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THE USE OF DYE EXTRACTED FROM ZOBO PLANT (*Hibiscus sabdariffa)* AS A COUNTER STAINING AGENT IN GRAM STAIN REACTION.

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Abstract

The potentials of extract solution of the zobo (*Hibiscus sabdariffa*) leaf as an alternative counter stain in Gram staining reactions were studied. The red part of zobo leaves was carefully picked and dried under the sun. The dye was extracted with soxhlet apparatus using methanol as the solvent. After drying to powder, the extract was dissolved in absolute ethanol 10% w/v. The staining solution was used to stain bacteria isolates of *E.coli* strains, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The experimental zobo extracts solution was used with as usual counter stain in place of safranin in Gram staining process. The results obtained therefore, showed that the zobo leave extract can be a substitute to the usual counter stains used in Gram staining reactions, as it clearly differentiated between the Gram positive and Gram negative bacteria. The study has demonstrated the use of dyes from plants as a suitable substitute for staining microorganisms; in a very simple, cheap, easy and readily available procedure.

Keywords: Dyes, Plants, Extracts, Staining, Microorganisms.

INTRODUCTION

Dyes are substance of natural or synthetic origin, soluble in a medium which is usually used to impart a desired colour to a non-food material like paper, leather, wood, textiles and even cosmetics in a process known as dying. Dyes are also referred to as stains and can be used to add colour to tissues, blood cells or organelles within individual cells as well as microorganisms such as bacteria, fungi and yeast to make them optically distinct. The majority of natural dyes are derived from plant sources, roots, berries, bark leaves and wood, fungi and lichens (Gupta, 2009). Most dyes are synthetic i.e. are man-made from petrochemicals. Other than pigmentation, they have a range of applications, including organic dye lasers (Siva, 2007); optical media (CD-R) and camera sensors (Colour Filter array).

Archaeological evidence shows that, particularly in India and Phoenicia, dyeing has been widely carried out for over 5,000 years. Early dyes were obtained from animal, vegetable or mineral sources with no or very little processing. By far the greatest source of dyes has been from the plant kingdom, notably roots, berries, bark, leaves and wood only few of them are used on a commercial scale. The first synthetic dye, Mauveine, was discovered serendipitously by William Henry Perkin in 1856 (Hubner, 2006). The discovery of Mauveine started a surge in synthetic dyes and in organic chemistry in general. Other aniline dyes followed, such as Fuschine, Safranine and Indulines (Castillejo *et al*, 2008; Balter, 2009).

The first use of dye in histology was credited to Antonie Van Leewenhoek, the father of microbiology who worked with Saffron, a natural dye extracted from Saffron crocus. Microorganisms can be fixed as well as stained to increase visibility, accentuate morphological features and sometimes preserve them for further study.

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 resources of synthetic dyes are non-renewable. The requirement of a professional in preparing synthetic dyes due to the specificity of accuracy in measure has led to its unavailability when needed unlike natural dyes which are easy to prepare (Avwioro *et al,* 2005). Some synthetic dyes have been found to be lightly 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 and mostly evaluated. This work is aimed at extracting dye from the popularly known Zobo plant (*Hibiscus sabdariffa*) using different solvents and to evaluate the potential of the extracted dye as a microbial staining agent.

MATERIALS AND METHODS

Samples Collection and Extraction of Dye

The red part of Zobo leaves (*Hibiscus sabdariffa*) was carefully picked and dried under the sun. Dyes were extracted from zobo leave (*Hibiscus sabdariffa)* using soxhlet apparatus with methanol as solvent (Akinloye *et al.,* 2010). The extract was concentrated using a water bath and may further be dried into powder by drying in the oven (Okolie; 2008).

Extract Preparation

The concentrated extract in dried form was carefully picked using a spatula and weighed. 5 grams of the dried extract was dissolved in 50 percent ethanol by continuous stirring until all particles were completely dissolved. The dissolved extract was then sieved with a filter paper to remove tiny particles after which it was poured into a clean covered bottle. A clean dropper was used in applying the dye on each slide.

Staining Procedure (Gram Staining)

Identified bacterial isolates of *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis.* were obtained from NAFDAC laboratory Agulu, Anambra State. Smears of bacterial colonies were made on clean grease free slides which were clearly labeled. The smears were air dried and heat fixed. The heat fixed smears were stained with Crystal violet for 60secs, washed off with water and drained, then flooded with Lugols iodine for about 60secs and washed off gently with water and drained. The samples/slides were rinsed with ethanol for 30secs and were rinsed with water and drained. The slides were then counter stained with zobo extract for 1min (60secs) after which the stains were washed off with water. The slides were air dried. Gram staining was also carried out as comparative study and analysis. It was also served as control experiment.

Microscopy

Microscopically, observation of each slide was made using x100 objective lens. Oil immersion was used for a clearer view. The different features of the organism were recorded and compared with standard Grams staining techniques. Photomicrograph of each slides showing the microscopic features of each organism were taken using a photomicroscopic camera from microbiology laboratory, Science Laboratory Technology Department Federal Polytechnic Oko.

RESULTS

The results of the microscopic examination show that the dye could serve as counter stain in Gram staining procedure. The photomicrographs of the stained bacteria stained with the dyes are shown in Figure 1-3.

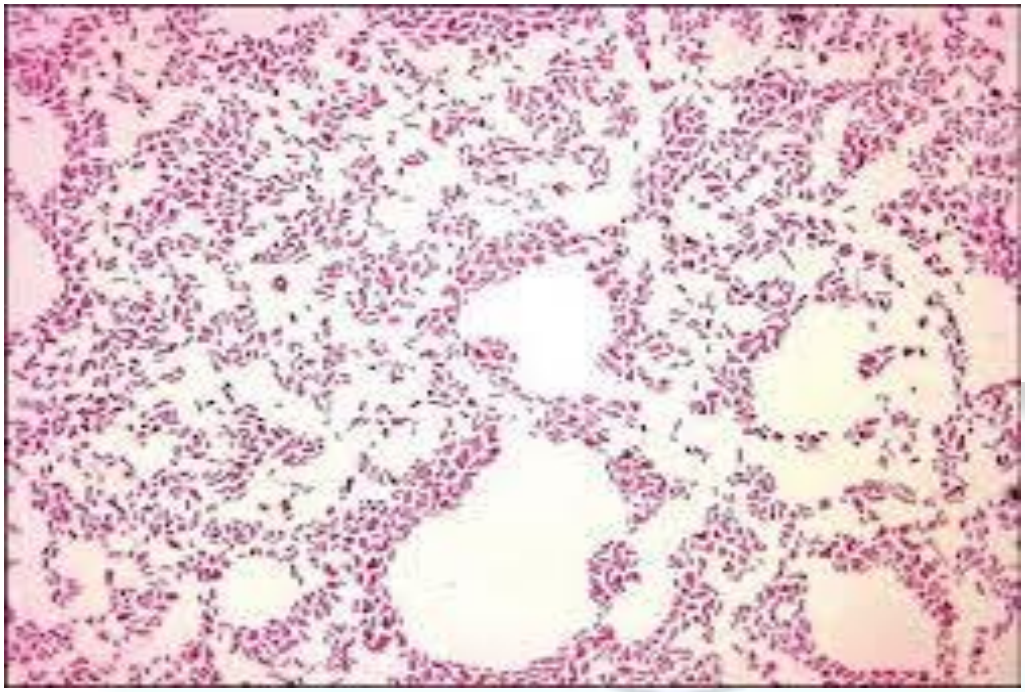

Figure 1: *E coli* counter-stained with the dye (*Hibiscus sabdariffa*)

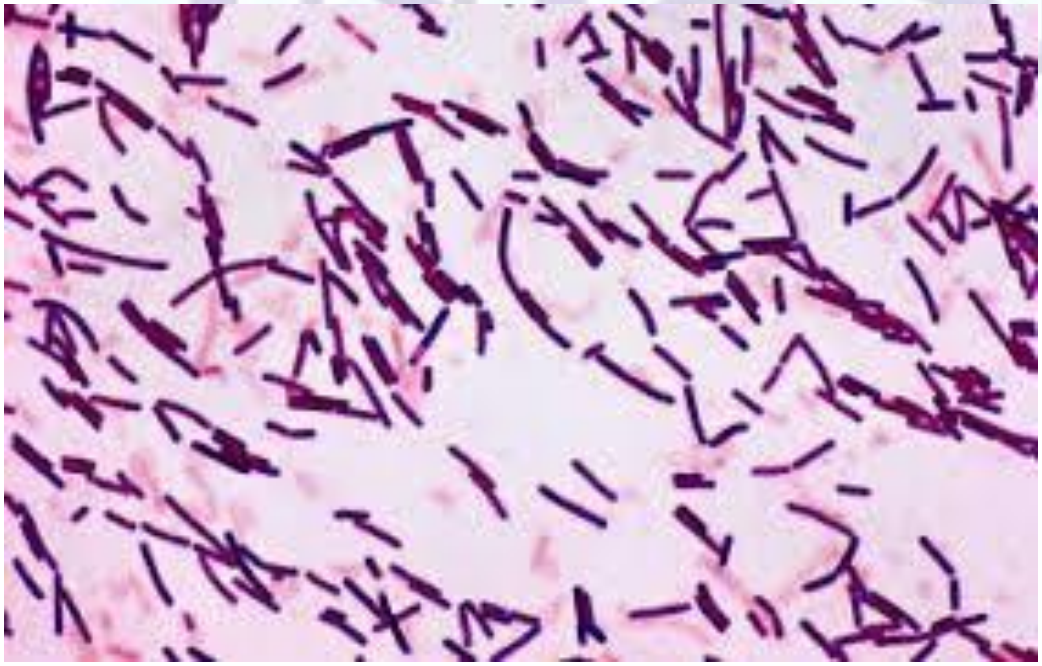

Figure 2: *Bacillus subtilis* counter-stained with the dye (*Hibiscus sabdariffa*)

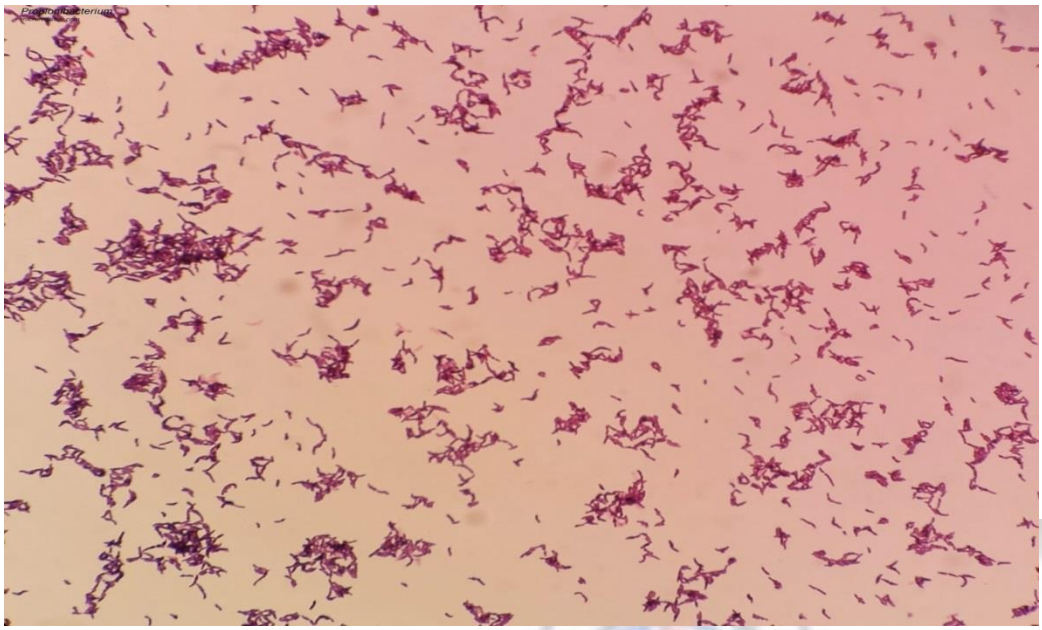

Figure 3: *Pseudomonas aeruginosa* counter-stained with the dye (*Hibiscus sabdariffa*).

DISCUSSION

Staining is an auxillary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes (Peter, 2011; Horobin and Kiernan, 2002; Prescott, *et al*, 2009).

In this study, the extract from zobo leaves (*Hibiscus sabdariffa*) imparted its dark red colour on the stained bacteria. The cellular morphology of each isolate was seen clearly. Dye extract from zobo leaves imparts its colouration on Gram negative organisms while the Gram positive bacteria tested retained its primary dye. It also crystallizes almost immediately when applied on a heat fixed smear. The bacteria appear distinct and clear when stained with extract from zobo leaves. In addition, the cellular morphology of the different organisms was also distinct. One could see the way the cells were arranged as observed with the usual Gram's staining method.

Several researchers have used so many local plants to stain both bacteria and fungi. Hafiz *et al*, (2012) used extract from henna plant (*Lawsonia inamis*) as a counter stain in Gram staining reaction. Braide, *et al*, (2011) also investigated the use of extracts from four local Nigerian plants for the staining of selected bacteria and moulds. Some have also been used as either as primary stain, counter stain or mordant of the Gram staining reagents (Chukwu *et al*, 2011).

Some of the advantages of using natural dyes are; they are more eco-friendly than the synthetic dyes, as the synthetic dying procedure can produce pollutants and certain diazo-dyes which are carcinogenic. Also natural dyes are free from carcinogenic components; they are

also known as antioxidants. Natural dyes are derived from natural sources unlike synthetic dyes; they are easily biodegradable and do not form recalcitrant by-products in the soil or in the environment (Adesokan *et al.*, 2007; Siva, 2007).

Finally, the procedures are simple and easy to apply. You do not need an elaborate process to carry it out. It is simple and most natural dyes from plants are used once and they are washed off and viewed under the microscope. Students will therefore find the procedure very friendly and one that can learn easily.

Conclusion

The result from this research work showed that the dye extract from zobo leaves, could be used as a suitable substitute for the usual stains used in Gram staining procedure in Nigeria and other countries where these plants are cultivated.

Recommendation

- The use of natural dyes in biological laboratories will facilitate the use of adequate protective measures for students and laboratory attendant. The applications of these natural dyes are found to be cheap, easy and more reliable. The extraction and application does not require specialized training. The dyes are also eco-friendly and biodegradable.
- With the increasing demand in the use of natural materials for environmental sustainability across the globe as well as the need to search for ways of remedying the impart of COVID-19 pandemic on the global economy, this plant is highly recommended for use as a counter staining agent in microbiological and other laboratories.

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