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Alternative Culture Medium for Bacterial Growth Using Chicory Roots Extract.

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Abstract:

This is the first study to test the using of hot water extract of Cichorium intybus (chicory) of roots as alternative medium for Bacterial growth.

The tested bacteria were: *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Acinetobacter spp*.The results showed heavy growth of all tested bacteria after incubation at 37^{o}C for 24hrs.

In conclusion, Cichorium intybus (chicory) roots medium is a good source of nutrients, in both solid and liquid media, due to it contains of proteins, fructoligosaccharide (inulin) and elements and can used as alternative medium.

Key words: *culture media, chicory roots, hot water extract.*

الخلاصة:

لأول مرة تم دراسة استخدام المستخلص المائي الحار لجذور نبات الهندباء كبديل للأوساط الزرعية التقليدية المستخدمة لتنمية البكتريا في المختبر. حيث استخدم الوسط المحضر لتنمية الأنواع البكتيرية التالية:

Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi and Acinetobacter spp.

أظهرت النتائج نمو جميع الأنواع البكتيرية المختبرة نموا ملفتا للانتباه بعد الحضن بدرجة حرارة 37م ولمدة 24 ساعة وفي كلا الوسطين: الوسط الزرعي السائل والوسط الزرعي الصلب المحضرة من المستخلص المائي الحار لجذور الهندباء.

نستنتج من هذه الدراسة، إن الوسط المحضر من المستخلص المائي الحار لجذور الهندباء يوفر وسطا زرعيا مناسبا لنمو الأنواع البكتيرية لاحتوائه على السكريات المتعددة والبروتينات والعناصر الضرورية للنمو.

Introduction:

Every organism must find in its environment all the substances required for energy generation and cellular biosynthesis. The chemical elements of this environment that are utilized for bacterial growth are known nutrients or nutritional requirements, these require ments must be met by a culture media [1].

In order to grow, the bacteria must have an energy sources, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature and pH [2].

A culture medium is a special medium used in microbiological laboratories to grow a different kinds of microorganisms, which composed of different nutrients that are essential for microbial growth [3,4]. Bacterial culture media can be classified in at least three ways. Based on consistency;liquid media, semisolid media and solid media [5].Or based on functional use, these include basal media, selective and enrichment media, differential media, transport and holding media [6].

Finally can be classify depend on nutritional component; as a simple, complex and synthetic. While most of the nutritional components are constant across various media, some bacteria need extra nutrients; those bacteria that are able to grow with minimal requirements are known fastidious and those that require extra nutrients are known to be fastidious [7].

Complex media such as blood agar have ingredients whose exact components are difficult to estimate. Synthetic or defined

media are specially prepared media for research propose where the composition of every component is well known [8].Screening of alternate media is found to be an important task [9].

A number of studies have been carried out to find alternative source of culture media to replace nutrient ager or broth. in a study was effectively used to replace nutrient agar for the growth of selected bacteria [10]. In another study vegetable was used as a nutrient source with agar for microbial growth [11].

In a pioneer study, I prepared alternative media using chicory roots. Chicory is the common name given to the flowering plants from the genus Cichorium of the family Asteriaceae; is a plant of great economic potential due to high concentrations of fructoligosaccharide, known as inulin in its roots [12]. Since the 16 th century the herbs has been used in food preparation. Root chicory has been cultivated in Europe as a coffee substitute for a long time. Fresh chicory root typically contains, by dry weight 68% inulin, 14% sucrose, 5% cellulose, 6% protein, 4% ash and 3% other components [13].

The aim of this study was to investigate the ability of both liquid and solid medium prepared from hot water extract of chicory roots to use as alternative medium to support the growth requirements of many types of pathogenic bacteria: *Klebsiella pneumonia. Staphylococcus aureus, Streptococcus pneumonia, Protens vulgaris, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter sp., Salmonella typhi,*

Materials and Methods:

Growth and maintenance of test bacteria: Bacterial cultures of *Staphylococcus aureus, Streptococcus pneumoniae Escherichia coli, Salmonella typhi, Acinetobacter sp, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae* were obtained from hospitals laboratories, all these isolates were identified and checked, for its purity using the methods described in [14,15].

The bacteria were maintained in test tube slants of Muller-Hinton agar at 37^{o}_{C} for 24hrs, and then stocked at 4^{o}_{C} Subcultures were prepared from stocks for assays.

Collection of plant Roots of *Cichorium intybus* (chicory) used in this study was collected and confirmed by National Herbarium of Iraq (Abu Garib).

Preparation of extracts:

The dried roots were finally powdered using electric blender and extracted with hot water according to Harborne, 1973 [16]. With a slightly modify. Distilled water (250 ml) was added to the roots powder (50 gm), the mixture was heated to 100^{o}_{C} for 30 minutes. The mixture was then allowed to cool and the extract was filtered using sterile muslin, then it was filtered by using Whitman filter paper. After filtration, the extract was sterilized by using Millipore filter (0.22Mm).

The sterile extract was putted in sterile container and preserved in refrigator (4^{o}_{C}) until used for preparation of alternative media. The pH of extract was 6.

Preparation of alternative medium:

Both of liquid and solid media were prepared from chicory root extract.

1- The extract as liquid medium: The sterile extract of chicory roots were dispensed in to sterile tubes (5ml of the extract in each tube).
2- The extract as solid medium: solid medium was prepared by adding sterile agar-agar to the extract, this done by dissolving 2 gm of agar-agar in 20ml distilled water and sterilized by using autoclave (121 ^{o}C, 15 Ib for 15 minutes), then left it to cool at 45^{o}_{C} to add it to 80ml of sterile chicory root extract. Approximately 20 ml of the sterilized prepared medium (chicory roots extract agar medium) was distributed in to each the sterile petridishes.

Bacterial inoculation:

The overnight culture broth of the following bacteria was used in this study: Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Proteus vulgaris, Acinetobacter sp.and Pseudomonas aeruginosa. A loopfull of each type of the tested bacteria was inoculated in to the liquid chicory roots extract medium, while the solid chicory roots extract medium was swabbed with tested bacteria.

The controls were both liquid and solid chicory roots extract media without inoculation. The inoculated tubes and plates of prepared extract media were incubated at 37 ^{o}C for 24hrs.

After incubation period, the bacterial growth and pH were detected and compared with controls.

To confirm the growth of tested bacteria on prepared media, a loopfull of the growth from both liquid and solid chicory roots extracts media was transfer to Muller Hinton agar plates, and they incubated at 37 ^{o}C for 24 hrs After incubation period, biochemical tests to identify the bacterial growth was done.

Results:

The present research showed that the chicory roots extract media supported the growth of all tested bacteria, due to its containing of the nutrients essential for their growth as sugars, proteins and other elements. Examination of bacterial growth in both liquid and solid chicory roots extract medium was done after incubation period; the pH of liquid medium was increased from 6 to 5 after the growth of tested bacteria.

In liquid chicory extract medium, the bacterial growth was active, and detected by the turbidity appeared within the test tubes which inoculated with different types of bacteria (figure-1) the test tubes were compared with control tubes, which contain the liquid chicory roots extract medium without bacterial inoculation. Control tubes were clear after incubation period. The turbidity appeared in test tubes is an indicator for bacterial growth (figure-2).

In the solid chicory roots extract medium, the bacterial growth was detected by presence of bacterial colonies on the surface of the solid chicory roots extracts medium (figures,4,5,6,7,8), this was compared with the control plates which contain solid chicory roots extract without bacterial inoculation (figure-3). The results of incubate control plates was no bacterial growth after incubation period.

The results of the subcultures done for bacterial growth from chicory roots extracts media in both liquid and solid media, showed that the growth is for the tested bacteria which confirmed by doing biochemical test.

Figure-1: The liquid medium after cultivation. From left side: control, *S. aureus, S. pneumonia, p. vulgaris, Ps. aeruginosa, E. coli, Acinetobacter sp., S. typhi, K. pneumonia.*

Figure-2: Turbidity liquid medium after cultivation with *P. vulgaris* compare with control. From left: control, *P. vulgaris*.

Figure-3: Chicory roots extract agar medium without cultivation.

Figure-4: C**ultivated solid medium Up: *E. coli,* Down: *K. pneumonia***

Figure-5: **Cultivated solid medium up: *S. pneumoniae,* down: *S. aureus***

Figure-6: **Cultivated solid medium up: *P. vulgaris,* Down: *K. pneumoniae*.**

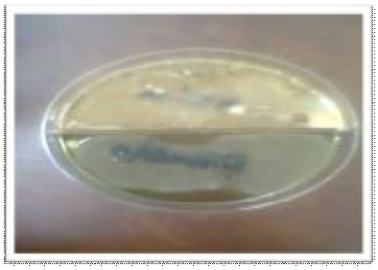

Figure-7: **Cultivated solid medium up: *Acinetobacter sp.* Down: *Ps. aeruginosa***

Figure-8: **Cultivated solid medium *Salmonella typhi***

Discussion:

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce.

In the environment, microbes have adapted to the habitats most suitable for their needs. In the laboratory, however, these requirements must be met by a culture medium. The culture medium should contain amounts of all basic nutritional requirements such as salts, amino acids, vitamins and carbon source. Hence an alternative media using hot water chicory roots extract which supply the growth requirements, was designed in the present study, and the ability of both liquid and solid chicory roots extract media to support the growth of the test bacteria was investigated, the results showed that this prepared medium support the bacterial growth due to its contain of the requirements for growth like protein, sugars and elements. In a study showed the chemical, elemental and types of sugars content in chicory roots, Chicory roots contain protein, oil, ash and contain Ca, Na, K, P, Mg, also contain inulin, glucose and fructose [17].

The presence of sugars such as inulin[18] and the elements presence is very important for living mater [19], therefore, the chicory roots extract could be a good medium for bacterial growth.

In this study, filtration with Millipore size 0.22 Mm was used to sterile the extract, therefore the bacterial growth was obtained is by inoculation with tested bacteria. Also

the growth was sub cultured in to Muller-Hinton ager medium, their identified by using biochemical tests.

Agar-Agar was used as a solidifying agent to investigate the ability of prepared solid medium to supply the requirements for bacterial growth Agar-Agar is a natural polysaccharide produced by marine algae and is the most commonly used solidifying agents added to media, therefore it has been used in the present study.

In conclusion, the relative performance of bacterial growth on the prepared media, when compared with the congenital media, illustrated a good growth for all bacteria were used. Hence, the prepared media produced from chicory roots extract can be used for the cultivation of microbes. It should done more studies for this extract and more test micro organisms should be used to identify the suitability of using chicory roots extract medium as an alternative general purpose medium.

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