Evaluation of the Antioxidant Activity of Novel Synthetic Chalcones and Flavonols

Venkatachalam H., Yogendra Nayak, and B. S. Jayashree

***Abstract*—In the present study, newer chalcones and flavonols were synthesized by Algar-Flynn-Oyamada method, purified and characterized by spectral methods. The pure test compounds were evaluated for in vitro antioxidant activity using four models namely 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•), Nitric oxide (NO) and Lipid Peroxidation scavenging methods. This study indicate that, amongst the ten evaluated chalcones and flavones, five test compounds showed activity with IC_{50} value lesser than that of the standard ascorbic acid at 91.21 µM for 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging.**

***Index Terms*—Antioxidant, Chalcones, Flavonols.**

I. Introduction

Many present day diseases are reported to be due to an impaired balance of the pro-oxidant - antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either on account of increased generation of free radicals caused by excessive oxidative stress, or due to poor scavenging in the body caused by depletion of the dietary antioxidants. Reactive oxygen species differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission, modification of polypeptides, lipid peroxidation etc [1].

Antioxidants can be defined as "any substance which significantly delays or inhibits oxidative damage to a target molecule". Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health. The need for antioxidants becomes even more critical with increased exposure to free radicals. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection [2].

In the present study we have undertaken the evaluation of antioxidant activity of novel flavonols obtained from various chalcones which are well known for their antioxidant activity. Antioxidants are compounds capable of preventing the damage caused in human tissue by the normal effects of physiological oxidation. Research has shown that antioxidants can play a role in preventing the development of chronic diseases such as cancer, diabetes and cardiovascular diseases.

Chalcones basic structure includes two aromatic ring bound by an α, β-unsaturated carbonyl group, a unique template associated with very diverse application [3]. Due to the presence of the reactive keto, vinylenic group, chalcones and their analogues have been reported to be antioxidant [4]. Hydroxyl and phenyl substitutents are associated with antioxidant properties. When chalcones get cyclised they form flavones. Flavones are also prominent plant secondary metabolites that have been found in dietary components, including tea and red wine. They express various biological activities such as anticancer, antitumor, antiprotozoal and antioxidant [5].

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II. Materials and Methods

A. Synthesis

The synthesis of the test compounds were undertaken by Algar-Flynn-Oyamada method.

The synthesized test compounds were purified by recrystalization and characterized by UV-Visble, Infra Red, NMR and Mass spectroscopy.

B. Antioxidant activity

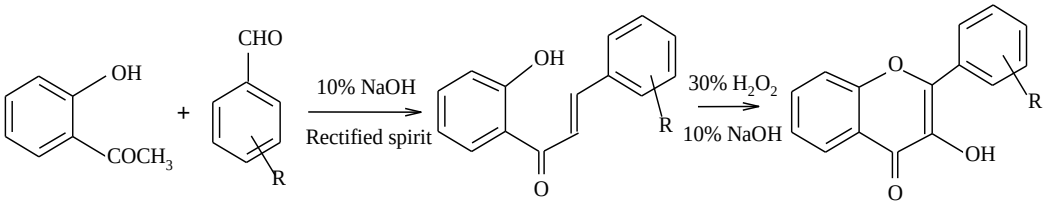
The following antioxidant methods were used to evaluate the antioxidant properties of our test compounds.

1) DPPH• Scavenging Activity

DPPH is a stable free radical that can accept anelectron or hydrogen radical to become a stable diamagnetic molecule. Due to its oddelectron, the methanolic solution of DPPH shows a strong absorption band at 517 nm. DPPH radical reacts with various electron donating molecules (reducing agents or antioxidants). When electrons become paired off, bleaching of the DPPH solution is the result. This results in the formation of the colourless 2,2'-diphenyl-1-picryl hydrazine. Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517 nm.

Procedure: Equal volumes of 100 µM 2,2'-diphenyl-1-picrylhydrazyl (DPPH) in methanol was added to different concentrations of test compounds (0 – 200 µM/ml) in methanol, mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured using the spectrophotometer UV-1650, Shimadzu [6]. Plotting the percentage DPPH• scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of blank-Absorbance of test}}{\text{Absorbance of blank}} \times 100$$



Algar-Flynn-Oyamada method

Aryl aldehyde = 3,4-(methylenedioxy)benzaldehyde (JVC1,JVF1)
4-benzyloxybenzaldehyde (JVC2, JVF2)
4-[(2-cyanoethyl)methylamino]benzaldehyde (JVC3, JVF3)
4-[(2-pyridyl)benzaldehyde (JVC4, JVF4)
4-(methylthio)benzaldehyde (JVC5,JV F5)

IC_{50} was obtained from a plot between concentration of test compounds and % scavenging. Ascorbic acid was used as standard for comparison.

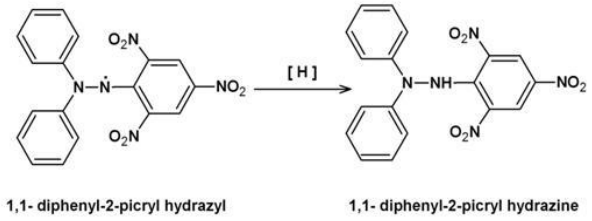


Fig. 2. Structural changes of DPPH during oxidation

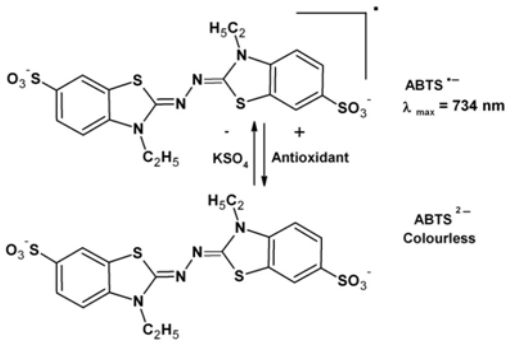


Fig. 3. ABTS Radical formation

2) ABTS radical scavenging assay

ABTS is chemically 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid). ABTS assay is based on the inhibition of the absorbance of the radical anion $ABTS^{\bullet-}$. $ABTS^{\bullet-}$is generated by addition of potassium per-sulphate to the colourless solution of $ABTS^{2-}$. The radical anion ($ABTS^{\bullet-}$) has ablue-green colour and characteristic long wavelength absorption spectrum λ max of 734 nm. The ability of the test compounds to scavenge $ABTS^{\bullet-}$ is measured by noting the fall in absorbance at 734 nm. [7]

Procedure: To the reaction mixture containing 0.5 ml of different concentration (2-200 µM/ml) of compounds, 1.7 ml of phosphate buffer (20 mM) and 0.3 ml of 100 µM $ABTS^{\bullet-}$(prepared by mixing 2 mM ($ABTS^{2-}$) with 0.17 mM potassium persulphate in 20 mM phosphate buffer pH 7.4; kept overnight before use) was added. Immediately, the decrease in absorbance was measured at 734 nm. Ascorbic acid was used as standard for comparison. Plotting the percentage $ABTS^{\bullet-}$ scavenging against concentration produced the standard curve. The % scavenging and the IC_{50} values were calculated as mentioned in the DPPH assay.

3) Nitric Oxide Scavenging Activity

Nitric oxide (NO) will be generated by sodium nitropruside in solution. In the presence of an antioxidant or nitric oxide scavenger the amount of NO generated will be less. The excess NO will be estimated by Griess reagent is the mixture of sulphanilic acid and naphthylethylenediamine dihydrochloride. The nitric oxide will give pink colour complex estimated at 540 nm.

Procedure: To a reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, 1.0 ml) and 1.0 ml of different concentration of test compounds/standard were incubated at 25 ℃ for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed and 1.0 ml of sulphanilic acid was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then 1.0 ml of naphthylethylenediaminedihydrochloride was added, mixed and allowed to stand for 30 min in dark at room temperature. The absorbance of these solutions was measured at 540 nm against corresponding blank solution without sodium nitroprusside [8]. The % scavenging and IC_{50} values were determined as explained in DPPH assay.

4) Lipid peroxidation assay

Lipid peroxidation is initiated by abstraction of a hydrogen atom from bis-allylic methylene carbon positioned on a polyunsaturated side chain of a lipid molecule. These carbons are attacked preferentially over the secondary -C-H bonds because of the production of a resonance-stabilized carbon-centered radical. As the name peroxidation indicates, the auto-reaction initiation follows the carbon radical to be stabilized by a molecular rearrangement to form a conjugated diene (Figure 4). Further, a variety of propagation reactions may takes place. The most likely reaction is between lipid radical and oxygen. After the addition of molecular oxygen to the lipid radicals, which is very fast in aerobic environment, the resulting peroxyl radical can attack neighboring polyunsaturated fatty acids or undergo intramolecular cyclization followed by breakdown to compounds such as, ethane, n-pentane and MDA.

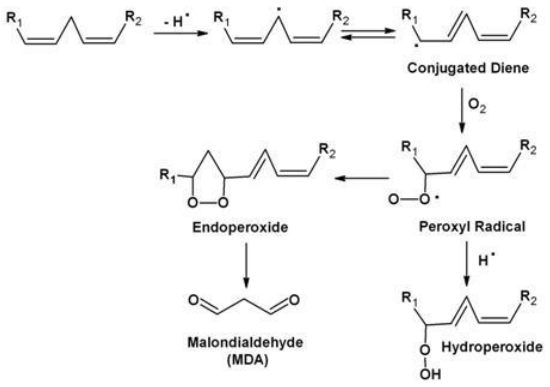


Fig. 4. Malondialdehyde (MDA) formation from lipid peroxidation

MDA serves as a convenient index for determining the extent of peroxidation reactions both in vitro and in vivo. In the assay system, MDA reacts with TBA to form thiobarbituric acid reactive substances (TBARS). The extent of lipid peroxidation was assayed by estimating the pink chromogen (TBARS) at 535 nm. Moreover, TBARS can be detected flourimentrically by excitation at 515 nm and emission at 553 nm [9].

In the assay we followed, ferrous sulfate stimulated lipid peroxidation in the rat brain homogenate resulting in the formation of MDA. Further, the MDA reacts with TBA to form a pink chromogen with absorption maximum at 535nm. In the presence of antioxidants, formation of this chromogen is reduced, which is expressed as antioxidant activity.

Procedure: One Wistar rat was sacrificed by cervical dislocation and the whole brain was dissected out and placed in ice cold 0.154 M potassium chloride immediately. The brain was blotted dry, weighed and added to nine times its weight of 0.154 M potassium chloride to prepare 10% homogenate. The tissue was then homogenized carefully in a homogeniser tube placed in an ice bucket to form a smooth homogenate without frothing.

The homogenate thus obtained was immediately centrifuged at 10000 RPM for 10 minutes at 4℃. The supernatant was used for the subsequent steps of the study. To 0.5 ml of rat brain homogenate, 1.0 ml solution of test compounds was added to yield a final concentration of 10 to 400 µM. Lipid peroxidation was stimulated by adding 0.5 ml of 400 µM ferrous sulphate (final concentration 100 µM). The reaction was stopped after 30 min by addition of 2 ml ice-cold TCA-TBA-HCl reagent [containing thiobarbituric acid (0.375% w/v) + trichloroaceticacid (15% w/v) + hydrochloric acid (0.25 N)]. The test tubes were heated in a water bath at 80 ℃ for 15 min and then further centrifuged at 10000 RPM for 10 min. The absorbance of the supernatant was measured at 535nm. Simultaneously, a blank absorbance was determined without the compounds. The % inhibition and IC_{50} were calculated as explained in DPPH assay method.

III. Result and Discussion

The results of antioxidant values expressed as IC_{50} with different antioxidant markers used are shown in Table 1. Out of 10 compounds tested using DPPH• scavenging method, JVF3 showed IC_{50} at 61.4 µM, when compared to that of the standard ascorbic acid at 54.08 µM. However, JVC1-JVC5 did not show any significant activity.

Further, from the antioxidant studies carried out using ABTS• scavenging assay for the 10 test compounds, JVC1, JVC3, JVC4, JVC5, and JVF2 showed IC_{50} values at 85.3, 53.76, 50.34, 83.15 and 89.12 µM respectively when compared to that of the standard ascorbic acid at 91.21 µM. However, JVF1 and JVF3 showed comparable IC_{50} value with that of the standard as shown in the Table I.

The antioxidant studies by NO scavenging method showed the IC_{50} values of all the test compounds and were greater than 300 µM except for JVC3 which showed its IC_{50} at 250.51 µM when compared to standard ascorbic acid at 89.33 µM.

Antioxidant studies by Lipid peroxidation method was undertaken for all 10 test compounds, JVC2 showed maximum inhibition of lipid peroxidation with IC_{50} value of 33.64 µM and JVF3 had IC_{50} at 358.47 µM when compared to that of the standard quercetin with IC_{50} at 320.36 µM.

TABLE I: Comparison of the IC_{50} of test Compounds Against Various Free-Radicals

Compounds	DPPH• Scavenging(µM)	ABTS•⁻ Scavenging(µM)	NO Scavenging Activity(µM)	Inhibition of Lipid Peroxidation(µM)
JVC1	NS	85.3	736.25	530.26
JVC2	NS	447.5	790.95	330.64
JVC3	NS	53.76	250.51	457.52
JVC4	NS	50.34	335.58	NS
JVC5	NS	83.14	315.93	NS
JVF1	159.07	109.89	305.71	NS
JVF2	123.28	89.12	NS	408.78
JVF3	61.4	100.5	639.94	358.47
JVF4	457.1	186.3	783.19	NS
JVF5	143.4	56.68	618.13	425.79
Standard	* 54.08	*91.21	* 89.33	#320.36

NS – Not Significant, *Ascorbic acid, #Quercetin

IV. Conclusion

The anti oxidant properties of the novel synthesized test compounds of chalcones and flavones when tested using 4 different anti oxidant evaluating methods it was found that, the ABTS method gave better antioxidant activity for both flavones and chalcones whereas, the DPPH• method gave antioxidant profile only for flavones. However, all the four evaluating *in vitro* methods showed that the test compounds such as JVC1, JVC2, JVC3, TVC4, JVC5, JVF2 and JVF3 had the maximum antioxidant properties. These methods have given us insight and the antioxidant profiles on few of the test compounds tested which will further provide scope for screening other related activities such as antimicrobial, anti-inflammatory and anticancer activities.

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