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Study of Antioxidant Activity in Vitro by Aqueous, Ethanolic and Ether Extract of Hibiscus Rosa-Sinensis Linn

KEYWORDS: Antioxidant, Hibiscus

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ABSTRACT *In the present study, antioxidant activities of water, ethanol and ether extracts of petals of Hibiscus rosa-sinensis L were determined by thiocynate method. Extracts neutralized the activities of radicals and inhibited the peroxidation reactions of linoleic acid emulsion. The antioxidant activities of water extract was noted to be higher in 500µg of abstract added to the linolic acid emulsion. Soxhlet method employed for ether extraction was not suitable. Total antioxidant activity was measured according to thiocynate method. A water soluble Ascorbic acid (vit C) was used as a standard antioxidant compound. Water extract and ethanol extract showed 47.06% and 17.37% respectively in 500µg of extract. On the other hand 500µg of ascorbic acid exhibited 70.59% inhibition on peroxidation.*

Introduction
Antioxidants are chemicals that reduces the rate of particular oxidation reactions in a specific context, where oxidation reactions are chemical reactions that involve the transfer of electrons from a substance to an oxidising agent. Antioxidants are particularly important in the con-text of organic chemistry and biology, all living cells contain complex systems of antioxidant chemicals and/or enzymes to prevent chemical damage to the cells' components by oxidation. Also, they can interfere with the oxidation process by reacting with the free radicals, chelating free catalytic metals and also by acting as oxygen scavengers (Larrauri et al., 1999). The antioxidant properties of plants could be correlated with oxidative stress defense in different human diseases including cancer, atherosclerosis, Alzheimer's and the ageing processes (Stajner et al., 1998; Malencic et al., 2000)

The main objective of this particular study was determination of antioxidant activities of various extracts of petals of *Hibiscus rosa-sinensis*. As some effects of this plant have been reported, this plant was chosen. Nowadays, natural antioxidants have become one of the major areas of scientific research (Demo et al., 1998; Sanchez-Morenoet al., 1999).Therefore it is important to exploit natural antioxidants, especially of plant origin which has increased greatly in recent years. There is a growing interest in natural additives as potential antioxidants (Grice, 1986; Moure et al., 2001; Oktay et al., 2003; Gülçin et al., 2005b).

Material & Method
Fresh *Hibiscus rosa-sinensis* flowers were collected and petals were dried at 50^0C or 48 hrs. in an incubator and powdered which used to prepare extract.

Water extract: 5 g dried sample was chopped into small parts in a blender and then extracted with 150 ml of boiled water by stirring for 30 min followed by filtration. Afterwards filtrate was dried by keeping on water bath.

Ethanol extract: 5 g dried sample was chopped into small parts in a blender and then extracted with 150 ml of ethanol by stirring for 2 hrs followed by filtration. Afterwards filtrate was dried by keeping on water bath.

Ether extract: 5 g dried sample was chopped into small parts in a blender and then extracted with ether in soxhlet apparatus until extraction solvent became colourless. It was followed by filtration. Afterwards filtrate was dried by keeping on water bath.

A 5 g dried sample was chopped into small parts in a blender and then extracted with ether in soxhlet apparatus until extraction solvent became colourless. It was followed by filtration. Afterwards filtrate was dried by keeping on water bath. The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. During the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{+2} to Fe^{+3}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. Solutions without added samples were used as blanks. All data on total antioxidant activity are the average of duplicate experiments. The percentage inhibition of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

Inhibition of lipid peroxidation (%) =100 - [($A_{Sample}/A_{Control}$) x 100]
Where, $A_{Control}$ is the absorbance of the control reaction

A_{Sample} is the absorbance in the presence of the sample of water extract or ethanol extract or ether extract

Antioxidant activity by thiocynate method.
Each sample (containing 500µg to 10000µg extract) in 0.5 ml of distilled water was mixed with 2.5 ml of linoleic acid emulsion (0.02M, in 0.04M pH 7.0 phosphate buffer).To this added 2 ml of phosphate buffer (0.04M, pH 7.0).Test tubes were incubated in darkness at 37°C.The amount of peroxide was determined by reading absorbance at 500 nm after colouring with FeCl3 and potassium thiocynate. Ascorbic acid was used as standard.

Observation and Results
The detailed study on Antioxidant activity of leaves of *Cydonia vulgaris* was done by Yildirim *et al* (2001).They showed that antioxidant activity of the water, as well as ethanol extracts of the leaves of *C.vulgari* increased with increasing amount of extract. But in case of *Hibiscus rosa-sinensis* such condition was not observed. Antioxidant activity of extract was irrespective of its concentration. Unlike *Cydonia vulgaris,* the ethanol extract had very less antioxidant capacity as compared to water extract and it was not

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also concentration dependent.

As can be seen in **Figure 10.5** the sample containing 500µg of water extract peroxidation is suppressed for about 5 hrs and after that it begins to increase. **Figure 10.6** shows that ethanol extract has very poor antioxidant activity. When different samples such as water extract, ethanol extract, standard antioxidant (ascorbic acid), and control containing

500 µg of extract had shown no much difference in std and water extract. In the sample containing 500µg of extract, peroxidation was supressed for 7 hrs where as in water extract for 5 hrs (as can be seen in **Figure10.7**).

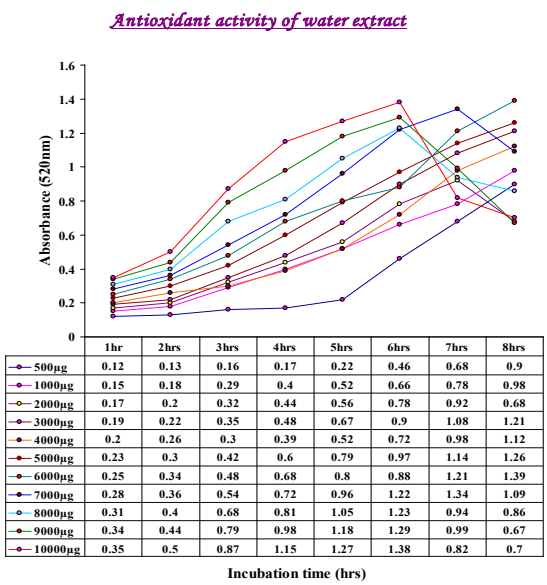
Conclusion

From the above discussion it can be concluded that, *Hibiscus rosa-sinensis* can used as a natural antioxidant source.The experiment of antioxidant activity further needs to be carried out over a more period of incubation. It may give more proper results regarding peroxidation.

Inhibition of lipid peroxidation in water extract was 47.06% whereas in ethanol extract it was 17.37% and that of standard was70.59%, from these values it was clear that, water extract had more antioxidant activity than ethanol extract (when compared to control) **(figure 10.8)**

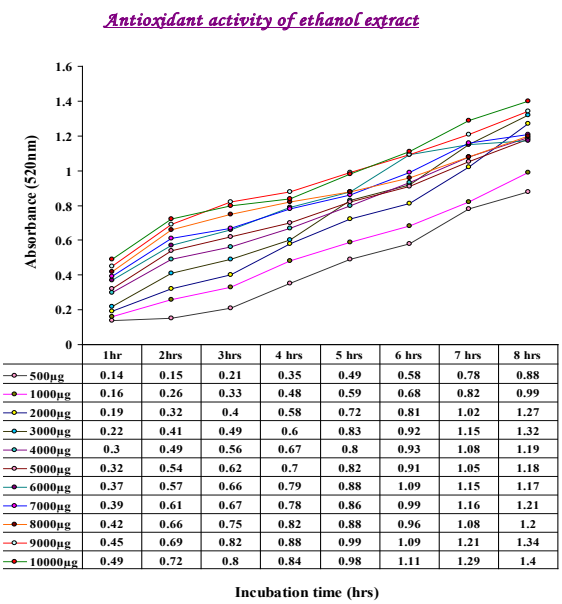
Antioxidant Activity

Figure 10.5 : Antioxidant activity of dried water extract of petals of Hibiscus rosa- sinensis. (Numbers following Ext indicates µg of extract added to the emulsion)



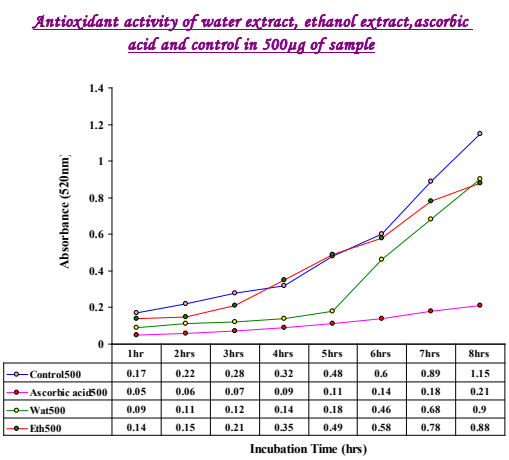
	1hr	2hrs	3hrs	4hrs	5hrs	6hrs	7hrs	8hrs
500µg	0.12	0.13	0.16	0.17	0.22	0.46	0.68	0.9
1000µg	0.15	0.18	0.29	0.4	0.52	0.66	0.78	0.98
2000µg	0.17	0.2	0.32	0.44	0.56	0.78	0.92	0.68
3000µg	0.19	0.22	0.35	0.48	0.67	0.9	1.08	1.21
4000µg	0.2	0.26	0.3	0.39	0.52	0.72	0.98	1.12
5000µg	0.23	0.3	0.42	0.6	0.79	0.97	1.14	1.26
6000µg	0.25	0.34	0.48	0.68	0.8	0.88	1.21	1.39
7000µg	0.28	0.36	0.54	0.72	0.96	1.22	1.34	1.09
8000µg	0.31	0.4	0.68	0.81	1.05	1.23	0.94	0.86
9000µg	0.34	0.44	0.79	0.98	1.18	1.29	0.99	0.67
10000µg	0.35	0.5	0.87	1.15	1.27	1.38	0.82	0.7

Figure 10.6 : Antioxidant activity of dried ethanol extract of petals of Hibiscus rosa- sinensis. (Numbers following Ext indicates µg of extract added to the emulsion)



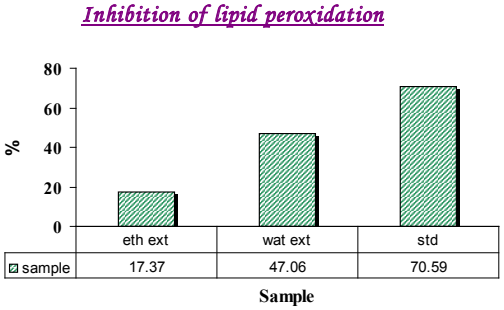
	1hr	2hrs	3hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs
500µg	0.14	0.15	0.21	0.35	0.49	0.58	0.78	0.88
1000µg	0.16	0.26	0.33	0.48	0.59	0.68	0.82	0.99
2000µg	0.19	0.32	0.4	0.58	0.72	0.81	1.02	1.27
3000µg	0.22	0.41	0.49	0.6	0.83	0.92	1.15	1.32
4000µg	0.3	0.49	0.56	0.67	0.8	0.93	1.08	1.19
5000µg	0.32	0.54	0.62	0.7	0.82	0.91	1.05	1.18
6000µg	0.37	0.57	0.66	0.79	0.88	1.09	1.15	1.17
7000µg	0.39	0.61	0.67	0.78	0.86	0.99	1.16	1.21
8000µg	0.42	0.66	0.75	0.82	0.88	0.96	1.08	1.2
9000µg	0.45	0.69	0.82	0.88	0.99	1.09	1.21	1.34
10000µg	0.49	0.72	0.8	0.84	0.98	1.11	1.29	1.4

Figure 10.7 : Antioxidant activity of dried water and ethanol extract of the petals of Hibiscus rosa-sinensis. In each there was 500µg of indicated dried extract or ascorbic acid while in control there was no extract. (Wat=water, eth=ethanol)
standard



	1hr	2hrs	3hrs	4hrs	5hrs	6hrs	7hrs	8hrs
Control500	0.17	0.22	0.28	0.32	0.48	0.6	0.89	1.15
Ascorbic acid500	0.05	0.06	0.07	0.09	0.11	0.14	0.18	0.21
Wat500	0.09	0.11	0.12	0.14	0.18	0.46	0.68	0.9
Eth500	0.14	0.15	0.21	0.35	0.49	0.58	0.78	0.88

Figure 10.8 : Inhibition of lipid peroxidation in water extract, ethanol extract and



	eth ext	wat ext	std
sample	17.37	47.06	70.59

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