ISOLATION OF FLAVONOID COMPOUND FROM IRAQI AWSAJ PLANT (*LYCIUM BARBARUM L.*) FRUITS AND THE STUDY OF ITS ANTIBACTERIAL ACTIVITY

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Abstract

This study was performed to evaluate the *in vitro* antimicrobial activity and the flavonoid content of Iraqi awsaj plant *(Lycium barbarum L.)* fruits. The fruits extracts contain important amounts of flavonoids. The results obtained in the antimicrobial tests revealed that flavonoid compound was more active than alcoholic extract both for Gram-positive and Gram-negative bacterial strains. The results suggest that these species are valuable sources of flavonoids with relevant antimicrobial activities.

Keywords: Awsaj, flavonoid, antimicrobial, herbal medicine

Introduction

Consequently, recent important epidemiological studies have concluded that certain natural foods and medicinal plants helps to prevent or hinder the development of different diseases (Dahech *et al*., 2013 and Vlase *et al*., 2013). Thus, the interest in developing natural nutritional antioxidants is increasing due to its well-documented impact on human health (Laurian *et al*., **2014).**

The importance of plants belonging to the genus *Lycium* L. (*Solanaceae*) has increased rapidly in the last few years due to their traditional usages in Chinese herbal medicine. They are considered by most researchers as functional foods with a large variety of beneficial effects (Li *et al*., 2007 and Qian *et al*., 2004). A sweet tonic decoction made from the fruits is used to lower blood pressure and blood cholesterol levels (Tahraoui *et al*., 2007). However, it acts mainly on the liver and kidneys. The fruit is taken internally for the treatment of high blood pressure, diabetes, poor

eyesight, vertigo, lumbago, impotence, and menopausal complaints. The fruit is harvested when fully ripe and is dried for later use. The root bark is a bitter, cool, and is an antibacterial herb that controls coughs and lowers fevers, blood pressure, and blood cholesterol levels**(**Goji, 2014**).**

Materials and Methods

Plant Material & Chemicals

The Awsaj plant (*Lycium barbarum L.*) fruits were purchased fresh from Abi –Algaseb and classified by the lush in the biology department of the College of Science at the University of Basra, Iraq .The fruits of the Awsaj plant were dried and turned to powder with the help of an electric grinder. All of the chemicals were purchased from Sigma- Aldrich Co. (St. Louis, MO, USA). Also, the solvents were obtained from E. Merck (Darmstadt, Germany). Furthermore, all of the reagents were prepared in deionized distilled water.

Preparation of Extracts

Alcoholic Extract

20.000gm of Awsaj fruits powder soaked in 300ml (80% ethanol) was stirred using Magnetic stirrer for 24 hours, and was filtered through a filter paper (whatman No.541).The filtrate was placed in a Petri dish at room temperature until it dries up. Thus, the weight of the amorphous brown powder that was formed was 7.053gm.

Flavonoid Compound

25.000gm of Awsaj fruits powder was soaked in 300ml (80% ethanol) by a Magnetic stirrer for 24 hours, and the extract was filtered through a filter paper (whatman No.541). 2% aqueous lead acetate was then added until flocculent and brown precipitate was formed. Hence, the precipitate was separated by a filter paper (whatman No.532). Then, it was washed with water, methanol, and ethyl acetate consecutively.

The salt that was produced was converted to chloride by dissolving it in 30ml acetone and 10ml 2N HCl; also, it was filtered using a filter paper (whatman No. 540). The filtrate was placed in Petri dish at room temperature until it dries up. However, the weight of amorphous brown powder that was formed was 1.895 gm.

Preliminary Qualitative Test

Preliminary tests were carried out on the alcoholic extract and flavonoid compound as shown in table (1). (Harbone, 1984)

Thin Layer Chromatography

Thin Layer Chromatography (TLC) were carried out on the alcoholic extract and flavonoid compound (Benzene: DMSO: Water: Acetic acid (4:0.5:0.2:1)) .

Physical and Group Activity Test

The physical and group activity test is usually carried out on the flavonoid compound.

Sepectroscopy

Ultra violet and visible spectra, UV-visible spectrum of the flavonoid compound using ethanol as the solvent, and the spectrum was recorded with a computerized thermos spectronic model LR 115161(England).

Infra-red spectrum FT-IR spectrum was recorded with FT-IR 8400SSHIMADZU–Japan.

The Determination of the Antimicrobial Activity of Alcoholic Extract and Flavonoid Compound Cultures

The Cultures of the bacteria (*Escherichia coli* Atcc25922 and *Staphylococcus aureus* ATCC25923) that were used was procured from the Department of Biology. Cultures were maintained on the medium suggested by the respective laboratory, and sub-culturing was done fortnightly.

Antibacterial Activity of Flavonoid Compound and Alcoholic Extract

In this study, the experiments were performed to check the antibacterial properties of flavonoid compound and alcoholic extract.

The disc diffusion method was used to evaluate the antibacterial activity (Elgayyyar *etal*. 2001 and Andrews, 2001). Mueller Hinton agar was prepared in the plates as the media for the test microorganisms. Sterile filter paper discs (Whatman No. 1mm) were impregnated with 100 µl of each of the extracts (10mg/ml) and left to dry under the laminar flow cabinet overnight. The bacterial inoculum was spread evenly on the surface of the Mueller Hinton agar plates using a sterile glass L-form rod before the extract discs were positioned on the inoculated agar surface.

Each extract was assayed in triplicate. Thus, sterile distilled water served as a negative control. Furthermore, all the plates were incubated for 24 hr at 37°C. In addition, the antibacterial activity was interpreted from the size of the diameter of zone inhibition which was measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs.

The alcoholic extracts and flavonoid were dissolved in DMSO to a final concentration of 10mg/ml for disc diffusion assay. In addition, various

types of reference strains of gram positive and gram negative bacteria (Escherichia coli Atcc25922 and Staphylococcus aureus ATCC25923) were tested using plates of muller Hinton agar. The antimicrobial activity was defined as the clear zone of growth inhibition.

The minimum inhibition concentration of the alcoholic extract and flavonoid compound (MIC), was estimated against deferent types of reference strains of bacteria with deferent concentration of the alcoholic extract and flavonoid compound ranging from 1000 to 150µg/ml (Collee et al., 1996).

Results and Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Andrews, 2001).

The alcoholic extract and flavonoid compound were isolated in a yield of 35.265% and 7.580% respectively from the dried fruits.

Table (1) indicates the preliminary phytochemical analysis for alcoholic extract and flavonoid compound. The results revealed that there were alkaloids, carbohydrate, glycosides, amino acid, and flavonoid in alcoholic extract. Thus, no saponin was present. The flavonoid compound contained only flavonoid compound. TLC procedure was applied for these compounds and the results are shown in table (2).

Table (1): Preliminary qualitative test for petroleum ether and flavonoid extract

Phytochemical	Alcoholic extract	Flavonoid compound
Flavonoid test	+	+
Carbohydrate test	+	-
Alkaloids test	+	-
Glycoside test	+	+
Amino acid test	+	-
Saponin test	-	-

Absence = - ; Presence = +

Table (2): TLC for preliminary qualitative test for petroleum ether and flavonoid extract

Reagent / Sample	$FeCl_3$+ $K_3Fe(CN)_6$	Ninhydrin	Folin reagent	Drangdroff	UV-Lump	Vanilin	visible
Alcoholic extract	0.23	0.48 0.40 0.3	0.23	0.06	0.23	0.23	0.23
Flavonoid compound	0.23	---	0.23	---	0.23	0.23	0.23

Table (3): Activity test for flavonoid compound

Test / Sample	amine	Aldehyde& ketone	Carboxylic Acid	alcohol	Double bond	Phenols
Flavonoid Compound	---	+	---	---	+	---

Table (4): Physical test for flavonoid compound

Test	Result
Physical	Brown amorphous
Melting point	176-175^{0}C
Solubility test	Soluble in water ,ethanol, and DMSO

Fig (1, 2) shows the UV spectrum and IR- spectrum. The UV spectrum shows maximum absorption at 340nm due to $\pi \rightarrow \pi^*$ transition which is the characteristic of unsaturated double bond. The visible spectrum also shows max absorption at λmax= 520 nm due to the $n \rightarrow \pi^*$ transition, and due to the presence of pairs of electrons. Furthermore, it shows the most important absorption peaks of the functional groups which belonged to the IR- spectrum of isolated flavonoid compound. From IR- spectrum, we can conclude that the isolated compound has aromatic structure containing phenolic hydroxyl groups, ether, and carbonyl groups within its structure Table (5).

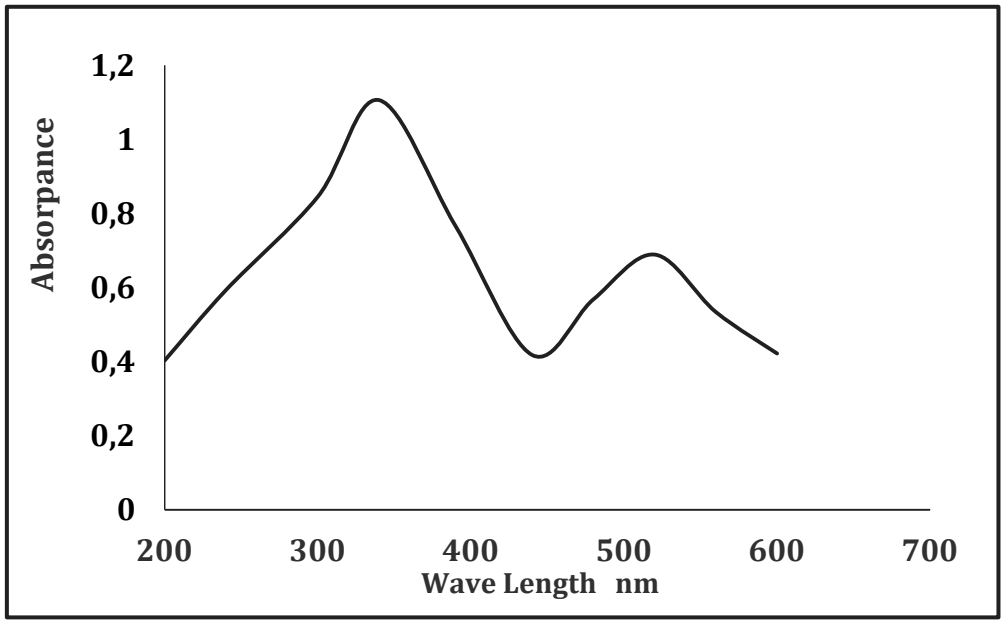

Fig. (1) shows the UV spectrum of flavonoid compound

Table (5) shows the full scan of IR spectrum of flavonoid compound

Wavenumber (cm^{-1})	Band shape	Band	Functional group
3500-3200	Broad	O-H	Stretching of phenolic-OH
1348	Broad	O-H	Bending of phenolic -OH
1612	Medium, broad	C=O	Stretching of ketone carbonyl
1620	Strong, sharp	C=C	Stretching of olfenic C=C
1039	Sharp	C-O-C	Stretching of ether

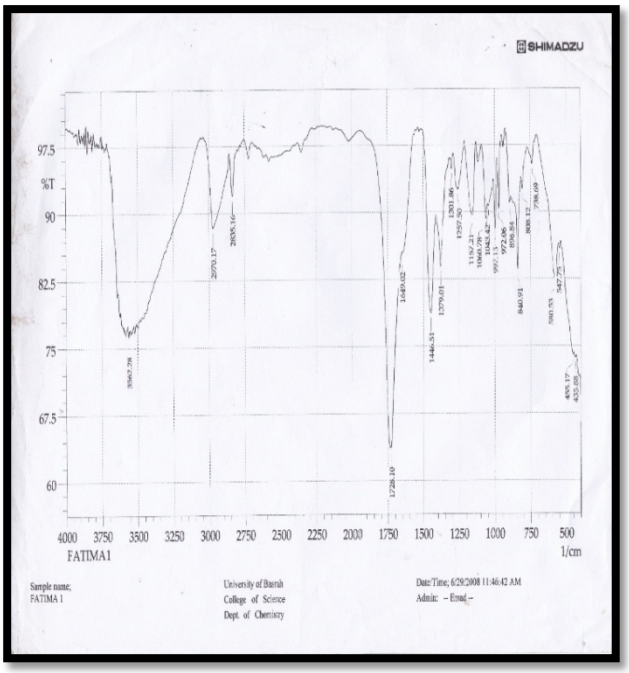

Fig. (2) shows IR for flavonoid compound

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. However, flavonoids belong to the polyphenol family. Flavanoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge. Thus, this forms a third middle ring, which can be five or six membered.

Furthermore, the flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins, and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables, and herbs (Harborne, 1994 and Harborne et *al*., 2000).

The results of testing the *Lycium barbarum L*. extracts for antimicrobial activities for both Gram-positive and Gram-negative bacteria

were summarized in Table (6). Results obtained in the present study relieved that *Lycium barbarum L*. flavonoid compound was found to be more active than alcoholic extract against both Gram-positive and Gram-negative bacterial strains. The MIC to flavonoid compound is 150µg/ml.

Generally, plant extracts are usually more active against Gram positive bacteria than Gram negative bacteria (Lin *et al*., 1999). The potent antibacterial activity of extracted flavonoid suggested that these extracts may have high total flavonoid content (Dijpa and Delmee, 2000). For phenols and phenolic compounds, an injury of membrane functions has been reported as a mechanism of action (Davidson *etal*., 1981, Sashidhar, 2002 and Abdul-Aziz , 2005).

The presence of alcoholic groups (-OH) in the structure of the flavonoid increase the activity of the plant extract to inhibit the microbial growth. So, the alcoholic compounds and their derivatives were considered as an antiseptic agents(Dey, and Harborne,1997).Thus, this agents are changing the cell protein nature and increasing the permeability of the cell membranes (Feeny, 1998).

Table (6) antimicrobial activities against both Gram-positive and Gram-negative bacteria for flavonoid test

Bacterial strains	Alcohol extract	Flavonoid test
S. aurens	**0**	**15mm**
E.coli	**0**	**12mm**

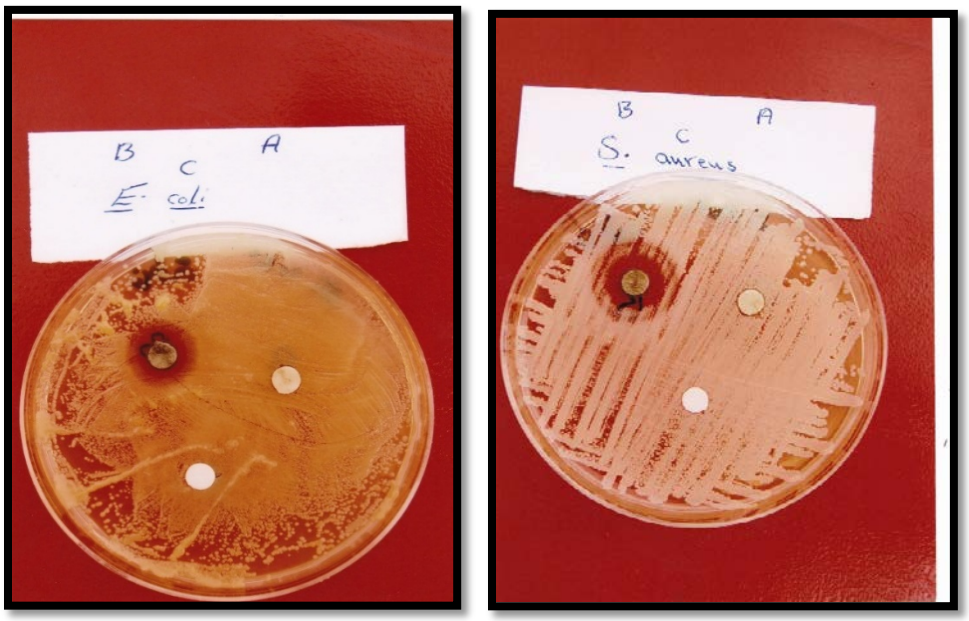

Figure (3) :The antibacterial activity of flavonoid pigment(B) and alcoholic extract (A) against *Staphylococcus aureus* and *Escherichia coli* Bacteria.

References:

Dahech, I.; Farah, W.; Trigui, M.; Hssouna, A.B.; Belghith, H.; Belghith, K.S.; Abdallah, F.B. Antioxidant and antimicrobial activities of *Lycium shawii* fruits extract. *Int. J. Biol. Macromol.* **2013**, *60*, 328–333.

Vlase, L.; Pârvu, M.; Pârvu, E.A.; Toiu, A. Chemical Constituents of Three *Allium* Species from Romania. *Molecules* **2013**, *18*, 114–127.

Laurian Vlase, Dan Cristian Vodnar, Cristina Bischin, Daniela Hanganu ,Ana-Maria Gheldiu , Radu Oprean , Radu Silaghi-Dumitrescu and Gianina Crișan. Polyphenolic Content, Antioxidant and Antimicrobial Activitiesof *Lycium barbarum* L. and *Lycium chinense* Mill. Leaves Andrei Mocan *Molecules* **2014**, *19*, 10056-10073,

Li, X.-M. Protective effect of *Lycium barbarum* polysaccharides on streptozocin-induced oxidative stress in rats. *Int. J. Biol. Macromol.* **2007**, *40*, 461–465.

Qian, J.-Y.; Liu, D.; Huang, A.-G. The efficiency of flavonoids in polar extracts of *Lycium chinense* Mill fruits as free radical scavenger. *Food Chem.* 2004, *87*, 283–288.

Tahraoui A, El HJ, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south eastern Morocco (Errachidia province). J.

Ethnopharmacol. (2007), 110: 105-117.

Plants For A Future (2014). Lycium barbarum Goji, Box Thorn, Matrimony vine PFAF Plant Database.html.

Harbone, J. B. (1984). *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*. (2nd edn). Chapman and Hall, London, pp. 1, 19, 37 – 168.

Elgayyyar, M; Dranghon, F.A.; Golden, D. and Mount, J.R. (2001). Antimicrobial activity of essential oils from polanrs against selected pathologic and saprophytic microorganisms. J. Food Prot., 64(7): (1019-1024).

Andrews, J.M. (2001). Standardized disc susceptibility testing methods. J. Antimicrob. Chemother., P.:(48-57).

Harborne, J.B.(Ed.)(1994). The flavonoids, Advances in research Since, 1986. 1994, Chapman &Hall, London, Pp.(378382).

Harborne, J.B. and Williams, Ch.A. (2000). Advances in flavonoid research sinc.1992. Phytochemistry, 2000, 55: (481504).

Lin, J.; Opaka, A.R.; Geheeb-Keller, M.; Hutehings, A.D.; Terblanche , S.E. and Jager, A.K. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol, 68:(267-274).

Dijpa, C.D.;and Delmee, M.(2000). Antibacterial activity of bark extracts of Zyzygium jambos (L.) Alston (Mytaceae).J. Ethnopharmacol, 71:(307-31).

Davidson, P.M. and Branen A.L.(1981). Antibacterial activity of non-halogenated phenolic compounds. J. Food Prot., 44: (623-632).
Sashidhar, N.S.(2002). Studies on bioactive natural compounds for their antimicrobial and antioxidant properties, Ph.D Thesis, Submitted to Osmania University, Hyderabad, India.
Abdul-Aziz, J.R.M. (2005). The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agent. Indian J. of Pharmacol., 37(1): (26-29).
Dey, P.M. and Harborne, J.B. (1997). Plant biochemistry. Academic press. London. P. 554.
Feeny, P. (1998). Inhibitory effect of oak leaf tannins and the hydrolysis of proteins by tyrosine. J. photochemistry, 8: (21162129).

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