans to add colors in their life. Using synthetic pigments for the purpose of coloring food, clothes, fruit juices, paints are accepted worldwide previously but due to hazardous impact of synthetic colors on to the environment and also on human health made to go for alternative sources for the pigments which are safe to use. Isolation of natural pigment is the alternative solution which will make the pigment availability from natural sources without harming to the environment as well as on to the health. Natural sources like plants and microorganisms are used for the pigment production process. Pigment extraction from the plant will be somehow difficult and expensive as compared to the pigment extraction from the microorganisms. Microorganisms uses various cheap sources for growth and it make it easy to grow them on controlled condition and production of pigments in larger amount. Pigments are produced by growing microbes on solid nutrient agar medium and then it is purified by using centrifugation and filtration form the microorganisms while pigment isolation from plants requires extract preparation and then isolation of pigments using various solvents. This paper will describe about various different microbial and plant pigments and their various applications. As pigments are nowadays extracted from the biological sources it is known as "biocolors".

Keywords: *Pigments, Medicine, Food, Environment, Synthetic*

[I] INTRODUCTION

1.1 History of pigments

Nature is rich in colours which are obtained from the various sources like plants, fruits, vegetables, roots, microorganisms like bacteria, fungi. As the colours are originated from the biologic materials it is referred as "BIOCOLOURS". Addition of colour to the processed food is very old practice. Saffron, turmeric, and vegetable dye is used for colour food, (Joshi *et al*., 2003). As people wearing clothes they want their clothes to be more attractive and more pleasing than others. In earlier days most dyes and pigments are isolated from the plants and animals which are available in ranges from ordinary to exotic. Dyes were made with natural pigments mixed with water and oil and then applied on clothes, ornaments, and used to decorate skin. As various researches was being carried out in this field had led to use different kinds of materials available to use as colouring agents. The different colourings of the flowers were identified by biochemists that it is for to attract insects which act as the important part in pollination. As they are used in dying clothes playing an important role in providing an intact colour to the fabric and also work as antibacterial and antifungal agent and provides protection to the fabric against bacterial and fungal infections. Natural pigments isolated from the plants, animals, and insects were colourents used since prehistoric times (Venil *et al*., 2003).

1.2 Classification of pigments

Pigments are classified into organic pigments and inorganic pigments or natural and synthetic pigments.

ORGANIC PIGMENTS – These are based the pigments based on carbon chain and carbon rings and they also contain inorganic elements that helps to stabilize the properties of organic components.

INORGANIC PIGMENTS – These are the chemical compounds which are not based on carbon and these are metallic salts which are precipitated from the solution.

SYNTHETIC PIGMENTS – These pigments are synthesized in laboratories through chemical manufacturing. These types of pigments had hazardous effects.

NATURAL PIGMENTS – Natural pigments are obtained from the animals, plants, and microorganisms. Some of the important natural pigments are carotenoids, flavonoids, and some tetrapyrroles.

PIGMENT CAN BE FURTHER CLASSIFIED ON THE BASIS OF THEIR SYNTHESIS.

EXOGENEOUS PIGMENTS – Pigments which are coming from the outside of the body are known as exogenous pigments.

ENDOGENEOUS PIGMENTS – Pigments which are synthesized within the body are known as endogenous pigments.

Some of the pigments are the normal constituents of the cell and some are abnormal and accumulate in cell under several special condition.

1.3 Factors affecting microbial pigment production

A colour of interest can be obtained by keeping in mind the chemical nature of the pigment which includes the optimum pH, optimum temperature, selected source for the carbon and nitrogen.

TEMPERATURE

The production of the microbial pigment is greatly affected by the incubation temperature and the type of strain or type of the microorganism used (Joshi *et al*., 2003). Different microorganisms require different temperature range.

pH

Type of pigment produced will be affected by the pH of the medium on to which organism is growing. Minor changes in the pH may lead to change in the type of pigment going to produced. pH requirements will be change from organism to organism. Neutral to slight alkaline pH favours lycopene formation while acidic pH favours β carotene synthesis (Joshi *et al*., 2003).

TYPE OF FERMENTATION MEDIA

Majorly two types of fermentations processes are used for the microbial pigment production, which are 1) solid fermentation, 2) submerged fermentation.

Solid state fermentation process yields more pigment production than the submerged fermentation process (Joshi *et al*., 2003). β carotene, riboflavin, phycocyanin are the fermentative products used in the food industries (Jacobson *et al*., 1997).

CARBON SOURCE

Majorly used carbon sources are glucose, fructose, galactose and maltose. Mycelia growth is affected by the carbon source used. Glucose and its oligosaccharides are the better source used for the pigment production and growth. The type of sugar used greatly affects the shade of the pigment (Joshi *et al*., 2003).

NITOGEN SOURCE

The type of nitrogen source used will be affects the pigment production. Organic nitrogen sources will give higher mycelia growth rates. Yeast extract was found to yield maximum pigment than other nitrogen sources on *Monascus purpureus* (R.S Subhasree*et al*., 2011).

MINERALS AND METALS

Microbes interact with minerals and metals in natural environments as well as synthetic environment (G.M Gadd *et al*., 2010). A mineral plays an important role in pigment production only on solid medium. Sometimes Zn acts as an inhibition of growth and increases pigment production by increase in uptaking of pigment production (Joshi *et al*., 2003).

1.4 Industrial application of microbial pigments

FOOD INDUSTRY

Nowadays development of different varieties of food in an attractive appearance is a major goal in the food industry. Due to toxic impact of synthetic dyes, interest has been increased towards using natural food colour.

PHARMACEUTICAL INDUSTRY

Most studies on the microbial pigments concluded that the pigments isolated from the bacteria possess antibiotic, anticancer and immunosuppressive characteristics. Pigments are used for treatment of several diseases like leukemia, diabetes etc. Anthocyanins are able to decrease the risk of cancer (Kim HW *et al*., 2012) and reduce inflammatory insult (Yuodin KA *et al*., 2002).

TEXTILE INDUSTRY

Textile industry uses and produces approximately 1.3 million tons of dyes, which are from synthetically manufactured. As synthetic dyes have hazardous effect on environment and also cause skin allergy and other harms to human body (Abhishek kumar*et al*., 2015). Biosynthesis of colours is increased interest in past few years.

[II] MATERIALS AND METHODS

2.1 Procurement of pigment producing microorganism

Pure culture was procured from the Department of Biotechnology, Veer Narmad South Gujarat University, Surat. Pigment extraction from microorganisms was done by solvent extraction method. Pigment producing culture was transferred to the pre sterilized nutrient broth (Himedia) and then incubated with shaker at 30^{o}C for 24 hrs of the incubation. One percent of the above cell suspension was used as inoculums for pigment production in 100 mL nutrient broth containing 1 % glycerol(standard glycerol1mL and Distilled water 9mL) which was added in the media for the enhancement of the pigment production (Mehta Mansi *et al*., 2016).

2.2 Extraction of pigment from the cultured broth

Extraction of the pigment was done by the modified method given by P.Gunasekuran, 2005.

2.3 Extraction procedure for microbial pigment extraction

Nutrient broth having pigmented organism was taken in centrifuge tubes. Vortexed properly for 2 minutes. Centrifuged at 2000 rpm for 15 minutes. Proper mixing was done by vortexing for 2 minutes. 5 mL 95% Methanol was added to the thepellet.Vortexedproperly.Centrifuged at 2000 rpm for 15 minutes.Pellets were washed with 5 mL 95% Methanol until pellet appeared colourless. Coloured supernatant was collected and subjected to evaporation for 3 h at 60°C. After evaporation, pigment was re-dissolved in 2 mL 95 % Methanol and Filtered using Whatman filter paper no.1. Filtrate was collected in culture tube and Preserved in refrigerator at 4°C for further use.

2.4 Extraction of the pigment from the flower petal

Fresh flowers were cut into small pieces and macerated into a pulpy mass. It was transferred to a beaker and about 100 mL 2N HCl was added and stirred. Incubated at 60˚C for 1 hr. The red solution was filtered through cheese cloth or using filter paper. The filtrate was cooled and shaken with few ml of petroleum ether. Yellow colour ether layer was discarded. Red aqueous layer was further extracted with few mL of isoamyl alcohol. Pigments pass on to the alcohol layer which then becomes deep red colour. Layer was separated and concentrated to dryness by keeping in a water bath. Residue was dissolved in a few mL of alcohol containing 2 drops of 2N HCl.

2.5 To check Stability of the pigments at different Temperature

a) Stability of microbial pigment v/s plant pigment

To check the stability of the pigments at different temperatures the extract of the both plant pigment and microbial pigment was taken and added in the different test tubes. That test tubes were further placed at different various temperatures that are -20^{o}C, Room Temperature, 37^{o}C, and 60^{o}C.

2.6 To check Stability of the pigment at different pH

Stability of Microbial pigments v/s plant pigments

To check the stability of pigments at different pH the pigment extract of plant and microorganisms were placed at different pH conditions. pH condition was maintained in the cuvett box containing 1 mL of buffer solution and loop full amount of pigment was added to check its stability at various pH conditions and that are 3, 4, 5, 6, 7, 8.

2.7 Antimicrobial activity of pigment extracts

The antimicrobial activity of the pigment extract was done on nutrient agar media by well diffusion technique against *Bacillus subtilis, Staphylococcus aureus*. 24 hrs broth culture of targeted strain were inoculated in the previously sterile nutrient agar media using sterile swab. Four wells were made and 100µL of the pigment extract were added in the respective three wells and one well was added with methanol as control. Plates were further placed in the incubator for the

24-48 hrs at 37°C and zone of inhibition were measured. Cleared zone of inhibition that formed around the well indicates the presence of antimicrobial activity of the pigment extract of plant and microbial.

[III] RESULTS AND DISCUSSION

3.1 Isolation of pigment extract from the microorganisms

Red colored colony producing microorganisms named *Serratia marcescens* were collected from the Department of Biotechnology Veer Narmad South Gujarat University Surat. Gram staining was done to check purity of the given culture.**(Fig. 1.1 a and b)**

3.2 Isolation of pigment extract from the flower

Plant pigment was successfully extracted from the flower petals. In this method the fresh flower was plucked out from the garden of Veer Narmad South Gujarat University Surat. Then 10 g floral petals are weighed and then it is converted into pulpy mass by grounded it in using mortal and pestle. Pulpy mass of the petals was then further placed in the 2N HCl solution containg 50 mL of flask. Flask were further placed in the water bath at the 70-80°C for 1 hour. After removing it from the water bath flask was allowed to cool at room temperature and further it was filterd by using whatmann filter paper no 1 of 6 mm diameter. The filtrate was further used for pigment extraction method by using solvent extraction method.

In the Solvent Extraction Method, the filtrated was treated with the few mL of petroleum ether and shaken vigorously for 15-20 minutes. Then two separate layers were formed from which upper layer was discarded and the bottom red aqueous solution was further treated with the few mL of isoamyl acohol for 15 to 20 minutes until two separate layer formed. Pigments were passed on to the deep red colored layer. That layer was then separated and concentrated by placing flask on the water bath at higher temperature for evaporation. Once the solution completely evaporated residues were dissolved in the few mL of alcohol which were containing 2N HCl for further use.

different Temperatures From the results it was observed that the extraction of the floral pigment will be depends upon the type of reagents used for the extraction. Pigment extraction was done by using petroleum ether which is a one of the best used solvent used for the plant pigment extraction methods. The 10 grams of red petals of *Hibiscus rosasinesis* containing 10 mg/mL concentration of the pigment.**(Fig 1.1 c and d)**

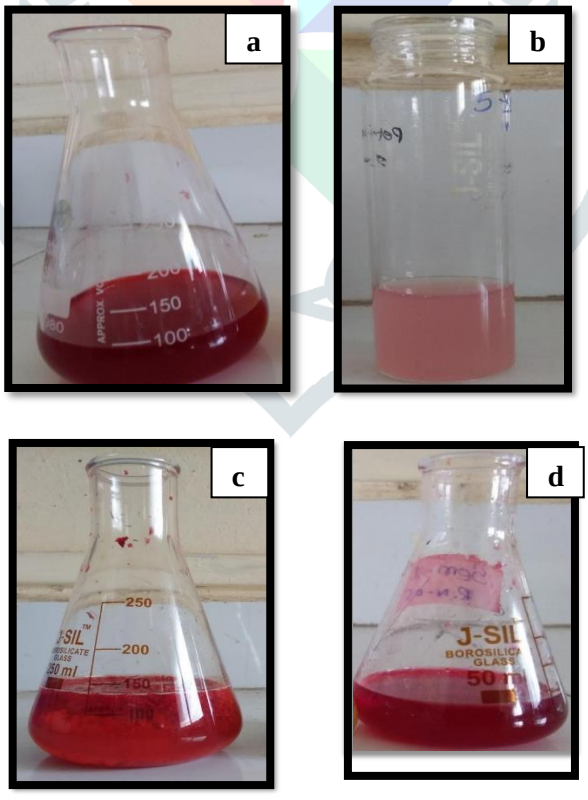

Fig. 1.1 a) Shows the pure culture of microorganism, b) Pigment extract from microorganism, c) flower petals placed in 2N HCl, d) Pigment extract from floral petals.

3.3 Stability of microbial pigment v/s plant pigments at different Temperatures

Extracted red pigments from the microorganism had higher stability at various temperatures as compared to the red pigment extract isolated from the flower. The microbial pigment was best stable at the room temperature and at 37°C and plant pigment was also best stable at room temperature and at 37°C. As temperature increased than this two temperature extract color becomes slight changes in the shade as compared to its original shade in both plant extract and also in the microbial extract. At -20°C microbial pigment changes its shade color while plant pigment had the original shade.**(Table 1)**

Temperature	Plant Pigment	Microbial Pigment
-20 °C	Light shade	Stable
Room Temperature	Stable	Stable
37 °C	Stable	Stable
60 °C	Evaporated at faster rate	Colour retain for longer time

Table 1- Shows the stability of pigment extracts at different Temperature

From the results it was observed that the among the both extract microbial pigments were more stable at various temperature conditions as compared to the plant pigments.

3.4 Stability of microbial pigments v/s plant pigments at varying pH

Extracted red pigment from the microorganisms and from the flower was tested for stability at various pH ranging from acidic 3 to basic 8 by using phosphate buffer. At low pH the red pigment extract from the microorganism changes its shade from dark pink to slight light pink in color while plant pigment had also given the slight light shade as compared to the original shade. At higher pH the pigment becomes colorless while plant pigment had no such changes in its shade at higher pH.**(Table 2)**

pH	Plant Pigment	Microbial Pigment
3	Light Colour	Light Colour (pink)
4	Light Colour	Light Colour
5	Light Colour	Medium Colour
6	Original Shade	Original Shade (red)
7	Dark Shade	Original Shade
8	Dark Shade	Light Colour (yellowish)

Table 2- Stability of Pigment Extracts at different pH conditions

Microbial pigments were more stable at various conditions as compare to the plant pigments so they are having good applications in various areas and have good stability.

3.5 Antimicrobial activity of pigment extracts

Antimicrobial activity of pigment extract was checked against test organisms. Complete zone of inhibition was observed in both the extract. 3.5 mm zone of inhibition in diameter was found from the microbial pigment extract plate and 3 mm zone of inhibition in diameter was measured in plant pigment extract. **(Table 3)**

Test Microorganism	Plant Pigment	Microbial Pigment
Staphylococcus aureus	**3 mm**	**3.5 mm**
Bacillus subtilis	**2.5 mm**	**3.5 mm**

Table 3- Shows the Antimicrobial activity of the pigment extract

[IV] CONCLUSION AND FUTURE PROSPECTS

From the results it can be concluded that the plant and microbial pigments both are a very good sources of pigments as the demand of colors is increasing nowadays it will fulfill the all the requirements of the dinaturalcolors. Both sources have

its own advantages and disadvantages as well. The source used for pigment will be depends on the individual's requirements.

Studies should be concern especially on finding the easiest method for harvesting bacterial pigments in order to increase their industrials applications.Also there is a need to look on various operational parameters that may cause a variation due to change and develop a new low cost process for the production of bacterial pigments by using agro-waste as substrate in the future. Future investigation on various technologies that would reduce the cost and increase yields for large scale production.

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