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Designing and Synthesis of Flavonoids Derivatives and Screening of their Antioxidant Activity

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ABSTRACT

The flavonoids present in red wine were responsible this low cardiovascular mortality rate. Epidemiologic studies further suggest that dietary flavonoids are useful to control and protect the CHD. The flavonoids are yellow color substance (pigments) and the name given on the basis of Latin term Flavus which means yellow color. Flavonoids are derivatives of benzo-pyrone. Banzopyrone is a group of heterocyclic aromatic oxygen containing compounds. Finely powdered zinc chloride (8.25) was dissolved in glacial acetic acid (18ml) by heating on sand bath then dry resorcinol (appx.5.5 gm) was added with continuous stirring to the mixture at 140^{0}C. Antioxidant Screening by hydrogen peroxide scavenging assays. Hydrogen peroxide solution (40 mini moles) was prepared with standard phosphate buffer of pH 7.4. Different concentration of the compound stock solution and 4ml distilled water was added to 0.6 ml of hydrogen peroxide solution. UV absorbance was determined at the wavelength of 230 nm after 10 min with a blank solution containing phosphate buffer without H_2O_2. Take 4 ml different concentration of sample solution and 1ml sodium nitroprusside solution, added and incubated for 2.5 hrs at 37^{0}C. After incubation baseline was taken with methanol and 1ml sodium nitroprusside solution as blank solution. Griess reagent and methanol was added immediately before recording of readings. The readings were recorded at 546nm wavelenth. In the series of synthesized and evaluated compounds of Flavanoid electron withdrawing group at position four shows good activity. 2,3-dihydroflavan-3-ol derivatives showed lower activity than that of 3-hydroxyflavone derivatives. The 4-oxo (keto double bond at position 4 of the C ring), especially in association with the J2-J3 double bond, increases scavenger activity by delocalizing electrons, 3-hydroxy group on the C ring generates an extremely active scavenger; the combination of J2-J3 double bond,3-hydroxy group and 4-oxo group appears to be the best combination for potent antioxidant activity.

Keywords: Flavonoids, Antioxidant activity, Hydrogen peroxide scavenging, free radicals

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INTRODUCTION:

The research based on flavonoids show on the basis of the discovery of the French paradox for example the low cardiovascular mortality rate identify in Mediterranean people in association with red wine intake and a high saturated fat consumption. The flavonoids present in red wine were responsible this low cardiovascular mortality rate. Epidemiologic studies further suggest that dietary flavonoids are useful to control and protect the CHD. However, information about the mechanisms of action of flavonoids was scant till 50 yrs. ago. In year nineteen thirty three some new compound was identify and isolate from oranges, which was supposed to be a member of a new class of vitamins, and was called as vitamin P. When it became clear that this particular compound was a flavonoid known as rutin, a numbers of pharmacological screening began in an attempt to isolate the various flavonoids. Since then numerous flavonoids were isolated and studied for their method by which flavonoids show their activity and extended further to synthetic expedition. The research has shown new diversified action of flavonoids. In-vitro studies also showed that flavonoids possess antioxidant activity.

The study of flavonoid chemistry has observed, like that they are most useful natural compounds obtain from natural sources in the search of some newer compounds and show useful pharmacological properties. The flavonoids are yellow color substance (pigments) and the name given on the basis of Latin term Flavu**s** which means yellow color. Flavonoids are derivatives of benzo-pyrone. Banzopyrone is a group of heterocyclic aromatic oxygen containing compounds. Flavonoids are chromene having basic heterocyclic ring system of benzo-4-pyranone.

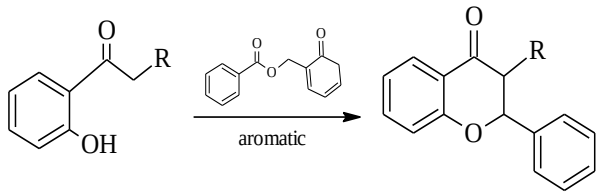
Table 1: Main classes of flavonoids, their individual compounds and food sources

Group	Compound	Food sources
Flavones	Apigenin	Apple skins
	Chrysin	Berries
	Luteolin	Celery
Flavonol	Kaempferol	Broccoli
	Myricetin	Fruit peels
	Rutin	Cranberries
	Sibelin	Grapes
	Quercetin	Lettuce
		Olives
		Onions
		Parsley
Flavanones	Fisetin	Citrus fruit
	Hesperetin	Citrus peel
	Narigin	
	Naringenin	
	Taxifolin	
Flavanol	Catechin	Red wine
	Epicatechin	Tea
	Epigallocatechin gallate	

EXPERIMENTAL WORK:

Flavones can be synthesized in various ways. Robinson's synthesis, Auwer's synthesis, Baker –venkataraman synthesis etc are route for synthesis.

Scheme



O-hydroxyarylketone flavone

In Allan-Robinson reaction is the chemical reaction of o-hydroxyaryl ketones with aromatic anhydrides to form flavones.

2, 4-dihydroxyacetophenone

Finely powdered zinc chloride (8.25) was dissolved in glacial acetic acid (18ml) by heating on sand bath then dry resorcinol (appx.5.5 gm) was added with continuous stirring to the mixture at 1400C. The solution was heated until the solution just begins boil and kept it for 20 min at 150^{0}C temperatures. Dilute HCl was added to mixture and cooled at the temperature of 5^{0}C then filter & washed with dil. Hydrochloric acid (1:3) and crystallized from hot water containing a little HCl.

Table 2: Synthetic Work Up

Hydroxy acetophenone	Aromatic aldehyde	Chalcone	Flavonol	2,3-dihydroflavan- 3-ol
2-hydroxyacetophenone	Salicylaldehyde	J1	V1	R1
2-hydroxyacetophenone	4-Isopropylbenzaldehyde	J2	V2	R2
2-hydroxyacetophenone	4-Methylbenzaldehyde	J3	V3	R3
2,4-dihydroxyacetophenone	Salicylaldehyde	J4	V4	R4
2,4-diydroxyacetophenone	4-Isopropylbenzaldehyde	J5	V5	R5
2,4-diydroxyacetophenone	4-Methylbenzaldehyde	J6	V6	R6

Chalcone synthesis

Procedure:

A solution of Appx. 2.2 g. of NaOH in 196 ml. of water and 122 ml of 95 % alcohol were placed into closed vessel. The mixture was placed in ice bath and stirred continuously. 0.42 moles of Hydroxy acetophenone was poured in above mixture while stirring. subsequently 0.42 moles benzaldehyde derivative was added. The temperature of mixture was maintained between 20-30^{0}C. Mixture was stirred(2-3 hours) till it became thick. Mixture was kept overnight in ice chest. The mixture became thick paste composed of small shot-like grains suspended in an almost colorless liquid. It was cooled in a freezing mixture, filtered and washed with water until the washings are neutral to litmus, and finally washed with 20 ml of 95 per cent alcohol, which was previously been cooled to 0°.

Cyclization of chalcone to flavonol

Procedure:

To a suspension of chalcone (0.01mole) in ethanol (85ml) was added 20% aqueous sodium hydroxide (10ml) with stirring , followed by careful addition of 20% hydrogen peroxide (18ml)over a period of half hr.The reaction mixture was stirred for 2-3 hrs. at 280^{0}c and poured onto crushed ice containing 5N HCL .The precipitate was filtered, washed, dried and crystallized from chloroform: methanol [9:1] .

Pharmacological Screening

Antioxidant Screening by Hydrogen peroxide scavenging assays:

Hydrogen peroxide solution (40 mini moles) was prepared with standard phosphate buffer of pH 7.4. Different concentration of the compound stock solution and 4ml distilled water was added to 0.6 ml of hydrogen peroxide solution. UV absorbance was determined at the wavelength of 230 nm after 10 min with a blank solution containing phosphate buffer without H_2O_2. The percentage scavenging activity at different concentrations of the different derivatives compared with the standard of vitamin C.

Nitric oxide scavenging assay

The Griess reagent was freshly prepared at the time of checking UV absorbance by following procedure.

Procedure:

Take 4 ml different concentration of sample solution and 1ml sodium nitroprusside solution, added and incubated for 2.5 hrs at 37^0C. After incubation baseline was taken with methanol and 1ml sodium nitroprusside solution as blank solution. Griess reagent and methanol was added immediately before recording of readings. The readings were recorded at 546nm wavelenth.[1]

% Inhibition = [Blank – Test]/Blank ×100

Table 3: Preparation of Griess Reagent

Sr. No.	Reagent	Preparation
1.	Griess reagent	0.665ml H_3PO_4 + 0.25g sulfanillic acid + 0.025g α- naphthyl-ethylenediaminedihydrochloride in 25ml distilled water
2.	Sodium nitroprusside solution (10mM)	0.065g in 25ml phosphate buffer (pH-7.4)
3.	Phosphate buffer (pH-7.4) KH_2PO_4 (0.2M) NaOH (0.2M)	2.718g in 100ml Distilled water 0.8g NaOH in 100ml Distilled water (50 ml 0.2M KH2PO4 + 39.1 ml 0.2M NaOH)

RESULT AND DISCUSSION:

Antioxidant Screening:

Table 4: Hydrogen Peroxide Scavenging Activity

Concentration	50 µg/ml	100 µg/ml	200 µg/ml
J1	40.2	60.40	75.27
J2	35.8	53.63	83.27
J3	19.41	21.53	30.00
J4	35.5	50.80	88.28
J5	30.45	40.52	84.24
J6	35.51	50.80	88.48
V1	44.23	65.75	73.43
V2	50.87	72.99	90.67
V3	48.02	70.34	89.80
V4	19.09	21.93	66.00
V5	32.90	46.15	80.70
V6	49.02	70.34	89.80
R1	27.72	32.48	68.36
R2	26.33	30.82	66.32
R3	17.52	30.47	65.12
R4	19.46	22.03	49.58
R5	21.87	26.83	72.00
R6	17.52	28.47	66.32
Ascorbic acid	49.41	61.32	79.96

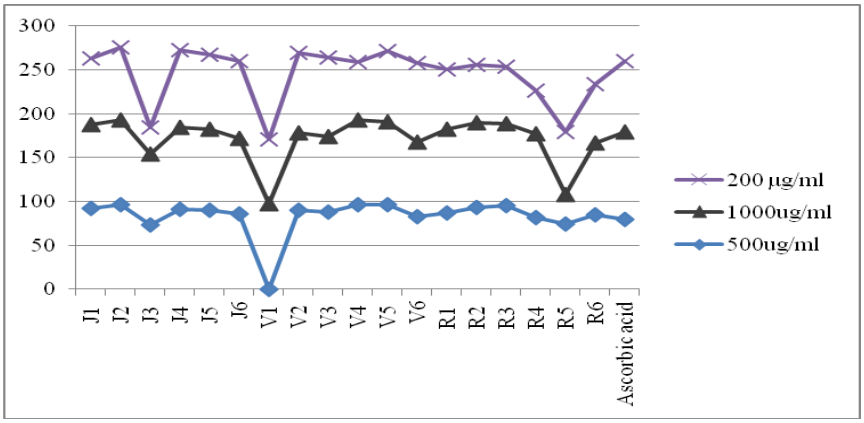


Figure 1 Hydrogen Peroxide Scavenging Activity

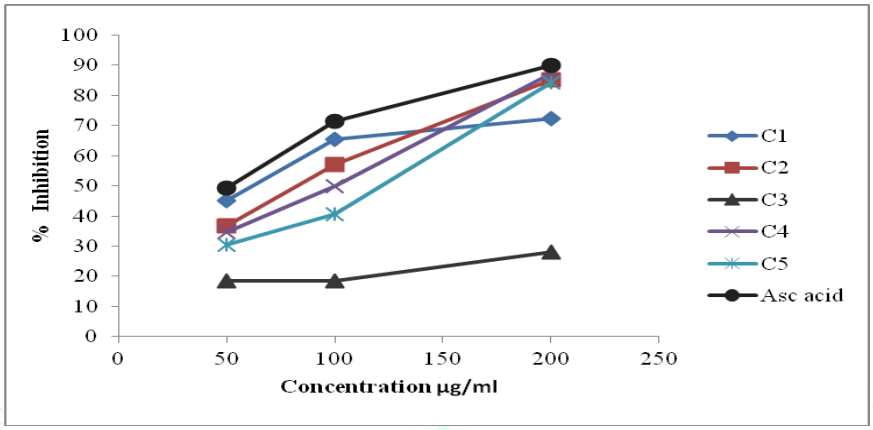


Figure 2 % Inhibition of chalcone derivatives

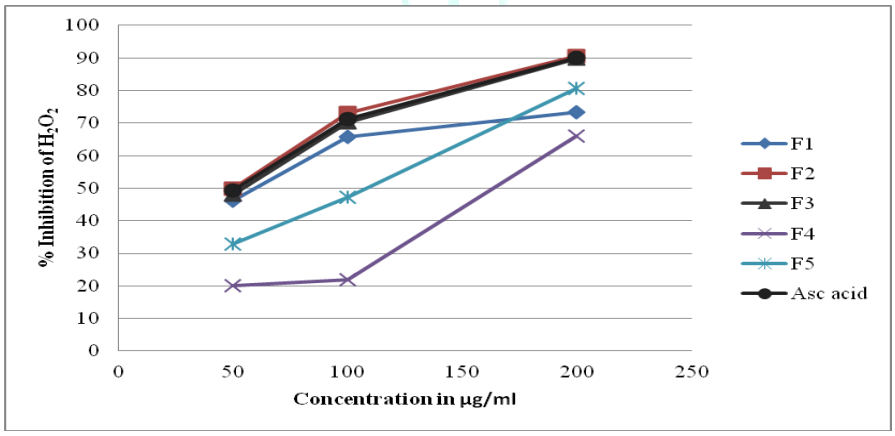


Figure 3 % Inhibition of 3-hydroxy flavone derivatives

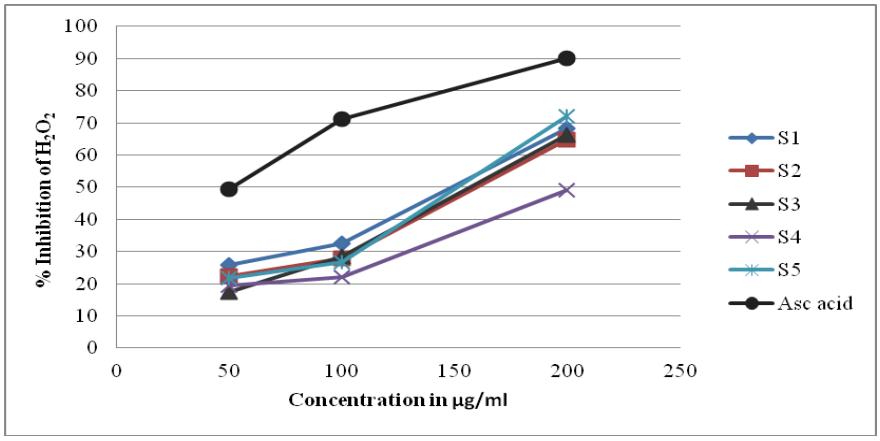


Figure 4 % Inhibition of 2,3-hydroflavan-3-ol derivative

Table 5: Nitric Oxide Scavenging Activity

compound	200ug/ml	500ug/ml	1000ug/ml
J1	79.61	92.72	95.22
J2	78.93	91.56	95.97
J3	65.96	73.08	81.01
J4	79.83	91.56	93.18
J5	77.96	89.78	92.94
J6	76.4	85.85	87.24
V1	74.79	97.71	97.42
V2	77.95	84.87	88.77
V3	77.44	87.85	89.24
V4	80.04	96.36	96.14
V5	79.67	93.12	95.02
V6	77.41	82.85	85.24
R1	71.14	87.09	95.17
R2	79.11	93.81	95.62
R3	78.48	91.08	93.57
R4	74.91	81.52	95.35
R5	71.11	74.43	76.34
R6	77.42	84.85	82.24
Ascorbic acid	74.02	79.61	99.98

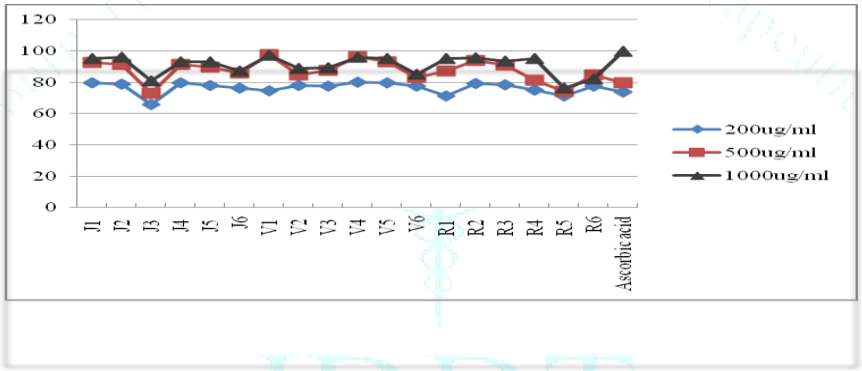


Figure 5 Nitric Oxide Scavenging Activity

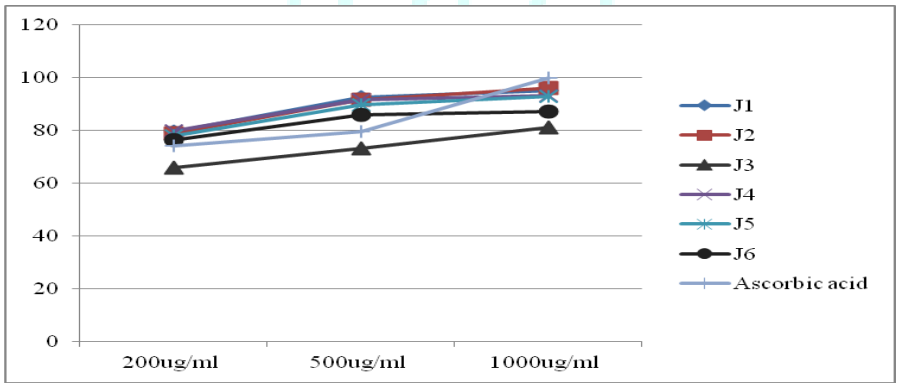


Figure 6 Nitric Oxide inhibition of chalcone derivatives

CONCLUSION:

The results of antioxidant screening showed that flavone derivatives have better antioxidant activity than their corresponding Chalcones. In the series of synthesized and evaluated compounds of Flavanoid electron withdrawing group at position four shows good activity. 2,3-dihydroflavan-3-ol derivatives showed lower activity than that of 3- hydroxyflavone derivatives. The 4-oxo (keto double bond at position 4 of the C ring), especially in association with the J2-J3 double bond, increases scavenger activity by delocalizing electrons, 3-hydroxy group on the C ring generates an extremely active scavenger; the combination of J2-J3 double bond,3-hydroxy group and 4-oxo group appears to be the best combination for potent antioxidant activity.

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