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PHYTOCHEMICAL SCREENING, EXTRACTION AND ISOLATION OF NEW COMPOUND FROM THE *bauhinia blakeana* LEAF EXTRACT

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ABSTRACT

The current study entails extracting phytochemicals from bauhinia blakeana leaves using various solvents and analysing their phytochemical screening results. Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, Tannins, Resin, Phenols, and Carbohydrate are among the secondary metabolites identified by phytochemical investigation. Using column chromatography, the novel phytochemical (7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one) was extracted from the methanol extract. HNMR, CNMR, LC/Ms, and IR analyses were used to confirm the structure of the molecule.

Keywords: *bauhinia blakeana,* Phytochemical, Isolation, NMR, Mass, Characterization

INTRODUCTION

The Hong Kong orchid tree, *Bauhinia blakeana (B. blakeana),* is a hybrid leguminous tree of the genus Bauhinia **[1]**. Large, thick leaves and beautiful purplish red blooms characterize this plant. The fragrant, orchid-like flowers bloom from early November through the end of March, and are 10 to 15 centimeters (3.9 to 5.9 in) across. Although it is currently grown in many places, it was first planted in Hong Kong in 1880, and it appears that all cultivated trees are descended from one that was grown at the Hong Kong Botanical Gardens and widely planted in Hong Kong beginning in 1914 **[2]**. In India, the Bauhinia genus of the Fabaceae family contains roughly 15 species.

A few are climbers, while others are shrubs or trees **[3]**. India is a developing country known for its biodiversity in terms of species, genetics, and habitat. Flowers are employed in all cultural events in poor countries like India **[3]**. Flowers are well-known and widely used for their aesthetic value as well as the colour they emit. Since the dawn of time, medicinal plants have been used in the treatment of ailments in the form of traditional medicine. *B. blakeana* is a modest evergreen tree utilised as livestock feed by Indian tribes **[4]**. Bauhinia species are known for a variety of biological activity. *B. blakeana* is a common species used to cure a variety of diseases in traditional medicine **[5]**. Antidiarrhoeal, anticancer, and thyroid gland stimulating activities have been described for *B. blakeana* **[6, 7]**. Because the blooms are high in phytoconstituents, they are used extensively in the pharmaceutical and nutraceutical industries. All valuable medical remedies were traditionally produced from plants, whether in the form of simple plant components or more complex crude extracts. As a result, the goal of this research was to examine phytochemicals in a dried sample of *B. blakeana* leaf and isolate a new phytocompound.

MATERIALS AND METHODS

Chemicals. All the chemicals used in this investigation were of analytical grade and were obtained from Sigma Chemicals.

Plant collection and identification

The fresh leaves of *B. blakeana* were collected form Trichy local area, Tamilnadu, India. The plant part was authenticated in the Botanical laboratory of the Department of Botony, St.Joseph's College (Autonomous), Tiruchirappalli-620 002, Tamilnadu. The leaves are washed and air-dried, ground in to fine powder for phytochemical analysis.

Sample Preparation

Fresh samples are washed with distilled water and dried in the open air. The dried plant material (leaves) is ground into a fine powder with a laboratory pestle and mortar and an electric grinder, then put into a clean sample container, labelled, and stored for future use. Extractions were performed using the traditional procedure, which involved soaking powdered materials in solvent in order of increasing polarity. Using the cold soaking procedure, a total of 1 kg of powdered sample was extracted. This was accomplished by soaking the ground plant material in non-polar, medium polar, and polar solvents, in that order, as the polarity of the solvents increased. *B. blakeana* was taken from its dried and powdered leaves. The sample was soaked in hexane at a 1:3 ratio in

a 5 litter Erlenmeyer flask for 72 hours at room temperature. The resultant hexane solution was then filtered through filter paper, and the residue was re-extracted for 72 hours with fresh hexane and filtered again. To get hexane crude extract, the extract was mixed and concentrated using a rotary evaporator (type Heidolph Laborota 4000 efficient) under reduced pressure. To obtain dichloromethane, ethyl acetate, chloroform, and methanol crude extracts, the leftovers were re-extracted using a similar technique with dichloromethane, chloroform, ethyl acetate, chloroform, and methanol crude extracts. The dry weight and yield of each crude extract were determined at the end of the extraction process. The study, however, employed dichloromethane extract.

Phytochemical Evaluation

Standard techniques [8-9] for identifying the presence and/or absence of phytochemicals were used to conduct phytochemical analysis of the drug's aqueous and alcoholic extracts: The following tests are carried out.

Alkaloids

(Dragendorff's test): Dissolve a few ml of alcoholic or aqueous extracts of drug in 5 ml of distilled water, add 2 ml HCL till an acid reaction starts, then add 1 ml of dragendorff's reagent, and an orange or orange red precipitate forms quickly.

(Wagner criterion): A yellow or brown precipitate is generated when 1 ml of alcoholic extract of the medication is acidified with 1.5 percent v/v HCl and a few drops of wagner reagents.

Flavanoids

(Shinoda test): In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dil HCl followed by small pieces of mg in the presence of flavanoid pink, reddish pink, or brown colour is produced.

Tri- terpenoids

(Libarmann Burchard test): a violet coloured ring is created when 2 ml of acetic anhydride solution is added to 1 ml of petroleum ether extract of drug in chloroform, followed by 1 ml of conc. H_2SO_4, indicating the presence of triterpenoids.

Carbohydrate

(Anthrone test): Add 0.5 ml of aqueous extract of medication to 2 mL of anthrone test solution; a green or blue hue shows the presence of carbohydrate.

(Fehling test): To 2 mL of aqueous drug extract, add 1 mL of a mixture of equal parts Fehling solution a and B, then boil the contents of the test tube for a few minutes

until a brick red precipitate appears. is created.

Protein

(A red or violet colour is obtained by adding 5-8 drops of 10% w/v NaoH solution to 1 ml of hot aqueous extract of drug, followed by 1-2 drops of 3 percent w/v CuSO4 solution (millon test): dissolve a small quantity of aqueous extract of drug in 1 ml of distilled water and 5-6 drops of millions reagent. On heating, a white precipitate forms, which turns crimson.

Resin

The aqueous extract was dissolved in 1 mL acetone and put into 5 mL distilled water; turbidity shows the presence of resin.

Saponins

(Foam tests): add drops of sodium bicarbonate to a test tube containing about 5 ml of an aqueous extract of the medication, shake rapidly, and let for a few minutes to create a honey comb-like structure.

Tannins

A few drops of 5 percent FeCl3 solution were added to 1-2 ml of drug extracts, resulting in a greenish colour for gallotannin and a white brown tint for tannin.

Steroids

(Salkowski tests): Carefully add 1 ml of concentrated H2SO4 to 2 ml of chloroform extracts of the drug; a red colour is formed in the chloroform layer on the test tube's side.

Compound Purification

Column Chromatography

The 70 % ethanol extract of *B. blakeana* plants was chromatographed on a silica gel column (60120 mesh) and eluted with various percentages of solvent solutions containing petroleum ether, chloroform, and methanol. The final methanol fraction was eluted with a 3:1 mixture of methanol and acetonitrile for compound purification. Using a vacuum evaporator, the eluting solvent was collected and concentrated. TLC (methanol:acetonitrile:formic acid (80:20:0.1 percent (v/v)) with 0. 49 Rf value proved the purity of the final single active ingredient. Ethanol was used to recrystallize the resultant eluent (Room Temperature). Melting point of this compound is 70-72°C.

Chemical Structure Elucidation

Various spectroscopy methods, including Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR), and Fourier Transform Infrared spectrometry, were used to identify the isolated secondary metabolite (FTIR). The chemical structure of the isolated secondary metabolite was determined using data received from several spectroscopy

methods as well as a comparison to previously published information.

Result and Discussion

Preliminary Photochemical screening

The phytochemical features of *B. blakeana* leaves were investigated in this work, and a new active phytochemical was isolated from this extract. Preliminary phytochemical screening revealed the presence of carbohydrates, phenolic chemicals, flavonoids, alkaloids, proteins, saponins, sterols, and tannins in the methanolic extract of the medications **(Table 1)**, suggesting that the medication could be effective for treating a variety of ailments.

Purification new compound

The compound 1 was isolated from methanol leaf crude extract of *B. blakeana..*The obtained yellow color extract from the methanol:acetonitrile (3: 2) solvent ratio was concentrated a subjected to TLC analysis for confirm the single fraction compound. The TLC analysis of the fraction was carried out in the methanol:acetonitrile:formic acid (80:20:0.1% (v/v). Its shows single compound under UV light with 0.49 Rf value **(Figure 1)**. This suggest that the TLC shows a pure compound and was named as a Compound 1 with 12.5 mg.

Structure Elucidation

The compound 1 was isolated from methanol leaf extract of *B. blakeana.* It was eluted with methanol:acetonitrile (3: 2) with its physical appearance as light yellow colour powder.

IR (υmax, cm^{-1}, KBr): 3208 (OH, Strong, broad), 3181 (OH, Strong, broad) **(Figure 2)** ^{1}H NMR (400 MHz, DMSO): δ 0.69-0.70 (6H, d, *J* = 6.6 Hz, Two methyl group), 1.44 (2H, dt, *J* = 7.7 Hz, 1-methylene), 1.67 (1H,t, 1-methine), 3.01 (2H, t, *J* = 7.5 Hz, 1 methylene), 6.2 (1H, s, 1-ethylene), 6.9 (1H, d, *J* = 8.4 Hz, 1 benzene), 7.01 (2H, ddd, *J* = 8.3, 1-benzene), 7.14 (1H,m, 1-benzene), 7.6 (2H, ddd, *J* = 8.3, 1-benzene), 9.45 (2H, s, two OH) (Figure 3). ^{13}CNMR (400 MHz, $CDCl_3$): 176 (1-Carbonyl), 163 (1-ethylene), 157 (1-benzene), 157(1-benzene), 153 (1-benzene), 128 (1-benzene), 128 (1-benzene), 125 (1-benzene), 122 (1-benzene), 118 (1-benzene) 115 (1-benzene), 115 (1-benzene), 110 (1-benzene), 106 (1-benzene), 105 (1-ethylene), 30 (Aliphatic C), 28 (Aliphatic C), 28 (Aliphatic C), 22 (Aliphatic C), 22 (Aliphatic C) (Figure 4). LC-MS (ESI) *m/z* (% of relative abundance) calculated for $C_{20}H_{20}O_4$: 324.37, Found $C_{20}H_{20}O_4$ $^{+1}$:325.14 [M+1] **(Figure 5)**. The purity of the isolated compound was confirmed by HPLC with RT 18.42 **(Figure 6)**. The complete assignments

of IR, NMR, Mass data of compound 1 was confirmed as a 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *B. blakeana* **(Figure 7)**. From the report of PubChem data base and literature survey for this compound 1, revealed that the 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one (IUPAC Name of Compound 1) was not reported anywhere.

Table 1: Preliminary phytochemical analysis of *bauhinia blakeana*

Phytochemicals	PET Ether	Chloroform	Acetone	Ethanol	Methanol
Alkaloids	High	Slightly present	High	Very high	Very high
Flavonoids	-	Slightly present	Slightly present	High	Very High
Saponins	-	-	-	High	High
Steroids	Slightly present	Slightly present	High	Slightly present	High
Terpenoids	-	-	High	Slightly present	High
Tannins	High	Slightly present	Slightly present	Slightly present	High
Resin	High	-	High	-	-
Phenols	Slightly present	High	High	Very High	Very High
Carbohydrate	-	High	High	High	High

Figure 1: TLC image of the Compound 1

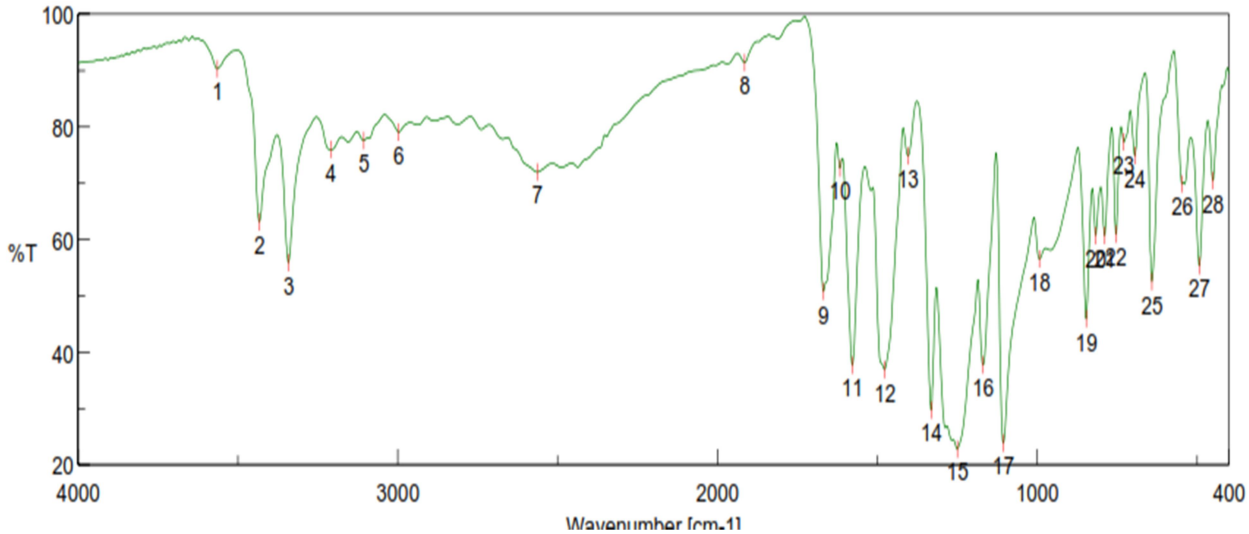

Figure 2: IR spectrum of the isolated 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *bauhinia blakeana*

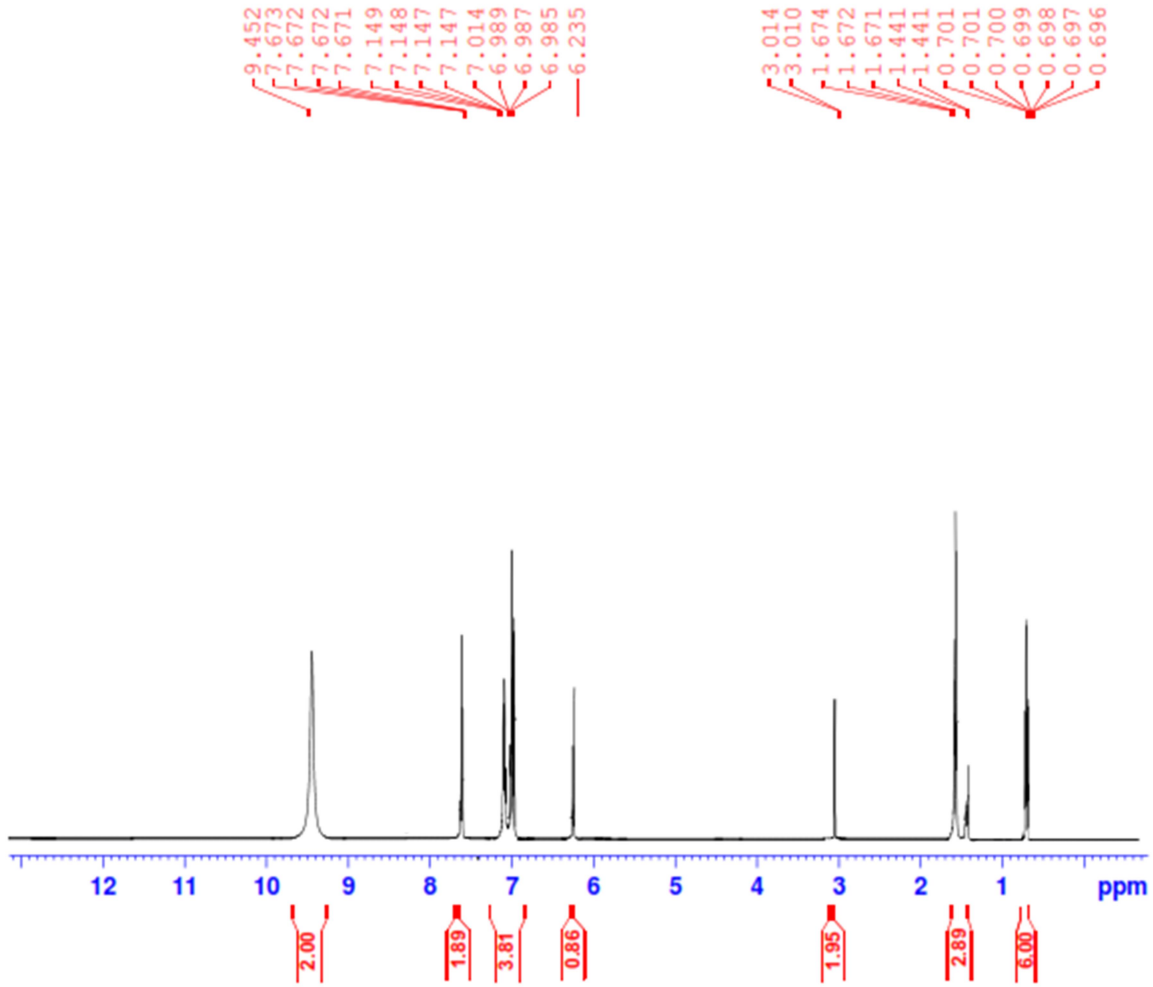

Figure 3: ^{1}H-NMR spectrum of the isolated 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *bauhinia blakeana*

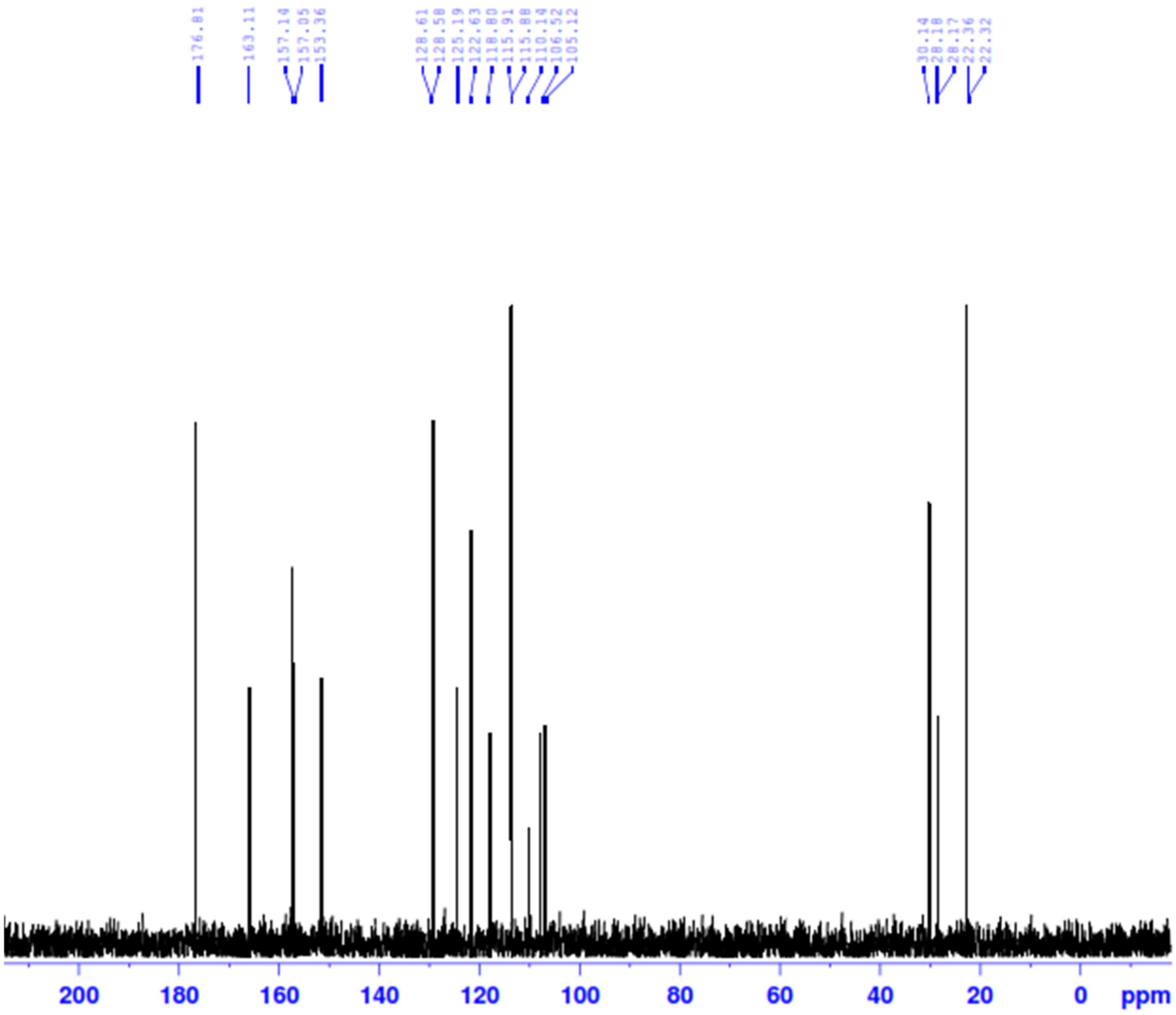

Figure 4: ^{13}C-NMR spectrum of the isolated 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *bauhinia blakeana*

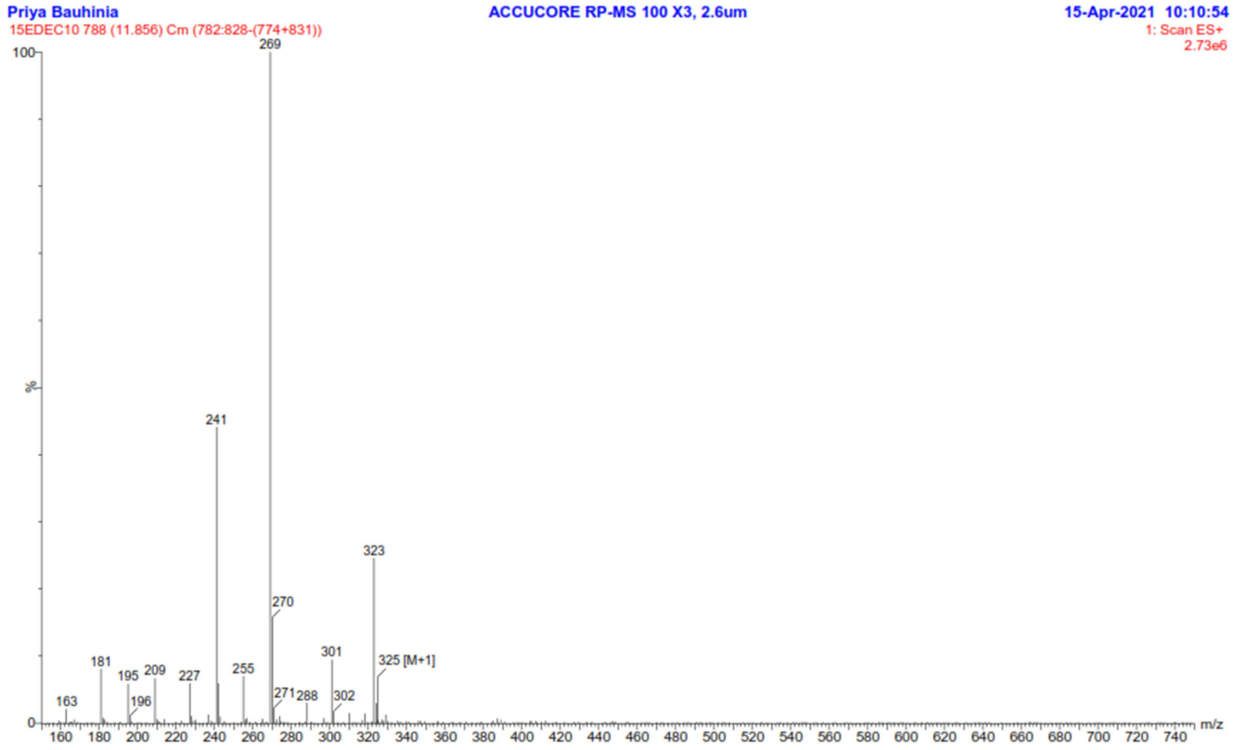

Figure 5: LC/MS analysis of the isolated 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *bauhinia blakeana*

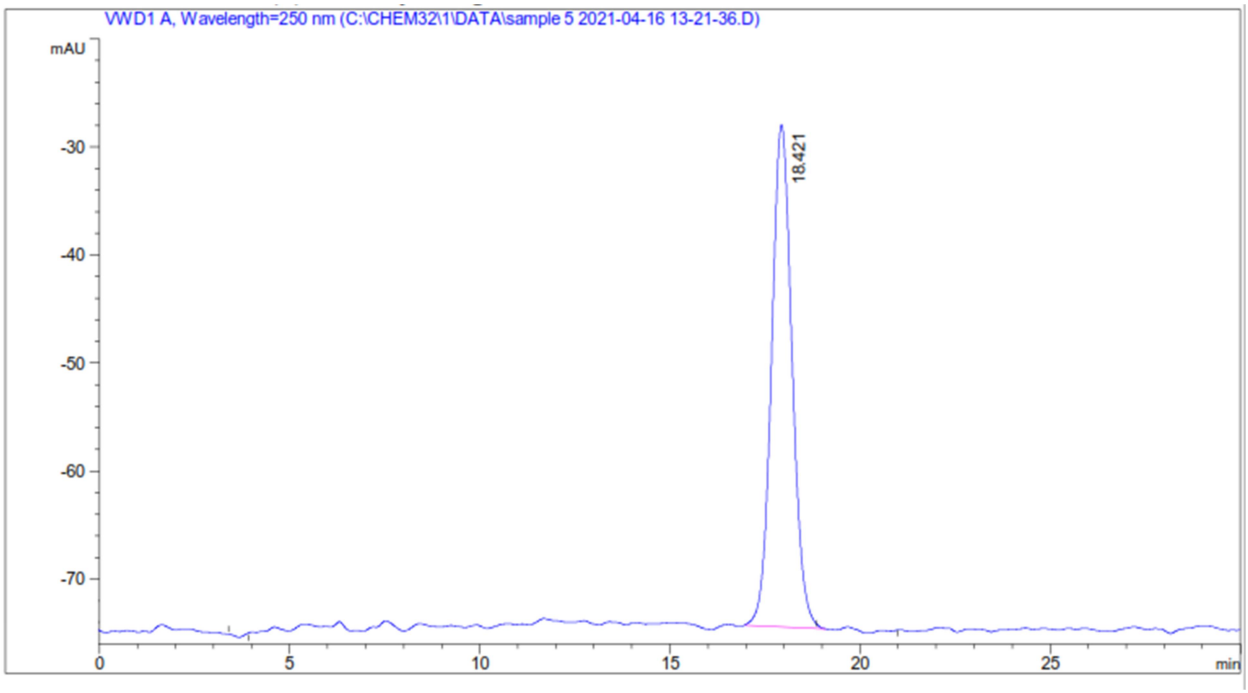

Figure 6: HPLC analysis of the isolated 7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbutyl)-4H-chromen-4-one from *bauhinia blakeana*

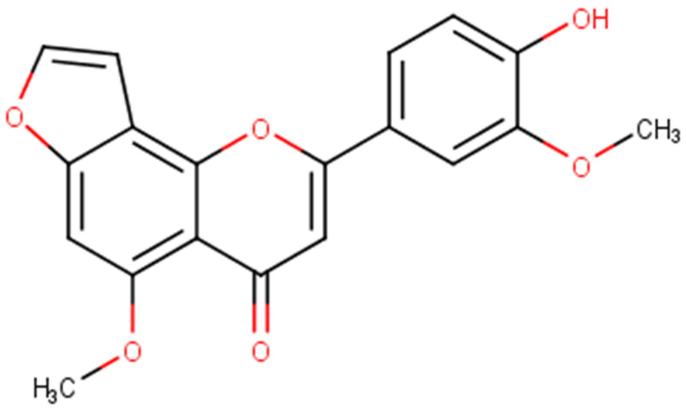

Figure 7: Molecular structure of Compound 1 (2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h]chromen-4-one)

CONCLUSION

B. blakeana Linn is a valuable medicinal plant that is widely used in traditional medicine for the treatment of a variety of ailments. According to a literature review, there has been relatively little studies on this type. The phytochemical study reveals the presence of numerous phytoconstituents in this plant. Using chromatographic and spectroscopic approaches, the structure of the pure and novel 2-(4-hydroxy-3-methoxyphenyl)-5-methoxy-4H-furo[2,3-h] chromen-4-one) chemical was obtained from this crude extract. The current investigation yielded positive results; however, the efficacy of the products can only be determined by pharmacology.

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