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Preparation of a cheap culture medium from pollen of *Typha domingensis* Pers. for the cultivation of Gram-negative and Gram-positive bacteria

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Abstract

Objective: The study aims to find a natural, low-cost culture medium that is available instead of the conventional chemical culture media for cultivation of Gram-negative and Gram-positive bacteria. Methods: In the current study, the male inflorescence of the Typha domingensis Pers plant, which is locally called the Kurat, was used to grow Gram-positive and negative clinical isolates and compare the growth with media commonly used for bacterial growth as a nutrient and blood agar. The possibility of using the locally prepared medium in the liquid form was also studied and compared with the liquid routine media used for the cultivation of bacteria as the nutrient broth. Results: the results showed that Kurat media was able to grow both Gram positive and negative bacteria in both forms (solid and liquid), when kurat was used instead of one of the main component of nutrient agar or both (peptone and/ or yeast extract), adding a colour to clarify the growth by using safranin to the medium, the growth was not affected by dye and it was similar to that resulted by using nutrient agar. Conclusion: Although the growth was slightly less or as same as the commercially used media but the idea of using Kurat media is to use cheaper media and to try using food product as culture media for bacteria, which suggest using the food wastes as a media for growing bacteria to minimize waste products and diminished the cost of the expensive media.

Keywords
Kurat, Bacterial culture media, cheap culture media, pollen of Typha domingensis Pers

The culture media is defined as a group of natural or manufactured materials that support the growth and reproduction of microorganisms outside the living body and are either in liquid or solid form [1].

Scientists need to study germs outside the living body to know their characteristics and ways to fight them, it was necessary to find components on which germs grow, multiply and live for long periods. The growth of microorganisms in the culture media depends on several factors such as moisture, heat, pH, nutrients, the most important of which are carbon, nitrogen, oxygen, phosphorous, sulfur and growth factors such as amino acids and vitamins [2].

The first culture medium for the development of bacteria was found by the scientist Louis

Pasteur in 1860, which consists of yeast, ash, candy sugar and ammonium salt 3, since then scientists have tried to develop culture media and add other components to the media to increase its efficiency and to grow bacteria that are difficult to grow in the detected media Previously [4,5].

The use of natural ingredients to grow bacteria in the laboratory is common, such as the use of egg agar medium for the long preservation of Streptococcus pneumoniae [6] and for the preservation and transmission of sensitive bacteria Neisseria meningitidis and Haemophilus influenzae Type b [7].

The gooseberry plant, or what is called locally currant (Gooseberry), was also used in the cultivation of many bacteria and fungi such as Mucor sps, Rhizopus sps, Fusarium sps, Trichoderma sps and Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia, Shigella sps, and proteus [8].

As a result of the increase in the prices of ready-made chemical culture media, there has been an increase in the demand for the manufacture of cheap and available alternatives for the cultivation of microorganisms. Plant residues were used as raw materials for the growing of bacteria as culture media, such as pistachios [9], sorghum extract [10,11], local food residues [12], cassava serum extracted from trees growing in Africa [13], three-leaf potato [14], corn and bean [15], avocado and pear [16].

In the current study, the pollen grains of Typha domingensis Pers plant that grows in the marshes of Dhi Qar Governorate, from it the local people are extracted the male inflorescence containing pollen grains to manufacture a candy known locally as the Kurat, was used as a natural culture medium for the cultivation of pathogenic bacteria isolated from humans.

The order Typhales and the Typhaceae family includes one genus, the Papyrus Typha, which includes 12 species, the most famous of which is T. domingensis Pers. Which grows in the province of Basra in the marshes and the area of Qurna, Karma and Abu al-Khasib and uses pollen by mixing it with sugar to prepare a dessert called the Kurat [17].

Papyrus is a perennial plant that blooms for two years, and its flowering period usually begins in April to July, with long, thick, spongy leaves [18].

The papyrus plant has many names, where it is called in Britain the name of pond weed Reed mace and in France it is called cattails, while it is called Butt in Egypt, they are biennial plants with a height of between 3 and 4 m, respectively, and their leaves are long, and they are anchored plants, the plant has its roots in the water, while most of the plant's body is outside the water [19].

The economic importance of the papyrus plant lies in the fact that it is included in many well-known local industries such as the manufacture of beds, chairs and hats from compressed sedge [20-22], and its use in the paper industry as raw materials [23,24], in addition to its use in the medical fields, as the efficiency of female floral inflorescences has been proven in treating wounds and burns [25].

The papyrus plant also has an effective contribution to the environment, if its efficiency is proven to remove many pollutants, especially in the aquatic environment, such as removing the elements of silver, nickel, cobalt and cadmium, and purifying water from them [26,27].

Or with bread 17, in addition to the use of pollen in the manufacture of pastries and sweets in Europe [28].

Material and methods

Isolation and identification of clinical isolates used in the study Isolation

Isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were obtained from Al-Bayan Laboratory and diagnosed using the Vitech device.

And the isolates of Klebsiella pneumonia and Acinetobacter baumannii were obtained by graduate student Raghad Jamil (University of Basrah, College of Science, Biology department), which was isolated from burn patients and diagnosed using the Vitech device.

Bacillus sp isolated from the environment, Streptococcus pneumonia has been isolated from the paranasal sinuses, using a cotton swab for the period from (the date of their isolation from and to) and it was planted on the nutrient, mannitol, and MacConkey agar according to the instructions of the World Health Organization [29].

Diagnosis

After cultivation of the specimens taken from the patients, obtaining pure colonies and recording the phenotypic characteristics of the isolates, the colonies were activated on the newly prepared nutrient agar. The dishes were incubated for 24 hours at 37° C. The purified young colonies were used for diagnostic purposes. Biochemical tests were performed to diagnose the isolated bacteria as mentioned at [30–32].

Gram stain

The smears were prepared on glass slides, stained with a Gram stain, and examined under a light microscope.
After obtaining the staining result, the Gram-positive isolates were diagnosed by catalase and coagulation enzyme tests in addition to the ability to ferment sugar mannitol, while Gram- negative isolates were tested IMViC tests, oxidase, urease and the ability to produce hydrogen sulfide gas.

production of the oxidase enzyme

This test was carried out on a sterile Whatman No.1 normal filter paper (7 cm in diameter) placed in a Petri dish and saturated a portion of this paper with 1-2 drops of 1% Tetramethyl-P-Phenylene diamin-dihydro-chloride solution. Then the colonies were transferred by means of wooden toothpicks (Wooden sticks) and placed on a filter paper impregnated with the reagent quickly before it dries.

Catalase production

The colonies were transferred with wooden sticks and placed on a drop of 3% hydrogen peroxide H2O2 placed on a glass slide.

The culture media used to compare with the media of Kurat

Nutrient agar (LAB (U.K))
Nutrient Broth (LAB (U.K))
Blood agar base (Himedia (India))
The culture media used for comparison with the locally prepared medium was prepared according to the instructions of the supplying companies and was sterilized using an autoclave at a temperature of 121 ° C and a pressure of 1 atmosphere.
The components of the nutrient agar medium from which the blood agar medium was prepared, with the addition of blood at a ratio of 5 ml per 100 ml of the nutritional agar, were as follows
For every liter of distilled water added
5 gm of peptone
5 gm of sodium chloride
3 gm of yeast extract
15 gm of agar

The locally prepared media of Kurat

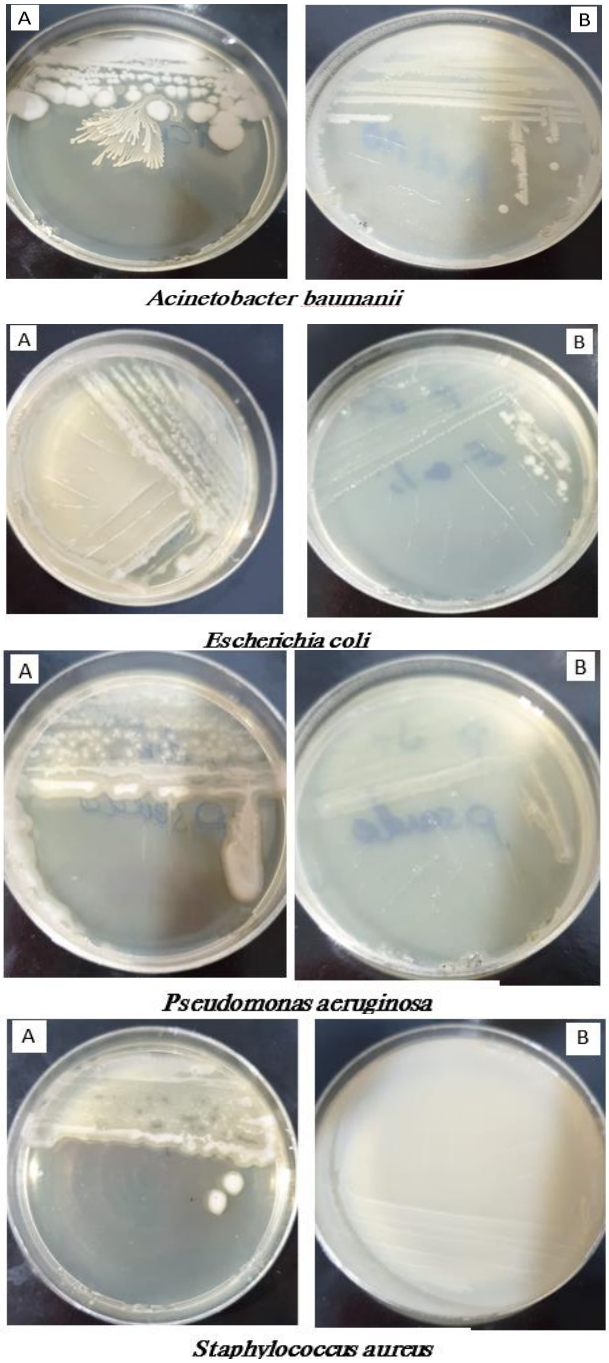
Preparation method for solid and liquid medium

The solid medium of the mixture was prepared according to the nutrient agar medium, where 5 g of peptone, 3 g of yeast extract, 5 g of NaCl and 15 g of agar were added, but the powder of Kurat was used once instead of the peptone and again instead of yeast extract.
The liquid mixture medium was also prepared, using Kurat instead of peptone and yeast extract used to prepare the nutrient broth, but without adding agar to it. The prepared media was filtered by filter paper to clear all insoluble or clumped material, and then autoclaved at 121 ° C and a pressure of 1 atmosphere for 15 minutes.
· When using the method of adding Kurat instead of peptone, the color of the medium was pale, so a 1ml of crystal violet dye or Safranin dye used in Gram stain was added for every 100 ml of the locally prepared medium of Kurat. The isolates were cultured on the two media and it was determined whether adding the dye affected the growth of the isolates.
The blood was added to the locally prepared medium of Kurat, to which the Kurat was added instead of peptone, as its color was close to the medium of the blood agar. The growth of hemolytic isolates was assayed on it and their ability to analyse blood fully and partially was recorded.
Measure the pH of Kurat media
The pH of the locally prepared medium was measured using a pH-meter (Inolab, Germany), where the pH was 4 for the medium prepared by using Kurat instead of peptone and instead of yeast extract and instead of both.

Results

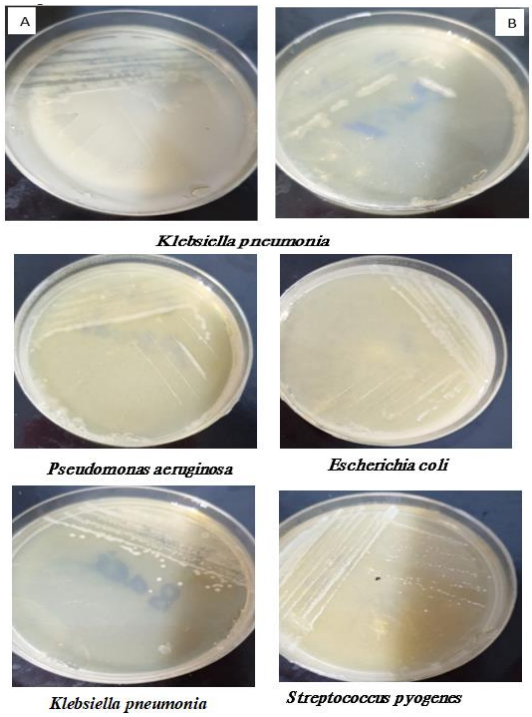
Comparison of growth of clinical isolates cultured on routine media with locally prepared solid media

The growth on the locally prepared medium was compared with the growth on the nutrient agar medium using Kurat instead of peptone or instead of yeast or instead of the two, for each of the clinical isolates isolated in the study. When used instead of peptone, it gave a growth similar to or slightly less than what was given by the growth of isolates on the nutrient agar media as in panel No. 1.



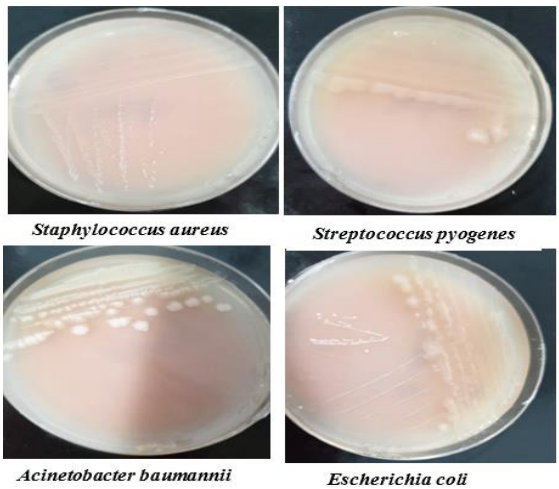
Panel 1 Comparison of the growth of clinical isolates on the prepared medium when using Kurat instead of peptone and the standard nutrient agar medium.

A- The medium of the laboratory prepared Kurat B- The medium of the nutrient agar.
While the growth when using Kurat instead of yeast was slightly less than it was in the nutrient agar, as shown in panel No. 2



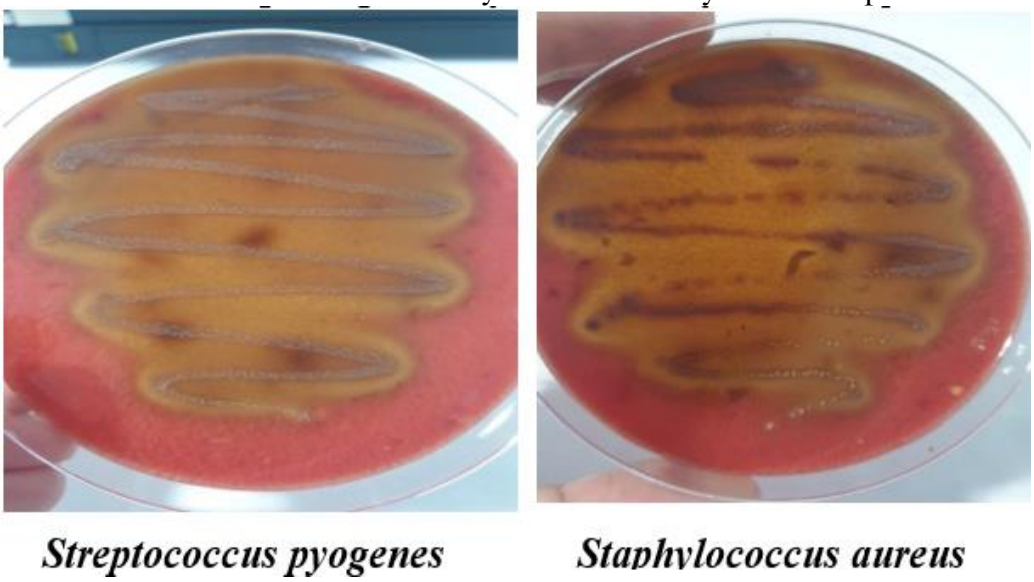
Panel 2 shows the growth of clinical isolates on the prepared medium when using Kurat instead of yeast extract.

And for the transparency of the color of the medium and the difficulty of distinguishing bacterial growth on it, safranin was added to add color to the medium, and the colored media gave a growth similar to what appeared on the nutrient medium panel 3.



Panel 3 shows the growth of clinical isolates on Kurat medium with adding Safranin dye.

The prepared medium also gave a tremendous result when used as enrichment medium by adding blood to it at 5% and growing the fully blood hemolytic isolates panel 4.



Panel 4 shows the growth of blood haemolytic clinical isolates on Kurat medium with blood adding.

Kurat media also gave a high efficiency when using it as a liquid medium to stimulate the growth of Gram positive and negative isolates used in the research, where the growth density was measured using a spectrophotometer as shown in table 1

Table 1: Optical density (OD 600) measurement of the liquid form of Kurat media

type of bacteria	type of medium	optical density measurement
E. coli	Kurat instead of Peptone broth	0.207
Staph. aureus	Kurat instead of Peptone broth	0.179
تكتب aureus E. coli	Kurat instead of Yeast extract broth	0.384
Staph. aureus	Kurat instead of Yeast extract broth	0.659
E. coli	Nutrient broth	0.114
Staph. aureus	Nutrient broth	0.291

Discussion

In recent years, the trend has become to use natural materials as part of the sustainability of the environment. As a result of this development, the world has become in a race to use materials that have the least impact on the environment, with less expense and less waste when destroyed. Therefore, the medical and environmental trend in all fields was to use natural materials.

As part of these attempts, the use of culture media prepared from natural foodstuffs or food waste materials containing nutrients necessary for the growth of microorganisms was considered.

In a previous study, the Kurat medium was used to grow the fungi [33], so, in our study the medium was assayed to grow different species of bacteria and the growth was compared with the routine bacterial media using in bacteriology laboratories.

Comparison of the growth of clinical isolates isolated on routine media with the locally prepared medium

The growth on the locally prepared medium was compared with the growth on the nutrient agar medium using Kurat instead of peptone or instead of yeast or instead of the two, for each of the clinical isolates isolated in the study. When used instead of peptone, it gave a growth similar to or slightly less than that given by the growth of isolates on the nutrient agar media, and the reason for this may be due to the fact that peptone is the main component of the protein and amino acids that produce organic nitrogen essential for the growth of bacteria. Al-Saeed study in 2012 proved that the fruits of Typha domingensis Pers was free of protein when conducting an analysis of the chemical components of the fruits of the papyrus plant [34], while Al-Musa and Abu Mijdad 2013 reported

that the culture medium prepared from the flowering inflorescence of the papyrus plant (Typha domingensis Pers) used for cultivation of fungi showed an intense growth of the fungi compared to the routine media used for the cultivation of fungi, and they attributed one of the reasons for the quality of Typha domingensis Pers medium It contains a high protein component, which provides the fungi with the organic nitrogen necessary for their growth [33].

The quality, density, and speed of growth of microorganisms depend on the components of the culture medium, and this is what the scientist Robert Koch observed in his attempts to grow bacteria on the culture media. It was thicker by using the medium of the meat broth and attributed this to the diversity of organic ingredients and their abundance in the meat broth and beef serum [35].

Variation in peptone as a constituent of broth buffered peptone water BPW has a significant effect on growth and enumeration of bacteria [36].

Conclusion

The locally prepared medium of Kurat is efficient for cultivation of clinical isolates and can be used instead of the expensive nutrient agar medium. The study showed the ability of Gram positive and negative bacteria to grow on Kurat media in solid and liquid form, which indicate the efficiency of this nutritional agent to be used as a commercial media in bacteriological studies

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