Water, Alcoholic and Etheric Extraction Method for Some Active Materials from Linden Plant, So Antibacterial and Antitumor Activity Studies

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الخلاصة

تضمن بحثنا هذا عزل بعض المواد الفعالة من نبات الزيزفون *(Tilia cordata)* كالتانينات والسابونينات والزيوت الطيارة وكانت نسبتها المئوية في النموذج (59.2%) , (31.1%) , (9.7%) على التوالي وكذلك تعيين بعض العناصر المعدنية في الزيزفون كالصوديوم والكالسيوم والبوتاسيوم وكان تركيزها في النموذج (86 ppm)، (65 ppm) ، (467 ppm) على التوالي باستعمال قياس طيف الانبعاث. كذلك أنجزت دراسة الفعالية المضادة للبكتريا للمستخلصات من نبات الزيزفون باستخدام نوعين من البكتريا المرضية وهي (*Escherichia Coli* و*Staphylococcus aurous*) حيث أظهرت الدراسة قدرة تثبيطية مختلفة للمستخلصات المختلفة وبأقطار تثبيط مختلفة تختلف باختلاف المواد الفعالة وتراكيزها وجنس البكتريا. كما تم استخدام نوع واحد من الخطوط الخلوية السرطانية لدراسة مدى تأثير مستخلصات الزيزفون على نمو الخلايا في المختبر وبالتالي معرفة مواصفات المستخلصات كمضادة للأورام وخط الخلايا المستخدم هو ($L_{20}B$) وهي خلايا فأر متحولة (Mice Transformed cell Line).

Abstract

The aim of this study is to isolate some active substances from the linden plant (Tilia cordata) as tannins and saponins and volatile oils. The extraction method shows the percentage of the isolated materials was ((31.1%) (59.2%) and (9.7%) respectively, as well as the appointment of some mineral elements in the linden as sodium, calcium, potassium,was (ppm 86), (ppm 65), (ppm 467), respectively,by application atomic emission spectroscopy. In addition study effectiveness of the anti-bacterial plant extractants of linden using two types of pathogenic bacteria it (Escherichia Coli and Staphylococcus aurous), where the study showed the ability of inhibition of the different extractants and different inhibition diameters vary according to different active substances and concentrations and type of the bacteria. Used one type of cancer cell lines to study the effect of extracts of lime on the growth of cells in the laboratory and thus know the specifications of the extractants anti tumor cell line used is (L20B) a mutant mouse cells (Mice Transformed cell Line).

Keywords: *Tilia cordata ,antibacterial, antitumor, extracts.*

Introduction

The medicinal plants, an important source of materials, effective intervention in the preparation of many drugs has been proven scientifically that the active substance manufacturer vitro does not cause the same effect as the physiological role of the active ingredient extracted from medicinal plants as well as the side effects left by the article prepared on the body, which may not appear until After a period may be long, and the linden leaf of plants belonging to the family (Tiliaceae) Type (Tilia cordata) whose scientific name (Tilia species) [1]. Linden tree and plant perennial wooden leg and smooth rind many twigs and leaves her big heart of a diagonal-like serrated leaves and color of the olive and silver flowers clustered white or blonde aromatic smell good. Used for the treatment of many diseases where the flowers are used for neuropathic pain, chest and to treat colds and bronchitis and to remove intestinal antiseptic, upset stomach, and the branches of linden wood

is used to treat sores and wounds in the shallow end of the skin [2]. Contains leaf linden in the installed material is effective as starch and cellulose, sugar, fiber, the active substances biologically tannins and glycosides and saponins and alkaloids, gums, resins, volatile oils and flavonoids (compounds hysperidine , cuerstine , estraligine and Kampferol) also contain acids, phenolic and fluid materials and sterols (beta sterols and segma sterols) and turbines Triple (squaline) [3], and the chemical content of the linden flowers include flavonoids (compounds isoquerstine , estragaline, and Kampferol querstine) and hydroxy coumarin (as compounds canthozide and squaline). Has been selected isolates of each bacterium (Escherichia Coli and Staphylococcus aurous) and that of medical importance to humans because it is one of the negative and positive bacteria to Gram-causing nature of many diseases [4].

Materials and methods

This study focus on used the flowers of linden plat which is obtained from the local market in the province of Anbar. Collected flowers, plant linden (Tilia cordata) from one shop in Fallujah city, individual solutions and kept at room temperature until the use has been diagnosed in the lush of the Iraqi National - General Authority for examination and certification of seeds - Ministry of Agriculture, and for preparation aqueous taken (40) g of linden flower powder placed in a conical flask with solution of extractant (200) mL distilled water, then mixed by a blender magnetic for (30) minutes and centrifuge for 15 minutes then put the clear solution in the oven and a temperature of 35 ° C and extractant at concentrations (25,15,10,5,1)%.

The alcoholic solutions of extractant was obtained from (50) gram of flowers powder, then prepared linden flower powder . Soxhelt extraction unit and added (350) ml of absolute ethanol and continued the process of extraction for a period of (12) hours at 40 ˚C using an evaporator rotor Vacuum Rotary Evaporator and temperature (35) ˚C, 4 concentrations and attended the same way as attending the concentrations of aqueous extract.

The extracted oil was obtained by adding (350) ml of petroleum ether at (60-40) ˚C to a continuous extraction and followed the steps above in the preparation of alcoholic extract [5].

Isolation of The Active Ingredients

Tannins: Tannins Was isolated from linden flowers, and by adding (75) mL of distilled water to (0.5) g of powder linden flowers, and put the mixture in a water bath boiled for (30) minutes and put the mix in centrifuge at speeds (200 cycle \ minutes for (20 minutes). transfer clear solution to volumetric flask (100) ml and complete the volume to the mark with distilled water then added (20) ml of 4% lead acetate with shaking continued and then take the precipitate to dried at a temperature (70)˚C in oven [6].

Saponins: To (10) grams of powder added (50) mL of (20% ethanol) and heated in water bath for 30 min. at 55 ˚C with stirring after filtered ,the solution separated was added to it (100)

ml of ethanol and then heated the solution using a water bath at (90) ˚C even become final solution volume (40) ml, where the transfer of filtrate and added to it (20) ml of ether in a separating funnel then separated layer of water and neglected layer ether was added to the layer of water (10) ml of (n-butanol) and evaporation the resulting solution in a bath of water and dry the solution for saponins [7,8].

Essential oil (Volatile oil): Extracted volatile oils by (Soxhlet), using ether as organic solvent where for (5) grams of powder added (150) ml of ether were carried out by the process of extraction for a period of (24) hours after the separation of the solvent for volatile oils [9].

Determination of Elements in The Linden Flowers

By used atomic emission Spectroscopy (Flame photometer) to estimate some elements using a type GENWAY PFP7 , for the analysis taking (1) g of powdered lime and melted in (20) ml of aqua regia (HNO_3 + 3HCl) and leave for 30 min. after the filtered the mixture and then complete the solution to (100) ml with distilled water. Where she attended a series of standard solutions were then measured the intensity of the emission standard solutions prepared and solutions of models [10].

The Study of Effective Anti-Bacterial Pathogenesis

Followed the method of (Agar-well diffusion method) depending on how you can use the way kirby Baauer [11] in the measurement of the sensitivity of the bacteria used in the search for the concentrations of various substances derived from the extract where to get bacteria (Escherichia Coli and Staphylococcus aurous) isolated and diagnosed in a laboratory culture the children's hospital in Ramadi, was also used center (Mueller Hinton ager) to test the sensitivity of bacteria to extracts of flowers, linden and attended as instructed by the company processed, then put dishes in the incubator at a temperature (37) ^{o} C for a period of (24) hours and was then measure the diameter of inhibition (Inhibition Zone) [12, 13] in each hole mediated by the ruler and record the results.

Preparation of Standard Solutions of The Substances Isolated from Linden

Been preparing a series of solutions of the extracts of different concentrations (1%, 5%, 10% 15% 25%) mg / ml.

Test the effect of extractants against cancer cells: Using one type of cancer cell lines to study the impact of the extracts under study linden extracts on the growth of cells in the laboratory and thus know the specifications anti extracts of tumors and was working at the Department of Cancer Research in Bio-technology Research Center at the University of Al-Nahrain.

Cell line used is (L20B) a mutant mouse cells (Mice Transformed cell Line). In this way is calculated the proportion of the number of cells within the optimal conditions for growth without the addition of extracts in question and then the output is the control group (control).

Then extracts are added for the purpose of knowing their effects on cell growth in the fonts elected.

Results and Discussion

Table 1 shows the percentages of the components of effective have been isolated from linden flowers (Tilia cordata) and the percentage of Tannins and saponins and volatile oils are (59.2%) (31.1%), (9.7%), respectively, where it is noted that the tannin has recorded the highest percentage in the linden flowers, followed by saponins and volatile oils.

Table 1: Percentages of Active Ingredients in The Linden Flowers (Tilia Cordata)

Active Substance	Percentages
Tannins	59.2%
Saponins	31.1%
Volatile Oils	9.7 %

Study also showed qualitative analysis of the active compounds contained in the linden to the presence of flavonoids and carbohydrates, tannins and intense catechol and anthocyandine and saponins.

Results at **Table 2** shows the amount of mineral elements in Linden, sodium (ppm 86), calcium (ppm65) and potassium (467 ppm) which is importance of functional and metabolic in the body, sodium play an important role in maintain the balance of fluids outside the cell in the body as well as the pH of these fluids and is also involved with the potassium in the organization of the movement of muscles involuntary, such as heart rate, calcium promotes strong bones and teeth and contributes to the transfer directives nerve and organization of the heartbeat also introduces magnesium in building the body's tissues which is an important component of more from (300) enzyme in the body with important metabolic functions and biology [14].

Table 2: The Amount of Mineral Elements in The Linden Measured By Flame Spectrometer Technology

Element	Symbol	Concentration (ppm)
Sodium	Na	85
Calcium	Ca	65
Potassium	K	467

Results at **Tables 3 and 4** show effective of anti-bacterial extractant of linden where the study of the effectiveness of these extracts separately and in different concentrations and using two types of pathogenic bacteria (Escherichia Coli and Staphylococcus aurous) have shown aqueous extracts of the latency of the highest effectiveness when the concentration is

(25) mg / ml, where inhibition was radii (20) mm for the bacteria aurous) Staphylococcus) and (1.5) mm for the bacteria (Escherichia Coli), followed by the rest of the varying concentrations and rates **Table 3**.

Table 3: The Effect of Cold Aqueous Extract of Linden In Different Concentrations on The Growth of Bacterial Pathogenesis Races

Inhibition Zone Diameter (mm)		**Concentration (mg\ml)**
Escherichia Coli	Staphylococcus Aurous	
14	20	25
12	15	15
10	14	10
8	11	5
7	9	1

Table 4: The Effect of Cold Alcoholic Extract of Linden And Different Concentrations on The Growth of Bacterial Species Pathological

Inhibition Zone Diameter (Mm)		**Concentration mg\ml**
Escherichia Coli	Staphylococcus Aurous	
9	10	25
7.5	7	15
3	4. 1	10
1	2. 1	5
1	1.1	1

The act inhibitory of the extracts of water is due to contain tannins, which include some phenolic compounds such as (Gallic acid) acid and tannic (Tannic acid) and who have a biological influence against many species of bacterial because of the presence of aggregates hydroxyl (-OH), which have the ability to form bonds of hydrogen between the hydroxyl group in these compounds and the water molecules in the bacterial cell and that the water (90%) by weight which disables the metabolism in the bacterial cell [15], as these compounds (Gallic acid, Tannic acid) as the phenolic compounds have the ability coagulation proteins on the bacterial cell and destroy the enzymes that participate in the manufacture of amino acids necessary to increase cell division [16].

In general, and **Tables 3 and 4** of the extracts of all and in all concentrations prepared to effect against bacteria (Staphylococcus aurous) is higher than against bacteria (Escherichia Coli) and found that inhibition zone of aqueous extracts cold is greater than it is for the rest of extracts other is due to the varying proportion of materials effective in the extracts of different terms found by searching the tannins and phenolic compounds different is responsible largely for the opposite effect of microorganisms compared to other components in linden, and water is one of the best solvents used in extracting tannin from its own sources of plant exclusive of other solvents [17] , It is known that there are two types of tannins biodegradable and Tannins extensive advantage of the first type biodegradable to components of origin when exposed to

high temperatures [18] or polymerization at temperatures higher than 60 °C [19]. For this reason, attributed the weak of effectiveness of the aqueous extract warm compared to cold water extract.

As for the alcoholic extract of the reason for its weak biological activity compared with cold water extract was due to the dissociation of tannin when exposed to solvents, so it's best to exclude alcoholic alcohol to extract tannins [18].

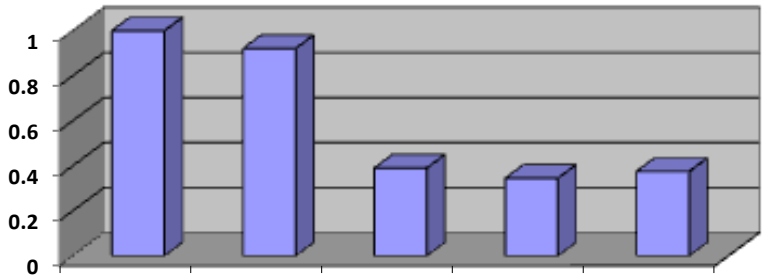
Test the effect of extracts against cancer cells

Been using a type of cancer cell lines to study the effect of extracts of lime on the growth of cells in the laboratory and thus know the specifications of the extracts anti tumors. Cancer cell line used is (L20B) a mutant mouse cells (Mice Transformed cell Line).

In this way is calculated the proportion of the number of cells within the optimal conditions for growth without the addition of extracts in question and then the output is the control group (control). Then extracts are added for the purpose of knowing their effects on cell growth in the fonts elected.

Extracts were divided into five groups, which included a cold aqueous extract of the first and the second group included a hot water extract, while the third group was cold alcoholic extract, and the fourth included the hot alcoholic extract, while the fifth group expanded to extract oil of the linden.

The analysis of the results obtained statistically significant way (one way ANOVA) she stated the following results, as planned (1) which demonstrates the impact of extracts on the ratio of the number of cells when using the line cell (L20B), it is clear that alcoholic extract hot have had the greatest influence on the proportion of the number of cells developing The effect was significant ($P < 0.05$) This result is identical to what is published in the literature (23-20). As was the effect of aqueous extract of a significant effect ($P < 0.05$) but the percentage of inhibition - as in the figure - less effective than alcoholic extract. Extracts and other effects of inhibition on the growth of mutated cells.



Scheme 1: Effect of Extracts on The Growth of Cells in The Cell Line (L20B)

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