Isolation and identification of bioactive flavonoids (genistein, rutin) from *Portulaca oleracea* L. cultivated in Iraq.

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ABSTRACT
Portulaca oleracea L. belongs to portulacaeae family which is succulent shrubs contains about 20 genera and 500 species. *P. oleracea* L. is one of the green vegetables cultivated and wild grown in Iraq and used largely as food. It contains many active constituents like: alkaloids, vitamins and minerals such as (potassium, magnesium, omega 3, calcium and iron), saponins, cardiac glycosides, flavonoids (genistein, rutin, quercetin) . In this study genistein and rutin were isolated from *P. oleracea* L. by the aid of high performance liquid chromatography (HPLC), and preparative thin layer chromatography (TLC), and identified by FT-IR, and measurement of melting point (M.P).

فصل وتشخيص الفلافونيدات النشطة بيولوجيا (الجنيستين ، الروتين) من البقلة المباركة المزروعة في العراق

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الكلمات المفتاحية: البقلة المباركة ، جنيستين ، روتين.

الخلاصة

البقلة المباركة تعود إلى عائلة portulacaeae التي هي شجيرات عصارية تحتوي على حوالي 20 جنسا و 500 نوعا. البقلة المباركة هي واحدة من الخضروات الخضراء المزروعة و البرية النمو في العراق و تستخدم إلى حد كبير كغذاء. أنها تحتوي على العديد من المكونات النشطة مثل : قلويدات ، فيتامينات ومعادن مثل (البوتاسيوم، المغنيسيوم ، الأوميجا 3 ، الكالسيوم والحديد) ، الصابونين ، جليكوسيدات القلب ، الفلافونويد (جينيستين ، روتين ، كيرسيتين) . في هذه الدراسة تم عزل الجينيستين و الروتين من البقلة الحمقاء بمساعدة كروماتوغرافيا الأداء العالي السائلة (HPLC) ، و كروماتوغرافيا الطبقة الرقيقة (TLC) ، و حددت من قبل IR -FT ، وقياس درجة الانصهار (MP).

1. INTRODUCTION:

The term *Portulaca* was originated from the Latin word “portare” which mean to carry and “lac” mean milk, and this refer to milky sap of the plant.[1] It has the following taxanomy:
Kingdom: Plantae
Phylum: Spermatophyta
Class: Dicotyledonae
Order: Caryophyllales

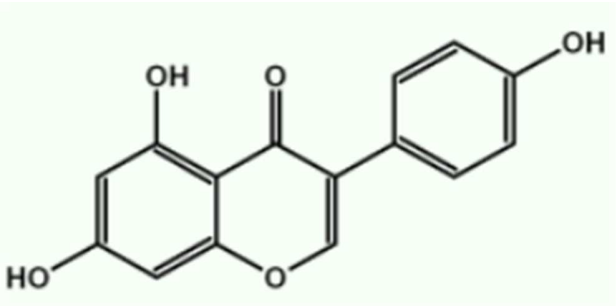
Family: Portulacaceae
Genus: Portulaca
Species: oleracea

Preferred Scientific Name
Portulaca oleracea Linnaeus
Preferred Common Name
Purslane .[2,3]

As *P. oleracea* L. contains many flavonoid compounds such as (genistein, rutin, quercetin), so it act as antioxidants , anti-inflammatory , anti-diabetic, anthelmintic , antibacterial , antiscorbutic, diuretic and as pain reliever in hemorrhoids and whitlow.[4]

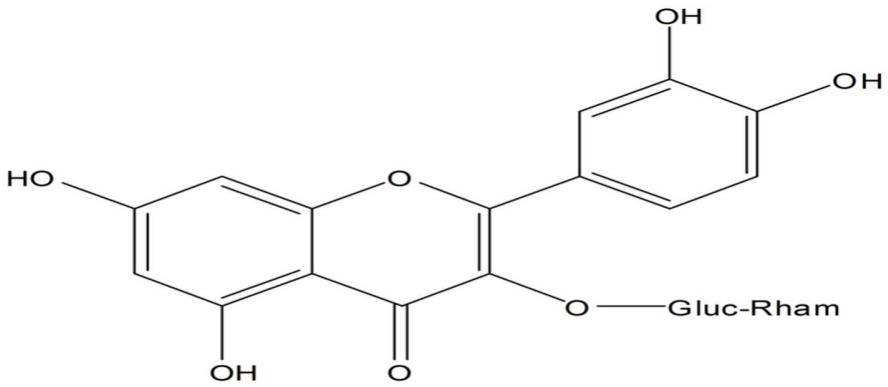
Genistein is isoflavonoid type of flavonoid group. It's a phytoestrogen which has selective estrogen receptor modulator properties. Genistein is effective to prevent chronic diseases like: osteoporosis, and hormone-related cancer due to its anti-tumor, antimutagenic, antifungal .[5,6,7]
As genistein (figure **1**) which is [5,7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one] undergo hydrolysis by enzymes in vivo, its cleaved to give the glycon part and aglycon part which is responsible for its activity toward the estrogenic receptor and make this compound of interest in reducing many symptoms that are associated with menopause.[4, 8,9]

Figure (1): chemical structure of genistein



Rutin is a flavonol type of flavonoid group [10]. It is rhamnoglucoside of the flavonoid quercetin,[11] so it's called (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside, it's the main flavonol in fruits and vegetables.
Rutin is the 3-O- rhamnoglucoside of quercetin, as shown in figure (2), this rhamnose moiety didn't absorb in the small intestine when rutin is hydrolyzed by bacteria possess rhamnosidase activity that made rutin absorbed more slowly than quercetin in the small intestine. It has many pharmacological activities like:
Anti-oxidative, anti-allergic, antifungal, anti-inflammatory and antimicrobial [10]. Also its used in the treatment of many chronic diseases, such as cancer, hypertension, hypercholesterolemia, and diabetes. It is a nontoxic flavonoid [12].

Figure (2): chemical structure of rutin.



(**rutin**, quercetin-3-rutinoside)

2.MATERIAL AND METHODS

The different measurements were used in this study are: HPLC (Shimadzu), FT-IR (Shimadzu-84005), Melting point, UV-visible spectrophotometer (Cary). All solvents and chemicals used are of analytical grade. Standard genistein and rutin were obtained from Chengdu Biopurify phytochemicals, Ltd.

Plant material

The aerial parts of *P. oleracea* L. was collected from two local locations at Baghdad and Karbala. These aerial parts wash with water to remove impurities then the leaves split off from the stems and dried separatory in an oven at 105-135ºC or at shade ventilation place, then grind by mechanical grinder to fine powder and weighed.

Extraction and isolation:

Fifty grams of powdered aerial parts of *P. oleracea* L. are placed in a suitable thimble and extracted by soxhlet with 600 ml of 75% Ethanol at (40ºC) for 10 hours, then the ethanolic extract cooled at room temperature and filtered. The clear filtrate evaporate to dryness under reduced pressure by rotatory evaporator at temperature between (40-50)ºC to give crude extract which was weighed (17.47g) and subject to isolation and identifications.[13]

Identification of isolated genistein:

The isolated genistein and rutin were identified by HPLC method and compared with reference standard one using an isocratic conditions for genistein:
Column: NUCLEODUR C18 (250 mm x 4.5 mm, 5 µm particle size), Column temperature at 25ºC with a flow rate of 0.7 ml /min, injection volume (20 µl), injection concentration (1mg /ml), and detected at 261 nm[14], mobile phase was methanol (HPLC grade).

For rutin:
Column :NUCLEODUR C18 (250mm x 4.5mm, 5µm particle size), Column temperature at 25ºC, with a flow rate of 1ml /min, Injection volume (20 µl), Injection concentration (1mg /ml), detection wavelength at 365 nm, mobile phase was methanol (HPLC grade).

TLC and paper chromatography

The isolated genistein and rutin were compared with standard genistein and rutin respectively by using TLC method: a precoated aluminum sheet with silica gel GF254 was used with paper chromatography, Whatman N0.1 filter paper as a stationary phase and purified by preparative TLC with the following mobile phases:

S1 : n-butanol- glacial acetic acid –water (4:1:1) [15]
S2 : n-butanol- glacial acetic acid-water (4:1:5) [16,17]
S3 : Ethylacetate-ethanol-formic acid-water (100:11:11:27) [16,18]
S4 : Ethylacetate-glacial acetic acid -formic acid-water (124:8:8:10) [19].

Spectrophotometric analysis

The isolated genistein and rutin were dissolved in absolute ethanol to determine its maximum wavelength by UV absorption and compare this wavelength with that one of the reference standard of genistein and rutin respectively. Infrared spectrum of the isolated genistein and rutin were determined by using KBr disc.

3.RESULT AND DISCUSSION

The isolated genistein show a melting point at (295-297) ºC where the standard genistein show melting point at (297-298) ºC. The HPLC of the extraction method and of standard and isolated genistein are identical by comparison their retention time (t_R) it was found that the crude extract (17.47)g of *P.oleracea* .L. contains 1.22% of genistein as shown in figures (3,4) and the IR spectrum show peaks at 3410, 3115, 1653 and 1039 which return to O-H stretching of phenolic group, C-H stretching of aromatic ring, and C=O stretching of ketone in conjugated system, C-O-C stretching of ether respectively as show in figure (**5)** while the Rf values of isolated and standard one in the four different mobile phases are shown in table (**1).**

Table(1):Rf values of standard and isolated genistein in four different solvent

Solvent systems	**S1**	**S2**	**S3**	**S4**
Rf value of genistein standard	0.91	0.66	0.43	0.93
Rf value of isolated genistein in extract	0.90	0.67	0.42	0.94

Figure (3): HPLC chromatogram of the standard genistein

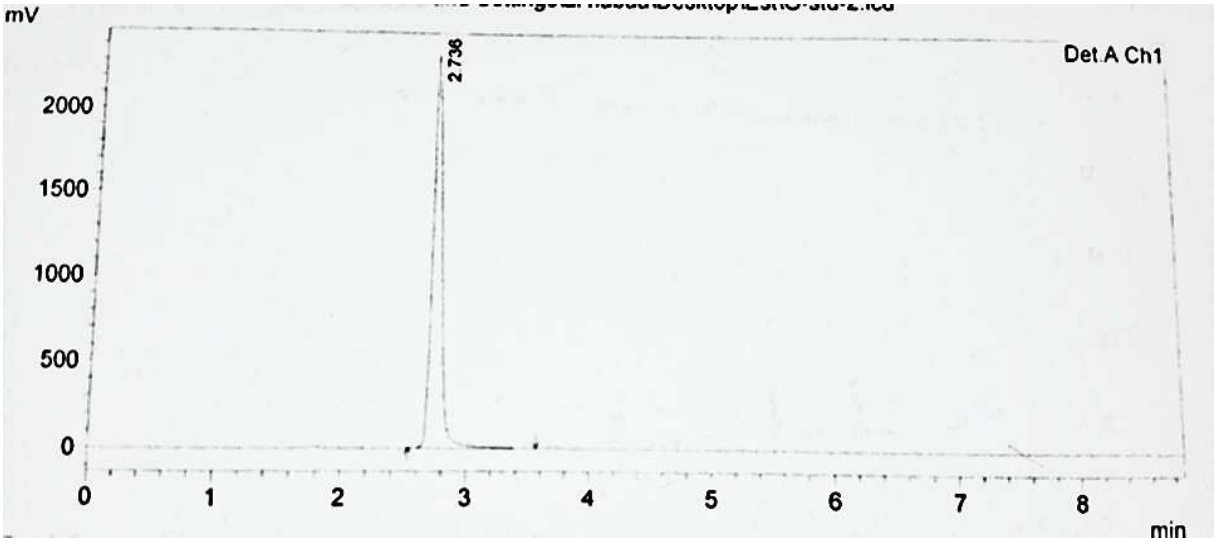


Figure (4): HPLC chromatogram of the isolated genistein

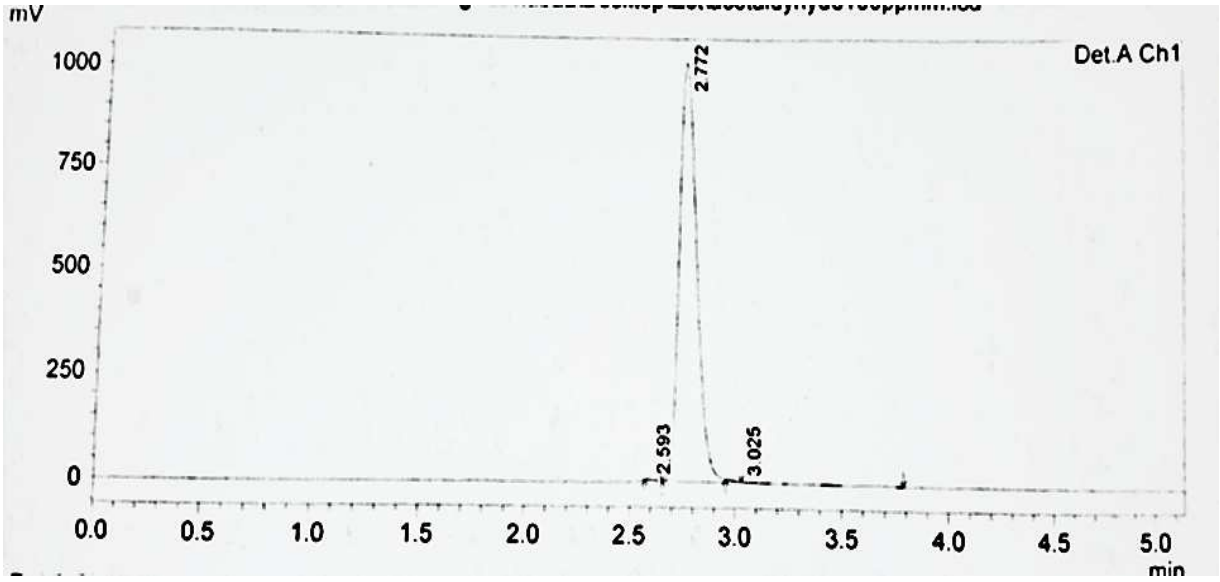
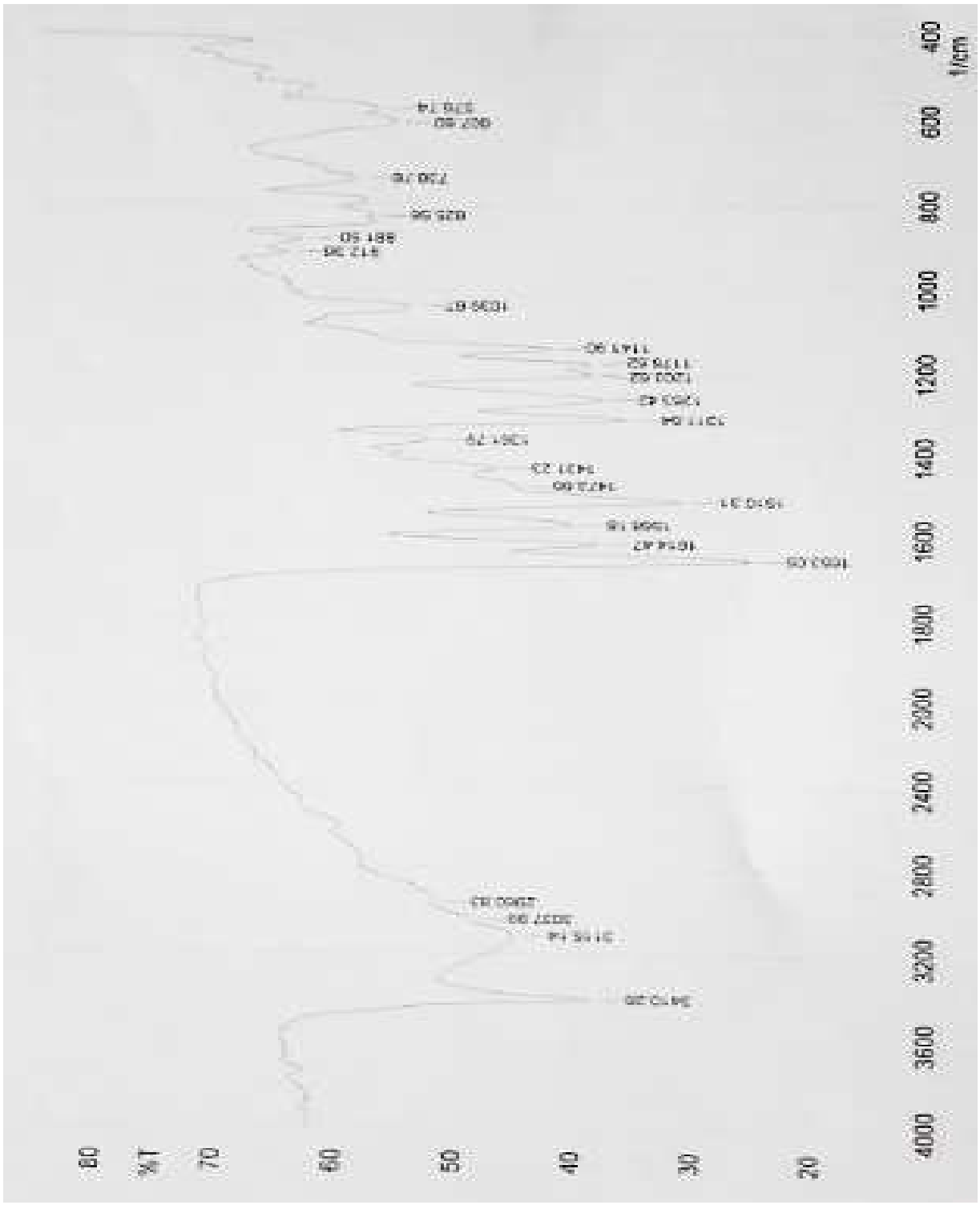


Figure (5) :IR spectrum of isolated genistein.



While the isolated rutin show a melting point at (193-195) ºC where the standard rutin show melting point at (195) ºC. The HPLC of the extraction method and of standard and isolated rutin are identical by comparison their retention time (t_R) as shown in figures (6,**7**) and the IR spectrum show peaks at 3427, 3367, 1654, 1155 which return to O-H stretching of phenolic group, C-H stretching of aromatic ring, and C=O stretching of ketone in conjugated system, C-O-C stretching of ether respectively as shown in figure (8) while the Rf values of isolated and standard one in the four different mobile phases are shown in table (**2).**

Table (2):Rf values of standard and isolated rutin in four different solvent

Solvent systems	**S1**	**S2**	**S3**	**S4**
Rf value of rutin standard	0.52	0.50	0.39	0.19
Rf value of isolated rutin in extract	0.53	0.51	0.40	0.20

Figure (6): HPLC chromatogram of the standard rutin

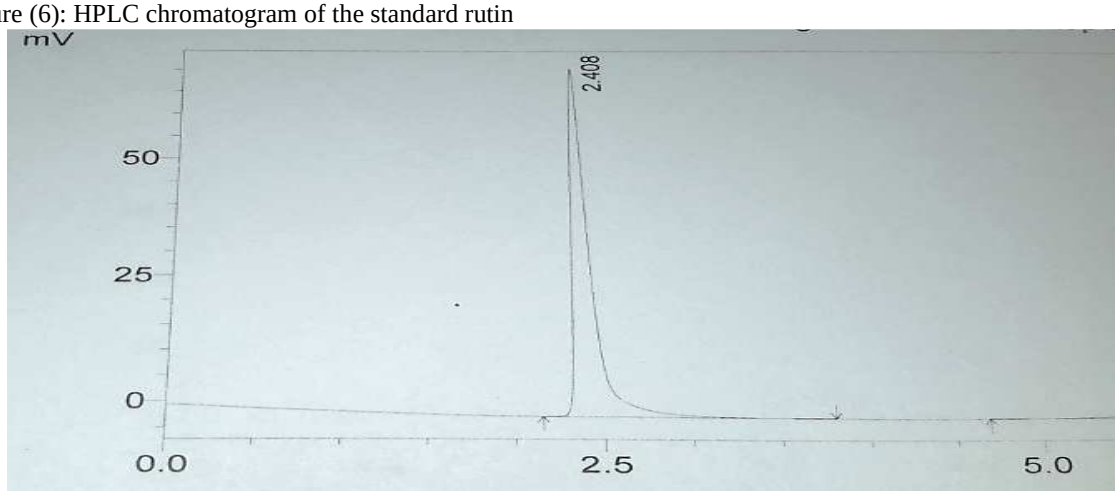


Figure (7): HPLC chromatogram of the isolated rutin

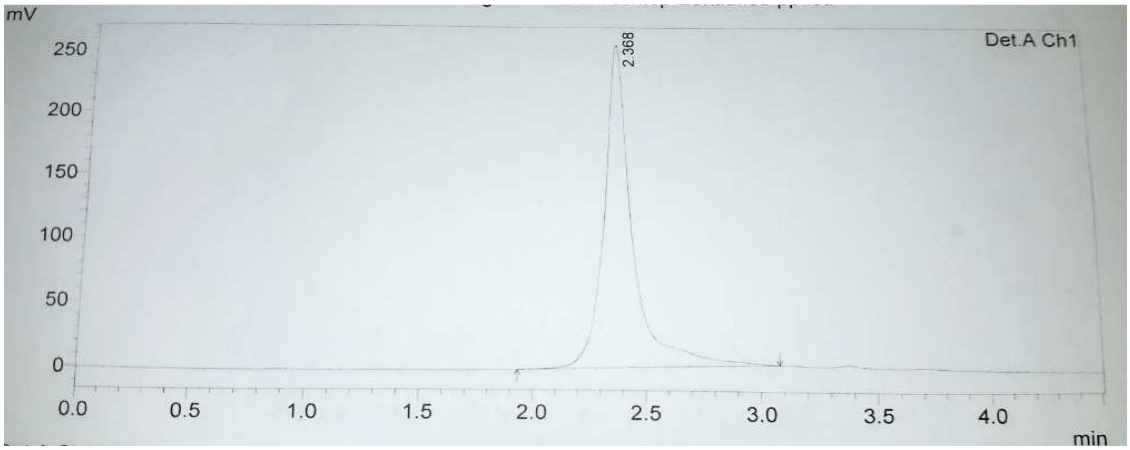
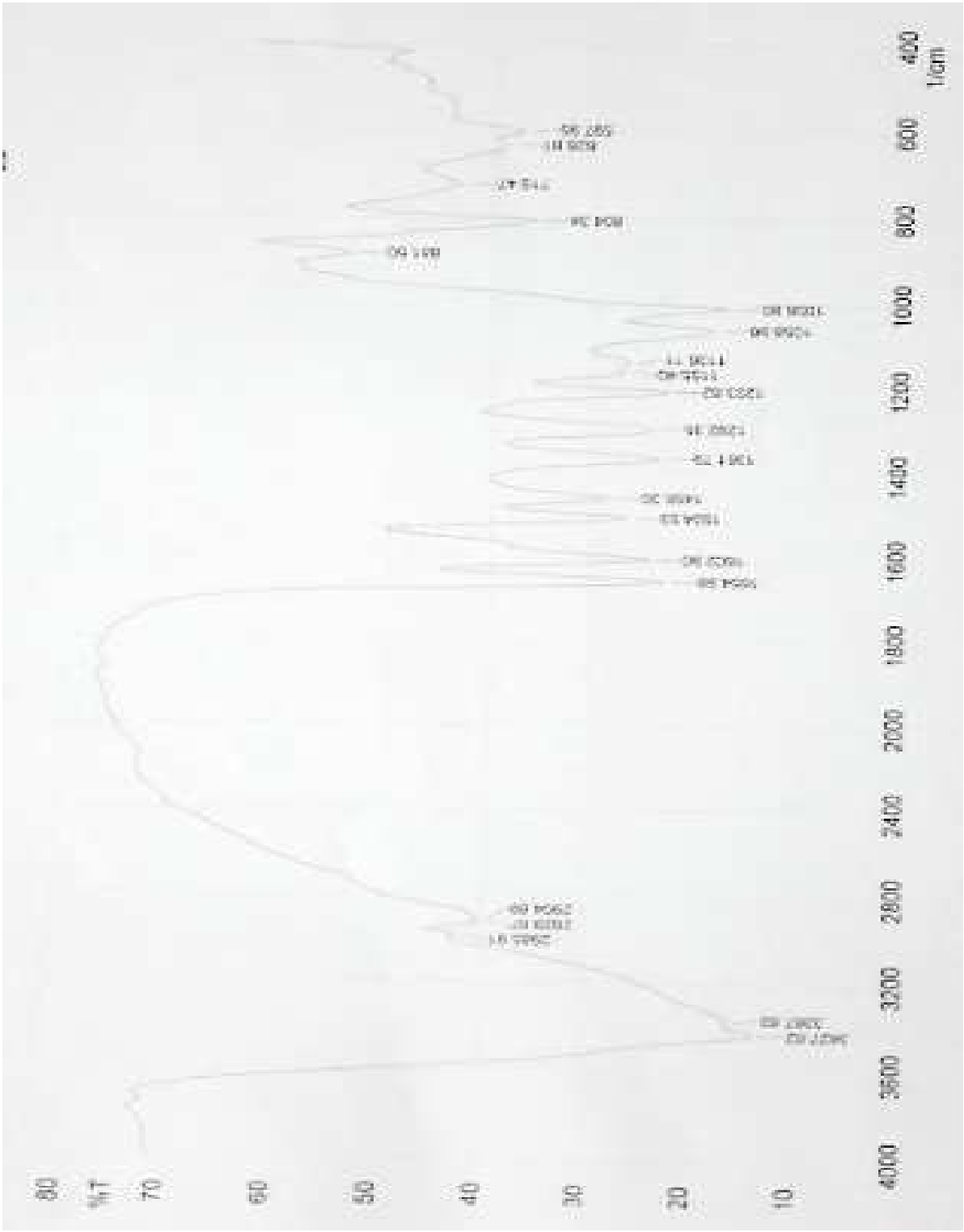


Figure (8) :IR spectrum of isolated rutin.



4.CONCLUSION:

The isoflavone (genistein) and flavonol (rutin) can be isolated and purified from the natural sources such as *Portulaca oleracea* L. depending on HPLC and preparative TLC technique with the aid of IR and UV techniques.

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