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Isolation and characterization of active components derived from whole plant of *Ionidium suffruticosum* (Ging.)

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ABSTRACT

The scope of the present investigation was to isolate the active components present in whole plant of *Ionidium suffruticosum.* The plant were extracted with various solvents(pet. ether, ethyl acetate and methanol), methanol was found to be more active among them. The preliminary phytochemical results revealed that coumarins, flavonoids and amino acids as active constituents in methanolic extract of *Ionidium suffruticosum*. The methanolic extract of *Ionidium suffruticosum* was undergone column chromatography with different solvent fractions. Despite, two compounds were isolated from methanolic extract of *Ionidium suffruticosum* with the compound 1 was eluted with benzene: Chloroform 70:30, v/v and compound 2 was eluted with ethyl acetate: methanol, 80:20, v/v. The structures of the two isolated compounds were characterized by using FT-IR, NMR and Mass spectrophotometric methods. Thus, the compound 1 was characterized as 3-amino-6-hydroxy-4-(4-methylphenyl)-2*H*-chromen-2-one($C_{16}H_{13}NO_3$), the compound 2 was characterized as 1-amino-1-ethoxypropan-2-ol ($C_5H_{13}NO_2$) and the compound 3 was characterized as methyl-2-hydroxy-4-methoxy benzoate. Obviously, this is the first report of occurrence of 3-amino-6-hydroxy-4-(4-methylphenyl)-2*H*-chromen-2-one in nature as well as the amino ester in this plant. Furthermore, pharmacological studies required for the isolated compounds.

Keywords: Ionidium suffruticosum, column chromatography, FT-IR, NMR.

1. INTRODUCTION

Ionidium suffruticosum (Ging.) it belongs to the family Violaceae known as Lakshmisheshta, Padmacharini or Purusharathna in Sanskrit, is an important plant in the Indian system of medicine. It is widely used as traditional healers for the treatment of diseases like diabetes[1], male sterility[2], urinary tract infections and water retention[3]. The tender leaf stalks are used as demulcent; the roots are antigonorrhoeic, diuretic, bowel complaints and urinary problems[4]. It is also attributed to its anti-inflammatory, antitussive, antimicrobial and antiplasmodial action[5,6]. It is used as anticonvulsant and free radical scavenging activity[7]. The aqueous and methanol leaf extracts possessed hypoglycemic activities[8]. Hence, the aim of the present investigation was to isolate active components derived from whole plant of *Ionidium suffruticosum* by using FT-IR, NMR and mass spectrophotometric methods

2. EXPERIMENTAL SECTION

2.1. Plant material

The whole plant of *Ionidium suffruticosum* (Ging) collected at Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Ionidium suffruticosum* (Ging), 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 Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[9] for 24 hours. Then the marc

was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc was subjected to Methanol for 24 hours. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. All the three extract were stored in screw cap vial at 4^{0}C until further use.

2.3. Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The various extracts of *Ionidium suffruticosum* was subjected to the following chemical tests such as tests for Alkaloids10, test for Carbohydrates[10], tests of Glycosides[10], tests for Phytosterol[11], test for Coumarins[11], test for Flavonoids[12,13], test for Tannins and Phenolic compounds[14], tests for Proteins and Amino Acids[10], test for Saponins[10], test for Fixed Oils[10].

2.4. TLC characterization of methanolic extract of *Ionidium suffruticosum*

The principle of separation is either partition or adsorption. The constituent which is having more affinity for mobile phase moves with it, while the constituent which is having more affinity for stationary phase gets adsorbed on it. This way various compounds appear as a band on the TLC plate, having different R_f values. The methanolic extract of *Ionidium suffruticosum* was subjected to thin layer and high performance thin layer chromatographic studies for the separation and identification of their components.

2.5. Preparation of plates

100g of silica gel G was weighed and made into a homogenous suspension with 200 ml of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10X2, 10X5, 30X5, 20X10 cm etc,). The coated plates were allowed to dry in air, followed by heating at 100-105°C for 1 hour, cooled and protected from moisture. Before using, the plates were activated at 110°C for 10 minutes.

2.6. Separation of components

The methanolic extracts of *Ionidium suffruticosum* was dissolved in methanol separately and spotted using a capillary tube on TLC plates 2 cm above from the bottom of the plate. The selection of solvent systems was based on increasing the order of polarity. The different spots developed in each system were detected by means of iodine staining.

2.7. Isolation of methanolic extract of *Ionidium suffruticosum* by using Column Chromatography

The 20gms of methanolic extract of *Ionidium suffruticosum* was admixed with 20gms silica gel (60/120 meshes) to get uniform mixing. 200gms of silica gel (70/325 meshes) was taken in a suitable column and packed very carefully without air bubbles using hexane as filling solvent. The column was kept aside for 1 hour and allowed for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by the eluting with various solvent mixtures with increasing order of polarity. All the column fractions were collected separately and concentrated under reduced pressure. Finally the column was washed with ethyl acetate and methanol.

2.8. Characterization of isolated Compounds

FT-IR

IR spectra of the compounds isolated from the methanolic extracts of *Ionidium suffruticosum* were recorded using a Nicolet 170SX. The spectral resolution for the Nicolet 170SX was 0.25cm^{-1}, and the spectral data were stored in the database at intervals of 0.5 cm^{-1} at 4000-2000 cm^{-1}, and of 0.25 cm^{-1} at 2000-400 cm^{-1}. Liquid samples were measured with liquid film method, and solid samples were measured by using KBr disc methods.

^{1}HNMR

^{1}HNMR spectra of the compounds isolated from the methanolic extracts of *Ionidium suffruticosum* was recorded using a JEOL AL-400 (399.65 MHz). The measuring conditions for the most of the spectra were as follows: flip angle of 22.5-30.0 degrees, pulse repetition time of 30s. The long pulse repetition time and small flip angle is used to ensure precise relative intensities. The ^{1}H NMR chemical shifts were referred to TMS in organic solvents and TSP in D_2O.

^{13}CNMR

^{13}CNMR spectra of the compounds isolated from the methanolic extracts of *Ionidium suffruticosum* was recorded with a Bruker AC-200 (50.323 MHz). The measuring conditions for the most of the spectra were as follows: a pulse flips angle of 22.45-45 degrees, a pulse repetition time of 4-7 seconds, and a resolution of 0.025-0.045 ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities, and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

Mass Spectrum

Mass spectra of the compounds isolated from the methanolic extracts of *Ionidium*

suffruticosum was recorded with JEOL JMS-700 by the electron impact method where an electron is accelerating voltage 75eV and an ion accelerating voltage of 8-10nV. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

3. RESULTS AND DISCUSSION

The various extracts of *Ionidium suffruticosum* (Ging.) were subjected to screening for its phytochemical constituents. The phytochemical screening results are shown in Table 1. The petroleum ether extract of *Ionidium suffruticosum* (Ging.) was contains phytosterols, fixed oils & fats. Ethyl acetate extracts containing Alkaloids, protein and amino acid compounds. The Methanolic extracts containing Alkaloids, Carbohydrates and glycoside, Protein and amino acid, Saponins, flavonoids, coumarins, Phenolic compounds and tannins.

Petroleum ether, ethyl acetate and methanol were used individually as solvent for the extraction of *Ionidium suffruticosum*. The methanolic extract of *Ionidium suffruticosum* was found active among them. Therefore, the methanolic extract of *Ionidium suffruticosum* was subjected to the TLC chromatographic profile and column chromatographic separation. The methanolic extract of *Ionidium suffruticosum* dissolved in their mother solvent was taken in a capillary tube and spotted on TLC plates 2cm above its bottom. Most of the sample for application were between 0.1 – 1%. The applied spots were of equal size as far as possible and diameter ranging from 2-3mm. The solvent system for methanolic extracts was developed by trial and error method using various solvents which were differing in polarities.

The methanolic extract of *Ionidium suffruticosum* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g methanolic extract of *Ionidium suffruticosum*) was adsorbed on silica gel (20 g) and transferred to a column of silica gel (200g equilibrated with benzene). Two compounds were isolated in column chromatography with different solvents. Obviously, the compound 1 (145 mg) was eluted with benzene: Chloroform 70:30, v/v and compound 2 (135mg) was eluted with ethyl acetate: methanol, 80:20 v/v and the compound 3 (110mg) was eluted with methanol 100 %. This active fraction was used to identify the chemical tests showed the presence of amino acid and flavonoids as active compounds. The actual compounds were isolated from column chromatography as mentioned in the experimental section. The spectra (IR, ^{1}H & $^{13}CNMR$ and Mass) of these compounds as mentioned in the experimental section.

3.1. Characterization of compound 1

The spectral data IR, $^{1}HNMR$ & $^{13}CNMR$ and Mass of the compound 1 are good in agreement with the structure proposed for the compound. The melting point of the compound 1 was found as 149-150ºC. The IR spectrum of the compound 1 was analysed from the IR data. The presence of –NH group known from the

Table - 1: Phytochemical analysis of various extracts of whole plant of *Ionidium suffruticosum* (Ging.)

Test	Petroleum ether	Ethyl acetate	Methanol
Alkaloids	-	+	+
Carbohydrates and glycosides	-	-	+
Phytosterols	+	-	-
Fixed oil and fats	+	-	-
Saponins	-	-	-
Phenolic compounds and tannins	-	-	+
Protein and Amino Acid	-	+	+
Coumarins	-	-	+
Flavonoids	-	-	+

+ Positive; - Negative

Table - 2: TLC profiles of methanolic extracts of *Ionidium suffruticosum*.

Solvent system	No. of Spot	Rf Value
Benzene : Chloroform (90:10)	2	0.40, 0.30
Benzene : Chloroform (80:20)	2	0.40, 0.60
Benzene : Chloroform (70:30)	2	0.50, 0.30
Ethyl acetate: Methanol (70:30)	2	0.60, 0.50

Ethyl acetate: Methanol (50:50)	2	0.60,0.40

absorption at 3320cm^{-1}. Absorption at 2920 cm^{-1} shows the presence of –C-H (aromatic) group. A strong band at 1664 cm^{-1} is due to the presence of –C=O group. The presence of –C=C (aromatic) indicates in the absorption at 1611 cm^{-1}.The ^{1}H and ^{13}CNMR spectral data of compound 1 are analyzed. Based on the ^{1}HNMR chemical shiftvalues and ^{13}CNMR chemical shift values of the compound 1 are found to be 4-phenylcumarine derivative (Fig 1&2).The mass spectral analysis of compound 1 led to the molecular peak *m/z* 288(M+9), which indicated the molecular formula $C_{16}H_{13}NO_3$.

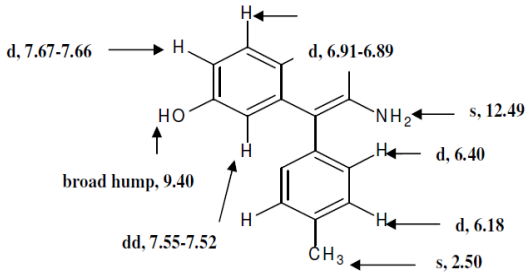

Figure - 1: ^{1}HNMR Spectral data and ssignment.

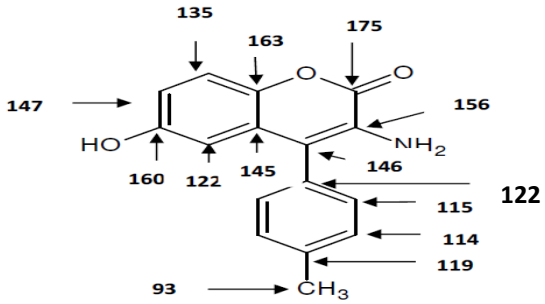

Figure - 2: ^{13}CNMR Spectral data and Assignment.

Thus, the compound 1 was characterized as 3-amino-6-hydroxy-4-(4-methylphenyl)-2*H*-chromen-2-one were given in Fig 3. The Molecular Formula of the compound 1 was deduced as $C_{16}H_{13}NO_3$. This is the first report of occurrence of this compound in nature as well as the amino ester in this plant

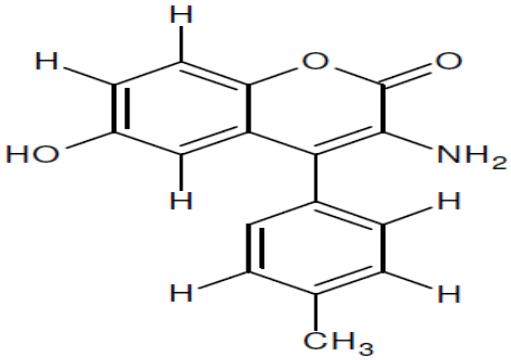

Figure - 3: Structure of Compound 1(3-amino-6-hydroxy-4-(4-methylphenyl)-2*H*-chromen-2-one).

3.2. Characterization of compound 2

The spectral data of IR, 1H &13CNMR and Mass of the compound 2 are good in agreement with the structure proposed for the compound. A broad band at 3405cm^{-1} is due to the presence of –OH &NH groups. Whereas, a strong absorption at 1096cm-1 indicates the presence of –C-O-C stretching.^{1}HNMR and ^{13}CNMR Spectra of this compound are given in the fig. ^{1}HNMR and ^{13}CNMR data and the corresponding assignments are given in the structure proposed to the compound (Fig 4&5).The mass spectrum of the isolated compound 2 is presented in the fig. the m/z value of isolated compound of the molecular ion is found as 119(M+3) which includes the isotopes of corresponding atoms. Thus, compound 2 was characterized as 1-amino-1-ethoxypropan-2-ol is given in Fig 6. The Molecular Formula of the compound 2 was proposed to $C_5H_{13}NO_2$.

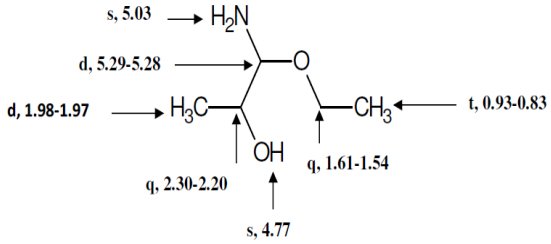

Figure - 4: ^{1}HNMR spectral data of compound 2 and correspon ignments.

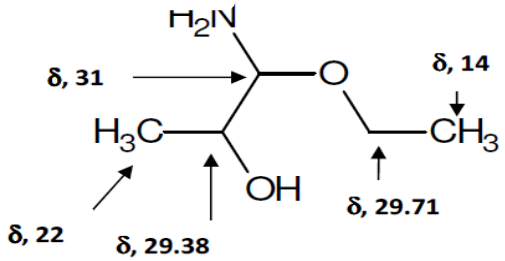

Figure - 5: ^{13}CNMR spectral data of compound 2 and corresponding assignments.

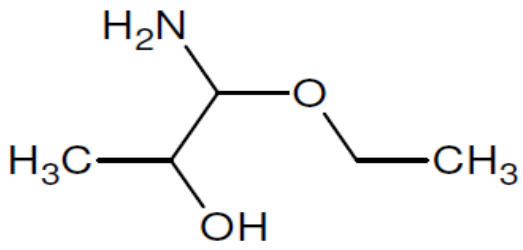

Figure - 6: Structure of Compound 2(1-amino-1-ethoxypropan-2-ol).

3.3. Characterization of compound 3

The spectral data of IR, 1H &13CNMR and Mass of the compound 3 are good in agreement with the structure proposed for the compound. The IR spectrum of the isolated compound was shown in the fig no: 5.16. There is an intense, broad band near 3372 cm^{-1}. This may be due to the hydrogen bonded O-H stretching vibration. The prominent absorption at 1700 cm^{-1} indicated C=O stretching vibration. The bands at 1244 and 1063 cm^{-1} are due to the presence of ester group (-$COOCH_3$). Absorption at 1158 cm^{-1} exhibits the C-O-C stretching.^{1}H NMR Spectrum of the compound was shown in the chemical shift appeared as singlet at δ 9.90 ppm equivalent to 1H is due to the O-H (Phenolic) proton. MUltiplet at δ 7.77-7.61 ppm corresponds to aromatic 2H present in C-5 and C-6 positions. Doblet at 7.46-7.44 equivalent to 1H exhibits the aromatic proton at C-3 position. A singlet at δ 3.80 ppm equivalent 3H exhibits the methyl protons which are bonded with oxygen. A singlet at δ 2.59 ppm equivalent to 3H indicates the methyl protons which is present in the ester group.^{13}C NMR spectrum of the compound was shown in the chemical shift values as δ 169 (C of C=O), 161 (C_2), 159 (C_1), 153.89 (C_6), 153.27 (C_5), 132 (C_3), 123 (C_4), 40 (OCH_3) and 39 ($COOCH_3$) ppm .Mass spectrum of the compound was shown in the fig no: 5.20 The observed mass for the parent molecular ion m/z is 183 (M^++1). (Where as the formula mass for the molecular formula $C_9H_{10}O_4$ is 182).Based on the spectral (IR, ^{1}H NMR, and MS) data, the structure proposed for the isolated compound is methyl-2-hydroxy-4-methoxy benzoate

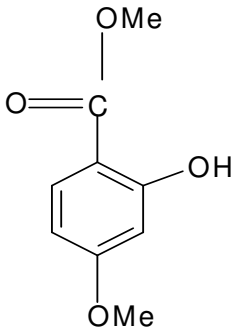

Figure - 7: Structure of Compound 3(methyl-2-hydroxy-4-methoxy benzoate)

4. CONCLUSION

From the above reports, three compounds were isolated from methanolic extract of *Ionidium suffruticosum.* This is the first report of occurrence of 3-amino-6-hydroxy-4-(4-methylphenyl)-2*H*-chromen-2-one and in nature as well as the amino ester in this plant. Furthermore, pharmacological studies required for the isolated compounds.

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