UDK 577

CORRELATION OF ANTIOXIDANT ACTIVITIES WITH THEORETICAL STUDIES FOR HESPERETIN SCHIFF BASES

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Schiff bases are a class of compounds with unique biological, analytical and industrial properties. The active pharmacophore (-N=CH-) of Schiff bases plays a major role in these significant biological activities. However, the attached neighbouring groups may also affect the activity.

Hesperetin (5,7,3'-trihydroxyl-4'-methoxyl-flavanone) is a kind of flavonoid which occurs ubiquitously in plants, fruits, flowers and foods of plant origin. Hesperetin has

multiple biological and pharmacological activities, including inhibition of cancer development, effects on the blood-brain barrier, signal transduction pathways, etc. However, antioxidant activity of hesperetin is insignificant, therefore three hesperetin hydrazones (HTSC, HIN and HHSB) (Fig. 1) were synthesized in order to increase the biological properties including antioxidant activity.

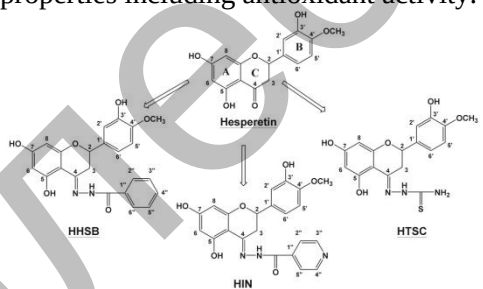

Fig. 1. The studied compounds: hesperetin, Schiff bases (HHSB, HIN, HTSC)

Free radical scavenging capacity of (poly)phenols is generally attributed to the hydrogen atom lability of the OH groups attached to aromatic rings (A-OH). However in some other antioxidants such as Schiff bases, NH and SH groups may provide labile hydrogen [1]. The formal H-atom abstraction from standard (poly)phenols described by:

$$A - OH + R^{\bullet} \rightarrow AO^{\bullet} + R - H$$

is known to involve complex processes. It has been recognized that this reaction proceeds via at least three different mechanisms: single-step hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET). These mechanisms may co-exist, and they depend on solvent properties and radical characteristics. The net result from all mechanisms is the same, i.e., as given in reaction.

DPPH$^{\bullet}$ assay is routinely practised for assessment of free radical scavenging potential of an antioxidant molecule and considered as one of the standard and easy colorimetric methods for the

evaluation of antioxidant properties of the compounds. DPPH$^{\bullet}$ is a stable radical in solution and appears purple colour absorbing at 515-520 nm in different solvent systems. In contact with antioxidant molecule, the radical DPPH$^{\bullet}$ reduces to $DPPH_2$ and the purple colour changes to yellow. The colour is monitored by spectrophotometrically and utilised for determination of parameters for antioxidant properties. The original DPPH$^{\bullet}$ assay procedure has beed adopted in different labs but with modifications for convenience. However, the most of the studies are based on fixed reaction time ranging from 20-30 min instead of total reaction time that is actually required to attain steady state to complete this redox reaction. Thus, the DPPH$^{\bullet}$ assay appears simple in nature but due to its stable nitrogen radical and, many antioxidants might react with different kinetics or might not react at all.

The aim of the present work was to ascertain correlations between the experimental radical scavenging activity of hesperetin Schiff bases by using DPPH$^{\bullet}$ assay and the geometry optimizations of parent molecules, radicals and radical cations were using PM3/DFT(B3LYP) method with the use of 6-31G(d,p) basis set. In the presented study the two methods in methanol medium, (a) steady state reaction time and (b) fixed reaction time, to simultaneously estimate kinetics and effective concentration of DPPH$^{\bullet}$ scavenging by a number of antioxidants were performed. The comparison of these two methods in this study revealed expected noticeable differences. The biological measurements on mitochondrial cells were also performed.

The reaction between DPPH$^{\bullet}$ and antioxidant is basically a kinetic driven process. Kinetic spectra were performed for individual compounds till steady state saturation was attained. The steady state analysis were performed in an excess of DPPH$^{\bullet}$ radical in order to exhaust the H-donating capacity of (poly)phenols as it is performed in fixed reaction time method. Fig. 2 presents the kinetic scans of hesperetin and its derivatives.

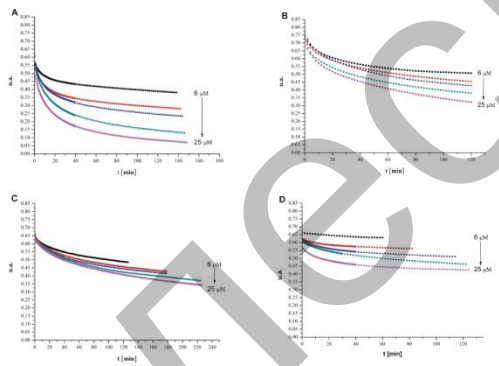

Fig. 2. The dependence of absorbance at 516 nm at a different concentration of antioxidant in the reaction mixture of DPPH$^{\bullet}$ (56 μM)
in MeOH:DMSO medium (97.5%:2.5% (v/v))

None of the studied compounds did not reach the completion of the reaction in 30 min. This time duration (20-30 min) is usually considered by most authors in fixed reaction time mode for estimation of antioxidant properties. All tested compounds were considered under slow kinetics.

EC_{50} values at fix time for HTSC, HIN, HHSB and hesperetin were 25.66±2.03, 29.89±2.77, 57.46±3.65 and 32.28±4.31 μM respectively. While, at steady state, EC_{50} for HTSC, HIN, HHSB and hesperetin were 19.03±2.66, 59.63±10.86, 228.41±11.22 and 68.99±15.72 μM respectively.

The DFT(B3LYP)/6-31G(d,p) calculations of reaction enthalpies related to several possible mechanisms for free radical scavenging by hesperetin and its derivatives (HTSC, HIN, HHSB): HAT, SET-PT and SPLET indicated that the compounds studied can react with DPPH$^{\bullet}$ via at least two mechanisms: HAT and SPLET. SPLET mechanism is more preferable in polar solvents that suport ionization, while in nonpolar solvents and in the gas phase the HAT mechanism is thermodynamically more preferable. Our calculations showed that the HAT and SPLET mechanism are both associated with the most stable 3'-O$^{\bullet}$ radicals. These radicals may be formed by single hydrogen atom abstraction from 3'-OH group (HAT) or they may be formed as a result of charge redistribution and resonance stabilization of the 7-OH phenoxide anions in methanol (SPLET). It is assumed that in DMSO the additional reaction pathway involving radicals formed by the N-H cleavage of the keto form of HIN, HHSB and HTSC may also contribute to the complex mechanism of DPPH$^{\bullet}$ radicals scavenging by the compounds studied.

HIN and HTSC prevented the development of oxidative stress and protected mitochondrial membranes from oxidative damage more effectively than HHSB and hesperetin, significantly reducing levels of TBARS and partially restoring the content of GSH.

Table – Reduced gluthatione (GSH), lipid peroxidation products (TBARS) levels in rat liver cell mitochondria.

Parameters	Compounds [average ± SD]						
	Control	tBHP	Hesp	HHSB	HIN	HTSC	Control+HIN
GSH [nmol/mg protein]	14.04±0.68	3.57±0.214*	3.47±0.19*	3.46±0.21*	4.20±0.12*#	3.83±0.18*	13.19±0.19
TBARS [nmol/mg protein]	0.028±0.003	0.084±0.004*	0.063±0.009*	0.062±0.010*	0.043±0.007*#	0.050±0.005*#	0.022±0.002

The list of references

1. Anouar, E. H., Raweh, S., Bayach, I., Taha, M., Baharudin, M. S., Di Meo, F., Hazizul Hasan, M., Adam, A., Hadiani, Ismail N., Weber, J.-F. F., Trouillas, P., Antioxidant properties of phenolic Schiff bases: structure–activityrelationship and mechanism of action. J. Comput. Aided Mol. Des., 2013. – V. 27. – P. 951-964.