Original Article

Isolation of Quercetin-3-O-β-D-Glucoside from *Azadirachta indica*

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ABSTRACT

The present work discusses new and simple preparative TLC method for isolation of quercetin-3-O-β-D-glucoside from aqueous extract of leaves of *Azadirachta indica*. The purity of isolated quercetin-3-O-β-D-glucoside was 98.23 %. Structure of isolated quercetin-3-O-β-D-glucoside was established by various spectral techniques such as IR, NMR and mass spectroscopy. Isolated quercetin-3-O-β-D-glucoside can be used as marker compound for marker-based standardization of extracts and formulations containing *Azadirachta indica* leaves.

Keywords: *Azadirachta indica*, Meliaceae, Neem, Preparative TLC, Quercetin-3-O-β-D-glucoside.

INTRODUCTION

Neem tree, *Azadirachta indica* belonging to mahogany family Meliaceae is one of the most beneficial natural or Ayurvedic medicine growing in tropical and semi-tropical regions. The genus name *Azadirachta* is derived from the Persian name of the tree that is *Azad-darakhat-e-hind* (noble/free tree of India).[1]

It is known as Arista, Neem and Margosa tree in Sanskrit, Hindi and English, respecytively.[2] Parts used of this tree are fruits, seeds, kernels, flowers, leaves, twings and bark.[3] Leaves of this tree contain flavonol glycosides mainly quercetin-3-O-β-D-glucoside (Figure 1), myricetin-3-O-rutinoside, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, kaempferol-3-O-β-D-glucoside and quercetin-3-O-α L-rhamnoside.[4,5]

Many methods have been reported for isolation of quercetin-3-O-β-D-glucoside from different medicinal plants but these methods use lengthy and expensive techniques such as vacuum liquid chromatography, gel filtration chromatography, high speed counter current chromatography and/or column chromatography.[6-27]

The present paper discusses a new and simple method for isolation of quercetin-3-O-β-D-glucoside from aqueous extract of leaves of *Azadirachta indica* using solvent extraction technique and preparative TLC.

EXPERIMENTAL

Reagents and standards

Standard of quercetin-3-O-β-D-glucoside (Purity 90.0 % w/w) was purchased from Sigma-Aldrich. All the solvents used for isolation studies were of AR grade.

Plant materials

Leaves of *Azadirachta indica* were obtained from Valsad district of Gujarat in August 2011 and authenticated at Botanical Survey of India (BSI), Pune under voucher specimen number AIGSOD3. Leaves were dried in oven with air circulation at 35 ^{0}C, for seven days, powdered, sieved, stored in air tight container and used for further study.

Preparation of extract

Dried and powdered leaves (20 g) were macerated for 24 h with 200 ml distilled water. The aqueous extract thus obtained was fractionated with petroleum ether (60-80 ^{0}C) (3 X 50 ml) to remove fatty material. Petroleum ether layer was discarded. The aqueous extract was then extracted with 3X50 ml of ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated to dryness using rotary evaporator at 40^{0}C.

TLC studies of ethyl acetate extract

TLC studies of ethyl acetate extract of leaves were carried out. Several mobile phases were tried and following mobile phase was selected which gave maximum resolution:

Stationary Phase: Precoated plates of Silica gelGF_{254} (E. Merck).

Mobile phase: Ethyl acetate: formic acid: acetic acid: water (10: 1.1:1.1:2.7, v/v/v/v).

Chamber Saturation Time: 15 min

Development Technique: Ascending development.

Sample: Ethyl acetate extract of leaves of *Azadirachta indica*.

Procedure: Ethyl acetate extract was dissolved in minimum amount of methanol and spotted in the form of bands on silica plates. The plates were developed using mobile phase, ethyl acetate: formic acid: acetic acid: water (10: 1.1:1.1:2.7, v/v/v/v). After development, the plates were observed under white light, short (254 nm) and long (366 nm) wavelength. The plates were derivatised with vanillin sulphuric acid reagent (VSR).

Isolation of quercetin-3-O-β-glucoside

Ethyl acetate extract was subjected to preparative TLC for isolation of quercetin-3-O-β-glucoside using same chromatographic as used for TLC studies of ethyl acetate extract and spot corresponding to quercetin-3-O-β-glucoside was scrapped, treated with methanol and filtered through Whatman filter paper. The procedure was repeated using several silica plates. The resulting methanol solution was evaporated to dryness to give quercetin-3-O-β-D-glucoside.

Spectral studies of isolated quercetin-3-O-β-D-glucoside

TLC and HPLC studies of isolated compound were carried out to confirm the purity of isolated quercetin-3-O-β-D-glucoside and structure was confirmed by spectral techniques like IR, NMR and mass spectrometry.

RESULTS AND DISCUSSION

In the present work, quercetin-3-O-β-D-glucoside, a marker from aqueous extract of leaves of *Azadirachta indica* was isolated using simple preparative TLC technique. TLC studies of ethyl acetate extract revealed the presence of quercetin-3-O-β-D-glucoside at Rf of 0.53 (Figure 2).

Quercetin-3-O-β-D-glucoside was isolated from ethyl acetate extract of *Azadirachta indica* by preparative TLC. Yield of isolated quercetin-3-O-β-D-glucoside was 0.042 % w/w. TLC studies of isolated quercetin-3-O-β-D-glucoside revealed the presence of single isolated spot at R_f of 0.53 which was compared with reference standard (Figure 3).

Before carrying out spectral studies, purity of isolated quercetin-3-O-β-D-glucoside was checked by HPLC. The purity of isolated quercetin-3-O-β-D-glucoside was found to be 98.23 %. Characterization of the isolated quercetin-3-O-β-D-glucoside was carried out by spectral studies such as IR, NMR, mass spectroscopy and spectra were obtained (Figures 4, 5 and 6, respectively).

In IR spectroscopy, peaks at wave numbers of 3200.29 cm^{-1} and 1660.41 cm^{-1} confirmed the presence of OH stretching and –C=O group, respectively. Proton NMR exhibited signal at δ 1.305 (dd, 2H) indicating presence of methylene protons at 6" position. Signals between δ 3.310-3.698 indicated the presence of protons on glucose moiety at carbon 5", 4", 3" and 2". In addition, aromatic protons produced signals at δ 6.214 (d, H-8) and 6.402 (d, H-6). Peaks at δ 6.890 (d, H-5'), 7.65 (d, H-6') and 7.712 (d, H-2') also confirmed the presence of another trisubstituted benzene. Mass spectrum confirmed the presence of M^+ peak at m/z value of 464.3 confirming the molecular weight (molecular weight of quercetin-3-O-β-D-glucoside: 464 g/mole) of quercetin-3-O-β-D-glucoside. Peak at m/z value of 302.5 was due to loss of glucose moiety from the molecule. Based on spectral studies, it was confirmed that the isolated compound was quercetin-3-O-β-D-glucoside.

CONCLUSIONS

Based on chromatographic and spectral studies isolated compound was identified as quercetin-3-O-β-D-glucoside. Thus, quercetin-3-O-β-D-glucoside was successfully isolated from leaves of *Azadirachta indica.*

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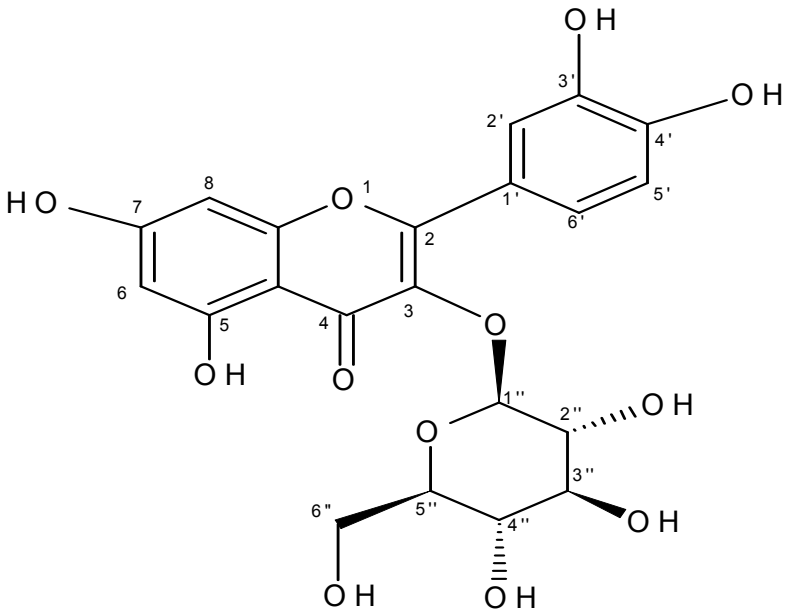
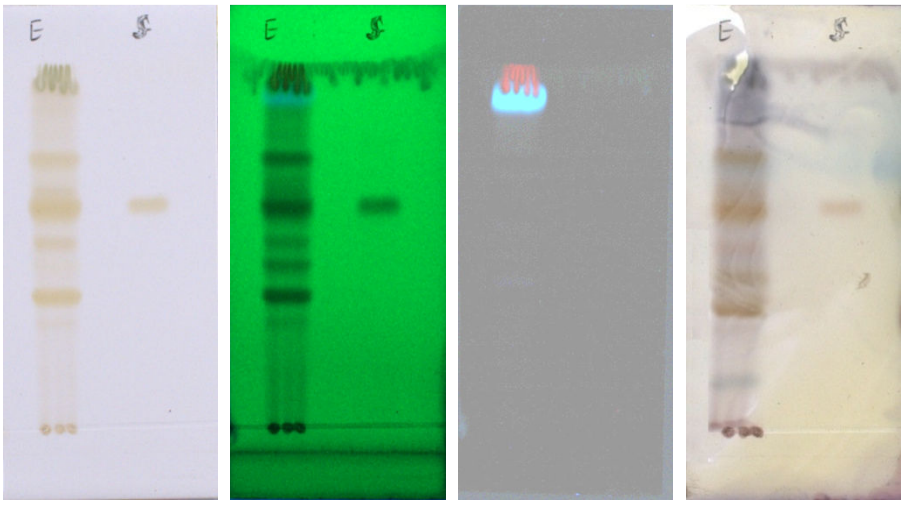


Figure 1. Structure of quercetin-3-O-β-D-glucoside



White light 254 nm 366 nm VSR

Figure 2. TLC profiles of ethyl acetate extract of *Azadirachta indica*

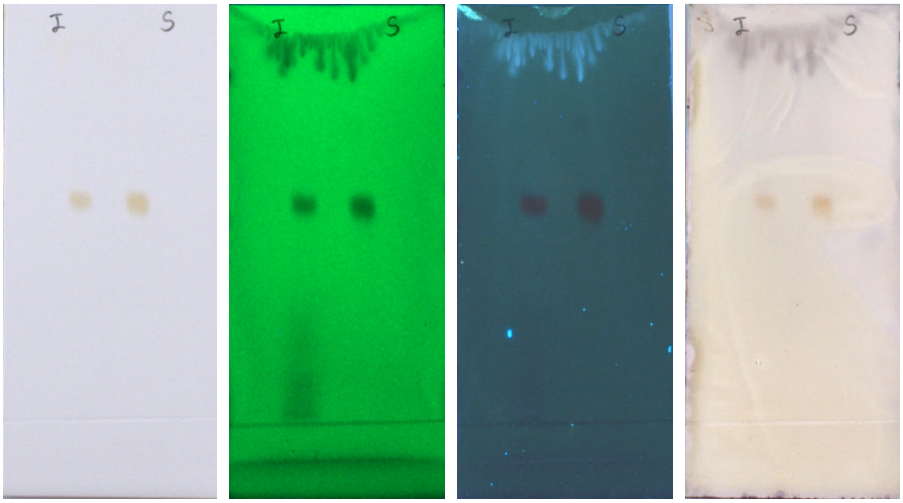


Figure 3. TLC profiles of isolated compound

I=Isolated compound, S= Standard of quercetin-3-O-β-D-glucoside

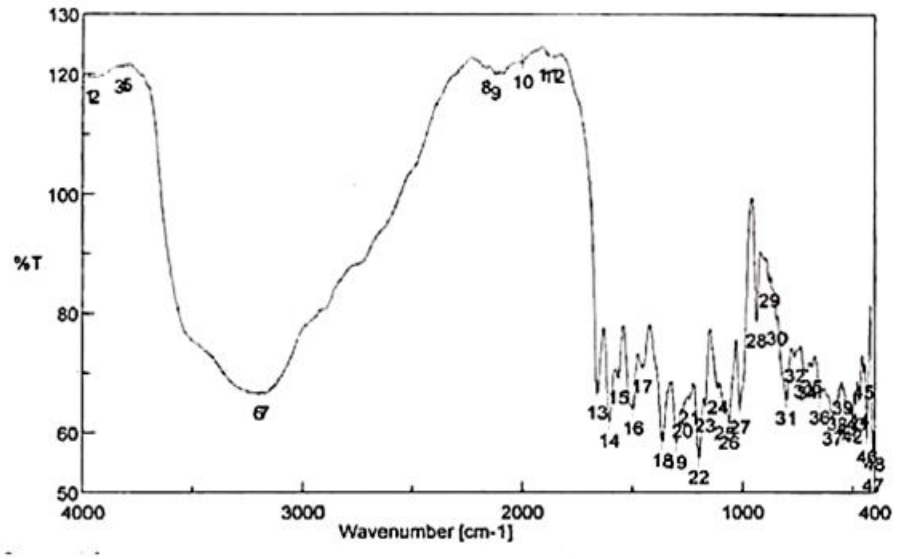


Figure 4. IR spectrum of isolated quercetin-3-O-β-D-glucoside

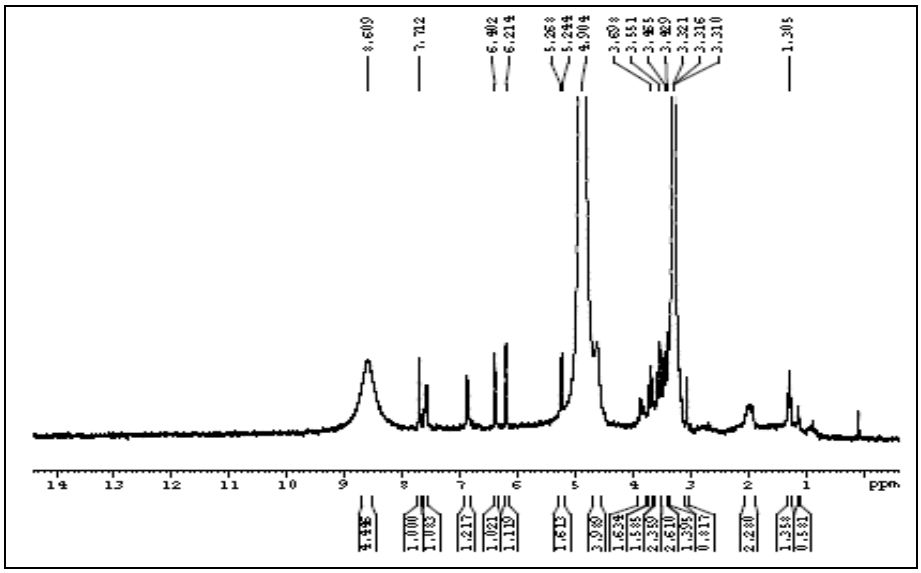


Figure 5. NMR spectrum of isolated quercetin-3-O-β-D-glucoside

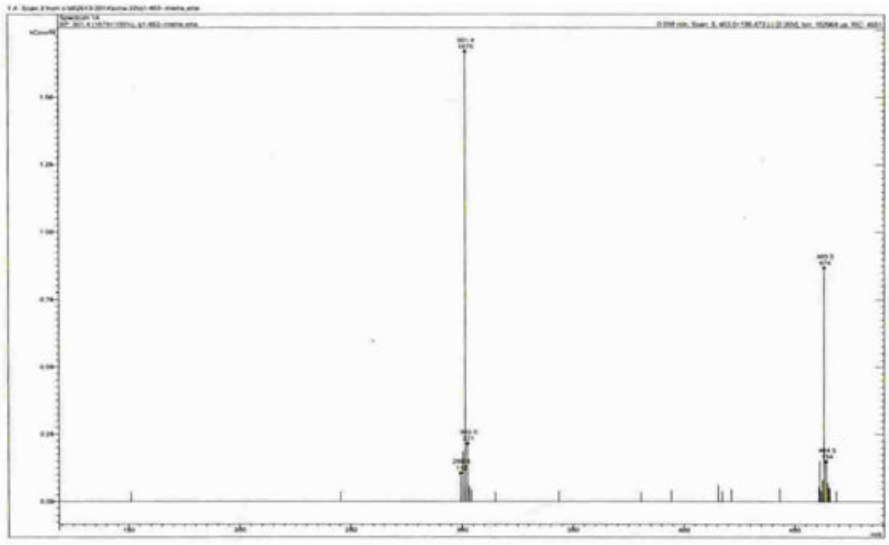


Figure 6. Mass spectrum of isolated quercetin-3-O-β-D-glucoside