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Full Length Original Research Paper

Preparation of a Microbial Stain from the natural product Kumkum for Pharmaceutical Applications

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Abstract:

This study was aimed at investigating the possibility of staining of microbes with a stain prepared from kumkum. A filtered water extract of kumkum, Glycerine (propane- 1, 2, 3-triol) and sodium bicarbonate made. Simple staining procedures to stain *E.coli (Escherichia coli)* were then carried out. Positive results were observed. *E.coli* microbes were stained in a light red hue imparted by kumkum which was further fixed by sodium bicarbonate ($NaHCo_3$).This study concluded that the cheap and easily available kumkum stain can be used to stain microbes and study them morphologically. Thus, such novel stains can be prepared and used for staining purposes in microbiology laboratories.

Keywords: Kumkum, Simple staining, *E.coli*.

Introduction

Kumkum (or kumkuma) is a red colored powder used for religious and purposes in India. Turmeric (*Curcuma longa*) or saffron (*Crocus sativus*) can be used to make kumkum. The rich yellow powder is turned into a bright red color as the turmeric is dried and powdered with slaked lime ($Ca(OH)_2$) along with the addition of water.(1) The usage of kumkum in India is immense, and no Hindu religious festival is complete without its usage. Kumkum is used on the forehead and head by people of all ages. It is also used by married woman on their head, amidst their hair parting to symbolize their marriage; however kumkum is not used by widows for spiritual and religious reasons. (2) Kumkum is applied at the centre of the forehead by Indians because of the belief that "the human body is divided into seven vortices of energy, called chakras, beginning at the base of the spine and ending at the top of the head. The sixth chakra, also known as the third eye, is centered in the forehead directly between the eyebrows and is believed to be the channel through which humankind opens spiritually to the Divine". (3)

Escherichia coli commonly abbreviated *E. coli* used for the staining in this study, is a Gram-negative, facultative, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).(4) *E.coli* is very widely used as a model organism due to its rapid growth rate, simple nutritional requirements, and knowledge of its entire genomic sequence.(5)

Cells and its components can be better visualized and observed under a microscope by a process known as cell staining. Different stains can be used to visualize the different components of a

cell. Preferential staining of cell components such as nucleus, DNA, RNA, cell wall or the entire cell can be done using a process called staining. Staining can be used to visualize both living and non-living cells, however only a few stains can be used for living cells and is usually done for non-living cells which are fixed. Staining is done to improve the visibility of the cells and its components, enhance the contrast between the cells and their background, to facilitate the identification of metabolic processes and to determine the cell viability of the culture. (6,12)

The isolation of new vitamin, antibiotic and enzyme producing microorganisms from soil, marine samples and water, are prime to pharmaceutical industries and research institutes. Basic staining processes are the first step leading to identification and classification of novel microorganisms.(7)

Many of the stains and dyes used for staining of microbes are extremely harmful to humans and other animals. Toxicity, carcinogenicity, genotoxicity, immunotoxicity are only some of the harmful effects of the standard stains and dyes available to us. (8,11,13)For example, crystal violet, a common stain used in microbiology labs for staining is a possible carcinogen (as proved by a study conducted on mice) and also very toxic to aquatic organisms. (9) So there is a necessity to develop stains which are efficient and safe.

Hence the present study was conducted to synthesize a safe as well as an efficient stain for microscopic purposes.

Materials and Methods

Preparation of the stain 0.2g of kumkum was added to 3ml of water in a clean test tube which was then subjected to a vortex to mix the contents thoroughly. The contents of the test-tube were then filtered and a clear solution was obtained. To this solution, 2.5ml of Glycerine (propane- 1, 2, 3-triol) was added as a ligating agent between the cell wall and the stain and the mixture was subjected to a vortex again. To this vortexed mixture, a pinch of sodium bicarbonate ($NaHCo_3$) was added, which makes the stain taken up by the cells more prominent, and the mixture was subjected to a vortex again.

Preparation of smear A loopful (0.1 ml of E.coli culture) *E.coli* culture was smeared on a clean sterilized slide in the laminar air flow chamber using an inoculation loop. The smear was then heat fixed.

Staining the smear The prepared stain was added to this smear and kept for a duration of 5 minutes. The stain was then washed off distilled water. The slide was then observed under the microscope.

Result

This study ended in a positive result. The staining procedure resulted in reddish- pink stained cells which were clearly distinct against the white background.

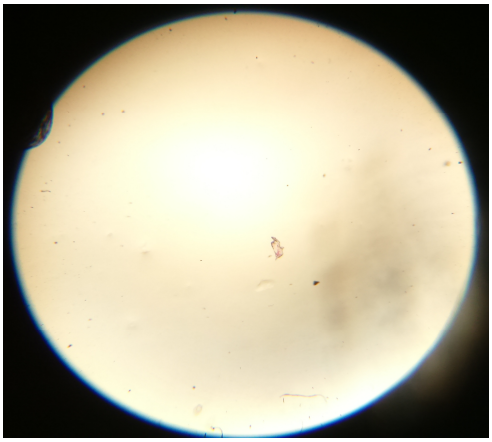

Fig 1: Single microbe stained reddish pink

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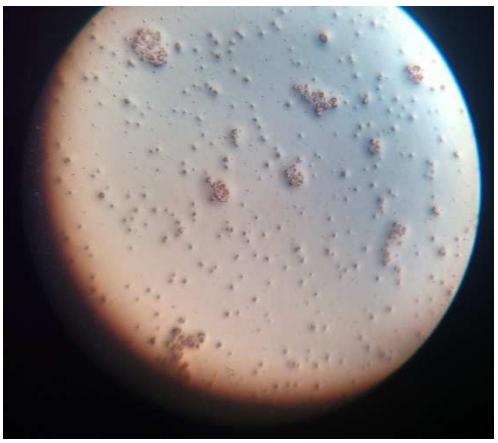

Fig 2: *E coli* culture stained reddish pink

Discussion

Glycerin (propane- 1, 2, 3-triol) makes the mixture viscous so that it doesn't runs off the slide, it also acts as a ligating agent between the cell and stain, hence the cells get stained.

Sodium bicarbonate ($NaHCo_3$) acts as a mordant and fixes the stain. As we know a mordant sets the stain in bacterial cell wall by forming an insoluble compound with the stain added prior to it. (10)

Kumkum imparts the reddish pink color to the bacterial cells and hence stains them visibly. Also, the intensity of the kumkum stain can be altered according to our need. This can be done in two ways. First, by increasing the quantity of kumkum used in the preparation of the stain. But care should be taken to properly vortex and filter the stain, as suspended particles of kumkum in the stain may hinder the process of visualisation of the microbes. Second, it can be done by increasing the quantity of turmeric used during the manufacture of kumkum itself. This can then be used in the preparation of the stain.

Conclusion

In the light of the findings it can be concluded that safer stains can be used to stain bacteria. These stains, such as the kumkum stain, can easily be prepared and used. The use of such stains would eliminate the risk that is posed by the current stains available.

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Conflict of Interest

Declared none

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