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Research Article

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An efficient greener approach of antioxidant isolation from the fresh flowering buds of *Bauhinia tomentosa* Linn (FFBBT)

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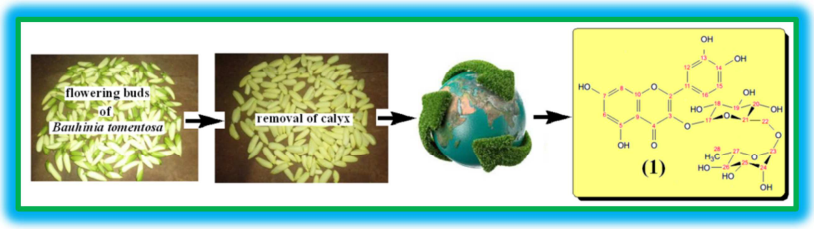
ABSTRACT

*A flavonoid glycoside, Rutin (**1**) a well-known natural antioxidant, is one of the medicinally important flavonoid, which was isolated from the flowering buds of Bauhinia tomentosa(FBBT) as greener manner with excellent yield. Many more studies had been done for the isolation of rutin by different methodology. The necessity of finding a better and/or cheaper commercial source for rutin and other related phenolic compounds is still valid for the utility of the compounds. During this study rutin was isolated from Bauhinia tomentosa by simple methodology, greener manner and excellent yield (6.5%). The structure of the compound was established by means of extensivechemical and spectral evidences.*



Keywords: *Bauhinia tomentosa*, Rutin, Flowering buds, Flavonoid glycosides and Leguminosae.

INTRODUCTION

Natural products have been an overwhelming success in our society. They have reduced pain and suffering, revolutionized medicine by facilitating the transplantation of organs. Natural products are the most important anticancer and anti-infective agents [1]. *Bauhinia tomentosa*(Linn.) (Family Leguminosae and sub-family Caesalpiniaceae) is an indigenous medicinal plant, commonly known as Kachnaar. Kokkumandarai, Tiruvaatchi and Manjal Mantharai in Tamil and Kachnaar in Hindi[2,3]. It is a medium size glabrous tree with a short bole and attaining a height of around 6m and is habitat in the littoral region of Southeast Asia. Traditionally, its bark is used as astringent; decoction of the root bark is prescribed for liver diseases and used as a vermifuge; seeds are used to treat wound healing. Seeds yield fatty oil called ebony oil, a water soluble mucilage and saponins [3]. Seed paste made with vinegar is an efficacious application to wounds inflicted by poisonous animals. The bruised bark is externally applied on tumours and wounds. Dried buds and young flowers are prescribed in dysenteric affections [4].

Rutin and isoquercitrin are flavonoid glycosides are most found and that the rhamnoglucoside and glucoside of the quercetin.

Rutin have many beneficial effects on human health and its also used as a coloring agent for food additives and for various purposes in cosmetics. It possess multiple biological functions including anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic, anti-atherosclerotic, anti-carcinogenic, anti-thrombotic, vaso-protective and cyto-protective properties [5].

Previous phytochemical investigation of sundried flowers (collected during Jul-Sep) [6] and fresh flowers (collected in Jan) [7] of this species showed flavonoids like Rutin (4.6%) and Isoquercitrin (6%) respectively. It is very common in phytochemical analysis, reinvestigation of a same species collected from different regions, different parts, different conditions and different seasons may result in different constituents due to changes in climatic conditions and environmental conditions. Based on this scenario, in the present work, we report the isolation and characterization of compound (**1**) (**Fig. 1**) from the ultrapure aqueous ethanolic extract of the flowering buds of *B. tomentosa*(collected in Dec)as simple methodology, greener manner and good yield (6.54% (dry basis), 1.29% (wet basis)).

EXPERIMENTAL SECTION

2.1 General Methods:

UV-Visible absorption spectra were recorded in JASCO- spectra manager (V-550) double beam spectrophotometer and FT-IR spectra were recorded on JASCO spectrophotometer using KBr pellets. NMR spectra (^{1}H-NMR, ^{13}C-NMR, H,H-COSY, C,H-COSY, HMBC and DEPT-135) were recorded in $CDCl_3$ and DMSO-d_6 on Bruker DRX-300 (300 MHz) Ultra Shield TM spectrometer using TMS as an internal standard. Chemical shifts (δ) are expressed in ppm, coupling constants (*J*) are expressed in Hz, and splitting patterns are described as follows: s = singlet; d = doublet; t = triplet; brs = broad singlet; m = multiplet; dd = doublet of doublet. Verification of the product and its purity analysis were carried out on a Shimadzu System (Shimadzu, Japan) equipped with a column (Shimadzu RP-18 LC-10A Pump). The purity analysis was performed using the following spectroscopic gradient: from 0 to 1 min in methanol, then from 1 to 35 min 90% methanol and 10 % H_2O. Injection Volume is 5 µl, flow rate is 0.30 mL/min and the oven temperature is 30 °C. The compound was analyzed by a PDA-UV-Vis detector at 254 nm.

Electrospray ionization mass spectrometry (ESI-MS) analysis was performed in the positive ion mode and negative ion mode on a liquid chromatography-ion trap mass spectrometer (LCQ Fleet, Thermo Fisher Instruments Limited, US). The collision voltage and ionization voltage were 33 V and 4.98 kV, respectively, using nitrogen as atomization and desolvation gas. Helium was used as the nebulizer at 40 psi. The desolvation temperature was set at 300 ^{o} C. The scan range of mass spectrum was 150–1000 m/z. For TLC, silica gel-G and Whatman No. 1 filter paper was used and the plates were visualized using iodine/UV lamp in long and short wavelength.

2.2 Plant material:

The flowering buds of *B. tomentosa* were collected from the vicinity of our resident, Madurai, Tamilnadu, India in December 2012 and identified by Dr. G. V. S. Moorthy (Botanical survey of India, Coimbatore, Tamilnadu). A botanically identified voucher specimen (voucher No: 34556) was deposited over the herbarium of Botanical survey of India, Coimbatore, Tamilnadu.

2.3 Extraction and isolation:

The fresh flowering buds of *B. tomentosa* (100.0 g) were homogenized with ultrapure aqueous ethanol (95:5) (3 x 200 mL). The combined extracts were filtered through a muslin-cloth and then on concentration *in vacuo* yielded a pale brownish yellow residual solution, which was successively filtered with high vacuum pump. Compound **1** was isolated as pale yellow color compound at 99.9% pure form. The aqueous fraction on concentration did not deposit any worthwhile compound.

RESULTS AND DISCUSSION

The homogeneity of compound **1** was ascertained by giving only one spot when examined by paper chromatography on a Whatman No. 1 filter paper, using n-butanol - water (4:6). The Purity of the compound **1** was further supported by its HPLC chromatogram (**Fig. 2**) and LC chromatogram data (**Fig. 3**). Compound **1**, isolated as pale yellow

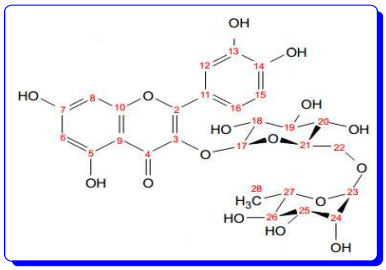
powder, gave green color with alcoholic ferric chloride indicating the presence of a chelated hydroxyl in it. It gave pink color with Mg / HCl indicates the flavonoid nature. Its UV absorption (λ_{max} 264, 299, 361) (**Fig. 4**) was reminiscent of that of a hydroxy-flavone. When the compound added to the alcoholic α-napthol/H_2SO_4, the violet ring was formed, this indicates the glycoside unit in it. That compound **1** was further supported by its IR, NMR and ESI-MS spectroscopic data. The IR spectra exhibit the characteristic patterns of the compound **1,** that generate bands about 3,000-3,330 [υ(OH)] and 1,655 [υ(C=O)] cm^{-1} (**Fig. 5**). The ^{1}H NMR spectrum of compound **1** displayed five doublet at δ 6.23, 6.40, 6.90, 7.62, 7.69 (**Fig. 6**) for five aromatic protons was due to quercetin moiety, the presence of which was supported by a two one-proton doublet at δ 6.23 (*J*= 2.1 Hz) and 6.40 (*J* = 2.1 Hz) was ascribed to H-6 and H-8 respectively. The up field three-proton doublet at δ 1.12 was due to methyl group. The downfield singlet (1H) at δ 12.27 was ascribed to the chelated hydroxyl (5-OH). Compound **1** was recognized as flavonoid by the characteristic complex ^{1}H NMR signals. The multiplicity of signals in the ^{13}C NMR spectrum of compound **1** (**Fig. 7**), shows twenty seven signals, which clearly indicates the compound **1**havingtwenty seven carbon atoms which are grouped into ten quaternary carbons, one methyl, five aromatic methine, ten aliphatic methine and one methylene carbons based on DEPT-135 subspectra. The aliphatic methyl signal resonated at δ 16.24. The downfield signal at δ 176.46 was due to the carbonyl carbon of the flavone moiety. The signal at δ 66.09 was assigned to methylene carbon. The ^{1}H NMR values for all the protons were assigned on the basis of the H, H-COSY (**Fig. 8**)spectra and were in good agreement with the proposed structure. Positive and negative modes of ESI-MS were used to identify the compound **1.**The representative MS spectra of rutin under positive mode and negative mode was illustrated in (**Fig. 9**). The positive ESI mass spectrum of compound **1** (m/z 611.25 $[M+1]^+$, m/z 633.33 $[M+Na]^+$ and m/z 649.33, $[M+K]^+$) confirm that the protonated, sodium and potassium-adducted compounds were observed under the positive mode respectively (**Fig. 9A**). The negative ESI mass spectrum of compound **1** (m/z 609.25 $[M-1]^+$) confirm that the deprotonated molecule could be observed under the negative mode (**Fig. 9B**). Based on the above spectral data, compound is assigned as rutin.

3.1 Hydrolysis of Rutin

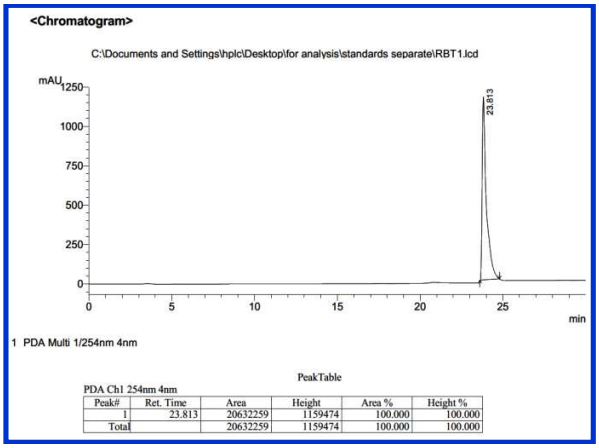
A solution of rutin (500 mg) in 5% methanolic sulphuric acid was heated on the water-bath for 15 min and then preceded with usual workup. The aglycone, quercetin (225 mg), which was separated as a yellow precipitate, m. p. 300-310^{o}C, was filtered off, washed, and dried. The occurrence of glucose and rhamnose in the sugar moiety of rutin was established by paper chromatography on Whatman No. 1 filter paper alongside glucose and rhamnose as controls.

To the best of our knowledge, this is the first report of the isolation of a compound **1** from the flowering buds of *B. tomentosa* in the greener manner and excellent yield. Compound **1** was identified as Rutin by comparison of their spectroscopic and chemical data with the data reported for the authentic compound [8].

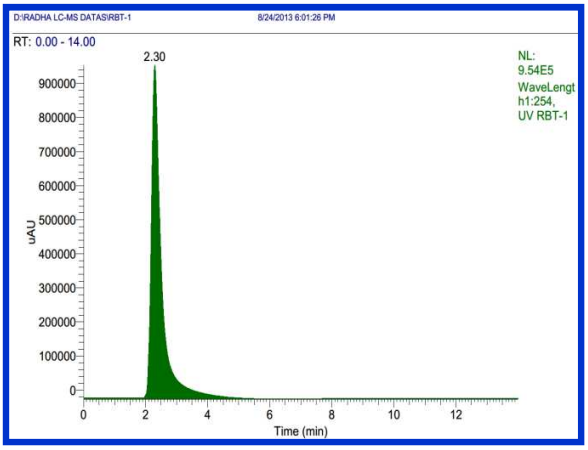
3.2 Rutin (1): Pale yellow powder, M.p. 188-190^{o}C; HPLC (R_t- 23.81min)UV (CH_3OH) λ_{max} (nm): 264, 299, 361; IR (KBr) υ_{max} (cm^{-1}): 1655 (>C=O) (s), 3000-3300(>O-H) (b);^{1}H NMR (300 MHz, $CDCl_3$ and DMSO, (d_6)) δ 12.27 (1H, s, 5-OH), 10.51 (1H, brs, 7-OH), 9.35 (1H, brs, 4'-OH),8.81 (1H, brs, 3'-OH), 7.67 (1H, d, *J* = 2.1 Hz, H-6'), 7.62(1H, dd, *J* = 2.4, 2.4 Hz, H-5'), 6.90 (1H, d, *J* = 8.4 Hz, H-2'), 6.40 (1H, d, *J* = 2.1 Hz, H-8), 6.23 (1H, d, *J* = 2.1 Hz, H-6), 5.38 (1H, s, aliphatic-OH), 5.09 (1H, d, *J* = 7.2 Hz), 4.90 (2H, s, aliphatic-OH), 4.52 (1H, s, aliphatic-OH), 4.24 (2H, s, - aliphatic-OH), 4.08 (1H, q, *J* = 7.2 Hz, H-5'"), 3.77 (1H, d, *J* = 10.8 Hz), 3.64 (1H, s), 3.34 (9H, brs, aliphatic-Hs), 2.02 (1H, s), 1.24 (1H, t), 1.12 (3H, d, *J* = 6.3 Hz, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$andDMSO, (d_6)) δ 176.46 (C=O, C-4), 163.19 (C, C-7), 160.29 (C, C-5), 156.38 (C, C-8a), 155.54 (C, C-3'), 147.22 (C, C-4'), 143.26 (C, C-2), 133.03 (C, C-3), 120.83 (C, C-1'), 120.26 (CH, C-6'), 115.55 (CH, C-2'), 114.22 (CH, C-5'), 103.01 (C, C-4a), 102.25 (CH, C-6), 99.58 (CH, C-8), 97.82 (CH, C-1'"), 92.55 (CH, C-1"), 75.82 (CH, C-5"), 74.47 (CH, C-3"), 72.95 (CH, C-2"), 71.37 (CH, C-2'"), 69.79 (CH, C-4'"), 69.31 (CH, C-3'"), 68.52 (CH, C-4"), 66.67 (CH, C-5'"), 66.09 (-CH_2-), 16.24 (-CH_3); DEPT-135 (75 MHz, $CDCl_3$and DMSO, (d_6)) δ127.05 (CH, C-6'), 121.59 (CH, C-2'), 120.18 (CH, C-5'), 108.49 (CH, C-6), 105.80 (CH, C-8), 103.98 (CH, C-1'"), 98.79 (CH, C-1"), 81.89 (CH, C-5"), 80.61 (CH, C-3"), 78.98 (CH, C-2"), 77.52 (CH, C-2'"), 75.94 (CH, C-4'"), 75.39 (CH, C-3'"), 74.58 (CH, C-4"), 72.97 (CH, C-5'"), 71.97 (-CH_2-), 22.57 (-CH_3); LC-MS (relative intensity) (Positive mode)[m/z] [611.10 ($[M+1]^+$, 100.0%), 633.24 ($[M+Na]^+$, 90.0%), 649.18 ($[M+K]^+$, 10.0%)(Negative mode)[m/z] [609.31 ($[M-1]^-$, 100.0%)] (calcd for $C_{27}H_{30}O_{16}$ 610.15).



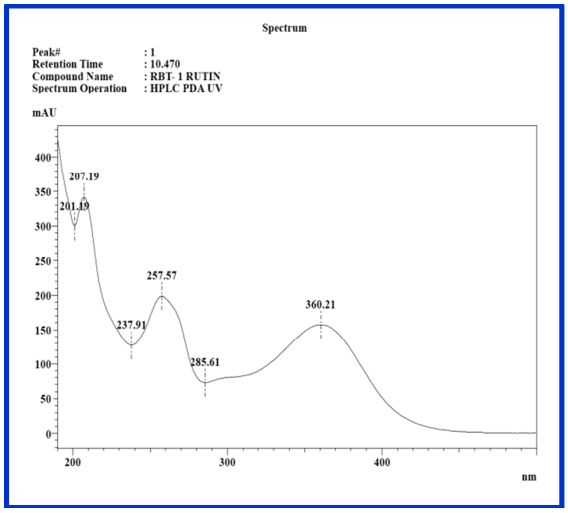
(Fig. 1)Structure of compound 1



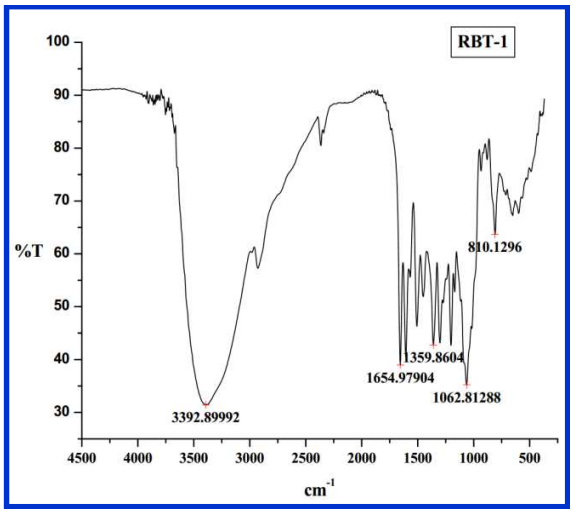
(Fig. 2) HPLC chromatogram of compound 1



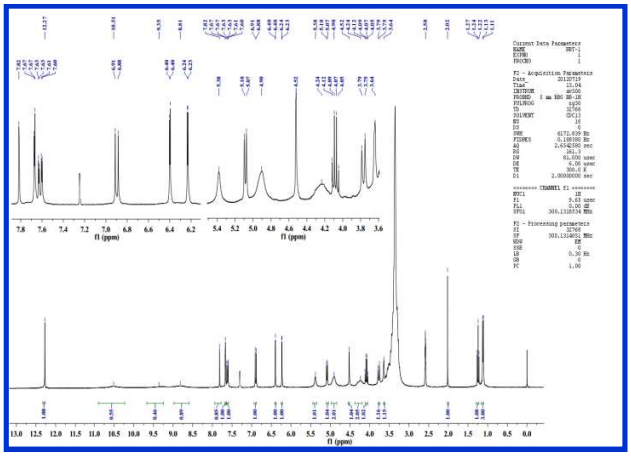
(Fig. 3) LC chromatogram of compound 1



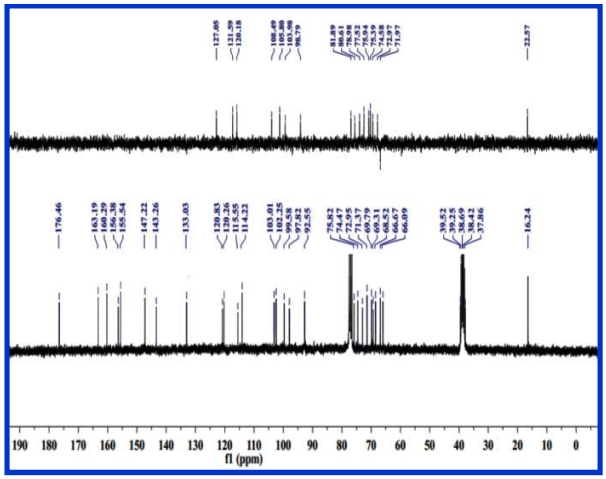
(Fig. 4) UV- Vis absorption spectrum of compound 1



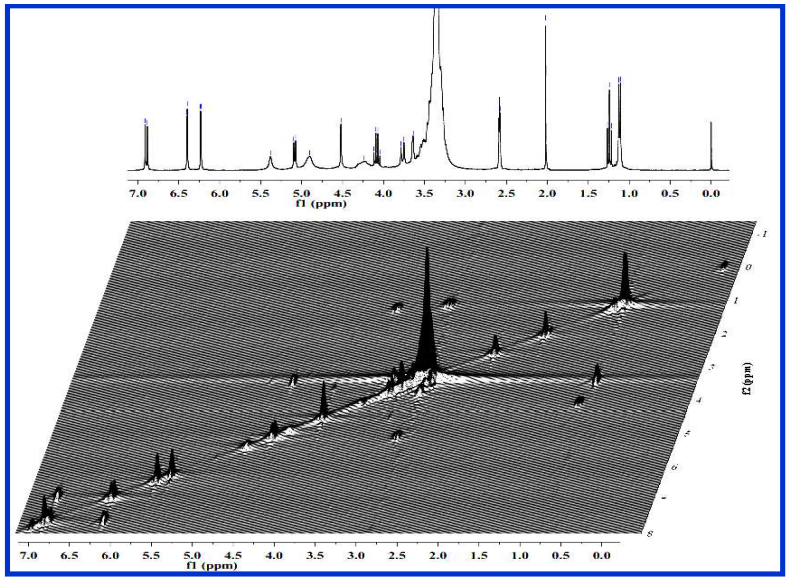
(Fig. 5) IR spectrum of compound 1



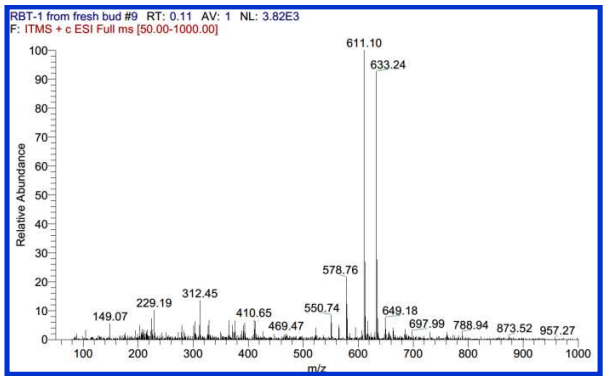
(Fig. 6)^{1}H NMR spectrum of compound 1



(Fig. 7)Comparison of ^{13}C NMR and DEPT 135 spectrum of compound 1



(Fig. 8) 2D-view of H,H-COSY spectrum of compound 1



(Fig. 9) (A) Positive Mode ESI-MS spectrum of compound 1

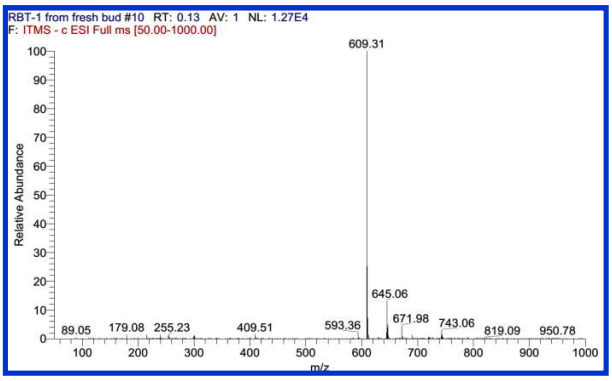


Fig. 9)(B) Negative Mode ESI-MS spectrum of compound 1

CONCLUSION

In conclusion, to the best of our knowledge there has been no prior report on the phytochemical constitution of the flowering buds of *B. tomentosa.* From this study, isolated phytochemical was flavonoid glycoside and we have developed the new methodology to isolated that under greener medium and excellent yield. It is noteworthy that this methodology is very simple, less time, reliable, eco-friendly solvents like water and ethanol. The natural availability of rutin could warrant further studies to assess their potential as effective natural remedies. The economical and environmental advantage of their protocol adds practical value for the industrial applications.

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