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Bioanalytical applications of some Iraqi wild herbal Awsaj (*Lycium shawii*) extracts

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Abstract

The goal of this study was to extract phytochemical contents of Awsaj (lycium shawii) fruits using Ethanol, Ethyl acetate, and hexane, respectively, by Soxhlet apparatus, with yields of (2.57gm/50gm) for ethanol(1.51gm/50gm) for ethyl acetate and (0.7gm\50gm) for hexane solvents. The antimicrobial, antioxidant, and anti-inflammatory properties of the three extracts were investigated. To test antibiotic activity, the agar well diffusion method was used against four bacterial species: two Gram-positive bacteria *(Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella sp*.) as well as one yeast (*Candida albicans*). The present investigation found that lycium shawii ethyl acetate extract inhibited the examined microorganisms, with an inhibition zone extending between (13-21mm), especially toward Staphylococcus epidermidis and Escherichia coli with a 21 mm diameter inhibition zone, hexane had aninhibition zone towards Staphylococcus epidermidis with a 10mm, while Ethanol extract had no inhibition zone. Atomic Absorption Spectrometry (AAS) revealed the presence of 5.9 g/dl Cu, 15.1 g/dl Zn, 4.2 g/dl Pb, 0.11 g/dl Cd, 0.08 g/dl Cr, 0.009 g/dl Ni, and 0.006 g/dl Mn, , Gas Chromatography-Mass Spectroscopy (GC-MS) analyses verified the presence of 13 compounds . chemicals Standard techniques were used to confirm the presence of alkaloids, tannins, terpenoid, saponins, glycosides, resins, and flavonoids, among other phytochemicals. Total phenol content and radical scavenging activity were quantified using the Folin-Ciocalteu and 2,2-diphenylpicrylhydrazyl (DPPH) techniques, respectively. And the extract has a high concentration of phenolic compounds (0.67mg/mL) and a high amount of antioxidant chemicals, according to the DPPH assay for ethyl acetate extract. The effect of lycium shawii ethyl acetate extract on cyclooxygenase (COX-2) enzyme activity was investigated, and it was discovered that the L. shawii extract inhibits COX-2 activity more effectively than anti-inflammatory drugs (aspirin). The data suggest that L. shawii ethyl acetate extract could be used as an antimicrobial, antioxidant, and anti-inflammatory agent.

Keywords: lycium shawii fruits extract, antimicrobial activity; antioxidant activity; Cyclooxygenase (COX-2).

1. Introduction

Herbal medicine is the oldest kind of healthcare, and it has been practiced in both developing and industrialized countries for decades. Food, shelter, clothes, and medicine were all provided by nature to primitive people. These folks were able to discern between plants that were beneficial and those that were inert or poisonous [1]. Approximately 50,000 plant species are said to have therapeutic characteristics in the literature [2]. As a result of scientific confirmation of herbal medicine, modern pharmaceutical medications such as aspirin, morphine, digi toxin, and quinine were produced [3] [4]. Plant-based drug awareness grew over time laying the groundwork for many traditional medicine systems around the world. Thorny perennial shrub Lycium shawii (Awsaj) belongs to the Solanaceae family. It grows in the ridges of sandstone. It bears red and purple blooms as well as vicious thorns. The leaves are oval and clumped together [5]. Flowers are produced in natural environment from March to April and in irrigated soil all year. The berries are globular, numerous-seeded, red-to-orange berries that are delicious and sweet. It supplies wild bees with honey, as well as food and shelter for wild birds and animals [6]. The roots of Lycium shawii are boiled, and the decoction is used to treat mouth sores, livestock in Tanzania. Constipation and stomach discomfort are treated with the leaves [7]. Secondary metabolites from Solanaceae plants have a wide range of biological activities, including nutritional antioxidants, antimicrobials, anti-inflammatory, anti-cancer, and cytotoxic characteristics, allowing them to play important roles in food and pharmaceutical applications [8] [9]. Recent phytochemical investigations have confirmed that Lycium species

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(Solanaceae family) have a wide range of biological activities due to their abundance of ingredients such as terpenoids, alkaloids, and flavonoids. [10] Many Lycium species' fruits, flowers, and roots have long been used as food and/or medicine sources [1]. To the best of the authors' knowledge, there have been no papers to date investigating Lycium shawii leaf extract as a natural antioxidant and antibacterial agent against Multidrug Drug Resistance (MDR) or Pan-Drug Resistance (PDR) pathogens of burn wound infections. As a result, the goal of this study was to assess the novel pharmaceutical potential of the traditional medicinal plant, L. shawii, as an active antimicrobial against MDR/PDR clinical pathogens of burn wound infections, an antioxidant agent, and toxicological safety using acute oral toxicity and hemolytic activity in order to complete its evaluation as a new leading structure in the pharmaceutical field. Lycium species have a wide range of biological activity, including antiviral properties. as antiviral, antioxidant [2][1], antimicrobial [1][3], antidiabetic [4][5], hepatoprotective [6], anticancer [7], hypolipidemic, hypotensive, glucosidase inhibition, and antiaging [8] activities. In addition to some biological activities as antioxidant, cytotoxic and hepatoprotective, laxative, diuretic, hypotensive, antidiabetic and cure jaundice. [5] [8] [9]. In Saudi Arabia L. shawii is used to treat mouth ulcer [10]. In addition, anti-diabetic, anti-toxoplasma [21], antiplasmodial, antitrypanosomal [21], and antibacterial actions were found in the aerial portions [13]. Phytochemical constituents were extracted from L. shawii fruits by Soxhlet instrument using solvent extraction methods such as ethanol and ethyl acetate and hexane. These techniques, however, have a number of flaws. In terms of time, cost, and the possibility of contaminating the environment by mixing the leftovers of the utilized solvent with the final extract and the waste product of the solvent.[8] The Soxhlet device, which is simple to operate and reduces chemical waste and pollution, is one of the traditional extraction processes. The antimicrobial , antioxidant, and antimicrobial properties of the lycium shawii plant were then investigated. COX2 is a pleiotropic and multifunctional enzyme that has a role in the genesis or promotion of carcinogenesis as well as cancer cell resistance to chemo and radiotherapy. COX2 is secreted into the tumor microenvironment (TME) by cancer-associated fibroblasts (CAFs), macrophage type 2 (M2) cells, and cancer cells. COX2 enhances apoptosis resistance, proliferation, angiogenesis, inflammation, invasion, and metastasis of cancer cells via inducing CSC-like activity. COX2-mediated hypoxia within the TME, as well as its favorable interactions with antiapoptotic mediators, are all factors that contribute to cancer cell resistance to chemotherapeutic treatments. COX2's metabolite, prostaglandin E2, performs the majority of the activities. In specific circumstances, COX2 may act as an anticancer enzyme. COX2's actions on cancer cells and its control are influenced by a variety of signals. COX2 inhibitors given before surgery may minimize the likelihood of cancer patients developing metastases. COX2 inhibition also makes cancer cells more sensitive to therapies like radiotherapy and chemotherapy. COX2 activity is negatively induced by chemotherapeutic drugs. As a result, picking the right chemotherapeutic medications, as well as adjusting the type and dose of COX2 inhibitors based on the type of cancer, would be a successful adjuvant strategy for cancer treatment. [19]

2. Experimental

2.1 Collection and preparation of L.shawii extract:

The plant was collected from the south of Baghdad, the Mada'in area, Baghdad, Iraq in November 2021. The red fruits were washed with water, dried, then crushed and stored. A quantity of 40 grams of gelatinous powder was taken against 400 mL of solvents (ethanol, hexane, and ethyl acetate) and extracted for 12 hours in the Soxhlet device. The biologically active components in the plant's fruits were extracted, then the plant was filtered to remove the remaining particles with (Whatman Grad No. 16) filter paper before being evaporated to dryness under vacuum and temperature of 50 °C. The extract was weighted, extract stock solution was made by weighing one gram of extract and diluting it with 10 mL of ethanol, ethyl acetate and hexane, respectively. A series of diluted solutions were made from the stock solution in the range of (0.00312 – 0.00625, 0.0125 – 0.025, 0.05,) mg/mL.

2.2 Determination of antimicrobial activity

The antimicrobial activity of L.shawii Fruits extract was tested using the agar well diffusion which was adopted From A.W. Bauer[11]using two gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two gram-negative bacteria (*Escherichia coli* and *Klebsiella* sp.) as well as *Candida albicans* (yeast) provided by Mustansiriyah University's Department of Biology. The antimicrobial activity of L. shawii extracts was examined using a simplified agar-well diffusion experiment. In sterile Petri dishes (90 mm), 15mL of molten agar at (45 °C) were put in. Cell suspensions were made and 100 µl of each were equally distributed onto the surface of Mueller-Hinton agar (Oxoid, UK) for bacteria and potato dextrose agar medium (Oxoid, UK) for fungal agar plates. With a sterile Pasteur pipette, 6 mm wells were punched into the agar after the plates had been aseptically dried. The various extracts (10 mg/mL) were dissolved in water and 80 µL were poured into the wells, and the plates were incubated for 24 hours at 37°C for bacterial strains and 72 hours at 30°C for fungi. As a

positive control for bacteria, gentamicin (10 µg/well) was utilized. The diameter of the circular inhibition zones around the well was used to assess antimicrobial activity. The tests were done in threes, and the results are the averages of three replicates.

2.3 Qualitative and quantitative analysis of chemical plant extract components

2.3.1 Qualitative L. shawii extract phytochemical analysis

Phytochemicals were analyzed qualitatively for alkaloids, tannins, terpenoids, steroids, saponins, carbohydrates, resins, and flavonoids using a standardized approach.[12] These active substances protect plants from damage and infections. Also, contributes to the scent, flavor, and color of the plants. Phytochemicals are the scientific term for them. Phytochemicals have been scientifically proven to provide health benefits for humans throughout time [12].

2.3.2 Atomic absorption spectroscopy (AAS) analysis and Gas chromatography-mass analysis (GC-MS)

Using an acid digestion technique, AAS was employed to measure the quantity of trace elements in a plant extract [14]. Trace elements play a very important role in the formation of the active chemical constituents present in medicinal plants and they are, therefore, responsible for their medicinal as well as toxic properties (Abugassa et al., 2008). Some metals are essential nutrients (zinc, magnesium, copper, chromium, and nickl) as shown in Table (3), and only become toxic at high concentrations, while others (lead and cadmium) have no known beneficial properties and are hence exclusively toxic. The chemical constituents in plants, including metal ions, are partially responsible for their medicinal and nutritional properties as well as the toxic ones. As trace elements, they play an important role in the plant metabolism and biosynthesis as cofactors for enzymes. Use of herbal medicines to relieve and treat many human diseases is increasing around the world due to their mild features and low side effects. The chemical consistency of the plant extract was determined using GC-MS technology Agelint (7820A) USA [15]. as shown in Table (4) and figure 1.

2.3.3 Determination of total phenolic compounds

A 100-liter aliquot of the extract was transferred to a volumetric flask containing 46 milliliters of distilled water (H_2O) and 1mL of Folin–Ciocalteu reagent. After 3 minutes, 3 mL of 2% Na_2CO_3 was added, and the mixture was incubated for 2 hours at 25°C.At λmax=760 nm, the absorbance was measured. For the calibration curve, gallic acid (Sigma-Aldrich, 0.2–1 mg/mL gallic acid) was employed as the standard, and total phenolic levels were represented as mg gallic acid equivalents per mL[16].

2.3.4 Determination of free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure antioxidant levels quantitatively. Two milliliters of a 0.0094 percent methanol solution of DPPH were mixed with 2mL of various ethyl acetate dilutions of the extract (Sigma-Aldrich/germany). As a synthetic reference, vitamin C (1 mM) (Sigma-Aldrich) was used. The absorbance was measured against a blank at λmax=517 nm after 30 minutes of incubation at room temperature. The percentage (I %) of inhibition of free radicals by DPPH was determined as follows:

$$\%\ \mathbf{Inhibition} = \frac{\mathbf{Ablank - Asample}}{\mathbf{Ablank}} * \mathbf{100}[17]$$

Where A sample is the absorbance of the tested molecule, and A blank is the absorbance of the control reaction (which contains all reagents except the test compound). The tests were done three times [25].

2.4 Estimation of the COX-2 activity in thyroid cancer patients

An assay for peroxidase activity in the serum of thyroid cancer patients based on a colorimetric technique was used to evaluate the activity of the COX-2 enzyme [26]. This process is based on monitoring the blue color of tetramethyl phenylenediamine (TMPD) oxidation by hydrogen peroxide, which is catalyzed by enzymes at λmax=610 nm. Under test conditions, one unit of activity is defined as the amount of enzyme necessary to convert 1 mole of hydrogen peroxide to the product[18].

2.4.1 Studying the effect of L.shawii and anti-inflammatory drugs on the activity of COX-2 in sera of thyroid cancer patients

After preparing the extract and drug at different concentrations, the effect of L. shawii extract and anti-inflammatory medicine (Aspirin) on COX-2 activity is investigated. As follows, a stock solution and serial diluents have been prepared:

1. To make sequential dilutions (0.05, 0.025, 0.0125, 0.00625, and 0.00312) g/mL from the stock solution, aspirin (0.5 gm) was mixed in 1 mL of ethyl acetate and the volume was completed to 4 mL by deionized D.W as a stock solution of 0.1 g/mL.
2. L. shawii extract (0.5 gm) was mixed in 1 ml of ethyl acetate and the amount was raised to 4 mL with deionized D.W as a stock solution (0.1 g/mL), then serial dilutions (0.05, 0.025, 0.0125, 0.00625, 0.00312) g/mL were then created in a 20 mL volumetric flask from the

stock solution .The COX-2 activity in the patient's serum was determined as follows: 2.74 mL of tris buffer and 40 mL of each inhibitor in various concentrations were added to 100 L of serum (5 patient samples), and the process was followed as described in section (2.4). The inhibition percentage was estimated by comparing activity under the identical conditions with and without inhibitors using the equation:

$$\% \textbf{ Inhibition} = \textbf{100} - \frac{\textbf{The activity in the presence of inhibitor}}{\textbf{The activity in the absence of inhibitor}} * \textbf{100} [20]$$

* The activity in the absence of inhibitor=5.24

2.5 Statistical analysis

All experimental results are reported as the mean ± SD of three different samples. The antimicrobial activity was determined as a mean of triplicate experiments and assessed in terms of zone of inhibition. The data is reported as a percentage of free radical scavenging, and the percentage of COX-2 inhibition was calculated using excel analysis at various doses of the extract. The standard curve was used to calculate the total phenolic content.

3. Results and Discussion

3.1 Antimicrobial activity

The antimicrobial effect of *ethyl acetate, ethanol* and *hexane* of L. Shawii fruit extracts was investigated. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Klebsiella sp.,* and *Candida albicans* were all tested as part of the initial screening. The ethyl acetate extract (S1), at a dosage of 0.1 gm/mL(higher concentration), had an effect on all of the bacteria examined. Overall, as indicated in Table 1, and was the most affected microorganism, while hexane extract shows a resistance just on *Staphylococcus epidermidis*, whereas the ethanol extract had no effect on the microorganisms tested.

Cell membrane damage, protein synthesis inhibition, and disruption of cell biological activities and cell membranes by specific enzymes are all examples of microbe inhibitory strategies [27]. Furthermore, the high lipid content of gram-negative bacteria cell walls (up to 20%) compared to 0–2 percent for gram-positive bacteria is responsible for germs like E. coli's resilience [2]. bacteria are more sensitive to the antimicrobial chemicals present in L. shawii, whereas gram-negative bacteria are less sensitive. [25]. It is concluded that L.shawii ethyl acetate extract has antimicrobial activity against gram-positive bacteria and gram-negative bacteria [2]. While Staphylococcus epidermidis showed a higher sensitivity to ethyl acetate extract ranging from 15-21 mm diameter, ethyl acetate was chosen to be used in further experiments.

3.2 Phytochemical compounds of L.shawii fruits extract

To investigate the existence of phytochemical components in L.shawii ethyl acetate extract, a standard qualitative chemical analysis (as described in table 2) was carried out to provide a good base for a better understanding of the L.shawii extract chemicals. The ethyl acetate extracts of L.shawii fruits contain alkaloids, terpenoids, steroids, saponins, glycosides, resins, flavonoids, and tannins, Herbal sedatives and stomachic combinations, for example, are mostly composed of aromatic plant species with therapeutic essential oils that have antibacterial, stomach-soothing, and antispasmodic qualities. The findings of this investigation are consistent with those of a previous study [21] [22]. Antibacterial, anti-inflammatory, and analgesic properties are all biological properties of an alkaloid present in the ethyl acetate extract. All substances with antihypertensive action on blood pressure and serum analysis of hypertensive patients are steroidal substances. Glycosides are also utilized to treat congestive heart failure and cardiac arrhythmia. Coughs, asthma, and hay fever are all treated with terpenoids found in the extract, as well as membrane disruption. Saponins have long been employed as detergents and insecticides, and they're also known to protect against hypercholesterolemia and have antibacterial characteristics. Aside from their industrial uses as foaming and surface-active agents, they also have health benefits [27]. Tannins, which serve as antioxidants similar to phenols, are antimicrobial, wound-dressing, anti-inflammatory, and inhibit ulcer development. [24].

Table 1: Mean and standard deviation (SD) values of ethyl acetate extract from fruits of L. shawii against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella sp. and Candida albicans.

Sample No.	Gram-positive				Gram-negative				Fungi	
	Staphylococcus*a ureus*		Staphylococcus *espidermis*		*Escherichia coil*		*Klebsiella sp*.		Candida albicans	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S1	11.33	0.43	21.2	1.19	15.11	1.22	17.56	1.0	20.12	1.27
S2	-	-	11	0.5	10	1.2	10	0.4	12.5	1.7
S3	-	-	-		-					
S4	-	-	-	-	-	-	-	-		
S5	-	-	-	-	-	-	-	-		

Table 2: Qualitative phytochemical analysis by standard procedures for L.shawii ethyl acetate &ethanol extract.

Phytochemicals	Reagents	Remarks	Ethyl acetate Extract
Alkaloids	Picric acid	The appearance of yellow colour	ND
Tannins	Lead acetate reagent Ferric Chloride	The appearance of gelatin solution The appearance of yellowish colour	+
Terpenoids and Steroids	Glacial acetic acid Sulphuric acid	The appearance of brown colour The appearance of dark blue colour	+
Saponins	Vigorous shaking	The appearance of white colour	ND
Glycosides	Benedict reagent	The appearance of brown colour	+
Resins	Ethanol	Turbidity	+
Flavonoids	Ethanol + KOH	The appearance of yellow colour	+

ND: not detection

Table 3: Concentration of trace elements in L.shawii extracts

Trace elements	Cu	Zn	Pb	Cd	Cr	Ni	Mn
Concentration in µg/dl	150	280	6.1	0.08	0.09	0.0012	0.027

3.4 GC-Mass analysis

The GC-MS study of L. shawii ethyl acetate extract revealed a variety of phytocomponents. The GC-MS chromatogram verified the presence of 13 compounds with different retention periods, as shown in figure 1.

The major compounds identified in the L.shawii fruits extract are shown in figure (1) and listed in table (4), that indicate the presence of compounds such as oxirane , androstan , silyloxcy , imidazole, adamantyl , vaccenic acid , heneicosene, octadecadien , linoleate , tricosonol , oleic acid,, as their active compounds. Due to a lack of library data for related substances, several GC-MS peaks remained unidentified.[14]

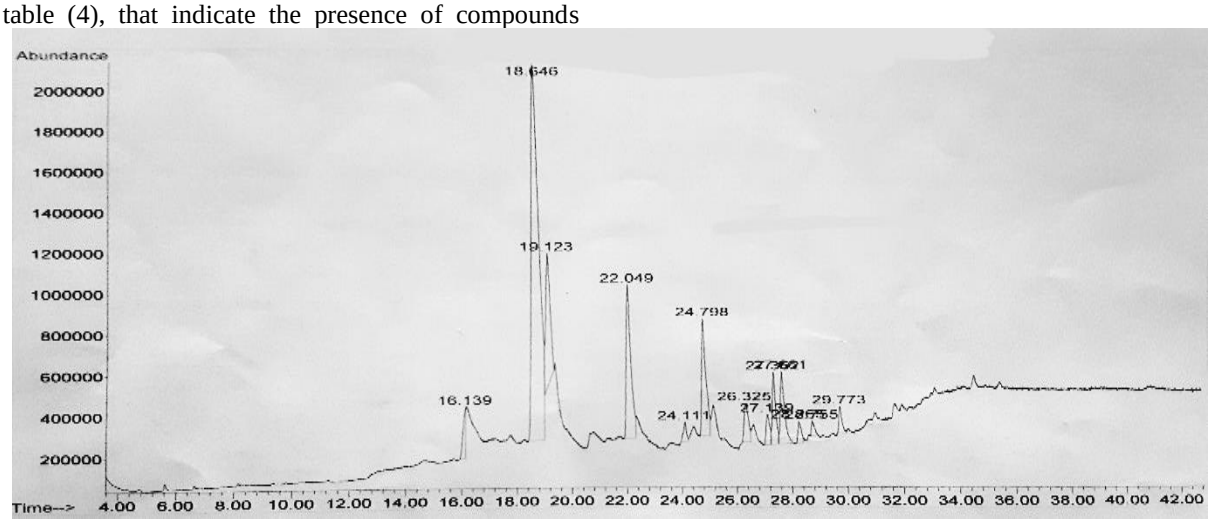

Fig. 1. GC-MS chromatogram investigation of L.shawii extract

Table 4: GC–MS analysis revealed the presence of active compounds in L. shawii fruits extract.

Peak	Retention Time(min)	Area%	Name of the compound	Formula	Mol. Wt.(g/mole)
1	16.13	2.55	DiChloroacetic acid, 3-tetradecyl ester	$C_{16}H_{30}Cl_2O_2$	325.3
2	18.64	48.93	Phenol, 2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.3239
3	19.12	20.23	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.32
4	22.04	24.87	1-nonadecene	$C_{19}H_{38}$	266.5
6	24.79	17.27	Trifluroacetic acid ,pentadecyl ester	$C_{17}H_{31}F_3O_2$	324.4
7	26.23	5.98	10-heneicosene	$C_{21}H_{42}$	294.6
9	27.36	7.94	1-tricosanol	$C_{23}H_{48}O$	340.6
10	27.66	10.39	Octacosyl acetate	$C_{30}H_{60}O_2$	452.8

3.5 Total phenolic contents

Antioxidant action is linked to phenolic compounds. As a result, quantifying TPC and determining its contribution to antioxidant activity is critical. Clearly, phenolic compounds' antioxidant activity is closely linked to their redox potential, which allows them to operate as potential metal chelators, hydrogen donors, reducing agents, and single oxygen quenchers. [28]

Table 5: Total phenolic of L. shawii fruits extract

Concentration (gallic acid) mg/mL	Absorbance	Total phenolic content in L.shawii extract mg/mL
0.2	0.921	-
0.4	1.163	-
0.6	1.346	-
0.8	1.599	-
1	1.798	-
Extract	1.400	0.67

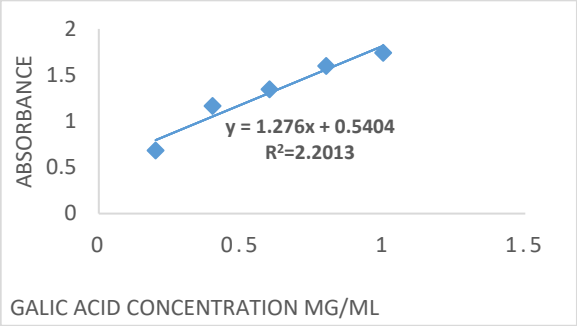

Fig. 2. Total phenolic concentration of L.shawii extracts

Total phenolics were determined using the gallic acid and L. shawii extract calibration curves, as indicated in table (5) and figure (2), and expressed as gallic acid equivalents per mL. The overall phenolic concentration was 0.67mg/mL, indicating that ethyl acetate extracts have high phenolic acid levels. This has to do with the extraction of soluble phenolic acids that are both nonpolar and semipolar. It's thought that the antibacterial activity is due to phenolic chemicals, which are antioxidants.

3.6 Free radical scavenging activity (qualitative and quantitative determination)

Free radicals are well-known contributors to biological destruction. The DPPH radical-scavenging assay is a commonly used approach to assess plant extracts' ability to scavenge free radicals produced by the DPPH reagent because of the high concentrations of phenols and flavonoids, which give high concentrations of antioxidants [28]. When DPPH, a stable free radical with a purplish color, reacts with an antioxidant, it transforms into a stable yellow molecule, DPPH's reduction capacity was measured by the antioxidant's ability to diminish its absorbance at 517 nm [29]. Table (6) shows the radical scavenging activity of different concentrations of extract as measured by DPPH radicals. When compared to the control (vitamin C), which is a powerful antioxidant, the concentration of the extracts influences the increase in antiradical activity against DPPH.

Table 6: Determination of DPPH free radical

The concentration of the extract (µg/mL)	DPPH scavenging effect (%)	Mean of DPPH scavenging effect (%)	Standard Deviation of DPPH scavenging effect (%)
Vitamin C	86.77	86.66	0.07
0.00312	51.16	51.08	0.05
0.00625	56.99	56.53	0.32
0.0125	58.89	58.79	0.07
0.025	60.52	60.50	0.01
0.05	77.11	77.02	0.06

3.7 The inhibition percentage of the L. shawii extract and anti-Inflammatory drug in the activity of COX-2 enzyme

The inhibitory impact of anti-inflammatory medications like aspirin and L. shawii extract on COX-2 was examined by preparing different concentrations of each drug and applying them in the enzymatic procedure. The study's findings revealed that each medicine has a different inhibitory percentage on COX-2 activity, as indicated in figure (3).

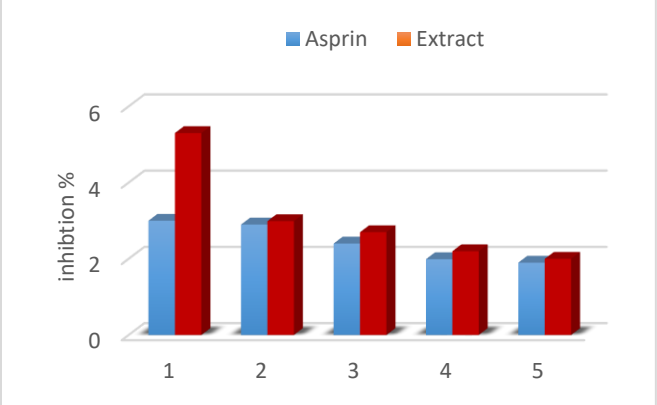

Fig. 3. The inhibition percentage of the L.shawii extract and aspirin drugs on the activity of the COX-2 in sera of thyroid cancer patients

Table 7: The inhibition percentage of the L.shawii extract and some anti-inflammatory drugs on the activity of the COX-2 in sera of thyroid cancer patients

inhibitor	Concentration (ug\ML)	COX2 Activity U\L	Inhibitors %
Aspirin	0.0158	3	42.7%
	0.0062	2.9	44.6%
	0.0031	2.4	54.1%
	0.0015	1.99	62%
	0.00077	1.9	63.7%
Extract	0.0158	5.4	4.5%
	0.0062	1.3	75%
	0.0031	1.7	67.5%
	0.0015	1.9	63.7%
	0.00077	1.1	80%

Conclusion

Antioxidants, that exist in phytochemicals, or bioactive compounds found in plants can neutralize free radicals. As a result, the progression of many chronic diseases linked to oxidative stress and reactive oxygen species (ROS)is slowed. Natural antioxidant usage has been linked to a lower risk of cancer, coronary heart disease, diabetes, and age-related disorders. The current findings indicate that ethyl acetate L.shawii extract has antimicrobial activity, with *Staphylococcus epidermidis* being the most affected pathogen. Using both traditional procedures, the extract provided improved anti oxidative activity. In comparison to synthetic antioxidants, which are rich in phenolics, terpenes, flavonoids, and a variety of trace elements, tested using standard techniques for determining their medicinal benefits by AAS, and GC-MS verified the presence of 13 compounds. The extract had a strong inhibitory effect on human COX-2 activity and thus also appeared to have an anti-inflammatory effect. As a result, the L. shawii extract can be used as a natural antioxidant and health-promoting agent with a wide range of therapeutic applications.

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