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Review Article

COUMARIN DERIVATIVES WITH ANTIOXIDANT AND ANTICANCER POTENTIAL: A REVIEW

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Abstract :

Coumarin (2H-1-benzopyran-2-one) is a plant derived natural product known for various biological activities such as anti-inflammatory, antibacterial, antifungal, anticancer, antitubercular, and antioxidant amongst a few. The phenolic OH group can be attributed mostly for the antioxidant activity. Several studies have revealed that various substituents on the coumarin core structure influence different biological activities. The current review highlights the antioxidant potential of coumarin derivatives by the DPPH, superoxide, hydroxyl and peroxyl radicals assay. Further the radical scavenging ability can be correlated to its anticancer activity. Thus designing new coumarin derivative with enhanced antiradical activity may lead to the development of drug with anticancer potential.

Keywords*:* Coumarin derivatives, Reactive Oxygen Species (ROS), Antioxidant and anticancer activity and DPPH assay.

INTRODUCTION

ROS which include hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\cdot}$), and several other free radicals produced by the cells cause damage to the lipids, proteins and DNA.[1] An imbalance in the generation of ROS species and antioxidant mechanism leads to oxidative damage of tissues and biomolecules, leading to disease conditions such as carcinogenesis, drug-associated toxicity, inflammation, atherogenesis and some degenerative diseases. [2] Thus, the significance of free radicals and ROS in the pathogenesis of these different diseases has attracted considerable attention. Antioxidants are currently fabricated as the drug candidates to counter these diseases. Minor dietary compositions have been seriously considered to combat the ill effects of free radicals and ROS. About three-quarter of the world population relies upon traditional remedies for the health care of its people according to World Health Organization (WHO). [3]

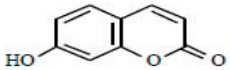
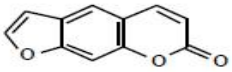
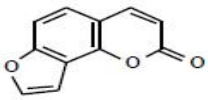
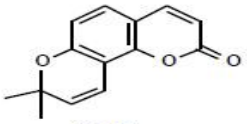
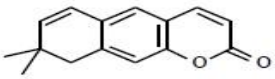
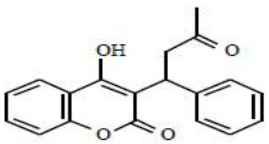
Coumarin (2H-1-benzopyran-2-one, Fig. 1a) obtained from various part of plant like fruits, nuts and seeds are known to have better dietary value and more than 1300 coumarins have been identified from natural sources[4]. They are classified into 4 major classes viz. Simple Coumarins, Furanocoumarins, Pyranocoumarins and Pyron-substituted Coumarins (**Table No.1**).

The biotransformation of coumarin into all derivatives are mentioned in **Scheme 1**. [5]

Coumarins with styryl carbonyl group have been found to be very important in scavenging of reactive oxygen species (ROS), thus contributing to the prevention of oxidative damage caused by free radicals.[6] Among the different existing active skeletons of coumarins, those

compounds with a 7-benzyloxy-coumarin core structure (Fig. 1b) have been found to possess good inhibitory activity and selectivity towards monoamine oxidase-B (MAO-B).[7] 1,5 substituted benzothiazepine[8] with a sulfonamide group are well known for their diverse therapeutically properties like antimicrobial[10] , antihypertensive[11] , calcium channel blocker[10] , blood platelet aggregation inhibitory[12] , and coronary vasodilatory effects [13]. While the isoxazoline derivative of coumarins and chalcones possesses antibacterial activity against gram +ve and -ve bacteria and antifungal activity. [14] The 3-bromoacetyl coumarin with thiazo group (Schiff bases) possess a broad spectrum of biological importance [15]. The coumarins containing Schiff bases are expected to have enhanced antitumor and other biological activity. Several hydrazone compounds containing this active moiety have shown to exhibit good anticancer activities [14]. Thus, a great deal of effort has been devoted to design and synthesize the functional coumarin derivatives known to display different biological and pharmacological activities. [15]

Table No. 1: Classification of coumarin derivatives [5]

Classification	Features	Examples
SIMPLE COUMARINS	Hydroxylated, alkoxylated or alkylated on benzene ring	 7-hydroxycoumarin
FURANOCOUMARINS	5-membered furan ring attached to benzene ring. Linear or Angular	 Psoralen  Angelicin
PYRANOCOUMARINS	6-membered pyran ring attached to benzene ring. Linear or Angular	 Seselin  Xanthyletin
PYRONE-SUBSTITUTED COUMARINS	Substitution on pyrone ring, often at 3-C or 4-C positions	 Warfarin

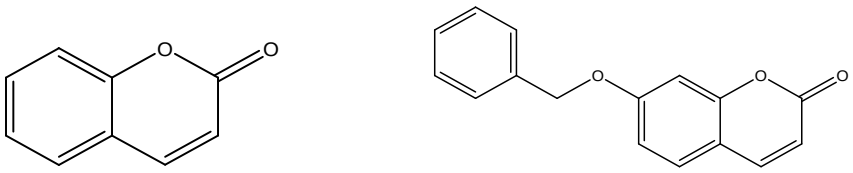
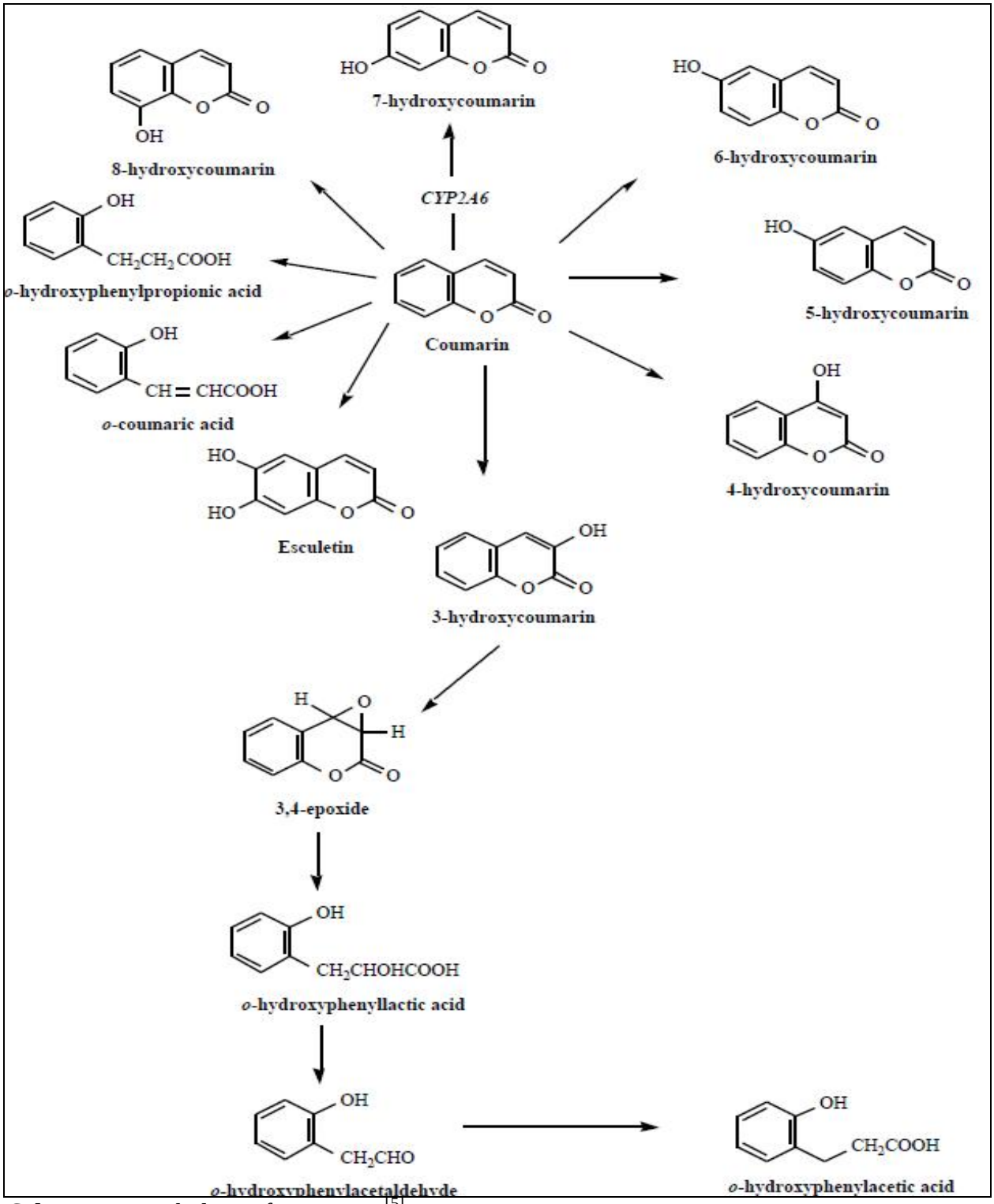


Fig 1: Chemical structure of (a) Coumarin and (b) 7-Benzyloxycoumarin



Scheme 1: Metabolism of coumarin. [5]

The major challenges about coumarins include the translation of current knowledge into new potential lead compounds and the repositioning of known compounds for the treatment of oxidative disorders [30]. In present review, various coumarin derivatives are evaluated for in *vitro* anticancer and antioxidant activity by DPPH, and several other super oxide free radical scavenging assay methods.

Photoreactive and fluorescent properties :

Coumarins such as 7-hydroxycoumarin (umbelliferone), 7-hydroxy-6-methoxycoumarin (scopoletin) and 6,7-dihydroxycoumarin (esculetin) emit blue fluorescence when excited in the UVA and UVB spectral regions. There have been interests in exploiting the fluorescence behavior of suitable coumarins as fluorescent whitening and brightening agents .The steady state fluorescence spectra of umbelliferone, 4- hydroxycoumarin and 7-hydroxy-4-methylcoumarin in protic solvents such as alcohol or water have been reported previously in the presence of added acid or base [16-18]. In these solvent systems, significant shifts and changes in the absorption and fluorescence spectra are observed. In a basic environment, the absorption and fluorescence spectra have additional bands at longer wavelengths than those associated with the neutral molecule which are attributed to the absorption by the ground state and emission from the first excited singlet state of the deprotonated, anionic forms of the hydroxycoumarins.

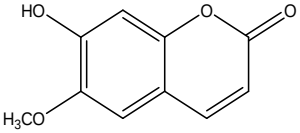


Fig 2a: Scopoletin

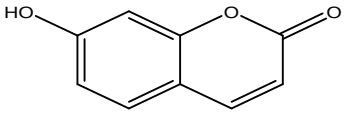


Fig 2b: 7-hydroxycoumarin

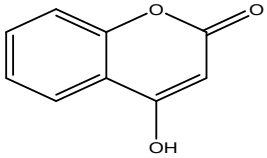


Fig 2c: 4-hydroxycoumarin

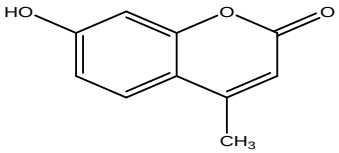


Fig 2d: 7-hydroxy-4-methylcoumarin

In order to simplify the interpretation of the absorption and fluorescence spectra as well as the primary photochemical reactions associated with 7-hydroxycoumarins, Smith et al. have systematically studied the absorption and fluorescence spectra of umbelliferone, scopoletin and esculetin in dry and aprotic solvents such as dimethylsulfoxide (DMSO), tetrahyrofuran (THF) and acetonitrile (ACN) . For all the three 7- hydroxycoumarins, besides the significant shifts and changes in the principal absorption and fluorescence spectra, minor absorption and fluorescence bands are observed at slightly longer wavelength than the principal bands in DMSO which are not present in the other two solvents. This can be ascribed to the deprotonated forms of the hydroxycoumarins with the deprotonation being facilitated by the greater proton affinity of DMSO than the other two solvents studied. Furthermore, as for the photostability of esculetin and scopoletin under UVA irradiation in all the three solvents, the former is less stable than the latter which is due to the more acidity of esculetin and is consistent with the initial step in the photodegradation with the deprotonation of the hydroxycoumarins being involved. The difference between esculetin and scopoletin is due to the presence of substitution group at position 6, i.e. hydroxyl group for esculetin while methoxyl group for scopoletin. Deprotonation of the two hydroxyl groups of the esculetin monomer is believed to be responsible for the minor absorption and fluorescence spectra. [19]

Intracellular ROS:

Intracellular ROS production is associated with a number of cellular events including activation of NAD(P)H oxidase, xanthine oxidase (XO), and the cellular mitochondrial respiratory chain. [20]

Xanthine Oxidase (XO)

Xanthine Oxidase (XO) is an important source of free radical and has been reported in various physiological and pathological models. This enzyme reduces molecular oxygen; leading to the formation of the superoxide released by processes such as oxidative phosphorylation and is first converted to hydrogen peroxide which is further reduced to give water. XO catalyses the oxidation of hypoxanthine and xanthine to uric acid yielding superoxide and raises the oxidative level in an organism [21]. The coumarins have the ability to inhibit Xanthine oxidase activity and the potent superoxide suppressive agent. Among tested compounds the esculetin (Fig. 3a) and 4-methylesculetin (Fig. 3b) with two hydroxyl moieties on the benzene ring are found to be the most effective radical scavengers. [22]

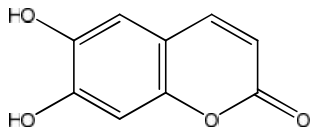


Fig 3a: Esculetin

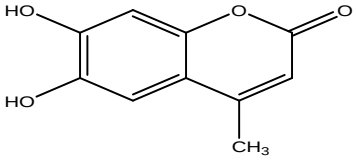


Fig 3 : 4-Methylesculetin

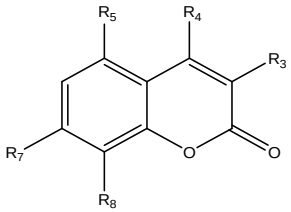
Monoamine oxidase-B (MAO-B)

Mono amine oxidases (MAOs) are flavoenzymes bound to the outer mitochondrial membrane and are responsible for the oxidative deamination of neurotransmitters and dietary amines. [23] Two isoforms, namely MAO-A and MAO-B, have been identified on the basis of their amino acid sequences, three dimensional structure, substrate preference and inhibitor selectivity. [24] MAO-A has a higher affinity for serotonin and noradrenaline whereas MAO-B preferentially deaminates phenylethylamine and benzylamine. These properties determine the clinical importance of MAO inhibitors.

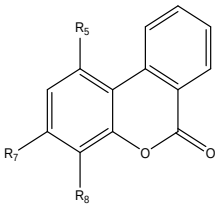
New series of coumarin derivatives with 4-methyl or cycloalkene or benzene ring condensed in the 3,4 position were synthesized. The substituents were introduced at the 5, 7 and/or 8 positions of the coumarin moiety. The synthesized compounds **1-13** were evaluated as MAO A and B inhibitors using clorgyline and selegiline as reference inhibitors. Compounds **6** (IC_{50} = 1.18 nM) and **10** (IC_{50} = 1.48 nM), show higher activity than selegiline (IC_{50} = 19.60 nM), and high MAO-B selectivity with 100-fold and 1600-fold inhibition levels, with respect to the MAO-A isoform. The activities were obtained in the nanomolar range [25]

Antioxidant activities- of coumarin and its derivatives:

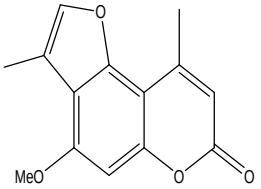
Many coumarin derivatives have special ability to scavenge reactive oxygen species (ROS)—free radicals, such as hydroxyl radicals, superoxide radicals or hypochlorous acid, and to influence processes involving free radical-injury. The inhibition of LDL peroxidation by the supplementation of antioxidants becomes an attractive therapeutic strategy to prevent and possibly treat atherosclerosis and related diseases in humans [26]. The human body is equipped with certain enzymatic and non-enzymatic antioxidant which can counteract the deleterious action of free radicals and radical induced cellular and molecular damage [27]. Disruption of this sensitive balance between the free radicals and the antioxidants may lead to cellular damage and trigger carcinogenesis [28]. Several antioxidant assays have been designated for the ROS estimation. Some of them are discussed below:



(1-7, 11, 12)



(8-10)

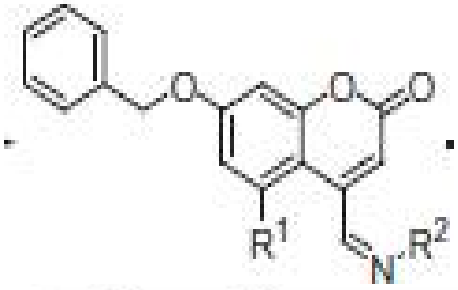


(13)

Compound	R_3	R_4	R_5	$R_7/(CH_2)_n$	R_8
1	$-(CH_2)_3-$		H	OH	Me
2	$-(CH_2)_4-$		H		
3	$-(CH_2)_3-$		H		
4	$-(CH_2)_4-$		H		
5	$-(CH_2)_4-$		H		
6	$-(CH_2)_3-$		H		
7	$-(CH_2)_4-$		H		
8	Ph		H		
9	Ph		H		
10	Ph		H		
11	H	CH_3	OH		
12	H	CH_3	$MeCOCH_2O$		
13	-		-		

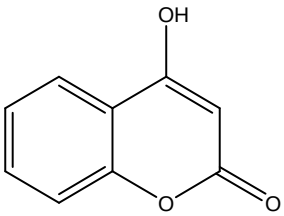
DPPH assay

4-Schiff base-7-benzyloxy-coumarins Fig. 5a1–5h2 and its derivative were synthesized by Zhang et.al to explore their antioxidant activities. The multifunctional and conjugation-effective salen group was designed to introduce at position 4 of 7-benzyloxy-coumarin structural core to enhance the donor–acceptor electronic effect and thus increase the electronic fluidity. Compounds 5b1, 5d1, 5f1, 5f2, 5g1 and 5g2 showed better radical scavenging activities than the synthetic commercial antioxidant butylated hydroxytoluene (BHT) , with IC_{50} values of 57.72, 10.51, 36.63, 6.91, 33.29 and 16.21 μM, respectively. In addition, compounds 5d1, 5f2 and 5g2 exhibited better DPPH radical scavenging activities than both ascorbic acid and butylated hydroxyanisole (BHA). [29]

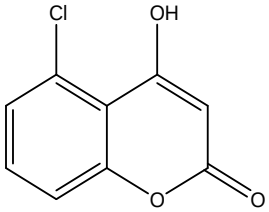


	R^1	R^2
5a$_1$	H	NH_2
5a$_2$	OCH_2Ph	NH_2
5b$_1$	H	C_6H_5NH
5b$_2$	OCH_2Ph	C_6H_5NH
5c$_1$	H	*p*-NO_2-C_6H_4NH
5c$_2$	OCH_2Ph	*p*-NO_2-C_6H_4NH
5d$_1$	H	*o*-NO_2-*p*-NO_2-C_6H_3NH
5d$_2$	OCH_2Ph	*o*-NO_2-*p*-NO_2-C_6H_3NH
5e$_1$	H	*o*-NH_2-C_6H_4
5e$_2$	OCH_2Ph	*o*-NH_2-C_6H_4
5f$_1$	H	*p*-OH-C_6H_4
5f$_2$	OCH_2Ph	*p*-OH-C_6H_4
5g$_1$	H	*o*-OH-C_6H_4
5g$_2$	OCH_2Ph	*o*-OH-C_6H_4
5h$_1$	H	OH
5h$_2$	OCH_2Ph	OH

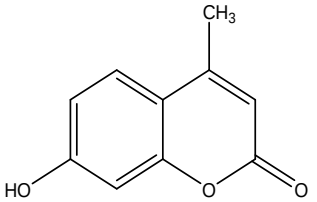
Three coumarin compounds (Fig. 6a-c) were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The IC_{50} value for coumarin compounds (6a-c) were found to be 799.83 µM, 712.85 µM and 872.97 µM respectively which were comparatively lower than the IC_{50} (829.85 µM) of ascorbic acid except for compound 6c. [30]



4-hydroxycoumarin



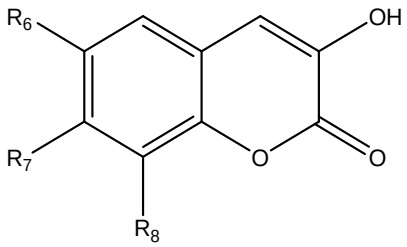
5-chloro-4-hydroxycoumarin



7-hydroxy-4-methylcoumarin

Fig 6: Hydroxycoumarin derivatives

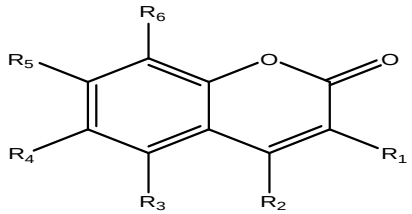
The five 3-hydroxycoumarins coumarins (Fig. 7a-e) were evaluated by their DPPH radical scavenging ability. The compound 7c and quercetin were the most powerful DPPH radical scavengers with comparable scavenging parameters (ECR_{50} around 0.25 and logZ around 2.60). Hydroxycoumarin 7e was a bit less reactive towards DPPH radical (ECR_{50} = 0.55; logZ = 2.20). [31]



7a: R_6=R_7=R_8= H
7b: R_6=R_7= H, R_8= OH
7c: R_6=R_8= H, R_7= OH
7d: R_7=R_8= H, R_6= OH
7e: R_6=R_7= OH, R_8= H

Fig 7: 3-hydroxycoumarin derivatives

The results of the DPPH radical scavenging activities of the coumarin derivatives 8.1 -8.15 are summarized in **Table 2**. [32], The order of reactivity was found to be 8.6 (95%) > 8.7 (88%) > 8.5 (82%) > 8.8 (69%). The remaining coumarin derivatives showed activity in the range of of 67% at 100 mM as compared to ascorbic acid (95%).

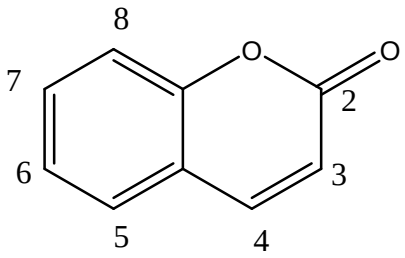


Structure No.	R_1	R_2	R_3	R_4	R_5	R_6	Structure No.	R_1	R_2	R_3	R_4	R_5	R_6
8.1	OH	H	H	H	H	H	8.9	H	CH_3	H	OH	H	H
8.2	H	OH	H	H	H	H	8.10	H	H	CH_3	H	OH	H
8.3	H	H	H	OH	H	H	8.11	H	CH_3	OH	H	OH	H
8.4	H	H	H	H	OH	H	8.12	H	OH	H	CH_3	CH_3	H
8.5	H	H	H	H	OH	OH	8.13	H	CH_3	H	H	OH	CH_3
8.6	H	CH_3	H	H	OH	OH	8.14	C_6H_5	CH_3	H	H	OH	H
8.7	H	H	H	H	OCH_3	H	8.15	C_6H_5	OH	OH	H	OH	H
8.8	H	OH	H	CH_3	H	H							

Table No. 2: Profile of DPPH radical scavenging activity of selected coumarin derivatives

Structure number	Concentration(µM/ml)	DPPH scavenging activity(%)	Structure number	DPPH scavenging activity(%)
8.1	10	46.78+0.56	8.9	26.31±0.13
	50	58.47±0.83		28.65±0.26
	100	67.83±0.50		30.40±0.52
8.2	10	34.50±0.78	8.10	3.74+±.09
	50	40.35±0.38		11.35±0.18
	100	53.81±0.20		13.35±0.18
8.3	10	9.94±0.38	8.11	23.65±0.74
	50	11.11±0.77		29.25±0.36
	100	14.61±0.48		36.14±0.26
8.4	10	19.38±0.32	8.12	21.06±0.30
	50	22.22±1.11		26.31±0.39
	100	38.01±1.15		37.42±0.28
8.5	10	51.46±0.66	8.13	21.83±0.09
	50	78.94±0.60		24.01±0.49
	100	82.45±0.59		27.32±0.22
8.6	10	63.75±0.39	8.14	243+0±11
	50	90.39±0.23		6.5+0. ±8
	100	95.63±0.33		30.13±.08
8.7	10	32.74±0.2647	8.15	17.42±.31
	50	47.35±0.85		19.17±0.36
	100	88.30±0.61		62.44±0.05
8	10	28.65±0.50	Ascorbic acid	66.81±0.34
	50	52.74±0.18		94.32±0.12
	100	69.00±0.27		95.98+0.32

Esculetin(6, 7 dihydroxy coumarin, Fig. 3a) is one of the well known antioxidant from coumarin family. At 50 min, DPPH radical scavenging rates of esculetin at different concentrations (0.02, 0.05, 0.08, 0.1 mg/ml) and BHT (0.5 mg/ml) were 28.13, 59.94, 79.68, 100, and 47.6%, respectively. [33]. The data obtained for the DPPH activity by Kim et.al were 8% at 0.1 µg/mL, 28% at 1 µg/mL, and 77% at 10 µg/mL [34]



Coumarin derivatives	C_4	C_6	C_7	C_8
Scopoletin (6-methoxy-hydroxycoumarin)	-	OCH_3	OH	-
Scoparone (6, 7-dimethoxy-coumarin)	-	OCH_3	OCH_3	-
Fraxetin (7, 8-dihydroxy-6-methoxycoumarin)	-	OCH_3	OH	OH
4-methylumbelliferone (4-methyl-7-hydroxycoumarin)	CH_3	-	OH	-
Esculin (6, 7-dihydroxy-8-O-glucosylcoumarin)	-	OGlu	OH	-
Daphnetin (7, 8-dihydroxy-coumarin)	-	-	OH	OH

Fig 9: Coumarin derivatives

4-methyl-umbeliferone (Fig 9) alone showed radical scavenging activity in the DPPH assay (EC_{50} = 0.218 μM), but its effects were limited compared to the reference antioxidant compound, quercetin[35]. Al-Ayed et.al evaluated the DPPH scavenging activity for (Fig 10), wherein Fig 10d exhibited maximum activity. [36]

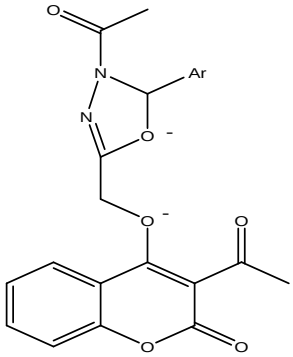


Fig 10:

10a Ar = 4-Bromophenyl

10b Ar = Beta-naphtyl

10c Ar = 3, 4-dimethylphenyl

10d Ar = 4-hydroxyphenyl

10e Ar = 4-methylphenyl

The comparative study exhibit that the coumarinic chalcone 11c is inactive even at a concentration of 3.77 mM. Compounds 11e and 11i was found to be the most active compounds in this study (**Table 3**). Comparing the activity of compounds 11f–h it was concluded that there is not a substantial difference when nature of the substituent Ar is changed. [37]

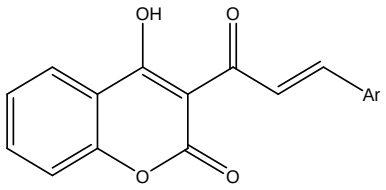


Fig: 11

11a-i	Ar
a	C_6H_5
b	PF-C_6H_4
c	$POCH_3C_6H_4$
d	$PMeC_6H_4$
e	$PBrC_6H_4$
f	$PNO_2C_6H_4$
g	3, 4, 5 $OCH_3C_6H_2$
h	$C_{10}H_7$
i	$PN(Me)_2C_6H_4$

Table 3: DPPH radical scavenging activity of coumarin derivatives

Compounds 11a-i	EC_{50} (µM)
11a	2.39
11b	2.34
11c	3.03
11d	2.24
11e	2.10
11f	2.16
11g	2.42
11h	2.29
11i	2.18

ABTS radical assays:

The free radical scavenging activities of coumarin derivatives are relevant to the number and position of the hydroxyl group on the benzenoid ring of the coumarin system [30]. Antioxidant activity of coumarins and their derivatives as well as their pharmacological and biochemical properties depend on their structure feature. [38]

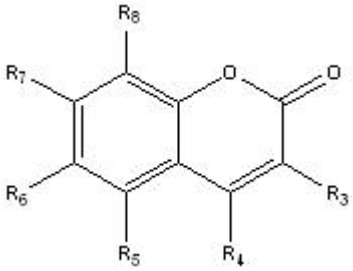


Fig 12: Methylcoumarin

Scopoletin bears a good scavenging ability against $ABTS^{\bullet+}$, but was not effective against DPPH, superoxide, hydroxyl radicals.[39] Compounds 10 a-e was also evaluated for their ABTS radical activity, wherein compounds 10a-d exhbit higher activity as compared to the synthetic antioxidant trolox.[36]

Superoxide Dismutase (*SOD) assay:*

A 7, 8(ortho)-dihydroxy compound shows good SOD mimetic capacity, 3-ethoxycarbonylethyl substituted compound shows slightly lower SOD mimetic activity [40].

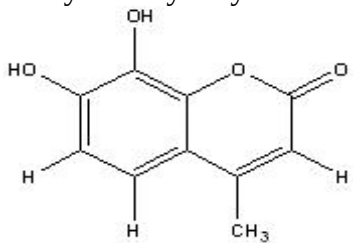


Fig 13: 7, 8(ortho)-dihydroxy coumarin

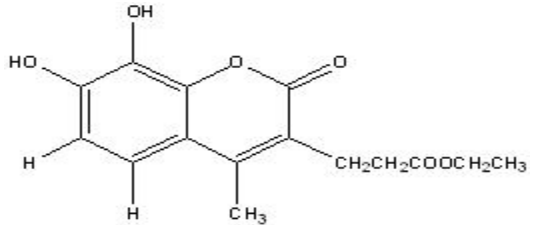


Fig 14: 3- ethoxycarbonylethyl substituted compound

Compounds like 4-hydroxy coumarin, 5-chloro-4-hydroxy coumarin and 7-hydroxy-methylcoumarin shows potent superoxide radical scavenging activity compared to the ascorbic acid at low IC_{50} studied by SOD assay[41]. The IC_{50} for Compound 6a-c were 641.21µM, 722.77µM and 2079.69 µM respectively and for ascorbic acid, it was 2051.16 µM. [fig. 6a-c] [30]. Umbeliferone was found to be effective in only $O_2^{\bullet-}$ quenching [41].

Other radical assays:

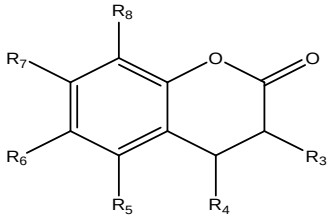
The intracellular ROS scavenging activity of esculetin in V79-4 cells after H_2O_2 treatment was detected by the DCFDA assay. The data revealed that the intracellular ROS scavenging activity of esculetin was 1% at 0.1 µg/mL, 40% at 1 µg/mL, and 75% at 10 µg/mL[34] .The V79- 4 cells exposed to H_2O_2 had increased lipid peroxidation, as evidenced by the generation of TBARS. However, esculetin inhibited the H_2O_2-induced peroxidation of lipids.

The superoxide anion and hydroxyl radical scavenging tests were best exhibited by the compounds 7c and 7e. The presence of the catechol moiety in 7e must can be attributed to its superior superoxide scavenger ability. While for the compound 7c the hydroxyl group on C-7 position is conjugated with the other hydroxyl group on C-3position in a manner similar to a hydroquinone system. The radical formed upon reaction with the superoxide anion is expected to be particularly stabilised by delocalisation over the cyclic structure. The contribution of this hydroquinone is essential to explain the position of 7c as the best hydroxyl and peroxy- nitrite scavenger and inhibitor of LDL oxidation. [31]

The protective effects of the compounds 7a-e were tested against peroxynitrite damage and expressed as the concentration giving 50% inhibition of the peroxynitrite induced oxidation of dihydrorhodamine 123(IC_{50}). Reference compounds, that is, trolox, quercetin, vitamin C, and 7c rapidly inhibited DHR 123 oxidation with half maximal inhibitory concentrations around 1.0µM. Compounds 7e (ED_{50} of 1.94µM), 7b (ED_{50} of 3.44µM) and 7d (ED_{50} of 5.49µM) were observed. Compound 7a was almost unreactive. [Fig 7a-e] [31]

The eight methylcoumarins tested included 5-dihydroxymethylcoumarins and 3 diacetoxy-methylcoumarins. Dihydroxy-4-methylcoumarins included 6,7-dihydroxy (ortho, C2), 7,8-dihydroxy (ortho, C3) and 5,7-dihydroxy (meta, C9). Furthermore, 7,8-dihydroxy-4-methylcoumarins with ethoxycarbonylmethyl (C4) and ethoxycarbonylethyl substitutions (C5) at position C3 were also tested for comparison. Also 7,8-diacetoxy-4-methylcoumarins with ethoxycarbonylmethyl (C7) and ethoxycarbonylethyl substitutions (C8) at position C3 were included in the study. The activities of all these coumarins in different chemical assays were compared with that of the natural coumarin esculetin, as the reference compound (C1) (Table 1). [42] The presence of a methyl or hydrogen atom at position 4 of the coumarin ring

does not influence the activity, because the electron-donor effect on the double bond is very weak, while substitution in the C3 position does not affect the antioxidant properties in the DPPH and FRAP assay.

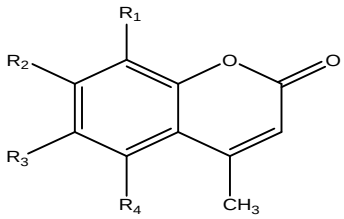


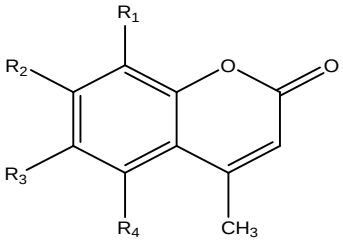
Compounds		R_3	R_4	R_5	R_6	R_7	R_8
15.1	6,7(ortho)-Dihydroxy-	H	CH_3	H	OH	OH	H
15.2	7,8(ortho)-Dihydroxy-	H	CH_3	H	H	OH	OH
15.3	7,8(ortho)-Dihydroxy-3-ethoxycarbonylmethyl-	$CH_2COOCH_2CH_3$	CH_3	H	H	OH	OH
15.4	7,8(ortho)-Dihydroxy-3-ethoxycarbonylethyl-	$CH_2CH_2COOCH_2CH_3$	CH_3	H	H	OH	OH
15.5	7,8(ortho)-Diacetoxy-	H	CH_3	H	H	$OOCCH_3$	$OOCCH_3$
15.6	7,8(ortho)-Diacetoxy-3-ethoxycarbonylmethyl-	$CH_2COOCH_2CH_3$	CH_3	H	H	$OOCCH_3$	$OOCCH_3$
15.7	7,8(ortho)-Diacetoxy-3-ethoxycarbonylethyl-	$CH_2CH_2COOCH_2CH_3$	CH_3	H	H	$OOCCH_3$	$OOCCH_3$
15.8	5,7(meta)-Dihydroxy-	H	CH_3	OH	H	OH	H

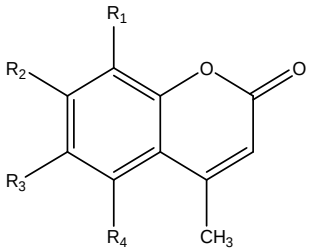
4-methylcoumarins showed some antioxidant activity in DPPH, $ABTS^{\cdot+}$ and FRAP assays except the compound 15.9 [5,7 (meta)-dihydroxy] that appeared almost completely devoid of antioxidant capacity. The antioxidant capacity of 15.3 against NO is significantly increased by the introduction of an ethoxycarbonylmethyl moiety at the same position (15.4).In the NO test the efficacy order was 15.4 > 15.1 > 15.5 > 15.6 > 15.8 > 15.3 > 15.2 > 15.7 while in the HClO assay was 15.3 > 15.4 > 15.5 > 15.1[15.2> 15.8 > 15.6 > 15.7; 15.9 hardly showed any activity in all three tests [42] The protective effect of these coumarins in a model system consisting of dipalmitoylphosphatidyl- choline/linoleic acid (DPPC)/linoleic acid (LA) LUVs incubated in the presence of the water-soluble azo compound AAPH as a free radical generator which induces a large increase in the accumulation of LOOH formed from LA peroxidation. In this assay the 6,7- and 7,8-dihydroxy- (15.2 and 15.3) and 7,8-dihydroxy-3-ethoxycarbonylethyl- (15.5) 4-methylcoumarins show a strong antioxidant capacity, that is better than that of esculetin and of the 7,8-dihydroxy-3-ethoxycarbonylmethyl-4-methylcoumarin (15.4) (efficacy order: 15.5 > 15.3 > 15.2 [15.4 > 15.1), while the other tested coumarins were completely ineffective.

The six novel 4-methylcoumarins bearing different functionalities such as amino, hydroxy, *N*-acetyl, acetoxy and nitro have been synthesized. Their effect on NADPH dependent liver microsomal lipid peroxidation *in vitro*, and the results were compared with other model 4-methylcoumarin derivatives to establish the structure–activity relationship. Ortho hydroxy-amino
coumarins 6, 7 and 12 were identified as potent inhibitors of lipid peroxidation, better than those of α-tocopherol demonstrated that amino group is an effective substitute for the hydroxyl group for antioxidant property and produced a dramatic inhibition of lipid peroxidation by Tyagi et.al. [43] The IC_{50} of compounds 6a-c were 743.02, 716.14 and 648.63

respectively which were found to be lower than IC_{50} of ascorbic acid (3083.18 µM).{Fig 6a-c] [30]







R2=OAc, R1=NHAc, R3=R4=H R3=OAc, R2=R4=NO2, R=H R3=OH, R2=R4=NH2, R1=H
R3=OAc, R4=NHAc, R1=R2=H R1=R2=OAc, R3=NO2, R4=H
R3=OAc, R2=R4=NHAc, R1=H

The free radical scavenging and lipid peroxidation assays revealed that five phenolic coumarins, scopoletin (1), aesculetin (2), fraxetin (3), umbelliferone (18) and daphnetin (19), possessed considerable antioxidant activities. the radical scavenging activities of the isolated coumarins against four free radicals, including DPPH, superoxide, hydroxy, ABTS•+ radicals and a reactive oxygen species HOCl. Among the test compounds, aesculetin (2), fraxetin (3) and daphnetin (19) were found to possess significant scavenging properties. Aesculetin showed the strongest quenching capacities against DPPH, OH• radicals, followed by daphnetin. These coumarins also exhibited the highest scavenging capacity against the ABTS•+ radical, stronger than those of caffeic acid and ferulic acid, two reference substances. Fraxetin was less active than 2 and 19 in DPPH, OH• and ABTS•+ radical scavenging, but was more potent in the bleaching effects against O2 •− and HOCl. Scopoletin (1) manifested a good scavenging ability against ABTS•+, but was not effective against the others. Similarly, umbelliferone (20) was found to be effective in O2•− radical quenching only[41].

ANTICANCER ACTIVITY:

Esculetin scavenges the hydroxyl radical produced by Fenton process observed by the ESR spin trap technique. The hydroxyl group present in esculetin binds efficiently with the XO enzyme and thus causes inhibition. Apart from *in vitro* studies esculetin also shows protective effect through scavenging of intracellular reactive oxygen species (ROS) in Chinese hamster lungs fibroblast (V79-4) cells through DCFDA assay and related techniques. Treatment with esculetin at 10µg/mL in V79-4 cells did not show cytotoxicity, compared with the control. Combination with esculetin at 10 µg/mL and H_2O_2 increased 87% in the cell survival rate,compared with 64% in H_2O_2 treated cells. The findings suggest that esculetin results in the protection of cell viability by inhibiting the damage of the cellular components induced by H_2O_2. The results presented in this report indicate that esculetin efficiently attenuated oxidative stress-induced cell damage via its anti-oxidant properties. Thus, esculetin might be useful in the development of functional food and raw materials of medicine.[34]

Esculetin added at the beginning of culture of Concanavalin-A stimulated lymphocytes showed a dose-dependent cytotoxic effect. Complete cytotoxicity was seen at 62.5 µM esculetin treatment, and the lethal concentration fifty (Lc50) was 7.5 µM. A less amount of 0.25 µM esculetin showed 6.6% cytotoxicity compared to the control. With CTLL-16, an IL-2 dependent cytotoxic cell line, no significant cytotoxicity was observed in the presence of esculetin. Rather, this cell line showed 8% growth stimulation at 0.25 µM esculetin treatment. Although esculetin is known to inhibit Lipooxygenase enzyme (LO) involved in leukotrien synthesis and affect cell proliferation it is unclear whether the inhibitory effect, seen at low doses of esculetin, on the generation of effector cytotoxic T cells may be related to leukotrienes inhibition as described by Al- Mustansiriya et.al.[44]

Esculetin has the ability to easily reduce oxidative stress in liver and kidney tissues of streptozotocin-induced diabetic rats and provides evidence that the protective effects are possibly due to significant increase potential probably by attenuating the hyperglycemia-mediated oxidative stress, decreased lipid peroxidation and preserving the structural and functional integrity of hepatic and renal tissues. Esculetin at a dose of 40 mg/kg BW showed a more pronounced effect than the other two doses 10 and 20 mg/kg BW.[45] Esculetin possesses inhibition effect on human HT-29 cells in a dose-dependent manner in vitro. These results make a strong case for expanding the investigations of natural antioxidants from purified compounds derived from Chinese traditional medicinal herbs. They also provide useful information on pharmacological activities associated with antioxidants of compounds [33]. There is a definite role of antioxidants in the regulation of certain molecular risk factors These agents appear to affect the activation of protocogenes, loss of antioncogenes suppressor genes and stimulation of cell signalling systems which can result in abnormal cell proliferation and differentiation. But, from an epidemiological perspective, the antioxidant defense system is more than just the sum of the individual parts. Future research will elucidate the concurrent application of safe, appropriate doses of antioxidants that could exert a potent synergistic chemopreventive effect. This preventive course of action continued for an extended period of time may reduce cancer risk without toxicity [46].

A recent study has shown that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is overexpressed in many types of cancer. This knowledge may lead to its use in cancer therapy. Esculetin inhibits growth and cell cycle progression by inducing arrest of the G1 phase in HL-60 leukaemia cells, resulting from the inhibition of retinoblastoma protein phosphorylation. [47] Recent studies investigating the potential of flavonoids as therapeutic agents have suggested they may have use in various therapeutic settings ranging from leukaemia treatment to the treatment of patients with HIV. Genistein is a well-known isoflavone and is a tyrosine kinase inhibitor. Studies have indicated that genistein elicits inhibitory effects on cell growth of various carcinoma cell-lines and may be a potential candidate for cancer therapy.

The 3, 7-dihydroxycoumarin derivatives Fig 16 and Fig 17 were found to be potent antioxidant compounds comparable to quercetin or vitamin C. Their antioxidant activities can be related to their abilities to give stable semiquinonic radicals for Fig 16 and non-classical radicals for Fig 17 [31].

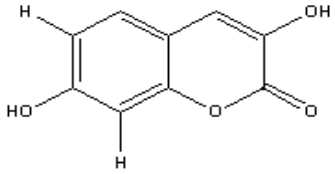


Fig 16: 3, 7-dihydroxy-2H-chromen-2-one

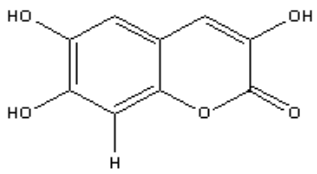


Fig 17: 3, 6, 7-trihydroxy-2H-cromen-2-one

These 3-hydroxycoumarins are worth being evaluated more accurately for their pharmacological properties [31]. The results obtained in this study confirm the good antioxidant activity of the 7, 8-dihydroxy-4-methylcoumarins. In general their activity is not significantly affected by the introduction of an ethoxycarbonylmethyl or an ethoxycarbonylethyl moiety at the C3 position. A discrete antioxidant activity is retained also by the 7, 8-diacetoxy-4-methylcoumarins, although they are less efficient than the corresponding 7, 8-dihydroxy compounds [42]. Most of coumarins grandivittin, agasyllin, aegelinol benzoate, and osthol from *Ferulago campestris* plant exhibit cytotoxic activity against the A549 lung cancer cell line [48]. The synthesis of coumarin derivatives containing pyrazole, pyrazolone, thiazolidin-4-one, 5-carboxymethyl-4-thiazolidinone and 3-acetyl-1,3,4-oxadiazole ring is reported to posses better antioxidant and anticancer potentials.[49]

a = CH_3, b = H, c = F

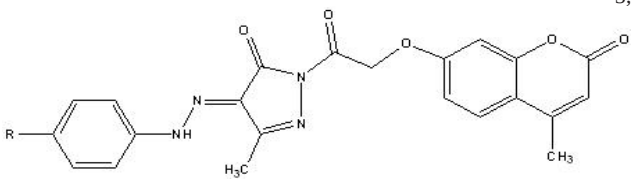


Fig 18a-c: {1-(4-methylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted phenyl) hydrazono-2-pyrazolin-5-ones}

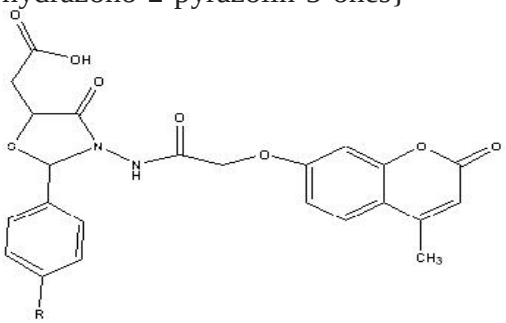


Fig 19a-c: {2-(substituted phenyl)-3-(4-methylcoumarinyl-7-oxyacetamido)-5-carboxymethyl-4-thiazolidinone}.

The compound 18b exhibit cytotoxicity against DLA cells at 100 µg mL^{-1} concentration. Compound 19b had highest free radical scavenging activity (95%). The derivatives 18b and 19b are found to be potent cytotoxic agents against EAC cells at a 50 µg mL^{-1} concentration. Also compound 19b can act as ideal antineoplastic agent[49].

The profile of cytotoxicity of the coumarin derivatives against selected cancer cell lines HeLa B75, HL 60 and HEP 3B were evaluated. The data observed is summarized in **Table 4**. However the compounds 8.9, 8.10, 8.12, 8.14 and 8.15 can be considered as lead molecules for designing novel cytotoxic agents as these molecules were found to be active cytotoxic agents (IC_{50} < 8.0 mM and $p < 0.05$) against most of the selected cell lines [32].

Table 4 : Profile of cytotoxicity of selected coumarin derivatives (IC_{50} µM) against selected cancer cell lines

Structure number	Cytotoxicity (IC_{50} µM)		
	Hela+B75	HL+60	HEP+38
C_1	7.51*	9.71	10.04
C_2	9.93	7.68*	17.32
C_3	10.19	9.69	10.07
C_4	9.69	10.05	7.56*
C_5	9.95	17.37	17.39
C_6	17.4	9.93	7.66*
C_7	7.55*	17.35	10.00
C_8	9.99	17.39	7.59*
C_9	7.52*	7.60*	10.00
C_{10}	7.60*	17.46	7.64*
C_{11}	17.4	17.35	10.27
C_{12}	7.61*	9.80	7.90*
C_{13}	9.90	17.39	17.33
C_{14}	7.60*	17.41	7.52*
C_{15}	9.85	7.65*	7.53*
Methotrexate	5.43*	7.55*	4.33*

Results are expressed as the mean values of two parallel experiments
*p< 0.05 when compared with control.

CONCLUSIONS:

A comprehensive study reveal that the phenolic coumarins possess considerable antioxidant activities against free radicals such as DPPH, superoxide, ABTS and other radicals. Esculetin amongst them has shown the ability to bind to the XO enzyme and cause inhibition. The data further shows that esculetin is able to reduce oxidative stress induced cell damage via its antioxidant properties. These findings hopefully encourage the scientific researchers to undertake further work on coumarins as antioxidant and anticancer drugs. The investigations can be further extrapolated to design new synthetic antioxidants that might be useful in the development of functional food and medicines.

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