BIOL MOL CHEM

Research Article

Biological and Molecular Chemistry

Determining the Antioxidant Properties of Synthesized Quercetin and Comparing it With Its Standard Sample in Oak

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ABSTRACT: A distinctive flavonol with five hydroxy groups (-OH) based on the chemical makeup of the flavone backbone is quercetin (3,3',4',5,7-hexahydroxyflavone). Here, we present a succinct, effective, and scalable synthesis of quercetin, an essential biological compound with antibacterial and antioxidant properties, in oak. With a 95% total yield, the synthesis was accomplished in multiple phases using inexpensive and readily available basic ingredients. The ^{1}HNMR, ^{13}CNMR, and UV spectra of this synthetic substance have verified its chemical structure. The results indicate that when compared to control molecules, synthesized quercetin exhibits the highest level of antioxidant activity.

KEYWORDS: Flavonol, Quercetin, Antioxidant, Oak.

Introduction

The oak tree, which is often grown in Iran's west, southwest, north, and northwest, is a member of the genus Quercus and family Fagaceae [1]. The fruit of the oak tree is rich in minerals, iron, manganese, copper, potassium, magnesium, and dietary fiber, among other beneficial substances. In addition, it

Received: Feb 28, 2024
Revised: Mar 10, 2024
Published: Mar 23, 2024

DOI: 10.22034/bmc.2024.449698.1032
Biol. Mol. Chem. 2024, 2, 23-32

contains significant amounts of unsaturated fatty acids, vitamins E and C, tannins, ellagic acid, gallic acid, quercetin, different galloyls, and derivatives of hexahydroxydiphenyl [2]. As a result, it can be regarded as a good source of antioxidant-active chemicals in food that have numerous beneficial impacts on human health [3]. Despite having less protein than cereal grains, oak fruit has unique nutritional qualities and is regarded as having biological value and an essential amino acid index similar to that of egg protein [4]. This valuable fruit can therefore be used for many purposes in pareshki, especially given the high annual output of the fruit in the nation and its lack of practical application, as well as the industry's propensity to use as many herbal and natural chemicals as possible in many disciplines [5]. Oak, with its high tannin content, possesses remarkable potential as an antibacterial agent against a wide range of microorganisms [6]. Its astringent qualities also aid in wound healing. In other words, eating oak fruits can aid in the removal of bacteria from the body since they also impede the growth and dissemination of germs and bacteria, preventing the spread of bacteria further [7].

The majority of fruits, vegetables, leaves, and seeds contain the flavonoid quercetin [8]. It can also be found in wines, meals, and dietary supplements [9]. A class of flavonoid chemicals known as flavonols share the 3-hydroxyflavone structure. Numerous fruits and vegetables contain these substances [10]. Yellow pigments are produced by a class of secondary plant compounds called flavonoids[11] . With about 4000 components, flavonoids are a broad class of polyphenols that have heart-protective, anti-inflammatory, cancer-preventive, and antioxidant properties in humans [12]. They also play an antioxidant function in photosynthesis in plants. The flavonoid molecule quercetin is used to combat cancer cells and viruses. One flavonoid that has anti-inflammatory and antioxidant qualities is quercetin [13]. One of the common food flavonoids, quercetin, has several beneficial effects on the heart and blood circulation system, including dilatation of conduction and resistance channels [14]. Furthermore, the endothelium's altered functions in diabetes mellitus enhance the vascular response to contractile agonists. Onions, apples, and other foods naturally contain quercetin, an antioxidant [15]. The antihistamine properties of quercetin are demonstrated by the findings of several investigations. According to study findings, this substance lessens the inflammatory response in the airways, which helps mice's allergic reactions [16]. Higher levels of quercetin absorption are linked to lowered blood pressure and a lower risk of heart disease. Although quercetin is present in most plants, apples, cranberries, cocoa, onions, and capers are the best food sources [17]. There are, of course, further supplements. Among the benefits of quercetin are: Reducing symptoms of allergies and asthma assisting males with prostate issues; lowering fatigue, anxiety, depression, and pain associated with arthritis; According to recent research, quercetin can delay the onset of cancer signs [18]. Slowly Quercetin also causes dizziness, nausea, and vomiting as side effects. In severe situations, headaches and vertigo may also result. Sweating and heat flushes typically accompany all of these symptoms [19]. Stomach distress is one of the negative effects of quercetin dihydrate that those with weak or sensitive digestive systems should be aware of. Heartburn, reflux, indigestion, and upset stomach are all possible side effects of quercetin [20]. This situation occurs especially when it is taken on an empty stomach. As a result, when one intends to take any quercetin supplement, it is important to do so with meals [21]. In this way, a person can avoid this negative reaction. In some cases, if quercetin is not taken in the correct amount, then it can also lead to a pro-oxidant effect. It means that the positive effect that quercetin is supposed to have, such as reducing inflammation, actually turns into a negative effect [22]. Therefore, instead of reducing inflammation, quercetin may cause inflammation. In this way, it invalidates the purpose of prescribing and consuming this medicine. Therefore, to avoid this effect, make sure that quercetin is taken in small amounts [23].

DOI: 10.22034/bmc.2024.449698.1032
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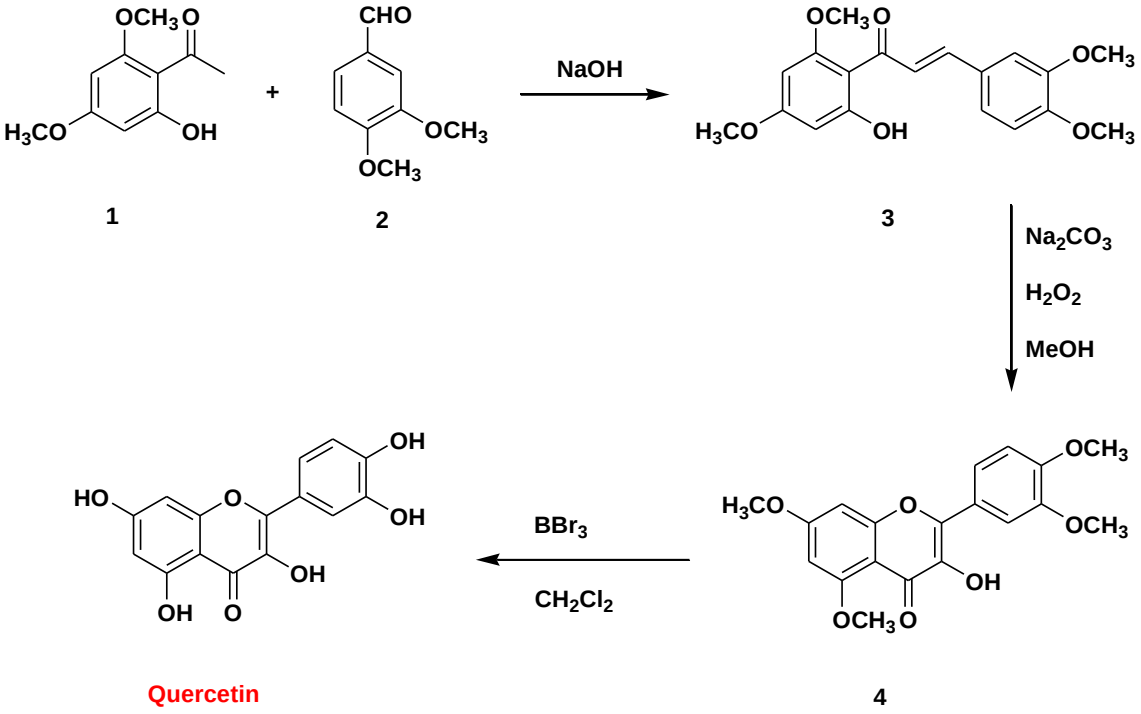
In this work, we report the successful, effective, and scalable production of quercetin in oak, a vital biological substance possessing antioxidant and antibacterial qualities. The synthesis was done in multiple steps with a 95% total yield, using accessible and reasonably priced starting ingredients. The outcomes demonstrate that the synthesized quercetin exhibits the highest level of antioxidant activity when compared to standard molecules.

Results and Discussion

Scheme 1 describes the synthetic process used to manufacture quercetin. In order to produce the chalcone with a 92% yield, the synthesis starts with an aldol-type condensation reaction between ketone 1 and aldehyde 2 in the presence of NaOH as a base. Nonetheless, we were able to effectively accomplish the oxidative cyclization of 3 to 4 in the presence of Na_2CO_3 as a base and hydrogen peroxide (H_2O_2) as an oxidant. Target quercetin was synthesized with an overall yield of 95% with the use of boron tribromide in a demethylation process.



Scheme. 1. Synthetic process used to manufacture quercetin.

Synthetic quercetin's UV–vis absorption spectra were captured in MeOH, DMSO, and PBS. Its maximal absorbance in MeOH is 365 nm, in DMSO it is 373 nm, and in PBS it is 376 nm, as shown in Figure 1. As the solvent's polarity increased, redshift or bathochromic shift in absorbance maxima was seen, which can be explained by the solvents' polarization effects. More so than the ground state, the excited state is stabilized by the polar solvents. By doing this, the HOMO-LUMO energy gap can be reduced, which causes a redshift or bathochromic shift in the synthetic quercetin's UV–vis spectra.

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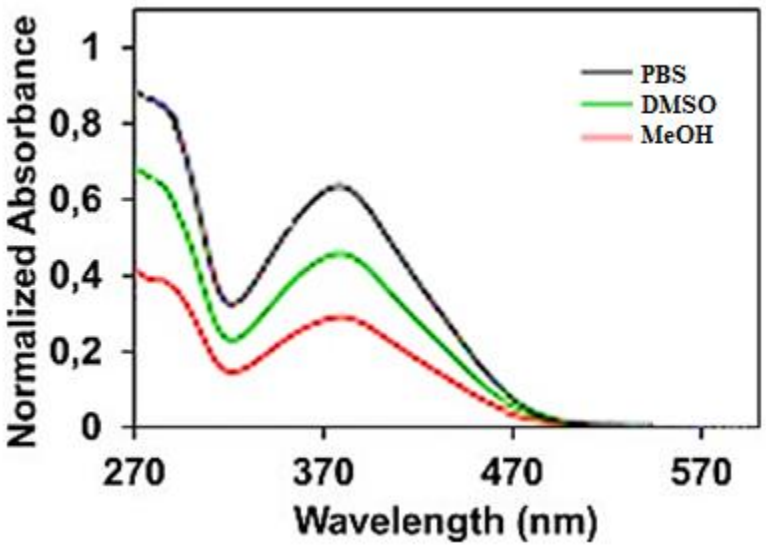

Figure 1. UV–vis absorbance spectra of synthetic quercetin in MeOH, DMSO and BPS.

The ^{1}H NMR and ^{13}C NMR techniques were used to confirm the structure of quercetin. Five hydroxyl proton signals were detected at δ 12.48 (s, 1H), 10.81 (s, 1H), 9.65 (s, 1H), 9.32 (s, 1H), and 9.31 (s, 1H) in the ^{1}H NMR spectra of target quercetin. Five aromatic proton signals were detected at δ 7.64 (d, 1H), 7.54 (m, 1H), 6.88 (d, 1H), 6.41 (d, 1H), and 6.18 (d, 1H). Furthermore, the synthetic quercetin's ^{13}C NMR spectrum showed fifteen carbon peaks, which corresponded to fourteen distinct kinds of carbons in the compound's chemical structure. With respect to the carbon in the carbonyl functional group, the signal at δ 178.12 matches.

Antioxidant activity

In this study, two antioxidant methods based on inhibition of DPPH and ABTS free radicals were used to investigate the antioxidant activity of ethanol extract of oak leaves. **Figure. 2** The antioxidant activity of the extract was equal to 53.67% based on DPPH free radical inhibition and 62.50% based on ABTS free radical inhibition.

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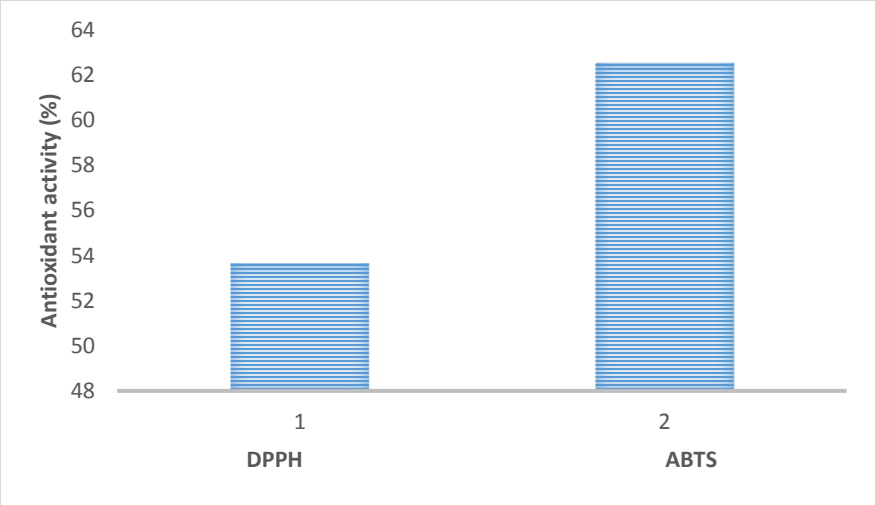
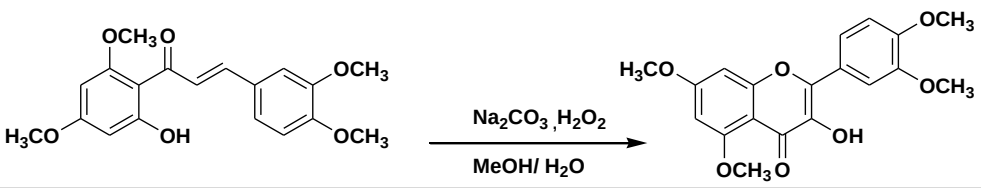


Figure 2. The amount of antioxidant activity of quercetin oak leaves based on DPPH and ABTS free radical inhibition methods.

Optimal circumstances were used to carry out this reaction in order to generate a better product. (**Table 1**). In a methanol/water mixed solvent with hydrogen peroxide present, a range of organic and inorganic bases were screened at ambient temperature. With a 95% yield, sodium carbonate was the most effective base. Flavanones are produced instead of flavonols when O-Michael addition is promoted by weak bases like sodium acetate and sodium bicarbonate. These were most likely brought on by weak bases' incapacity to start the reaction. However, the main compounds that were most likely generated were 2-hydroxy-6-methoxybenzoic acid and 4-methoxybenzaldehyde when strong bases like KOH, $N(Et)_3$, DBU, and TBD were used. Next, we ran the process using various solvents. Among the other solvents we investigated, we discovered that a 2:1 methanol/water mixture worked the best. In an effort to further refine the ideal circumstances for flavonoid production, the sodium carbonate and hydrogen peroxide equivalents were examined. The optimal mixture was 2.5 equivalents of hydrogen peroxide and 5 equivalents of sodium carbonate. We discovered that chalcone was converted to flavonone rather than flavonol by reducing the equivalents of hydrogen peroxide and sodium carbonate, although an increasing amount of the byproducts 2-hydroxy-6-methoxybenzoic acid and 4-methoxybenzaldehyde were formed.

Table 2. Optimizing the synthesis of quercetin[a]



Entry	Base (5 eq)	Solvent	H_2O_2(eq)	Yield (%) [a]
1	NaOAc	EtOH/H_2O	5	No
2	K_2CO_3	EtOH/H_2O	5	24
3	NaOH	EtOH/H_2O	5	19
4	DBU	EtOH/H_2O	5	38

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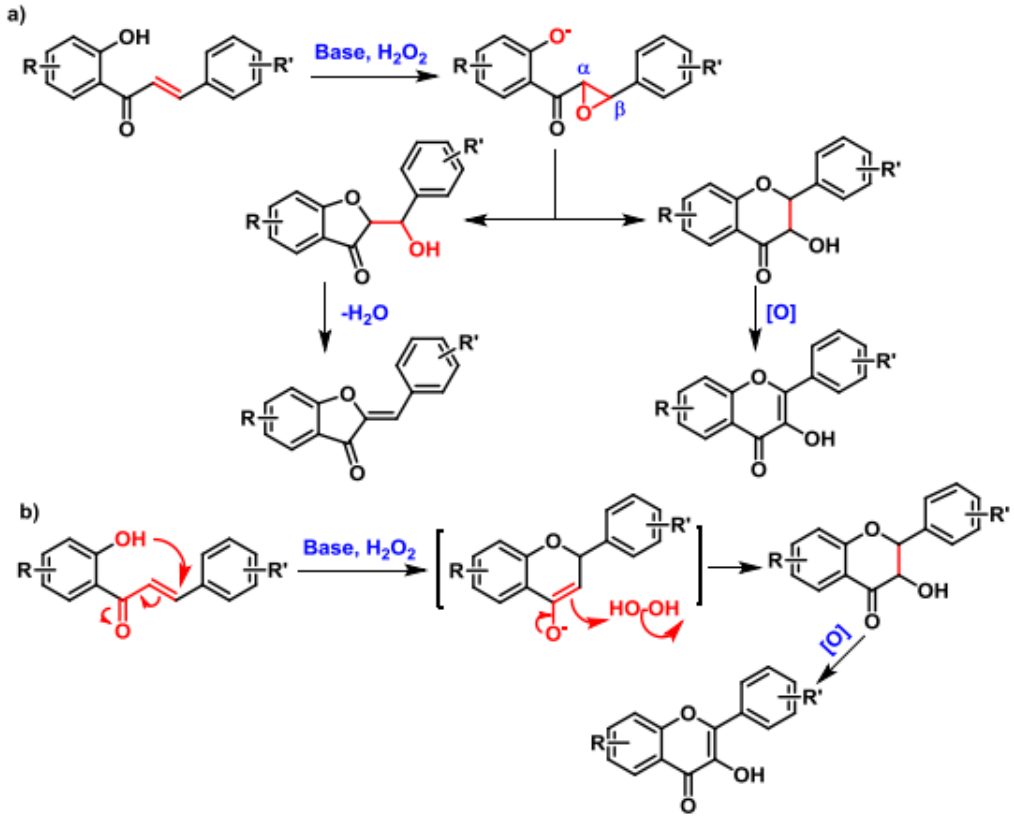
5	TBD	$EtOH/H_2O$	5	69
6	$N(Et)_3$	$EtOH/H_2O$	5	8
7	KOH	$EtOH/H_2O$	5	71
8	Na_2CO_3	DMSO	5	68
9	Na_2CO_3	DMF	5	41
10	Na_2CO_3	H_2O	2.5	62
11	Na_2CO_3	EtOH	2.5	63
12	**Na_2CO_3**	**$EtOH/H_2O$**	**2.5**	**95**
13	Na_2CO_3	$EtOH/H_2O$	7.5	25
14	Na_2CO_3	$EtOH/H_2O$	10	16

[a] Isolated yields

Two theories were put forth regarding the reaction's mechanism. In the first one (Scheme 2a), 2-hydroxychalcone is transformed into chalcone epoxide. Epoxide may subsequently cycle at the a-orb-position, the aurone, or the flavonol, in that order. The alternate theory (Scheme 2b) for the creation of flavonol begins with the cyclization of the chalcone anion, which is then followed by an electrophilic assault by hydrogen peroxide on the anion's C–3 carbon atom and more flavonol formation. As an alternative, the two steps might be combined in a concerted process. Neither process has been supported nor an epoxide intermediate has been described by experimental data yet.



Scheme. 2. Proposed mechanism for quercetin.

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■ Conclusion

As a result, a concise, efficient and scalable synthesis of the biologically important flavonol quercetin has been achieved in several steps from starting materials 1 and 2 in an overall yield of 95%. Analytical characterization of synthetic quercetin has been performed by ^{1}H NMR, ^{13}C NMR with high resolution and UV-vis. Synthetic quercetin has the highest antioxidant activity compared to standard quercetin antioxidants. We believe that further studies are needed to clarify the exact mechanism of action of quercetin's antibacterial activity.

■ Experimental

Materials and devices used

The Fluka, Aldrich, and Merck businesses provided all of the chemicals and solvents required for the reactions, which were employed without being purified. An electrothermal apparatus was used to measure the melting point, and the results are presented uncorrected. With the use of Nicollet 800 and potassium bromide tablets, infrared spectra were captured. The thin layer chromatography method was used to track the development of the tetrazole production processes. The Bruker Avance instrument was used to record the magnetic resonance spectra of the hydrogen and carbon nuclei at 500 and 125 MHz, respectively. DMSO was always utilized as the internal standard.

Synthesis of Chalcone

0.1 mmol of the appropriate aldehyde was added to 1 mmol of O-hydroxy acetophenone, 10 mL of 95% alcohol, and 10 mL of 30% base (NaOH). After at least three hours of stirring the reaction mixture, crimson liquid chalcone production was observed.

Synthesis of Quercetin

Na_2CO_3 (5 mmol) and H_2O (20 mL) were added to a solution of chalcone (1 mmol) in MeOH (40 mL), and the reaction mixture was agitated at room temperature for 30 minutes. After carefully adding 30% H_2O_2 (2.5 mmol) to the mixture and stirring it for 30 minutes, the temperature was gradually raised to 27 °C. For a full day, the stirring was maintained. TLC was used to track the reaction's development (PE/EA ¼ 3/1). Following the reaction's completion, the mixture was gradually acidified with 1 M HCl (aq) until pH ¼ 5, at which point it was diluted with water. EtOAc was used to extract the product. Water was used to wash the organic layer, and anhydrous Na_2SO_4 was used to dry it. Following the solvent extraction, the crude residue was refined using column chromatography and petroleum ether-ethyl acetate (3:1, v/v) to produce pure flavonol.

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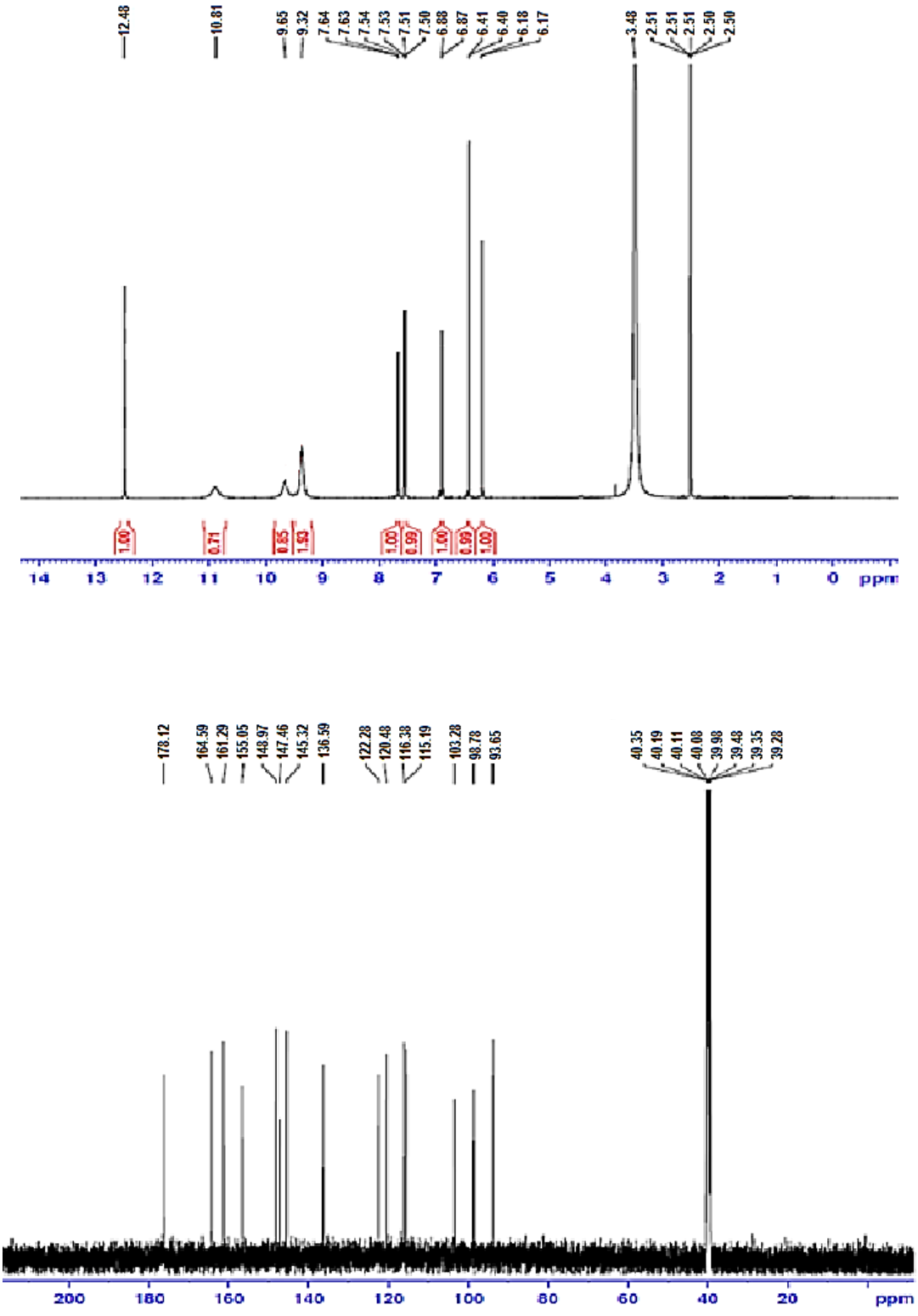
NMR spectral data



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