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### ISOLATION, PURIFICATION AND STRUCTURAL ELUCIDATION OF BIOACTIVE POLYPHENOLIC COMPOUND (CATECHIN) FROM *CRATAEVA NURVALA* BUCH- HAM STEM BARK CHLOROFORM FRACTION

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**Abstract:** 2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (Catechin), a bioactive polyphenolic compound acts as free radical scavenger in both plant and animals possibly via antioxidant activity. Literature reveals that majority of the herbs containing high level of polyphenolic compounds are traditionally being used to treat neurodegenerative disorders mediated through reactive oxygen species (ROS). With this background, current study was undertaken to isolate bioactive polyphenolic compounds from *Crataeva nurvala*, a well explored traditional Indian medicinal plant with historical evidence of efficacy in the treatment of neurological and antioxidant deficiency related disorders. In this study, chloroform fraction of ethanolic extract of *Crataeva nurvala* stem bark was purified by column chromatography (gradient elution technique) to isolate bioactive polyphenolic compound. The structure of the isolated compound was determined by various spectrophotometric analysis like UV, IR, <sup>13</sup>C and <sup>1</sup>H NMR and mass spectroscopy. Mass spectrum of isolated compound showed a parent molecular ion (M<sup>+</sup>) peak at m/z 289.0 gm/mol correspond to the molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>. In the <sup>1</sup>H NMR spectrum, multiplet (m) at δ<sub>H</sub> 5.7769-7.9909 correspond to 5H of aromatic ring; another multiplet (m) appearing at δ<sub>H</sub> 3.8645 correspond to 1H at H-2; multiplet (m) appearing at δ<sub>H</sub> 4.5351 depicted 1H at H-3, the multiplet (m) appearing at δ<sub>H</sub> 8.5715-8.9525 clearly indicated presence of 4H of Ar. –OH; another multiplet (m) appearing at δ<sub>H</sub> 2.5633 & 2.7871 were accounted for 2H at H-4α & H-4β respectively; the multiplet appearing at 2.4021 accounting for 1 H can be assigned to aliphatic –OH. Moreover, <sup>13</sup>C NMR spectrum showed presence of total 15 carbon atom in the isolated compound. Based on physical and spectral characteristics, the isolated compound was identified and reported (Catechin).

**Keywords:** *Crataeva nurvala*; Isolation; Catechin, Spectrophotometric analysis



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## INTRODUCTION

Catechin, a bioactive polyphenolic phytomolecule routinely used as herbal supplement in various diseases associated with reactive oxygen species (ROS) such as cancer, cardiovascular and neurodegenerative diseases due to its antioxidant potency <sup>[1]</sup>. Consumption of catechin through regular diet is considered as beneficial as it elevates plasma antioxidant potency and fat oxidation where as provide resistance of LDL to oxidation <sup>[2]</sup>. Moreover, epidemiological and *in-vivo* studies revealed anticancer and anti-angiogenic potency of catechin via modulating the activity of mitogen-activated protein kinases (MAPKs), IGF/IGF-1 receptor, Akt, NFκB and HIF-1α <sup>[3]</sup>. Catechin has also shown to be hypocholesterolemic activity and thus prevent the development of atherosclerotic plaques <sup>[4]</sup>. Among age-associated pathologies and neurodegenerative diseases, catechin has shown significant protection against Parkinson's disease, Alzheimer's and ischemic damage <sup>[5]</sup>. The tanning property of catechin in human skin may be an active ingredient for the treatment of leucoderma <sup>[6]</sup>. It has also shown *in-vivo* antidiabetic activity and promotes energy expenditure <sup>[7]</sup>.

*Crataeva nurvala* (*C. nurvala*) Buch-Ham (Family: Capparidaceae) commonly known as Varuna, is a well explored traditional Indian medicinal plant used to treat various ailments in particular urolithiasis and neurological disorders <sup>[8-9]</sup>. Traditionally the stem bark is also used as stomachic, laxative, anthelmintic, expectorant and anti-pyretic <sup>[10]</sup>. Moreover, pharmacological study reveals multidirectional potentiality of *C. nurvala* extract and its active principle, particularly lupeol as diuretic, anti-inflammatory, antioxidant, cardio-protective, hepatoprotective, lithonotriptic, anti-rheumatic, anti-periodic, contraceptive, anti-protozoal, rubifacient and vesicant <sup>[11]</sup>. Since no study has ever conducted to isolate bioactive polyphenolic compounds, the present study was designed to isolate and characterize bioactive polyphenolic compound from chloroform fraction of stem bark of *C. nurvala*.

## MATERIALS AND METHODS

### Collection and authentication of plant material

The stem bark of *C. nurvala* was collected from the stream sides of Westernghat, India and authenticated by Dr. K.V. Nagalakshamma, Professor and Head, Department of Biotechnology (UG) of St. Aloysius College, Mangalore, India. The herbarium (voucher specimen no. NGSMIPS/Hb-04/2011) was preserved in the institutional department.

### Extraction and fractionation

1 kg coarsely powdered raw material of *C. nurvala* stem bark was extracted by cold maceration with ethanol and concentrated through rotary flash evaporator at 40°C under reduced pressure and stored in deep freezer at -20°C <sup>[12]</sup>. The yield was found to be 17 % w/w. The concentrated

ethanolic extract (60 gm) was fractionated in the similar manner described in our previous published articles <sup>[13, 14]</sup>. Further, chloroform soluble part of ethanolic extract was chosen for isolation of bioactive polyphenolic compounds.

### Isolation and purification of compounds

Chloroform fraction (10 gm) was subjected to purification by silica gel column chromatography using ethyl acetate: methanol solvent system with increased order of polarity (from 0:100 to 100:0, v/v) <sup>[15]</sup>. The progress of separation was monitored by TLC (silica gel G 60 F254 plates, Merck). The eluents were collected into twelve different fractions. Based on their homogeneity on TLC plate, fraction IV-VI were combined together and subjected for re-chromatography where eluting with ethyl acetate: methanol (4:6) resulted a crude amorphous yellowish white substance. After being washed with methanol, it was converted to pearl white crystalline substance (40 mg). The crystals were further analyzed spectrophotometrically to elucidate the structure. TLC chromatogram developed with chloroform: ethyl acetate: methanol (1:4:6) was found to be homogenous with  $R_f$  0.46. Further, the chemical nature of the compound was evaluated by standard qualitative phytochemical screening methods.

### Qualitative analysis

#### 5% alcoholic $FeCl_3$ Test

On addition of 5% alcoholic  $FeCl_3$  to the ethanolic solution of isolated compound, deep blue black color appeared <sup>[16]</sup>.

#### Tests for alcohol

4 gm of ceric ammonium nitrate was dissolved in 10ml of 2N  $HNO_3$ , on mild heating. A few crystals of CN-01, CN-02 and CN-03 were dissolved in 0.5ml of dioxane and added to 0.5ml of ceric ammonium nitrate reagent. The developed yellow to red color indicates the presence of an alcoholic hydroxyl group <sup>[16]</sup>.

### Structural characterization of compounds

The UV spectra were recorded in the wavelength range 200-800 nm with Shimadzu UV-1700 Pharmac-spec UV-Vis spectrophotometer (Japan). The signals were acquired four times and the mean signals were taken as the best value of the UV spectra. Before every measurement the blank spectrum was also recorded, and automatically subtracted from the sample spectrum by the instrument software using the signal background ratio.

The IR spectra were recorded in the wavenumber range  $400-4000\text{ cm}^{-1}$  with a resolving power of 0.5/cm on a Alpha-Bruker IR spectrophotometer (Karlsruhe, Germany) from  $CH_2Cl_2$  sample

solution. The signals were acquired four times and the mean signals were taken as the best value of the FT-IR spectra. Before every measurement the blank spectrum was also recorded, and automatically subtracted from the sample spectrum by the instrument software using the signal background ratio.

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectrum was recorded on a Bruker Advance II 400 NMR spectrophotometer (Karlsruhe, Germany) in deuteriochloroform solutions.  $^1\text{H}$ -NMR chemical shifts are given in ppm from using tetramethylsilane (TMS) used as internal reference and  $^{13}\text{C}$ -NMR chemical shifts (in ppm) are given using DMSO and were taken from fully decoupled spectra.

Low resolution and HR electron ionization (EI) MS were recorded by the Waters Q-TOF (Micromass, Altrincham, UK) and mass spectrometer connected with a GC system HP 6890 series (Hewlett Packard, Palo Alto, CA, USA). LR-EI-MS (resolution power 1500) and HR-EI-MS (resolution power 8000, 10% resolution valley definition) were performed under the following experimental conditions: electron beam energy 70 eV; source temperature 210 $^{\circ}\text{C}$ ; source pressure 10 $^{-7}$  Torr; trap current 250  $\mu\text{A}$ ; emission current 2.3  $\mu\text{A}$ ; accelerating voltage 8.0 kV. Gas-chromatographic conditions were: injector temperature 290 $^{\circ}\text{C}$ ; column ATTM-5 (Alltech, Deerfield, IL, USA), film thickness 0.25  $\mu\text{m}$ , length 30 m, ID 0.25 mm, carrier gas (helium) flow 1.0 mL/min, isotherm at 120 $^{\circ}\text{C}$  (5 min), ramp 120–240 $^{\circ}\text{C}$  (20 $^{\circ}\text{C}/\text{min}$ ), isotherm 240 $^{\circ}\text{C}$  (9 min).

## RESULTS

From the positive qualitative analysis, the isolated compound was assumed to be flavanoid in nature. The melting point was observed at 175 $^{\circ}\text{C}$ ; UV spectroscopic analysis revealed the  $\lambda_{\text{max}}$  at 275 nm. IR spectrum showed presence of OH (amide) str. at 3610  $\text{cm}^{-1}$ , CH and CH<sub>2</sub> str. at 2874  $\text{cm}^{-1}$ , Ar. C=C str. at 1708  $\text{cm}^{-1}$ , Ar. C=C bend at 1609  $\text{cm}^{-1}$ , OH bend at 1264  $\text{cm}^{-1}$ , C-O str. at 1037  $\text{cm}^{-1}$ , CH bend at 870-675  $\text{cm}^{-1}$ . The spectral data of the isolated compound was summarized in table 1.

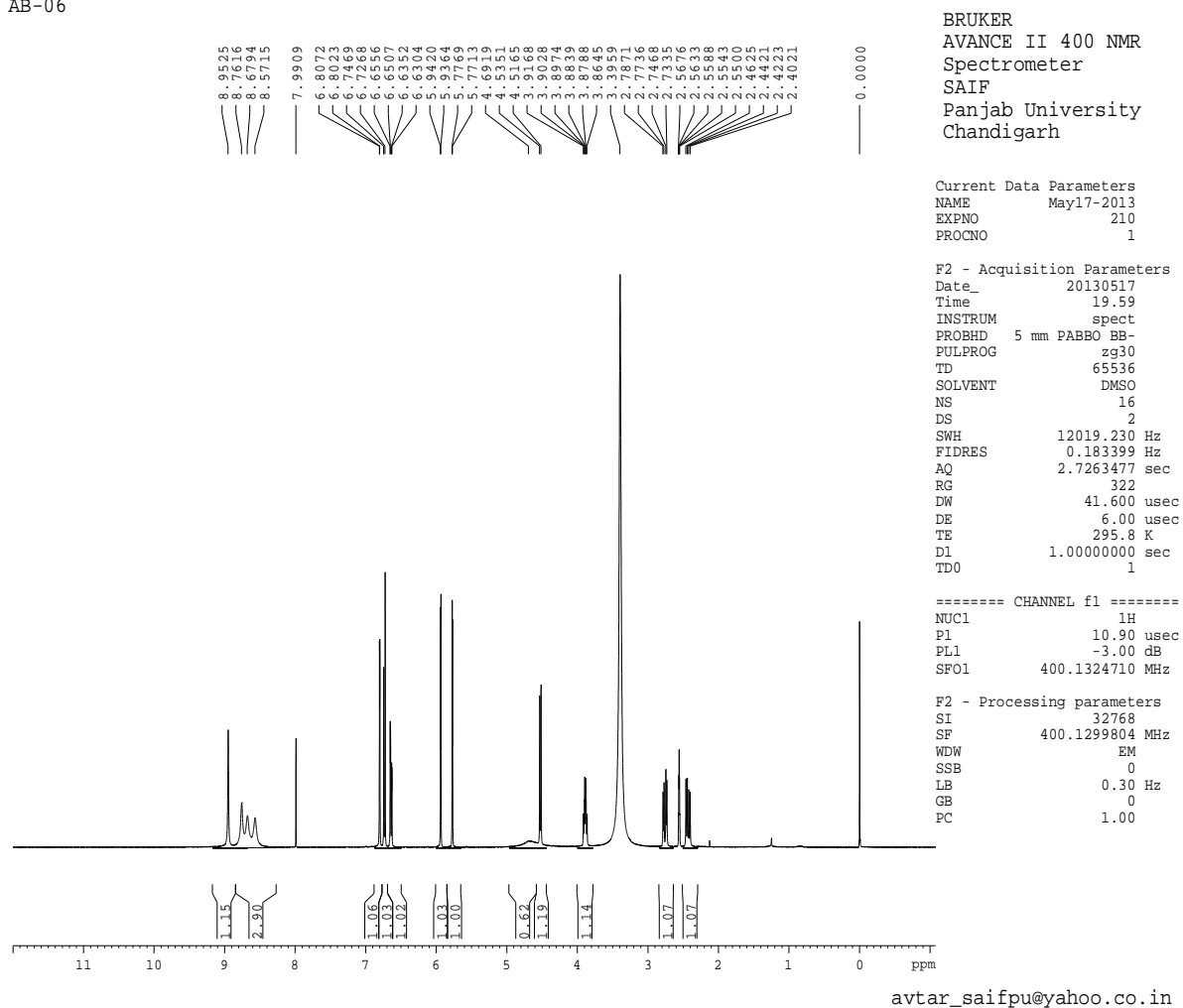
The  $^1\text{H}$  NMR spectrum in DMSO showed a sharp multiplet at  $\delta_{\text{H}}$  5.7769-7.9909 clearly indicated presence of 5 aromatic protons. Another multiplet appeared at  $\delta_{\text{H}}$  4.5351 were accounted for 1 H at H-3. Multiplet signals appearing at  $\delta_{\text{H}}$  8.5715-8.9525 accounted for 4 H of aromatic -OH group. The multiplet signals appearing at  $\delta_{\text{H}}$  2.5633 & 2.7871 were accounted for 2 H at H-4 $\alpha$  & H-4 $\beta$  respectively of the flavanoid. Further, multiplet signal appearing at  $\delta_{\text{H}}$  2.4021 correspond to 1 H, aliphatic -OH group of flavanoid (Figure 1, 2 and 3).

Moreover,  $^{13}\text{C}$  NMR spectrum revealed the presence of total 15 carbon atom in the molecule; The spectra revealed the presence of one methylene carbon at  $\delta_{\text{ppm}}$  27.53, seven methine carbons were accounted at  $\delta_{\text{ppm}}$  81.20, 66.53, 95.10, 93.88, 130.45, 114.89 and 118.28. Further,

seven quaternary carbons were accounted at  $\delta_{\text{ppm}}$  156.52, 155.98, 155.18, 99.03, 130.45, 144.65 and 144.63 (Figure 4, 5 and 6).

Mass spectroscopy revealed molecular ion peak and base peak at  $m/z=289.0$ . Further, relative intensity of different fragments were summarized (Figure 7, 8 and 9). These assignments revealed the molecular formula of the isolated compound as  $C_{15}H_{14}O_6$ . By comparing IR, TOF MS ES,  $^1\text{H}$  &  $^{13}\text{C}$  NMR data with existed literatures the isolated compound was identified as flavan-3-ol compound i.e. catechin<sup>[17-18]</sup> (Figure 10).

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Figure 1:  $^1\text{H}$  NMR spectra of isolated compound ( $\delta$  ppm = 0-10)

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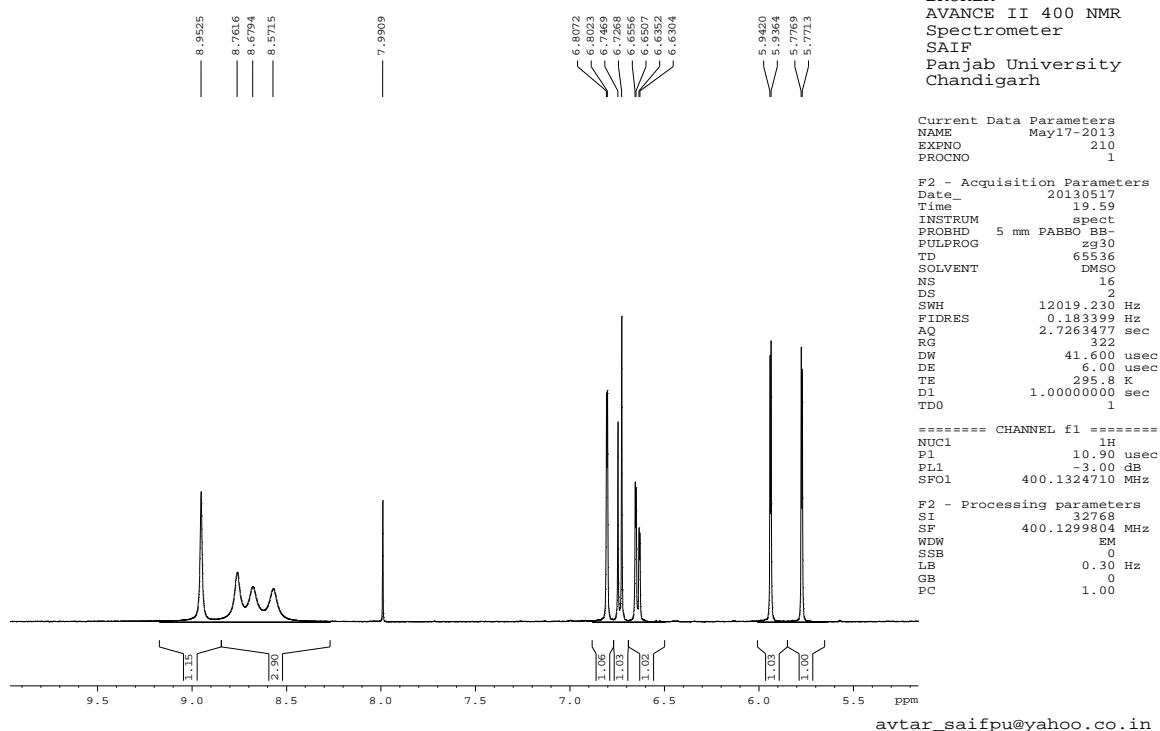


Figure 2: <sup>1</sup>H NMR spectra of isolated compound ( $\delta$  ppm = 5-10)

AB-06

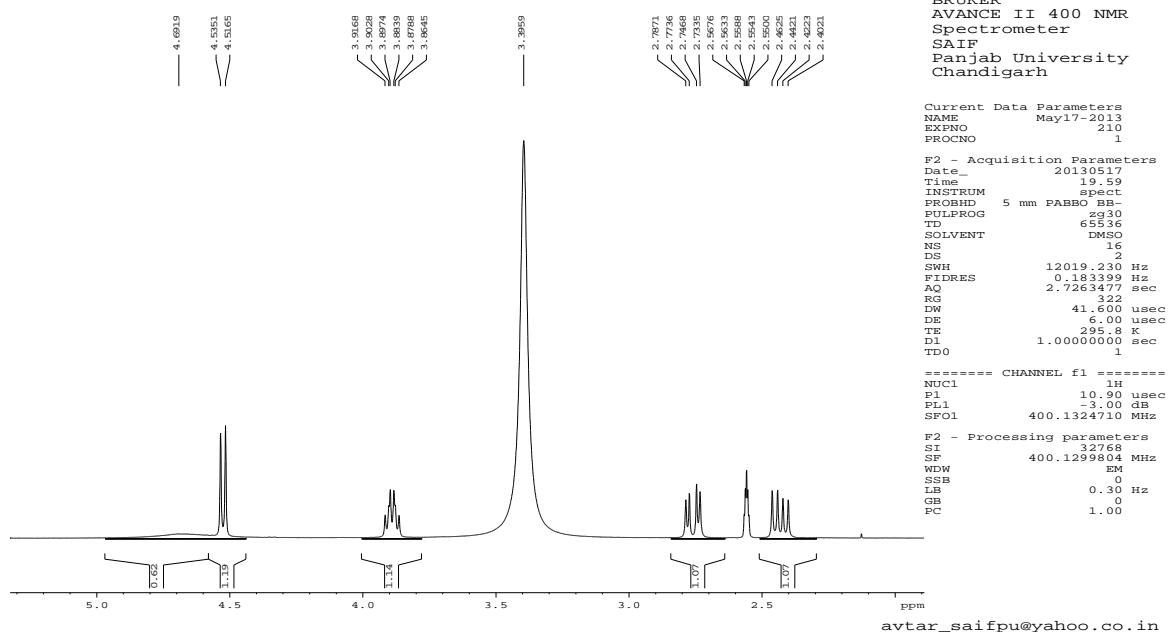


Figure 3: <sup>1</sup>H NMR spectra of isolated compound ( $\delta$  ppm = 2-5)

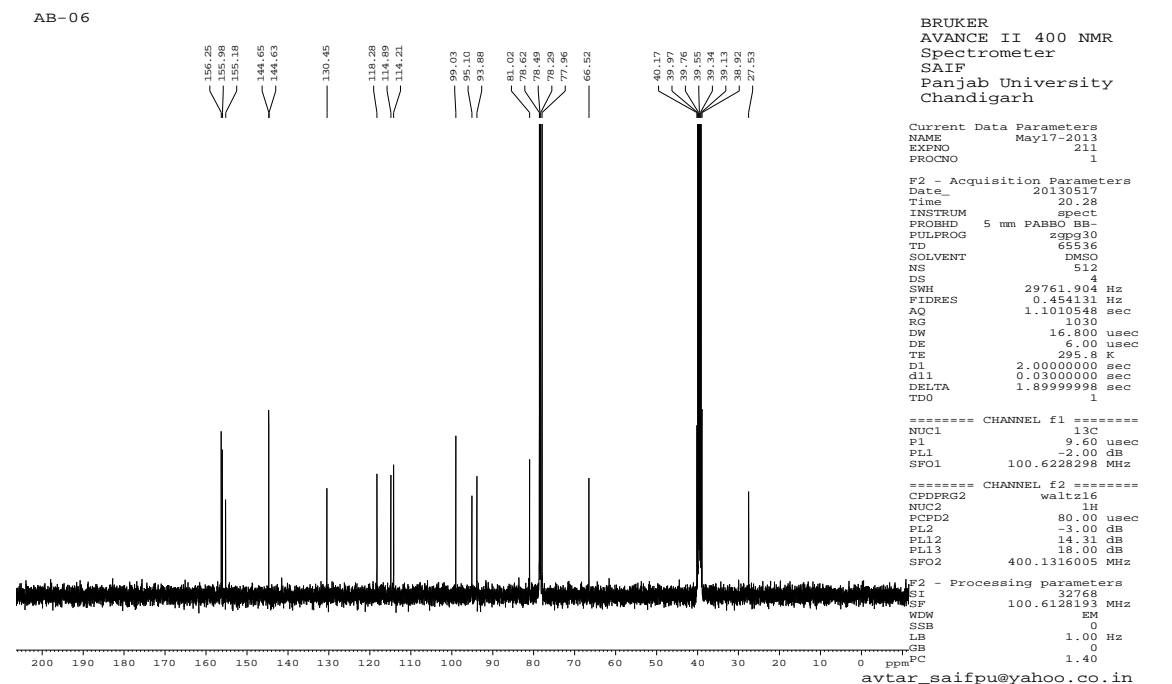


Figure 4: <sup>13</sup>C NMR spectra of isolated compound ( $\delta$  ppm = 0-200)

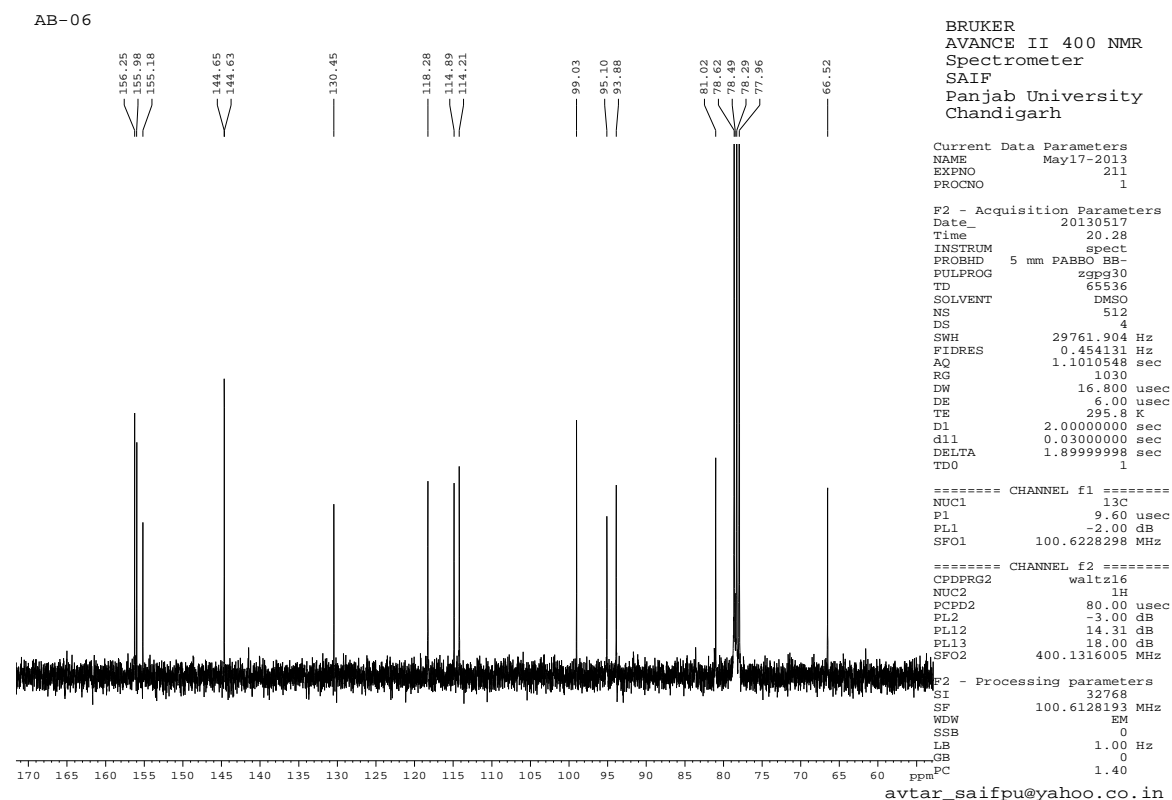


Figure 5: <sup>13</sup>C NMR spectra of isolated compound ( $\delta$  ppm = 50-170)

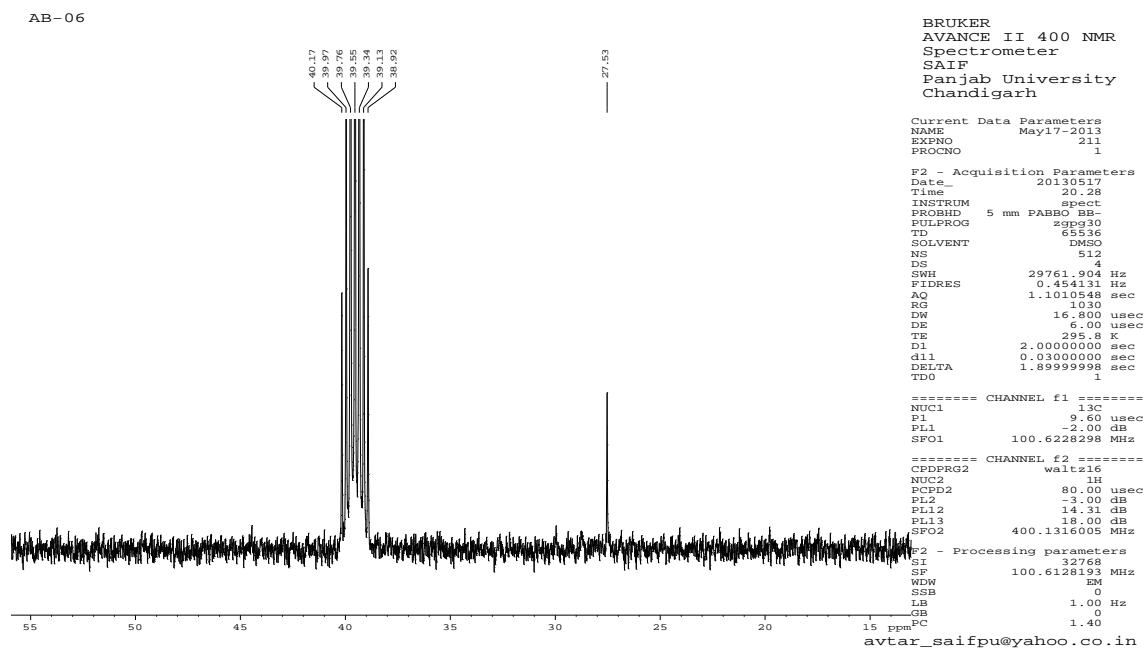


Figure 6:  $^{13}\text{C}$  NMR spectra of isolated compound ( $\delta$  ppm = 10-60)

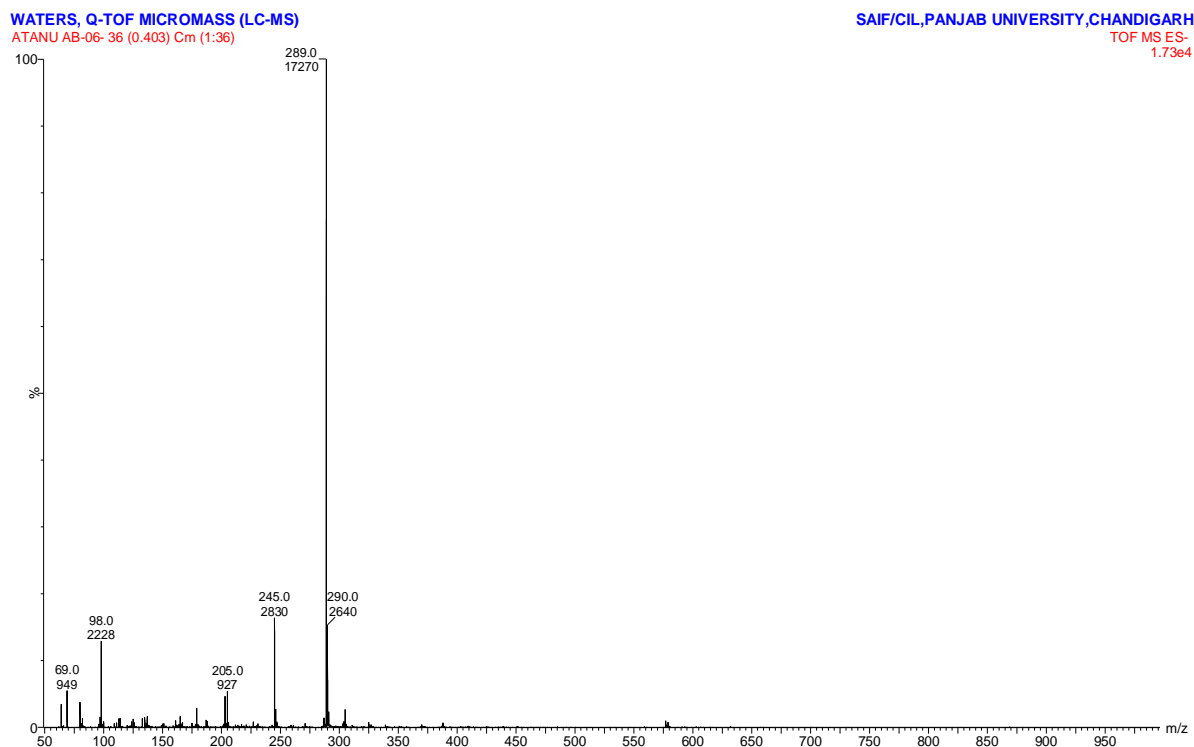


Figure 7: Mass spectrum of isolated compound ( $m/e = 50-1000$ )



WATERS, Q-TOF MICROMASS (LC-MS)  
ATANU AB-06- 36 (0.403) Cm (1:36)

SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH  
TOF MS ES-  
5.56e3

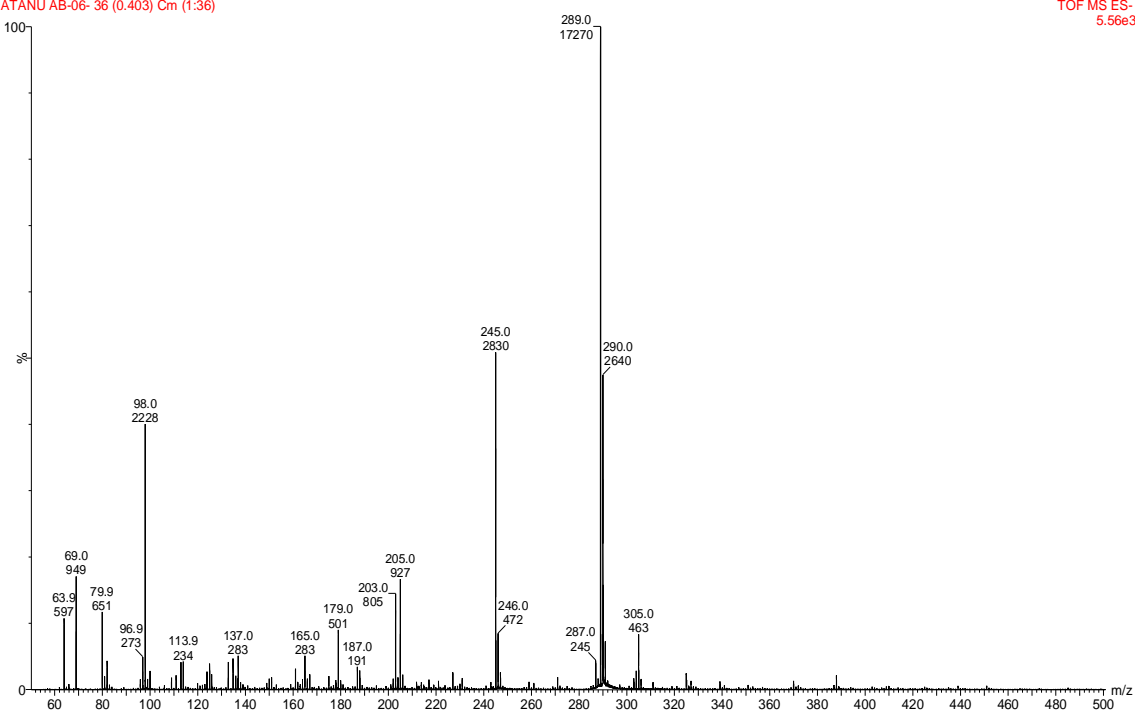


Figure 8: Mass spectrum of isolated compound (m/e = 50-500)

WATERS, Q-TOF MICROMASS (LC-MS)  
ATANU AB-06- 36 (0.403) Cm (1:36)

SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH  
TOF MS ES-  
6.15e3

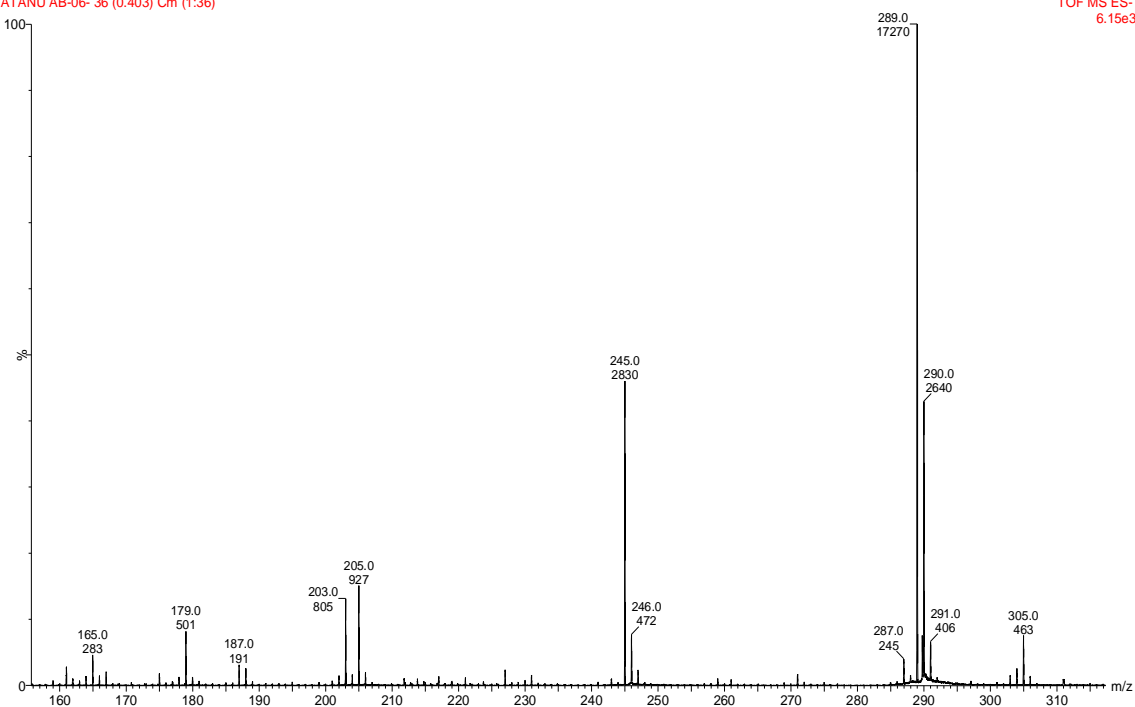


Figure 9: Mass spectrum of isolated compound (m/e = 150-310)

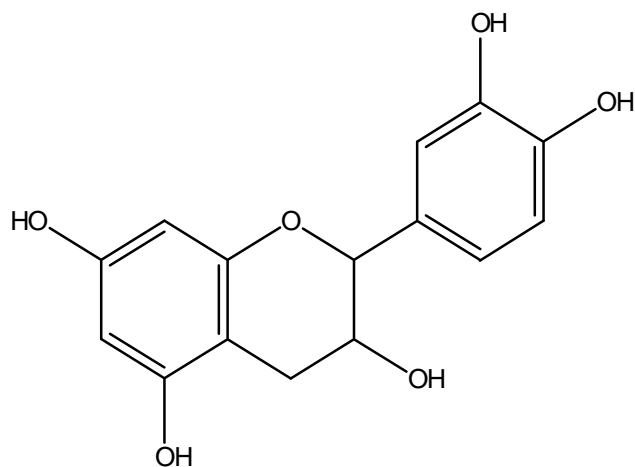


Figure 10: Catechin

Table 1: Spectral characteristic of isolated compound

IR (ATR)	3610 $\text{cm}^{-1}$ (-OH str.), 3074, 2945, 2874 $\text{cm}^{-1}$ (-CH and -CH <sub>2</sub> str.), 1708 $\text{cm}^{-1}$ (Ar. C=C str.), 1609 $\text{cm}^{-1}$ (Ar. C=C bend), 1448 $\text{cm}^{-1}$ (-CH bend), 1264 $\text{cm}^{-1}$ (-OH bend), 1037 $\text{cm}^{-1}$ (C-O str.), 870-675 $\text{cm}^{-1}$ (-CH bend)
<sup>1</sup> H NMR $\delta$ (ppm)	$\delta$ 5.7769-7.9909 (m, 5 H, Ar. H), $\delta$ 3.8645 (m, 1 H, H-2), $\delta$ 4.5351 (m, 1 H, H-3), $\delta$ 8.5715-8.9525 (m, 4 H, Ar. -OH), $\delta$ 2.5633 (m, 1 H, H-4 $\alpha$ ), $\delta$ 2.7871 (m, 1 H, H-4 $\beta$ ), $\delta$ 2.4021 (m, 1 H, aliphatic -OH)
<sup>13</sup> C NMR $\delta$ (ppm)	81.20 (C-2), 66.53 (C-3), 27.53 (C-4), 156.52 (C-5), 95.10 (C-6), 155.98 (C-7), 93.88 (C-8), 155.18 (C-9), 99.03 (C-10), 130.45 (C-1'), 114.21 (C-2'), 144.65 (C-3'), 144.63 (C-4'), 114.89 (C-5'), 118.28 (C-6')
EIMS (m/z) (%)	289.0 [M <sup>+</sup> ], (100 %),
Relative intensity	63.9 (12 %), 69.0 (17 %), 79.9 (14 %), 98.0 (42 %), 137.0 (8 %), 165.0 (8 %), 179.0 (9 %), 203.0 (14 %), 205.0 (18 %), 245.0 (50 %), 289.0 (100%), 305.0 (10%)

## DISCUSSION

**Catechin**, a flavan-3-ol compound in higher plants is a potent antioxidant and free radical (ROS) scavenger. Catechin exerts antioxidant effects via rapid electron transfer to ROS-induced radical sites on DNA, metal chelating property and reducing lipid peroxidation *in-vivo* [19]. Catechin prevents oxidative stress *in-vitro* by inhibiting the influx of calcium ions into the cell which is

pivotal to the neurodegenerative cascade as it triggers calcium dependent restriction endonuclease initiating apoptosis <sup>[20]</sup>. Further, catechin is a selective monoamine oxidase inhibitors (MAOIs) of type MAO-B. Hence, it might be used to reduce the symptoms of neurodegenerative disorders like Parkinson's and Alzheimer's <sup>[21]</sup>. The immune-modulating potentiality of catechin is associated with its histidine decarboxylase inhibitor activity and thus, it reduces of potentially damaging, histamine-related local immune response <sup>[22]</sup>. The anti-inflammatory activity of catechin might be due to their nitric oxide (NO) scavenging and reduction of NO synthase (NOS) activity <sup>[23]</sup>. Further, astringent activity of catechin favors its wound healing potential. Moreover, it exhibits cardioprotective activity which might be associated with its hypocholesterolic activity <sup>[24]</sup>. Recently, antimalarial and health tonic property of catechin has also been established <sup>[25]</sup>. Catechin have been found to have complement-inhibitory activity which might be useful in the treatment of neuro-inflammatory disorders associated with CNS like Alzheimer's, Parkinsonism and other cognitive dysfunction <sup>[9]</sup>.

## CONCLUSION

A new method of isolation for novel bioactive polyphenolic compound i.e. catechin had been developed which might be extremely suitable as marker compounds for standardization of commercial extract and herbal-preparation containing *C. nurvala*. In addition, these results will explore a new pre-clinical aspect to find the utility of *C. nurvala* against neurological disorders associated with the generation of free radicals like Alzheimer's.

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## CONFLICT OF INTEREST:

We declare that we don't have any conflict of interest

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