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Isolation and identification of two active compounds in Gotu kola extract and estimation of its effectiveness against pathogenic bacteria

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Abstract :

The current study was aimed to isolation and identification of two active compounds in Gotu kola extract and estimation of its effectiveness against pathogenic bacteria. The methods used in the current study included the following main axes: extraction of active compounds from medicinal plants using a series of solvents, purification of active compounds in medicinal plants using chromatography methods, identification of purified compounds using analytical methods (IR, NMR, C^{13}NMR, GC-Mass), and measurement of the effectiveness of compounds against cancer cells outside the cells. The plant extract was then used to study its effect on bacteria growth. When analyzing the plant extract GK-1 by gas chromatography-mass spectrometry, it was found that the compound is: Bis(2-ethylhexyl) phthalate found in the table with a probability of 90% where a band appeared within the range (1672-1731) cm-1) that is attributed to the carbonyl group in the two ester groups, as well as the appearance of the band at the range (1557-1623) attributed to the C=C bonds in the aromatic ring,When analyzing the plant extract GK-2 by gas chromatography-mass spectrometry, it was found that the compound is: N-Benzyl-N-ethyl-p-isopropylbenzamide Where showed absorption at the peak of 1630 cm^{1-} which indicates the amide carbonyl group C=O that appears in the range between 1630-1690. The compound also shows absorption at 1500-1600 cm^{1-} indicating the distinctive aromatic C=C bond that usually appears in this range. The compound also shows vibrations at 2900 representing the aliphatic alkyl groups.The ethanolic extract Gotu Kola showed a higher inhibitory effect against Staphylococcus aureus, especially at a volume of 100 microliters (20.48±2.71) compared to the same volume of the extract against Escherichia coli (17.4±2.85). the ethyl acetate extract of the Gotu Kola showed a higher inhibitory effect against Staphylococcus aureus, especially at a volume of 100 microliters (19.17±3.31) compared to the same volume of the extract against Escherichia coli (16.6±1.42). Based on the results of the current study, Gotu kola extracts have antibacterial properties, especially against Gram-positive bacteria

Keywords: C. asiatica, Gotu kola, E. coli, S. aureus.

عزل وتشخيص المركبات الفعالة من مستخلص نبات سرة الارض وتقدير فعاليته البيولوجية

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مستخلص:

هدفت دراستنا الحالية الى عزل وتشخيص مركبين فعالين نبات سرة الارض وتقييم فعاليتهما البيولوجية ضد البكتريا المرضية وتضمنت الطرق المستخدمة في الدراسة المحاور الرئيسية التالية: استخلاص المركبات الفعالة من النباتات الطبية باستخدام سلسلة من المذيبات وتنقية المركبات الفعالة باستخدام طرق الكروماتوغرافيا وتشخيص المركبات النقية باستخدام الطرق التحليلية (FTIR,H^1NMR,C^{13}NMR,GC-Mass) ومن ثم تم قياس فعالية المركبات ضد الخلايا السرطانية خارج الجسم كما تم استخدام مستخلص النبات لمعرفة تاثيره على البكتريا المرضية وكانت النتائج كما يلي:

وعند تحليل المستخلص النباتي باستخدام تقنية كروماتوغرافيا الغاز - مطياف الكتلة وجد ان المركبات GK-2, GK1 هو على الترتيب N-Benzyl-N-ethyl-p-isopropylbenzamide , Bis(2-ethylhexyl) phthalate

حيث اظهر المركب GK-1 حزمة ضمن نطاق (1731–1672) التي تعزى إلى مجموعة الكربونيل في مجموعتي الإستر ، وكذلك ظهور حزمة في النطاق (1623–1557) المنسوب إلى روابط C = C في الحلقة العطرية واظهر المركب GK-2 حزمة امتصاص في ذروة 1630 مما يشير إلى مجموعة كربونيل الامايدية C = O التي تظهر في النطاق بين 1690–1630. كما اظهر المركب امتصاصا عند 1600–150 مما يدل إلى الرابطة العطرية المميزة C = C كما يظهر يظهر المركب أيضا اهتزازات عند 2900 تمثل مجموعات الألكيل الأليفاتية. كما اظهر المستخلص الايثانولي لنبات سرة الارض تاثير تثبيطي اعلى ضد بكتريا المكورات العنقودية خاصة عند الحجم 100 مايكروليتر (20.48±2.71) مقارنة ب (17.4±2.85). لبكتريا الاشريكية القولونية عند نفس الحجم كما اظهر مستخلص الاثيل اسيتيت تاثير تثبيطي اعلى ضد بكتريا المكورات العنقودية بلغت (19.17±3.31) خصوصا عند الحجم 100 مايكروليتر مقارنة ب (16.6±1.42) لبكتريا الاشريكية القولونية عند نفس الحجم وبناءً على نتائج الدراسة الحالية فان مستخلصات نبات سرة الارض تمتلك خصائص مضادة للبكتريا وخصوصا ضد البكتريا الجرام الموجبة .

الكلمات المفتاحية: العشبة الاسيوية ، سرة الارض ، الاشريكية القولونية ، المكورات العنقودية الذهبية.

Introduction

Medicinal plants are important sources of active ingredients with preventive and therapeutic effects due to their possession of many antioxidant properties and their ability to treat many diseases, which makes them an effective alternative in treatment to the use of traditional medicines (1). Medicinal plants have been found to contain many effective therapeutic compounds. The World Health Organization confirmed that 80% of the world's population has begun using medicinal plants to treat many diseases that affect the body, including bacterial, fungal and viral diseases, as well as chronic diseases, autoimmune diseases, and diseases. Alzheimer's disease and cancer that result from various causes (2,3). Gotu kola is one of the most popular herbs in the world that has been used as a folk medicine, especially in Southeast Asia. The plant is a perennial creeping, thin-stemmed herb, belonging to the Umbelliferae family. Originally, the plant was botanically identified as Hydrocotyle asiatica Linn., but later, it was named Centella asiatica. (4) Centella asiatica Also known as gotu kola, C. asiatica is also known as Vallarai in India, and Di Chien Tsao in China. It is believed that all have the same active compounds and may have good antimicrobial activity to prevent the growth of certain microorganisms that are believed to be the causative microorganisms of oral diseases (5). The plant was found to contain volatile fatty acids consisting of stearic, palmitic, oleic, lignoceric, linolenic and linoleic acids (6) and was found to contain glycosides and flavonoids including 3-glucosylquercetin, 3-glucosylkaimferol, and 7-glucosylkaimferol. The plant is reported to contain amino acids such as aspartic acid, glycine, glutamic acid, l-alanine, and phenylalanine in addition to tannins, sugars, and inorganic acids (7). The current study was aimed to isolation and identification of two active compounds in Gotu kola extract and estimation of its effectiveness against pathogenic bacteria and the aim of our current study was to isolate and characterize the active compounds from the hexane extract of the plant and its biological activity.

Materials and Methods

Preparation of Plant Crude Extracts

The plant extracts were prepared by following the method mentioned by (8). This method depends on the nature of the active components separated from the plants and the nature of the solvent used in the separation process and using the successive solvent system. Therefore, three different polar solvents were used in the extraction process, namely petroleum ether (60-80) °C, ethyl acetate and ethanol 99%. The extraction process was carried out at different temperatures according to the boiling point of each solvent, provided that it does not exceed 80°C. 45 g of plant powder was placed in the continuous extraction device using 500 cm3 of the solvent. The solvent was concentrated using the rotary evaporator RVE at 40°C. Then the raw extracts were placed in opaque, tightly sealed bottles and kept in the refrigerator until later use.

Purification of the Active Compounds

Detection of active compounds and identification of the mobile phase of the separation column (silica gel)

Using Thin Layer Chromatography (TLC) technology, Silica Gel plates prepared by Mercke were used with a thickness of 0.25 mm and dimensions of 20 x 20 cm. The samples were loaded on one end of the plate and in the form of spots along the starting line by means of a capillary tube and the plate was placed in the tank in a vertical manner so that the end loaded with the samples was at the bottom and not in contact with the Solvent System, which is chosen with a specific polarity based on obtaining the best separation of the extract whose components are to be separated. The container was covered with its special cover and left at the laboratory temperature until the separation solution rose a distance before the end, after which the plate was lifted from the container and dried by means of nitrogen gas, then The spots were shown by washing them with Phosphomolybdic acid hydrate detector as well as Uv The same method was used to determine the mobile phase of the separation column for all the separated compounds (9).

Identification of active compounds

The purified compounds from the Goto cola plant and the catfish were

identified in the laboratories. Gas chromatography device connected to the mass spectrometer type GC-MS QP210 Ultra from the Japanese company SHIMADZU, which contains a capillary column with dimensions of 30 meters in length and a diameter of 0.23 and the thickness of the stationary phase is 0.25 micrometers and the mobile phase is the carrier gas of high purity helium The separation process was carried out according to the thermal system at a temperature of 40 m for one minute and then it starts to increase until it reaches 150 m for one minute at a rate of 5 m per minute and stabilizes at 280 m and then the automatic injection process of 1 microliter was carried out in the GC-MS device. After Obtaining the results is processed by the GC-MS Solution program to view the charts and separated picograms based on the database of the library spectra.

FTIR Spectroscopy

This analysis is used to determine the active groups in plant extracts and was done by placing a small amount of the sample between two calcium bromide discs [10].

Separation of the compounds

Separation of the compound (GK**-1)**

Separation of the compound (GK-1) from the hexane extract of the catnip plant and its purification:

2 g of the hexane extract, coded GK-1, of the catnip plant were dissolved. The components of the extract were separated using a separation column packed with silica gel of the type Silica gel 60A for flash Chromatography, and 30 g of the gel was saturated with a mixture solvent (Hexanol).

Separation of the compound (GK-2)

Separation of the compound (GK-2) from the ethyl acetate extract of the catnip plant and its purification using a chromatographic separation column. eparation of the compound (GK-2) in its pure form from the extract using a chromatographic separation column packed with silica gel of the type Mesh size 60A

The test of antimicrobial activity for extracts

The antibacterial activity on agar was measured using the diffusion technique [11]. Using the McFarland opacity standard, a bacterial strain culture was created at a concentration of 1×10^{6}

cells per milliliter. The Mueller Hinton agar plates were filled with 0.1 ml of each bacterial isolate (1×106 cells per ml) using a procedure called the spreading method. As a control, 10 ml of extract was added to the wells. Petri plates were held at 30 minutes prior to being placed in the incubator device. Each petri dish was incubated at 37°C for a full day. Measurements of the inhibitory zones were conducted.

Results and Discussion

This aspect includes the separation and identification of a number of active compounds in groups and in a pure form from the plants under study, as a number of active compounds were separated from the plants using a chromatographic separation column (CC) and using Silica gel as a packing material (mesh 120-60) after the raw plant extracts were prepared using the successive solvent system using the continuous extraction device Soxhlet and different polar solvent systems as explained in the paragraph of materials and methods of work,

The separated active compounds were identified using chromatographic diagnostic techniques such as the separation column and TLC, the nuclear magnetic resonance device NMR and C13 NMR and infrared spectrum IR.

1- Identification of compound (GK-1) from the hexane extract of the Gotu Kola

A- Spectral diagnosis by infrared spectroscopy (I.R.)

Curve (1) shows the infrared spectrum of the compound, where a band appeared within the range (1672-1731) cm-1) that is attributed to the carbonyl group in the two ester groups, as well as the appearance of the band at the range (1557-1623) attributed to the C=C bonds in the aromatic ring, as well as the peak at (1083-1240) confirms the presence of C-O bonds in the ester, and the bands were consistent with the literature.

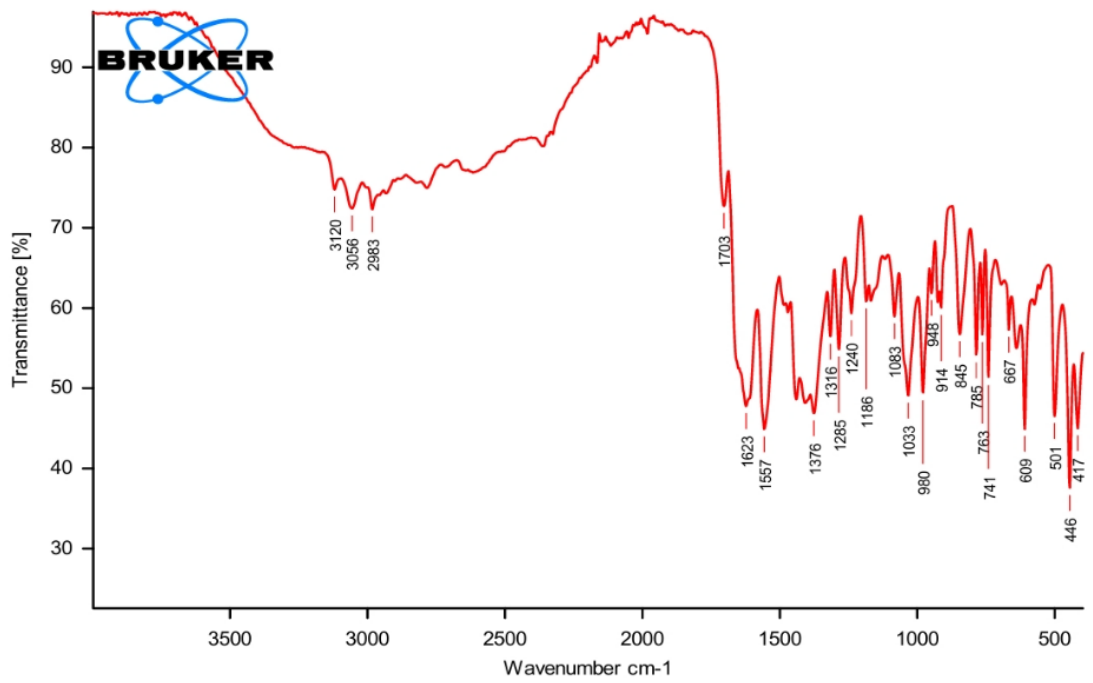
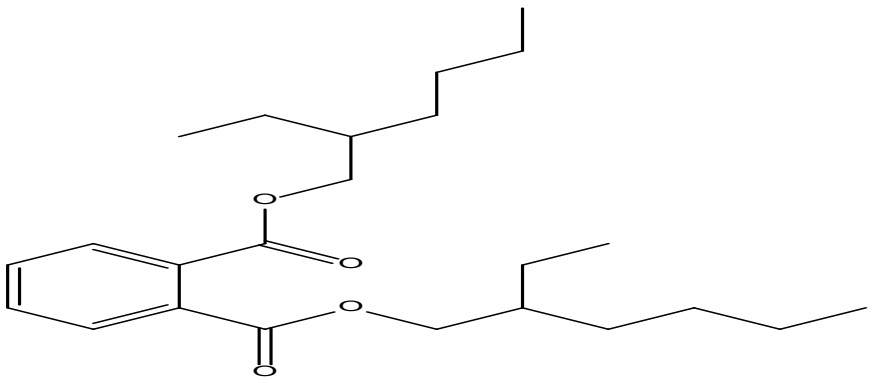


Figure (1): Spectral diagnosis by infrared spectroscopy for GK-1.

Diagnosis by gas chromatography-mass spectrometry:

When analyzing the plant extract GK-1 by gas chromatography-mass spectrometry, it showed the series of compounds attached in the following table. When analyzing the compounds in the table and matching them with the spectra, it was found that the compound is:

Bis(2-ethylhexyl) phthalate found in the table with a probability of 90



bis(2-ethylhexyl) phthalate

2. Identification of the compound (GK-2) from the ethyl acetate extract of the plant Gotu Kola

A. Spectral diagnosis by infrared spectroscopy (I.R.)

The spectrum shown in Figure (2) showed absorption at the peak of 1630 cm1- which indicates the amide carbonyl group C=O that appears in the range between 1630-1690. The compound also shows absorption at 1500-1600 cm1- indicating the distinctive aromatic C=C bond that usually appears in this range. The compound also shows vibrations at 2900 representing the aliphatic alkyl groups. The compound also shows absorption at 3340 cm1- indicating the distinctive N-H bond that usually appears between 3300-3500 cm1-

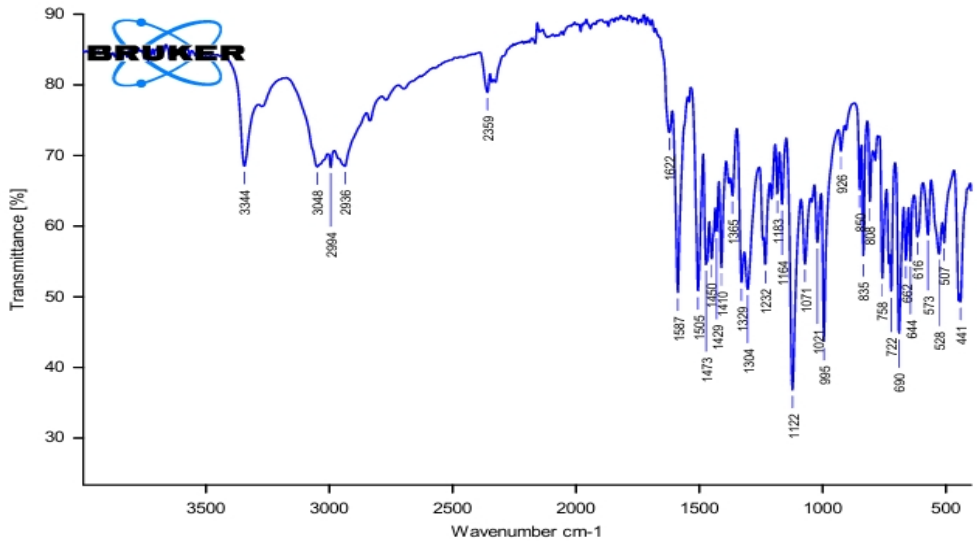
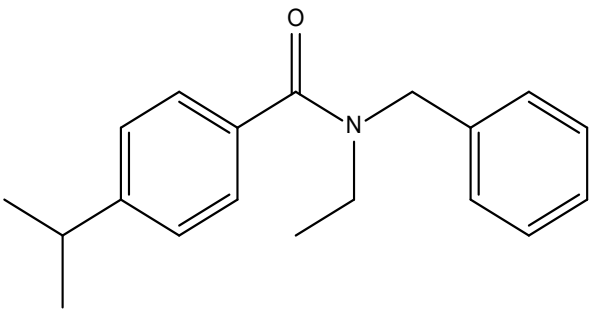


Figure (2): Spectral diagnosis by infrared spectroscopy for GK-2

B. Diagnosis by gas chromatography-mass spectrometry:

When analyzing the plant extract GK-2 by gas chromatography-mass spectrometry, it showed the series of compounds attached in the following table. When analyzing the compounds in the table and matching them with the spectra, it was found that the compound is: N-Benzyl-N-ethyl-p-isopropylbenzamide



N- benzyl- N-ethyl-p-isopropylbenzamide

Sensitivity test of extracts against bacteria

Table (1) shows the inhibitory ability of extracts of both Gotu Kola against two types of Gram-negative and Gram-positive bacteria. The Gotu Kola extract showed a higher inhibitory effect against Staphylococcus aureus, especially at a volume of 100 microliters (20.48±2.71) compared to the same volume of the extract against Escherichia coli (17.4±2.85).

Table (1): Inhibitory effect (mm) of the alcoholic extract of the Gotu Kola plant and the saltwort at concentrations against two types of bacteria

Treatment / Bacteria type	*Centella asiatica*	
	50ul	100ul
E.coli	0.6 7.37±	2.85 ± 17.4
S. aureus	9.5±1.2	20.48±2.71

On the other hand, the extract of the Gotu Kola plant showed a higher inhibitory effect against Staphylococcus aureus, especially at a volume of 100 microliters (19.17±3.31) compared to the same volume of the extract against Escherichia coli (16.6±1.42) table (2) and figure (1).

Table (2): Inhibitory effect (mm) of ethyl acetate extract of the Gotu Kola plant at concentrations against two types of bacteria

Treatment / Bacteria type	*Centella asiatica*	
	50ul	100ul
E.coli	0.5 5.17±	1.42 ± 16.6
S. aureus	8.46±1.19	19.17±3.31

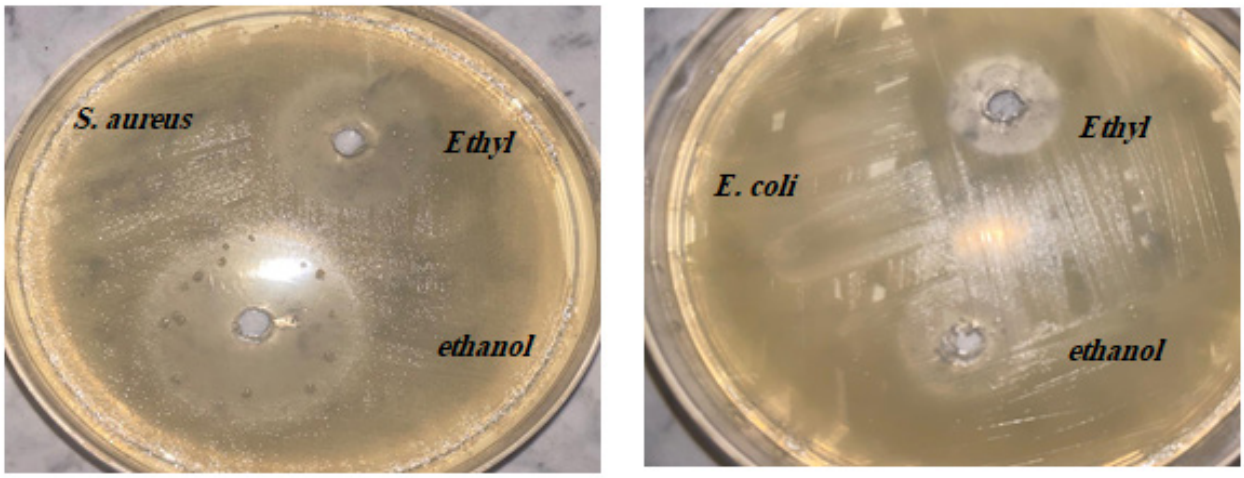


Figure (1) : Zone of inhibition of ethanolic and ethyl acetate extracts of Gotu kola plant against the studied bacteria.

The present study showed that the extract of Gotu kola plant had a higher inhibitory effect against Staphylococcus aureus than Escherichia coli. In a study conducted by Talabani [12], phytochemical analysis of Gotu kola plant revealed the presence of several secondary metabolite chemicals, including saponins, steroids, tannins, alkaloids, and flavonoids. Gotu kola leaves contain chemicals with antibacterial properties. Thus, they may be able to inhibit the growth of Staphylococcus aureus. Inhibition of energy production and nucleic acid synthesis are just two of the many targets that the active ingredients in Gotu kola leaves pursue. These substances can also alter the structure and function of cell membranes, leading to the degradation and damage of membrane proteins. This explains the results of the present study in terms of the role of Gotu kola extracts against the studied bacteria. The results of the current study are also supported by the inhibitory role of Gotu kola extracts, as it contains many active compounds, as tannins have antibacterial effects by preventing the formation of bacterial cells by interfering with reverse transcriptase and DNA [13]. Bacterial cell membranes are damaged when flavonoids combine with external proteins to form molecules, which are then degraded inside the cell, preventing energy synthesis, and saponins increase

permeability, causing cell leakage and decomposition of intracellular chemicals [14]. The difference in the structure of the bacterial wall plays an important role in the susceptibility of bacteria to infection [15]. According to several researchers, Gram-negative bacteria have an outer membrane made of lipopolysaccharides (LPS) that limits the diffusion of hydrophobic compounds [15,16]. In addition, the peribacterial region contains enzymes capable of destroying foreign molecules introduced from the outside [17], which makes these bacteria generally less sensitive to plant extracts than Gram-positive bacteria [18].

Conclusions

Based on the results of the current study, Gotu kola extracts have antibacterial properties, especially against Gram-positive bacteria. This may be due to the nature of the cell wall structure of the bacteria.

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