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ANALYTICAL CHARACTERISATION OF *RHODODENDRON GRIFFITHIANUM* USING SPECTROSCOPIC TECHNIQUES

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ABSTRACT
Background: The *Rhododendron griffithianum* have been used in traditional medicine for the treatment of inflammatory conditions, pain, gastro-intestinal disorders, common cold, asthma, skin disease, etc. **Purpose:** The focus of this present research is based on the analytical methodologies, which include the isolation of active ingredients from *Rhododendron griffithianum*. This study focused on isolation and characterization of *Rhododendron griffithianum* active constituent by column chromatography. **Methodology:** Various solvent at different polarity were pre-tested in TLC for developing solvent system. The eluted fractions were run in TLC mobile phase with the solvent Toluene: Ethyl acetate: Acetic acid (8:2:0.4). The isolated compounds were characterized by modern analytical techniques such as UV-visible Spectroscopy, FTIR (Fourier Transform Infrared spectroscopy), NMR Spectroscopy and Mass spectroscopy. **Results:** Spectral analysis confirmed, the existence of coumarin in Fraction A of RGE (F3). More studies are needed in the same lines to evaluate other phytoconstituents of this plant.

KEYWORDS: Isolation, Spectroscopy, Chromatography, *Rhododendron*

INTRODUCTION

Medicinal plants or plant-based medicine has been used cost-effectively throughout the world to prevent and/or treat diabetes[1]. In fact, many developing countries rely on plant-based medicine to treat people with diabetes and other conditions. Consumers all over the world are becoming more conscious of the nutrition value, health benefits and safety of their food and its ingredients. In addition, there is a preference for natural functional food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts[2] Several pharmaceuticals commonly used today are structurally derived from natural compounds that are found in traditional medicinal[3-4]. For example, the anti-hyperglycemic drug called metformin, currently used to treat diabetes, can be traced back to the traditional use of *Galega officinalis* to treat diabetes [5-6]. Most commonly used medicinal plants and vitamins with hypoglycemic activities to improve the immune system and manage blood sugar levels in humans include *Allium sativum* (garlic), *Momordica charantia* (Bitter Melon), *Hibiscus sabdariffa L*[7] (Roselle Plant), *Zingiber officinale Rosc* (Ginger), and Vitamins C, D, and E[8].

Given that many medicinal plants are easily accessible, cheap, and useful for the management of diabetes, many developing countries and a few wealthy countries use medicinal plants to meet their healthcare needs[9-10]. The genus *Rhododendron L.* (Ericaceae) comprises 8 subgenera with over 850 species[11-12]**.** The majority of the species grow in the Himalayan region, South-East Asia and Malesia, with 650 species in China and 155 species endemic to New Guinea[13-14]. Traditional uses of selected Rhododendron species point to their importance especially in the treatment of inflammation, pain, common cold symptoms, skin ailments, and gastrointestinal disorders. Some species have also been utilized as insecticides. Most of the ethnomedical Rhododendron species are employed in Traditional Chinese Medicine for the treatment of arthritis, acute and chronic bronchitis, asthma and pain[15-23]. *Rhododendron dauricum* and *Rhododendron* molle are listed in the Chinese Pharmacopoeia and indicated against cough, migraine, swelling and pain associated with injuries from falls, and stubborn tinea[24].

MATERIALS AND METHODS

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study was of analytical grade.

Extraction of plant by soxhlet extraction method

Coarsely powered leaves of *Rhododendron griffithianum* was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus[25].

Formula;

$$\%\ yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x}100$$

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the extract of *Rhododendron griffithianum*[26].

Qualitative chromatographic analysis:-

Preliminary Thin layer chromatography:-

In this method, the mobile phase travels upward through the stationary phase. The solvent travels up the thin plate soaked with the solvent by means of capillary action. During this procedure, it also drives the mixture priorly dropped on the lower parts of the plate with a pipette upwards with different flow rates. Thus the separation of analytes was achieved. This upward travelling rate depends on the polarity of the material, solid phase, and of the solvent[27].

$$\mathbf{R_f\ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Fractionation and Isolation of bioactive compound:-

In order to facilitate isolation, 8g of hydroalcoholic extract of *Rhododendron griffithianum* was dissolved in distilled water (100 mL) and successively fractionated with petroleum ether, chloroform, ethyl acetate, Methanol and water yielding respectively 0.180 g, 0.208 g, 2.200 g, 5.800 and 3.018 g of fractions after evaporation to dryness. The highest yield fractions were selected for the isolation of bioactive constituent by column chromatography.

Active constitutes isolated from RGE extract by column chromatography. Part of Methanol fraction of *Rhododendron griffithianum* (RGE (F3)) was subjected to silica gel column chromatography. 10 fractions were collected and combined on the basis of their TLC profiles Solvent system developed in preliminary TLC for RGE (F3) extract in which the maximum spots were visible is Toluene: Ethyl acetate: Acetic acid (8:2:0.4) mobile phase with std. Phenol. So that Toluene: Ethyl acetate: Acetic acid (8:2:0.4) solvent was taken as mobile phase for column chromatography[28].

Spectroscopic characterization: -

UV - Visible Spectroscopy

The isolated fraction of sample was diluted with the same solvent. The extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1800) and the characteristic peaks were detected and recorded[29].

FT-IR spectroscopy-

To establish the presence of the functional groups, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer [30].

NMR spectroscopy-

^{1}H and ^{13}C-NMR spectra were recorded on a FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) using TMS as an internal standard. The solvents used were Hexane: Acetone (8: 2) and DMSO. Chemical shifts ware shown in δ values (ppm) with TMS as an internal reference[31].

Mass spectroscopy-

The mass spectrometer used for the identification of the molecular weight of the compound was Bruker Daltonik, Benchtop easy-to-use, high performance Electrospray Ionization Quadrupole time-of-flight LC MS spectrometer[32].

RESULTS AND DISCUSSION

Table 1 Phytochemical analysis of *Rhododendron griffithianum* Extract

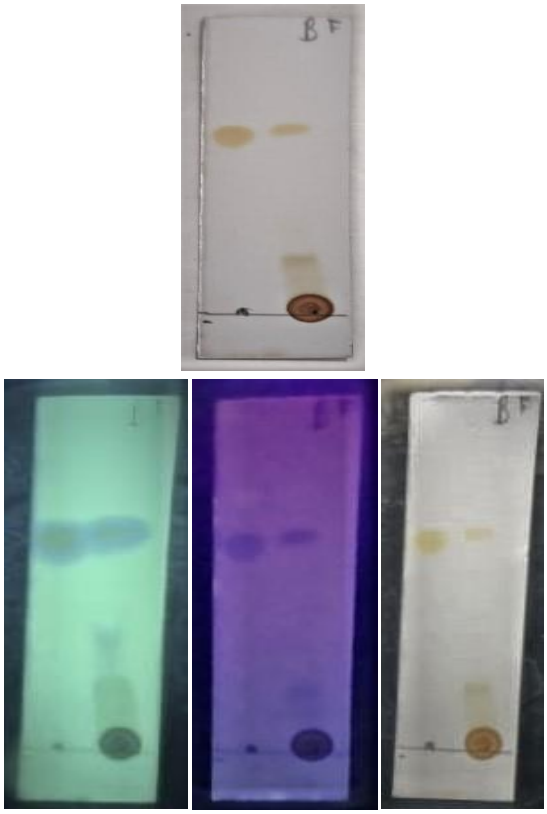
Identification Test	**Observation**	
	Petroleum ether	Hydroalcoholic
Test for Carbohydrates		
Molisch's Test	-	+
Fehling's Test	-	+
Benedict's Test	-	+
Bareford's Test	-	+

Test for Alkaloids		
Mayer's Test	-	-
Hager's Test	-	-
Wagner's Test	-	-
Dragendroff's Test	-	-
Test for Terpenoids		
Salkowski Test	-	+
Libermann-Burchard's Test	-	+
Test for Flavonoids		
Lead Acetate Test	-	+
Alkaline Reagent Test	-	+
Shinoda Test	-	+
Test for Tannins and Phenolic Compounds		
$FeCl_3$ Test	+	+
Lead Acetate Test	+	+
Gelatine Test	+	+
Dilute Iodine Solution Test	+	+
Test for Saponins		
Froth Test	+	+
Test for Protein and Amino acids		
Ninhydrin Test	-	+
Biuret's Test	-	+
Million's Test	-	+
Test for Glycosides		
Legal's Test	-	-
Keller Killani Test	-	-
Borntrager's Test	-	-

Table 2: Rf values of RGE (EtOH) extract & Std. Phenol

S. No.	**Solvent system**	**No. of spots**	**Color of spots at Wavelength (254 & 365nm)**	**Rf value (Extract)**	**Rf value (Std)**
`1.	Pet. Ether: Ethyl acetate: Acetic acid (7:2:1)	05	Dark Brown (std) Brown Purple Blue Brown	- 0.20 0.31 0.67 0.74	0.85
2.	Toluene: Ethyl acetate: Acetate acid: Water (3.3:0.8:0.2)	03	Brown (std) Purple Blue	- 0.09 0.24	0.29

3	Toluene: Ethyl acetate: Acetic acid (8:2:0.4)	07	Purple (std) Fluorescence Light Green Green Blue Purple (std) Fluorescence	- 0.54 0.06 0.12 0.25 0.49 0.54	0.49



Short-UV **Long-UV** **Visible**

Figure 1: TLC estimation by UV lamp for RGE (F3) with Std. Phenol Toluene: Ethyl acetate: Acetic acid (8:2:0.4)

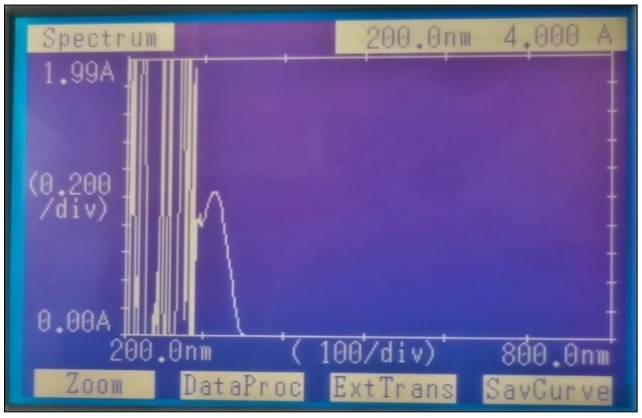


Figure 2: Active constitutes estimation By UV- Spectra of A fraction of RGE (F3) extract after column chromatography

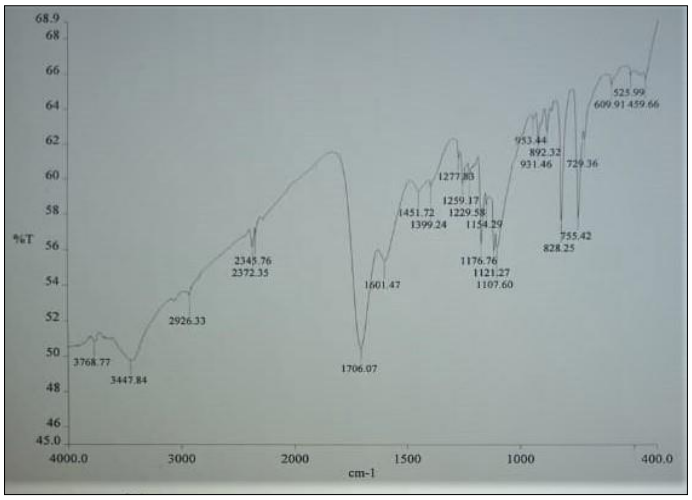


Figure 3: IR spectra of the isolated compound (Fraction A) of RGE (F3)

Table 3: FTIR- Spectrum Frequency Range of the isolated compound (Fraction A) of RGE (F3)

Sr. No.	**Fraction**	**Frequency Range**	**Group Absorption (cm^{-1})**	**Appearance**	**Group**	**Compound Class**
1	**A**	4000- 3000 (cm^{-1})	3447.84	Strong, broad	O-H stretching	Hydroxyl Group
		1670- 1600 (cm^{-1})	1601.47	Medium	C=C stretching	conjugated alkene
		3000- 2500 (cm^{-1})	2926.33	Medium	C-H stretching	Alkane
		1400- 1100 (cm^{-1})	1107.60	Weak	C-C stretching	Alkane
		2000- 1600 (cm^{-1})	1706.07	Medium	C-O stretching	Carbonyl group
		1600-1400	1451.72	Strong	C=C stretching	Benzene Ring
		900-700 (cm^{-1})	729.36	Strong	C-H bending	Aromatic Structure

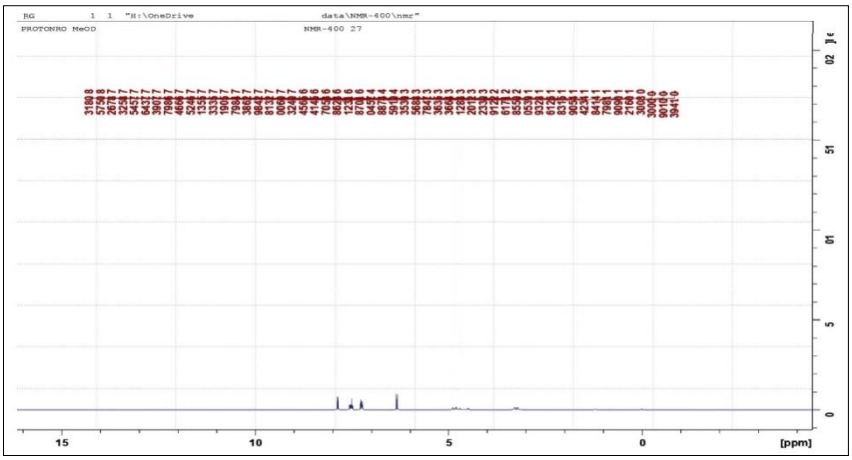


Figure 4: ^{1}H-NMR spectra **of the** isolated compound (Fraction A) of RGE (F3)

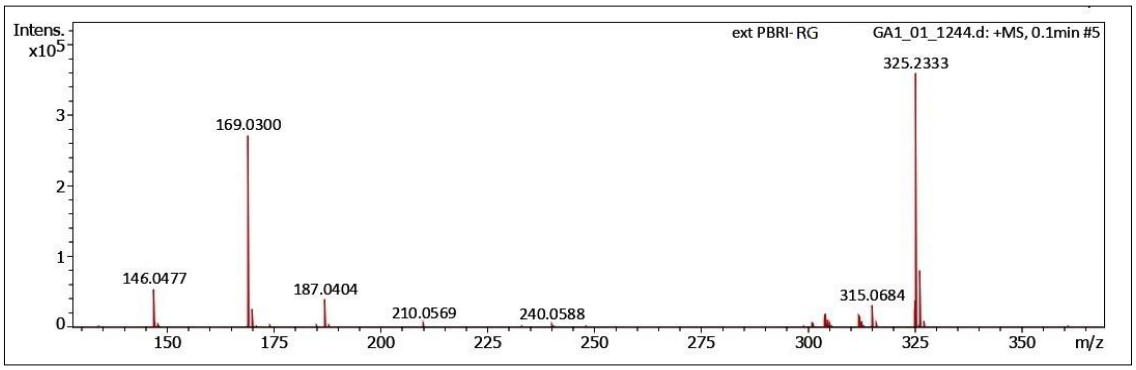


Figure 5: **Mass spectra of the** isolated compound (Fraction A) of RGE (F3)

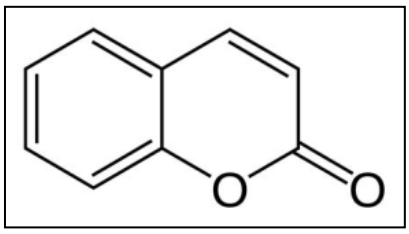


Figure 6: Coumarin

Qualitative phytochemical screening of *Rhododendron griffithianum'ss* hydroalcoholic extract unveiled the presence of diverse phytoconstituents, including terpenoids, flavonoids, alkaloids, glycosides, steroids, and phenols. To isolate and characterize these compounds, thin-layer chromatography (TLC) was employed with solvent systems chosen based on existing literature. The most effective separation was achieved with Toluene: Ethyl acetate: Acetic acid (8:2:0.4) as the mobile phase, resulting in clearly visible bands for RGE (F3). The Rf values obtained were 0.48 for RGE (F3) and 0.49 for the standard Phenol.

This optimize mobile phase, Toluene: Ethyl acetate: Acetic acid (8:2:0.4), was subsequently used for column chromatography to isolate active constituents from RGE (F3), yielding Fractions 01-02 (a), 03 (b), 04 (c), 05 (d), 06 (e), 07 (f), 08 (g), 09 (h), and 10 (i). To confirm the presence of active constituents in Fraction A of RGE (F3), TLC estimation was performed

using the same mobile phase, Toluene: Ethyl acetate: Acetic acid (8:2:0.4), and compared with the standard Phenol. The collected fractions underwent UV spectrum analysis, recording UV spectra in the range of 200-800 nm. The λmax for Fraction A of RGE (F3) was determined to be 320 nm (Figure 2). The structure of these compounds was elucidated by FT-IR H^1NMR and Mass spectroscopy.

In the IR spectra of isolated Fraction A of RGE (F3), distinct peaks included a broad O-H peak at 3447.84 cm-1 (indicative of a Hydroxyl Group), a C-H stretching peak at 2926.33 cm-1 (associated with Alkane), C=C absorption peaks at 1451.72 & 1601.47 cm-1 (related to the Benzene ring & conjugated alkene), a C-C stretches peak at 1107.60 cm-1 (representative of Alkane), a C-O peak at 1706.07 cm-1 (indicative of the Carbonyl group), and a C-H bending peak for the Aromatic Structure at 729.36 cm-1 (Figure 3, Table 3).

Additionally, in the ^{1}H-NMR spectra of isolated Fraction A, proton signals included 1H-1 at 6.35 (d) ppm, 1H-2 protons in the range of 7.20-7.40 ppm (7.23 (ddd), 7.24 (ddd)), 1H-2 protons in the range of 7.60-7.89 ppm (7.68 (ddd), 7.85 (ddd)), and 1H-1 at 7.87 (d) ppm (Figure 4).

Furthermore, mass spectra of Fraction A displayed molecular ion [M+] peaks at m/z 146.0477, corresponding to the molecular formula $C_9H_6O_2$, as inferred from their fragments (Figure 5). Overall, the comprehensive physical, chemical, and spectral investigations collectively confirmed the presence of Coumarin in Fraction A of RGE (F3). Hence, this research has demonstrated that *Rhododendron griffithianum* is a good source of coumarin.

CONCLUSION

Spectral analysis confirmed, the existence of coumarin in Fraction A of RGE (F3). More studies are needed in the same lines to evaluate other phytoconstituents of this plant. These studies not only enhance our understanding of *Rheum palmatum's* phytochemical but also identify Coumarin as a significant active compound in Fraction A of RGE (F3). This knowledge paves the way for potential applications and further research in diverse scientific contexts.

Conflict of interest: Nil

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