Extraction and application of plant dye in microbial staining

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Abstract: Microbial cell staining is a key step use to identify material under microscope. To increase visibility, emphasize morphological traits and preserve cells it becomes necessary to be fixed and dyed it. The study was aimed to observe bacterial cells staining efficacy of natural dyes extracted from different plants viz. *Tagetes erecta, Rosa indica, Punica granatum, Beta vulgaris* and *Citrus sinensis*. Gram positive *Bacillus spp.* and *Escherichia coli* bacterial cells were stained by applying acetone and methanol extract of petals of flowers obtained from titled plant species.

Keywords: ***Tagetes erecta, Rosa indica, Punica granatum, Beta vulgaris, Citrus sinensis, extract, natural dye.***

Introduction:

Around the world, dyeing has gained importance in social culture. In addition to beautifying an item, dyes are used to represent the culture and regions around the globe. it also provides evidence on the discrepancies between previous civilizations too. Natural dyes are derived from many natural sources includes plants, minerals, insects and animals. They are non-toxic and majority of cases allergy-free. (S.M. Amir-Al Zumahiab; September 2020, e05104).

Perkin's invention of the major synthetic dye has changed the situation. As a result, synthetic dye manufacturing grew at a rapid rate as they became popular as substitutes to natural dyes in meals, non-linear optical motions, cosmetics, and material commercial enterprises due to their ease of dyeing and low cost. Experts and manufacturers have placed a greater emphasis on shading materials with natural dyes in order to save energy and protect the environment. Researchers are now striving to improve pigment extraction from plant components such petals, leaves, bark, and seed, in order to increase the percentage of output. (S.M. Amir, AlZumahiab; September 2020, e05104).

Dyes are compounds of natural or synthetic origin that are soluble in a media and are used in the dying process to give a desired colour to non-food materials such as paper, leather, wood, textiles, and even cosmetics. Dyes, often known as stains, are used to impart colour to tissues, blood cells, or organelles within individual cells, as well as microorganisms like bacteria, fungus and yeast in order to make them optically distinguishable. (Stella Adeyemo; January 2018).

The majority of stains currently in use are chemically produced from low-cost petroleum by products have been found to be detrimental to human health. Because several synthetic dye components are carcinogenic or at the very least highly allergic, they are being phased out as their danger is known. The widespread use of synthetic dyes has resulted in pollution of water, land and air, disrupting the earth's ecological balance and posing health risks. As a result, some countries, including India, the Netherlands, and Germany, which were among the first to create synthetic dyes, have prohibited their production. (Stella Adeyemo; January 2018).

This has promoted researchers to look for less expensive, eco-friendly, and biodegradable dyes for staining microbial cells, food samples, tissues and other things. Natural dye from plants and animals, which has become a hot issue due to an increase in environmental awareness aimed at lessening the harmful effects of toxic synthetic dyes on living things, is one of the ways to provide an alternative to synthetic dye. (Stella Adeyemo; January 2018).

Several synthetic dyes (e.g., dyes with azo bonds nitro- or amino-groups) contain toxic heavy metals such as chrome, copper and zinc which are known to be carcinogenic; it also causes allergic-like symptoms. Synthetic dyes have been implicated in being non-biodegradable and producing harmful waste to the environment which is toxic to man. The sources of synthetic dyes are non-renewable. The requirement of a professional in preparing synthetic dyes due to the specificity of accuracy in measurement has led to its unavailability when needed unlike natural dyes which are easy to prepare. Some synthetic dyes have been found to be highly inflammable which have resulted in fire outbreak in some laboratories and industries. Therefore, there is a need for alternative source of dye which is easily available from plants which are eco-friendly, biodegradable, non-toxic to man and easy to produce (Stella Adeyemo; January 2018).

Using chosen microorganisms, the staining capability of extracts of fruits and flowers from five plants was investigated, including *Tagetes erecta* (marigold plant), *Rosa indica* (red rose), *Punica granatum* (pomegranate), *Beta vulgaris* (beetroot), and *Citrus sinensis* (orange). These plants can be found in India and are readily available. The extract's efficacy was also compared to that of synthetic staining reagents. The therapeutic properties of these plants, as well as their safety for human ingestion, have made them extremely valuable.

The goal of this study is to add to the growing body of knowledge about the value of employing natural dyes instead of synthetic dyes in the identification and characterization of microorganisms. It will also inform microbiologists and pathologists on the efficacy of natural dyes in staining gram positive and negative organisms, cells and tissues. It will demonstrate how simple it is to tint microorganisms with natural colours. (Stella Adeyemo; January 2018).

MATERIALS AND METHODS:
Materials Required
Source: Marigold flower petals, Rose flower petals, orange fruit pulp, Pomegranate fruit seeds, Beetroot fruit.
Substrate: Gram positive Bacillus spp., Gram negative Escherichia coli
Chemicals: Acetone, Methanol, Water
Instruments: Hot Air Oven, Spectrophotometer

Methods:
Extraction of the Natural Dyes: Dyes were extracted from petals of marigold and rose flowers, pomegranate seeds, orange pulp and beetroot fruit. Firstly, experimental part of the desired plant was crush finally by using mortar and pestle. For the extraction, 5 g of each plant material was taken in 50 ml of solvent. (Solvent for marigold, rose, orange and pomegranate are acetone and for beetroot is methanol). After extraction, filter the extract using filter paper in a beaker to remove all non-soluble plant material. The extracts were collected in beaker and left in the hot air oven to evaporate the solvent. (Sanjeet Kumar Sharma Date uploaded on Jun 09, 2020)

Measurement of Absorbance of Dyes: The concentrated extract of each plant material in dried form was carefully placed in Eppendorf tube. It is then dissolved it in its respective solvent and then transferred volume up to 1 ml in a glass cuvette and absorbance was measure.

Extract Preparation: The concentrated extract in dried form was carefully picked using a spatula. Dried extract was dissolved in water by continuous stirring until all the particles were completely dissolved. The dissolved extract was then sieved to remove tiny particles. A clean dropper was used in applying the dye on each slide (Stella Adeyemo; January 2018).

Staining Procedure and Slide Preparation: The extracted dyes from the selected plant were tested on a bacterial culture that was 18 -24 hours old and contained gram positive *Bacillus spp.* and gram negative *Escherichia coli*. To introduce distilled water to a grease-free slide, a loop was used; the loop was sterilised by passing it through a blue flame and then allowed to cool. The sterile loop was used to select a distinct colony from the fresh culture and then applied it to the distilled water on the slide to create a thin film smear. The thin film smear was gently passed through the flame one or two times and allowed to dry before applying the dye to the slide with a dropper and allowing it to dry. (Stella Adeyemo; January 2018).

Microscopy each slide was examined under the microscope with an x100 objective lens. The various characteristics of the organisms were recorded and compared to a standard staining technique (Gram's staining). A photomicrograph of each slide displaying the microscopic features of each organism was taken. (Stella Adeyemo; January 2018). Gram's Staining was also carried out using the method as comparative study and analysis. This also served as control experiment. (Stella Adeyemo; January 2018).

Result and Discussion:
Absorbance of Pigments the absorbance of each pigment was taken by spectrophotometer at different wavelengths.

Table 1: Absorbance of Pigments

Sr. No.	Pigment	Wavelength	Absorbance
1	Lutein	460 nm	1.874
2	Anthocyanin Rosa indica Punica granatum	 500 nm 480 nm	 1.904 1.864
3	Beta-carotene	450 nm	1.848
4	Betanin	450 nm	1.848

The plates below show the photomicrograph of the cells of the different microorganisms when stained with different natural dyes and synthetic dyes and viewed under the microscope.

it was observed that, Gram positive *Bacillus spp.* is perfectly stained with anthocyanin pigment of *Rosa indica* and *Punica granatum*, beta-carotene pigment of *Citrus sinensis* and betanin pigment of *Beta vulgaris* except lutein pigment of *Tagetes erecta*; and Gram negative *Escherichia coli* is perfectly stained with anthocyanin pigment of *Punica granatum*, lutein pigment of *Tagetes erecta*, beta-carotene pigment of *Citrus sinensis* and betanin pigment of *Beta vulgaris* except anthocyanin pigment of *Rosa indica*.

Conclusion:
According to the findings, dyes extracted from five different plants could be used as a suitable substitute for some synthetic dyes. Furthermore, the use of natural dyes in biological laboratories will make it easier to use adequate protective measures for students and laboratory attendants, as well as proper effluent treatment and disposal of natural and synthetic dyes. These natural dyes have

been found to be less expensive, easier to use, and more dependable in their applications. The extraction and application do not necessitate any specialised knowledge. The dyes are also environmentally friendly and biodegradable.

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