In vitro Evaluation (Antioxidant Activity) of coumarin derivatives

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Abstract

Coumarin is a heterocyclic molecule associated with beneficial human health effects such as to reduce the risk of cancer, diabetes, cardiovascular and brain disease. These effects are thought to be related to free radical scavenging due to their antioxidant properties. Coumarin is a substance that has been synthesized in many of its derivatives in recent years. This entity is a major source of interest for many medicinal chemists to explore its various pharmacological possibilities, especially anticoagulant activity.

The antioxidant activities of two synthesized coumarin derivatives namely 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene) acetohydra zide [M1] and 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2 hydroxy phenyl) ethylidene) acetohydrazide [M2] were studied with the DPPH method. Structure of two coumarin synthesized compounds is proposed on the basis of spectroscopic evidence.

Keywords: Coumarin, Antioxidant, DPPH, Synthesis, Ascorbic acid.

Introduction

Coumarin is one of the potent secondary metabolites of plants[1,2] and fungi[3] and is characterized by multiple pharmacological properties[4]. Like declucin and declucinol, these coumarins have a pyranocoumarin moiety isolated from the medicinal plant Angelica[5]. Many of these compounds are antibacterial[6-8], antifungal[9], anti-inflammatory[10], anticoagulant[11], anti-HIV[12] and antitumor[13].

It is shown that coumarin is commonly used as an additive in foods, perfumes, cosmetics[14], pharmaceuticals and optical brighteners[15] and disperses fluorescent and laser dyes[16]. Coumarin also has excellent optical properties such as superthermal stability, extended spectral sensitivity, high quantum yield and excellent optical stability[17].

The anti-inflammatory activity of coumarin-derived compounds has been extensively reviewed and a structure-activity relationship (SAR) has been established with anti-inflammatory effects when aromatic groups are directly fused at the 3-position of the coumarin nucleus or bound via intermediate bonds induces. Many of these derivatives also have antioxidant activity due to the scavenging mechanism[18]. A coumarin agent consisting of a fused ring of benzene and pyrone (known as 1,2-benzopyrone) is abundant in plants and more than 1300 coumarins have been identified from natural resources[19].

The synthesis of coumarins and their derivatives has received considerable attention from organic and medicinal chemists over the years, as many natural products contain this heterocyclic core[20].

Therefore, the synthesis of this heterocyclic core is very interesting. Coumarin has been synthesized by several routes including the Pechmann[21], Parkin[22], Knoevenagel[23], Reformatsky[24] and Wittig[25] reactions. Coumarin also has anticoagulant properties, some of which are commonly used as anticoagulants such as warfarin and acenocoumarol[26-29].

Antioxidants have the ability to protect organelles from damage caused by oxidative stress caused by free radicals. The free radicals used include hydroxyl radicals, superoxide anion radicals and hydrogen peroxide. Reactive free radicals formed by exogenous chemicals, stress, or food systems can oxidize biomolecules, causing cancer, coronary artery disease and hypertension[30].

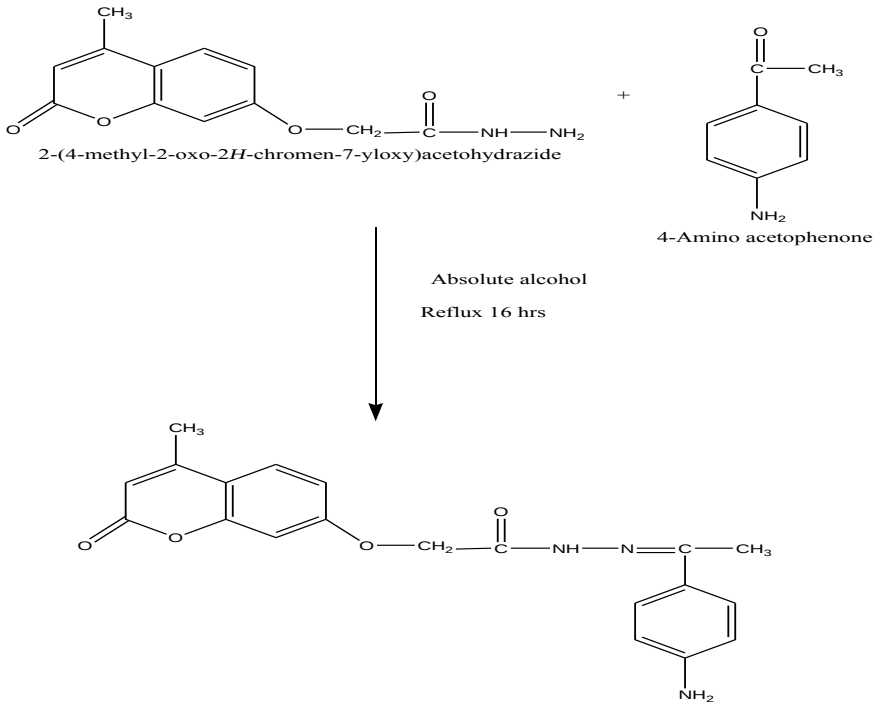
In general, most of the metabolically produced free radicals are removed by endogenous defense systems such as catalase, superoxide dismutase and the peroxidase-glutathione system[31].

Material and Methods

Materials: FTIR spectra of the samples are performed by FTIR Spectrophotometer MODEL-8300 of SHIMADZU, in the region of 400 4000 cm^{-1}. Melting points were recorded by the melting point apparatus. All reactions and product purity were examined by thin layer chromatography (TLC) on aluminum back panels coated with silica gel.

All chemicals were purchased from Aldrich Sigma Company. NMR spectra of the synthesized compounds were recorded in DMSO (dimethyl sulfoxide) using BROKE JEOL Model AV300 at 300 MHz spectrometer.

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)-ethylidene)-acetohydrazide [M1][32]: A mixture of 2-3 drops of glacial acetic acid in compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mol), 4-aminoacetophenone (0.0008 mol) and anhydrous alcohol (60 mL) was refluxed on a water bath for 16 hours. The reaction mixture was poured into crushed ice. It is filtered, dried and recrystallized from ethanol to give compound (M1).

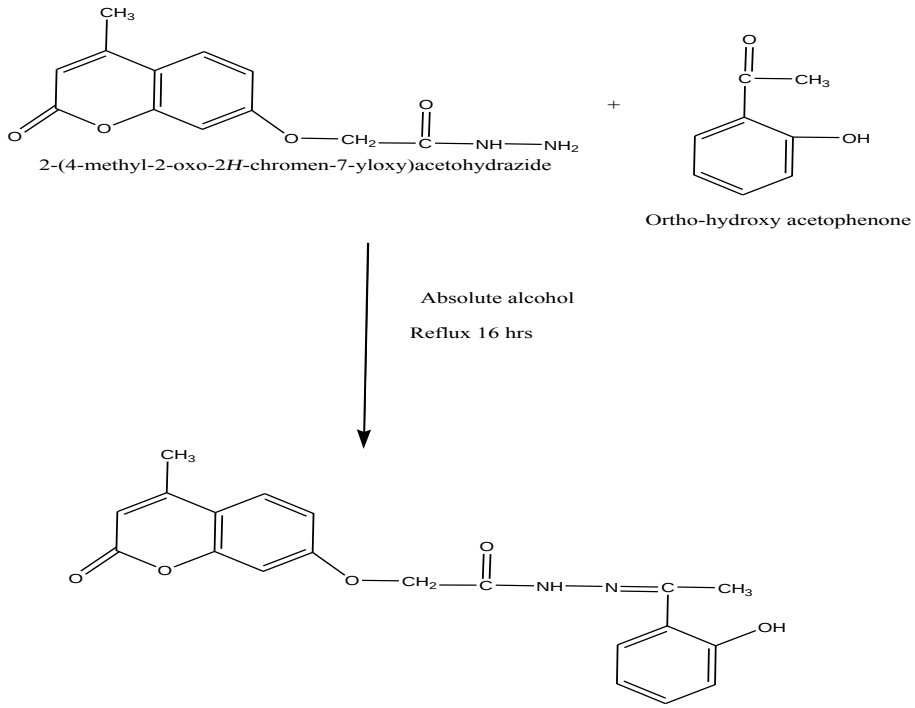


Scheme 1: 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl)ethylidene)acetohydrazide

Physical features:
a) Percentage yield: 72%
b) Melting point: 215^{0}C
c) Rf value: 0.32
d) Mobile phase: Ethyl acetate: Hexane (1:1).

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2 hydroxy phenyl) ethylidene) acetohydrazide [M2][32]: A mixture of 2-3 drops of glacial acetic acid in compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide (0.0008 mol), o-hydroxyacetophenone (0.0008 mol) and anhydrous alcohol (60 mL) was refluxed on a water bath for 16 hours. The reaction mixture was poured into crushed ice. Filter, dry and recrystallize from ethanol to give compound (M2).

Physical features:
a) Percentage yield : 76%
b) Melting Point : 264 ^{0}C
c) Rf value : 0.24
d) Mobile Phase : Ethyl acetate: Methanol (1:1)



Scheme 2: 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene)acetohydrazide

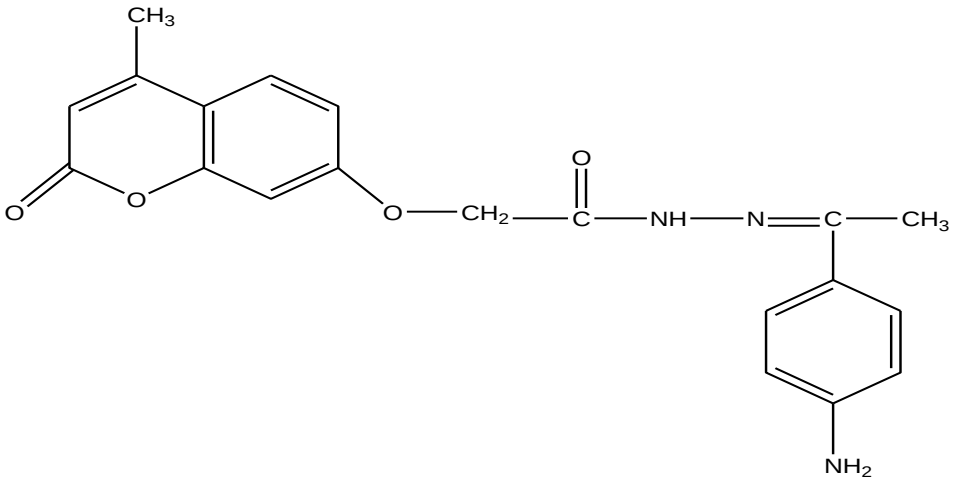
Antioxidant activity

DPPH Assay: The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on a DPPH free radical. Different concentrations (20, 50, 100, 200 and 400 g/mL) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 2mL of DPPH solution (0.002% in methanol) was mixed with 2mL of different concentrations of compounds and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517nm using UV-Visible Spectrophotometer. The absorbance of the DPPH control was also noted[33,34]. The scavenging activity was calculated using the formula: Scavenging activity (%) = (absorbance of control — absorbance of sample / absorbance of control) × 100.

Results and Discussion

Two 7-hydroxy 4-methylcoumarin derivatives were prepared according to the scheme. Yields for all compounds were calculated. Their physical constants and thin layer chromatography confirmed the purity of the synthesized compounds. The structure of the synthesized compound was confirmed by IR and 1H NMR spectroscopy.

The IR spectrum is obtained by preparing KBr pellets with a Shimadzu FTIR spectrophotometer 8300 and is expressed as a wave number in cm^{-1}. The 1H NMR spectra of the synthesized compound were recorded in DMSO using an AV300BROKEJEOL on a 300MHz spectrophotometer.



Structure of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(4-aminophenyl) ethylidene) acetohydrazide

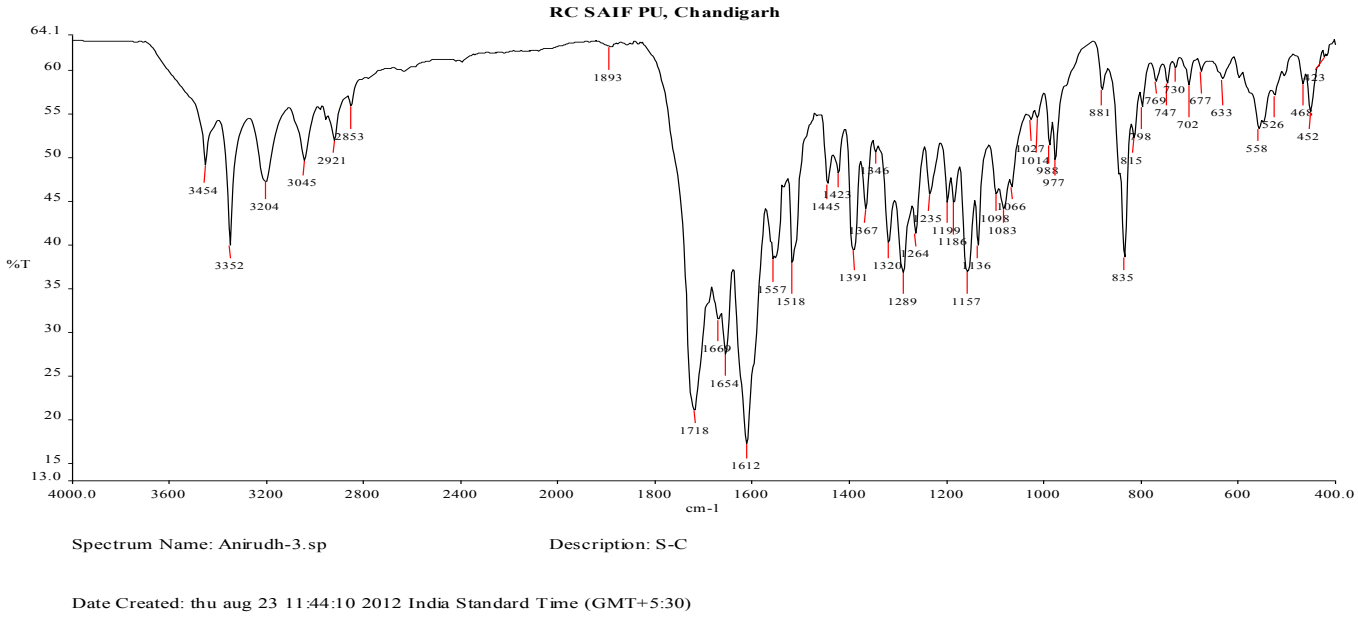


Figure 1: IR Spectra of 2-(4-methyl-2-oxo-2H-Chromen-7-yloxy)-N-(1-(4-aminophenyl) ethylidene) acetohydrazide(M1)

Table 1
IR Spectra value of M1

S.N.	v (cm^{-1})	Functional Group Assignment
1.	3045	C-H str. Aromatic
2.	3352	N-H str.
3.	2921	C-H str. Aliphatic
4.	1718	C=O str.
5.	1669	C=O str.
6.	1612	C=C str. aromatic
7.	1518	C=N str.
8.	1391	C-N str.
9.	1083	C-O str. in C-O-C

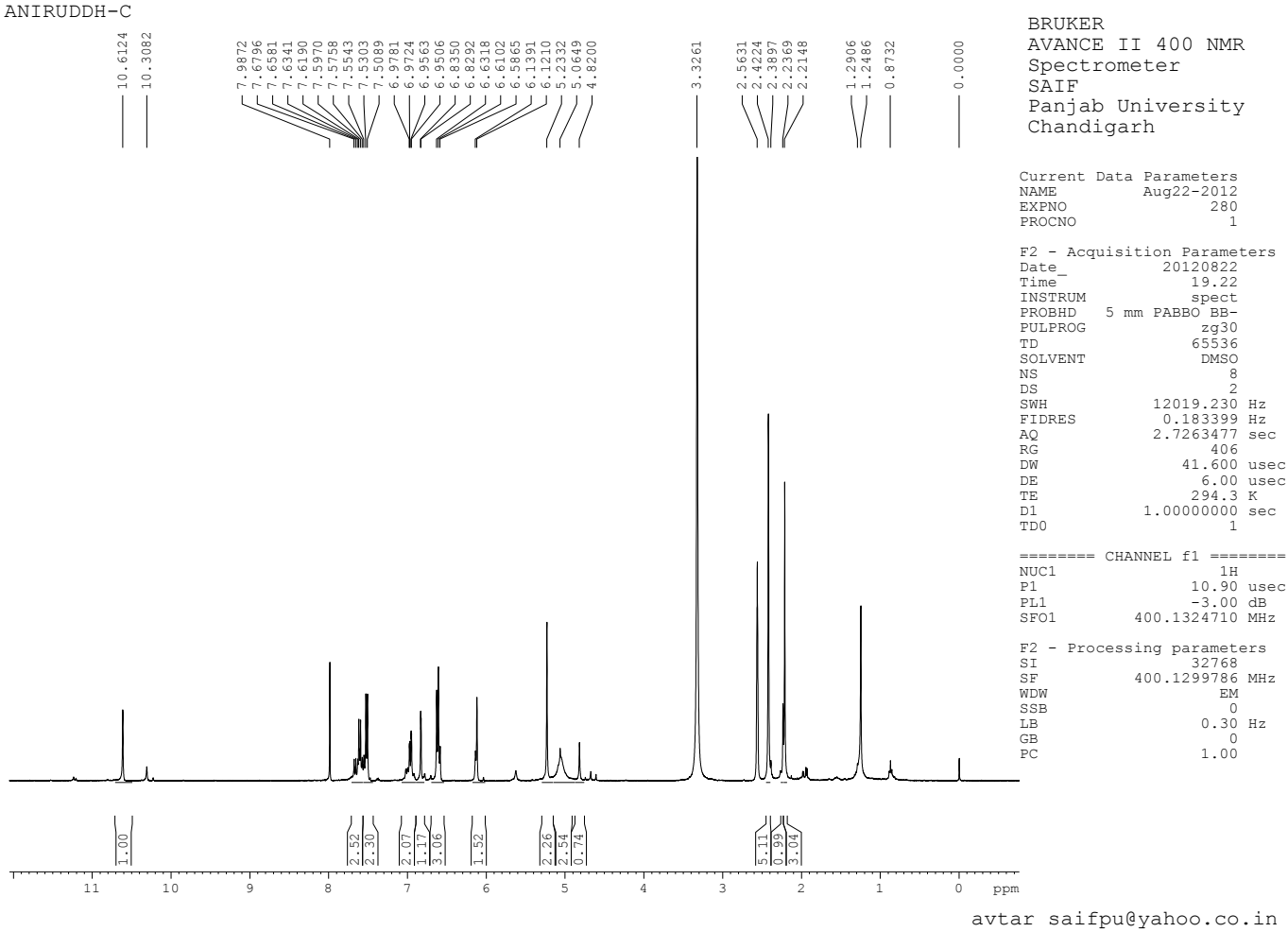
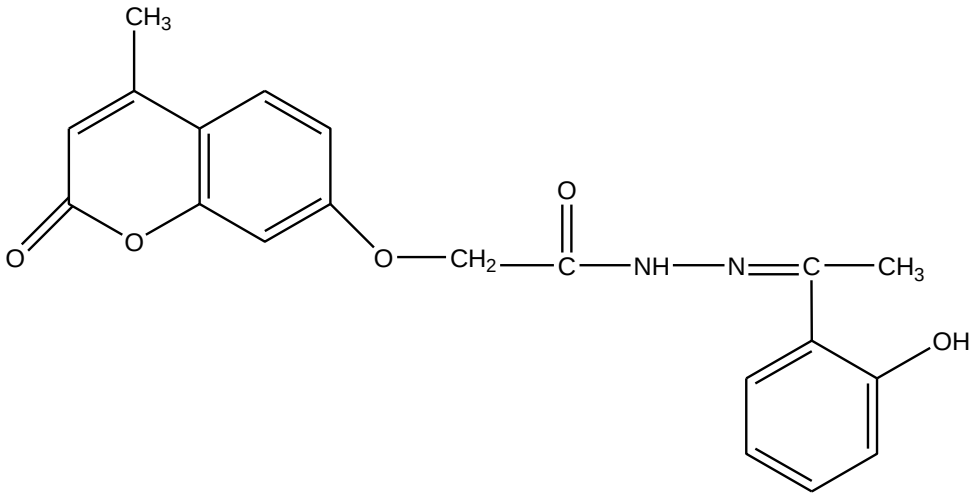


Figure 2: NMR spectra of (M1)

Table 2
NMR spectra value of (M1)

S.N.	Chemical shift(δ)(ppm)	No. of protons	Inferences
1.	10.61	1H	-NH
2.	6.12-7.98	8 H	Aromatic
3.	5.06	2 H	NH_2
4.	5.23	2H	$-OCH_2$
5.	3.32	3H	CH_3 attached to coumarin ring
6.	2.23	3H	CH_3



Structure of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene)acetohydrazide

2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N'-(1-(2-hydroxyphenyl)ethylidene)acetohydrazide

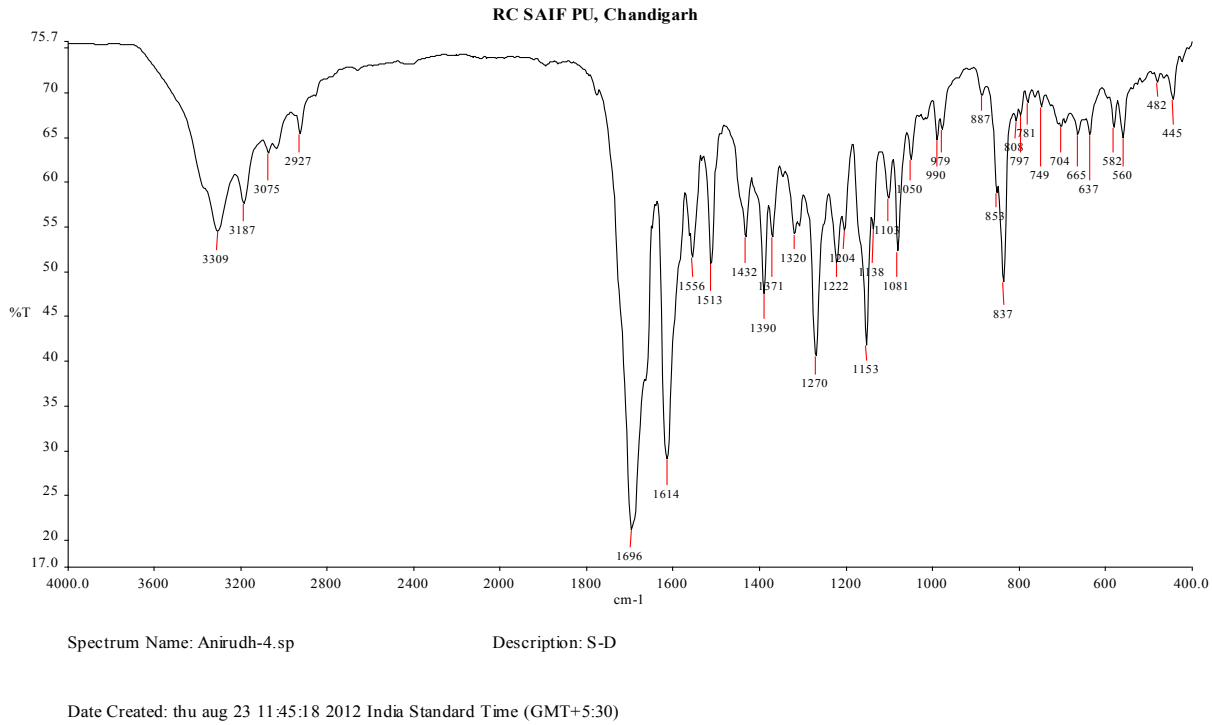


Figure 3: IR Spectra of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(1-(2 hydroxyphenyl)ethylidene) acetohydrazide(M2)

Table 3
IR Spectra value M2

S.N.	ν (cm^{-1})	Functional Group Assignment
1.	3309	N-H str.
2.	3075	C-H str. Aromatic
3.	2927	C-H str. Aliphatic
4.	1696	C=O str.
5.	1614	C=O str.
6.	1556	C=C str. aromatic
7.	1513	C=N str.
8.	1390	C-N str.
9.	1081	C-O str. in C-O-C

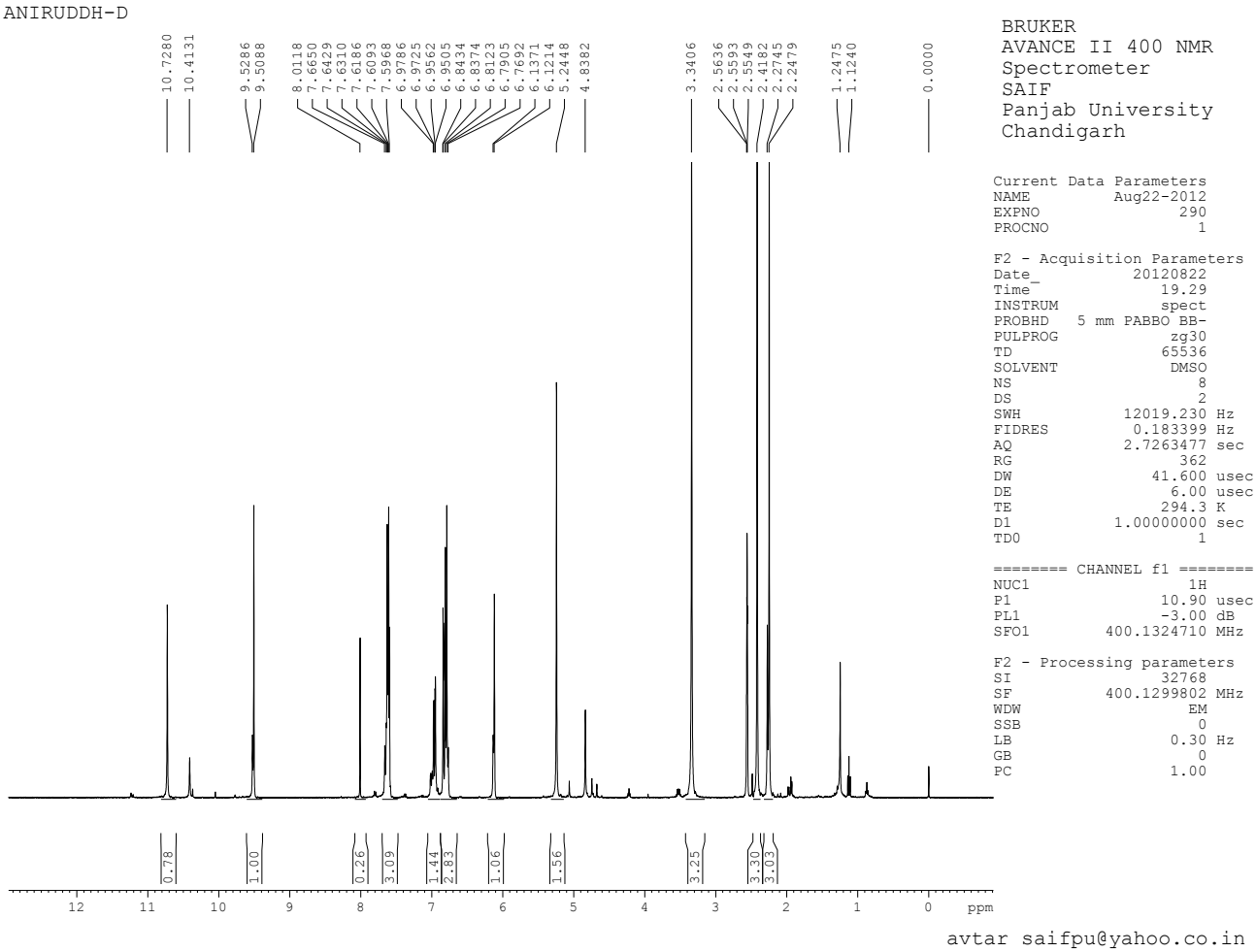


Figure 4: NMR spectra of (M2)

Table 4
NMR Spectra value (M2)

S.N.	Chemical shift(δ)(ppm)	No. of protons	Inferences
1.	10.72	1H	-OH
2.	10.41	1 H	-NH
3.	6.12-8.01	8 H	Aromatic
4.	5.24	2H	$-OCH_2$
5.	3.34	3H	CH_3 attached to coumarin ring
6.	2.41	3H	CH_3

Table 5
Scavenging activity of different concentration in %

S.N.	Scavenging activity of different concentration (µg/ml)				
	20	**50**	**100**	**200**	**400**
Control	79.34	82.54	89.21	95.45	97.54
Test (M1)	82.98	89.39	85.42	92.87	95.75
Test (M2)	83.65	81.47	91.45	96.07	99.04

Antioxidant Activity: The antioxidant activity at different concentrations 20, 50, 100, 200 and 400 µg/ml of the synthesized compound and ascorbic acid was tested on the basis of the radical scavenging effect of the stable DPPH free radical assay. The obtained results were recorded in table 5 In this study, the absorbance was found to increase with the dose of compound and standard.

Conclusion

Coumarin derivatives play an important role for several of biological activities. We synthesised two coumarin derivatives M1 and M2 characterised by chromatography, melting point, FTIR and NMR. The synthesised compounds were screened for antioxidant activity.

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