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ISOLATION AND CHARACTERIZATION OF SECONDARY METABOLITE FROM *IMPATIENS BALSAMINA* LINN

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Abstract

The present study was designed to isolate the flavonoid from the ethylacetate fraction of ethanol extract of *Impatiens balsamina (*Balsaminaceae) by coloum chromatography using gradient elution method. The isolated flavonoid was characterized by spectral studies. The compound identified as (3, 5, 7 - trihydroxy-2 - (3, 4-dihydroxyphenyl) -4H-chromen-4-one) Quercetin ($C_{15}H_{10}O_7$).

Keywords: *Impatiens balsamina,* Quercetin.

Introduction

The genus *Impatiens Balsamina* (Balsaminacea) is distributed in the tropical and sub-tropical part of India. The Annual herb of *Impatiens Balsamina* issued to emetic, cathartic, diuretic, in Hawaii Island used for ulcers extract as anticancer and flower as cooling, tonic, antiseptic.[1] Although many compounds have been reported from the genus, *Impatiens Balsamina* previous phytochemical investigation with the occurrences of saponins, Apigenin, flavonoids, napthaquinone, glycosides, kaempferl 3 –rhamnosyl glycoside, kumarin.

In our previous study, the phytochemical investigations of ethylacetate fraction of *Impatiens Balsamina r*evealed the presence of flavonoid by preliminary test and TLC studies, and exhibited antioxidant activity. Hence the current protocol is designed for isolation and characterization of flavonoids from ethanol extract of *Impatiens Balsamina*.[2]

Methodology

The plant specimen *Impatiens balsamina* Linn (Balsaminaceae) collected from Kolli Hills, Namakal, identified and authenticated by the Botanist, Botanical Survey of India, TNAU, Coimbatore, and Tamilnadu Ref.No: BSI/SC/5/23/10-11. 500 gm of whole powdered plant material of *Impatiens balsamina* Linn *(*40# size) was extracted with ethanol in a soxhlet extraction apparatus.[3] The extraction was carried out exhaustively and the solvent was recovered by distillation under reduced pressure on a steam bath for 72 hours. The extract was concentrated and a greenish brown mass was obtained. The above mass was taken in 200 ml of water and transferred

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to a separating funnel. The liquid was then extracted with petroleum ether (60-80°C) for five times (5 x 60ml) and extracts were collected together. This combined extracts, evaporated to dryness. The aqueous phase obtained after petroleum ether extraction was extracted with ethyl ether in the same manner and ethyl ether extract was evaporated to dryness and kept it inside refrigerator. The aqueous phase obtained was then acidified with 7% sulphuric acid and extracted with ethyl acetate. The ethyl acetate extract thus obtained was evaporated to dryness and stored in a refrigerator for further use and the aqueous acidic layer was discarded. The ethyl acetate fraction was tested for qualitative test - flavonoid.

About 120 ml of activated silica gel (60-120 mesh) was measured and made into thin slurry with initial hexane and ethyl acetate: Methanol (30; 70) solvent system. The excess solvent on top of the column was allowed to flow out and 10 ml of ethyl acetate fraction of ethanol extract was carefully layered on the top of the column and the solvent was allowed to flow out slowly till the solution was adsorbed on the top of the silica gel G layer. The rate of elution was adjusted to 20 drops per minute. The solvent eluting through the column was collected in flasks with a time gap of 10 minutes per fraction. Each collected fraction was tested for the presence of various constituents by TLC for number of types of constituent and similar fractions was pooled together. The fractions 94-100 showed single spot with TLC plate sprayed with ammonia vapour, iodine vapour and 5% ethanol ferric chloride reagent.[4, 5] The isolated compound recrystallized in ethanol gave a pure compound. The percentage yield was found 0.30% w/w with respect to dry powdered plant material. The isolated material, further characterized by spectral analysis and physico-chemical properties.

Results

Phytochemical test for isolated flavonoid

- Shinoda test (Mg-Hcl reduction test): Isolated sample in alcohol, fragments of magnesium metal and concentrated hydrochloric was added drop wise along the tube, which gave pink to red colour indicating the presence of flavanoids.
- Zn-Hcl reduction test: 1ml test sample mixed with zinc dust and few drops of concentrated Hydrochloric acid was added dropwise along the sides of the tube, red colour indicating the presence of flavanoids .
- Ferric-Chloride test: 1ml test sample, freshly prepared ferric chloride solution was added, which showed bluish green to black colour.

Characterization of flavonoid Compound

Spectral data

Structure of isolated compound were established based on UV,IR, 1HNMR and mass spectral studies.The isolated compound shows Yellow crystals, 0.30%w/w. R_f 0.70,Melting point found 313^{o}C, the absorption maxima were obtained at 369 nm. IR spectrum of isolated compound showed characteristic absorption band at 3406 cm-1 due to hydroxyl group. Another band at 1609 cm-1 attributed to stretching frequency of carbonyl group. The CH=CH stretching peak appeared at 2912 cm-1. The 'H NMR spectrum of isolated compound showed signals at δ 5.95 (d, 1H, J=2Hz, H-6 & 8), δ 6.69 (d, 1H, J=2Hz, H-6') δ 6.60 (d, 1H, J=2Hz, H-2') and at δ 6.51 (d, 1H, J=2Hz, H-5') of the CH groups. The signals at δ 5.00 (d, 1H, J=2Hz, H-5, 7, 3' & 4') show the presence of 4 aromatic C-OH groups. The other enol group appear at δ 15.01 (d, 1H, J=2Hz, H-3). This shows presence the flavonoid moiety- quercetin.

The Mass spectrum of isolated compound showed molecular ion peak at m/z at 302 which corresponds to its molecular formula (C_{15} H_{10} O_7) and molecular weight. All the above spectral data were consistent with matched by using Dr. Duke's Phytochemical and Ethno botanical Databases and it was confirmed as Quercetin.

Discussion

The isolated compound from *Impatiens balsamina* Linn showed positive results for flavonoids by qualitative analysis. The isolated material showed identical physiochemical properties as recorded in the literature of Quercetin. The spectral studies like UV, IR, NMR and MS found to be identical with that of Quercetin. All these data conclusively prove the identity of the isolated compound from ethanol extract of *Impatiens balsamina* as Quercetin.

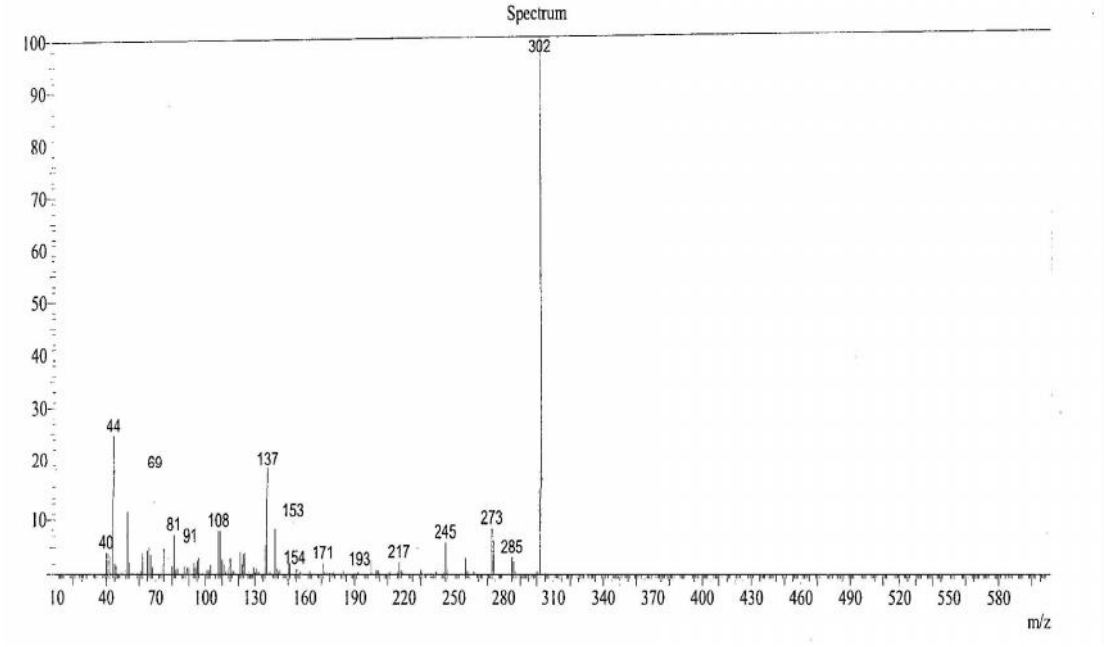

Fig. No. 01: The Mass spectra of isolated compound

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