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A Novel Yellow Dye Phytoconstituent from The Leaves of *Rhus Parviflora*

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Abstract: Present study is revealed the phytochemical analysis of *Rhus parviflora* leaves. There are more than 250 species make up the genus Rhus (family: Anacardiaceae, order: Sapindales), which is found in temperate, subtropical, and tropical climates. In the past, Rhus species extracts and products have been widely employed in traditional textile dyeing techniques and medicine to cure bacterial, fungal, and protozoal illnesses in both humans and animals. They are also considered to be significant remedies. The majority of biological activities are ascribed to phytochemical constituents found in the different Rhus species, such as flavonoids, phenolic acids, and terpenoids. The current investigation utilized some advanced spectroscopical techniques like UV-Vis, FT-IR, EI-MS, ^{1}H-NMR, ^{13}C-NMR, DEPT-NMR, COSY-NMR and HMBC-NMR to isolate and characterize as naringenin-4'-methyl ether-7-O-β-D-xylopyranoside, a novel phytochemical ingredient derived first time from the leaves of *Rhus parviflora* collected from Pauri (Garhwal), Uttarakhand, India.

Keywords: Phytochemical analysis . *Rhus parviflora* . UV-Vis . FT-IR . EI-MS . ^{1}H-NMR . ^{13}C-NMR . 2D NMR

Introduction

Medicinal plants store extractable organic matter in sufficient quantities to be used profitably as an active ingredient in medicines to treat a wide range of illnesses. These substances are what give plants their therapeutic properties and play a significant role in medicine. Their significance in terms of pharmacological (Anonymous, 2006) and subsequently economic value has not diminished to this day. Either primary or secondary metabolites can be made of these chemicals. Due to their function in basic metabolism, primary metabolites, which are often found in bulk volume and are concentrated in seeds and other vegetation storage organs, are essential to the physiological growth of the plant. Although secondary metabolites are not directly involved in a plant cell's regular growth, development, or reproduction, their involvement in protecting the cell from predators makes them more significant illnesses. The medicinal value of a plant is found in these secondary metabolites, which have a specific physiological effect on the human body. These are exclusively present in a few distinct plant organs and are produced in specialized cells from primary metabolites. Since ancient times, natural product-based remedies—primarily medicinal plants—have been used to heal human ailments. This is because the entire Garhwal Himalayan belt is a rich source of medicinal plants in India (Gour RD, 1999). The sub-deciduous shrub *Rhus parviflora Roxb.* (Belongs to the family Anacardiaceae) has spherical berries and irregularly crenate, trifoliate leaves (Fig. 1).

This plant species ranges in elevation from 700 to 1,600 meters and is found in India, Nepal, Bhutan, and Sri Lanka. It is frequently referred to as "sumac" or "Tung" in Uttarakhand, India. It is also referred to as "Tunn or Tungla" and is a frequent ingredient in Ayurvedic medicines that treat stomach and neurological disorders. When Bhakuni (Bhakuni DS et al., 1971) previously studied the native medicinal plant *Rhus parviflora*

Roxb, he found that it contained quercetin and isorhamnetin-3-arabinoside.


Figure 1. A plant and Shaded dried leaves of *Rhus parviflora*

In light of the previous finding of myricetin in Rhus species, the hunt for myricetin glycosides is still ongoing. Previously, *Rhus parviflora* leaves were analyzed for the study and there were three flavonols such as myricetin, quercetin, and kaempferol as well as two aromatic acids as p-coumaric acid, caffeic acid and 1-hydroxy-8-acetoxy-10α-11β-methyl eudesma-(5)-ene-12, 6-olide (Singh SV et al., 2021) were identified. The leaves also contained a variety of physiologically active substances, many of which have been demonstrated to possess antimicrobial qualities. Moreover, the fruits of *R. parviflora* were used to isolate chysoeriol-7-O-β-D-glucopyranoside, luteolin-7-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranoside, quercetin-3-O-β-Dgalactopyranoside, and quercetin-3-O-α-l-rhamnopyranoside (Shrestha S et al., 2012a). There are some flavonoids such as agathisflavone, fustin, taxifolin, aureusidin cupressuflavone and mesuaferrone B (Shrestha S et al., 2013a) have also been previously reported from this species. In addition, there are nine phenolic compounds (Shrestha S et al., 2013b): leonuriside A, 3-methoxy4-hydroxyphenol-1-O-β-D-glucopyranoside, p-hydroxybenzoic acid-4-O-β-D-glucopyranoside, phloracetophenone-4-O-β-D-glucopyranoside, and cis-p-coumaric acid and trans-p-coumaric acid and -4-O-glucopyranoside. The fruits of *R. parviflora* were used to isolate -4-O-β-D-glucopyranoside, trans-p-coumaric acid, 9-O-β-Dglucopyranoside, (–)-shikimic acid, and (–)-methyl shikimate. Certain Rhus species are utilized in traditional medicine as antibacterial mixtures or for their cytotoxic qualities, while other species have insecticidal qualities.

A number of Rhus species have also yielded previously isolated forms of several biflavonoids ((Shrestha S et al., 2012b; 2012c), including agathisflavone, rhusflavone, and mesuaferrone B. Sumac (*Rhus parviflora*) is one such plant that is widely used in Turkey and the Middle East and its crushed and dried leaves have been used as a tanning agent due to their high tannin content. Previous phytochemical studies from this plant species (Opiyo SA et al., 2021) have demonstrated the presence of flavones, tannins, anthocyanins, and organic acids in the leaves of this plant species.

Materials and Procedures

Identification of plant and collection of plant material

At an elevation of 1500 meters, leaves of *Rhus parviflora* were taken from the Pauri Garhwal region of Uttarakhand. The collection was recognized from the Botanical Survey of India, Dehradun, and was based on ethnophamocological and ethnobotanical literature. July was the month that the collection was made. For a week, the plants were left outside in the shade in the chemistry departmental laboratory at HNB Garhwal University, Campus Pauri, Uttarakhand, India.

Extraction of plant material

Six kilogram of powdered dry leaves under shade was thoroughly extracted using ethanol at 65–80 degrees Celsius. The plant extract was concentrated in a vacuum rotatory evaporator with decreasing pressure. After the crude mass dried, it was separated by using column chromatography onto silica gel (60–120 mesh) using methanol as a solvent with increasing polarity and chloroform to produce different fractions.

Compound isolation

Compound was isolate after utilizing MeOH and $CHCl_3$ as a mobile phase to fractionate the ethanolic extract on a 100 cm x 5 cm Sephadex LH 20 column, 80 fractions (15 ml each) were produced. The fractions (98:2-90:10) were chromatographed using column chromatography over silica gel that was eluted with $CHCl_3$ and MeOH by increasing polarity.

Spectroscopical analysis, Interpretation and Characterisation

The compound and its structure were verified through the use of contemporary EI-MS, ^{1}H, ^{13}C and 2D NMR (DEPT, COSY and HMBC like advanced techniques) instruments such as the Brukar NMR spectrophotometer and the EI-Mass spectrophotometer. By comparing the obtained data with existing literature, it was determined that only the compound identified as compound RP-2 from this species with that polarity was novel.

Results and Discussion

Following various spectroscopical approaches for spectroscopical investigation, the following spectral data were observed:

A pale yellow needles [MeOH]; Molecular Formula: $C_{21}H_{22}O_9$; Molecular weight: 418.

UV Spectral data: λ_{max} (MeOH) nm: 286, 332

FT-IR Spectral data: ν_{max} (KBr) cm^{-1}: 3410, 1695, 1580, 1515

EI-MS *m/z*: 435 $[M+H]^+$, 457 $[M+Na]^+$, 273 $[Aglycon + H]^+$ (Fig. 2):

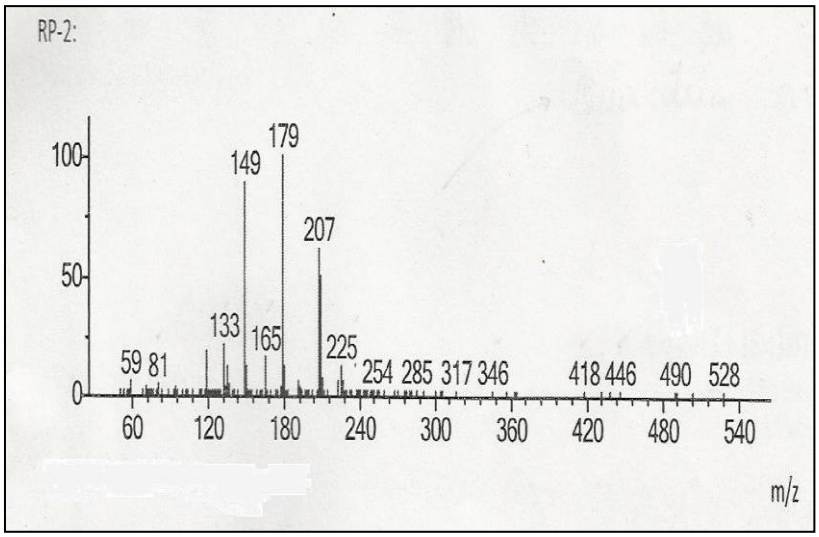

Figure 2.EI-MS Spectra of Compound RP-2

^{1}H NMR spectral data (600 MHz, DMSO-*d6*) : Aglycon d 12.06 (1H, *brs*, 5-OH), 9.61 (1H, *brs*, 4`-OH), 7.33 (2H, *d*, *J*= 8.5 Hz, H-2`,6`), 6.81 (2H, *d*, *J*= 8.5 Hz, H-3`,5`), 6.16 (1H, *d*, *J*= 2.1 Hz, H-8), 6.14 (1H, *d*, *J*= 2.1 Hz, H-6), 5.51 (1H, *dd*, *J*= 12.7/3.0 Hz, H-2), 3.35 (1H, *dd*, *J*= 17.1/12.7 Hz, H-3ax), 2.76 (1H, *dd*, *J*= 17.1/3.0 Hz, H-3eq); sugar moiety d 4.97 (1H, *d*, *J*= 7.7 Hz, H-1``), 3.68 (1H, *dd*, *J*= 11.8/3.3 Hz, H-6b``), 3.46 (1H, *dd*, *J*= 11.8/5.8 Hz, H-6a``), 3.39 (1H, *m*, H-5``), 3.28 (1H, *t*,

J= 9.2 Hz, H-3``), 3.23 (1H, *dd*, *J*= 7.7/9.2 Hz, H-2``), 3.16 (1H, *dd*, *J*= 9.2/9.1 Hz, H-4``) as in the Fig. 3-4.

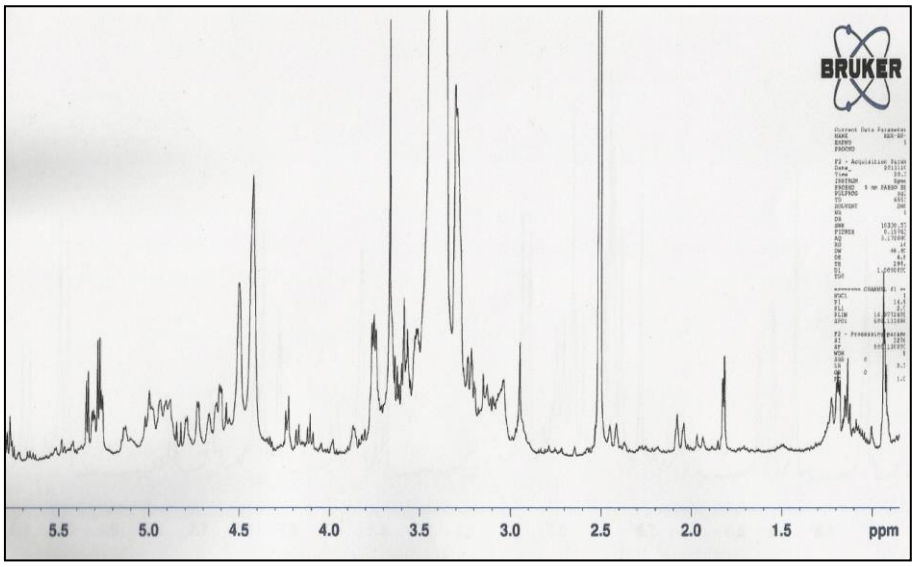

Figure 3. ^{1}H-NMR Spectra of Compound RP-2

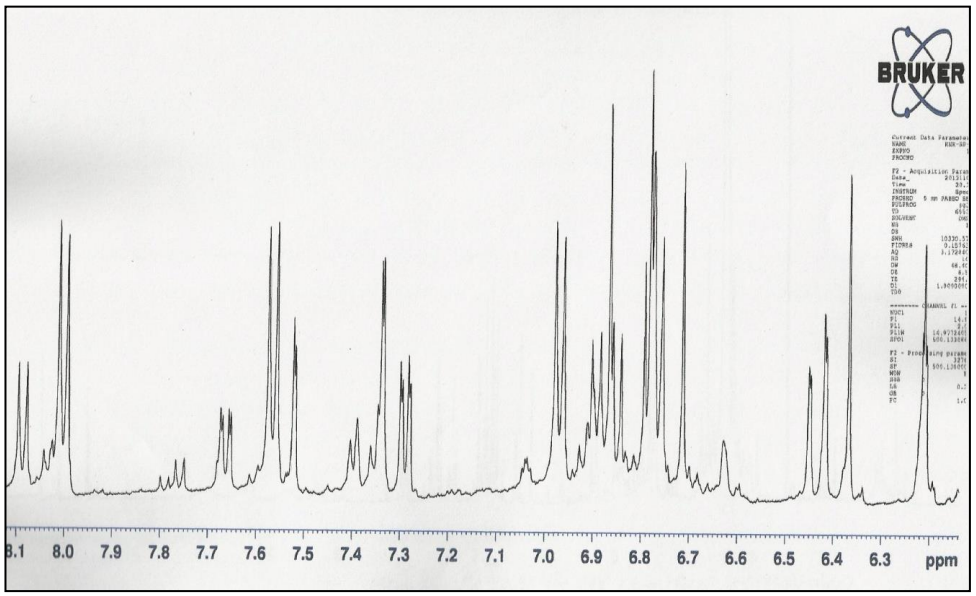

Figure 4. ^{1}H-NMR Spectra of Compound RP-2

^{13}C NMR spectral data (100 MHz, DMSO-*d6*) : Aglycon d 197.18 (*s*, C-4), 165.28 (*s*, C-7), 162.91 (*s*, C-5), 162.71 (*s*, C-9), 157.77 (*s*, C-4` ), 128.59 (*s*, C-1`), 128.40 (*d*, C-2`,6`), 115.16 (*d*, C-3`,5`), 103.22 (*s*, C-10), 96.46 (*d*, C-6), 95.41 (*d*, C-8), 78.63 (*d*, C-2), 42.03 (*t*, C-3); sugar moiety d 99.57 (*d*, C-1``), 77.04 (*d*, C-5``), 76.28 (*d*, C-3``), 72.98 (*d*,C-2``), 69.46 (*d*, C-4``), 60.53 (*t*, C-6``) as reveals from the Fig. 5.

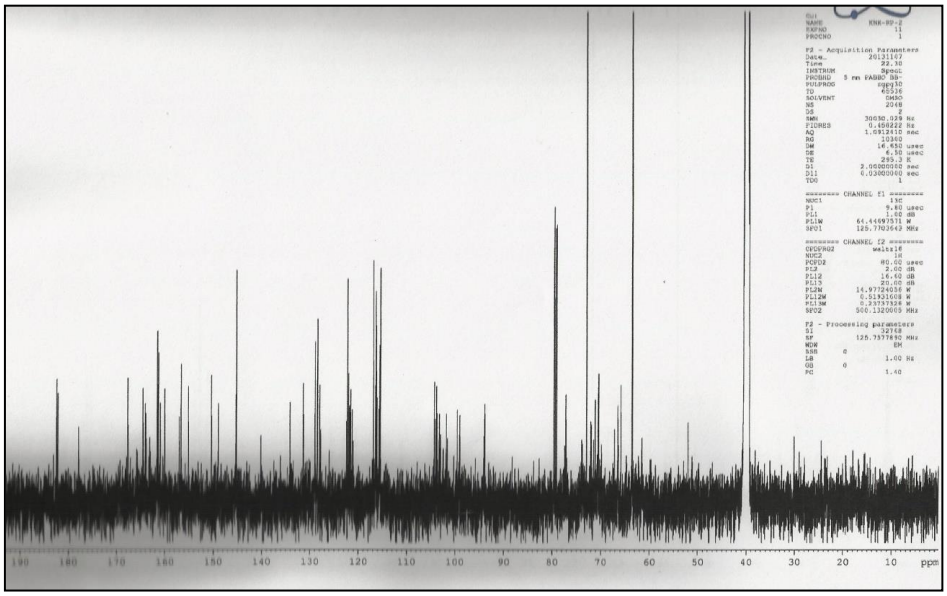

Figure 5. ^{13}C-NMR Spectra of Compound RP-2

At δ 98.00 and 102.00, the deshielded anomeric carbons were measured, whereas the methyl carbon of xylose was detected at δ 17.50 and 18.00 ppm. Until recently, homonuclear spin decoupling was a major factor in the understanding of proton spin-spin coupling patterns. Sadly, this can lead to incorrect interpretations due to instrumental anti facts. That being said, homonuclear decoupling is still very much relevant. The >CH2 and =CH-groups that are present in the current molecule are revealed by DEPT-NMR spectroscopy (Fig. 6).

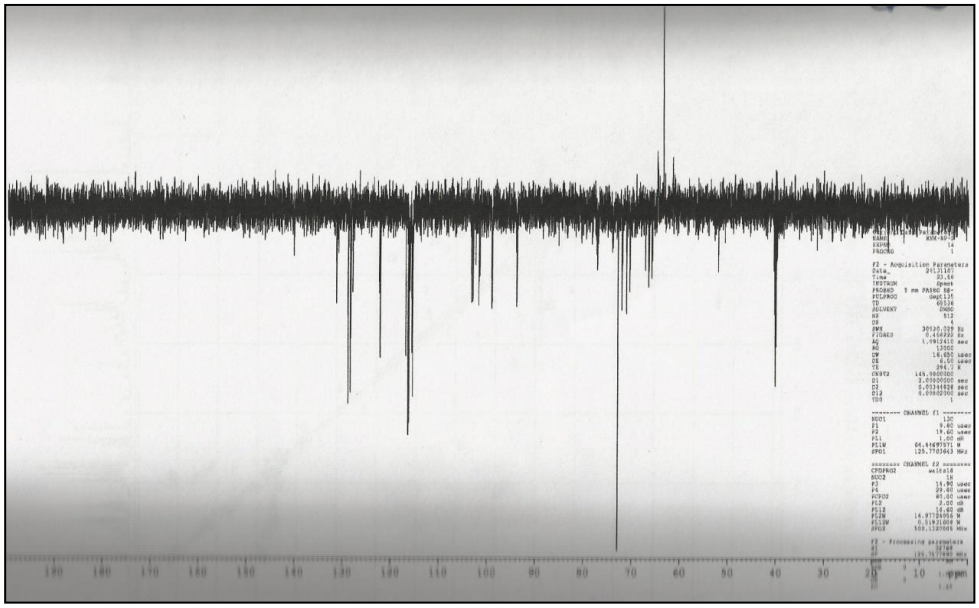

Figure 6. DEPT-NMR Spectra of Compound RP-2

More accurate and definitive results are frequently obtained with the 2D-NMR experiment known as homonuclear correlation spectroscopy (HOMCOR or COSY). An individual HOMCOR experiment, showcasing the whole proton-proton spin coupling and

functioning as a single decoupling experiment (Fig.7).

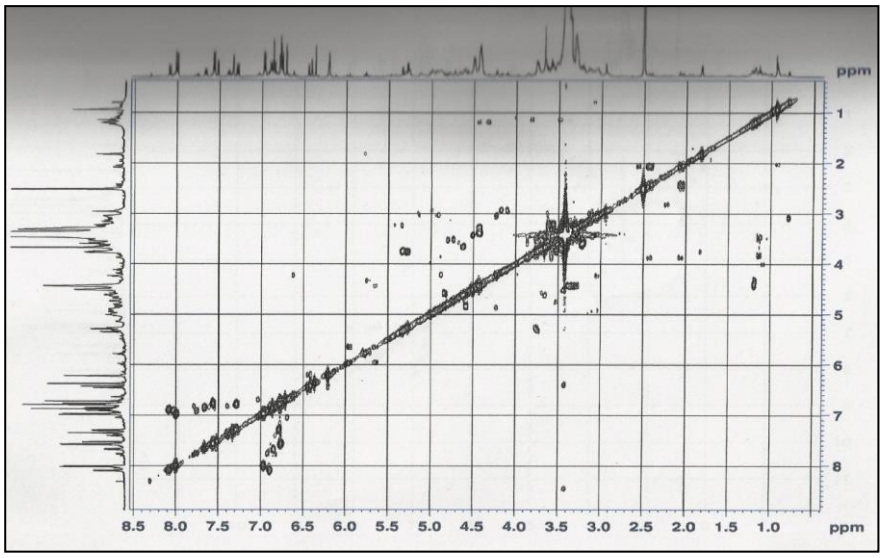

Figure 7. COSY-NMR Spectra of Compound RP-2

Strong coupling between 2', 6' and 3', 5' protons is revealed in ^{1}H-^{1}H COSY spectra of the compound, whereas the simple doublet nature of the 3', 5 protons facilitated glycosylation at 4' OH, and weak meta coupling was seen between the 6 and 8-positions. Because the C-H bonds are perpendicular to the π system, the only source of weak overlap is the C-bonds in their protons. The sugar methyl carbon and anomeric carbons exhibit long range couplings with the remaining sugar carbons, namely C-2, C-3, C-4, and C-5 carbons, in the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 8).

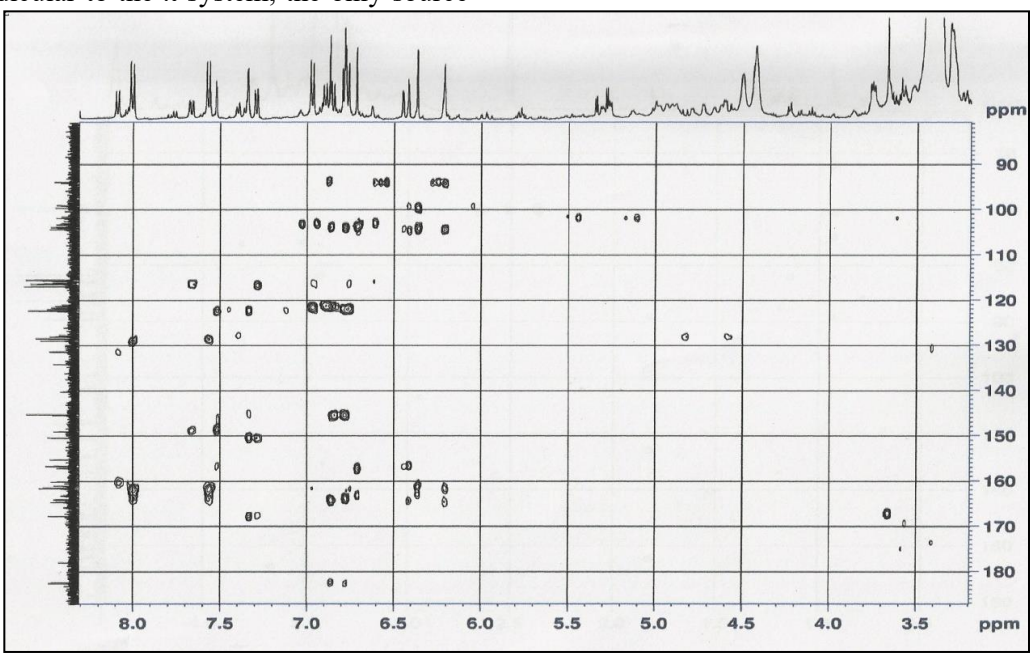

Figure 8. HMBC-NMR Spectra of Compound RP-2

Although certain impurities were present, the structure and naming of the isolated and described chemical compound RP-2, which is first reported from this species, are proven to of spectrum data.

be naringenin-4'-methyl ether-7-O-β-D-xylopyranoside (Fig. 9.) based on the interpretation

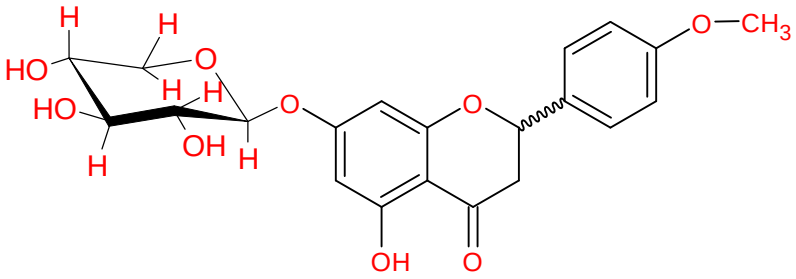

Figure 9. Characterised structure of Compound RP-2

Conclusion

It was concluded from the current study that the phytochemical investigation of *Rhus parviflora* leaves shown a new yellow coloured phytoconstituent after spectroscopical data interpretation, structure revealed named as 'naringenin-4'-methyl ether-7-O-β-D-xylopyranoside' that was identified and isolated for the first time from this species (Fig. 9.)

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