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Isolation of flavonoids and Pharmacognostical study of Iraqi *Albizia lebbeck* L.

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Abstract :

Introduction: The family leguminosae contains many genera, one of them Albizia which containing about 150 species all of these trees and shrubs grow in tropical and subtropical area of different continents like Asia and Africa. A. lebbeck is one of this genera and used in traditional medicine to treat different diseases and symptoms like inflammatory, arthritis, allergic rhinitis, bronchitis.

Objective: the design study is extracted the important active ingredient quercetin flavonoids and shown the phytochemical profile of active compounds in leaves and flower with anatomy study of plant tissue.

Method and material: the extraction of the active compounds by different solvents and exposed the plant leaves to the Pharmacognostical study and both leaves and flower for phytochemical screening. the isolation of quercetin flavonoids from ethyl acetate layer by preparative TLC and then purified material and analyzed by NMR.

Result and Conclusion: The microscopical examination results were referred to paracytic type of stomata with waves cell wall at upper and lower surface of leaves, multi-cellular unbranched covering of trichomes, stone cell, fibers with helix vessels were presented, the similarity between the isolated compound and the previous literature by resemblance with peak and comparison literature the fragment pattern m/z 302.95 and its spectrum corresponded, which confirms that isolated compound from preparative TLC is quercetin. According to the results as shown in Pharmacognostical and phytochemical studies, the expended of grown this plant in Iraqi environments is very necessary to improve quality and quantity of active compounds like flavonoids specially quercetin which isolated from plant flowers.

Keywords: . Albizia lebbeck, flavonoids, phytochemical study, NMR.

INTRODUCTION

Traditional medicine , may be called in different names like herbalism or called botanical medicine , in this field used medicinal plant or herbs for their therapeutics or for treatment some diseases , entire herb or part of plant valued for medicinal according to the groups of active compounds which contain in those part , today in the scientific research isolated many of those active compounds and purificated it for used in many pharmaceutical fields[1,2].

The family leguminosae contains many genera, one of them Albizia which belong to the family Leguminosa and include about 150 species all of these trees and shrubs grow in tropical and subtropical area of different continents like Asia and Africa [3]. A. lebbeck is one of this genera and used in traditional medicine to treat different diseases and symptoms like inflammatory, arthritis, allergic rhinitis, bronchitis [4]. There are many active compounds of A. lebbeck on of them is flavonoids .The pharmacological uses of flavonoids related with antioxidant activity due to ability to proton donated to the free radicals in the body and production it from formation of a reactive oxygen species (ROS) and inhibition the enzyme which involved in oxidative methods or by act as chelating metal traces and production the body from different diseases like cardiovascular and cancer [5, 6, 7]. Flavonoids are found in many fruits and vegetable of different medicinal plants and classified according to aglycone part of it one of the important one of them is Quercetin 2 (3, 4`,5`, 5, 7-pentahydroxyflavone) presents in many medicinal plants, is a act as antioxidant and has a wide range of biological activities. [8, 9].





Figure (1) leaves and flower of Albizia lebbeck L.

MATERIAL AND METHOD

Plant Sample Collection.

The plant *Albizia lebbeck* L. was authenticated by Assistant professor Ibrahim S. Abbas in Department of Pharmacognosy and Medicinal Plant , College Of Pharmacy , Mustansiriyah University and the leaves and flower were collected from medicinal plants garden of college of pharmacy of Mustansiriyah University in Baghdad, after collection we cleaned the part used (the aerial part) and extract the active compounds by different solvents and exposed the plant leaves to the Pharmacognostical study and both leaves and flower for phytochemical screening .

The Pharmacognostical study of the plant done by Macroscopic and Microscopic examination, used fresh leaves for evaluation the stomata and trichomes and dried leaves for anther microscopical examination for evaluation anatomical plant tissue [10,11].

Extraction of Plant Material Soxhlet extraction

The dried weight of leaves and flower of plant placing into a separated thimble, and the Soxhlet extraction processes using different solvents (ethanol 75%, hexane and water). 150 mL of solvent was added to the round bottom extraction flask and placed on the heating mantle, The extraction was carried out for 10 hours[12].

Phytochemical screening

For phytochemical study used different solvents for extract the active compounds from two parts of plant (leaves and flower) were carried out by standard procedure methods of phytochemical

screening such as mayers , dragendroffs test, for alkaloids , borntragers for glycosides . The foam test for saponins , lead acetate test, ferric chloride test, for tannins and NaoH for flavonoids test [13,14].

Extraction of Flavonoid.

Powdered Plant Material Hexane Soxhlet Marc Marc Methanol Soxhlet Total Methanol extract (TME) Concentrated under vacuum Partition with ethyl acetate (x3) Ethyl acetate layer (EAE) collected Evaporate under vacuum Weight & Examine

Scheme (1): Schemes for Extraction of Flavonoid.

Preparative Thin Layer Chromatography:

The ethyl acetate layer was purified by preparative TLC performed on silica gel GF254 pre-coated plates (0.5mm and 1mm thickness) and developed with mobile system (ethyl acetate: methanol: water, 15:1.25:1), the separated bands were visualized under UV light (254nm), bands at Rf= 0.5 were scrapped off and eluted with acetone. Then evaporated the solvent by rotary evaporator and the purified material analyzed by NMR.

RESULTS AND DISCUSSION

The microscopical examination results were referred to paracytic type of stomata with waves cell wall at upper and lower surface of leaves . multi-cellular unbranched covering of trichomes with fibers were presented at upper surface . stone cell , fibers with helix vessels were presented (figure 2,3,4,5,6).

The preliminary phytochemical results of active constitutes were referred to different active compounds were presented according to different part used and different solvents were used in this study . Ethanol 75% , water and hexane for flower extract were contained tannin , flavonoids and terpenoid while leaves extract carried out terpenoid only in all the solvents used [table 1, 2].

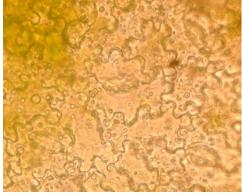


Figure (2) waves cell wall paracytic stomata

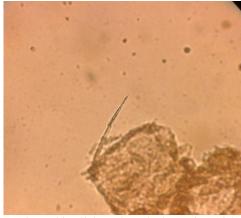


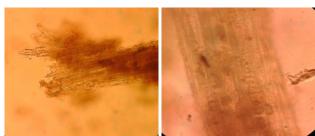
Figure (3) multi-cellular unbranched covering trichomes



Figure (4) fiber



Figure (5) stone cell



helix vessel 10x b- helix vessel 40x Figure (6)

The isolated compound was re-suspended in deuterated methanol for $^{1}\text{H-}$ NMR and ^{13}C NMR analysis that was performed on BRUKER 400 MHz using TMS as internal standard. As shown in figure (7,8,9) the similarity between the isolated

and comparison literature the fragment pattern m/z 302.95 and its spectrum corresponded . which confirms that isolated compound from preparative TLC is quercetin [15].

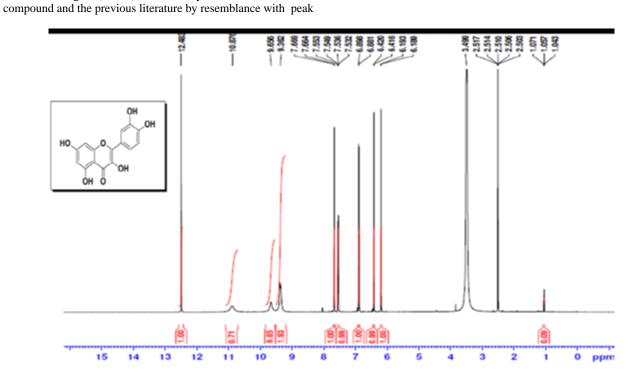


Figure (7) H¹ NMR Spectrum of the isolated of quercetin

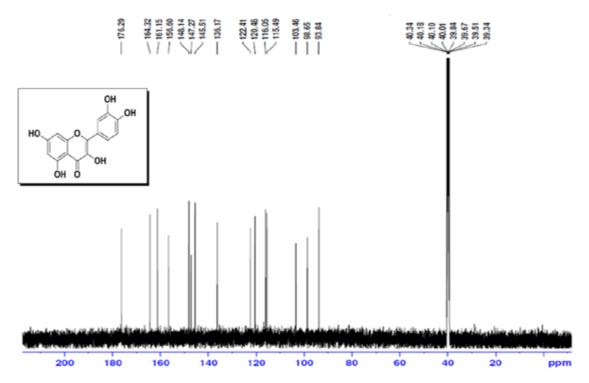


Figure (8) C13 NMR Spectrum of isolated of quercetin

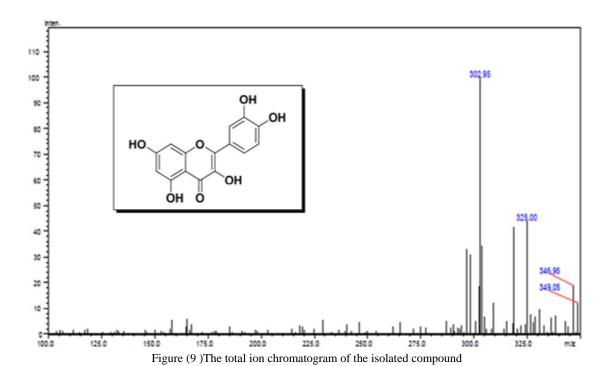


Table 11 Phytochemical constituents of flower of Albiria labback

Chemical	Flower extract		
test	Ethanol	Aqueous	Hexane
Tannins	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Alkaloids	-	-	-
Coumarin	+	+	-
Terpenoid	+	+	+

[Table-2] Phytochemical constituents of leaves of Albizia lebbeck

Chemical	Leaves extract		
test	Ethanol	Aqueous	Hexane
Tannins	+	+	-
Saponins	+	+	-
Flavonoids	-	-	-
Alkaloids	-	-	-
Coumarin	+	+	-
Terpenoid	+	+	+

CONCLUSIONS

According to the results as shown in Pharmacognostical and phytochemical studies, the expended of grown this plant in Iraqi environments is very necessary to improve quality and quantity of active compounds and the results were referred to different active compounds in different solvents and differences between the part used and found very important active compound like flavonoids specially quercetin which isolated from plant flowers

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