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Research Article

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Phytochemical, Antioxidant and Antibacterial Activity of *Lycium schweinfurthii* Leaves Extracts

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ABSTRACT

Lycium schweinfurthii (*L. schweinfurthii*) is a shrub belonging to the Solanaceae family which is widely distributed in North Africa and the Mediterranean region. The leaves and fruits have traditionally been used for gastrointestinal diseases. There are currently only a limited number of scientific studies investigating the antibacterial activity of *L. schweinfurthii*. This study aimed to investigate the antioxidant activity and antibacterial effect of *L. schweinfurthii* leaves extracts. Extraction was carried out using a cold maceration method by fractionation on different solvents (petroleum ether, ethyl acetate and methanol).

The phytochemical screening was performed qualitatively. An in-vitro analysis of antioxidant activity was applied using qualitative and quantitative DPPH scavenging activity test. *Lycium schweinfurthii* leaves extracts were tested at different concentrations against Gram-positive and Gram-negative bacteria isolated bacteria from Tripoli Medical Center (*Staphylococcus aureus & Escherichia coli*) zone of inhibition was recorded as the mean value. The phytochemical screening showed the presence of major secondary metabolites. However, the methanol fraction contained (flavonoids, phenolic compounds, alkaloids, tannins, saponins and carbohydrates). The methanolic extract showed good DPPH scavenging activity compared to the ascorbic acid which used as positive control. At 100 mg/ml concentration, petroleum ether fraction had the highest antibacterial activity against *Staphylococcus aureus*, followed by methanol and ethyl acetate fractions (8.33, 7.33 & 5 mm respectively). Methanol fraction at 100 mg/ml showed the largest zone of inhibition against *Escherichia coli* followed by ethyl acetate and petroleum ether leaves extract (9, 6.66 and 5.83 mm respectively). It can be concluded that the *Lycium schweinfurthii* leaves extracts have promising antioxidant and antibacterial activity against bacterial strains.

Keywords- Antibacterial; DPPH; Medicinal plants, Solanaceae.

INTRODUCTION

Lycium schweinfurthii is a shrub belonging to the Solanaceae family, commonly found throughout the Mediterranean region and North Africa.[1] It can be found blossoming throughout the year. It grows in sandy and stony areas along the coastal line and found in many countries including Libya, Tunisia, Egypt, Portugal and Spain.[1] Twenty- six known compounds and two new ones were isolated from *L. schweinfurthii*. Four showed cytotoxic effects against skin cancer cells, while three showed cytotoxic effects against colon cancer cells. All of them exhibited minimal toxicity against normal cells from the skin and colon, indicating their potential selectivity and safety as cytotoxic compounds.[2] A previous phytochemical screening reported a presence of important bioactive compounds in methanolic leaves different parts of *L. schweinfurthii* such as alkaloids, saponins, flavonoids and tannins. The total flavonoids and other contents were found to be more concentrated in the leaves than in other parts of the plant, and five flavonoids were isolated from the methanolic extract of the leaves using various chromatographic techniques. Neuropharmacological activity of the methanolic leaves extract was evaluated, as a result, it was found that that *L. schweinfurthii* has anti-depression, sedative and analgesic-like activities.[3] In other scientific reports which investigated the effect of *L. schweinfurthii* extract on the central nervous system in mice, including anticonvulsant, antidepressant and muscle relaxant activities suggested that the methanolic extract of *L. schweinfurthii* leaves has anticonvulsant and antidepressant-like activities without any muscle relaxant effect in mice.[4] Bacterial infection is a prevalent clinical disease that can affect many organs and tissues.[5] It may also be described as the spread of poisonous or hazardous strains of bacteria or toxins produced inside or on the body. Bacteria may harm any part of the human body, examples are food poisoning, meningitis, and pneumonia.[6] Bacterial resistance to antibiotics remains a major problem since organisms appear to acquire resistance to new medications as quickly as they are introduced.[7] Many current drugs are based on plants and plant-based remedies against a variety of diseases. Research into medicinal plants as natural products is becoming increasingly popular worldwide. These plants have active chemical constituents with

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significant antioxidant effects that assist in the prevention of many diseases.[8] However, there is no published data about the antibacterial activity of *L. schweinfurthii*. Thus, the aim of this study was undertaken for the first time to investigate the antibacterial effect of *L. schweinfurthii* leaves extracts against isolated bacteria.

MATERIALS AND METHODS

Plant collection

The fresh leaves *of Lycium schweinfurthii* (*L. schweinfurthii*) were collected from the city of Gharyan, Northwest Libya, in March 2024. Prof. Mohammed Makhlouf (Department of Botany, Faculty of Science, University of Tripoli, Libya) identified the plant, and a voucher number of 676271 was deposited in the University's herbarium.

Plant preparation and extraction

Plant leaves were washed, shade-dried at 24 ± 2°C for 7 days, ground into a fine powder and cold maceration extraction was employed using solvents in order of increasing polarity. Petroleum ether extract was firstly used on plant. The grounded leaves was macerated for 5 days, filtered using Whatman number 1 filter paper and evaporated under reduced pressure at 40°C to obtain crude extract. The remaining marc was extracted similarly by ethyl acetate then methanol in the same manner. The obtained crude extracts were stored at 4°C until use.[9]

Phytochemical screening

Qualitative phytochemical analysis was conducted on the extracts to detect major secondary metabolites following standard procedures.[10] The screened phytochemicals were flavonoids (Shinoda test), phenolic compounds (ferric chloride test), alkaloids (Mayer's and Dragendorff's reagents), tannins (ferric chloride test), saponins (froth formation test), carbohydrates (fehling's and Molish's tests), resins (Acetic anhydride test) and steroids (Salkowski test).

In-vitro analysis of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) was used for both qualitative and quantitative analysis of free radicals scavenging activity.

Qualitative analysis

Antioxidant activity of the plant leaves methanolic extract was initially screened using thin layer chromatography (TLC). Spots of methanolic extract from *Lycium schweinfurthii* leaves (1mg/ml) and a methanolic L- ascorbic acid solution (1mg/ml) were applied on a TLC plate. After spraying the plate with a methanolic DPPH solution, the appearance of pale-yellow spots against a purple background indicated the presence of antioxidant substances.[11]

Quantitative analysis

The quantitative DPPH scavenging activity was measured according to Kumar *et.al* 2014 and Kumarasamy *et.al* 2007 standard procedures with slight modifications.[11,12]

The methanolic solution of DPPH (80µg/ml) was prepared. *Lycium schweinfurthii* methanolic leaves extract stock solutions (1mg/ml) was prepared followed by concentrations of (0.5, 0.25, 0.125 mg/ml). One milliliter of the prepared DPPH solution was added to 1 ml of different concentrations of plant leaves extract and left for 30 min in cold dark place. UV absorbance of all samples was measured at 517 nm. A standard of L- ascorbic acid was used as a positive control. The experiment was performed in triplicate.

The results were expressed as a percentage of the inhibition of the DPPH radical and were calculated by the following formula:

% Inhibition of DPPH = Absorbance (control)- Absorbance (sample)/ Absorbance (control) X 100

Antibacterial activity testing

Isolated gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial strain from Tripoli Medical Center was used in this study.

The agar well diffusion method was used to evaluate antibacterial activity. Mueller-Hinton agar was prepared and bacterial suspensions were standardized to 1.5 × 10^8 CFU/ml using the 0.5 McFarland standard. Wells (6 mm) were made in the agar, and 100 µl of each extract at concentrations (100, 50, 25, and 12.5 mg/ml) in DMSO 2% were added. Plates were incubated at 37°C for 24 hours. Levofloxacin was used as positive control and DMSO 2% was used as negative control. Zones of inhibition were measured in millimeters, the diameter around cup cuts with no bacterial growth was measured and recorded.[13] The tests were carried out in triplicate.

Statistical analysis

Data were analyzed using SPSS (Version 29) and results were presented as mean ± standard deviation (SD). The data was statistically analyzed by independent-sample *t*-test. Non-parametric data were analyzed statistically by means of Mann-Whitney-*U* test. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Phytochemical Screening test results

The preliminary phytochemical screening of *Lycium schweinfurthii* leaves extracts revealed the presence of several secondary metabolites (Table 1). Flavonoids, phenolic compounds, alkaloids, tannins, saponins and carbohydrates were detected predominantly in the methanolic extract. Petroleum ether and ethyl acetate extracts contained limited phytochemical contents. Notably, steroids were absent across all solvent extracts.

DPPH scavenging activity test results

Qualitative analysis

Methanolic extract of *L. schweinfurthii* leaves was spotted

on TLC plates and sprayed with DPPH methanolic solution (Figure 1). The positive result of free radical scavenging activity was indicated by color change from purple to yellow. The clear observed color change of the extract showed a potential of the extract to contain antioxidant compounds and according to this result further quantitative assay was performed.

Quantitative analysis

After measuring the absorbance of different concentrations of *L. schweinfurthii* methanolic extract incubated with DPPH solution, the results (Figure 2) showed a good antioxidant activity with a maximum of DPPH scavenging activity of about 93.63% at a concentration of 0.125 mg/ml. *L. schweinfurthii* methanolic extract with no significant difference ($P > 0.05$) with ascorbic acid at all concentrations.

Antibacterial Activity test results

The antibacterial activity of the plant leaves extracts was assessed against two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the agar well diffusion method.

Petroleum ether leaves extract exhibited the highest zone of inhibition against *Staphylococcus aureus* (8.33 ± 1.44 mm) at 100 mg/mL, followed by the methanolic leaves extract (7.33 ± 0.28 mm) then ethyl acetate leaves extract (5.00 ± 0.00 mm) as shown in Table 2. In the same time, methanolic leaves extract demonstrated the largest inhibition zone against *Escherichia coli*, the (9.00 ± 1.00 mm) at 100 mg/mL, while petroleum ether and ethyl acetate leaves extracts showed moderate inhibition zones (6.66 ± 0.76 and 5.83 ± 0.28 respectively).

The positive control (Levofloxacin) consistently produced significantly larger inhibition zones (~30 mm) against both bacterial strains, while the negative control (2% DMSO) showed no antibacterial activity (Table 3).

Table 1: Phytochemical screening of *L. schweinfurthii* leaves extracts using different solvents: petroleum ether, ethyl acetate and methanol.

Constituents / Tests	Observations	Petroleum ether extract	Ethyl acetate extract	Methanol extract
Flavonoids (Shinoda)	Pink colour	-Ve	-Ve	+Ve
Phenolic compounds	Dark green colour	-Ve	-Ve	+Ve
Alkaloids:				
A- Dragendorff's reagent	Reddish-brown precipitate	+Ve	+Ve	+Ve
B- Mayer's reagent	Creamy-white precipitate	+Ve	+Ve	+Ve
Tannins	Green colour	-Ve	-Ve	+Ve
Saponins	Permanent foam	+Ve	+Ve	+Ve
Resins	Violet quickly.	+Ve	+Ve	-Ve
Steroids (Salkowaski)	Yellow-coloured ring	-Ve	-Ve	-Ve
Carbohydrates:				
A- Fehling's test				
(reducing sugars)	Brick-red precipitate	+Ve	+Ve	+Ve
B- Molish's Test	Violet ring at the interphase	+Ve	+Ve	+Ve

+Ve = presence of the constituent ; – Ve = absence of the constituent.

Table 2: The antibacterial screening of *L. schweinfurthii* leaves extracts (different solvents) against *Staphylococcus aureus* (Gram-positive).

Zone of inhibition mm ± SD			
The concentration (mg/ml)	Petroleum ether extract	Ethyl acetate extract	Methanol extract
100mg/ml	8.33 ± 1.44	5 ± 0.00	7.33 ± 0.28
50mg/ml	4.16 ± 3.61	- ve	3.83 ± 3.40
25mg/ml	3.50 ± 3.04	- ve	- ve
12.5mg/ml	2.83 ± 2.46	- ve	- ve
Levofloxacin (positive control)	20 ± 0.00	20 ± 0.00	20.66 ± 1.15

Data presented as mean ± SD (n = 3). - ve: No activity.

Table 3: The antibacterial screening of *L. schweinfurthii* leaves extracts (different solvents) against Escherichia coli (Gram-negative).

Zone of inhibition mm ± SD			
The concentration (mg/ml)	Petroleum ether extract	Ethyl acetate extract	Methanol extract
100mg/ml	0.28 ± 5.83	0.76 ± 6.66	1.00 ± 9.00
50mg/ml	1.44 ± 8.33	0.00 ± 5.00	0.28 ± 6.83
25mg/ml	0.00 ± 5.00	ve -	0.00 ± 5.50
12.5mg/ml	0.00 ± 2.50	ve -	0.00 ± 5.00
Levofloxacin (positive control)	0.00 ± 30.0	0.00 ± 30.0	0.00 ± 30.0

Data presented as mean ± SD (n = 3). - ve: No activity.



Figure 2: TLC for *L.schweinfurthii* leaves methanolic extract (1mg/ml) and Vitamin C (1mg/ml) sprayed with DPPH methanolic solution.

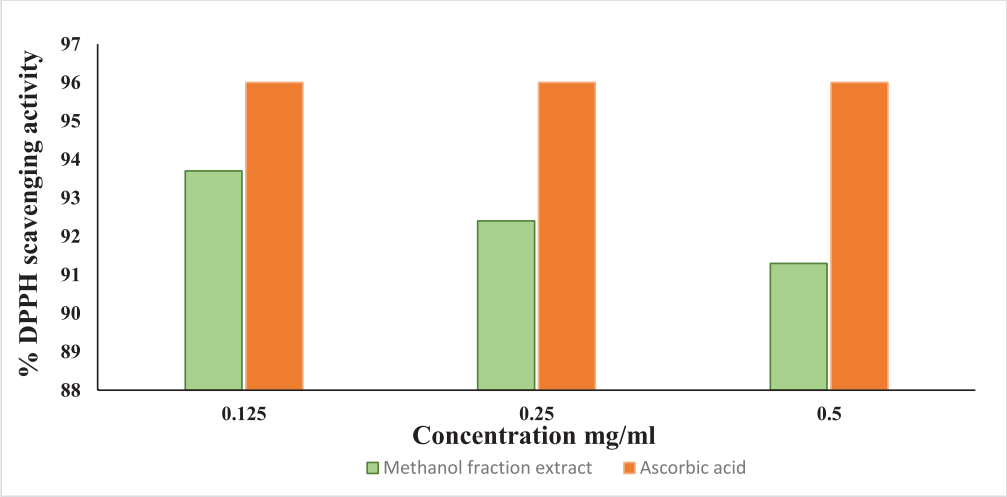


Figure 2: Percentage of DPPH scavenging activity of different concentration of *L. schweinfurthii* leaves methanolic extract with respect to ascorbic acid.

DISCUSSION

In the present work, phytochemical constituents, antioxidant and antibacterial properties of *Lycium schweinfurthii* leaves was tested and studied for a plant traditionally used in Libyan medicine. The results confirmed that the leaves extracts, particularly those obtained using methanol possessed significant antibacterial and antioxidant activities, likely due to the rich presence of secondary metabolites such as flavonoids, phenolic compounds, alkaloids, and saponins. These results suggest that the antioxidant activity of the methanolic leaves extract was attributed to the total flavonoid contents in the extract.[3] Interestingly, it was found that the low concentrations of methanolic leaves extract had higher DPPH scavenging activity, this might be illustrated that some compounds with antioxidant activity was unable to be dissolved in the solvent at high concentration leading to precipitating out from solution making them unavailable to react with DPPH. When diluted, they made them more available as they dissolved and showed their activity. That could explain higher activity in more diluted form.[14] Methanol leaves extract exhibited the strongest antibacterial effect against *Escherichia coli*, indicating the effectiveness of polar phytochemicals in disrupting bacterial cell processes. This is consistent with prior findings where methanol was found to be an effective solvent for extracting potent antibacterial compounds from medicinal plants.[12,15] Interestingly, petroleum ether extract was more effective against *Staphylococcus aureus*, suggesting that non-polar constituents such as certain lipophilic alkaloids and essential oils may contribute to its antibacterial action. The difference in activity between Gram-positive and Gram-negative bacteria could be explained by the structural differences in their cell walls, with Gram-negative bacteria typically being more resistant due to the presence of an outer membrane that limits the penetration of many antibacterial agents.[16] Although the inhibition zones produced by *L. schweinfurthii* extracts were smaller compared to the standard antibiotic (Levofloxacin), the observed antibacterial activity supports the traditional use of this plant for treating infections. It also suggests that *L. schweinfurthii* could be a valuable source of new antibacterial agents, particularly in an era of rising antibiotic resistance.[17]

CONCLUSION

Lycium schweinfurthii leaves extract exhibited promising antibacterial properties and antioxidant activity. Methanol extract, in particular, showed substantial activity against *Escherichia coli*. These findings support the plant's traditional medicinal use and highlight its potential as a source for novel antibacterial agents. Further studies are needed to isolate and characterize active compounds and assess their therapeutic potential, especially in light of rising antibiotic resistance.

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