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# Isolation and characterization of secondary metabolites from hexane:benzene:acetone (1:1:1) leave extracts of *Pithecellobium dulce* leaves.

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**ABSTRACT**
***Objective: The present study describes the isolation and characterization of valuable secondary metabolites from the leave of Pithecellobium dulce (P. dulce).***
***Methods: The dried leaves of P. dulce were collected, dried, grinded and extracted in soxhlet extractor with hexane: benzene: acetone (1:1:1). The same was subjected to qualitative TLC analysis and finally the isolated compounds were identified using IR, $^{1}$HNMR, $^{13}$C NMR, Mass spectroscopy[15] techniques.***
***Results: The result of the present investigation showed that the compound 2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4H-chromen-4-one was obtained which is recognized as rutin in methanol fraction. Its melting point is 315-320 $^{O}$C. Its molecular formula and weight is $C_{27}H_{30}O_{16}$.***
***Conclusion: The obtained leave extract is rich source of rutin which is having strong antioxidant power and can be used for medicinal purpose.***

**Keywords:** Pithecellobium dulce, leave extract,TLC, NMR and IR- Spectroscopy.

## INTRODUCTION

The seed, root, fruits, bark, leaf and stem of plant *Pithecellobium dulce* (P. dulce) have been known for their medicinal values(1). For long the leave of P. dulce has been used as antioxidant, anti-diabetic and antibacterial drug(1,2). Although, previous investigation have reported the presence of different types of steroids, terpenoids, flavonoids, steroids and their glycosides in the same(1,3). But their chemical nature, composition and protective efficacy differ with the extraction method(2,4). Moreover, different extraction medium contain different active constituents so medicinal properties of the same herb vary with extraction system. In present study, hexane:benzene:acetone (1:1:1) leave extracts of *P. dulce* was investigated for the presence of bioactive compound. So, in this chapter we have discussed the isolation and identification of compound from the hexane: benzene: acetone (1:1:1) extract of pithecellobium dulce (P. dulce) leaves.

## MATERIAL AND METHODS

**Collection, Extraction and isolation:**

The dried leaves of *P. dulce* were collected from D- 1 University campus, Dewas road Ujjain, M.P., India, and identified by Dr. Zia-Ul Hasan, Dept of Botany, Safia college of Science, Bhopal (M.P.) and the voucher specimen 439/ Bot /saf/13 was deposited in the Safia college of Science, Bhopal (M.P.). For the experimental use, the powdered leaves were exhaustively extracted in soxhlet extractor with hexane: benzene: acetone (1:1:1). Removal of solvent under reduced pressure afforded solid mass which was stored for chemical investigation.

**Investigation of hexane:benzene:acetone (1:1:1) extract of *P.dulce*:**

The hexane:benzene:acetone(1:1:1) extract was subjected to qualitative TLC analysis to revealed the presence of 4 compounds(2-4). The compounds were separated by column chromatography on silica gel grade III. The obtained eluents were further pooled and termed as P1, P2, P3, P4, P5 and P6 respectively. After that 10ml chloroform and 10 ml of distilled water was added in each pool fractions, which were separated by separating funnel. The chloroform layer was taken and termed as p2c, p3c, p4c, p5c, p6c. The p5c fraction has shown good UV results and re-column of p5c fraction with pure methanol and single compound was obtained, which led into the isolation of one compound in pure form designated as **$P_5C$**. The purity of the P5C compound was tested and confirmed by TLC. The P5C fraction has shown good UV results and column chromatography of p5c fraction with

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pure methanol yielded single compound in pure form designed as ($P_5C$). The compound was identified using IR, $^{1}$HNMR, $^{13}$C NMR, Mass spectroscopy[15] techniques.

**Column Chromatography**

Silica gel 60 (0.06-0.2 mm, 60-120 mesh) was used as stationary phase. Silica gel 40 g, were used for packing the column. The stationary phase was suspended in water and stirred gently until slurry suspensions were formed. Glass open column (2.5 cm inner diameter and 75 cm length) was cleaned and tightly clamped in upright position. Small pieces of glass wood were placed at the bottom of columns using glass rod. The suspensions were then poured carefully and slowly into the column. Solvent system were allowed to run out of the column slowly, not less than three hours to allow silica particles to pack. It was very important not to let the level of solvent system go below the surface of stationary phase since, if this occurs, air will enter the bed, it will lose its even packing and thus uneven flow of solvent system. The phytoconstituents were detected using spectroscopic analysis. The pools were subjected to further purification by successive solubility studies.

**Results:** The result of the present investigation showed that the compound 2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4H-chromen-4-one was obtained which is recognized as rutin in methanol fraction. Its melting point is 315-320 $^{O}$C. Its molecular formula and weight is $C_{27}H_{30}O_{16}$. The peaks were observed in the IR spectrum are 3418,2984, 1493,1360,1656, 1603, 1493and 806 $cm^{-1}$.

**$^{1}$H NMR spectra of $P_5C$ :** δ6.40(d,H-29), 6.21(d,H-27), 5.11(d,H-11), 4.52(d,H-2), 3.63(dd,H-3), 3.61(dd,H-8), 3.53(dd,H-4), 3.4(m,H-8,13,6,12), 3.31(m,H-14,5,9) and 1.12(d,H-18)ppm.

**$^{13}$C spectrum NMR (δ in ppm)** : 157.3 (C-2),134.1 (C-3), 178.2 (C-4), 157.5 (C-5), 99.5 (C-6), 164.9 (C-7), 94.5 (C-8), 162.1 (C-9), 104.8 (C-10), 122.5 (C-1'), 116.1(C-2), 145.6 (C-3'), 149.3 (C-4'), 117.1 (C-5'), 122.0 (C-6'),101.6 (C1-G), 74.9 (C2-G), 77.3 (C3-G), 72.7 (C4-G), 76.7 (C5-G), 67.9 (C6-G), 102.2 (C1-R), 70.8 (C2-R), 71.2 (C3-R), 71.4 (C4-R), 69.1(C5-R), 18.6 (C6-R).

**Mass Spectrum:** m/z 612,614, 449, 305, 626, 302, 615, 465, 611, 613, 301, 304, 303 and 466.

**Table- $^{1}$H NMR Spectrum of $P_5C$**

| S.No. | Shift (ppm) | H's | Type | J (Hz) | Atom | Range (ppm) |
|---|---|---|---|---|---|---|
| **1.** | 6.401 | 1 | D | 2.07 | H-29 | 6.37-6.43 |
| **2.** | 6.214 | 1 | D | 2.08 | H-27 | 6.19-6.24 |
| **3.** | 5.113 | 1 | D | 7.70 | H-11 | 5.09-5.14 |
| **4.** | 4.521 | 1 | D | 1.49 | H-2 | 4.50-4.55 |
| **5.** | 3.632 | 1 | Dd | 3.42, 1.86 | H-3 | 3.60-3.66 |
| **6.** | 3.612 | 1 | Dd | 11.02, 1.41 | H-8 | 3.76-3.85 |
| **7.** | 3.531 | 1 | Dd | 9.51, 3.45 | H-4 | 3.50-3.56 |
| **8.** | 3.447 | 4 | M | 1.16 | H-8,13,6,12 | 3.37-3.49 |
| **9.** | 3.313 | 3 | M | 2.10 | H-14,5,9 | 3.21-3.33 |
| **10.** | 1.124 | 3 | D | 6.22 | H-18 | 1.07-1.113 |

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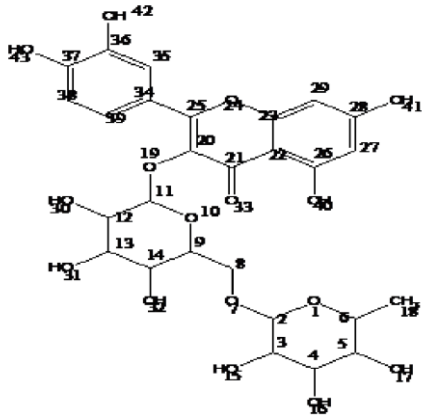

2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1? 6)-β-D-glucopyranosyloxy]-4H-chromen-4-one

**Table- $^{13}C$ spectrum NMR of $P_5C$**

| S.No. | Shift (ppm) | C's | Atom | Range (ppm) |
|---|---|---|---|---|
| **1.** | 178.2 | 4 | 4-C | 179.11-177.85 |
| **2.** | 164.9 | 7 | 7-C | 165.43-163.22 |
| **3.** | 162.1 | 9 | 9-C | 163.52-160.98 |
| **4.** | 157.5 | 5 | 5-C | 159.08-156.92 |
| **5.** | 157.3 | 2 | 2-C | 157.35-157.29 |
| **6.** | 149.3 | 4 | 4'-C | 150.13-148.78 |
| **7.** | 145.6 | 3 | 3'-C | 146.37-144.51 |
| **8.** | 134.1 | 3 | 3-C | 135.61-133.74 |
| **9.** | 122.5 | 1 | 1'-C | 123.16-120.89 |
| **10.** | 122.0 | 6 | 6'-C | 122.97-121.91 |
| **11.** | 117.1 | 5 | 5'-C | 118.05-116.02 |
| **12.** | 116.1 | 2 | 2-C | 116.87-115.92 |
| **13.** | 104.8 | 10 | 10-C | 105.37-103.57 |
| **14.** | 102.2 | 1 | 1-r-C | 103.11-101.23 |
| **15.** | 101.6 | 1 | 1-g-C | 101.88-100.21 |
| **16.** | 99.5 | 6 | 6-C | 100.13-99.38 |
| **17.** | 94.5 | 8 | 8-C | 95.78-92.76 |
| **18.** | 77.3 | 3 | 3-g-C | 78.94-77.22 |
| **19.** | 76.7 | 5 | 5-g-C | 77.15-75.38 |
| **20.** | 74.9 | 2 | 2-g-C | 75.74-73.81 |
| **21.** | 72.7 | 4 | 4-g-C | 73.71-72.05 |
| **22.** | 71.4 | 4 | 4-r-C | 72.01-71.31 |
| **23.** | 71.2 | 3 | 3-r-C | 71.30-70.97 |
| **24.** | 70.8 | 2 | 2-r-C | 70.91-69.82 |
| **25.** | 69.1 | 5 | 5-r-C | 69.76-68.64 |
| **26.** | 67.9 | 6 | 6-g-C | 68.91-66.33 |
| **27.** | 18.6 | 6 | 6-r-C | 19.57-17.68 |

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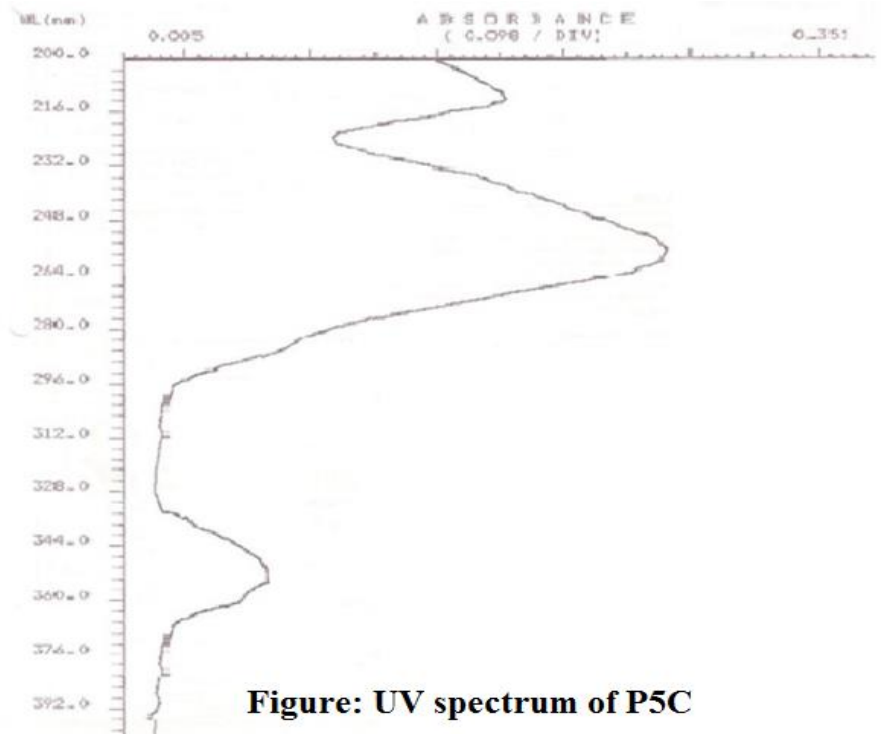


**Figure: UV spectrum of P5C**

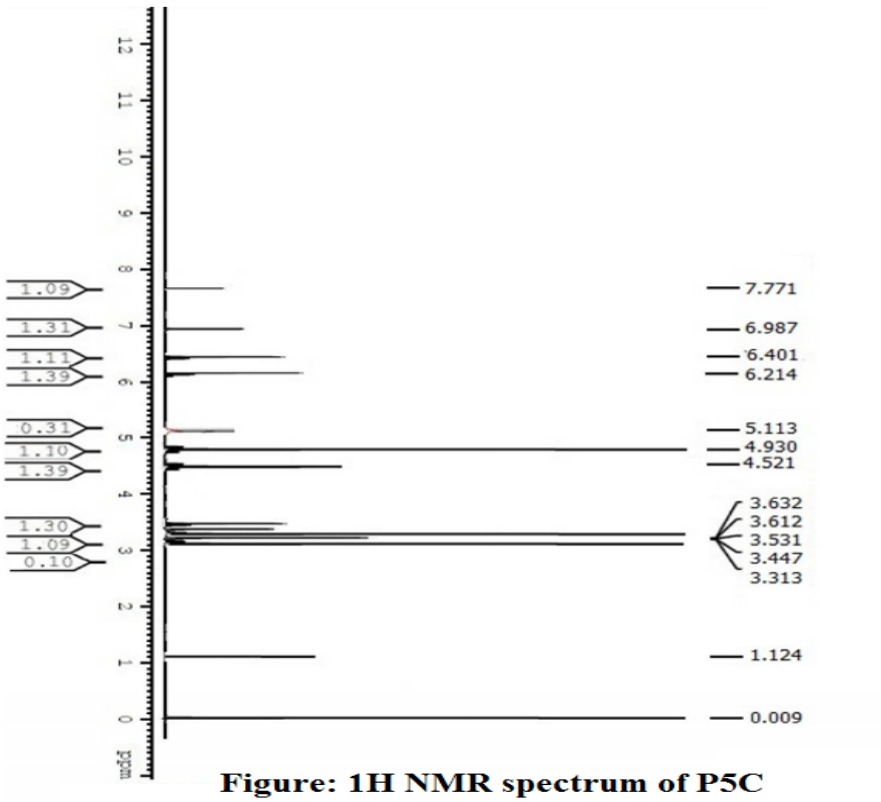


**Figure: 1H NMR spectrum of P5C**

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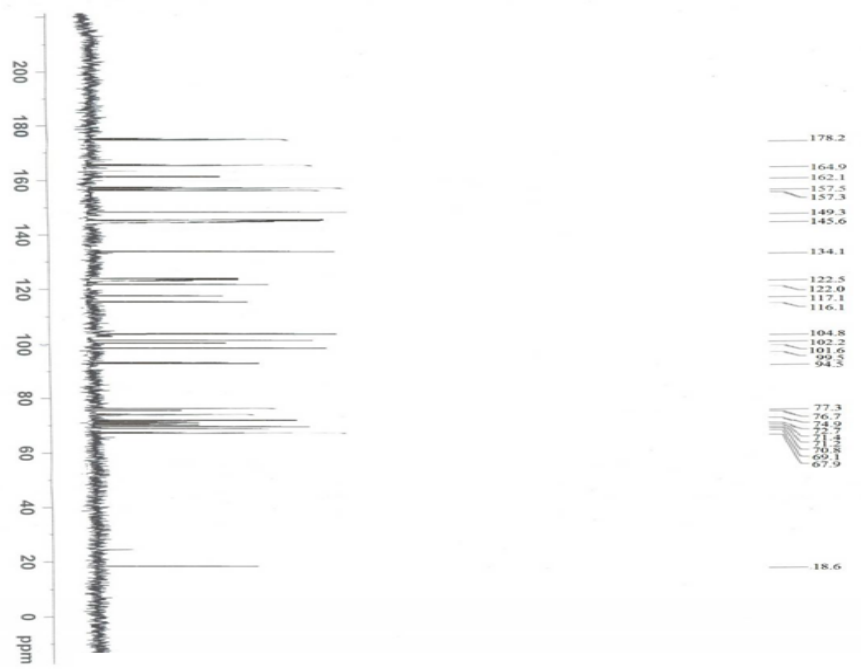


**Figure: 13C NMR spectrum of P5C**

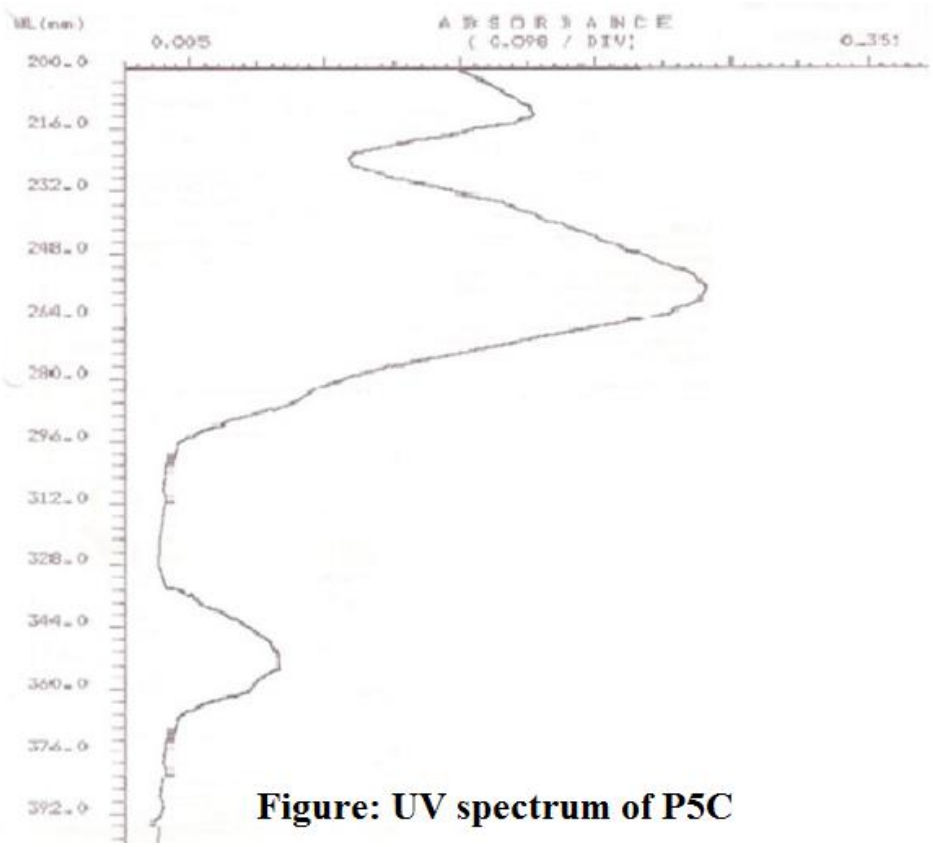


**Figure: UV spectrum of P5C**

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## DISCUSSION:

The IR spectrum of the compound P5C showed its structure as described above. The mass spectrum of the compound showed molecular ion peak at m/z 612 associated with the molecular formula $C_{27}H_{30}O_{16}$. The 1H NMR spectrum and 13C NMR spectrum revealed the structure of isolated compound that is the compound 2-(3, 4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4H-chromen-4-one was obtained which is recognized as rutin in methanol fraction. Which is a known antioxidant molecules(6-8). Whose melting point is 315-320 $^{O}$C. The peaks were observed in the IR spectrum are 3418,2984, 1493,1360,1656, 1603, 1493and 806 $cm^{-1}$. The further analysis revealed that it's a glycoside combining the flavonol quercetin(9,10). It a type of citrus flavonoid found in a wide variety of plants parts(1,5,11,12). The presence of steroid skeleton was confirmed by the 13C NMR signals.

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