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Research Article

Quercitrin from *Ixora coccinea* Leaves and its Anti-oxidant Activity

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Abstract

One method has been developed to isolate quercitrin from *Ixora coccinea* leaves, which depends on fractionation of defatted hydro-alcoholic extract by different polarity solvents followed by purification through column chromatography. Isolated quercitrin has been characterized by using UV, IR, Mass spectral data, NMR data and also confirmed by using HPTLC and elemental analysis. The isolated quercitrin was shown a challenging potency to scavenge DPPH free radicals and also nitric oxide free radicals with very low IC_{50} value.

Keywords: Quercitrin, *Ixora coccinea*, HPTLC, anti-oxidant, DPPH, nitric oxide

Introduction

Ixora coccinea is native to tropical south-east Asia, including Southern India and Sri Lanka. It has become one of the most popular flowering shrubs in South Florida gardens and landscapes.[1] The flower of Ixora traditionally used as anti–inflammatory, aromatic, antipyretic drug and useful in extensive thirst and fatigue according to Siddha literature. In the study presented, flowers of both the varieties of Ixora were pharmacologically tried in different conditions. The fresh juice of flower was used in 2 ml/100g oral dose in the studies.[2-5] Pharmacological evaluation revealed that the plant *Ixora coccinea* has alterative property in fatigue and thirst, protecting property against electro-convulsions, anti-inflammatory (both acute and chronic) property and haemostatic efficiency in animals. The plant does not have any effect in adjuvant induced arthritis, barbital induced sleeping time and yeast induced anti-pyretic activity in Albino rats. Generally all these disease can be occurred due to the presence of free radicals in our body and in major cases polyphenolic compounds are responsible to scavenge free radicals from our body.[3-10] As in qualitative chemical tests the presence of quercitrin was identified in different extracts of *Ixora coccinea* leaves, an attempt has been taken to isolate and characterize quercitrin and performed the in vitro anti-oxidant activity of the isolated quercitrin.

Materials and Methods

Materials

Petroleum ether (60-80° C), benzene and ethyl acetate were purchased from Merck Specialities Ltd., Mumbai, India. All other reagents were of analytical grade and used without further purification.

The plant *Ixora coccinea* was collected from Herbal Garden of Gupta College of Technological Sciences, Asansol, West Bengal, India and authenticated by the Head, Botanical Survey of India, Shibpur, Howrah, West Bengal, India vide reference number CNH/1-1/(201)/2007/Tech. II/2.

Isolation of Quercitrin

100g air-dried leaves of *Ixora coccinea* was powdered and defatted with 750 ml of petroleum ether (60-80°C). It was then extracted with 750ml distilled ethanol in a soxhlet for 16h. The extract was filtered and concentrated in a rotary flash evaporator at 60^0C. The concentrated ethanolic extract was poured into excess of distilled water with stirring and filtered. The filtrate that comprises water soluble portion of ethanolic extract was extracted in liquid-liquid extractor with petroleum ether (60-80°C), benzene and ethyl acetate. Ethyl acetate extract was concentrated to a small volume and was kept in a refrigerator for 2 days which yielded yellow crystals. These crystals were dissolved in ethyl acetate and were tested for the presence of flavonoid.

A column chromatography was performed to purify the isolated flavonoid with ethyl acetate, glacial acetic acid, formic acid and water (100:11:11:27) as solvent system. Isolated & separated compound was dissolved in methanol (pure) and evaporated it on the hot water bath. After 90% evaporation, crystals appeared, filtered to separate them. Then the compound was lyophilized and performed its spectral analysis.

Quercitrin

Yellow powder (MeOH), mp 181-182°C; 1H-NMR (400 MHz, CD_3OD) d: 7.57 (1H, d, J = 1.9 Hz, H-2'), 7.53 (1H, dd, J = 8.2, 1.9 Hz, H-6'), 6.77 (1H, d, J = 8.2 Hz, H-5'), 6.29 (1H, d, J = 1.8 Hz, H-8), 6.10 (1H, d, J = 1.8 Hz, H-6), 5.01 (1H, d, J = 2.0 Hz, H-1"), 4.22-3.14 (sugar H), 1.02 (3H, d, J = 6.0 Hz, H-6"). 13C-NMR (100 MHz, CD3OD): C2,158.1; C3,136.0; C4,179.3; C5,159.1; C6,99.5; C7,165.8; C8,94.3; C9,163.0; C10,105.5; C1′,122.5; C2′,116.2; C3′,146.2; C4′,149.5; C5′,116.7; C6′,122.7; C1″,103.4; C2″,71.8; C3″,72.0; C4″,73.1; C5″,71.7; C6″,17.5.

Antioxidant activity

DPPH Radical scavenging method[11-14]

0.1 mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of isolated compound solution in water at different concentrations (10-80 μg/ml). Thirty minutes later, the absorbance was measured at 517 nm, lower absorbance of the reaction mixture indicate higher free radical scavenging activity. Ascorbic acid was used as a standard. The difference in absorbance between test and control was calculated and expressed as percentage scavenging of DPPH radical.

Nitric Oxide scavenging method[15-17]

Nitric oxide was generated from sodium nitroprusside and messured by Griess reagent. Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4) saline was mixed with different concentration (10-80 μg/ml) of isolated compound dissolved in water and incubated at 25°C for 150 min. At interval, sample (1.5 ml) Griess reagent. The absorbance was read at 564 nm.

Results and Discussion

The elemental analysis report of isolated Flavonoid (1.33% yield) represents that there are 56.2% Carbon, 4.46% Hydrogen and no nitrogen present in its chemical structure. From the UV spectra it is clear that the fraction which has been collected by passing through the silica column, showed peaks at wavelengths 245, 288 and 325 nm. Flavonoids generally show two characteristic bands in the region and they are band 1 is between 300 to 340 nm and band 2 is between 240 and 280 nm.[18-20] As the column isolated compound shows peaks in the region of band 1 and band 2, so, it might be predicted that the compound is flavonoid. After performing FT-IR of the column isolated sample, almost 32 peaks were determined among which, some of the major peaks have been identified.[18-20] Peak at 3417.00 cm^{-1} broad represents Hydroxyl group, Hydrogen bonded, represents -OH stretch, peak at 2973.92 cm^{-1} represents C-H stretching (-CH_3), peak at 1371.50 cm^{-1} represents -CH_3 bending and peak at 1465.13 cm^{-1} represents bending of methylene and methyl group, peak between 1700cm^{-1} to 1740 cm^{-1} represents conjugated ketone (C=O), peak at 1415.31 cm^{-1} and 651.94 cm^{-1} represent aromatic ring system, peak at 1298.31 cm^{-1} represents C=C group, peak at 1076.82 cm^{-1} represents primary alcohol (-OH), peak at 1076.82 cm^{-1} represents ether link (-C-O-C-). Thus, by comparing the peak values of the column isolated sample with the standard values, it can be inferred that the column isolated sample contain the following groups: a) aromatic ring b) phenolic OH group with hydrogen bonding c) a Conjugated ketone d) C-H stretching (-CH_3) and -CH_3 bending e) bending of methylene and methyl group f) C=C group g) aromatic ring system and h) both primary alcohol (-OH) and ether link (-C-O-C-). From the above identified groups, we can predict that the sample has Flavonoidal structure. As R_f value (0.32) of this compound in TLC and melting point are similar to Quercitrin[12], the FTIR data of standard Quercitrin were compared and found to be very similar.[13-14] So now, we can predict that the isolated sample may be Quercitrin. The molecular weight of the isolated compound was found to be 448.41. After comparing R_f value, melting point, HPTLC report (Fig. 1), elemental analysis, UV spectra, IR spectra, ^{1}H-NMR, ^{13}C-NMR and mass spectra it can be concluded that the isolated compound is Quercitrin (Fig. 2).

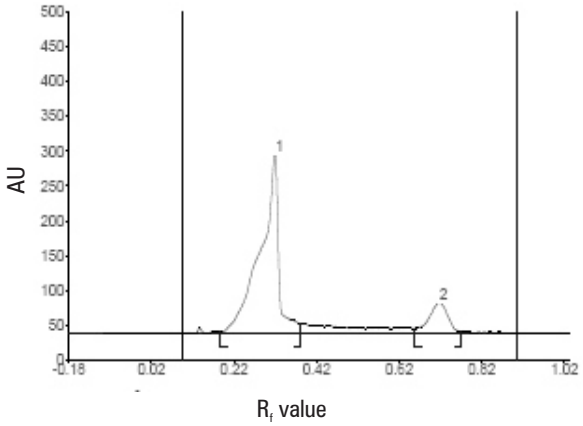

Fig. 1: HPTLC analysis of isolated quercitrin

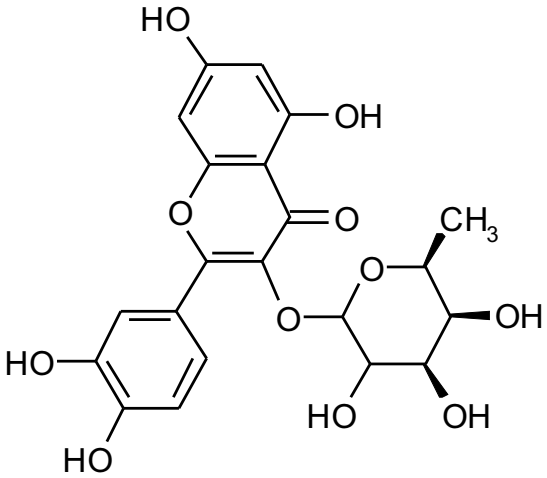

Fig. 2: Chemical structure of isolated quercitrin

A 80 μg/ml of isolated quercitrin and ascorbic acid exhibit 90.46% and 96.30% inhibition against DPPH respectively and IC_{50} value were found to be 6.167 μg/ml and 4.321 μg/ml for isolated compound and ascorbic acid respectively. The nitric oxide when react with oxygen from nitrite, which inhibited by antioxidants by competing with oxygen to react with nitric oxide. The % inhibition and IC_{50} values isolated quercitrin and ascorbic acid were found to be 86.23 % and 93.90 % and 20.38 μg/ml and 17.43 μg/ml respectively. The isolated quercitrin exhibited significant antioxidant activity with low IC_{50} values in these two models.

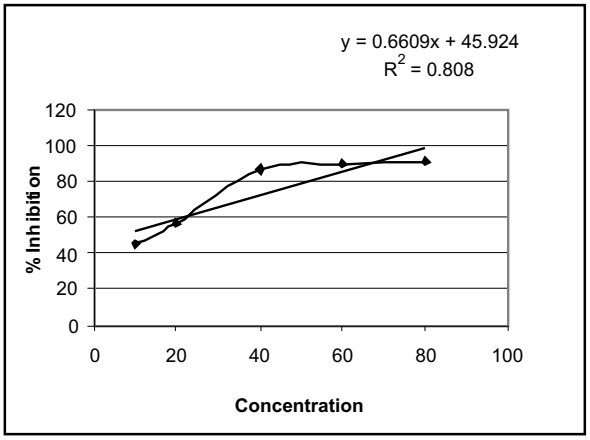

Fig. 3: DPPH radical scavenging activity of isolated quercitrin

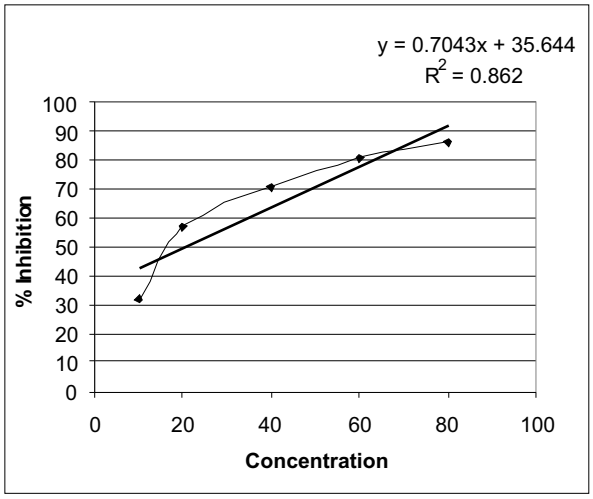

Fig. 4: Nitric oxide scavenging activity of isolated quercitrin

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