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Isolation and identification of flavonoidfrom *Borreriahispida(linn)*

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ABSTRACT

The objective of the present analysis was designed to isolate the active flavonoid component obtain in the whole plant of *Borreriahispida*.Methanolic extract from the leaves of *borreriahispida*was examined for the separation of flavonoids from a plant part. Phytochemical studies revealed the presence of flavonoid compounds in methanol extract. The separated flavonoid compoundwhere further purified by the TLC and Column chromatography technique. The structures of the isolated compounds were characterized by using UV-Visible, FT-IR,$C^{13,}$ and H^1NMR spectroscopy.Thus the isolated compound was identifiedas 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one i.e., Rutin.Further biological investigations need to be carried out for the isolated flavonoid present in this plant.

KEYWORDS:*Borreriahispida*, separation of flavonoids, UV-Visible,FT-IR,C^{13}& H^1 NMR.

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1. INTRODUCTION

The plant *Borreriahispida(linn)* belonged to the rubiaceae family and it comprises one of the largest angiosperm families, with 650 genera[1] and approximately 13,000 species[2] distributed mainly not only in tropical and subtropical regions but also reaching the temperate and cold regions of Europe and Northern Canada[3] In Brazil, this family comprises about 130 genera and 1500 species distributed across different vegetation formations, with a great occurrence in the Atlantic Forest,This family is currently classified into three subfamilies and over 43 tribes.[4,5]The main objective of the present investigation was to an isolation of active components mainly flavonoid from the whole plant of *Borreriahispida*and followed by characterization using spectroscopy methods such as UV-Visible, FT-FT-IR, and C^{13}& H^{1} NMR. From the review reports the medicinal value of the plant was very rich and not much work has been carried out in this plant.

2. EXPERIMENTAL SECTION

2.1. Collection of Plant material

The whole plant of *Borreriahispida*(Linn), were collected from Pachur, Thanjavur District of Tamil Nadu, India. Taxonomic identification was made from azymes biosciences by bangalore. The whole plant of *Borreriahispida*(Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

2.2. Extraction

The powdered plant materials were successively extracted with Hexane (35-65^{0}C) by hot continuous percolation method in Soxhlet apparatus for 48 hours. Then the extract was filtered and concentrated by using a rotary evaporator.Then the remaining marc was subjected to Chloroformand extracted for 48 hours as such hexane extract. Similarly, ethyl acetate and methanol extracts were collected and concentrated. All the extracts were subjected to freeze-drying in a lyophilizer until the dry powder was obtained and stored in screw cap vial at 4C until further use.

2.3. Separation of flavonoid from the methanolic extract of Borreriahispida

The methanol extract was further taken for separation process because of flavonoid test shows a positive result in methanol extract. Few spots of methanol extract were spotted on preparative TLC i.e. Preparative Thin Layer Chromatography. The mobile phase of the system is usedas methanol: glacial acetic acid: water (90:5:5) and benzene: acetic acid:water (60:30:10).The Rf value of the spot was noted and TLC was visualized in UV-Chamber for the identification of spots.

2.4. Isolation of flavonoid by using Column Chromatography:

The 20gms of methanolic extract of *Borreriahispida*was mixed with 20gms silica gel (60/120 meshes) to get uniform mixing. 200gms of silica gel (70/325 meshes) was taken then loaded in a suitable column and packed very carefully without air bubbles; petroleum ether was used for slurry preparation. The column was kept aside for 1 hour and allowed for close packing. The admixture was then added at the top of the stationary phase and started separation of compounds by the eluting with various solvent mixtures mentioned in the TLC. All the column fractions were collected separately and concentrated under reduced pressure. The concentrated fraction was further used for structural analysis.

2.5. Chemical identification of flavonoids:

The following tests were performed for the confirmation of the presence of flavonoid fraction.[6,7,8]

2.5.1. Shinoda Test

To a small amount of test solution inalcohol, magnesium ribbon was added followed byaddition of drops of concentrated hydrochloric acid;formation of pink colour confirms the presence offlavonoids.

2.5.2. Zn- Hydrochloride Reduction Test

To the test, solutionadds a mixture of zinc dust and concentrated hydrochloricacid. Heat the solution, after a few minutes, a color of thesolution changes to red.

2.5.3. Aluminum Chloride Test

To a small amount of testsolution, two drops of 1% aluminum chloride were added,yellow coloration indicates the presence of flavonoid.

2.6. Characterisation of Flavonoid

The separated compound was identified by a phytochemical screening test and further taken for spectral studies for the characterization purpose. The UV-visible Absorption spectrum of CD-1 was taken, and methanol was used as a reference solvent. Functional groups are identified by FT-IR and, it was conducted by a minimal amount of CD-1 mixed with spectroscopic grade KBr then well grounded before preparing the pellet. Proton NMR (H^1-NMR) and Carbon-13 NMR (C^{13}-NMR) were analyzed in Bucker NMR 200MHz spectrophotometer. DMSO and TMS were used as the solvent and internal standard respectively.

3. RESULT AND DISCUSSION

3.1. Separation of Flavonoids:

Figure1: TLC image of Methanol extract

Figure2: Column Chromatogram of methanol extract

The result of TLC shows yellow bands with Rf value 0.43 and 0.32 were obtained in solvent system of methanol: glacial acetic acid: water (90:5:5) and benzene: acetic acid: water (60:30:10). Using the column chromatography the flavonoid was isolated from crude methanol extract. The images of TLC and Column Chromatography were sown in figure-1 and 2.On the basis of phytochemical and spectroscopic studies the characterization and structural elucidation of the compound was done as follows. The final line of results shows in the pictures.

3.2. Characterisation of Flavonoid

The colour of the isolated compound was pale yellow or light yellow powder. The melting point of the isolated compound was 190^0C. The UV spectrum of this compound exhibited two major absorption peaks in the region 354nm and 288nm, which indicates the presence of flavonoid nucleus structure and it was shown in figure-3.

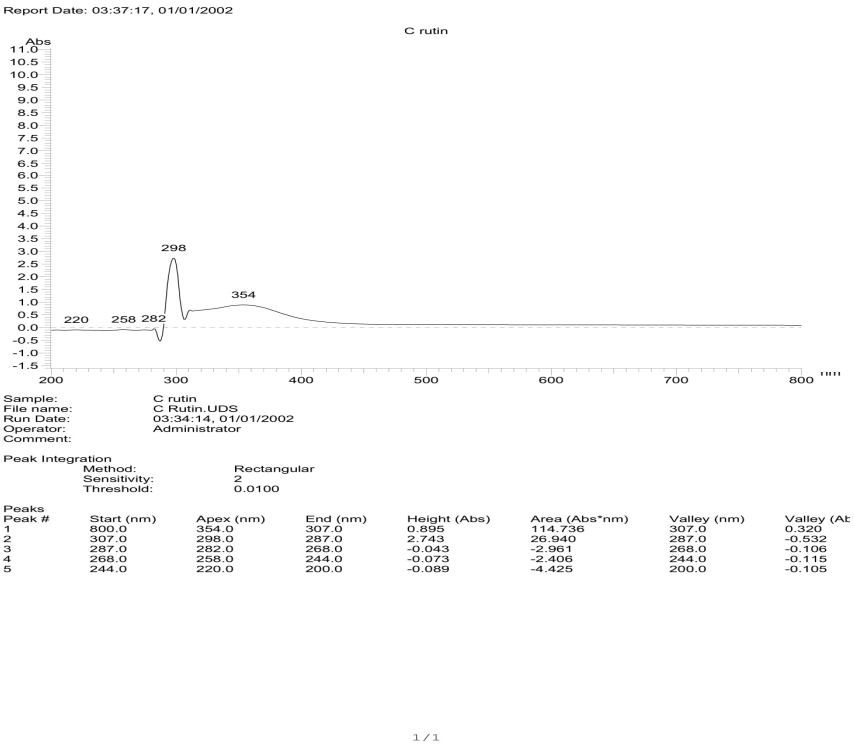

Figure-3: the UV - Visible spectrum of Flavonoid

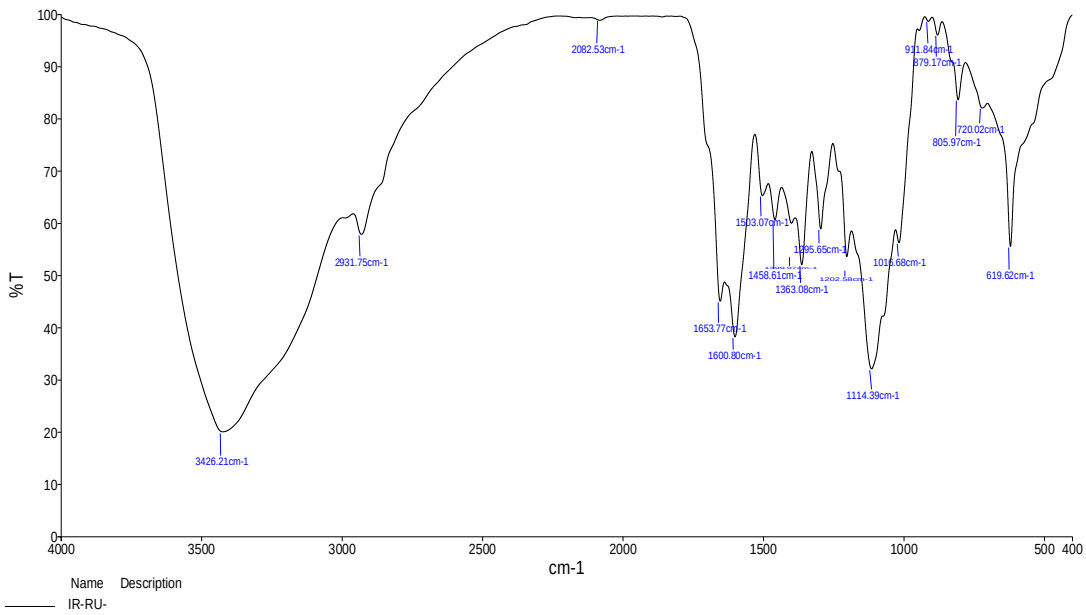

Figure-4: the FT-IR spectrum of Flavonoid

FT-IR spectra showed OH-stretching at 3426.21 cm^{-1},CH_2 stretching at 2931.75 cm^{-1},CH-bonding at 2082.53 cm^{1},COgroup at 1458 cm^{-1},C-OH vibration at 1363.07cm^{-1}. The FT-IR result of the flavonoid was showed in the fig-4.

^{1}H-NMR report reveals the peak with different δ values and its possible notations are explained and are (300 MHz, CD1) δ ppm = 6.84 (*d, J=2.0* HZ, 1H,H-6), 6.39 (*d, J*=2.0Hz,1H,H-8), 7.55 (*d,J*=2.05Hz,1H,H-2'), 6.88 (*d, J*=8Hz,1H,H-5'), 7.62 (*dd, J*=8.5 & 2.2Hz, 1H, H-6'), 5.11 (*d, J*=7.5Hz, 1H,H-1"), 4.39 (*d,J*=1.5Hz,1H,H-1"'), .99 (*d,J*=6.3Hz,3H,H-6"'), 3.32-3.86(m). The H^{1}-NMR of flavonoid shown in figure-5.

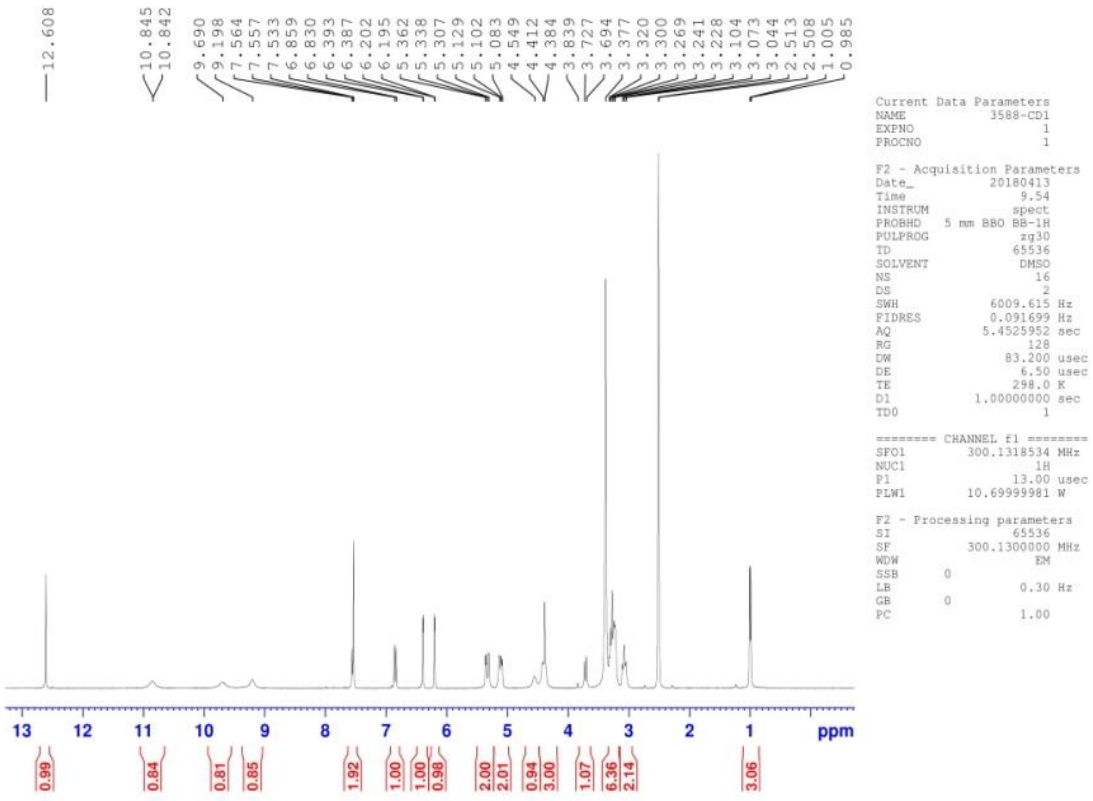

Figure-5: the H^{1} - NMR spectrum of Flavonoid

In ^{13}C-NMR reports and its interpretations are given below [(9)] and are (75.5 MHz,CD3OD) δppm=158.6 (C-2), 135.8 (C-3), 177.8 (C-4), 161.64 (C-5,) 101.4 (C-6), 164.5 (C-7), 94.1(C-8), 157.0 (C-9,) 104.4(C-10,) 121.8(C-1'), 116.7 (C-2'), 145.1 (C-3'), 148.8 (C-4') 116.7 (C-5'), 123.4 (C-6'), 104.4 (C-1'') 76.5(C-2''), 77.3 (C-3''), 70.9 (C-4''), 78.1 (C-5'') 68.7 (C-6''), 102.5 (C-

1’’’), 72.3 (C-2’’’), 72.2 (C-3’’’) 73.9 (C-4’’’), 68.7 (C-5’’’), 18.1 (C-6’’’). The result of the C^{13} – NMR was showed in figure-6.

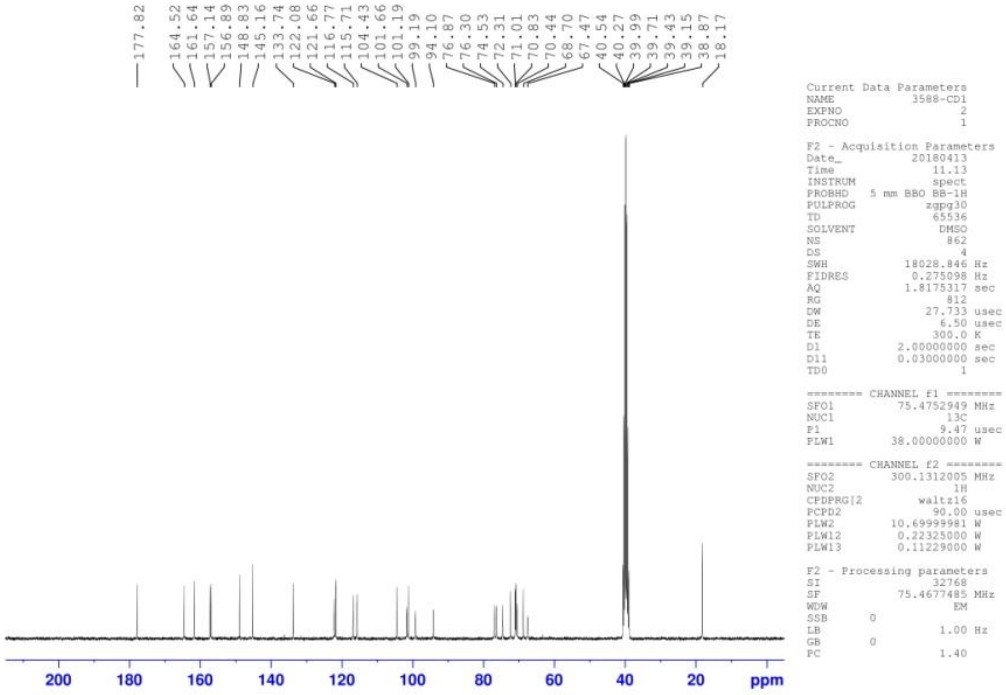

Figure-6:the C^{13} - NMR spectrum of Flavonoid

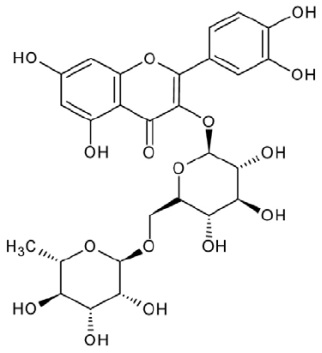

Figure-7: Structure of Rutin

From the physical, chemical and spectral characteristics it was confirmed that the isolated compound was 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl 6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside i.e., Rutin and the structure was given in figure-7.. The molecular weight of the isolated compound was 610.521 and its corresponding molecular formula was C_{27} $H_{30}O_{16}$.

4. CONCLUSION

This research work concluded that the methanol extracts mostly separate flavonoids from the plant materials and methanol solvent was a suitable solvent for extraction offlavonoids. The Chromatographic technique such as TLC and Column can be engaged for the isolation of flavonoids compounds. The study mainly reveals the occurrence ofRutinin this plant. It has been earlier reported in other plants but this is the first reportof evidence of Rutin in this plant[10]The advantages of Rutinwas, it protects against DNA damage.[11]It is protectiveagainst carcinogenesis and inhibits low-densitylipoprotein (LDL) peroxidationRutin possesses antioxidantactivityand also used in the treatment of various diseases such as capillary bleeding and moreover, it increased capillary fragilityand permeability.[12]Flavonoids are a part of human diet.The whole plant*Borreriahispida*contains high quantity of Rutinand synthesis of Rutin from *borreriahispida*may be ofeconomic benefit and easily available. The future scope of Rutin aimed to analyze the biological application in In-Vitro and In-Vivo studies.

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