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CHARACTERIZATION AND ANTIOXIDANT ASSAY OF NEW FLAVONE ISOLATED FROM *ACACIA NILOTICA* FRUIT

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ABSTRACT

A new flavonoid compound was isolated from the fruit extract of *Acacia nilotica*. The compound showed significantly high antioxidant properties in comparison with that of the standard used. The structure suggested as 5, 7, 4′, 5′-tetrahydroxy-3-methoxy-flavone (methyl derivative of quercetin) has been elucidated based on the FT-IR, ^{1}HNMR and ^{13}CNMR spectroscopic data analyses.

KEYWORDS

Acacia nilotica, Antioxidant property, *DPPH* radial scavenging activity, Reducing power activity and Methyl derivative of quercetin.

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INTRODUCTION

Acacia nilotica (AN) is a well-known traditional plant of Bangladesh which is locally known as “Babla”. It has a wide range of ecological amplitudes and is distributed in many regions like India, Ceylon, Baluchistan, Egypt, and tropical Africa[1]. It is grown in almost all districts, especially, northern part of Bangladesh. It has been cultivated in Iran, Vietnam, Australia and the Caribbean. *AN* is widely used in various ayurvedic formulations and have traditionally been proved for various diseases like cancer, cold, congestion, cough, diarrhea, dysentery, fever, hypertension, hemorrhoid, ophthalmic, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma

and menstrual problems[2]. Phytochemically tannins mucilage, amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids, fluoroacetate, gums, nonprotein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins have been isolated from different parts of *AN*. Pods and leaves of *AN* contain digestible protein and young seedless pods contain 18%-27% of tannins[3-6]. Moreover young seedless pods of *AN* contain flavonoids, minerals and higher quantity of tannins[7]. Different bioactive compounds, isolated from *AN*, which showed spasmogenic, vasoconstrictor, anti-hypertension, anti-spasmodic, anti-inflammatory and anti-platelet aggregatory properties, gastro protective effect, and antidiabetic property etc[8-11]. We reported the antioxidant, antimicrobial and insecticidal properties of three different fractions (chloroform, ethyl acetate, and methanol) of the fruit extract of *AN*[12]. In the present study, we report a new flavonoid compound isolated from a typical fractionate of petroleum ether fraction of fruit extract of the *Acacia nilotica*.

MATERIALS AND METHODS

Plant material and isolation process

Acacia nilotica (*AN*) matured fruits were collected from the Rajshahi University campus, Bangladesh, during the period of May, 2011. The fruits were washed thoroughly in tap water, and dried at ambient temperature (25°C). The dried fruit was powdered in an electric grinder and was stored in an airtight container. A plastic container was used in the extraction process using methanol (Merck, Germany) as solvent. The extract was filtered through a Whatman paper and it was concentrated under reduced pressure at 45°C using the Buchi Rotavapor (R-200). The concentrated extract (1.152 Kg) thus obtained was called crude extract. The crude extract was divided into two parts: one part was kept as stoke crude in refrigerator and other part was used to obtain water soluble component. The crude was taken in a 2.5 L reagent bottle and shacked slowly with water. Water soluble triturate was then separated out by filtration. The water soluble portion was passed through the dia-ion resin until the white color of the resin was turned to brown. The adsorbed components were then eluted with methanol. This process was repeatedly done to separate all the antioxidants components from the remaining water soluble part of extract. The methanol was removed from the elute using Rotavapor at 45°C and water was removed from the condensed eluted part by freeze dryer. Finally, 0.122 Kg powder component was found. This dried material was then used to fractionate into different fractions by triturating with different solvent. 55g dried material found from dia-ion resin column was taken for separating the polyphenolic compounds. This was mixed with 110g silica gel (Merck, 70-230 mesh) with the ratio1:2 and dried in air. This powder was then ready for column chromatography. A piece of cotton was placed in the bottom of the column and it was half filled with petroleum ether (40-60^{o}C). Then the silica gel was passed through the column slowly and finally the column was packed with 660g of silica gel. Elutes were collected in an amount of about 30-50ml in a series of conical flask and elutes of similar TLC behaviour were combined together. A typical fraction, yellowish in color, was isolated from petroleum ether; and after 38 days it was dissolved in n-hexane and methanol (1:1) in a conical flask. It was kept at room temperature and after two days later some needle shaped crystal was observed. It was stored another three days and then a good amount of crystal was found. The crystal was filtered and washed with n-hexane.

Determination of total antioxidant capacity

Total antioxidant capacity of the isolated pure compound-1 from *AN* was determined by the method described herein with some modifications[13]. 1.0 mg of the pure compound with different concentrations was taken in a test tube. 3.0 mL of reaction mixture containing 0.60 M sulphuric acid (Merck, Germany), 28.00 mM sodium phosphate (Sigma, USA) and 1% ammonium molybdate (Sigma, USA) was added into the test tube. The test tube was incubated at 95°C for 10 min. to complete the reaction. Then the absorbance of the solution was measured at 695nm by spectrophotometer (Shimadzu, Japan). The results were plotted as mean ±STD.

Determination of reducing power capacity

The reducing power capacity of the pure compound-1 from *AN* was determined by using the Oyaizu *et al*. method[14]. 1.0 mg of the compound-1 with different concentrations was taken in a test tube. 2.5 mL of potassium buffer (0.2M) and 2.5 mL of 1% potassium ferricyanide (Merck, Germany) solution were added into the test tube. The reaction mixture was incubated for 20 min. at 50°C to complete the reaction. The 10% 2.5 mL of trichloroacetic acid (Merck, Germany) was added into the test tube. The total mixture was centrifuged at 3000 rpm for 10 min. Then 2.5 mL supernatant solution was withdrawn from the mixture and mixed with 2.5 mL of distilled water. 0.1% 0.5 mL of ferric chloride (Sigma, USA) solution was added to the diluted reaction mixture. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer against blank. Higher the absorbance indicates higher the reducing power capacity.

DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) radical scavenging assay

DPPH (Sigma, USA) method was used to evaluate the free radical scavenging activity of the pure compound-1[15]. 1.0 mg of the pure compound-1 with different concentration was taken in a test tube. 3.0 mL of methanol (Sigma, USA) and DPPH were added into the test tube. The test tube was incubated at room temperature for 30 min. in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against a blank experiment. The blank solution contained all reagents except plant extract or standard solution. The percentage (%) inhibition activity was calculated from the following equation:

$$\% I = \{(A_o - A_1)/A_o\} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, A_o is the absorbance of the control, and A_1 is the absorbance of the extract/standard. Then % inhibitions were plotted against concentration and from the graph the IC_{50} value was calculated. Here, (BHT) *tert*- butyl-1-hydroxytoluene was used as standard.

RESULTS AND DISCUSSION

Total antioxidant capacity

The antioxidant capacity has been assessed as the ability to prevent from oxidation. Synthetic antioxidant such as tert-butyl-1-hydroxytoluene (BHT), butylated hydroxylanisole (BHA), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) have been widely used as peroxidation. Figure No.1 showed the total antioxidant capacity of the pure compound-1 isolated from the fruit extract of *AN*. From the Figure No.1, it is found that the antioxidant capacity of the compound-1 showed less antioxidant at the concentrations >20 to 100 µg/ml in comparison to the standard catechin. However, the antioxidant capacity is greater than the standard at the concentrations 5 to <20 µg/ml. However, at a certain concentration the antioxidant activity will follow the order: catechin>compound-1.

Reducing power capacity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-complex to the ferrous form by donating an electron to the Fe^{2+} complex. The compound isolated from fruit extract of *AN* was subjected to the free radical scavenging activity. Figure No.2 showed the ferric reducing power capacity of the compound-1 isolated from *AN* fruit. It is has been clearly shown that the compound-1 has a considerable amount of reducing activity. The total reducing activity of the compound is greater than the reducing activity of the standard ascorbic acid.

DPPH radical scavenging activity

The DPPH has been widely used to evaluate the free radical scavenging capacity of antioxidants. The DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. Figure No.3 showed the DPPH radical scavenging activity of the pure compound-1 isolated from the fruit of *AN*. The compound-1 showed the higher DPPH radical scavenging activity (94.91±0.91) at the concentration 12.5 µg/ml in comparison to BHT (63.96±1.02) at 12.5 µg/ml. The corresponding %

inhibitions, the IC_{50} value was found to be 2.25 for the compound-1 and that of the standard BHT 9.10.

From the analyses of the antioxidant assay, the compound isolated is a potential source of natural antioxidant. The present result showed that the compound contains significantly high polyphenolic with superior antioxidant as suggested by scavenging of the free radicals.

Characterization the compound-1

The compound-1 was obtained as yellowish colored solid needle shaped crystal. The TLC examination of the compound indicates that it was a single compound. The R_f value was calculated and was found to be 0.76 in the solvent ethyl acetate: petroleum ether (3:2). The melting point was recorded to be 200±0.7°C (Buchi melting point, B-545). In the IR spectrum (FT-IR spectrometer, PERKIN ELMER) (Figure No.4), five absorption bands were observed at 3240–3500 cm^{-1} region. The main band observed at 3411cm^{-1} was due to the –OH group confirmed by qualitative functional group analysis. Another strong band observed at ~1693 cm^{-1} owing to the>C=O stretching vibrations. The band at ~1618 cm^{-1} was due to the conjugated C=C bonds.

The bands observed at ~1535, ~1469 and ~1442 cm^{-1} were due to the bending vibrations of the $–CH_3$ bond. The bands ~1373 and ~1330 cm^{-1} was confirmed by the bending vibrations of C–O–H bonds.

^{1}H NMR spectrum (Bruker DPX-400, 400 MHz) of the pure compound-1 is shown in Figure No.5. The peak at δ=7.05 ppm suggesting three aromatic rings with 4′,5′-substituted aromatic band. The peak at δ=3.81 ppm could be the methoxyl proton at C-3 position. The peak at δ=4.91, 3.35 and 3.31 ppm were reasonably assigned to the hydroxyl proton at C-5,7,4′,5′ positions. From the ^{13}C NMR spectrum (Figure No.6), a downfield signal at δ=167.86 ppm was confirmed for the >C=O carbonyl carbon. The signal at δ=145.09 ppm has been ascribed for the C–O substituted carbon oxygen carbon and the signals at δ=138.45 and δ=120.09 ppm was due to the C=C double bonded carbon. Another signal at δ=108.84 ppm for the non-aromatic carbon-carbon double band and the signal at δ=51.09 ppm was assigned for the $–CH_3$ methyl carbon. On the basis of theft-IR, ^{1}H NMR and ^{13}C NMR spectroscopic analyses and comparison with the literature[16], the probable structure of the compound-1 has been suggested which is shown in Figure No.7.

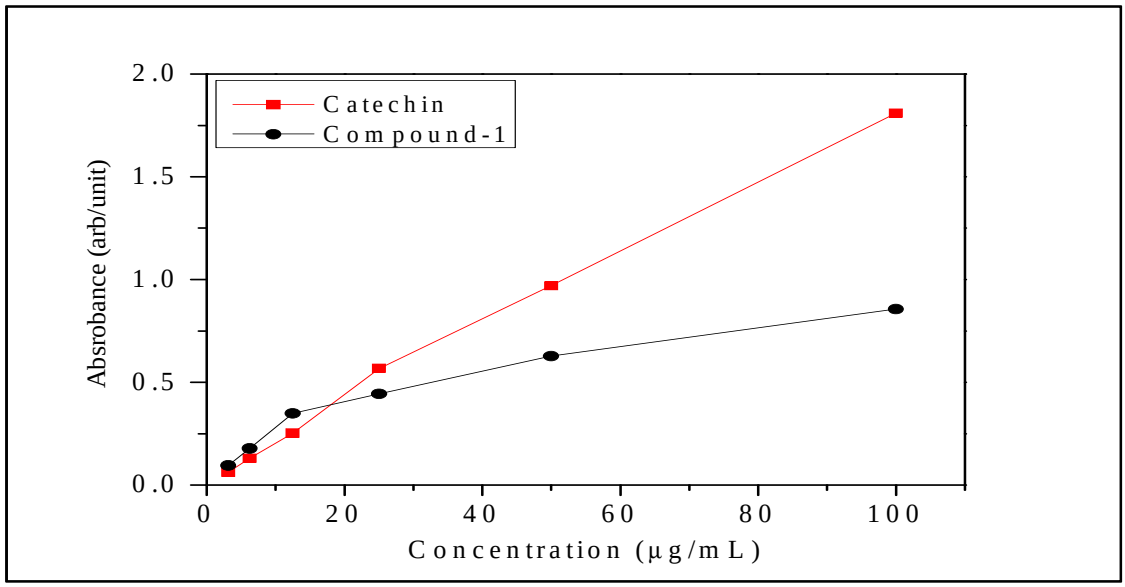

Figure No.1: Total antioxidant capacity of the pure compound-1 isolated from the fruit extract of *AN*. Catechin used as standard

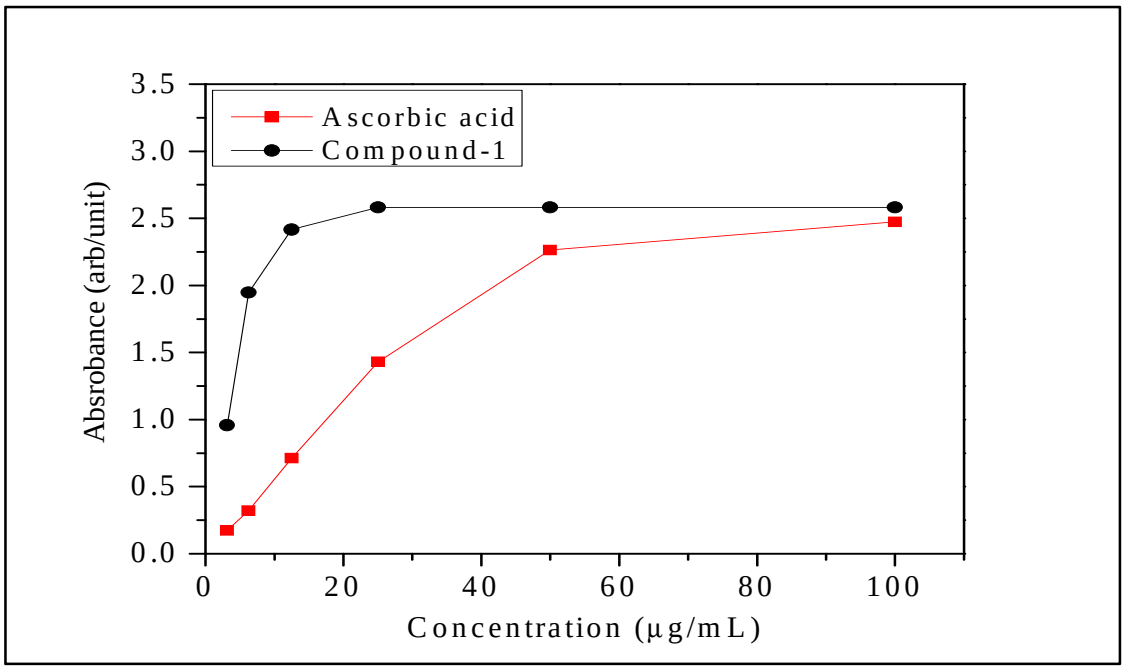

Figure No.2: Reducing power capacity of the pure compound-1 isolated from the fruit extract of *AN*. Ascorbic acid used as standard

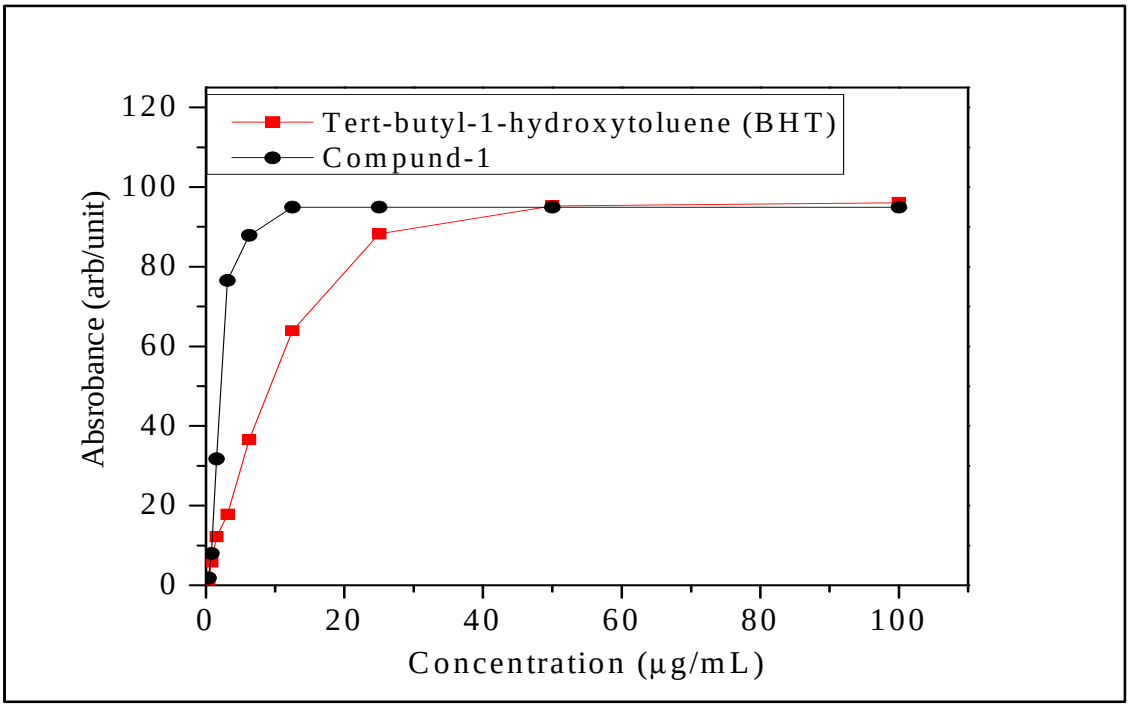

Figure No.3: DPPH radical scavenging activity of the pure compound-1 isolated from the fruit extract of *AN*. BHT used as standard

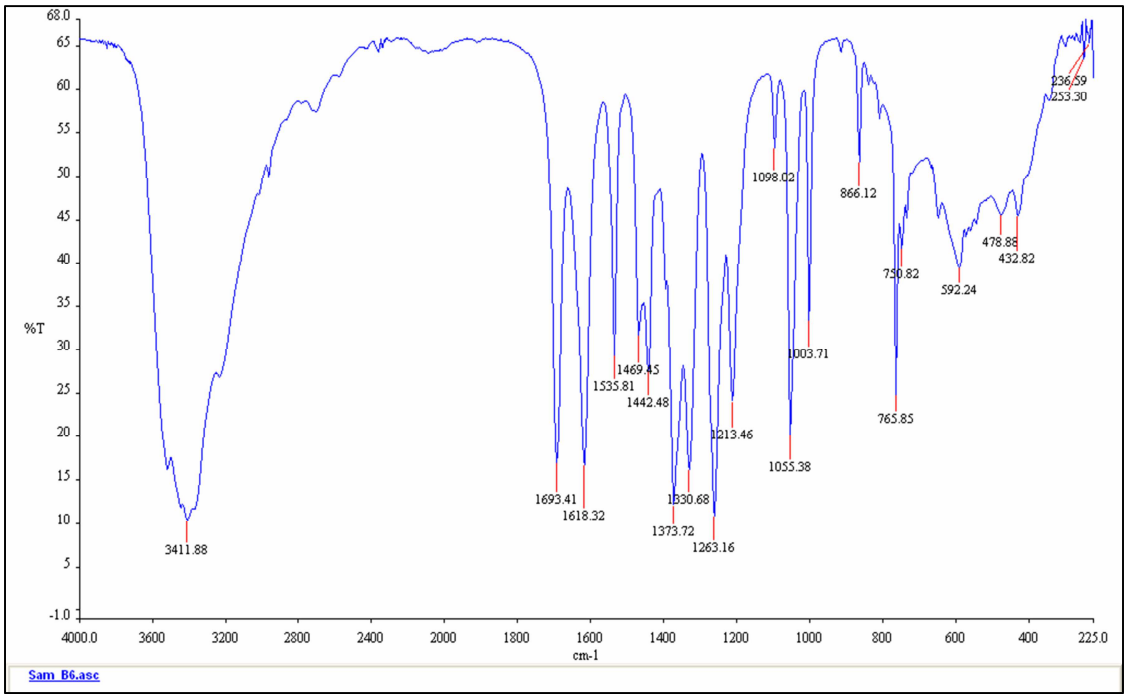

Figure No.4: IR spectrum of the pure compound-1 isolated from the fruit extract of *AN*

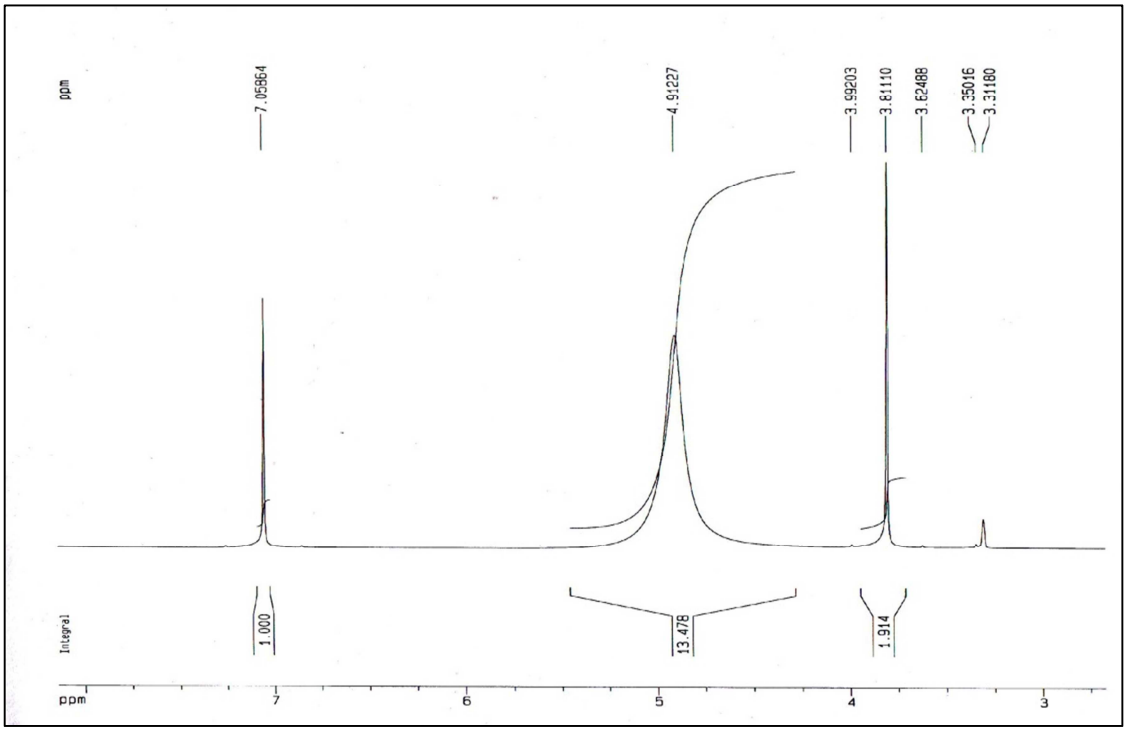

Figure No.5: ^{1}H NMR spectrum of the pure compound-1isolated from the fruit extract of *AN*

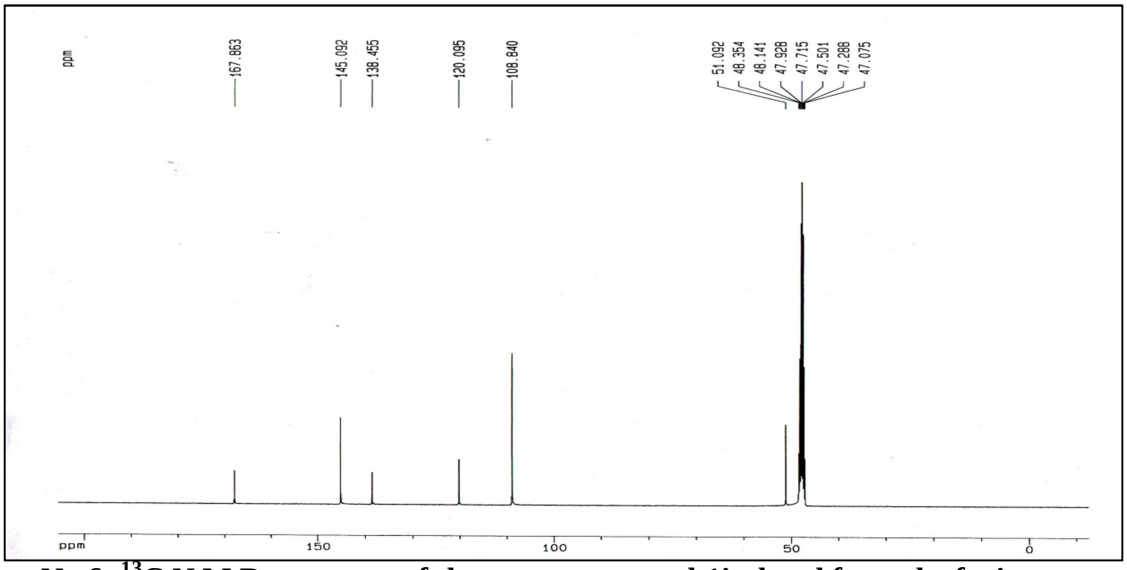

Figure No.6: ^{13}C N M R spectrum of the pure compound-1isolated from the fruit extract of *AN*

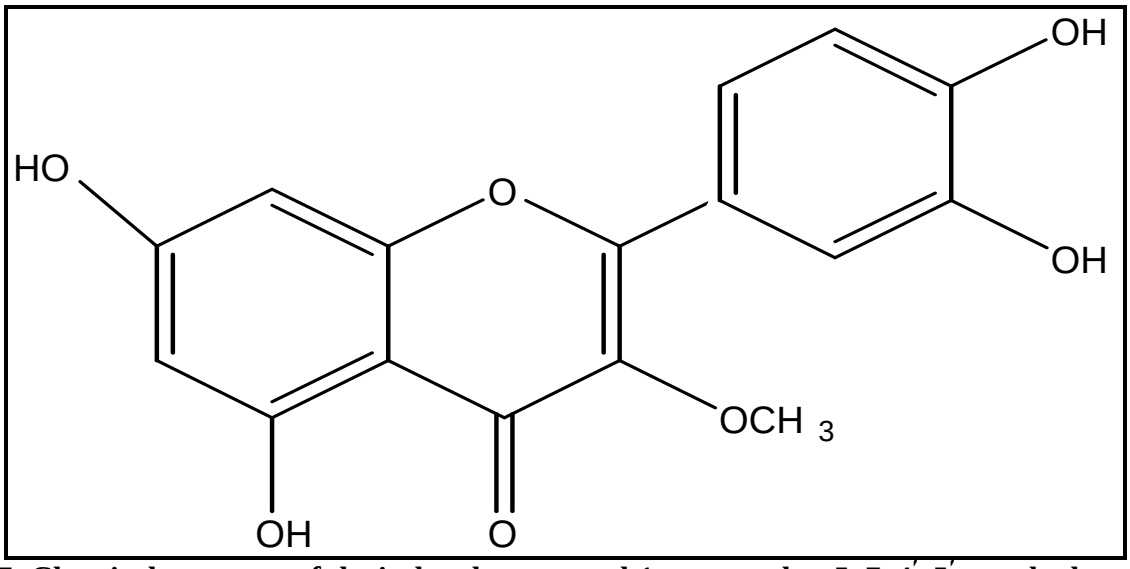

Figure No.7: Chemical structure of the isolated compound-1 suggested as 5, 7, 4′, 5′-tetrahydroxy-3 methoxy-flavone (methyl derivative of quercetin)

CONCLUSION

In this study, one new flavonoid compound has been isolated from the fruit extract of *AN* through systematic guided fractionation. The compound was found to be the highest antioxidant in comparison with that of the standard. The further research is under progress to explore the detail biological profile of the isolated compound.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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