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IN VITRO ANTIOXIDANT ACTIVITY OF THREE NOVEL HYDROXY FLAVONES

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ABSTRACT To investigate in vitro antioxidant and anti-inflammatory activities of 3 novel hydroxy Flavones (7 hydroxy flavone,7,3',4' tri hydroxy flavone and 7,8,3' tri hydroxy flavone). Methods used two methods DPPH scavenging activity and Nitric oxide scavenging activity both methods are most popular and both methods various concentrations (20,30,60,120,240µg /ml) dissolved in ethanol was prepared compared with ascorbic acid as a standard. All the flavonoids have offered significant antioxidant activity with log IC50 values of NO is 1.605, 1.706, 1.447 and 1.561/mL and DPPH is1.539, 1.65, 1.593 and 1.581respectively. The findings suggest that the 7 hydroxy flavones could be used as a source of natural antioxidants.

KEYWORDS : Flavones, Anti oxidant, DPPH, Nitrous oxide

INTRODUCTION:

Free radicals are produced by an important chemical process known as oxidation that in turn initiates chain reactions to damage the cells and originate oxidative stress. This process leads to the development of different disorders like Alzheimer's disease [2, 3], Parkinson's disease [4], the pathologies caused by diabetes[5,6], rheumatoid arthritis[7], and neurodegeneration in motor neurons[8].The antioxidants have been used specifically to stop the chain reactions by the removal of free radical intermediates and slow down other oxidation processes by oxidizing themselves and acting as reducing agents like polyphenols or ascorbic acid[9]. The small molecular weight antioxidants that are naturally found in skin include compounds synthesized by skin cells such as glutathione and ubiquinol as well as those assimilated from plant sources in the diet such as vitamin E, vitamin C, and retinoids. They function synergistically in some cases but also operate as part of independently-regulated systems to address challenges to the redox status of the cell or the tissue.

Since many relatively simple bioassays are readily available for assessment of antioxidant activity, a large number of plant compounds and extracts have been shown to act as antioxidants *in vitro*, and many have also demonstrated the capacity to reduce oxidative stress in skin in vivo as well as skin cells in vitro. This activity may well be expected to protect aging cells; however, as will be seen in the examples below, many antioxidant compounds display additional biological activities such as inhibition of inflammation or modulation of gene transcription that may not be exclusively related to antioxidant activity, and this uncertainty can thwart simple attempts to associate antioxidant activity in and of itself with a predictable clinical benefit.Naturally, a complex system of enzymes and antioxidant metabolites work in coordination to stop oxidative damage to the cellular components like proteins, DNA, and lipids by preventing the formation or removal of these reactive species before damaging the important components of the cells[10,11].

MATERIALAND METHODS:

Collection and Identification of flavonoids

This 7hydroxy flavone and its derivatives were selected for the study and these were obtained from Sigma Aldrich, the test compounds were prepared as a fine in power.

Drugs and chemicals:

India Diagnostics assay kits (Cayman., USA) were used for interleukin-1β, tumour necrosis factor, and cyclooxygenase assay. Celecoxib (Manufactured by Ipca Laboratories Pvt. Ltd. Mumbai, India) Aspirin(Wellona Pharma Private Limited Nana Varachha, Surat, Gujarat) Ascorbic acid (Facmed Pharmaceuticals PVT. LTD.New Delhi, India).

DPPH scavenging activity[12]

Stock solution of DPPH was prepared by dissolving 25mg of DPPH in 100ml of ethanol. 2ml of reaction mixture containing 1.9 ml of DPPH and 0.1 ml of different 7-hydroxy flavones (7 hydroxy flavone, 7,3',4'tri hydroxy flavone and 7,3,4'tri hydroxy flavone) of various concentrations (20,30,60,120,240µg /ml) dissolved in ethanol was prepared. Control without test compound was prepared in an identical manner. The reaction was allowed to be complete in the dark for about 20 mins. Then the absorbance of the test mixture was read at 517nm. The activity was compared with Vit E (1- 200µg/ml), which was used as a standard antioxidant. The percentage DPPH inhibition was calculated from the following formula % of DPPH inhibition = OD of control – OD of test / OD of control x 100. (Shimada et al., 1992)

Nitric oxide scavenging activity[13]

An aqueous solution of sodium nitroprusside spontaneously generates nitric oxide (NO) at physiological pH, which interacts with oxygen to produce nitrate ions which were measured calorimetrically. 3 ml of reaction mixture containing 2ml of sodium nitroprusside in phosphate buffered saline and 1 ml of various concentrations (20,30,60,120,240µg /ml) of the 7-hydroxy flavones dissolved in ethanol were incubated at 37° C for 4 hours. Control without test compound was kept in an identical manner. After incubation, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and test compounds. A standard antioxidant Vit E (1-200µg/ml) was used for comparison. The formula used for calculation was % nitric oxide inhibition = OD of control- OD of test / OD of control x 100. (Green et al., 1982)

RESULTS AND DISCUSSION

The in vitro enzyme inhibition potentials capacity of the flavones derivatives was determined and IC50 values are given in Table 1 and 2. It is evident that flavones (7,3',4' and 7,8,3') showed good activity in comparison with another flavone derivative (7-HF). These results suggest that a change in the position or additional moiety may increase or decrease the potency of individual flavones. The antioxidant capacity of the flavones derivatives was estimated with DPPH andNitric oxide scavengingsystems and results are shown in Tables 1 and 2. Concentration-dependent DPPH scavenging effects of flavones derivatives are given. Amtheall flavones, the maximum concentration dependent DPPH scavenging effects of 76.44 (< 0.001) at 240 g/mL were observed by 7,3',4'THF while mild effects of 71.08 (< 0.05) were produced by 7,8,3'THF ata high concentration of 240 g/mL and are comparable with standard ascorbic acid. A similar type of findings was observed using Nitric oxide scavenging system and is given in Table 1. It is evident from the results (Table 1) that nitrogen-containing flavone derivative (7,8,3') showed less activity in comparison with halogenated one (7,3',4'THF). thus suggesting that positioning of halogens may increase or decrease the antioxidant effects asevident from the findings. These findings may help future research and open a new window for the synthesis of potent antioxidants for the treatment ofa wide range of diseases associated with ROS. In normal situations, the free radicals as by-products are constantly formed by the body's cells from the cellular redox process using oxygen, an essential element of life [14]. These are generally called reactive oxygen species (ROS) and have a special attraction for proteins, carbohydrates, lipids, and nucleic acids [15]. It has been reported that ROS can be both beneficial and harmful based on the concentration and environment in the biological systems[16-17]. The beneficial effects involve defence against infections, the function of cellular signalling, and gene expression. On the other hand, ROS can

mediate injury to cell structures and is often referred to as an "oxidative stress" [18]. The harmful effects of ROS are counteracted by antioxidants, some of which are enzymes present in the body[19]. Natural antioxidants like -tocopherol, carotenoids, ascorbic acid, flavonoids, and other phenolic compounds might also play a significant role as physiological and dietary antioxidants[20,21]. The natural antioxidants are known to possess extensive biological effects that include anticancer, antiviral, antibacterial, anti-inflammatory, antithrombotic, and vasodilatory activities[22]. One method of estimating the antioxidant activity is based on the use of a stable free radical known as DPPH [23-25] and the electron donation ability of antioxidants can be determined by DPPH purple-coloured solution bleaching[26]. This method is based on scavenging of DPPH through the addition of an antioxidant that decolorizes the DPPH solution and the degree of decolourization is proportional to the free radical scavenging activity indicating its potency [27]

Table 1 Anti oxidant activity of flavones

Nitric oxide scavenging activity				
Concentration in µM	**Ascorbic Acid**	**7-HF**	**7,3',8THF**	**7,3',4'THF**
20	27.58	31.14	37.44	30.28
30	37.54	37.08	45.1 2	37.34
60	48.18	50.72	56.54	44.08
120	51.14	57.66	62.46	54.42
240	59.54	63.36	75.28	58.42
IC50	36.4	28.01	50.87	40.26
LoglC50	1.561	1.447	1.706	1.605

DPPH scavenging activity				
Concentration in µM	**Ascorbic Acid**	**7-HF**	**7,3',8THF**	**7,3',4'THF**
20	28.76	27. 16	34.12	27.64
30	37.82	36.44	43.38	37.28
60	49.36	51.34	56.62	52.44
120	61.74	67.16	64.78	64.34
240	72.28	72.74	76.44	71.08
IC50	34.6	44.62	39.21	38.06
LoglC50	1.539	1.65	1.593	1.581

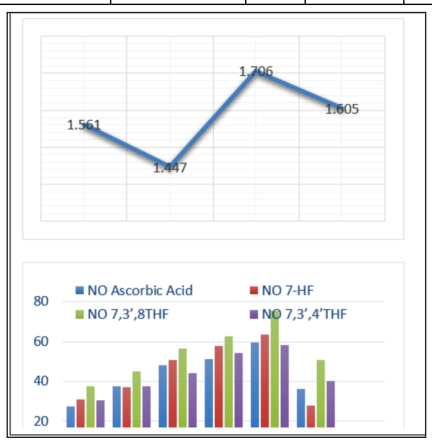

Figure 1 Nirous oxide scavenging activity

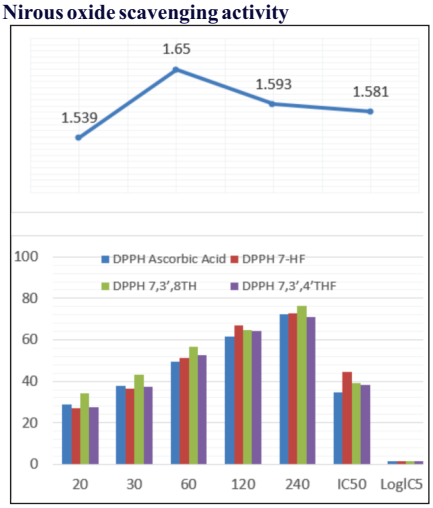

Figure 2 DPPH Antioxidant activity

CONCLUSION:

In conclusion, the present study confirms the enzyme inhibition and antioxidant activities of flavone derivatives. These findings will open a new channel to synthesize halogenated flavones and explore the development of synthetic flavones derivatives for the treatment of a wide range of diseases associated with ROS.

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