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Macro Coumarins as Novel Antioxidants

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ABSTRACT

The present work pointed at the design and synthesis of novel coumarin compounds as antioxidants. The alteration of 4-hydroxycoumarin by several reaction levels was performed to generate destination compounds. Spectroscopic methods like Infrared Spectra FT-IR, ^{1}H-NMR and ^{13}CNMR, elemental analysis techniques, melting point, and thin layer chromatography defined the structure of the prepared compounds. The antioxidant activities of individual compounds were tested against stable free radical 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), hydrogen peroxide (H_2O_2) and FRAP (ferric reducing antioxidant power) and the results were related to the Gallic acid, ascorbic acid and Trolox as standards. The effects revealed that most of the syntheses presented greater activity as an antioxidant than the standards in different concentration range. The distinguished competence inactivating action was observed for compound 2 (86.3±1%) followed by compounds 3 (85±0.5%) using DPPH method. The antioxidant mode of action of the prepared compounds was also investigated.

Keywords: Antioxidant, DPPH, FRAP hydroxycoumarin H_2O_2 in activators, Macromolecules.

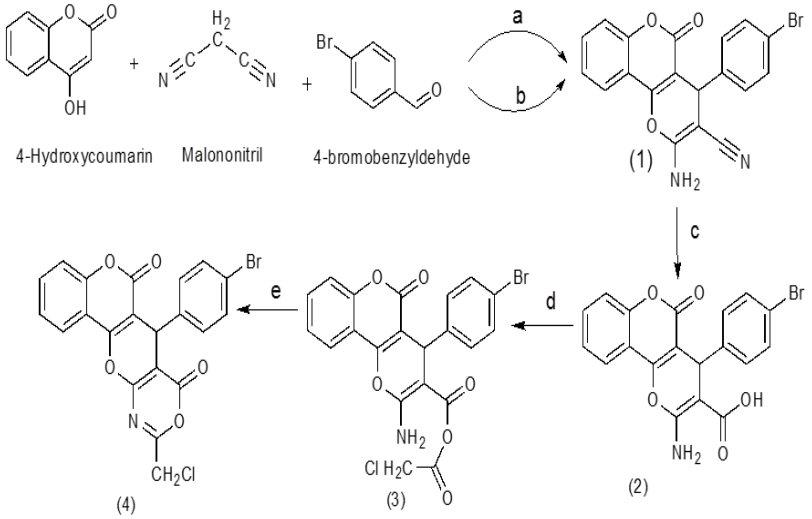
INTRODUCTION

Coumarin is a simple molecule that originates from lactones family. As it possesses a benzopyrone system, it isolates itself from plants. Also, it can undergo total synthesis that is conducted in the laboratory. Furthermore, coumarin can be synthesized from many established chemical reactions. Although coumarins can be obtained from various natural resources, new coumarin derivatives are continuously being discovered and synthesized on regular basis.

Coumarin and coumarin-related compounds have demonstrated much popularity in their used way back for a long time. This is because these compounds have shown significant potential in the production of therapeutic products. They are known for a variety of uses: physiological, antimicrobial[1,2], antioxidant[3,5], anti-inflammatory[6,7], anticoagulant and antitumor[8] activities. In recent years, there is much attention in the search for antioxidants from natural, industrial sources. As antioxidants inhibit and scavenge radicals, they play an important role in protecting humans from infections and degenerative diseases. As such antioxidants can be defined as any substrate that significantly delays or prevents oxidation. According to 9 in comparison with an

oxidizable substrate (lipid, protein, carbohydrate, or DNA) only low concentration of antioxidant is required to delay or prevent numerous diseases conditions such as swelling, carcinogenesis, atherogenesis and also in the aging process as well as after virulent exposure to xenobiotic[10-12].

Interest in the discovery of new antioxidant agents has surmounted increased, since the implication of oxidative damages in many pathology cases. The other class of compounds that has caught much attention is polyphenols. This group of molecules comprises flavonoids Flavonoids like flavonols, chalcones, flavanones, flavones, flavan-3-ols in addition to lignins, tannins and tocopherols and phenolic acid[13]. However, the flavonoids constitute the largest group of phenolic compounds with a wide range of biochemical and physiological actions like an antioxidant and free radical inactivates characteristics[14]. Furthermore, the phenolic molecules can serve as antioxidants by coordinating metal ions, counteracting the generation of radicals and stimulating the antioxidant endogenous system forward[15]. Originally, we were utilizing 4-hydroxycoumarin as a preliminary substance and all the integrated coumarins are manifested in Scheme 1.



Scheme 1. Transformation series of the prepared compounds Chemicals and Environments: a = Tetrabutylammonium bromide (TBAB)/reflux; b = diammonium hydrogen phosphate (DAHP)/reflux; c = hydrolysis; d = benzene/ reflux; e = H_2SO_4 / reflux

EXPERIMENTAL

General Chemistry

The compounds utilized throughout synthesis were provided by Sigma-Aldrich (Selangor, Malaysia). The infrared ranges were achieved on a Nicolet 6700 FT-IR spectrophotometer (Thermo Nicolet Corp., Madison, WI, USA), and the data are displayed in cm^{-1}. NMR bands were verified manipulating an AVANCE III 600 MHz spectrometer (Bruker, Billerica, MA, USA), DMSO used as a solvent and the data are revealed in δ ppm. CHN analysis was accomplished on an Elemental analyzer Carlo Erba 1108 (Vario El III, SpA, Rodano, Italy).

Chemical Synthesis

Synthesis of 2-amino-4- (4-bromophenyl)-5-oxo-4, 5-dihydropyrano [3, 2-c] chromene-3-carbonitrile (1)

Tetrabutylammonium Bromide (TBAB) as catalytic agent for synthesis of compound (1)

The reactants, 4-Hydroxy-1-benzopyran-2-one (0.162 g; 10 mmol) with 4-bromobenzaldehyde (5 mmol), malononitrile (15 mmol) and Tetra-n-butyl ammonium bromide (10 mol %) were agitated under reflux. Then, the combination was chilled to ambient temperature. The slurry was separated by filtration and desiccated then recrystallized from ethanol 16. Then the compound was characterized by spectroscopic and physical data yields 75%, m.p (223-225)°C.

Diammonium Hydrogen Phosphate (DAHP) as catalytic agent for production of compound (1)

In an aqueous ethanol solution (50%

H_2O: 50% EtOH) totally 50 ml starting compounds comprising 4-hydroxycoumarin (0.162 g; 10 mmol), 4-bromobenzaldehyde (10 mmol), malononitrile (12 mmol) and ammonium monohydrogen phosphate (26.4 mg, 10 mol %), were agitated for 4 h, at ambient condition. when the reaction complete, the slurry was separated by filtration and rinsed with ethanol[17]. Then the compound was characterized by spectroscopic and physical data., yields 70%., m.p (225-227°C); ^{1}H-NMR: δ 4.28 (s, 1H, CH), δ 7.11 and δ 7.83 (dd, 2H), δ 7.42-7.83 (m, 1H, C-H aromatic ring), δ8.49 (s, NH_2); ^{13}CNMR (DMSO): 40, 58.3, 108, 116.0, 125.1, 128.1, 152.1, 160.5, 161.1 and 165.5; FT-IR: 3385.8 cm^{-1} (NH_2), 3189.0 cm^{-1} (C-H aromatic), 1709.2 cm^{-1} (C=O, lactone), 2213 cm^{-1} (C≡N), 1638.0 cm^{-1} (C=C aromatic); CHN analysis: Calcd. for $C_{19}H_{11}BrN_2O_3$: C 57.74% H 2.81% N 7.09%, Found: C 57.54% H 2.1% and N 6.91%.

Synthesis of 2-amino-4- (4-bromophenyl)-5-oxo-4, 5-dihydropyrano [3,2-c] chromene-3-carboxylic acid (2)

Aqueous sodium hydroxide solution (1 M, 0.3 ml) was supplemented to compound (1) and the combination was warmed for 5 min. at 140^{o}C. hydrochloric acid (1 M, 0.3 ml) was then added to make solution neutral under these conditions we got compound (2) at 19–20 minutes After finishing the reaction, the slurry was separated by filtration and rinsed with ethanol, 18, yields: 50%, m.p (230-233°C); ^{1}H-NMR: δ 3.93 (s, 1H, CH), δ 7.10 and δ 7.81 (dd, 2H), δ 7.32-7.82 (m, 4H, C-H aromatic), δ 8.5 (s, NH_2) , δ 11.0 (s, OH); FT-IR: 3221(NH_2), 3307 cm^{-1} (OH); 3090.1 cm^{-1} (C-H aromatic), 1652 cm^{-1} (C=O, lactone), 1710 cm^{-1} (C=O, carboxylic), 1625.3 cm^{-1} (C=C aromatic); CHN analysis: Calcd. for $C_{19}H_{12}BrNO_5$: C 55.09% H 2.92% N 3.38%, Found :C 54.54% H 2.5% and N 3. 1%.

Synthesis of 2-amino-4- (4-bromophenyl)-5-oxo-4, 5-dihydropyrano [3,2-c] chromene-3-carboxylic 2-chloroacetic anhydride (3)

In 100 ml RBF, (0.02 mol, 9.81 g) of compound (2) in 4 ml ethanol was mixed in magnetic agitator for 10 minutes. Chlor-acetyl chloride (0. 02 mole, 4 equivalent) was supplemented gradually for 1 h, Reaction combination was maintained on stirring for 24-writhe reaction mixture was chilled, transferred into an ice water (50 ml) including a drop of pyridine and agitated till the oil solidifies. The crude result was filtrated, rinsed with ice-cold water and evaporated. The result was recrystallized from ethanol, yields 45%, m.p (244-246°C); ^{1}H-NMR: δ 2.1 (s, 1H, NH), δ 3.91 (s, 1H, CH), δ 7.2 and δ 7.82 (dd, 2H), δ 7.4-7.79 (m, 4H, C-H aromatic ring), δ8.4 (s, NH_2); FT-IR: 3433- 3328 cm^{-1} (NH_2), 3055.1 cm^{-1} (C-H aromatic), 1652 cm^{-1} (C=O) 1602.9 cm^{-1} (C=O, lactone); CHN analysis: Calcd. for $C_{20}H_{14}BrClN_2O_4$: C 52.03 % H 3.06 % N 6.07 %, Found: C 51.5% H 2.88% and N 5.81%.

Synthesis of 7-(4-bromophenyl)-10-(chloromethyl) -6H-chromeno [3', 4': 5,6] pyrano [2,3-d][1,3] oxazine-6,8(7H)-dione (4)

To a solution of 2 mmol, 0.941 g of compound (3) in 50 ml of dry methanol was added concentrated sulphuric acid (80 mmol). The combination was refluxed for (3-4) h, Following cooling; the reaction substance was transferred into an ice-water mixture. The solid particles were collected by percolation, rinsed with water and recrystallized from methanol to obtain compound (4) 19, yields 35%, m.p (217-219°C); ^{1}H-NMR: δ 3.9 (s,2H,CH_2Cl), δ 4.4 (s, 1H, CH), δ 7.1 and δ 7.8 (dd, 2H), δ 7.4-7.79 (m, 1H??, C-H aromatic ring) ; FT-IR: 3181.6 cm^{-1} (C-H aromatic), 1670.6, 1612.1 cm^{-1} (C=O, lactone), 1570.4 cm^{-1} (C=C aromatic); CHN analysis: Calcd. for $C_{21}H_{11}BrClNO_5$: C 53.36% H 2.35% N 2.96%, Found: C 52.64% H 2.1% and N 1.91%.

Antioxidant activity

(2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) (DPPH) free radical scavenging activity

Antioxidant properties of synthesized coumarin compounds (1-4) were tested spectrophotometrically utilizing 2,2-diphenyl-1-picrylhydrazyl radical,[20,21]. At first, 0.1 mL of different strengths of prepared compounds 0.25, 0.5, 0.75 and 1 mg/ml and standard ascorbic acid were combined 1 ml of 0.2 mM DPPH solubilized in CH_3OH. The reaction admixture was kept in the darkness for 30 min at 28^{o}C. The control experiment was carried out as above without test samples. The DPPH antioxidant activity was ascertained by determining the absorbance, at 517 nm operating the UV-VIS spectrophotometer. The reduction of DPPH radical in activator was computed relative to the measured absorbance of the control applying the subsequent equation (1):

$$\text{Scavenging effect \%} = \frac{Ao - A1}{Ao} * 100 \qquad (1)$$

Where A_o is the absorbance of the controller reaction, and A_1 is the absorbance in the existence of the tests or standards.

H_2O_2 Scavenging Activity

H_2O_2 solution (40 mM) was made in buffer phosphate (pH 7.4). Various dilutions (250, 500, 750 and 1000 g ml^{-1}) of the prepared compounds (or ascorbic acid) were supplemented to the H_2O_2 liquid (0.6 ml, 40 mM). The absorbance of H_2O_2 at 230 nm was revealed after 10 min. in contradiction of a blank solution having phosphate buffer lacking H_2O_2[22]. The H_2O_2 proportion inactivating activity was estimated applying equation (1).

(FRAP) Ferric Reducing Antioxidant Power Assay

The method suggested by Muss[23] was applied to establish the antioxidant action through FRAP with certain minor adjustments. The FRAP component was made fresh, as in the use of 300 mM acetate buffer, pH 3.6 (3.1 g $C_2H_3NaO_2$. $3H_2O$, with 16 ml glacial acetic acid is adjusted up to 1:1 with DW; 10 mM of TPTZ, in 40 mM HCl; and 20 mM $FeCl_3 \bullet 6H_2O$ are mixed in a ratio of 10:1:1 followed by incubation at 37°C for 10 min. prior to the analysis to allow the reagent working time. About 1 ml of FRAP substance was supplemented to 10 µl of each compound after the addition of the FRAP reagent, the plates were preserved for 30 min. at ambient condition and the absorbance of the blue complex (ferrous tripyridyltriazine) was formed from the reaction. All the data were taken in triplicates. The Trolox standard calibration curve was established by plotting the curves of various concentrations in methanol (0.00625–0.2 mg/ml) against absorbance (at 595 nm using UV-Vis spectrophotometer see Fig. 3.[26]). The calibration equation found for Trolox was y=ax+b (r^2 = 0.999), where y is the optical density at 593 nm and x is the concentration (mg/ml) a, b is the constant, r^2 is the confidence of coefficient. The findings were reported in mg Trolox equivalents/g of synthesis compound. The ferric reducing antioxidant power was estimated applying the subsequent method: FRAP= (C x V)/M where FRAP = the ferric reducing antioxidant power, mg/g compound, in TE; C = the concentration of Trolox ascertained from the standardization curve, mg/mL; V= the volume of compound solution, 0.1 mL; M = the weight of synthesis compound, 0.0001 gram.

Arithmetical analysis

Data obtained were presented as mean ± SD and the arithmetical significance of variances was established using one-way assay of variance (ANOVA) by the SPSS 17.0 arithmetical software package. Variances were reflected significant at $P<0.05$. The significances are represented as mean ± SD (n=3).

RESULTS AND DISCUSSION

Antioxidant assay

Antioxidant activity of prepared compounds was achieved manipulating different in vitro tests against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, hydrogen peroxide) inactivating and (FRAP) ferric-reducing antioxidant strength test.

DPPH Antioxidant Activity of Compounds (1-4)

The function of an antioxidant is to eliminate free radicals. One significant assumption of such elimination is by providing hydrogen to FR (free radicals) in its conversion to inactive type 20. The hydrogen addition would eliminate the single electron aspect, which is liable for radical reactivity. Free radicals have been a topic of considerable concern between specialists in the preceding decade. The antioxidant actions of compounds (1-4) were evaluated in vitro utilizing DPPH (1,1-Diphenyl-2-picrylhydrazyl radical radical inactivating methods. Gallic acid was applied as the standard. Donating hydrogen activity, as estimated using DPPH radicals as a hydrogen acceptor, revealed that considerable correlation might be observed linking the concentration of the recently synthesized compound and the inhibition rate. DPPH molecules (1-4) have been proved to diminish the established radical. As indicated by Fig. (1) the prepared compounds 1, 2, 3, and 4 possess 61±0.6%, 86.3±1%, 85±0.5%, and 71.2±0.8% DPPH radical-inactivating action. The compounds (1, 2, and 3) contain the N-H group, which enables hydrogen atom transfer (HAT) to the DPPH free radical to provide a resonance-stabilized radical. The scavenging influence enhanced with rising concentrations of samples. In the DPPH technique, the highest antioxidant effect was 90±1% at a strength 1000

µg/mL for compound (2), and the least inactivating power was 26.1±0.5% at 250 µg/mL concentration for compound (1). Fig. (2) shows the proposed mechanism for compounds, specifically, 2-amino-4-(4-bromophenyl)-5-oxo-4, 5-dihydro pyrano (3, 2-c) chromene-3-carboxylic acid (2), and 3-amino-2-((4-bromophenyl)(2-oxo-2H-chromen-3-yl) methyl) acrylic 2-chloro acetic anhydride (3).

Table 1: DPPH antioxidants activity of comp (1-4)

Comp NO	H_2O_2 inhibition %SD 100µg/ml	IC_{50}±SEM
1	61±0.6%	31±0.2%
2	86.3±1.0%	41.3±1%
3	85±0.5%	41±0.8%
4	71.2±0.8%	26.4±0.5%

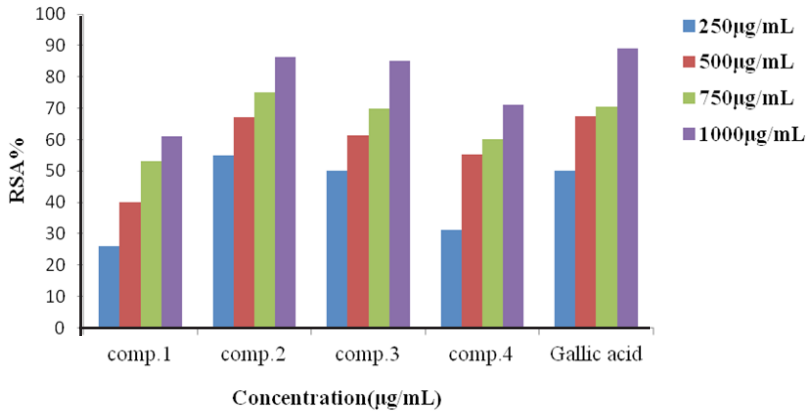


Fig. 1. Evaluation of antioxidant properties by DPPH assay for compounds (1-4)

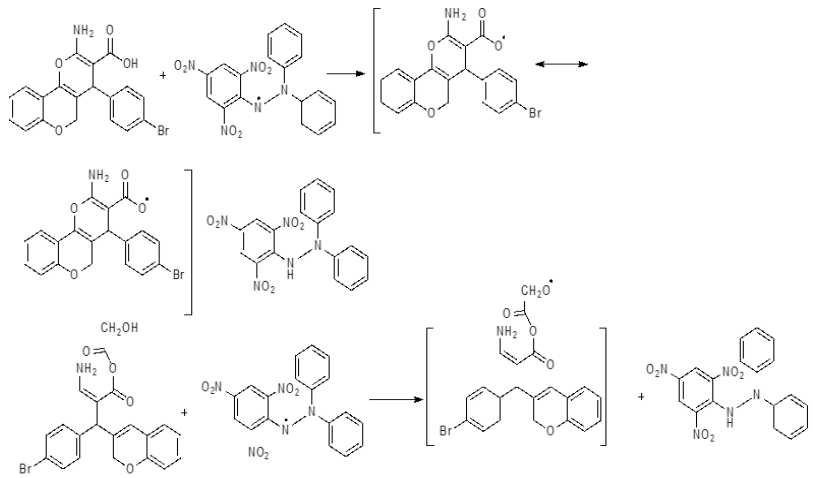


Fig. 2. The schematic mechanism between DPPH FR (free radicals) and compounds 2 and 3

H_2O_2 Scavenging Capacity

Hydrogen peroxide intact isn't extremely reactive, but the compound can occasionally be noxious to the cell due to the subsequent hydroxyl radical in cells[24]. Thus, the elimination of H_2O_2 is very significant for antioxidant protection in the cell. The inactivating ability of prepared compounds on H_2O_2 equaled with ascorbic acid as a reference compound is shown in Fig. 3 the coumarin compounds were able of inactivating H_2O_2 in a concentration-dependent fashion. Results display that H_2O_2 scavenging actions of compounds 2 and 3 are higher than that of the standard, and the values are 80±1.0%, and 81.5±1.5%, respectively. Compounds 1 and 4show scavenging activity on hydrogen peroxide with lower value compared with ascorbic acid.

Table 2: H_2O_2 scavenging activity of comp (1-4)

Comp NO	H_2O_2 inhibition %SD 100 µg/ml	IC50 ±SEM
1	60±0.5%	29±0.5%
2	80±1.0%	39.4±0.3%
3	81.5±1.5%	40±0.6%
4	54.2±1.1%	26.4±0.8%

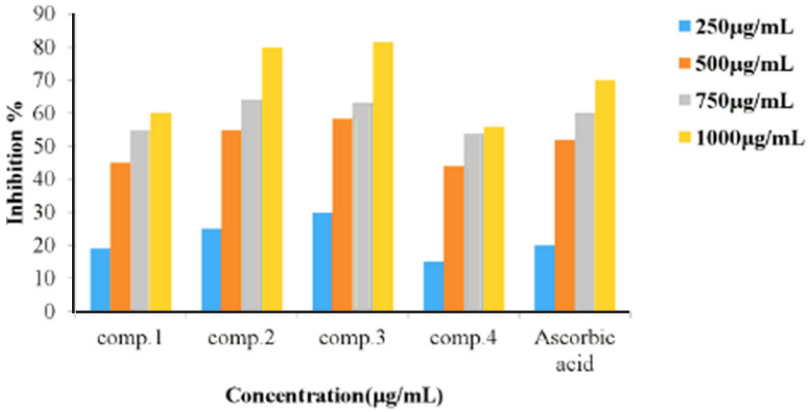


Fig. 3. Influence of compound 1-4 on hydrogen peroxide

Ferric Reducing Ability Power (FRAP)

The antioxidant potential of all compounds was ascertained by a FRAP test. The FRAP assessment was revealed as an equivalent of compounds (mg/g of standard antioxidant Trolox). The FRAP test estimated the potency of a compound to reduce the ferric 2,4,6-Tri(2-pyridyl)-s-triazine complex to the stained ferrous complexes compound. FRAP results are acquired by relating the absorbance alteration at 593 nm in assay reaction admixtures with those having ferrous ions in identified concentrations. In general, the reducing properties of these sets of compounds largely rely on the sum and position of hydrogen-donor hydroxyl clusters on the aromatic group of phenolic compounds; in addition, the properties are influenced by other aspects, such as H-donor groups (-NH, -SH)[25], which apply their effect by destroying the free radical series via providing a hydrogen atom[26,27]. The FRAP test considers the antioxidants specimen as a reluctant in a redox-associated colorimetric effect[28] following the equation:

FRAP: $Fe(TPTZ)_2^{3+} + ArOH \rightarrow Fe(TPTZ)_2^{2+} + ArO. + H^+$

FRAP: $[Fe(TPTZ)_2]^{2+}$ (Ferrous tripyridyl triazine cation)

Several synthetic and naturally existing coumarins display possible antioxidant activity. Therefore, the antioxidant influence of coumarin derivatives 1-4 was assessed utilizing FRAP test in contrast to Trolox. The linearity of FRAP (dose–response curve) for conventional solutions is presented in Fig. (4). The values of the FRAP test are stated in Table (1), and ferric reducing ability was represented as the concentrations of antioxidant as FRAP value (mg/g). Depending on results, compounds 2 and 3 revealed considerable antioxidant activity compared with Trolox owing to the existence of the NH_2 group, which donates a hydrogen atom, in the structure of these compounds. However, the remaining compounds show poor antioxidant power.

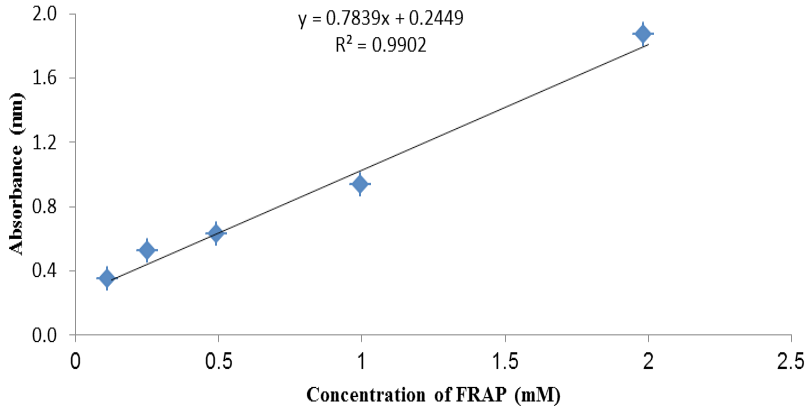


Fig. 4. Linearity of FRAP for stock solutions (Trolox)

Table 1: FRAP antioxidant activities of prepared compounds

Compound	FRAP value (mg/ g)
1	10.4
2	41.6
3	60.2
4	9.1

CONCLUSION

The preparation and characterization of coumarin derivatives are of considerable interest owing to their potential for various applications based on their biological activity. A series of 4-hydroxy coumarin compounds were successfully synthesized by conventional methods. The recently prepared molecules were described by using elemental analysis (CHN) and spectroscopic methods (IR and ^{1}H and ^{13}C NMR). The scavenging activities of prepared coumarins compounds were verified by utilizing hydrogen peroxide test (H_2O_2), DPPH antioxidant examines, and FRAP assess. Results indicate that DPPH give the best antioxidant activity observed for compound 2 (86.3±1%) followed by compounds 3 (85±0.5%). The new coumarins hold greater scavenging activity compared with vitamin C, gallic acid, and Trolox as standards.

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