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ANTIOXIDANT ACTIVITY STUDIES OF SOME HALOGEN SUBSTITUTED FLAVONE DERIVATIVES

Aseemuddin N, Chavan SB, Shaikh Nuzhat B and Baseer MA*

Organic Chemistry Research Laboratory, Yeshwant Mahavidyalaya, Nanded-431602, India.

ABSTRACT

A series of biologically active halogen substituted flavones (2a-j) were evaluated for their antioxidant potential using DPPH screening method. These compounds exhibited potent antioxidant activity, which is close to standard flavonoid quercetin.

Keywords: Halohydroxychalcones, Flavones, Antioxidant activity.

INTRODUCTION

Flavone derivatives exhibit a broad range of biological activities[1-3], such as Analgesic [4], Anti-inflammatory [5], Chemopreventive [6], Anti-cancer [7, 8], Antitumor[9], Antioxidant [10], Antiglycation [11], Vasorelaxant [12], Cyclo-oxygenase-2 (COX2) [13], Antiplaque [14] and Anti-tuberculosis[15]. It is well known that halogen substituted flavonoid compounds are also strongly biologically active [16]. On the other hand, halogen and methyl group substituted in pyron ring also exhibit biological activity [17]. Considering these observations, we report herein, the antioxidant activity of new flavones (2a-j), having chloro, bromo, iodo and methyl moiety in pyron ring. We have reported a new series of halogen substituted-chromen-4-ones as antimicrobial agents by the oxidative cyclization of 2'-hydroxy-substituted chalcones in the presence of few crystals of iodine in dimethyl sulfoxide (DMSO) [18].

MATERIALS AND METHODS

The Flavone compounds used for study were synthesized in same laboratory and reported [18] and their solutions were prepared in various concentrations with DMSO. Chemicals 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Quercetin were obtained from MP Biomedicals Ltd., USA. and Dimethyl sulphoxide, methanol were obtained from SD fine Chemicals Ltd.,India. The following Fig.1 explains the various substitution patterns in Flavones **2(a-j).**

Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH., which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured [19]. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present)[20].

The scavenging reaction between (DPPH.) and an antioxidant (H-A) was shown as-

$$(DPPH) + H\text{-}A \rightarrow DPPH\text{-}H + A$$
(Purple) (Yellow)

Corresponding Author: - **Mohammad Abdul Baseer** Email: dr.baseer.nanded@gmail.com

4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. 50 µl of various concentrations of Flavone compounds as well as standard compound (Quercetin) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min. at 517 nm using methanol as blank on UV-visible spectrometer Shimadzu, UV-1601, Japan.

The free radial scavenging activity of all the flavones 2(a-i) were evaluated through their ability to quench the DPPH (1,1-Diphenyl-2- picryl hydrazyl) [21] using Quercetin as reference.

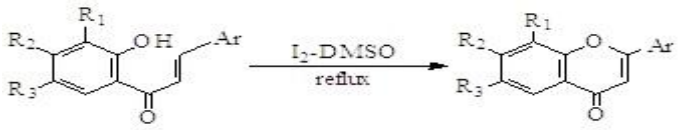
All the synthesized derivatives have shown different percentage inhibitions at concentrations of 50, 75 and 100µg/ml. The free radical scavenging activity [22] is expressed as follows.

$$S\ (\%) = \frac{100\ (A_0 - A_S)}{A_0}$$

Where A_0 = Absorbance of control
(Containing all reagents except the test compound)
A_S= Absorbance of the test Compound

In the present study the *in vitro* antioxidant model 1,1-Diphenyl-2- picryl hydrazyl (DPPH) scavenging activity (as it is model for Lipophilic radicals which initiate lipid peroxidation) was used.

Fig.1



	R_1	R_2	R_3	Ar
2a	I	CH_3	Cl	Ar_3
2b	I	CH_3	Cl	Ar_4
2c	Br	OH	Br	Ar_1
2d	Br	OH	Br	Ar_2
2e	Br	OH	Br	Ar_3
2f	Br	OH	Br	Ar_4
2g	I	H	Cl	Ar_1
2h	I	H	Cl	Ar_2
2i	I	H	Cl	Ar_3
2j	I	H	Cl	Ar_4

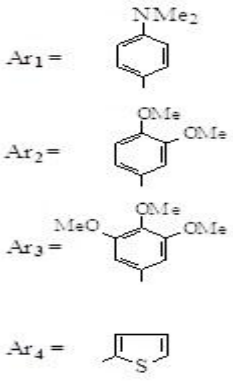


Table 1. Antioxidant activity of synthesized flavones (2a-j) and standard flavonoid Quercetin represented as % DPPH inhibition.

Concentration (µg/ml)	Series name / Products and Standard Quercetin										
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**Std. Quercetin**
50	28.74	33.12	39.63	41.30	47.23	41.34	75.24	79.42	63.51	59.36	**56.73 (5µg/ml)**
75	37.42	41.31	43.71	49.28	56.66	51.70	82.32	87.45	76.83	68.28	**74.96 (10µg/ml)**
100	42.28	53.60	56.63	61.91	67.50	70.14	91.52	94.73	88.51	74.85	**89.96 (15µg/ml)**

All the above values are representative of three independent replicates and calculated from as mean ± SEM of individual samples.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index .In the DPPH Free radical scavenging activity, ten Flavone compounds **(2a-j)** were evaluated for their free radical scavenging activity with Quercetin as standard compoundand summarized in Table 1.The scavenging effect increased with the increasing concentrations of test compounds. Flavones were recognized as possessing potent anti oxidant activity were also strong scavengers of DPPH. DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up. Substances capable of donating electrons/hydrogen atoms are able to convert DPPH (Purple) into their non radical form 1, 1-diphenyl-2- picrylhydrazine (Yellow), a reaction which can be followed/monitored spectrophotometrically. Free radical scavenging activity of the Flavone compounds is concentration dependent, as the concentration of the test compounds increases, the radical scavenging activity increases. From results, it may be postulated that all Flavone compounds were able to reduce the stable free radical DPPH to the yellow-colored diphenylpicrylhydrazine exhibiting better free radical scavenging activity than the standard antioxidant Quercetin. Structure activity relationship study showed that the anti oxidant activity of these Flavone derivatives could be attributed to electron donating nature of the substituents like –OH, $-CH_3$ and –Cl on Flavone scaffold, reduce free radical DPPH and prevent the damage of cell. The more hydrogen donors, the stronger is the anti oxidant activity. These anti oxidants should display anti oxidant activity if one or more the groups like –OH, $-CH_3$ are free, since they are known to be good hydrogen donors [24, 25].

CONCLUSION

To conclude, ten halogeno hydroxy subst. 2-phenyl-Cromene-4-ones (flavones) were evaluated for antioxidant potential using *in vitro* DPPH free radical scavenging method. It was found that compounds 2g, 2h and **2i** displayed strong antioxidant activity compared to the Quercetin and it suggested that these compounds could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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