

Isolation of coumarin from *Atriplex nummluria* of Iraq

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Abstract

Atriplex nummluria, a plant belong to the family Chenopodiaceae . It is characterized by a particularly wide spectrum of different plant constituents. Many compounds have been isolated and identified from the plant. These included coumarins, flavonoids, steroid, phthalic acid, saponine, and tannins. Coumarins are the major constituent of the plant. In this study coumarin was isolated from *Atriplex nummluria* widely grown in Iraq by Preparative TLC. The isolated coumarin was identified by, GC/MS, TLC, HPTLC and HPLC. Preparative TLC was performed to measure the percentage of coumarin in the Iraqi plant.

Keywords: atriplex ; Coumarin; preparative TLC

INTRODUCTION

Old man saltbush (*Atriplex nummluria* Lindl.), is a halophyte species and one of the most important forage shrubs suited to alkaline and saline lowlands .(1)

Atriplex nummluria Lindl. occurs spontaneously in areas and can be cultivated Plants of the *Atriplex* genus are perennial . (2)

Atriplex nummluria was evaluated for its secondary metabolite contents and its anti-bacterial activity. Many compounds were isolated from this plant and total extract and different plant fractions were screened for their anti bacterial activity. phytosterol like Beta sitosterol glucoside and Stigmasterol glucoside , flavenoid like quercetin glycoside , rutin (3) kaempferol glycoside (4) and apignin glycoside (5), Saponins (triterpenoid saponins).(6)(7), Coumarins and trace amont of Tannins.

Coumarins are the major constituent of *Atriplex nummlaaria*.

Pharmacological activity of plant: Antihyperglycemic (8). Antihyperlipidemic (9), Antioxidant effect(10) , antimicrobial activity(11) , and antifungal activity(12) . Coumarins (2H-1-benzopyran-2-one) consist of a large class of phenolic substances found in plants. (13) The coumarins are of great interest due to their pharmacological properties. In particular, their physiological, bacteriostatic and anti-tumor activity makes these compounds attractive backbone derivatisation and screening as novel therapeutic agents. (14) Coumarins are an important class of compounds of both natural and synthetic origin. Many compounds which contain the coumarin moiety exhibit useful and diverse pharmaceutical and biological activities. Some of these coumarin derivatives have been found useful in photochemotherapy, antitumor, anti-HIV therapy, as CNS-stimulants, antibacterial , anticoagulants , antifungal , antioxidant agents and as dyes . Natural, semi-synthetic and synthetic coumarins are useful substances in drug research .(15)

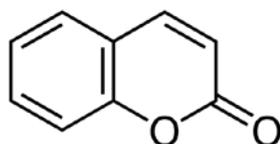


Figure 1: Coumarins (2H-1-benzopyran-2-one).

MATERIALS AND METHODS

Thin layer chromatography, GC-MS (Shimadzu), preparative thin layer chromatography, HPLC and IR.

All chemicals and solvents are of analytical grade. Standard coumarin was obtained from Sigma Aldrich Company.

Plant material

The aerial part of *Atriplex nummluria* was collected from area in Baghdad, The plant was identified and authenticated by National herbarium in Abu –Griab. The plant was dried in shade for several days at room temperature and then grinded as powder.

Extraction and isolaton of coumarin :

Powdered plant aerial part (1000g) was extracted by Soxhlet apparatus with methanol (80%) (1000 mL) till exhaustion. The extract was concentrated by evaporation under vacuum. Water (100ml) was added and the suspension was partitioned with petroleum ether (2x 100ml). The petroleum ether layer was discarded and the aqueous layer was extracted with chloroform (3x100ml). The combined chloroform layers were dried with anhydrous sodium sulphate, filtered and evaporated under vacuum. The collected fractions were analysed by TLC comparing with standard coumarin.

Preparative thin layer chromatography:

The R_f value of isolated coumarin was compared with standard coumarin. Preparative TLC was performed using 0.5 mm thickness silica gel. Elution of the isolated band was conducted with AR grade acetone.

RESULTS AND DISCUSSION :

Isolated coumarin was identified by melting point. 72°C which was identical with that reported in the literature. TLC of isolated comarin was compared with standard in three solvent systems (table 1). The occurrence of coumarin in the plant was confirmed by GC/MS (Figure 2). Retention time of coumarin was 14.7 min. Fragmentation pattern of isolated coumarin was comparedl with that reported in the literature (Figure 3) Isolated coumarin IR are shown in Figure 4. HPLC also support HPTLC also support the occurrence of coumarin in Iraqi atriplex nummluria .(Figure 6).

Table 1: Rf of standard and isolated coumarin in three solvent system

Solvent system	Rf of standard coumarin	Rf of isolated coumarin
Toulen:acetone: water(4: 5:1)	0.75	0.76
Tolune: acetone: chloroform (45:55:5)	0.66	0.67
Dichloromethane	0.36	0.39

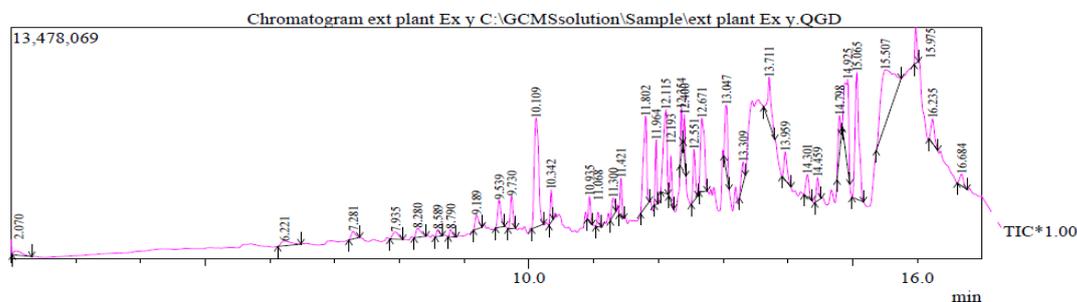


Figure 2: GC/MS analysis of hexane extract of *Atriplex nummularia*

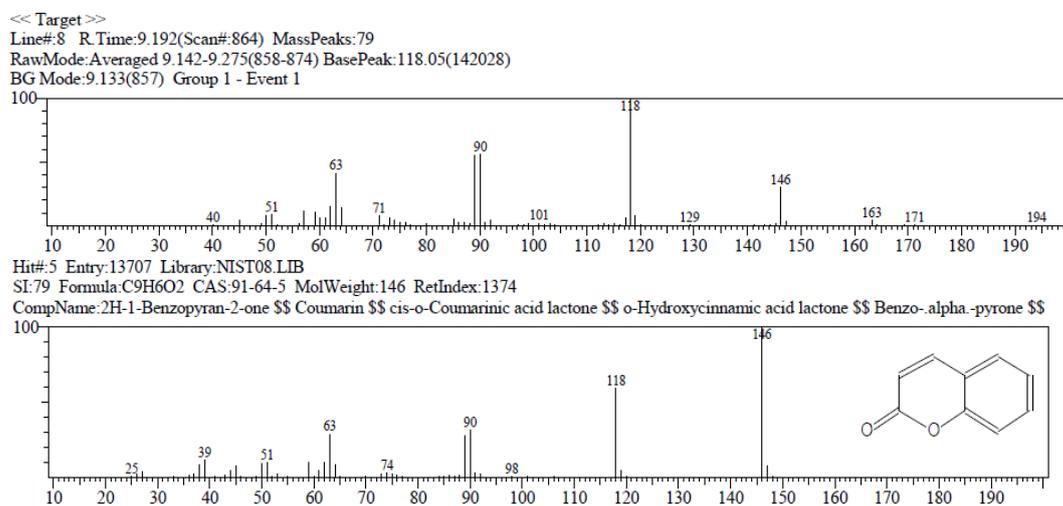


Figure 3: Fragmentation pattern of isolated coumarin

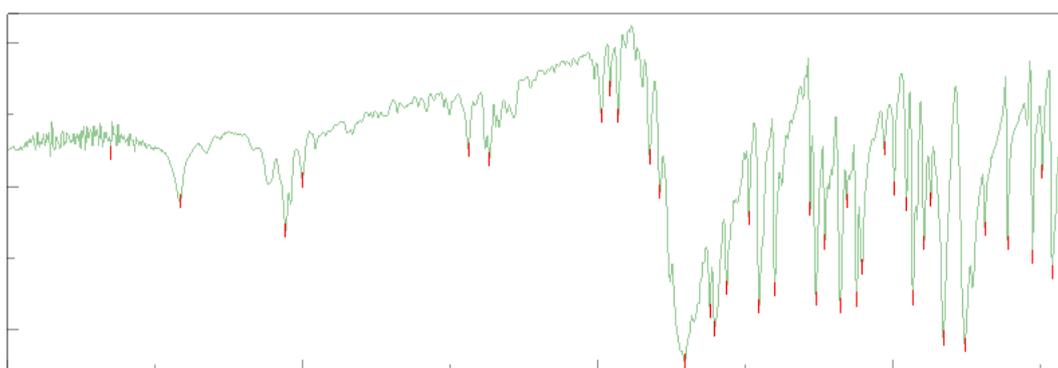


Figure 4: IR of isolated coumarin.

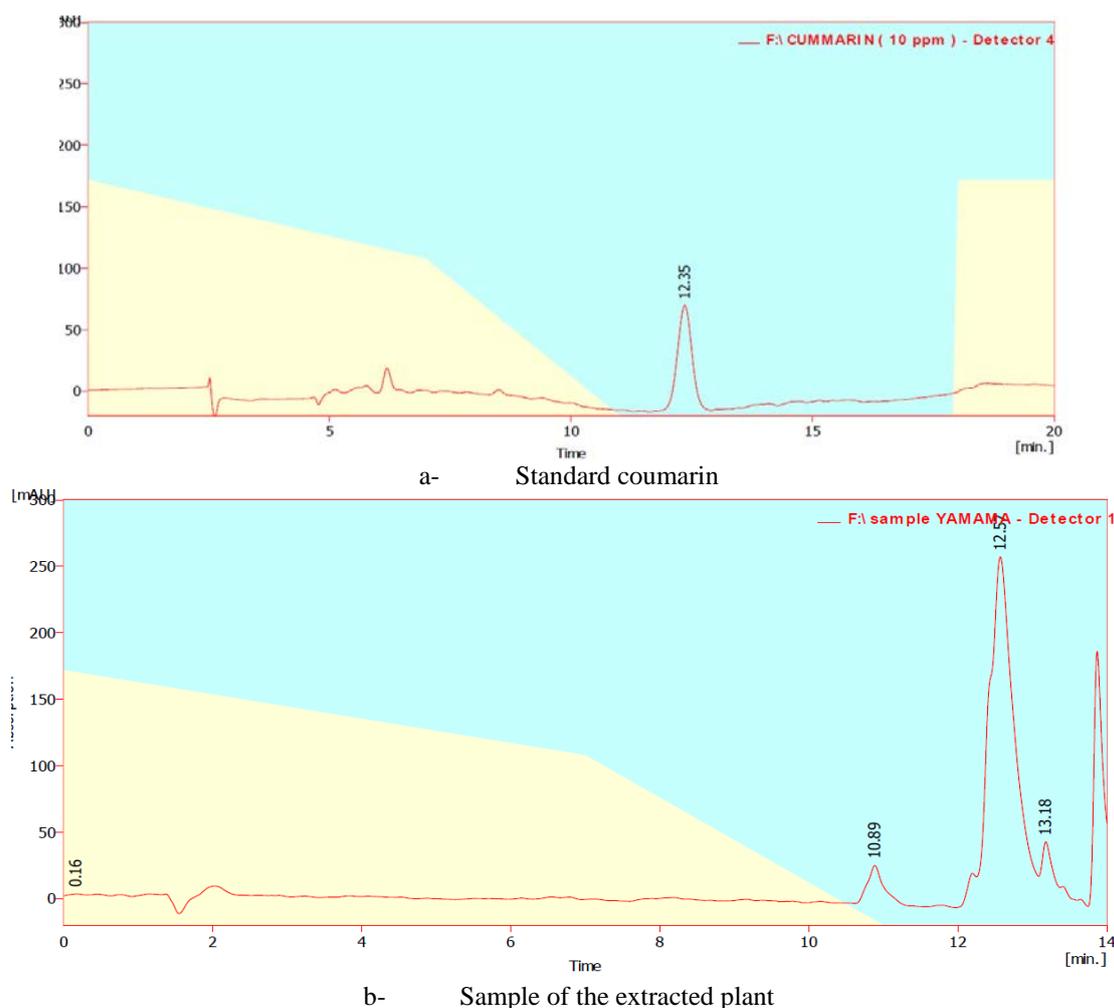


Figure 6 a-b: HPLC of standard and extracted plant .

CONCLUSION

The valuable Iraqi medicinal plant *Atriplex nummularia*, contains coumarin as a major active constituents. Coumarin is responsible for most medical and therapeutic function in this plant. The percentage of coumarin in *Atriplex* of Iraq was 0.62 %. This percentage indicates that *Atriplex nummularia* of Iraq is official herb (BP) since the percentage is more than 0.3% and less than 0.9%.

REFERENCES

1. Feedipedia S. Feedipedia. 2016;
2. de Souza ER, dos Santos Freire MBG, de Melo DVM, de Assunção Montenegro AA. Management of *Atriplex nummularia* Lindl. in a Salt Affected Soil in a Semi Arid Region of Brazil. *Int J Phytoremediation*. 2014;16(1):73–85.
3. Abd D, Raheim EL, Abd D, Raheim E. *Issn: 2277 – 4998 Nummularia Extracts*. 2013;2(6):1260–9.
4. Awaad AS, Maitland DJ, Donia AERM, Alqasoumi SI, Soliman GA. Novel flavonoids with antioxidant activity from a Chenopodiaceous plant. *Pharm Biol*. 2012;50(1):99–104.
5. GENETIC EVALUATION OF SOME RANGE PLANTS USED AS USED AS FODDERS IN THE NORTH WESTERN Department of Genetics Faculty of Agriculture -Ain Sham University. 2016;(September 2005).
6. Fayed AM, Essawy ME-, Eid EY, Helal HG, Abdou R, Shaer HM El. Utilization of Alfalfa and *Atriplex* for Feeding Sheep under Saline Conditions of South Sinai, Egypt. 2010;6(12).
7. Azeana Z. Chapter 2 2. General Chemical Aspects of Alkaloids. 2010;13–47.
8. Babbar N. An introduction to alkaloids and their applications in pharmaceutical chemistry. 2015;4(10):74–5.
9. Chikhi I, Allali H, El Amine Dib M, Medjdoub H, Tabti B. Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin-induced diabetic rats. *Asian Pacific J Trop Dis*. 2014;4(3):181–4.
10. Soliman GA, Abd El Raheim M. Antihyperglycemic, Antihyperlipidemic and Antioxidant effect of *Atriplex farinosa* and *Atriplex nummularia* in Streptozotocin-induced Diabetes in rats. *Bull Env Pharmacol Life Sci*. 2015;4(12):10–8.
11. Tahar SB, Hadj-Mahammed M, Yousfi M. Study of the antioxidant activity of phenolic extracts of *A. halimus* L and *Haloxylon scoparium* Pomel northern Sahara. *J Chem Pharm Res*. 2015;7(11):258–64.
12. Isolated G, Atriplex F, Herb L, El-aasr M, Kabbash A, El-seoud KAA, et al. Antimicrobial and Immunomodulatory Activities of Flavonol. 2016;8(10):1159–68.
13. Venugopala KN, Rashmi V, Odhav B. Review on natural coumarin lead compounds for their pharmacological activity. *Biomed Res Int*. 2013;2013(Table 1).
14. Jain PK, Joshi H. Coumarin: Chemical and pharmacological profile. *J Appl Pharm Sci*. 2012;2(6):236–40.
15. Salem M, Marzouk M, El-Kazak A. Synthesis and Characterization of Some New Coumarins with in Vitro Antitumor and Antioxidant Activity and High Protective Effects against DNA Damage. *Molecules* [Internet]. 2016;21(2):249. Available from: <http://www.mdpi.com/1420-3049/21/2/249>