Republic of Iraq
Ministry of Higher Education and Scientific Research

University of Basrah
College of Pharmacy

Pharmacognosy Department

Detects the active constituents and antibacterial activity for ethanolic extract of costus roots

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بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

(المجادلة : ١١)

صدق الله العظيم

الأهداء

إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلا بطاعتك ول اتطيب اللحظات إلا بذكرك.. ولا تطيب الأخرة إلا بعفوك.. ولا تطيب الجنة إلا برؤيتك الله جل جلاله إلى من بلغ الرسالة وأدى الأمانة.. ونصح الأمة.. إلى نبي الرحمة ونور العالمين سيدنا محمد صلى الله عليه واله وسلم إلى من كلله الله بالهيبة والوقا .. إلى من علمني العطاء بدون انتظار.. إلى من أحمل أسمه بكل افتخار.. وإلى الأبد والدي العزيز.. إلى معنى الحب وإلى معنى الحنان والتفاني..

إلى بسمة الحياة وسر الوجود إلى من كان دعائها سر نجاحي وحنانها بلسم جراحي إلى أغلى الحبايب أمي الحبيبة إلى منارة العلم والعلماء إلى الصرح الشامخ ....

جامعة البصره كلية الصيدله إلى الذين حملوا أقدس رسالة في الحياة إلى الذين مهدوا لنا طريق العلم والمعرفة..

أساتذتنا الأفاضل

الشكر والتقدير

في البداية ، الشكر والحمد لله ، جل في علاه ، فإليه ينسب الفضل كله في إكمال - والكمال يبقى لله وحده - هذا العمل . وبعد الحمد لله ، فإنني أتوجه إلى أستاذتي الدكتوره سبأ علي محمد بالشكر والتقدير الذي لن تفيه أي كلمات حقه ، فلولا مثابرتها ودعمها المستمر ما تم هذا العمل . وبعدها فالشكر موصول لكل أساتذتي الذين تتلمذت على أيديهم في كل مراحل دراستي حتى اتشرف بوقوفي أمام حضراتكم اليوم .

ABSTRACT

Plants are a mine of many medical substances, making them a pharmacy used by humans to treat any disease or develop new drugs. This research about **Costus** was chosen to study the phytochemical content and the biological activity against bacteria. Costus is a well-known and important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments, such as **asthma, inflammatory diseases, ulcer and stomach problems**. Costus has a higher concentration of **total phenolic content**, **total flavonoids**, **alkaloids**& **tannins**. Phytochemical tests illustrate this plant rich by flavonoids and polyphenol compounds. Biological activity of Costus extract detected by using a bacterial isolates of Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa, and the results showed a good antibacterial activity against gram positive bacteria **than** gram negative bacteria.

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Introduction:

●Plant materials are exploited in the pharmacy sector as both natural remedies and raw materials, and constituting a significant proportion of the world drug market. It has been estimated that the global commerce in herbal medications and phytochemicals was just about $ 75 billion within 2007 [1], further predicted to reach $262.9 billion by 2020. Conversely, the contribution of diverse regions to global trade is negligible. As a result, the study of these plants for medicinal and nutritional purposes opens up new possibilities for their future consideration [2].
●The Indian subcontinent has immense diversity of the flora because of their broad geographical, meteorological, and ecological circumstances. It contains about 15,000 flowering plants species and overall, six percent of all species in the planet. In India, these plants have been utilized for medicinal uses since ancient times and contain about 3000 scientifically documented plants having immense medicinal potential [3].

●The **Asteraceae** family contains roughly 1000 genera and 30,000 species that are dispersed globally, with approximately 177 genera and 1052 species native to India. There are over 300 species in the genus ***Saussurea,*** with roughly 61 species found in India. The most well-known ***Saussurea*** species is ***S. costus***, which has a variety of therapeutic benefits in various traditional healthcare systems [4].

●***Costus*** is a common name of *S. costus* in English having diverse vernacular names in India viz., Kuth (Hindi), Postkhai (Kashmiri), Kostum (Tamil), Kot (Punjabi), Kut (Gujrati), Kur (Bengali), Sepuddy (Malayalam), Kushta (Sanskrit), Kustam (Telgu), Kushta (Marathi) and Koshta (Kannada) (Madhuri et al., 2012). Despite several efforts to obtain therapeutics from medicinal herbs, only 25 % of approved bioactive molecules are of plant origin [5]. In India, the pharmaceutical sector ustilizes approximately 280 medicinal plants to make herbal medication, fragrances, and soaps, the bulk of which are found in the Himalayan mountains [6].
●*S. costus* is traditionally used as **anti-cancer**, **hepatoprotective, antiarthritic**, **anti-convulsant**, **antimicrobial**, **anti-viral activities**, etc. in different Indian indigenous systems, proved through different in-vitro and in-vivo approaches and offers a valid rationale to the ancient statements [7]. in recent times. *S. costus* has been successfully produced with in various states of India due to high demand at the national and international level. In the global market, *S. costus* (root oil and roots) becomes a crucial drug.

●*S. costus* is a major source of concern, as indicated by a number of recent studies conducted by various researchers. As a result, we decided to compile the most up-to-date and widely available information on *S. costus* botany, traditional applications, phytochemistry, and pharmacology to investigate its beneficial potential and new research options. As a result, this study may serve as a scientific base for future research on *S. costus* .

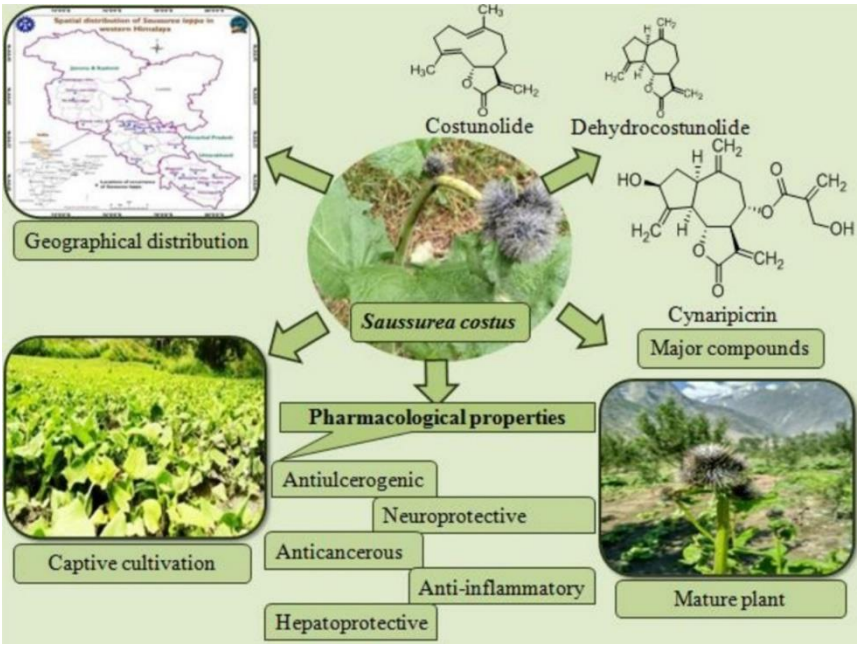


Figure 1/ General information about the plant

Plant Taxonomy [8]:

- ▼Kingdom: Plantae
- ▼Subkingdom: Virideplantae
- ▼Infrakingdom: Streptophyta
- ▼Division: Tracheophyta
- ▼Subdivision: Spermatophytina
- ▼Infradivision: Angiospermae
- ▼Class: Magnoliopsida
- ▼Superorder: Asteranae
- ▼Order: Asterales
- ▼Family: Asteraceae
- ▼Genus: *Saussurea*
- ▼Species: *S. Costus*

Figure 2/ Saussurea Costus Plant

Pharmacological Activity [9]:

- Digestive Disorders: The dried roots of *Saussurea costus* have been used in traditional medicine systems to aid digestion. It is believed to have carminative properties, helping to relieve indigestion, flatulence, and abdominal discomfort.
- Respiratory Conditions: *Saussurea costus* has been used to treat respiratory ailments such as cough, bronchitis, and asthma. It is thought to possess expectorant properties, helping to expel mucus and alleviate respiratory congestion.
- Aphrodisiac: In certain traditional practices, *Saussurea costus* has been used as an aphrodisiac. It is believed to have stimulating properties that enhance sexual vitality and performance.
- Anti-inflammatory: The root of *Saussurea costus* is sometimes used topically for its potential anti-inflammatory effects. It has been applied to the skin to reduce swelling and inflammation in conditions such as arthritis and rheumatism.
- Antimicrobial: In some traditional systems, *Saussurea costus* has been employed for its antimicrobial properties. It is believed to have activity against certain pathogens, making it useful in treating infections.
- Skin Disorders: *Saussurea costus* has been used in traditional remedies for various skin disorders. It is believed to have soothing and healing properties, assisting in the treatment of conditions such as eczema, dermatitis, and skin irritations.

It's important to note that these traditional uses are based on historical practices and anecdotal evidence. Further scientific research is needed to validate these claims and understand the specific mechanisms of action.

Active Constituents [10]:

i. Essential Oils: The roots of *Saussurea costus* contain essential oils that are responsible for its characteristic aroma. The essential oils of *Saussurea costus* are composed of various volatile compounds, including camphor, linalool, alpha-pinene, and beta-pinene. These compounds contribute to the aromatic and therapeutic properties of *Saussurea costus*.

ii. Coumarins: *Saussurea costus* contains coumarins, which are aromatic compounds with diverse pharmacological activities. Scopoletin and isoscopoletin are two coumarins found in *Saussurea costus*. Coumarins are known for their anti-inflammatory, antioxidant, and antimicrobial properties.

iii. Phenolic Compounds: *Saussurea costus* contains phenolic compounds such as caffeic acid and chlorogenic acid. These compounds are known for their antioxidant properties and contribute to the overall antioxidant capacity of *Saussurea costus*.

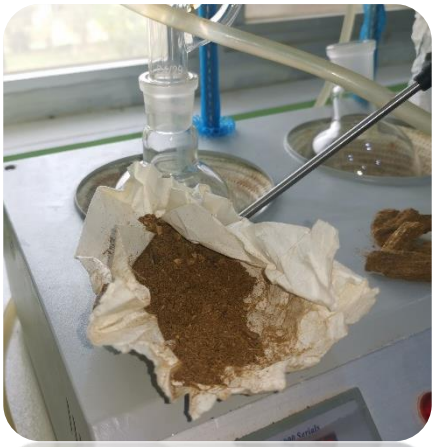
iv. Flavonoids: *Saussurea costus* is also rich in flavonoids, including kaempferol and quercetin derivatives. Flavonoids are bioactive compounds with antioxidant, anti-inflammatory, and immunomodulatory effects.

v. Alkaloids: *Saussurea costus* has been reported to contain alkaloids, although their specific identities and biological activities are less well-studied compared to other constituents.

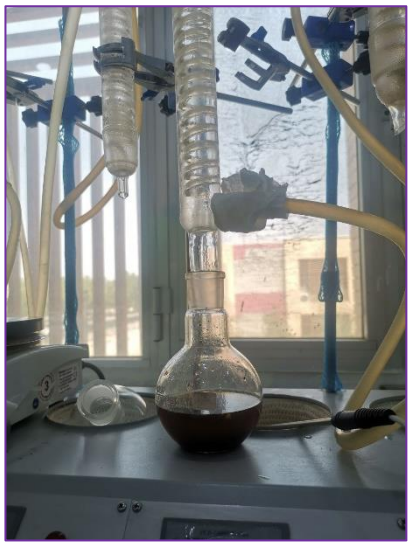
It's important to note that the chemical composition of *S. costus* can vary based on several factors. Further scientific research is needed to comprehensively identify and characterize the complete chemical profile of *S. costus* and investigate the specific effects of its individual compounds.

Materials & Methods:

Plant collection Plant roots were collected from the local Iraqi market then grind to a fine powder by an electric mill.



Extraction methods: Plant powder was extracted by reflex method using 10g of roots powder with 100ml of 80% ethanol for 2h, the extract was filtered by using Whatman number 4 filter paper. Then freed of solvent by evaporation at room temperature, the dried crude extract was stored at 20 C^0 [11].



Phytochemical tests [12]:

1-Tests for Alkaloids:

A -Dragendroffs test:

(potassium bismuth iodide)1ml of ethanolic extract was treated with few drops Dragendroffs reagent. Formation of reddish-brown precipitate indicated the presence of alkaloid.

B-Mayer's test: (potassium mercuric iodine solution)1ml of ethanolic was treated with few drops of Mayer's reagent. Formation of **creamy white** precipitate indicated the presence of alkaloids.

2-Test for Flavonoids:

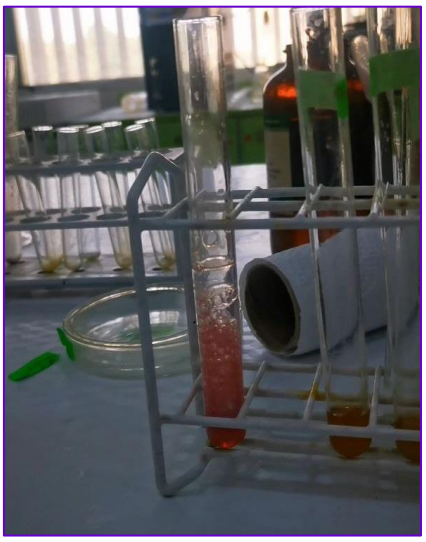
Shinoda test: To 1ml ethanolic solution of extract a few fragments of magnesium ribbon and concentrated hydrochloric acid were added. Appearances of red to pink color after few minutes indicated the presence of flavonoids.

3-Test for Polyphenols:

Ferric chloride 5%: To 1ml of ethanolic extract a few drops of 5% ferric chloride was added. Formation of red, blue, green or purple colors indicated presence of phenolic compounds.

4-Test for Tannins:

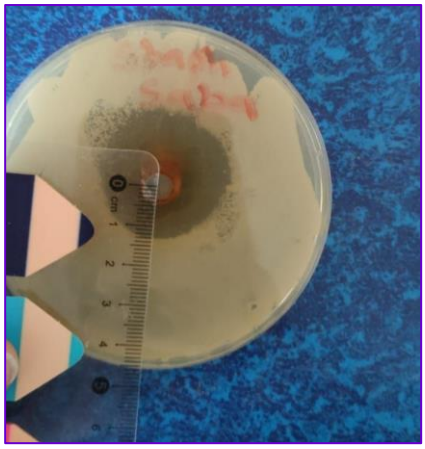
Ferric chloride 1% test: to extracts a few drops of 1% neutral ferric chloride solution was added. Formation of blackish blue color indicated the presence of tannins.



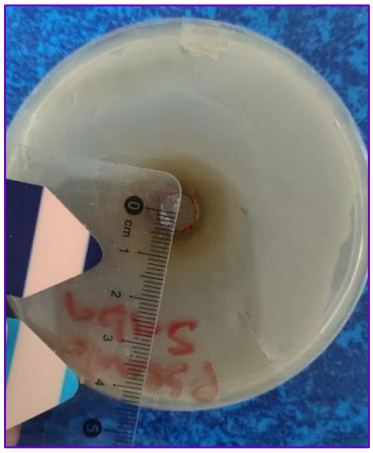
Antibacterial Activity of Costus Sp. :

The antibacterial activity of *Costus* crude extract was estimated qualitatively by using the agar well diffusion technique. The concentration of 1 mg/ml was used as a concentration detector for antibacterial activity assay. Local bacterial isolates were tested. The bacterial isolates include two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and one-gram negative bacteria (*Pseudomonas aeruginosa*), which were provided by the microbiology laboratory, Pharmacy College, Basrah University). To reach the stationary growth phase, bacteria were incubated at 37°C , for 24 h in Nutrient Broth (NB, Difco, MD, USA). Each plate (Mular Hinton agar) was inoculated with only one microorganism. well per plate was made using an 8 mm sterile cork borer. The well was inoculated with 100 µl of *Costus* extract solution. Then, bacterial plates were incubated for 18 h at 37°C. The diameter of clear zones around each well was measured, showing no bacterial growth [13].

activity against bacillus subtilis



activity against staphyllococus aureus



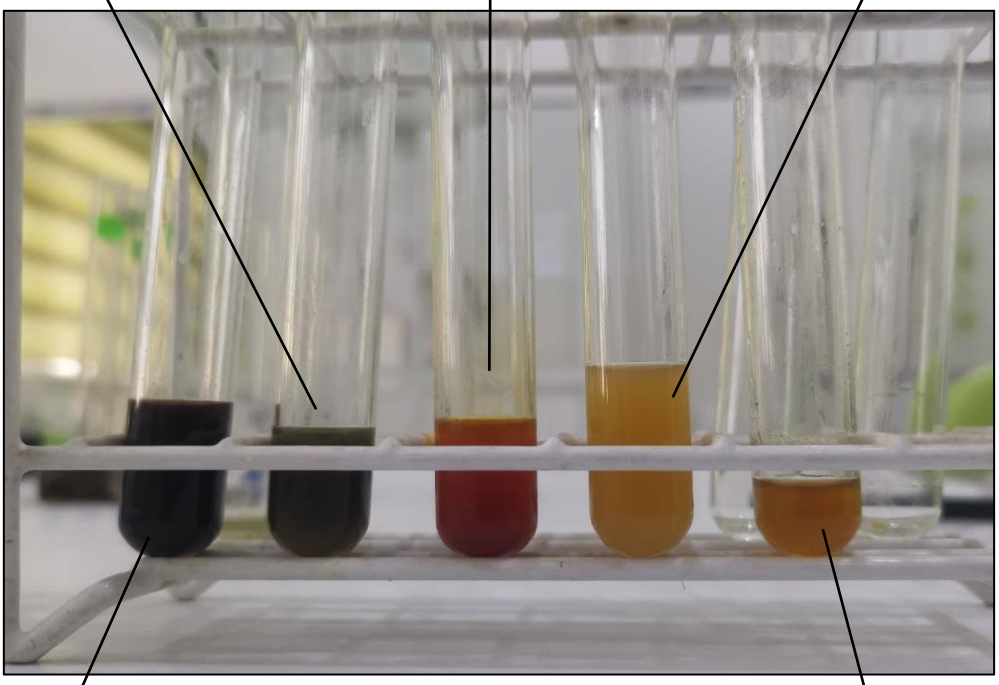
activity against pseudomonas aueroginosa

Results & Discussion:

Polyphenols test, When Saussurea costus extract is treated with a 5% ferric chloride solution result in formation of a dark green color which indicates the presence of polyphenols.

The Shinoda test is a colorimetric test used to detect the presence of flavonoids. When Saussurea costus extract is treated with Shinoda reagent, result in appearance of pink to red coloration which indicates the presence of flavonoids.

Mayer's reagent is another test used to detect alkaloids. Ethanolic was treated with few drops of Mayer's reagent, there was No changes in Color or formation of precipitate which indicated absence of alkaloids.



Tannins test, When Saussurea costus extract is treated with Ferric chloride solution result in formation of a dark blue color which Indicates the presence of tannins.

Dragendorff's reagent, When Saussurea costus extract is treated with Dragendorff's reagent result in formation of reddish brown precipitate which indicates the presence of alkaloids.

Results of phytochemical tests of costus extract.

The phytochemical tests provide qualitative indications of the presence of alkaloids, flavonoids, polyphenols, and tannins in *Saussurea costus*. They are commonly employed to assess the presence of these compounds in plant extracts and can aid in the initial screening of bioactive constituents. It's important to note that these tests provide preliminary information, and further analysis is needed to identify and quantify the specific compounds present.

Results of this study showed that the costus extract has antibacterial activity because **polyphenolic** and **alkaloids** contents and more effective on Gram positive than on Gram negative bacteria; these results may be due to the nature of bacterial cell membrane. The cell membrane of Gram positive bacteria contains mucopolysaccharides, protein and less amounts of phospholipids, while gram negative bacteria have a huge amounts of phospholipids, and more pores in cell envelope. So, the permeability, entrance and reaction of the most antibiotic and /or antimicrobial agents through cell envelope (the outer and cytoplasmic membrane) are highly efficient for Gram positive bacteria depending on reaction with the protein layer (**mucopolysaccharides** or **peptidoglycanes**) [14]

Conclusion:

Saussurea spp. is an important and widely recognized medicinal plant provided by the pharmaceutical systems of India, China, Korea and Pakistan. It is used to treat many diseases and disorders after the experiments conducted on it, such as headache, stomach pain, epilepsy, leprosy, typhoid, pneumonia, especially chronic bronchitis, and chemicals extracted from plant roots show many pharmacological activities such as anti-inflammatory, antimicrobial, etc. An examination of the literature on this plant concluded that it is medicinally important and endangered due to high demand and illegal consumption and exploitation. From various evidence it has been shown that *S. Costus* is safe and effective when used in conventional doses. It also shows some other properties except medicinal properties, such as perfume, antiparasitic and insecticide. Due to its long-standing important pharmacological and ethnological uses and the presence of several important bioactive substances that can lead to the extraction and identification of some new chemical compounds, it was concluded that *Saussurea spp.* could help in future clinical and chemical research.

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