

The Inhibitory Effect of some Plant Extracts on some Pathogenic Bacteria

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ABSTRACT

The purpose of the present study is to assay the inhibitory effect of five concentrations 10, 20, 30, 40 and 50 % of the aqueous and alcoholic extracts for leaves of the Sidr (*Zizyphusspin-csit*) and Eucalyptus leaves (*Eucalyptus camaldulensis*) *in vitro*, against the three types of the experimental bacteria, which *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* for their clinical importance as common, pathogenic in urinary tract infection by using the well diffusion assay method. The results showed that antimicrobial activity depends on kind of extract and tested microorganism. and the most bioactivity was done by methanol extract than the aqueous extract, the methanolic extract of Sidr leaves presented the inhibitory effectiveness of all the species used in this study, the highest inhibition was 36 mm at 50% concentration against *E. coli* and methanolic extract of eucalyptus leaves showed an inhibitory effect on the growth of all bacteria except *E. coli*, showed resistance to the extract, As for the aquatic extracts, all the bacteria species illustrated resistance to the aquatic extracts of the leaves of the Sidr except *Staph. aureus*. Inhibition rates were recorded at 17 and 15 mm at the concentration (30 and 20%), respectively, and the aquatic extracts of Eucalyptus leaves had no inhibitory effect on the growth of all the species used in this study, except *S. typhimurium* bacteria and all concentrations used. The result of the qualitative chemical detection of the active compounds found in the extract indicated that it contains, mainly like flavonoids, alkaloids, Tannins and phenols, Saponins, Glycosides. in aquatic and alcoholic extraction.

Introduction

In recent years, the world has turned its attention to the study of medicinal plants, many of which have been found to have inhibitory effect against pathogens. They have been used in the treatment of many diseases, even the most difficult and intractable ones, since they contain effective compounds that are inhibitory and free from side effects compared with the drugs used which have side effects on health with increased resistance towards it by time. This has called for urgent and continuous need to search for new antimicrobials as a result of increase in disease cases. The other reason is increasing resistance to antibiotics and on a continuous basis. Thus, researches are currently conducting new research on plants to overcome the resistance of microbes to these antibiotics and to obtain natural remedies to strengthen immunity. In addition, medicinal plants

treatment is economical and highly efficient. More than 50% of the medicines are of natural origin, and plant medicines account for more than 26 percent of the total [1]. you can check it again Medicinal plants contain various types of natural active substances used in traditional medicine (alternative medicine) to treat diseases around the world [2,3,4,5].

The efficiency of these medicinal plants or their extracts varies depending on the method of extraction, the type of solvent used in the extraction and the microscopic organism [6,7], of the most important medicinal plants at present time is the Sidr (*Zizyphusspin-csit*) because its leaves contain effective groups of therapeutic significance. It is used for the treatment of many cases or symptoms such as headache treatment, laxative for the abdomen, fever and skin purifier. Its leaves are used particularly for

the treatment of joints, malignant tumors, immune deficiency, stomachaches and malaria [8]. Sidr (*Zizyphusspin-csit*) also has high efficacy against the growth of microorganisms, inhibiting many Gram positive and negative bacteria growth. [9,10].

Other medicinal plants are eucalyptus (*Eucalyptus camaldulensis*), a tree widespread in Iraq with many species used extensively to treat respiratory infections accompanied by pus. It is also used for the treatment of middle ear infections (otitis media) and as an antibacterial, especially the positive gram stain types [11] and viruses. It is also used to treat sinusitis [12]. Therefore, the current study aimed at testing the effect of plant extracts (Sidr and Eucalyptus leaves) on the growth of some bacteria using water and methyl alcohol by studying the biological efficacy of these extracts to take advantage of the secondary metabolic materials of these plants' leaves as an alternative to antibiotics in the treatment of various diseases and employing it in the health and medical fields; with the possibility of their use in the treatment of many medical conditions caused by the types covered under the study.

Material of Methods

1. Collection and configuration of plant samples

The leaves of the plant used in this study consisted of Eucalyptus (*Eucalyptus camaldulensis*) and the Sidr (*Zizyphusspin-csit*) which are growing in Iraq. These plants are collected from different areas from the garden of the college of Agriculture / University of Tikrit. The leaves of the plant were washed under running tap water, and placed in shade to dry. The dried leaves were pounded in to a homogenous powder using electric blender. The plant powder was stored in the refrigerator for further use.

2. Preparation of plant extracts

Two different solvents (distilled water and methanol and distilled water) were used for extraction. 50g each of the dry plant materials (dried powder) were soaked in 500 ml of distilled water and methanol at room temperature for 24 hours. The extracts were filtered using three layers of muslin cloth whatman filter paper NO.0.1 and centrifuge of 3000 (g) cycles / min for 15 minutes and the supernatant was collected and dried using the oven at 40°C and then stored the dry powder in the refrigerator for further use to test the effectiveness against the bacteria used in the experiment (The dry weight of the plant sample was 15 g dissolved in 15 ml of distilled water to obtain the stock solution 100%)

The concentrations used in this study were (10, 20, 30, 40 and 50 %) of the aqueous extract and the extracts of methyl alcohol of the plant. When the extracts were used in the inhibition experiments, they were sterilized using Membrane filter with diameters of 0.45 µm. [13]

3- Bacterial species used in the study

The bacterial species used in the study were obtained from the college of Science at the University of Tikrit

((*Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*)).

4 – Antibacterial activity test of plant extracts

Antibacterial activity was determined by the Well diffusion assay. According to [14] each microorganism was inoculated by streaking the swab over surface of Mueller-Hinton Agar plates, and the inoculum was allowed to dry at room temperature for 5 min. Holes of 5 mm in diameter were made in the seed agar using Glass Pasteur pipettes. Each plant extract was checked for antibacterial activity by introducing 0.1 ml into the well. The plates were allowed to stand at refrigerator temperature at 2 hours for Extract to diffuse into the agar and then they incubated at 37 °C for 18-24 hours. The resulting inhibition zones were measured in millimeters (mm) [15,16]

5- The qualitative detection of the active compounds (phytochemical) in the extract (methanolic and aqueous extracts)

5-1-Detection of Saponins

According to [17] was used to detect the Saponins: 5 ml of the plant extract with drops of distilled water and the mixture was shaken vigorously (solidly), acupious foam formation was noticed which indicated the presence of saponin. If foam produced persists for ten minutes it indicates the presence of Saponins [18].

5.2 Detection of glycosides

Fehling's test is used for detection of glycosides by mixed was 5ml of the extract with 5ml of Fehling's solution A&B, and heated gently and boiling over a water bath (100°C for 10 minutes); formation of red precipitate indicates dark green colouration indicated the presence of glycoside [17,18,19, 20].

5.3 Detection of Tannins

According to [21] by taking drops of Lead acetate solution (1%) was added to 1.6 ml of the plant extract, Formation of a white precipitate indicated the presence of Tannins.

5-4 Detection of Alkaloids

Mayer's Test: Extracts were treated (1 ml) with Mayer's reagent (Potassium Mercuric Iodide) in a watch glass. Formation of a white coloured precipitate indicated the presence of alkaloids [18].

5-5 Detection of flavonoids

taking 10 g of plant extracts were dissolved in 50 ml of 95% ethyl alcohol. The solution was then filtered and the solution A was indicated, added 10 mL of ethyl alcohol at 50% to 10 mL of 50% potassium hydroxide solution. B, and then mixed equal amounts of A and B, Formation of yellow colour precipitate presents the presence of flavonoid [22].

5-6 Detection of Phenols.

Ferric Chloride Test: by Extracts (3ml) were mixed of with 2 ml of ferric chloride solution. Formation of bluish green colour indicates the presence of phenolic compounds [23,24].

Results

The methanolic extract of sidr (*Zizyphusspin-csit*) leaves showed 50% inhibitory activity for all types of bacteria under study. It recorded inhibition diameters

of 36,24 and 16 mm for bacterial types *E. coli*, *Staph. aureus*, and *S. typhiurium*, respectively (Figher 1,2,3), while methanol extract for eucalyptus leaves showed inhibitory activity for all concentrations used for bacterial types *S. typhiurium* (Figher 4) and *Staph. Aureus* (Figher 5), while *E. coli* showed resistance to all concentrations under study (Figher 6), as shown in Tables 1, 2 and 3.

As for the aquatic extract of the Sidr leaves, it elucidated an inhibitory effect to inhibit the growth of bacteria type *S. typhiurium* only(Figher 7) and did not affect the other bacterial types (species) under study (Figher 8 and 9) for bacterial types *E. coli* and *Staph. aureus*, respectively. The aquatic extract of eucalyptus leaves showed no inhibitory effect towards growth of *E. coli* (Figher 10) and *Staph. aureus* (Figher 11) for all concentrations, while it showed an inhibition activity towards bacteria *S. typhiurium* (Figher 12) for all concentrations used are as shown in Tables 4 and 5. The results of the qualitative chemical detection of the active substances in the plant extracts also confirmed that they contain many of it, the most important of which are the Tannins, phenols, flavonoids, glycosides and others about tannins the result shows a white precipitate , the Phenols test presents a formation of bluish green colour, for the flavonoids, the result showe is an s a formation of a yellow colour Precipitate indicated for the presence of flavonoid. The formation of red Precipitate with a dark green colouration expressed the presence of glycoside, a formation of a copious foam formation was noticed which refers to the presence of saponin. if foam produced persists for ten minutes it explained the presence of Saponins, a formation of a white coloured Precipitate the presence of alkaloids,as shown in Tables 5 and 6.

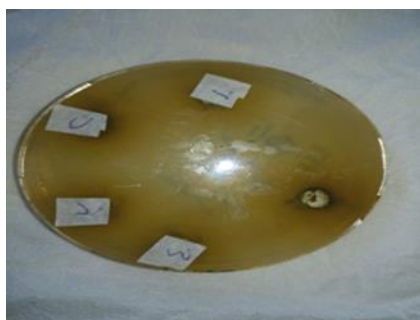


Figure (1) The inhibitory activity of the alcoholic extracts (*Zizyphusspin-csit*) on the growth of *E.coli* .



Figure (2) The inhibitory activity of the alcoholic extract (*Zizyphusspin-csit*) on the growth of *Staph. aureus*.



Figure (3) The inhibitory activity of the alcoholic extract (*Zizyphusspin-csit*) on the growth of *Salmonella typhiurium*.

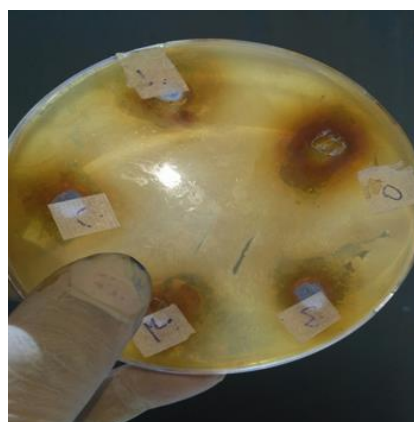


Figure (4) The inhibitory activity of the alcoholic extract (*Eucalyptus camaldulensis*) on the growth of *Salmonella typhiurium*.



Figure (5) The inhibitory activity of the alcoholi c extract (*Eucalyptus camaldulensis*) on the growth of *Staph. aureus*.



Figher (6) The inhibitory activity of the alcoholic extract (*Eucalyptus camaldulensis*) on the growth of *E.coli*.

Table (1) The inhibitory activity of the alcoholic extracts studied in the study of the growth of *Staphylococcus aureus* bacteria.

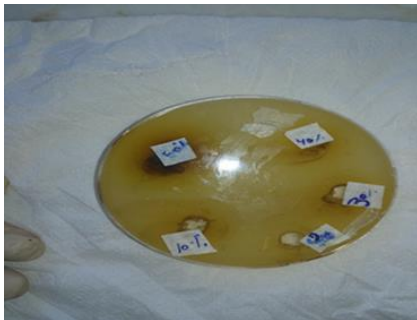
| concentration of extract Type of extract | Inhibition Zone (mm) | | | | |
|---|-----------------------|-----|-----|-----|-----|
| | 10% | 20% | 30% | 40% | 50% |
| <i>Zizyphusspin-csit</i> | 17 | | | | 16 |
| <i>Eucalyptus camaldulensis</i> | 1 | 13 | 15 | 20 | 20 |

Table (2) The inhibitory activity of the alcoholic extracts studied in the study of the growth of *Escherchia coli*.

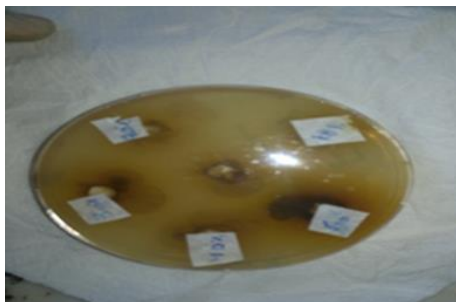
| concentration of extract Type of extract | Inhibition Zone (mm) | | | | |
|---|-----------------------|-----|-----|-----|-----|
| | 10% | 20% | 30% | 40% | 50% |
| <i>Zizyphusspin-csit</i> | | | | 17 | 36 |
| <i>Eucalyptus camaldulensis</i> | - | - | - | - | - |

Table (3) The inhibitory activity of the alcoholic extracts studied in the study of the growth of *Salmonella typhiurium*

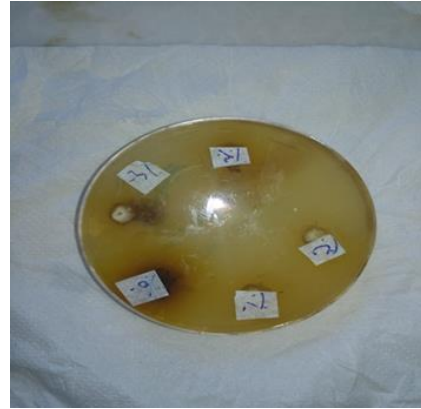
| concentration of extract Type of extract | Inhibition Zone (mm) | | | | |
|---|-----------------------|-----|-----|-----|-----|
| | 10% | 20% | 30% | 40% | 50% |
| <i>Zizyphusspin-csit</i> | 16 | 12 | 18 | 12 | 24 |
| <i>Eucalyptus camaldulensis</i> | 20 | 23 | 21 | 23 | 25 |



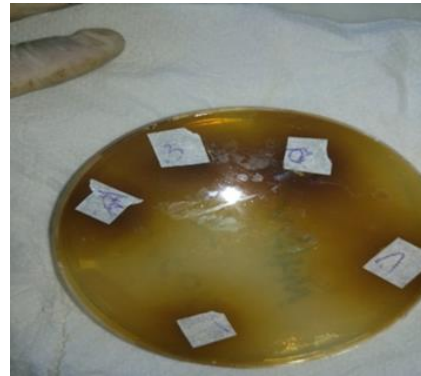
Figher (7) The inhibitory activity of the aquatic extracts (*Zizyphusspin-csit*) on the growth of *Salmonella typhiurium*.



Figher (8) The inhibitory activity of the aquatic extract (*Zizyphusspin-csit*) on the growth of *E.coli*.



Figher (9) The inhibitory activity of the aquatic extract (*Zizyphusspin-csit*) on the growth of *Staph. aureus*.



Figher (10) The inhibitory activity of the aquatic extract (*Eucalyptus camaldulensis*) on the growth of *E.coli*.



Figher (11) The inhibitory activity of the aquatic extract ((*Eucalyptus camaldulensis* on the growth of *Staph. aureus*.



Figher (12) The inhibitory activity of the aquatic extracts (*Eucalyptus camaldulensis*) on the growth of *Salmonella typhiurium*

Table (4) The inhibitory effect of the studied aquatic extracts on the growth of *Staphylococcus aureus*

| concentration of extract Type of extract | Inhibition Zone (mm) | | | | |
|---|----------------------|-----|-----|-----|-----|
| | 10% | 20% | 30% | 40% | 50% |
| <i>Zizyphusspin-csit</i> | - | 15 | 17 | - | - |
| <i>Eucalyptus camaldulensis</i> | - | - | - | - | - |

Table (5) The inhibitory effect of the studied aquatic extracts on the growth of *Salmonella typhiurium*.

| concentration of extract Type of extract | Inhibition Zone (mm) | | | | |
|---|----------------------|-----|-----|-----|-----|
| | 10% | 20% | 30% | 40% | 50% |
| <i>Zizyphusspin-csit</i> | - | - | - | - | - |
| <i>Eucalyptus camaldulensis</i> | 14 | 20 | 20 | 20 | 20 |

Table (6) Specific chemical analyzes of the active substances of the aquatic plant extracts covered in the study

| Type of plant | Active Substances | | | | | |
|---------------------------------|-------------------|------------|-----------|---------|------------|---------|
| | Saponins | Glycosides | Alkaloids | Phenols | Flavonoids | Tannins |
| <i>Zizyphusspin-csit</i> | + | ++ | V | - | - | ++ |
| <i>Eucalyptus camaldulensis</i> | - | + | V | + | V | ++ |

+ Positive result, - negative result, v divergent result

Table (7) Specific chemical data for the active substances of the alcoholic plant extracts covered in the study

| Type of plant | Active Substances | | | | | |
|---------------------------------|-------------------|------------|-----------|---------|------------|---------|
| | Saponins | Glycosides | Alkaloids | Phenols | Flavonoids | Tannins |
| <i>Zizyphusspin-csit</i> | ++ | ++ | V | ++ | V | V |
| <i>Eucalyptus camaldulensis</i> | + | ++ | ++ | + | V | + |

+ Positive result, - negative result, v divergent result

Discussion

The effectiveness of sidr (*Zizyphusspin-csit*) extracts is attributed to the presence of many active compounds that have inhibitory effect on positive and negative Gram-stain bacteria. It contains many active ingredients, including pseudo alkaloids such as spinanina and jujube, which are responsible for antimicrobial activity. Extracts are attributed to the presence of many active compounds that have inhibitory effect on positive and negative Gram-stain bacteria. It contains many active ingredients, including pseudo alkaloids such as *spinanina* and *jujube*, which are responsible for antimicrobial activity [25]. In addition, it includes all types of flavonoids such as antioxidant glycosides, sapindales, phenols, tannins, terpenes, pectin, fat, tannic acid and zizyphic acid. This is consistent with [26], especially phenols which work to change the nature of proteins and the damage caused it the membranes by being linked to the active sites of cellular enzymes by the groups of hydroxyl which have the ability to form hydrogen bonds with those sites more than the base material [27,28].

Thus, it inhibits a number of metabolic reactions that are controlled by those affected enzymes and which are necessary for the growth of the microorganism and responsible for the construction of different proteins. Fennema [29] and Cowan [30] pointed out that tannins had the potential to inhibit bacteria by binding to their cell walls, as well as inhibitory effect on transcriptase enzymes (Viral Reverse Transcriptases) and dysfunction of vector proteins, thereby inhibiting their growth. The study corresponds with the results of [13].

In addition, Mahasneh [32] and Ghazal [33] point out that flavonoids have an inhibitory effect against a wide variety of microbes and their inhibitory

effectiveness lies in their ability to bind to cellular proteins and the complex formation with the cell wall of the affected bacteria.

Chakravarty [34] confirmed that eucalyptus leaves contain many active compounds, including flavones such as *hyperoside* and *rutin* types, as well as containing phenols which play an important role in inhibiting the growth of bacteria by inhibiting the enzymes responsible for the basic metabolic reactions by their non-specialized interaction with proteins leading to the denaturation of the protein (35). Whereas Cowan [30] explained that the effectiveness of this extract is due to containing effective tannins which inhibit bacteria and viruses because of its ability to stimulate phagocytic cells and has the effect of destroying proteins and other structures that are present on the bacterial cell wall which are used by the bacterial cell for adhesion. In addition, it comprises the oil that contains the turbines which have an inhibitory effect on the bacteria and which work to rupture the cellular membranes by fat loving materials [36].

These results were in line with [37] findings on eucalyptus extracts and the effective effect of its essential (volatile) oils on the Gram-negative bacteria such as *E. coli* and the Gram- positive bacteria such as *S. aureus*, due to the presence of the two phenolic compounds *thymol* & *carvacrol* in their essential (volatile) oils. These results were identical with [38] on the effective effect of eucalyptus oil on resistant strains of negative bacteria such as *E. coli*. Our results were consistent with what [39] conducted concerning the alcoholic extract of the eucalyptus plant and its effect on the Gram -negative bacteria types such as such as *E.coli*, *Shigella sp*, *Klebsiella sp* and *Salmonella*

The response of *Staphylococcus aureus* bacteria to the effect of the plant extract may be due to the chemical structure of the cell wall in *S.aureus* bacteria, as well as the other Gram- positive bacteria, because these species lack the external membranes, which makes it more easier to penetrate into the cell [40]. The lack of impact of the alcohol and water extracts on *E.coli* bacteria may be attributed to the occurrence of genetic mutations that produced an enzyme that causes resistance to these bacteria [41], or this may be due to the fact that these bacteria contain an effective permeable membrane in the outer wall which acts as a buffer to entering these extracts into the cell, thus preventing its inhibitory effect on the Gram-negative bacterial types, including *E. coli* and *S.typhimurium* [42]. The reason may be that the selected concentrations are not sensitive enough to induce the desired effect because there is no or low concentrations of inhibitory active substances in these concentrations under experiment, especially in the

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- aqueous extract or the bacteria possess ferocity factors which protect it against natural antibacterial substances.
- Tables 7 and 8 clarify the results of the qualitative chemical detection of the active substances. These plant extracts contained many active ingredients, namely alkaloids, phenols, glycosides, tannins, flavones, saponins, etc These compounds have high inhibitory properties and have toxic properties to microorganisms. It has also been shown from the results of this study that the alcohol extract has actually given a more efficient antibiotic compared to the plant aqueous extract which may be due to the polarity of alcohol which plays an important role in the extraction of a number of effective compounds compared to other active solvents, which in turn lead to the precipitation of the largest possible amount of these compounds when extracted. In general, these active compounds operate on the bacterial cell in several inhibitory directions [43.28].
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التأثير التثبيطي لبعض المستخلصات النباتية على بعض انواع البكتيريا المرضية

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الملخص

هدفت الدراسة الحالية التحري عن الفعالية التثبيطية لخمسة تراكيز هي 10, 20, 30, 40, 50% من المستخلص المائي والكحولي لاوراق السدر *Zizyphusspin-csit* واوراق اليوكالبتوس *Eucalyptus camaldulensis* في تجارب مختبرية (*in vitro*) في تثبيط نمو بعض انواع البكتيريا الاختيارية والتي شملت *Escherichia coli* و *Staphylococcus aureus* و *Salmonella typhimurium* وذلك لاهميتها السريرية بصفاتها المسببة لالتهاب المجاري البولية, وباستعمال طريقة الانتشار بالحفر وقد تنوعت نتائج الفعالية التثبيطية للمستخلصات النباتية باختلاف نوع المستخلص والتركيز المستخدم واختلاف الكائن المجهرى المختبر, وقد اعطى المستخلص الكحولي تثبيطياً اعلى من المستخلص المائي حيث اظهرت المستخلصات الكحولية لاوراق السدر فعالية تثبيطية لجميع الانواع البكتيريا المشمولة بالدراسة وسجلت اعلى قطر تثبيطي 36 ملمتر عند تركيز 50% تجاه نمو بكتيريا *E.coli*, اما المستخلص الميثانولي لليوكالبتوس قد اظهر فعالية تثبيطية تجاه نمو جميع الانواع البكتيريا قيد الدراسة ما عدا بكتيريا *E.coli* فقد اظهرت مقاومة عالية تجاه المستخلص المذكور. اما بالنسبة للمستخلصات المائية فقد اظهرت جميع الانواع البكتيرية المختبرة مقاومة تجاه المستخلص المائي لاوراق السدر ما عدا بكتيريا *Staph. aureus* وبلغت اقطار التثبيط 17 و 15 ملمتر عند التركيز (30 و 20%) على التوالي, ولم يكن للمستخلص المائي لاوراق اليوكالبتوس تأثير تثبيطي تجاه نمو الانواع البكتيرية قيد الدراسة ما عدا بكتيريا *S. Typhimurium* ولجميع التراكيز المستعملة. كما بينت نتائج الكشف الكيميائي النوعي للمواد الفعالة في المستخلصات قد احتوى على العديد من المركبات الفعالة اهمها الفينولات والكلايكوسيدات و الفلويديات و الفلافونيدات الصابونيات بالاضافة الى التانينات ومواد اخرى.