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Evaluation of Antioxidant Activity of Chalcones and Flavonoids

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1. ABSTRACT: A series of 2'-hydroxychalcones and 3-hydroxyflavones were evaluated for in vitro antioxidant activity on four different models namely Diphenyl Picryl Hydrazine, Nitric Oxide, Hydrogen peroxide, and Phenyl hydrazine induced hemolysis of erythrocytes. The studies indicate that amongst evaluated 2'-hydroxychalcones, 2-Bromochalcone and 2-Chlorochalcones showed significant activity and amongst different 3-hydroxyflavone evaluated 3'-hydroxyflavones, 4'-methoxy and 4'-Chloroflavone showed significant activity.
KEYWORDS: Chalcones, Flavonoids, Antioxidant Activity.

2. INTRODUCTION AND EXPERIMENTAL

Antioxidants are compounds capable of preventing and even counteracting the damage caused in human tissue by the normal effects of physiological oxidation. A lot of research has shown that antioxidants can play a role in preventing the development of some chronic diseases. In addition to those mentioned previously, diseases such as atherosclerosis, emphysema, iron overload, malaria, muscular dystrophy, retinal degeneration, and rheumatoid arthritis are but a few examples where research has shown the likelihood of direct links and the possibility of positive dietary and perhaps even nutraceutical interventions.

Chalcones basic structure includes two aromatic ring bound by an α, β-unsaturated carbonyl group, a unique template associated with very diverse application[1] .Due to the presence of the reactive keto, vinylenic group, chalcones and their analogues have been reported to be antioxidant[2].Hydroxyl and phenyl substitutents are associated with antioxidant properties.

Flavones are group of aromatic oxygen-bearing heterocyclic pigments widely distributed among higher plants. They are prominent plant secondary metabolites that have been found in dietary components, including fruits, vegetables, olive oil, tea, and red wine Members of this class show wide variety of biological activities such as anticancer, antitumour , antiprotozoal and antioxidant[3].

A. Diphenyl Picryl Hydrazine (DPPH) Scavenging Activity:

Procedure: Antiradical activity of Compounds was performed by DPPH model. Stock solution of DPPH (1.3mg/ml) in methanol was prepared. Stock solution of DPPH 100µl was added in 3ml of methanol and absorbance was recorded at 516nm. The various concentrations of Compounds (25, 50, 75, 100, 125 µg/ml) were prepared. All sample solutions 1 ml each is diluted to 3 ml and 100µl of stock solution of DPPH was added then absorbances were recorded at 516nm and EC_{50} was calculated against methanol as a blank. [4, 5,10]

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

B. Nitric Oxide Scavenging Activity:

Reagents required:

Table:1. Preparation of reagents for activity.

Sr. No.	Reagent	Preparation
1.	GRIESS Reagent	0.665ml H_3PO_4 + 0.25g sulfanilamide + 0.025g α-naphthyl-ethylene dihydrochloride in 25ml distilled water
2.	Sodium Nitroprusside solution(10mM)	0.065g in 25ml phosphate buffer (pH-7.4)
3.	Phosphate buffer (pH-7.4) a) KH_2PO_4 (0.2M) b) NaOH (0.2M)	(Mix 50ml 0.2M KH_2PO_4 + 39.1ml 0.2M NaOH)

Method:

To each solution, 1ml Sodium Nitroprusside solution was added and incubated at 37^0 C for 2.5 hrs. After incubation baseline was taken with methanol and 1ml Sodium nitroprusside Solution was used as blank. GRIESS reagent and methanol was added immediately before recording of readings. Readings were recorded at 546nm. [6, 7]

% Inhibition = [Blank – Test]/Blank ×10

C: Hydrogen Peroxide Scavenging Activity:

Hydrogen peroxide scavenging activity was measured with titrimetric method of estimation.1ml of compounds concentration was mixed with 1ml of 0.1mM of H2O2 , 2 drops of 3% Ammonium molybate indicator ,10ml sulphuric acid and 7ml of 2M KI .The mixed solution was titrated with 5mM sodium thiosulphate until yellow color disappeared ,Ascorbic acid was used as positive control and percentage hydrogen scavenging was determined. [8,11]

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

D: Assay For Phenyl Hydrazine Induced Haemolysis of Erythrocytes

(Membrane Stabilization Study):

The erythrocyte suspension 20% PCV (packed cell volume) of human blood was prepared and assay was carried out according to the procedure described by Cazana et al. The method involves the incubation of mixture containing 1 ml of phenyl hydrazine hydrochloride (0.5 mM), different concentration of compounds and 0.1 ml of 20 % erythrocyte suspension and final volume made to 3.0 ml by phosphate buffer solution. The mixture was incubated at 37^0 C for 1 hour and then centrifuged at 1000 g for 10 min. The absorbance of supernatant was measured at 540 nm. Suitable blank was also carried out to nullify the effect of solvents and inherent haemolysis. α-tocopherol was used as a positive control. Percent inhibition was calculated using following formula. [3, 8.]

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

4. RESULTS AND DISCUSSION

A series of 3-hydroxyflavones and 2'-hydroxychalcones were found to possess antioxidant activity compared to that of standard [ascorbic acid and α-tocopherol] which was evaluated on four different methods namely.

1. DPPH [Diphenyl Picryl Hydrazine]
2. Hydrogen peroxide.
3. Nitric oxide scavenging model and
4. Phenyl hydrazine induced hemolysis of erythrocytes.

Generally Flavonoid has better antioxidant activity than their corresponding Chalcones.

Amongst series of evaluated 2'-hydroxychalcones, 1-[2'-hydroxyphenyl]-3-[2-chloropheyl] -2-propen-1-one, and 1-[2'-hydroxyphenyl]-3-[2-bromophenyl] -2-propen-1-one, showed good activity.

Amongst different, 3-hydroxyflavones 3-hydroxy-4'-methoxyflavone and 3-hydroxy-4'chloroflavone showed maximum activity compare to 3-hydroxy-3,'4.'5'-trimethoxyflavone, 3-hydroxy-2'-chloroflavone.

In the series of evaluated 3-hydroxyflavones flavonoid having electron withdrawing group at position four shows good activity.

Table.No.1

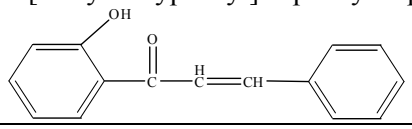
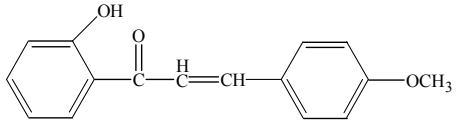
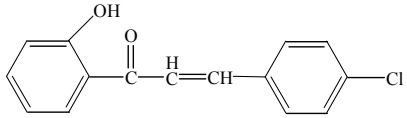
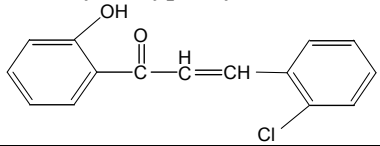
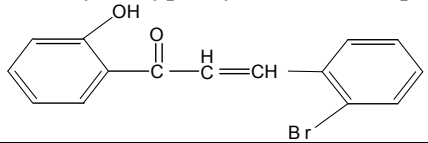
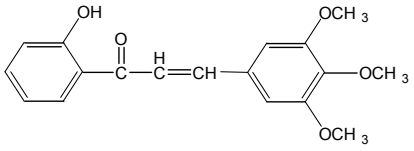
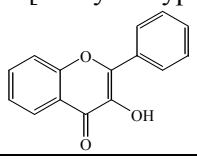
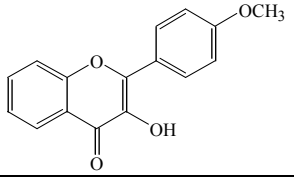
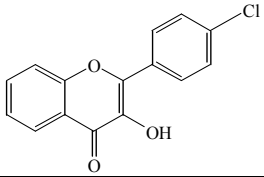
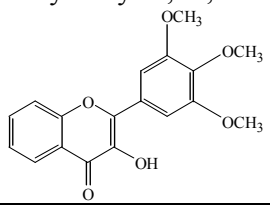
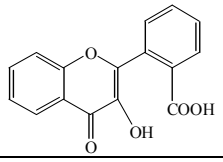
Compound code	2'-hydroxychalcones Series
A	1-[2'-hydroxyphenyl]-3-phenyl-2-propen-1-one 
B	1-[2'-hydroxyphenyl]-3-[4'-methoxyphenyl]-2-propen-1-one 
C	1-[2'-hydroxyphenyl]-3-[4-chlorophenyl]-2-propen-1-one 
D	1-[2'-hydroxyphenyl]-3-[2-chlorophenyl]-2-propen-1-one 
E	1-[2'-hydroxyphenyl]-3-[2-bromophenyl]-2-propen-1-one 
F	1-[2'-hydroxyphenyl]-3-[3, 4, 5-trimethoxyphenyl]-2-propen-1-one 

Table No.2

Compound code	3-hydroxyflavones Series
1	1-[2'-Hydroxyphenyl]-3-[2-Carboxyphenyl]-2-propen one 
2	3-Hydroxy-4'-Mehoxyflavone 

3	3-Hydroxy-4'-Chloroflavone 
4	3-Hydroxy-3', 4', 5'-Trimethoxyflavone 
5	3-Hydroxy-2'-chloroflavone 

Evaluation of Antioxidant activity:

Table No.3: Observation for antioxidant activity in terms of DPPH method.

Compound code	% Scavenging [mean ± SEM]					IC_{50} ug/ml
	25ug/ml	50ug/ml	75ug/ml	100ug/ml	125ug/ml	
A	12.61 ±06	22.31±0.026	25.41±0.098	36.97±0.15	47.32±0.026	137.67
B	18.06±0.01	28.56±0.06	30.91±0.05	52.75±0.12	60.20±0.14	96.38
C	21.21±0.12	31.37±0.09	38.22±0.24	43.44±0.15	56.53±0.16	107
D	12.39±0.05	25.60±0.025	37.24±0.16	56.52±0.052	63.70±0.01	88.41
E	8.83±0.098	14.95±0.026	45.00±0.023	56.24±0.05	65.01±0.11	86.70
F	17.26±0.25	29.56±0.13	40.40±0.16	51.50±0.09	57.47±0.12	96.24
1	14.10±0.13	32.12±0.25	41.51±0.12	52.97±0.01	62.29±0.11	98.30
2	35.00±0.12	58.23±0.01	65.31±0.18	75.00±.0.08	82.13±0.14	44.31
3	25.00±0.17	50.11±0.05	62.03±0.2	75.80±0.03	85.00±0.08	49.75
4	28.44±0.03	37.09±0.12	48.89±0.14	55.60±0.21	72.48±0.12	83.41
5	21.77±0.14	31.11±0.08	44.03±0.21	57.80±0.2	71.83±0.02	86.67
STD (Ascorbic acid)	22.28±0.12 5ug/ml	41.03±0.19 10ug/ml	52.06±0.2 15ug/ml	75.02±0.09 20ug/ml	96.10±0.18 25ug/ml	14.66

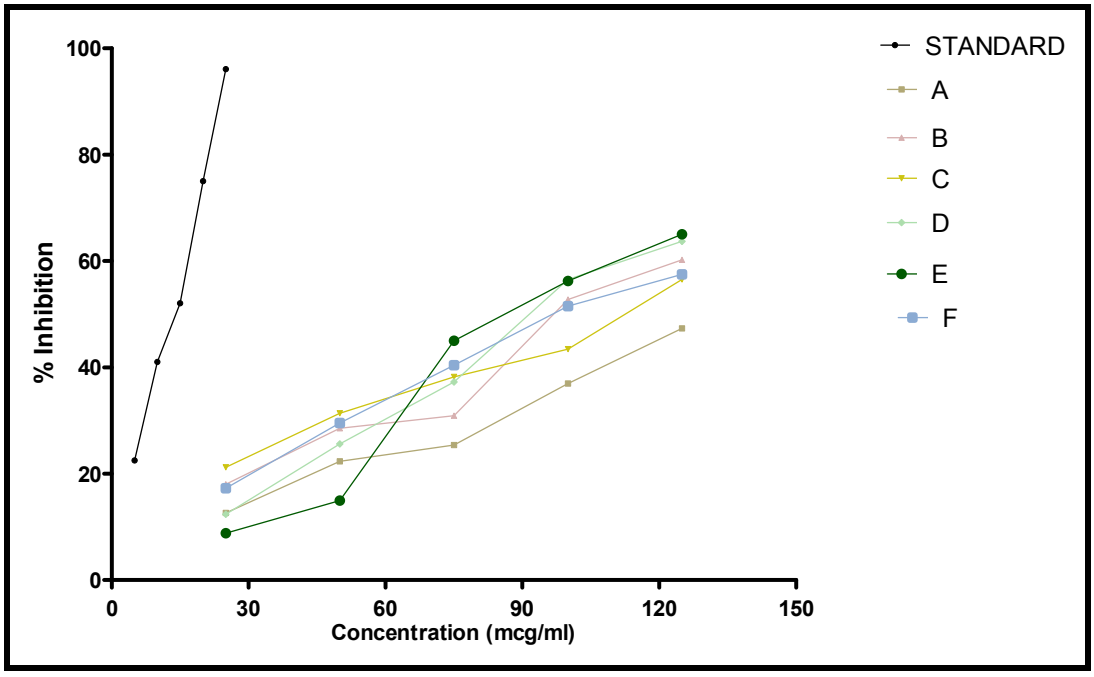


Figure:1.Graph showing DPPH scavenging activity of chalcones

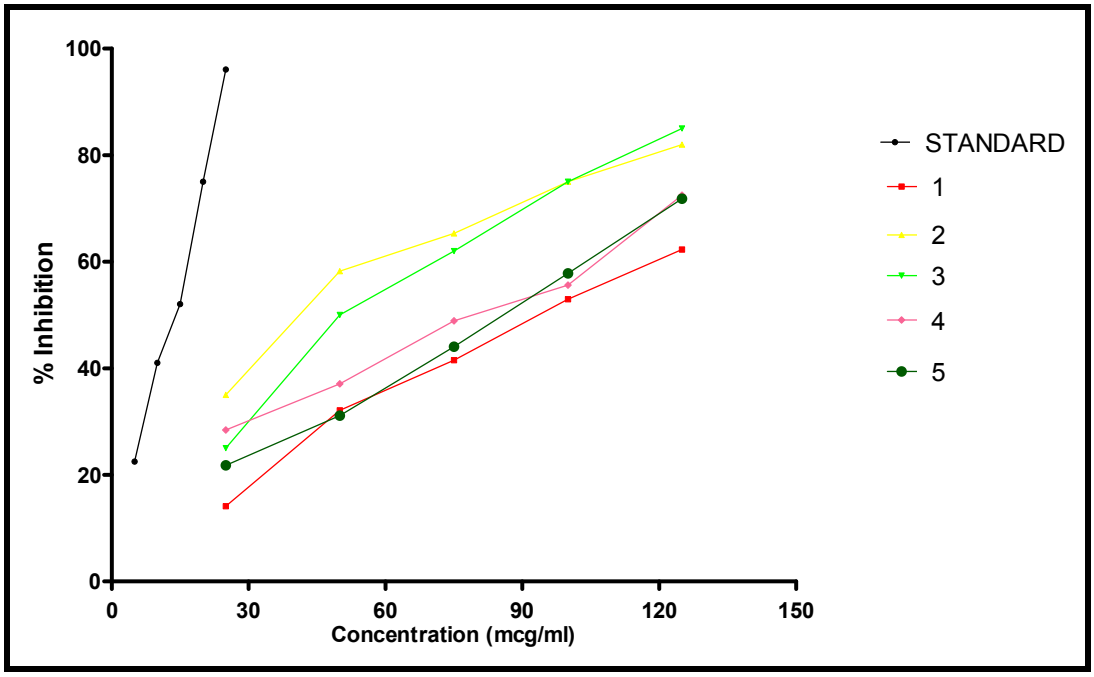


Figure:2 .Graph showing DPPH scavenging activity of flavonoid

Table No.4: Observation for antioxidant activity in terms of Nitric oxide method.

Compound code	% Scavenging [mean ± SEM]				IC50 ug/ml
	50ug/ml	100ug/ml	200ug/ml	400ug/ml	
A	40.01±0.023	47.05±0.21	59.03±0.11	78.08±0.016	158.12
B	37.10±0.05	42.07±0.12	60.90±0.17	64.12±0.01	159.42
C	22.04±0.098	33.05±0.11	55.01±0.13	69.05±0.11	180.71
D	16.04±0.2	36.12±0.11	71.03±0.01	88.08±0.15	141.90
E	20.05±0.15	39.08±0.052	66.06±0.12	84.10±0.17	135.50
F	28.07±0.06	35.12±0.04	48.14±0.08	57.00±0.13	248.98
1	25.04±0.16	40.05±0.15	61.12±0.012	78.00±0.05	145.12
2	35.14±0.13	54.06±0.21	71.16±0.23	84.03±0.14	85.46
3	35.14±0.06	49.06±0.25	72.05±0.12	88.04±0.15	103.19
4	27.13±0.12	37.16±.0.12	51.05±0.14	65.14±0.01	196.84
5	20.05±0.21	47.15±0.014	61.21±0.3	67±0.19	115.56
STD [Ascorbic acid]	49.12±0.16 25ug/ml	60.14±0.15 50ug/ml	73.08±0.23 75ug/ml	88.30±0.01 100ug/ml	33.48

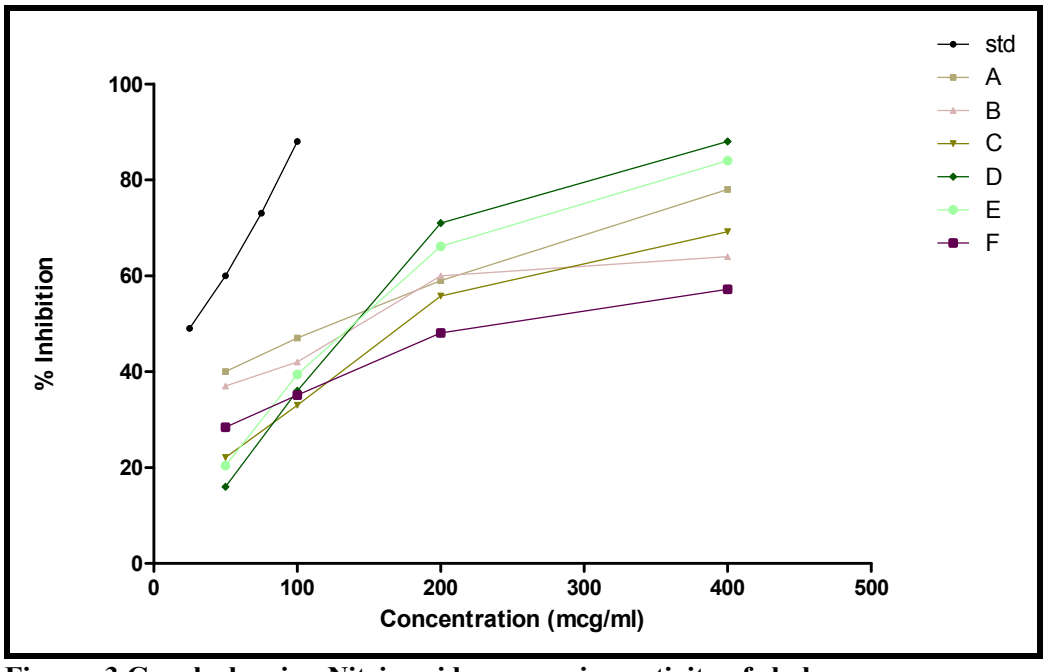


Figure: 3.Graph showing Nitric oxide scavenging activity of chalcones

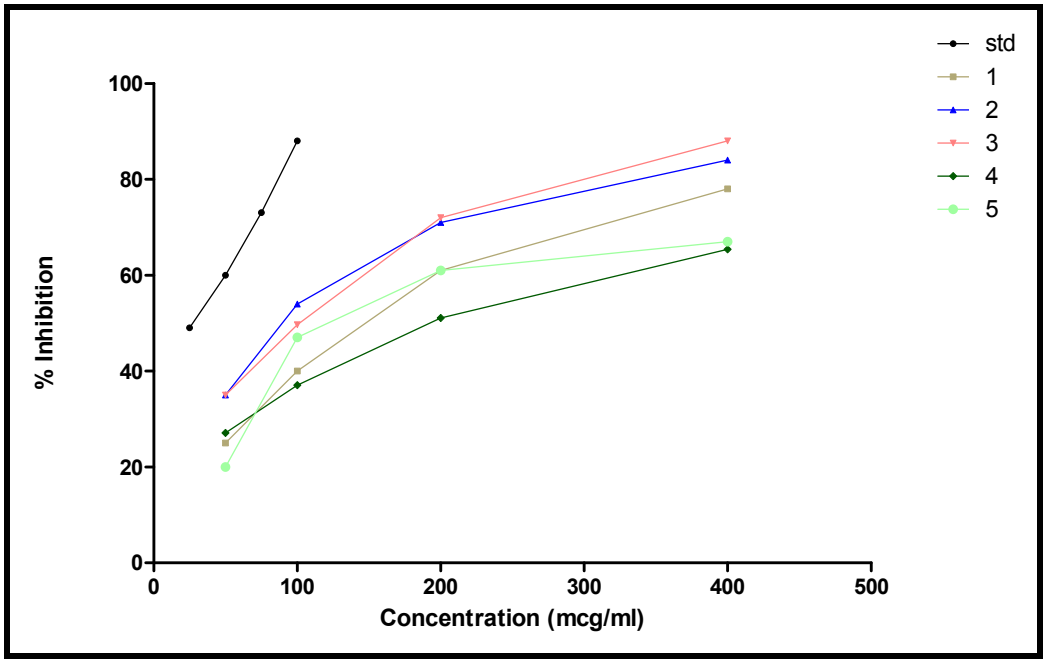


Figure: 4. Graph showing Nitric oxide scavenging activity of flavonoid

Table No.5: Observation for antioxidant activity in terms of hydrogen peroxide method.

Compound code	% Scavenging [mean ± SEM]				IC50 ug/ml
	50ug/ml	100ug/ml	200ug/ml	400ug/ml	
A	13.25±0.01	46.17±0.11	53.48±0.18	56.67±0.01	155.41
B	28.88±0.01	39.55±0.17	45.33±0.06	54.00±0.12	290.13
C	27.77±0.11	38.67±0.13	57.77±0.12	70.66±0.08	175.26
D	28.77±0.13	46.98±0.08	56.22±0.04	77.89±0.15	130.41
E	31.33±0.17	47.11±0.12	59.33±0.21	79.77±0.19	116.32
F	18.21±0.15	33.44±0.01	59.78±0.16	70.00±0.03	165.36
1	26.66±0.05	43.55±0.01	49.33±0.016	59.29±0.98	202.35
2	38.88±0.14	49.77±0.23	69.10±0.12	88.85±0.12	104.4
3	44.08±0.15	55.11±0.12	76.66±0.18	90.10±0.11	82.55
4	36.55±0.01	47.1±0.14	55.00±0.067	72.22±0.16	135.0
5	31.59±0.19	39.77±0.3	54.22±0.098	66.88±0.08	152.41
STD [Ascorbic acid]	33.33±0.15 25 ug/ml	66.00±0.23 50 ug/ml	78.66±0.19 75 ug/ml	90.85±0.21 100 ug/ml	35.41

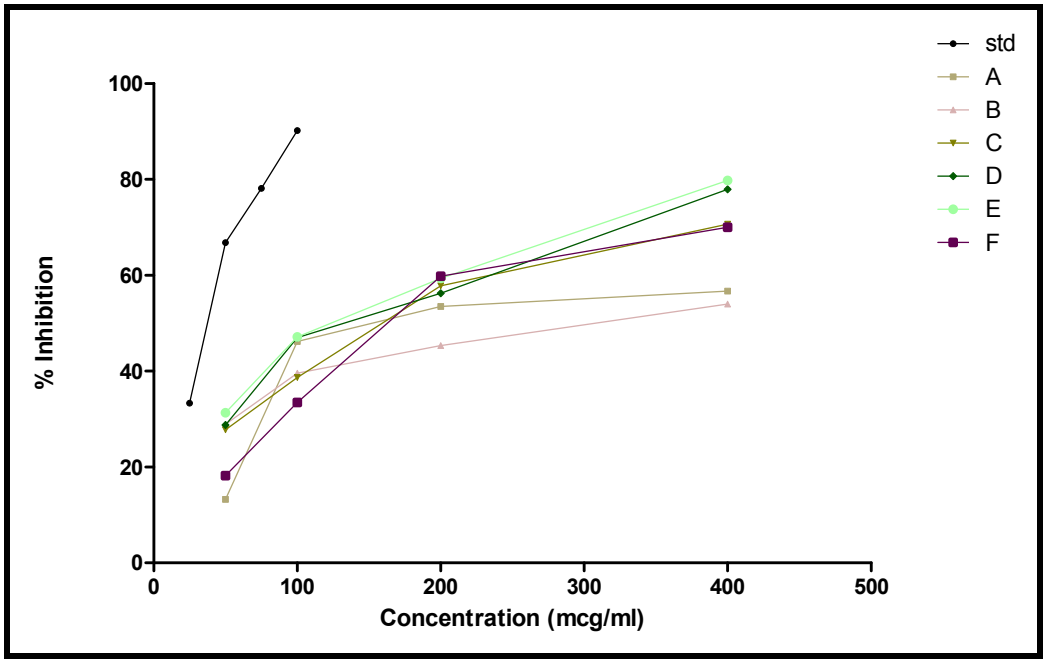


Figure:5.Graph showing hydrogen peroxide scavenging activity of chalcones

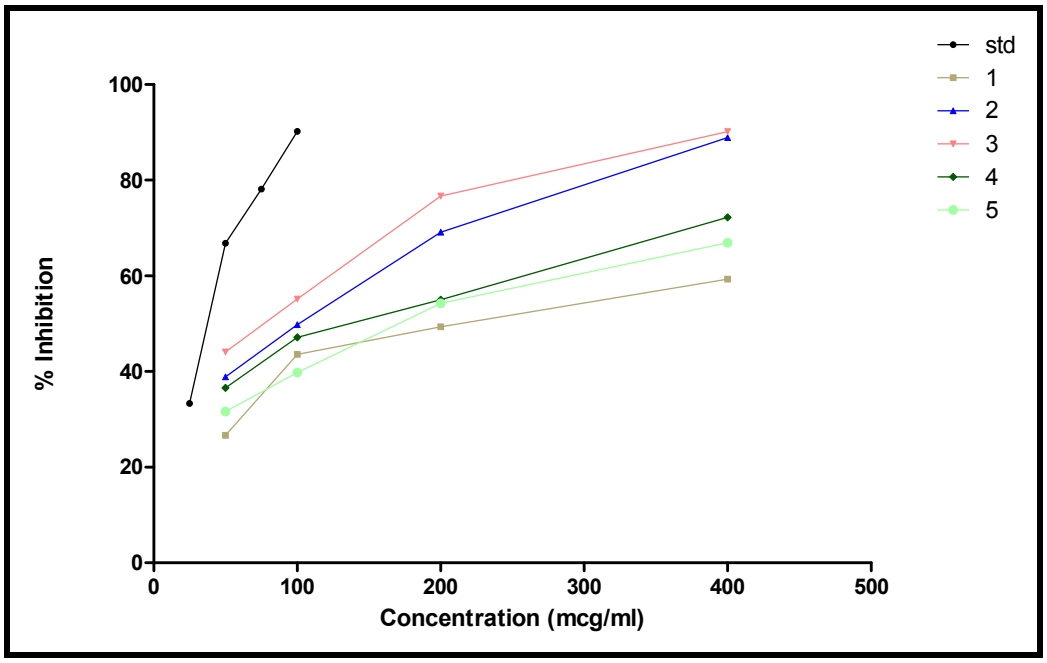


Figure: 6.Graph showing hydrogen peroxide scavenging activity of flavonoid

Table No.6: Observation for antioxidant activity in terms of phenyl hydrazine hydrochloride induced hemolysis method.

Compound code	% Scavenging [mean ± SEM]				IC50 ug/ml
	50ug/ml	100ug/ml	200ug/ml	400ug/ml	
A	9.18±0.18	18.86±0.016	27.56±0.17	39.60 ±06	470.31
B	2.97±0.06	20.53±0.01	23.66±0.016	34.82±0.01	500.26
C	4.91±0.04	19.41±0.13	32.92±0.01	54.12±0.12	330.49
D	22.32±0.12	32.92±0.11	42.96±0.15	59.28±0.05	288.28
E	22.65±0.21	32.47±0.17	49.77±0.11	66.74±0.098	208.13
F	16.74±0.16	30.24±0.15	25.22±0.17	51.45±0.25	380.84
1	16.18±0.016	29.58±0.05	41.85±0.25	49.66±0.13	407.43
2	27.34±0.12	41.07±0.14	51.78±0.05	59.93±0.12	196.95
3	26.22±0.18	39.95±0.15	52.34±0.14	57.47±0.17	180.52
4	10.73±0.067	16.07±0.01	27.34±0.15	38.39±0.03	478.38
5	16.18±0.098	27.00±0.19	40.84±0.016	45.20±0.14	438.16
STD [α-Tocopherol]	40.17±0.18 25ug/ml	55.47±0.23 50ug/ml	67.63±0.08 75ug/ml	75.89±0.17 100ug/ml	43.75

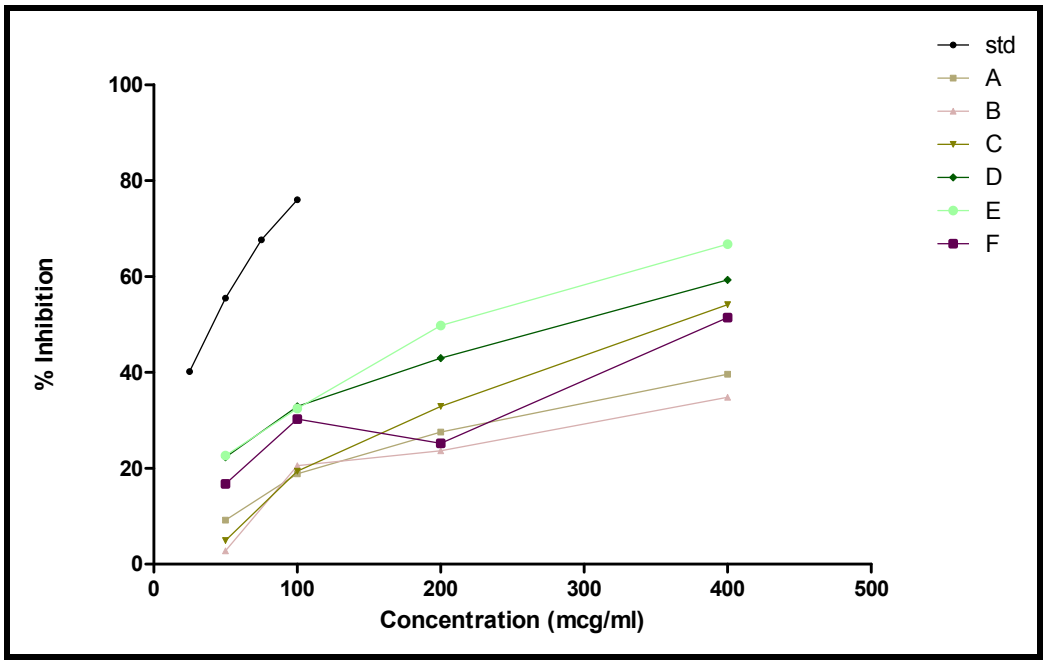


Figure:7. Graph showing Phenyl hydrazine assay scavenging activity of chalcones

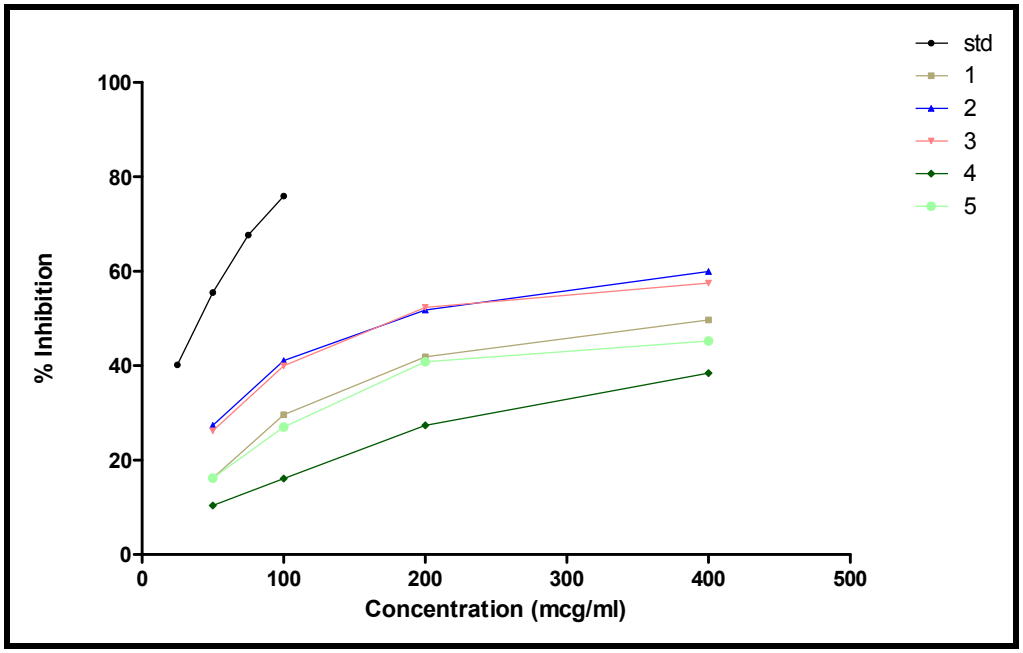


Figure:8. Graph showing Phenyl hydrazine assay scavenging activity of Flavonoids

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