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Chemical investigation of Methanolic Extract of *Piper betle Linn* (Leaf stalk)

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Research Article

Abstract

Natural products have been a major source of drugs for centuries. The leaf stalk of Piper betle is used traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts, and injuries. The main objective of this study is to carry out a phytochemical analysis of the methanol crude extract of the leaf stalk of P. betle. Phytochemical screening on this crude extract revealed the presence of phenols, alkaloids, steroids, terpenes, saponins, and flavonoids. After silica gel column chromatography, the crude extract led to the isolation of compound- 2-[4'-(3''-methylbut-2-en-oxy)-(3',5'-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4 (PBT-V). Characterization of this compound was achieved via spectroscopic methods (NMR, UV, mass spectroscopy, and IR).

Keywords: Piper Betle (leaf stalk), IR, 1H NMR, 13C NMR, Mass Spectroscopy

1. Introduction

Piper betle Linn. (Local name 'Pan') belongs to family *Piperaceae*, is a dioecious, perennial creeper. It climbs by many short adventitious rootlets, and is widely cultivated in hotter and damper parts of the country. It is widespread in damp forests and is cultivated in India and other countries in South East Asia, such as Vietnam and China. In Ayurveda, the leaf of *Piper betle* is used as acrid, healing, tonic, carminative, stomachic, and anthelmintic, aphrodisiac, laxative, and bronchitis, elephantiasis of the leg and to improve appetite. But it should not be taken in eye diseases, leprosy, poisoning thirst, alcoholism, and asthma. In the Unani system of medicine leaves are used to improve taste, appetite, tonic to the brain, in heart and liver diseases. It strengthens the teeth, and clears the throat. The juice of leaves is dropped into the eye for night blindness. In India, the Ayurveda system has described many such medicines based on plants or plant products. The determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action. However, a large number of tropical plants have been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing, etc.

2. Material and methods

The *Piper betle* plant material was collected from Kolkata (West Bengal). The leaf stalk studied was collected from plants grown in Kolkata, West Bengal. A voucher specimen has been deposited at the herbarium of Vikram University, Ujjain (M.P.). The taxonomic identification of

the plant material was obtained from the authorities of the institute of environment management and plant sciences, Vikram University, Ujjain (M.P.) India.

2.1 Extracion by Soxhlet Extractor

About 25 kg of shade-dried material of the plant was grinded or crushed in mechanical stirrer and squeezed to remove water. The squeezed material was dried and extracted with methanol either in cold condition or by Soxhlet extractor. The extract was dried in vacuum and subjected to TLC analysis.

2.2 Processing of *Piper betle Linn. (Leaf stalk)*

The extract was fractionated on a new technique, due to which the time and cost are reduced, in this technique the extract was coated with silica gel (60-120) mesh size in 500ml conical flask and eluted with different solvents in their increasing order of polarity. Due to these techniques, three fractions of different solvents namely n-hexane, benzene, and ethyl acetate are prepared. Since the yield of hexane fraction is not good and work on hexane, benzene extract was already done, so we have not taken it. The ethyl acetate fraction was taken up for the present work. The fractionated ethyl acetate was qualitatively analyzed by thin-layer chromatography (TLC) to know the number of compounds present in it. The ethyl acetate elute was separated by column chromatography using silica gel (60-120) mesh size (Merck) as an adsorbent. The elution of the column was carried out with various solvents and a mixture of solvents in increasing order of polarity.

2.3 Characterization of the compound- PBT-V:

The compound was isolated from chloroform: methanol (6:4 v/v) elutes. The Melting point was found to be 200^0C. The nature of the compound is a dark brown amorphous compound. The mass spectrum and other spectral data revealed its molecular weight of 412 m/z and molecular formula $C_{23}H_{24}O_7$. It is soluble in methanol and water.

2.3.1 IR- Spectrum (λ_{max}, KBr, cm^{-1})

The IR spectrum of **PBT-V** indicated the broad peak at 3350 cm^{-1} indicates the presence of –OH group. The peak at 1714 cm^{-1} due to keto- group (C=O) at 4H-Chromen. The peak at 1074 cm^{-1} is due to methoxy groups. The peak at 2359 cm^{-1} showed the unsaturation at C-2'' of the second molecule[12].

2.3.2 ^{1}H NMR Spectrum (300 MHz, $CDCl_3$, TMS, δ)

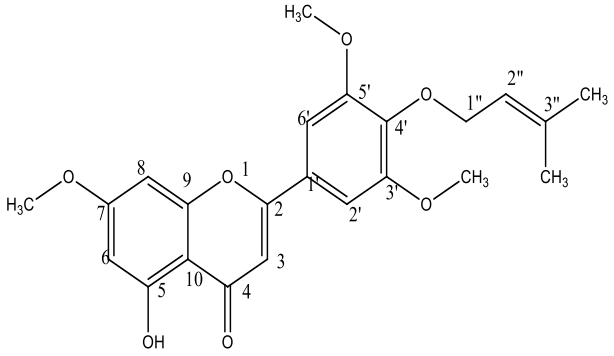
The ^{1}H NMR recorded in the $CDCl_3$ in 300 MHz. ^{1}H NMR δ3.757 showed the singlet nine methoxy protons at C-7, C-3' and C-5' positions. An intense singlet at δ 1.674 was assigned to rest of the two terminal methyl protons at C-3'''. A sharp singlet peak at δ 5.0 confirmed hydroxyl proton at C-5. A triplet at δ5.226 showed a single proton at C-2'' at the double-bonded carbon atom. The characteristic singlet signal at δ 7.225 was assigned to the C-2' and C-6' of the aromatic ring, respectively.

2.3.3 ^{13}C NMR Spectrum (500 MHz, $CDCl_3$, TMS, δ)

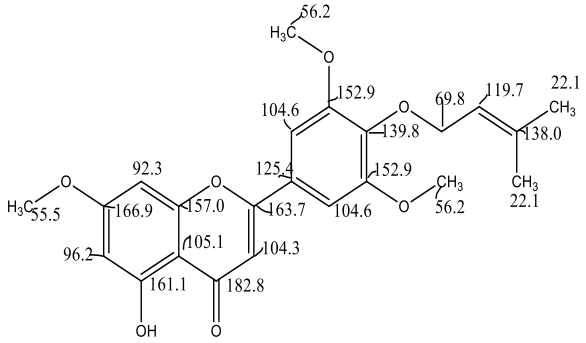
The ^{13}C NMR of the **PBT-V** showed the highest peak at δ182.8 of the carbonyl carbon of Chromen. Out of twenty-three carbons three are methoxy carbons having values δ55.5 (C-7), δ56.2 at (C-3' and C-5'), two are terminal methyl carbons having values at δ22.1 at C-3**'''**, six are methine carbons resonated at δ92.3 (C-8), 96.2 (C-6), 104.3 (C-3), 104.6 (C-6' and C-2'), 119.7(C-2'') and only one methylene carbon confirmed at 69.8 ppm at C-1 position as already discussed in table -7

2.3.4 Mass Spectrum (EIMS, m/z, rel. int. %)

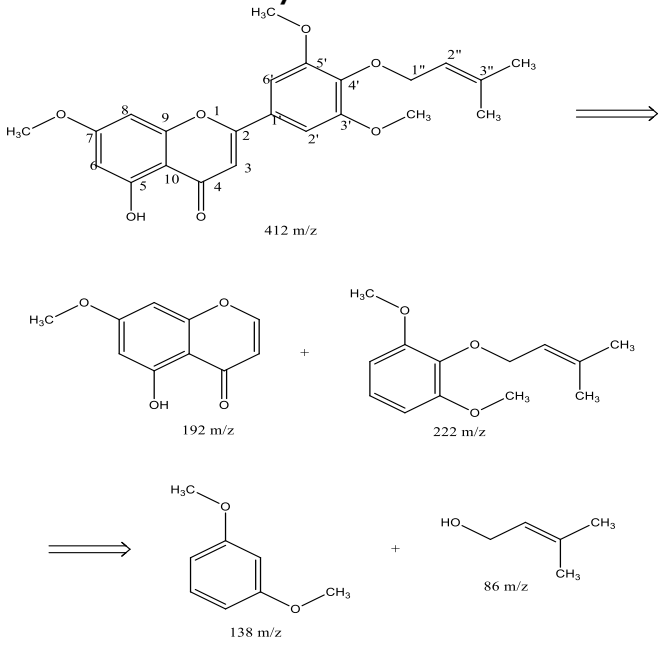
The mass spectrum of **PBT-V** indicated the molecular ion peak at 412m/z. The base peak at 79 m/z shows 100% abundance. Other abundant fragments are; 411m/z, 409 m/z, 379 m/z, 355 m/z, 315 m/z, 222 m/z, 192m/z, 138 m/z, 86m/z, 79 m/z, 64 m/z, and 39 m/z were in agreement with the proposed structure. Mass fragmentation of the compound **PBT-V** is given in the scheme-V.



2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4 (PBT-V)



^{13}C NMR spectral data of 2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4.

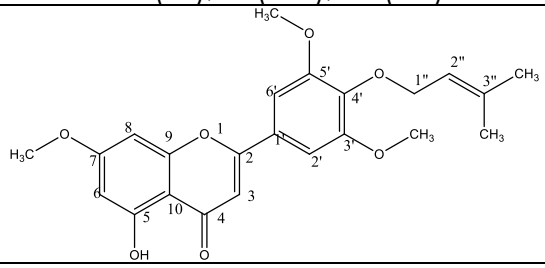


Scheme V: Mass fragmentation of 2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4.

3. Result and Discussion:

The isolation of compound 2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4. (PBT-V) were identified and characterized by using IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy besides by comparing the spectral data with those reported in the literature.

Table 1: Isolation of compound-2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4. (PBT-V)

Molecular formula	$C_{23}H_{24}O_{7.}$
Melting point	200^{0}C
Molecular ion peak	412 m/z
TLC solvent system	Chloroform: Methanol(9:1, v/v)
Recrystallization	Chloroform
Solubility	Methanol
State	Dark Brownish amorphous powder
IR- Spectrum (λ_{max}, KBr, cm^{-1})	3550(-OH) , 1714(C=O), 1074(Me-O-C), 2359(C=C) cm^{-1}
^{1}H NMR Spectrum (300 MHz, $CDCl_3$, TMS, δ)	δ 3.89(s,3H, 1xCH_3), 6.42(s,-CH), 6.237(d,1H,-CH), 6.457(d,1H, -CH), 7.255(s,1H, -CH), 3.757(s, 3H, -CH_3), 4.61(d, 2H,-CH_2), 5.226(d, -CH), 1.674(s,3H, _CH_3), 5.0(s,1H, -OH)
^{13}C NMR Spectrum (500 MHz, $CDCl_3$, TMS, δ)	55.5, 163.7, 104.3, 182.8, 161.1, 96.2, 166.9, 92.3, 157.0, 105.1, 125.4, 104.6, 152.9, 139.8, 152.9, 104.6, 56.2, 69.8, 119.7, 138.0, 22.1 ppm.
Mass Spectrum (EIMS, m/z, rel. int. %)	M+ 411(80), 409 (3.2), 293 (75), 179 (87), 101 (15), 79(100), 64 (1.1).
Structure of the compound	
IUPAC Name	*- 2-[4′-(3″-methylbut-2-en-oxy)-(3′,5′-dimethoxy)phenyl]-5-hydroxy-7-methoxy-Chromone-4.*

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Conflict of Interest: The author declares no conflict of interest.

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