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# Isolation and identification of rutin from tissues cultures of *Ruta graveolens* L.

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**Abstract:**

*Ruta graveolens* L. a member of Rutaceae family, it is known as medicinal plant. It contains more than 210 pharmaceutical active compounds related to different secondary metabolites.Rutin is one of these compounds, it is a kind of flavonol glycoside compound used to antagonize the increase of capillary fragility associated with hemorrhagic diseases or hypertension in human.

**Aim:** The aim of this study is to isolate and identify rutin from tissue culturs of *Ruta graveolens* L.(Rutaceae)

**Material and Methods:** *Ruta graveolens* L. seeds were collected from 2 year old plants grown at the botanical garden, they were washed and sterilized and germinated on agar-solidified MS medium as described by (Al-Mahdawe and Al-Mallah 2015). Callus culture were subcultured every 4 weeks intervals, *R. graveolens* plant were cultivated in the botanical garden, leaves and full ripe seeds were collected and stored at 30̊ c and mass calli production were collected for the determination of rutin content. The samples were dried and ground into powder. Rutin was extracted from 5g of the powdered seeds, leaves and 1g from the powdered callus to give yellow powder rutin according to (Hamad, 2012).

**Results and Discussion:** Rutin was identified by using infrared spectrum device (IR)and the main functional groups of rutin were recorded which were alcoholic and phenolic (-OH) group, alkene(C=C)the isomeric and nonisomeric ether (C-O-C), carbonyl(C=O) group and aliphatic and aromatic(C-H) stretching group. Rutin was also identified and quantitatively estimated by using High Performance Liquid Chromatography (HPLC).HPLC analysis was performed on a model UFLC (Shmadzu,Japan). Results obtained by using HPLC technique showed that the percentage of rutin in seeds was 73.6% whereas in leaves was 11.7%. It was recoded that the percentage of rutin was significantly higher in callus culture reaching 89.2%.

**Conclusion:** It is concluded that plant tissue cultures are preferred as a continuous source of rutin and perhaps other secondary metabolites.

**Key words:** *Ruta graveolens* , callus culture, Rutin, HPLC.

## INTRODUCTION:

*Ruta graveolens* L.(garden rue), amember of Rutaceae family, it is known as medicinal plant (1). The plant contains more than 210 pharmaceutical active compounds related to different secondary metabolites such as alkaloid, coumarins, flavonoids and essential oil( 2). Rutin is a kind of flavonol glycoside compound. The main biological property of rutin is to antagonize the increase of capillary fragility associated with some hemorrhagic diseases or hypertension in human(3). The amount of rutin in the cultivated plants varies from 1% to 2% (4).Plant secondary metabolites are often produced in mini-scale quantities, causing an ever-increasing demand. Furthermore, due to the many benefits, the consumption of herbal medicines is increasing worldwide, and many people rely on the use of traditional medical plants (5). Recent advances in plant biotechnology techniques have increased the potential of plant cell culture as a vital source of plant secondary products (6). Additionally, tissue culture technology provides many exclusive advantages for the generation of bioactive molecules, such as the optimization of the growth conditions for biomass production, the synthesis and accumulation of metabolites moreover, defining elicitation factors to determine their properties (7). Therefore, plant cell and tissue culturing are biotechnological methods that represent a viable renewable resource of various important plant secondary metabolites, and different strategies have been employed to improve the production of these compounds (8, 9, 10). this study was to investigate the accumulation of rutin glycoside in callus culture.

## MATERIALS AND METHODS

- **Callus induction**

*Ruta graveolens* L. seeds were collected from 2 year- old plants grown at the botanical garden (College of Education for Pure Sciences, University Diyala, Iraq), seeds were washed in running tap water, surface disinfected in a solution of NaOCl 3% for 5 min. and finally rinsed with sterile distilled water for three times. Sterilized seeds were germinated on agar- solidified MS medium (11) and maintained in dark for three days at 25±2̊ C. The produced plantlets were maintained at 25±2̊ C under 16h photoperiod with a photon flux density of 50 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps. Hypocotyl explants were excised from 21 days old plantlets, and sliced into lengths of 1.0 cm. Then they were placed horizontally on the surface of callus-induction MS basal medium supplemented with 0.25 mg. $L^{-1}$ 6-benzyladenine (BA) in combination with 0.75 mg.$L^{-1}$ 2,4-dichlorophenoxy acetic acid (2,4-D) (12). Callus culture were subcultured every 4 weeks interval.

- **Sample preparation and rutin extraction.**

**Plant material:** Garden rue plant were cultivated in botanical garden, leaves and full ripe seeds were collected and stored at 30 C̊ and mass calli production were collected for the determination of rutin content, these samples were dried and ground into powder.

**Extraction and isolation:** Rutin extracted from 5 g of the powder seeds, leaves and 1 g from the powder callus by Soxhlet apparatus with 250 ml of 80% ethanol till exhaustion. The extract was filtered and concentrated by evaporation under vacuum to about 10ml then mixed with 25 ml distilled water, and extracted with petroleum ether (50ml x 3), then with chloroform (50 ml x 3). After extraction, the aqueous layer was collected and left to stand in a cold place for 72 hours; a yellow precipitate separated out of the solution. The precipitate was filtered and washed with a mixture of chloroform : ethylacetate : ethanol (50:25:25). The un-dissolved part of the precipitate was dissolved in hot methanol and filtered, the filtrate was evaporated to dryness to give yellow powder rutin ( 13).

**Identification of isolated rutin by IR spectrum:** Put drops of plant extracts for each sample in a cell Infrared spectrum device (IR) and recorded graphs sites the main functional groups of the compound rutin which included site group a alcoholic(-OH)group, phenolic(-OH) group, alken(C=C), the isomeric and nonisomeric ether(C-O-C), carbonyl(C=O) group and (C-H) aliphatic and aromatic stretching group.

**Identification of isolated rutin by HPLC:** HPLC analysis was performed on a model UFLC(Shmadzu Japan).the analytical column was ODS-2(250×4.5mm ID) packed with 5µm particles, and a mixture of methanol: water (70:30 ratio) as a mobile phase. The flow rate and volume injection were 1.5ml/min and 20 µL respectively, and detection was performed at 350 nm. The

percentage of rutin can be calculated according to the following equation:

$$\text{Percentage(\%)of rutin} = \frac{T}{S} \times 100$$

T= represents the curves area that belongs to the isolated compound out of the tested samples.
S= represents the curves area that belongs to the standard compound.

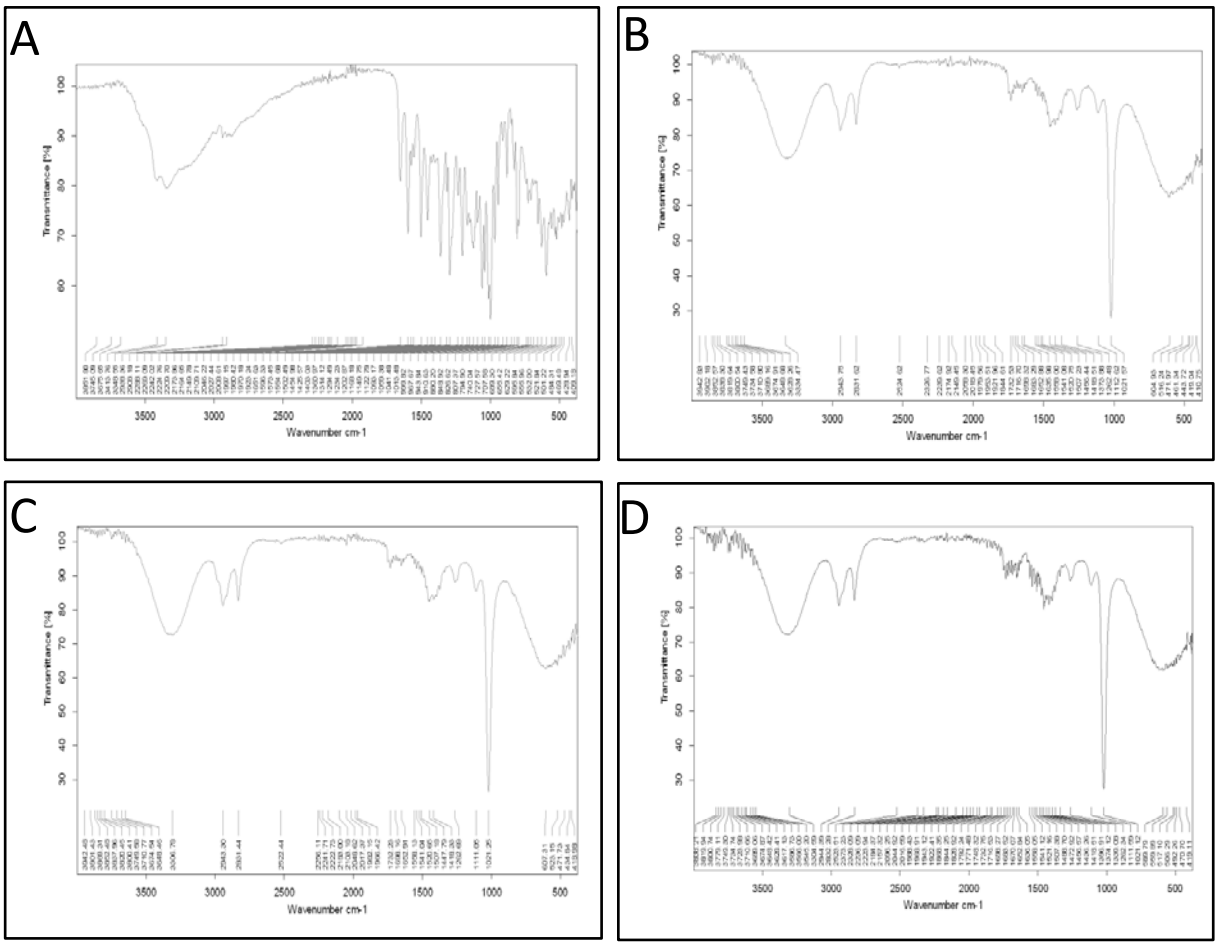
## RESULTS AND DISCUSSION:

- **Chemical and qualitative identification of rutin by using IR technique:**

The results of (IR) spectrum revealed the existence of the major functional groups of rutin compound which were very close to the values of these groups in the curve of standard rutin. The absorption beams appeared at the frequency range of 3304.5-3334.4 $cm^{-1}$ close to the beam frequency in the standard sample 3348.5 $cm^{-1}$ and it belongs to the frequency of stretching alcoholic hydroxyl(-OH) group. The absorption beams at the frequency range of 3545.2-3648.4 $cm^{-1}$ showed that they are close to the beam frequency of the standard sample 3413.7 $cm^{-1}$ that belongs to the stretching frequency of the phenolic hydroxyl (-OH) group. There is a clear peak at the frequency 2831.6-2944.3 $cm^{-1}$ which means the existence of stretching aliphatic (C-H) group, which was close to the frequency of the standard sample 2938.3 $cm^{-1}$. However, there was another peak at the frequency 3674.5-3674.9 $cm^{-1}$ which belongs to the stretching aromatic (C-H) group which has nearly the same frequency 3675.6 $cm^{-1}$ of the standard sample. There are beams belongs to the double bond that are among the structural formula of rutin formed at the range 1558.0- 1558.1 $cm^{-1}$ which was close to the frequency of alkene (C=C) group 1596.3 $cm^{-1}$ of the standard sample. The frequencies of the isomeric ether (C-O-C) group at the frequency of 1262.3-1262.6 $cm^{-1}$ and non-isomeric at the frequency 1111.0-1111.6 $cm^{-1}$, showed consistency with the frequencies of the standard sample. The concordance of the frequency of the carbonyl (C=O) group at the range 1652.8-1652.9 $cm^{-1}$ with the frequency 1651.6 $cm^{-1}$ which belongs to the standard sample, this indicates that rutin is the isolated flavonoid from all samples in sense of the matching of absorption sites of these spectra of the standard sample (Fig1.A) with the absorption spectra of the isolated substance from the seed extracts (Fig1.B), leaves of the seeding plants (Fig1.C) and from the callus that was derived from the hypocotyl stems(Fig1.D).

- **Identification and quantitative estimation of rutin by using HPLC technique.**

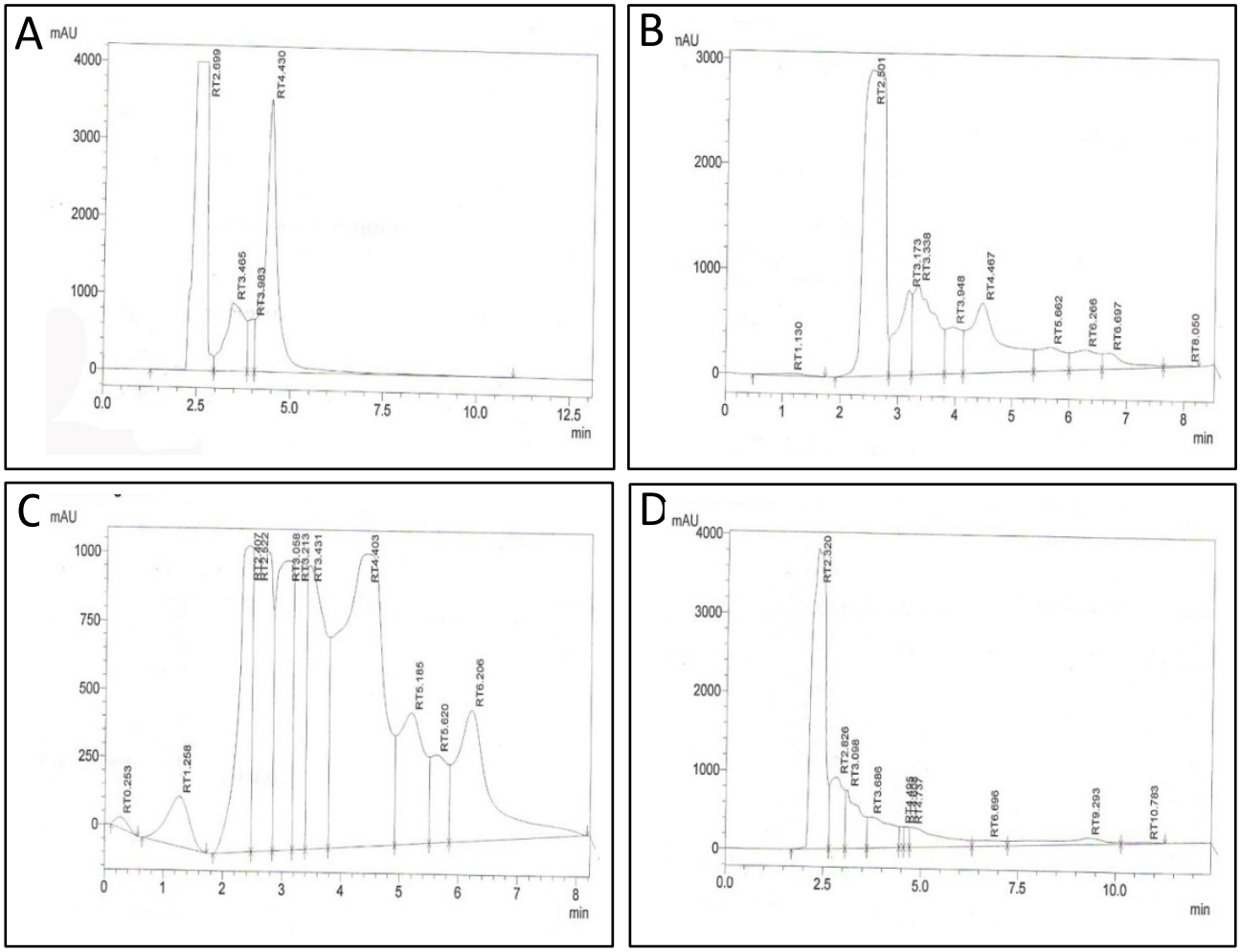
The recorded curves revealed that the seeds, leaves of the plant and the callus, contain rutin, depending on the values of retention time which were ranging from 2.3 to 2.5 minutes, that were matching the retention time of the standard rutin(Table 1).




Fig(1): Absorption curves of the (IR)spectra for the standard rutin (A). which was isolated from the seeds (B). Leaves (C). and the callus derived from the hypocotyl stems (D). of the plant *Ruta graveolens* L..

Table(1): Retention time of the isolated rutin and its percentage in seeds, leaves plants of *Ruta graveolens* L. and the callus derived from hypocotyl stems.

| Rutin source | Retention time (min.) | Curves area(%) | Rutin(%) |
|---|---|---|---|
| Standard rutin | 2.6 | 45.26 | 100 |
| Seeds | 2.5 | 45.55 | 73.6 |
| Leaves | 2.4 | 20.8 | 11.7 |
| Callus derived from hypocotyl | 2.3 | 50.57 | 89.2 |




Fig(2): Rutin's curves of the standard sample (A), the isolated rutin from seeds(B), Leaves (C) and from the callus derived from the hypocotyl stems (D) of *Ruta graveolens* L.

The results showed that there is a slight differences between the percentage of the curve's area of the standard rutin (Fig2.A) and the curve's area recorded for the isolated rutin from the seeds(Fig2.B), whilst the leaves greatly differ in terms of the percentage of the curve's area of isolated rutin from them (Fig2.C), from the curve's area of the standard sample.

The results of estimation of the percentage of rutin in these parts indicate its existence at 73.6% in the seeds this exceeds its percentage in the leaves. The recorded curves of rutin in the callus cultures that was derived form the hypocotyl (Fig2.D) indicate their prevalence as the percentage of the presence of rutin which was 89.2%.

It can be concluded from this study that the callus cultures represent desired small bioreactors for the production of the secondary metabolites, especially those of the pharmaceutical importance due to their high growth rate and the easiness of its culturing and their growth on the nutrition media and the potentiality of playing with their gens. The most important is the optimization of the conditions for the *in vitro* production of the secondary metabolites that are very difficult to be controlled in nature.

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