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Development of Sustainable Separation Method and Analytical Characterization of Bio-active molecule from Herb

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Abstract:

This research describes the sustainable separation and identification of Gallic acid (GA) from herb using circularly spreded silica gel layer by chromatographic separation method. The purity of isolated Gallic acid confirmed by reverse phase High-performance liquid chromatography (HPLC) which was 98.24%. Compound was isolated and identified as gallic acid through Ultraviolet–visible spectroscopy (UV-Vis.), Fourier-transform infrared spectroscopy (FTIR), HPLC, High-resolution mass spectrometry (HR-MS); ^{1}H, ^{13}C Nuclear magnetic resonance (NMR) and Differential scanning calorimetry (DSC) measurements. This method was reported first time for Gallic acid isolation from *F. arnottiana* leaves. Gallic acid is mainly used in synthesis of polymers and grafting process. Gallic acid exhibited higher levels of antioxidant activity hence it is an ideal choice for reducing the risk of cancer.

Keywodrs: Sustainable; HR-MS; NMR; HPLC; FTIR; DSC

1 Introduction

F. Arnottiana is a medicinal plant that is widely used in Indian medicines; genus of *Ficus* (Moraceae) comprises about 1200 species distributed mainly in tropical and subtropical regions **[1]**. It is commonly known as Paras Pipal. *F. Arnottiana* is distributed throughout India, mostly in rocky hills at 1350m elevation **[2]**. The *F. Arnottiana* plant extract is used in Indian traditional medicines to treat ulcer and diabetes **[3]**. The leaves

of plant are used for controlling fertility **[4]**. Leaves and bark extract are also used to evaluate the analgesic activity, anti-inflammatory activity, hypoglycemic and antioxidant activity **[5-7]**. A variety of chemical constituents, such as different types of triterpenes, flavonoids, sterols, alkaloids and other miscellaneous compounds have been reported in the *Ficus* species **[8]**. The chemical constituents of *F. Arnottiana* has not been thoroughly investigated specifically, therefore present research was carried out in order to have an insight into the chemical basis for some of the pharmacological properties reported for the plant in traditional medical system.

Generally, phenolic compounds have been investigated by gravity-flow column liquid chromatography (GCC), thin-layer chromatography (TLC), gas chromatography (GC) and HPLC, NMR, MS and electrochemical methods each with its own characteristics **[9-12]**.

Gallic acid is a phenolic acid found in many dietary substances. Gallic acid, a well-known powerful antioxidant, could be found in many plants and food substances such as blackberrys, strawberrys, walnuts, apples, grape seed, litchi fruit seed, and tea **[13, 14]**.

Gallic acid is a trihydroxybenzoic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid. The chemical formula is $C_6H_2(OH)_3COOH$. It is white, yellowish-white, or pale fawn-colored crystals soluble in alcohol and acetone. Thirty ayurvedic herbs and formulations have been screened for the presence of Gallic acid, which is already in use for treatments of different diseases over years **[15]**. There are various research available on green synthesis of nanomaterial using plant exact **[16]**, and their application towards bioplymres like BSA **[17,18]**, and DNA **[19, 20]** and anticancer activity **[21, 22, 23]**. Gallic acid is found in a variety of foods and herbs, which are well known as powerful antioxidants. Blueberries are used as a relaxant during childbirth and also as a good tonic for purifying the blood **[24]**. GA was applied to cuts and wounds to prevent infection, treat menstrual problems, colds **[25]**, intestinal disorders, bleeding, hematochezia and hyperhidrosis **[26]**. Gallic acid possesses good antioxidant activity, which is exerted through increase in the DNA damage and release of cytochrome c. It also decreased the glutathione and mitochondria potential of the cells **[14]**], antifungal property **[27]**, antimalarial **[28]**, to treat albuminuria and diabetes. Gallic acid has also been reported to inhibit several cancer cell lines through multitude of mechanisms **[29]**. In the present study, we have isolated Gallic acid from the *F. Arnottiana* leaves extract using chromatographic techniques and chemically characterized them using UV-Vis, FTIR, NMR, LC-MS and DSC analysis **[30, 31, 32]**.

2 Experimental section

Chemicals and reagents

All chemicals used were of analytical reagent grade. All solvents used for extraction and isolation of Gallic acid were of analytical grade. Milli-Q water from Millipore system was used as the solvent throughout the experiments.

Collection of plant material and Preparation of plant extracts

Collection of plant material and preparation of plant extracts was mention in previously published research paper **[18]**.

Preparation of Circularly Spreaded Silica Gel Layer

Silica gel was spreaded circularly and uniformly on cleaned glass plate and treated with hexane solvent, a central part of the layer of silica gel was removed for the addition of crud ethanolic *F. Arnottiana* leaves extract **[18]**.

Isolation of Gallic acid

Ethanol extract of *F. Arnottiana* leaves was subjected at the center of the Circularly Spreaded Silica Gel Layer on glass plate. On application of 1st mobile phase (petroleum ether), separation of dark yellow colored band was occurred from centre of silica gel circle. This band was removed by scratching, the scratched area was filled up with fresh silica gel again. Then 2nd mobile phase (petroleum ether and chloroform in 1:1 ratio), was used and dark yellow colored band was separated from centre of silica gel circle. 3rd mobile phase (Chloroform) was used to separate yellow colored broad circle from the middle of ethanol extract. This dark yellow colored part was scratched and pick up from the spreaded silica gel in circular form, and fresh silica gel was filled up again in the scratched area. 4^{rth} mobile phase (chloroform and acetone in 2:1ratio) was used to separate colorless broad circle from the middle of ethanol extract. This colorless part was scratched and picked up from the spreaded silica gel and the scratched area was filled up by aluminum oxide active base. Finally 5th mobile phase (acetone and methanol in 2:1ratio) was used and dark yellow colored circular band was separated from middle of ethanol extract. This dark yellow colored fraction was further subjected to pencil column using acetone: methanols (1:1) as a 6th mobile phase, from which three major fractions were separated out. Fraction 1 was white colored powder. Fraction 2 was Yellow colored sticky mass. Fraction 3 was yellow colored powder.

Characterization of Gallic acid

A chromatographic separation was carried out using circularly spreaded silica gel on glass plate. The white colored powder (Fraction 1) was further analyzed with RP-HPLC (Chromeleon, Dionex, 1996-2006) using Poroshell 120 Hilic (4.6×150) mm, 2.7µm particle size column (Agilent Technologies), with flow rate of 1.0 ml min^{-1} and detection at 210 nm. The mobile phase used was an isocratic of MeOH: Water, (95:05).

UV- Vis spectroscopic analysis was performed using *LAB* UV3000^{plus} at room temperature with quartz cuvette of 1 cm path-length as a sample holder (200 - 800 nm). FTIR spectrum was recorded on Perkin Elmer FTIR spectrometer Frontier using ATR transmittances at 4000 to 400 cm^{-1}. DSC instrument was used for measurement of melting temperature of Gallic acid with empty reference aluminum pan. Heat flow was measured by comparing the difference in temperature across the sample and the reference, temperature ramp 5°C/min in range from 30 to 400°C. ESI-MS data was obtained on a HR-MS which was performed with a

Bruker-Daltonics microTOF-Q II mass spectrometer (operating conditions Source Type: ESI, Ion Polarity: Positive, Set Nebulizer: 0.4 Bar, Focus: Active, Set Capillary: 4500 V, Set Dry Heater: 180 °C, Scan Begin: 50 m/z, Set End Plate Offset: -500 V, Set Dry Gas: 4.0 l/min, Scan End: 300 m/z, Set Collision Cell RF: 350.0 Vpp, Set Divert Valve: Waste). The mass of the purified Gallic acid was determined as 171 by LC-MS.

The Bruker AMX-400 MHz NMR spectrometer (France) was used to record ^{1}H NMR and ^{13}C NMR data. ^{1}H-NMR (400 MHz, MeOD) ppm: δ 6.97 (s, 2H, Aromatic); ^{13}C-NMR (100 MHz, MeOD) ppm: δ 173 (C=O), 148 (C-OH), 142(C-OH), 124 (C-H, aromatic), 112 (C-OH, aromatic).

3 Result and discussion

The compound Gallic acid was isolated from acetone and methanol fraction of ethanolic extract of the leaves parts of *F. Arnottiana*. The compound isolated by using circularly spreaded silica gel was in the form of white colored powder (**Fraction 1**).

The UV-Vis spectrum showed maxima at λmax 212 (abs = 1.10) and 266 (abs = 0.410) nm suggesting a Gallic acid chromophore (**Fig.1**). FTIR spectrum suggested that several signal including 3278 cm^{-1} (-COOH), 3005 cm^{-1} (C-H), 1707 cm^{-1} (-C=O), 1616 cm^{-1} (C=C), 1440 cm^{-1}, 1379 cm^{-1}, 1255 cm^{-1}, 1037 cm^{-1}, 704 cm^{-1} were obtained (**Fig.2**) **[11]**.

Melting point detected was 260.46°C using DSC (**Fig.3**). Peak purity of compound analyzed was confirmed by HPLC (retention time, 0.637 min) which was 98.24% shown in **Fig.4**. The compound Gallic acid was identified by HR-MS (**Fig.5**), ^{1}H (**Fig.6**) and ^{13}C NMR (**Fig.7**). The molecular formula $C_7H_6O_5$ was inferred from the HR-MS spectrum: $[M-H]^+$ peak at m/z 171. These data was conformed from the report of Kelebek **[9]**.

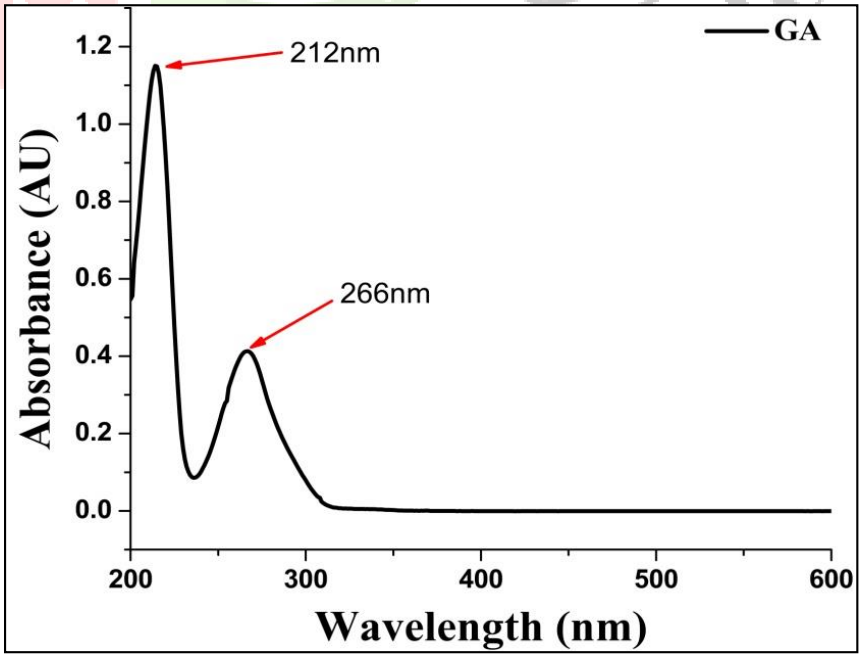

Fig.1 UV-Vis absorption spectrum of Gallic acid

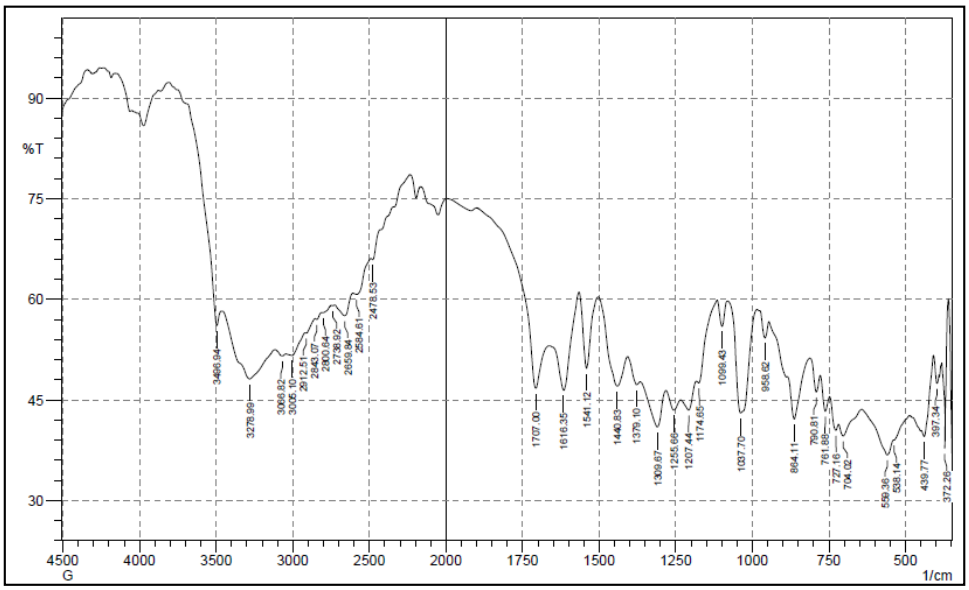

Fig.2 FTIR spectrum of Gallic acid

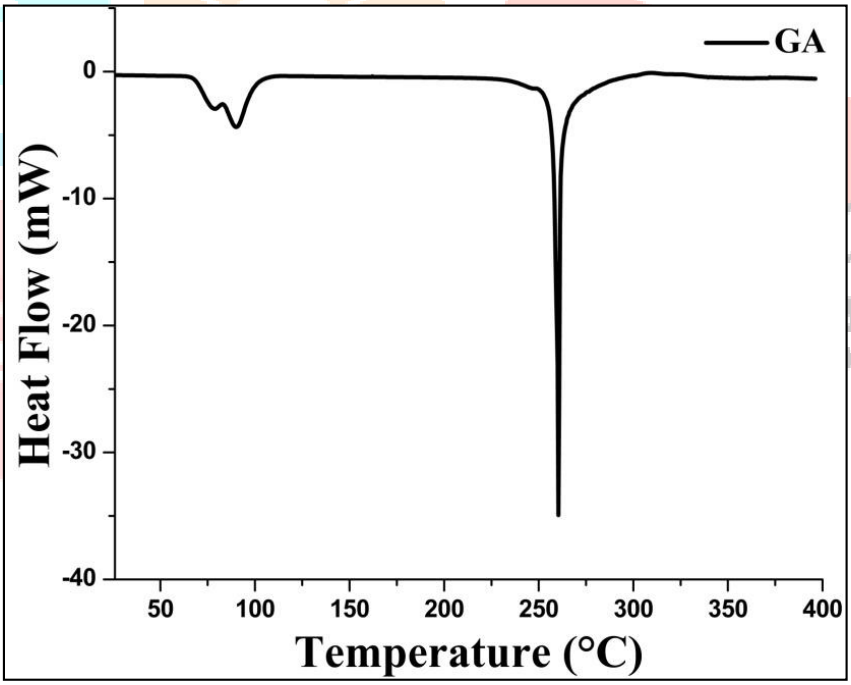

Fig.3 DSC thermogram of Gallic acid

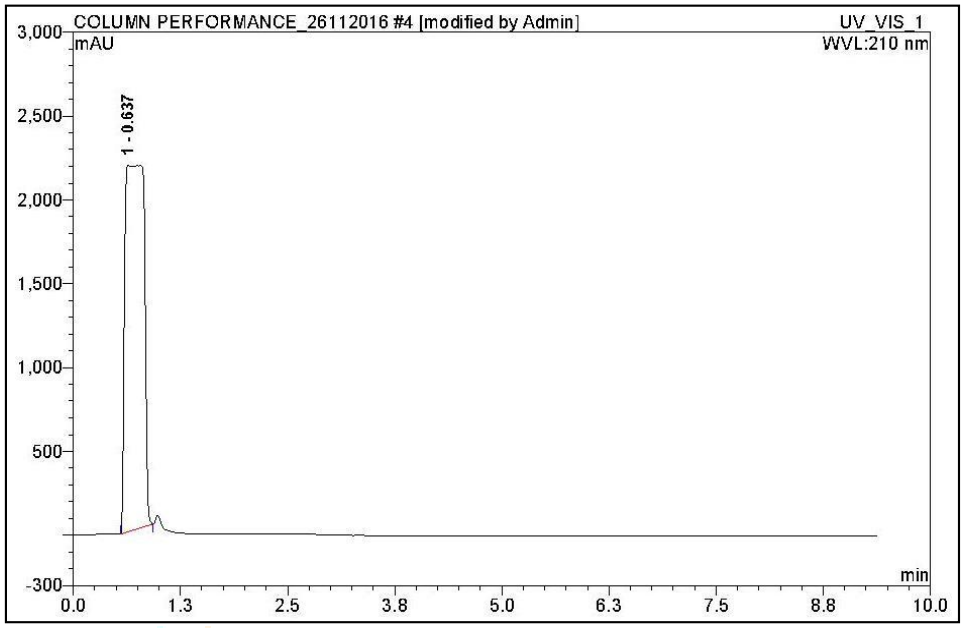

Fig.4 HPLC chromatogram of Gallic acid

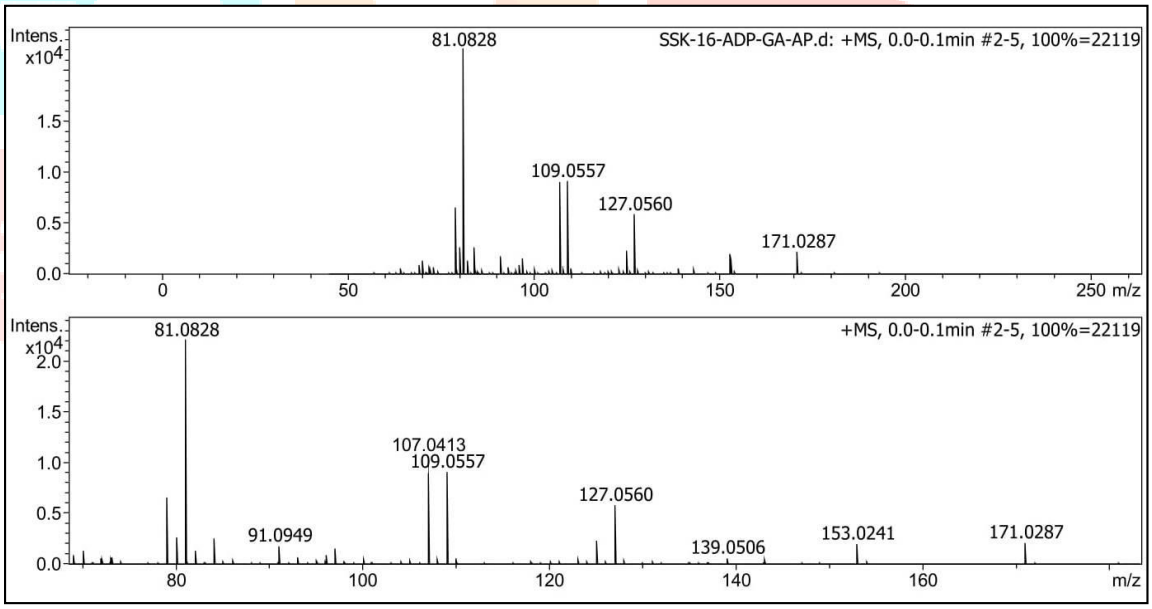

Fig.5 HR-MS spectrum of Gallic acid

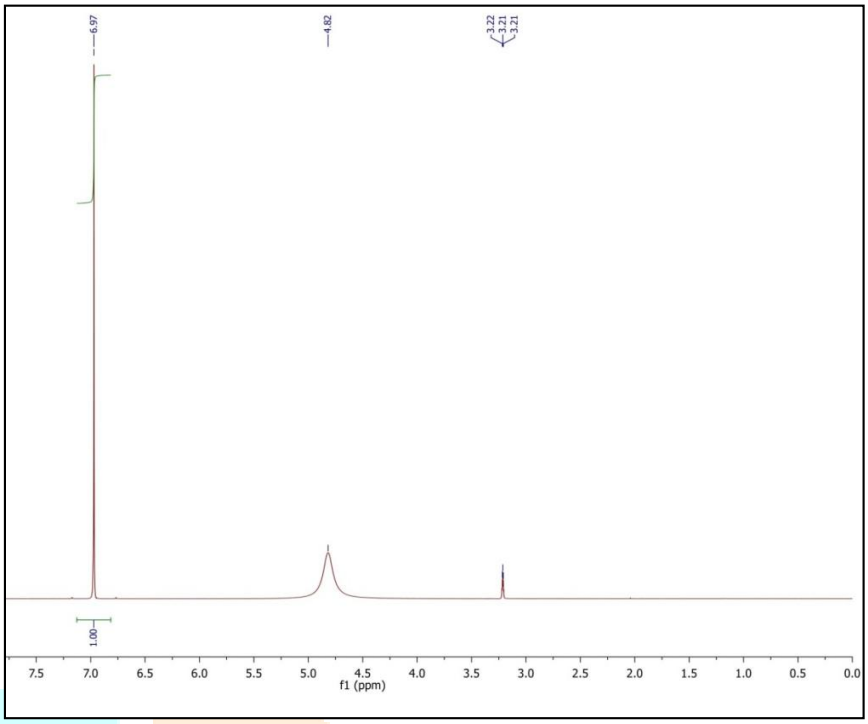

Fig.6 ^{1}H NMR spectrum of Gallic acid

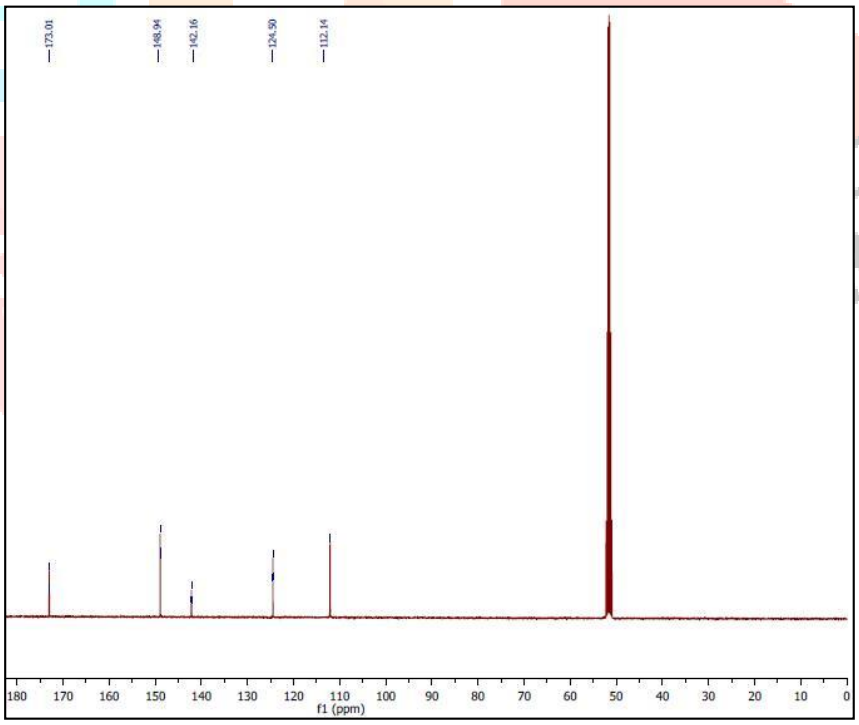

Fig.7 ^{13}C NMR spectrum of Gallic acid

The ^{1}H NMR of galic acid exhibited one singlet at δ 6.97 ppm for the two aromatic protons. Furthermore, the ^{13}C-NMR spectrum of the same showed the five signals, out of which, the signal at δ 173 ppm was assigned for the carbonyl carbon of carboxylic acid and other four signals resonated at δ 148, 142, 124, 112 ppm for the aromatic carbons. ^{1}H and ^{13}C NMR data was also similar to the reports of Kamatham et al **[11]**. On the

basis of above spectral and chemical evidences, the isolated compound identified was Gallic acid (**Fig. 4.8**).

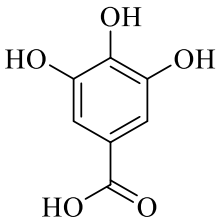

Fig.8 Structural formula for Gallic acid isolated from *F. Arnottiana*.

4 Conclusion

The compound Gallic acid was isolated as white colored powder from acetone and methanol fraction of ethanolic extract of the leaves parts of *F. Arnottiana*. The compound, Gallic acid has been identified in very few plant sources and extracted from *F. Arnottiana* first time. We have studied isolation of Gallic acid extracted from *F. Arnottiana* leaves by circularly spreaded silica gel layer technique as novel and green method of isolation which required less quantity of solvent than conventional route of column separation. Isolated compound was confirmed and characterized by using UV-Vis, FTIR, DSC, LC-MS and NMR. The above-mentioned characterization data of leaves extract also confirmed the purity of Gallic acid fraction. The presence of Gallic acid in *F. Arnottiana* leaves demonstrated that the plant may be a promising source of natural antioxidants and other bioactive compounds and may be the new horizon in food and pharmaceutical industries. This work of isolation and characterization of bioactive Gallic acid from the leaves of *F. Arnottiana* that can be of great help for its standardization, as it is a drug of controversial identity in the traditional system of medicine in the world.

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Conflicts of interest

The authors certify that there is no conflict of interest for our work

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