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Use of some plants color as alternative stain in staining of bacteria

Fouad H. Kamel[1] , Chnar Najmaddin[2]

[1]Erbil Medical Technical Institute / Hawler Polytechnic University

fhkamel2013@yahoo.com[1]

[2]Collage of science / University Salahddin Iraq

Chnar_Najm@yahoo.com[2]

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ABSTRACT

Natural dyes from plants such as Stigma (Isatis sp.), Myrtle (Myrtussp.), Rosella (Hibiscussp.) and crust of Walnut (Juglanssp.) fruits were extracted by 95% ethyl alcohol or distilled water. Myrtle and Stigma weremixed dye with ratio 1:2, respectively, also Rosellaand crust of nut fruits were prepared with same ratio. Mixeddyes or stains prepared as alternative of Gram stainfor staining Gram negative and Gram positive bacteria. The results showedthat the wall of bacteria were stained. This is well comparable to Gram stain in respect to clarity, differentiation, and economic cost.

Keywords: *Plants extracts, gram stain, Stigma, Myrtle, Rosella, Nut.*

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استخدام بعض الالوان النباتية كصبغة بديلة لصبغ البكتريا

فؤاد حسين كامل[١] ، جنار نجم الدين[٢]

[١]جامعة بولي تكنيك اربيل / المعهد التقني الطبي / اربيل– العراق

fhkamel2013@yahoo.com[1]

[٢]جامعة صلاح الدين / كلية العلوم / قسم علوم الحياة / اربيل – العراق

Chnar_Najm@yahoo.com[2]

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الملخص

اجري استخلاص الاصباغ الطبيعية من نباتات الوسمة والأس والكوجرات وقشرة ثمرة الجوز باستخدام الكحول الاثيلي ٩٥ % او الماء المقطر. مزج مستخلص صبغة الاس وصبغة نبات الوسمة بنسبة ١:٢ على التوالي وكذلك صبغة نبات الكوجرات وصبغة مستحضر قشرة الجوز بنفس النسب على التوالي . اعتمد مزيج الصبغات المحضرة كصبغة بديلة لصبغة جرام الشائعة الاستخدام في صبغ جدار البكتريا السالبة والموجبة لصبغة جرام . كانت النتيجة اصطباغ جدار الخلايا بشكل جيد تضاهي صبغة جرام الشائعة في الوضوح وتمايزها بالكلفة الاقتصادية.

الكلمات الدالة: مستخلص النبات، صبغة كرام، ستكما، ميرتل، الجوز

1. INTRODUCTION

Staining is an auxiliary 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.A stain is a discoloration that can be clearly distinguished from the surface, material, or medium it is found

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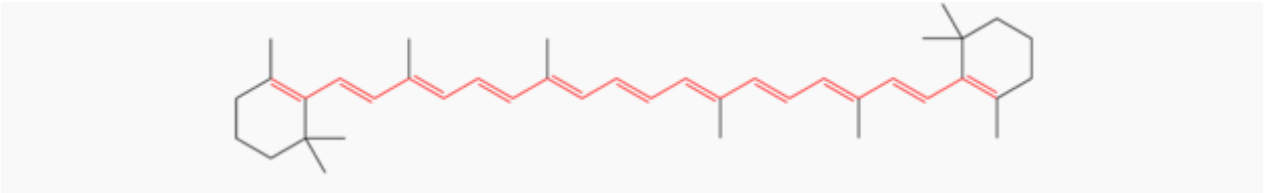
upon. They are caused by the chemical or physical interaction of two dissimilar materials. Staining is used for biochemical research, metal staining, and art e.g. wood staining, stained glass[1].

Carotene is an orange photosynthetic pigment, They are responsible for the colours of many roots, fruits and vegetables for example, carrot, sweet potatoes, chanterelle and orange cantaloupe melon. Carotenes are also responsible for the orange colors in dry foliage. In the lower concentrations impart the yellow coloration to milk-fat and butter [2].



Chemical structure of Carotenoid

Gram stain is named after its inventor, the Danish scientist Hans Christian Gram (1853–1938), who developed the technique in 1884 to discriminate between pneumococci and *Klebsiella pneumoniae*bacteria. Gram staining or the Gram's method is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls [3,4 ,5].

2. Materials and Methods

A-Extracted method.

Weight 20gm of plants (Stigma (*Isatis*), Myrtle (*Myrtus*), Rosella (*Hibiscus*) and crust of Walnut (*Juglans*) fruits) that bought from shop, and add 200ml of 95% ethanol, put in shaker for 1hr. then remain in refrigerator overnight at (4^{O}C), finallyfiltered by filter paper and dry in room temperature, then used this extract (dry weight)to prepare the stain[6].

B- Stain preparation

Two grams of plants extract (as in step A)of*Hibiscus* sp. (Rosella) and *Isatis* sp. (Stigma)were mixed in 100ml ethyl alcohol (70%) or distillated water. One gram of *Juglans* sp. (Walnut) extract were dissolved in 100ml ethyl alcohol (70%) or distillated water, then mixed (2:1) from

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(Rosella with Walnut) respectively before use. While *Myrtus* sp. (Myrtle)was prepared by dissolving two grams of extract in 100ml absolute ethyl alcohol then mixed (2:1) from (Myrtle with Stigma) respectively and 0.8gm ammonium oxalate wasadded [5].

C- Bacteria staining

The samples of bacteria obtained from Science collage-Biology department-Salahaddin University. The bacteria used were*Staphylococcus aureus* and Escherichia coli. Beginning with fixed the bacteria cells on the slide, then used the stain that mixed Myrtle with Stigma for 10min. after that washed by distilled water, the add iodine solution 20sec. after that washed by absolute ethyl alcohol 20sec. then add stain mixed Rosella with Stigma for 10min. and washed by distilled water, finally examine under microscope (oil emersion power).

3. Results & Discussion

Thestigma obtained blue color as a result of the plant containing a substance as flavonoids and fixed by addedMyrtleextractand add ammonium oxalate 0.8gmhas major role in the fixation of dye or stainfigure (1; A, B, C,) shows stainingwall of spherical Gram positive bacteria, where the degree of clarity of the dye is similar to laboratories gram stain.

Rosellaobtained red color as a result of the plant extract, the plant containing a substance as flavonoids,and add the outer shell or crust fruits of a walnut extract has role to fixed color of stain and stain the wall of negative Gram stain bacteria as in figure (1; D, E) showing theGgram negative bacteria.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria. Gram-positive bacteria retain the crystal violet dye, while a counterstain such assafranin of fuchsine, added after the crystal violet,theGram negative bacteria appear a red or pink coloring [3].The Gram stain is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic method in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to Gram variable and Gram indeterminate groups[8]

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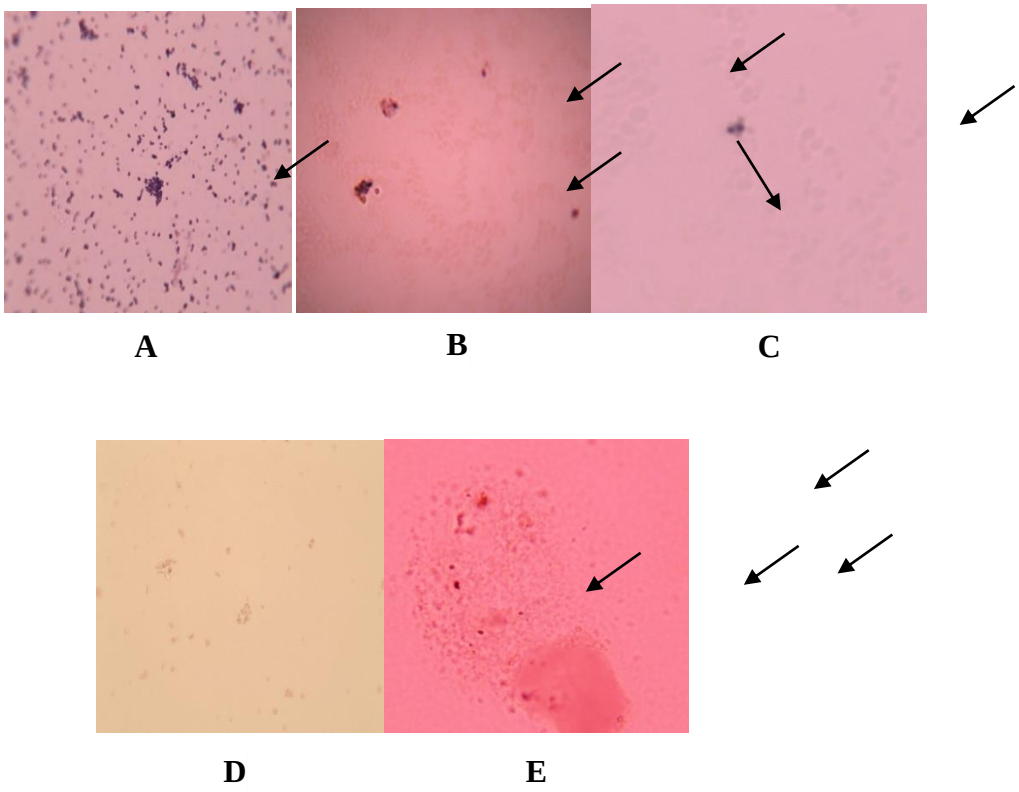

Fig. (1): shows the bacteria. A, D bacteria stain with gram stain. B,C,E bacteria stain bymixed extracted plant stain.

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