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Phytochemical Screening, Antioxidant and Antibacterial Properties of Alcoholic Extract of *Saussurea costus* Roots

Esraa M. Adel[1], Thekra S. Al-Tayawi[1], Farah H. Omer[1], Manal F. Mohammed[1]

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ABSTRACT

Background: Diseases in their essence are non-curable and the underlying pathogenesis is bidirectional of inflammation and oxidative stress. Fighting oxidative stress and inflammation should be the target for any first-line therapy, perhaps *via* chemical or herbal therapy. In the present study, we aimed to identify the antioxidant and antibacterial activities of *Saussurea costus* roots. The study aimed to harness *S. costus* plant as antimicrobial *via* assessment of its antibacterial efficacy against pathogens by disc diffusion and minimum inhibitory concentration (MIC) tests.

Methods: Alcoholic extract of *S. costus* roots was tested for antioxidant activity using diphenyl picrylhydrazyl (DPPH) and antibacterial effects on a few common bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus spp*.).

Result: The contents of *S. costus* extracts revealed of the presence of therapeutic principle components with variety of medicinal effects, including alkaloids, terpenoid, phenols and others. Different concentration of extracts have comparable antioxidant activity to ascorbic acid (81.96%). Similarly, different concentrations of the extract have confirmed antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* with no impacts on *Proteus* spp.

Key words: Antibacterial, DPPH, FTIR, *Saussurea costus, Saussurea lappa.*

INTRODUCTION

Medicines of plant origin remain an important resource in the fight against serious diseases, especially in developing countries (Ansari *et al.,* 2021). Traditional medicines are still used by 60-80% of the world's population for the treatment of common diseases (Oyebode *et al.,* 2016). Most patients with arthritis and such long-standing impairments are reported to be using alternative medicines for the reduction of symptoms and relieving pain, with traditional Chinese medicines based on herbs and traditional formulas being the most commonly used (Mehta and Shah Shahista, 2021; Singh and Chahal, 2018). The evidence of the use of medicinal plants for the management of the symptoms of any disease can be witnessed from five thousand years back through the written documents of early civilizations in India, China and the Near East (Petri *et al.,* 2015).

Although there is huge evidence of the benefits of plant-based medicines and their therapeutic effects, their use in the management of diseases and treatment had remained unexplored (Vijayalakshmi *et al.,* 2022). In recent decades, there has been increasing interest in studying natural products from various sources, especially from higher plants, to discover new antioxidant and antimicrobial agents such as flavonoids, tannins, alkaloids and terpenoids, which have been shown to have *in vitro* antimicrobial characteristics and properties (Deabes *et al.,* 2021). In recent years, infectious diseases have increased dramatically and antibiotic resistance has become a growing therapeutic problem (Aslam *et al.,* 2018). In the treatment of multiple infections such as urinary tract infections, respiratory tract infections, gastric issues and typhoid fever the use of these plants as therapeutic agents is extremely promising as it induces therapeutic effects and helps in curing the diseases (Mikawye *et al.,* 2017).

However, the use of complex plants as a source of novel drugs remains unexplored. Plants are composed of an extensive range of phytochemicals, including tannins, terpenoids, alkaloids and flavonoids, which exhibit powerful antibacterial properties against multiple microorganisms including, bacteria, fungi and others (Kumar *et al.,* 2024; Semwal *et al.,* 2020). They are much safer and result in fewer adverse effects as compared to synthetic drugs (George, 2011). The search for antimicrobial ingredients has become increasingly important in recent years due to worldwide growing concerns about the rapidly increasing infections and diseases which are resulted from microorganisms which are antibiotic-resistant (Ashry, 2019; Kumar and Pundir, 2022). Many components of the plants have the potential to be powerful remedies for a variety of diseases (Gurib-Fakim, 2006).

Due to the presence of several compounds in *Saussurea costus*, including Kaempferol, Cinnamic acid,

[1]College of Pharmacy, University of Mosul, Mosul, Iraq.

Corresponding Author: Thekra S. Al-Tayawi, College of Pharmacy, University of Mosul, Mosul, Iraq.
Email: thekra.siddeq@uomosul.edu.iq

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Taxifolin, Naringenin, Ferulic acid, Vanillin, Coumaric acid, Ellagic acid, Rutin, Pyro catechol, Syringic acid, Coffeic acid, Methyl gallate, Catechin and Chlorogenic acid) with some of them have been proved as having biological activity against cabcer cell lines (Deabes *et al.,* 2021). The objective of this study were to characterize the constituents present in the *S. costus* and assess their antimicrobial activities on common bacterial species in an attempt to overcome the problems of antimicrobial resistance through finding alternative remedies.

MATERIALS AND METHODS

Sample collection: Roots of *S. costus* were purchased from the Mosul Herbarium Market, the plant authentication has been previously described in Mosul city (Iraq) (Jabori *et al.,* 2023; Al-obaidy and Esmaeel, 2021). Four pathogenic microorganisms were kindly offered by Faculty of Science and University of Mosul: "Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Enterobacter faecium and Pseudomonas aeruginosa*)". This research was carried out at the "College of Pharmacy, University of Mosul."

Preparation of extract

Dried *S. costus* roots were ground to a powder. A total of 25 g of the powder was added to absolute ethanol (150 ml, stirred for 24 h with a magnetic stirrer, room temperature). The mixture was then filtered (Berreghıoua and Ziane, 2024).

Phytochemical screening

Phytochemical screening of extracts from the roots of *S. costus* was carried out using standard methods (Fig 1, Table 1), including.

Alkaloid testing

A small part of the sample was pipetted into a test tube and one milliliter of Mayer's reagent (mercury potassium iodide) was added and mixed. The presence of alkaloids was indicated by the formation of a yellow creamy precipitate (Thenmozhi *et al.,* 2023).

Terpenoid testing

The terpenoids in the sample were confirmed by 1-3 drops of chloroform to a small sample followed by one millilitre of concentrated hydrochloric acid, which was heated for two minutes to form a reddish-brown colour (Berreghıoua and Ziane, 2024).

Phenol testing

Two millilitres of $FeCl_3$ 5% solution were added to a small amount of the specimen and incubated for 5 minutes at room temperature. The phenol content is denoted by the formation of a blackish blue colour (Akshaya *et al.,* 2023).

Tannin testing

To do this test, two drops of 10% lead acetate were mixed with 1 ml of the plant extract leading to formation of yellow precipitate which indicates presence of tannic acid (Akshaya *et al.,* 2023).

Carbohydrate testing (Molish test)

The extract was placed in test tube and the sulfuric acid added against test tube wall resulting in violent ring at the contact site which do indicated the presence of carbohy drates (Thenmozhi *et al.,* 2023).

Saponins testing

To do this test, two drops of distilled water were mixed with 1 ml of plant extract, this has led to formation of foam which do indicates the presence of saponins (Berreghýoua and Ziane, 2024).

Table 1: Results of phytochemical analysis.

Phytoconstituents test	Reaction color produced	Test result
Alkaloids	Yellow	+
Terpenoids	Reddish	+
Phenols	Black bluish precipitate	+
Tannins	Yellow precipitate	+
Saponine	No Formation foam	-
Coumarin glycoside	Yellow precipitate	+
Flavonoids	Greenish blue	+
Quinine	Reddish brown	+
Steroids	Red	+
Protein	No Formation of reddish colour	-
Carbohydrate	No Formation purple ring	-

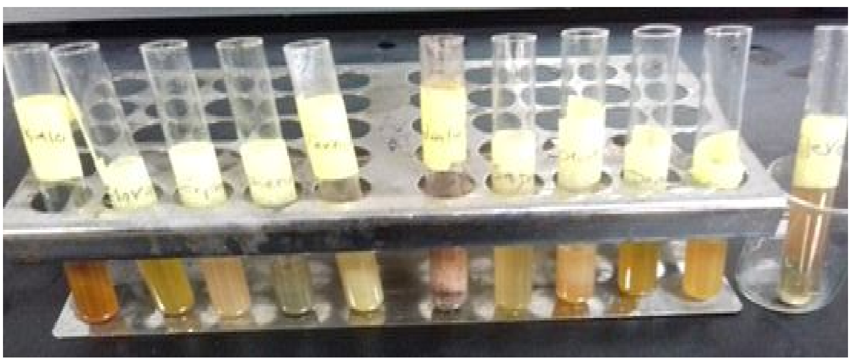

Fig 1: Results of phytochemical analysis.

Coumarin glycosides

The formation of yellow precipitate following mixing 2 ml of diluted HNO3 with equal amount of plant extract indicated the presence of Coumarin glycosides (Calimpang *et al.,* 2023).

Flavonoid test

A few drops of ferric chloride added to 1ml of the *S. costus* extract, the presence of flavonoids has vchanged the color of extract from green to black after addition of ferric chloride (Devi *et al.,* 2023).

Quinine test

After 2 min of incubating equal amount of one milliliter of sodium hydroxide and plant extract solution it will lead to formation of a reddish-brown colour indicated quinine (Devi *et al.,* 2023).

Protein analysis

In a test tube, small amount of sample was placed and a drop of concentrated HNO_3 was added. The light red colour formed indicates the presence of protein (Calimpang *et al.,* 2023).

Detection of steroids (Salkowaski reaction)

After 5 min of incubating equal amount of one milliliter of chloroform, concentrated H_2SO_4 and plant extract solution it will lead to formation of a red color in the lower layer indicates the presence of steroids (Devi *et al.,* 2023).

Radical scavenging effect of the extract on DPPH radical

The free radical scavenging activity of plant extracts was determined according to previous studies as follows (Afzal *et al.,* 2021). A known volume of plant extracts was added to the test tubes and gently mixed and then (1.0 ml) of DPPH solution (0.2 mM of ethanol) was added to each tube, mixed well (incubated at room temperature, 30 min) to reach a known volume (1.0 ml). The same protocol was applied for the negative control group (a sample with no plant extract). A 0.03% (w/v) solution of ascorbic acid was used as an additional control. Absorbance (A) at 517 nm for samples and control was measured (Jenway 6300 spectrophotometer). The percentage of DPPH radical inhibition was calculated using the following formula:

$$\text{Percentage} = \frac{(\text{Ac}-\text{As})}{\text{Ac}} \times 100$$

Compound identification

Fourier transform infrared (FTIR) spectrophotometer was used to analyze the extracts of the plants using (Bruker-Alpha ATR-FTIR (Germany).

The roots of *S. costus* were studied against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus spp*. The growth of four bacteria obtained from the University was studied. The antibacterial effect of the drug was studied by the disc diffusion method, in which various types of microorganisms were cultured in nutrient broth and incubated at 37°C for 24 hours. Then, the tested concentrations of different compounds, *i.e.* (200, 100, 50, 25, 12.5) mg/ml, were attended and one ml of each concentration was placed in test tubes containing 100 pieces of filter paper (Whatman No. 1) sterilized by autoclave with a diameter of (6) mm. Then, 0.1 ml of bacterial suspension was inoculated into several Petri dishes containing nutrient agar medium and sparing the surface of the nutrient agar, a disc was placed in each Petri dish for each concentration of the substance considered and the Petri dishes were placed in an incubator at 37°C for 24 hours. The diameter of the zone of inhibition around the disc was determined.

Minimum inhibitory concentration (MIC) assessment

The ethanolic extract of *S. lappa* roots was fixed at serial dilutions (25, 50, 100 and 200 mg/ml of supplement) followed by 5% dimethyl sulfoxide solution (DMSO). Then, 100L 106 cells/ml of four bacterial strains (*S. aureus, P. aeruginosa, Escherichia coli* and *Proteus spp.)* were cultured in a pre-arranged broth. After one day of incubation, the optical density (620 nm) of the cultured microorganisms was assessed using a spectrophotometer and the results were compared with negative control (broth medium without microorganisms) and positive control (microorganisms developed without any treatment). Then the serial dilution of the plant extract were used to prepare discs of different concentration. Once culture is ready these discs were incubated with bacteria in the cultured petri dishes, the zone of inhibition noticed after 2 days measured and calculated as zone of inhibition. For each tests a replicate of three tests were conducted and mean± standard deviation was calculated for each and compared to other concentations.

Statistical analysis

The collected data were grouped and represented as percentage of antioxidant activity. Diameter zone in millimeter for growth inhibition of affected bacteria caused by S. lappa roots extracts. Area under the curve considered for functional group characterization of the extract.

RESULTS AND DISCUSSION

Study settings: The present study was conducted in College of Pharmacy at University of Mosul during the period January 2023 to December 2023. The bacterial isolates were previously characterized in the Department of Clinical Laboratory Sciences in College of Pharmacy at University of Mosul. The plant species was characterized and authenticated by the Department of Pharmacognosy and Medicinal plants in College of Pharmacy at University of Mosul.

Characterization of bioactive compounds

Chemical analysis revealed the composition of active chemicals and other unknown substances in the ethanolic extract of *S. costus* roots. Alkaloids, terpenoids, phenolics, tannins, coumarins, glycosides, flavonoids, quinines and steroids were found in the samples.

DPPH radical scavenging activity

Table 2 outlines the output of the DPPH scavenging activity of flavonoids in ethanolic extracts of the roots of *S. costus* and compares them with the ascorbic acid as a positive control. The free radical scavenging potential of serial dilutions of the root extracts of *S. costus* was 71.9% at 62.5 ppm, 87.3% at 125 ppm and 89.3% at 187 ppm, which is comparable to ascorbic acid.

FT-IR

Table 3 and Fig 2 show the FT-IR results. The solutions were analyzed separately using an FT-IR spectrophotometer (Bruker-Alpha ATR-FTIR, Germany). According to FT-IR studies, the molecule includes multifunctional groups such as phenolic -OH, carbonyl (-C=O) and olefin C=C, *etc*.

Antibacterial activity

The antibacterial activity of *S, lappa* roots extract was tested against four different types of pathogens (Table 4 and Fig 3). The results showed that the extract had different antibacterial activities against each of the tested strains. Using the disc diffusion method, *S. lappa* root extract showed the widest zone of inhibition against *Staphylococcus aureus*. *Escherichia coli* and *Pseudomonas aeruginosa* showed less inhibition, but S. pyogenes check whether it is pyogenes or aureus showed higher resistance to *S. lappa* roots extract (Table 5). Assumption of MIC values: The MIC values of *S. lappa* roots extract varied from 0.125 to 0.5 mg/ml against all the tested bacteria, with Proteus spp. showing considerable resistance to this extract.

Over thousands of years, the effectiveness and beneficial effects of the plants have become known and plants have been considered one of the major sources of antibiotics (Dhakal *et al.,* 2021). The world's population still relies primarily on conventional remedies such as herbs to obtain medicines (Gul, 2020). Plants can also be used as alternatives to antibiotics and have fewer adverse effects than pharmaceutical drugs. The increase in antibiotic resistance and adverse effects of synthetic drugs have stimulated interest in the discovery of new antimicrobial compounds from plants. *S. lappa* is a well-known essential medicinal plant that is widely used in many

Table 2: Antioxidant effects of ethanolic extract of Saussurea costus roots against DPPH.

Treatment	Conc. (ppm)	Antioxidant activity (%)
Alcoholic	62.5	71.9
Saussurea costus	125	87.3
roots	187	89.3
Ascorbic acid	23	81.96

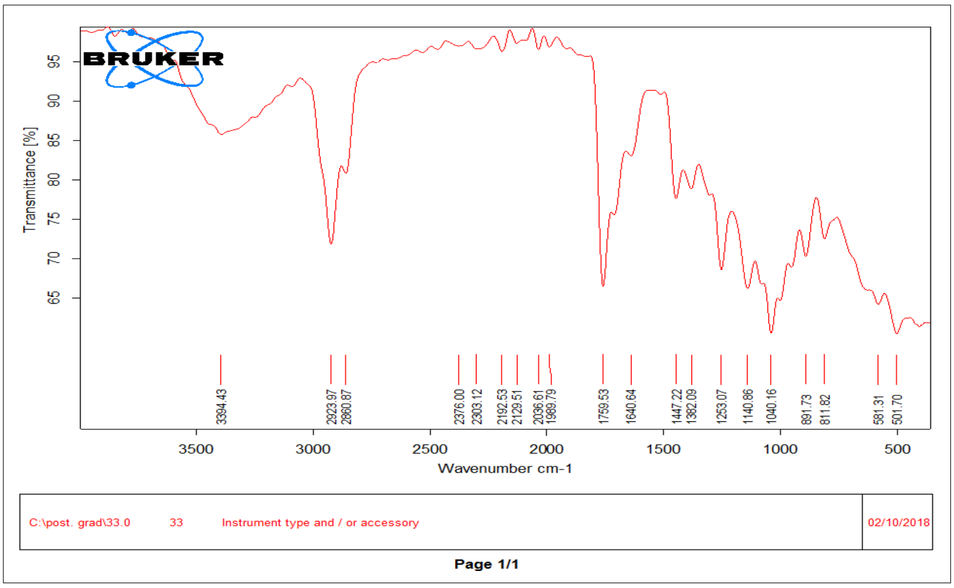

Fig 2: The result of FT-IR.

Table 3: The functional groups of the compounds from the IR-spectrum.

Wave no.1	Band shape	Band	Functional group
3394.43	Brand	OH	alcohol
2923.97	Sharp	-CH2-	Aliphatic stretch
1759.53	Sharp	C=O	Stretching C=O of-CHO
1442.22	Sharp	-C=C-	Stretching of olefinic
1040	Sharp	C-O	C-O Stretching

medicinal systems for curing and treating multiple different nature of diseases, including peptic ulcers, asthma and gastric disorders (Malik, 2020). The rhizomes and roots of *S. lappa* have bitter, astringent, purgative, anthelmintic, antioxidant, antitumor, digestive stimulant and stimulating properties.

Due to the active ingredients present in *S. costus* extract, with some of these biomolecules have been confirmed as having biological activity against cancer cell lines (Deabes *et al.,* 2021; Kumar *et al.,* 2024). *S. costus* extract has been demonstrated as having antimicrobial activity against Gram-positive (*Streptococcus pyogenes and Bacillus subtilis*) and Gram-negative bacteria (*Escherichia* coli and P. aeruginosa)(Al-Zayadi *et al.,* 2023), this finding is highly critical to conquer these bacterial resistance which do have the capacity to persist in bleak surroundings (Pourhajibagher *et al.,* 2016; Qi *et al.,* 2016; Gheorghe-Barbu *et al.,* 2022). Similarly, Al-Zayadi *et al.,* (2023) reported that their extract contents were congruent with compounds detected in the present study.

In this study, the bacterial inhibitory zone revealed weak or no impacts on proteus species and this result harmonized with the studies conducted by Deabes *et al.* (2021) and Abdallah *et al.* (2017), who have shown a better antibacterial effects of *S. costus* extract on growth inhibition of Gram-positive (*Streptococcus and Staphylococcus*) versus Gram-negative bacteria (*Salmonella and Escherichia coli*) with detected presence of approximately similar active biomolecules.

Struvite renal stone is a renal lithiasis formed due to continuous successive renal tract infections with gram negative bacteria and is also called infectious lithiasis (Polat and Eral, 2022; Bichler *et al.,* 2002). The dualistic mode of action of *S. costus* extract on struvite renal lithiassi which involve solubilization of the renal crystals alongside killing the causative agents of involved pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*) (Mammate *et al.,* 2023). Furthermore, Sadki *et al.* (2017), reported that

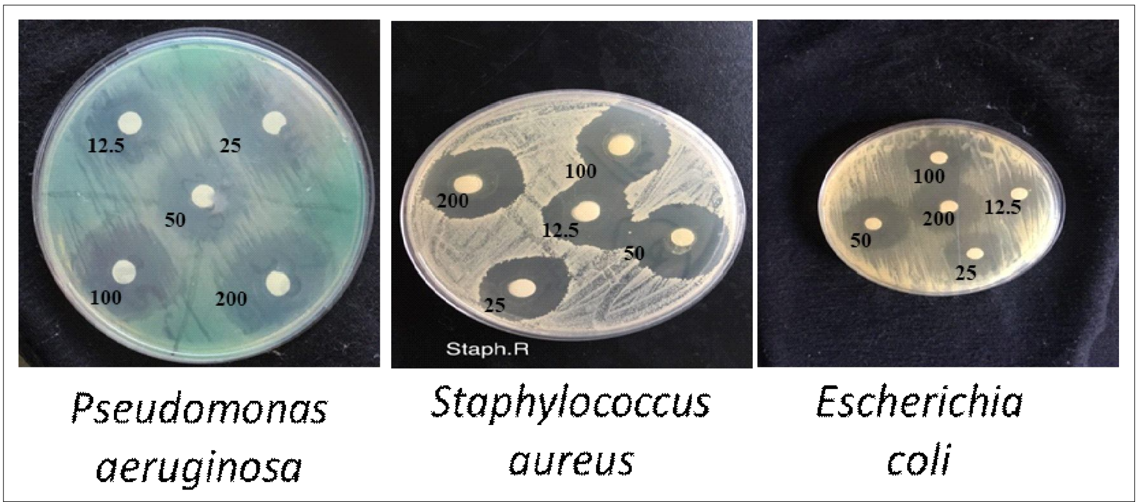

Fig 3: Inhibition zone of affected bacteria through agar disk diffusion method with *Saussurea lappa* roots extract.

Table 4: Diameter zone for growth inhibition of affected bacteria (mg/ml) caused by *Saussurea lappa* roots extracts in mm.

Concentration (mg/ml)	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus spp.*
200	26±1.5a	25±0.5b	23±1.5c	-
100	24±a	20±1b	20±0.5c	-
50	20±0.5a	18±1ab	18±1ca	-
25	18±0.5a	15±1ab	15±1ca	-
12.5	14±1a	11±1ab	12±1cb	-

Data expressed as mean±SD, One-way anova conducted to compare between groups followed by Post-hoc test to identify the statistically different group at p<0.05 and similar letter indicated non-significant differences while different letter indicates significant differences between compared group.

Table 5: Minimum inhibitory concentration (MIC) of *Saussurea lappa* roots extracts (mg/ml).

Bacterial species Extracted compound	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus spp.*
Saussurea lappa roots extract	0.125	0.5	0.25	-

S. costus inhibited the formation of ammonium–magnesium phosphate crystals which is the main component in renal lithiasis of struvite renal stone.

Several pharmacological experiments using *in vitro* and *in vivo* models have shown that *S. lappa* has anti-inflammatory, peptic ulcer, anticancer and hepatoprotective properties (Nadda *et al.,* 2020; Sundar *et al.,* 2018). The bioactive compounds extracted from this plant include dehydrocostusolide, Costunolide and Cynaropicrin. Given the impressive bioactivity of *S. lappa* and its compounds, it would be reasonable to develop them as drugs. The effect of ethanolic extract of *S. lappa* on the growth of *Streptococcus mutans* was examined in a dose-dependent manner (Nadda *et al.,* 2020). *S. lappa* has been used in the management of dental diseases because it is effective against cariogenic *Streptococcus mutans*.

CONCLUSION

Saussurea can be used medicinally for oxidative stress or as an antibacterial drug. The outcome was based on the experimental identification of therapeutic effects of *S.* using laboratory tests. The effects could be further confirmed by clinical application or experimental animal studies.

Informed consent

The present study was registered and approved by the Department of Clinical Laboratory Science in the University of Mosul (Iraq).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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