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## Isolation of Total Alkaloids and Evaluation OF Total Antioxidant Activity From The Red Flowers of Hibiscus Rosa – Sinensis Linn

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### ABSTRACT

Hibiscus rosa – sinensis Linn is certain to emerge in the near future as a major player in the growing field of herbal health supplements and medicines both in daily self care and in professionally managed health care system. All the parts of hibiscus rosa sinesis Linn are used as medical properties throughout world. Flowers containing chemical constituents are used as anti-tumor, anti fertility, anti ovulatory, anti implementation, anti-inflammatory, analgesic, antiestrogenic, antipyretic, antispas modic, antiviral, antifungal, antibacterial, hypo glycaemic, spasmolytic, CNS depressant, Hypo tensive and juvenoid activity. In the present study, total alkaloids are isolated from the flower and evaluated for their antioxidant activity by using ferric thiocyanate method and thiobarbituric method. The yield of total alkaloid from the red flower of hibiscusRosa

sinensis is 12.5%(W/W). It elicited statistical significant antioxidant activity when compared with standard antioxidant drug vitamin E.

**Keywords:** Hibiscus Rosa Sinensis Linn , Red Flowers, FTC Method, TBA Method, And Antioxidant activity.

## INTRODUCTION

The red flowers of hibiscus rosa sinensis (family :malvaceae) has been reported in the ancient Indian medicinal literature with beneficial effects in heart diseases(Nadakanj). The flowers are ascribed as emollient , refrigerant, aphrodisiac, demulcent, emmenagogue, homeostatic, brain tonic and cardio tonic (Shanmugasundaram etal 1991). Non polar benzene extract of flowers of hibiscus rosa-sinensis showed anti fertility effect in laboratory rats[kho khar, I and Ahmed A, 1992, Kholkute, S.D., etal 1976) flowers collected in winter season showed maximum post-coital antifertility activity[kholkute, S.D etal 1977] and flowers were studied for the treatment of diabetes mellitus as temporary measure to bring down sugar level(Alam MM etal 1990). The extracts isolated from the flowers were already reported to have significant analgesic, antipyretic and antinflammatory activities (Kumara guru.S etal 2001). Acetone soluble fraction of ethanolic extract of flower produced statistical significant anticonvulsant activity against electroshock and pantylene tetrazole induced convulsion in mice (kasture vs etal 2000). Free medicals are unpair electron containing species that produce unwanted effects to plants, animals and humans. Antioxidant prevent the formation of free radicalnd inhibit lipid proxidation(Halliwell B., Gutteridge JMC, 1999)

## EXPERIMENTAL

### Isolation of Alkaloids

2 kg of dried and coarsely cut red flowers of *Hibiscus rosa-sinensis* Linn. were extracted with 95% ethanol. First. the plant material was immersed in alcohol and then heated to 60°C and then was kept over night at room temperature. It was then filtered and the process was repeated for 3 times. The combined alcoholic extract was concentrated at 55°C. The thus obtained extract concentrate was termed as the Total Alcoholic Extract Concentrate which was then weighed and was found to be 200.40 gm. 100 gm of the total alcoholic extract concentrate was extracted with

0.1 NHCL by allowing it to stand for about 5 hours. The aqueous acid extract was partitioned with 100 ml of chloroform.

100gm of the total alcoholic extract concentrate was extracted with 0.1 N HCl by allowing it to stand for about 5 hours. The aqueous acid extract was partitioned with 100 ml of chloroform in a separating funnel. This procedure was repeated for 2 more times and the combined chloroform layer was rejected. The aqueous layer was basified with ammonium hydroxide to pH 9.0 and again partitioned with chloroform. The aqueous layer was rejected while the chloroform layer was collected and evaporated to dryness. The total alkaloidal substance was tested for alkaloids by using the 3 alkaloidal testing reagents such as **Dragendorff's reagent**, **Wagner's reagent** and **Mayer's reagent**. The total yield of the alkaloidal substance was 12.5gm. The code TK was prepared using this total alkaloidal material.

### **Preparation and use of alkaloidal testing reagents**

The following alkaloidal testing reagents were prepared and used for testing routinely in the laboratory.

#### **Dragendorff's reagent (Chemical Abstracts, 1925)**

This reagent was synonymously known as "Potassium Bismuth Iodide Reagent". 8 gm of bismuth subnitrite was dissolved in 20 ml of concentrated nitric acid with a specific gravity of 1.18. It was slowly added to a solution prepared by dissolving 22.7 gm of potassium iodide in 20ml distilled water. The solution was allowed to stand until potassium nitrite was precipitated. The solution was filtered and the clear filtrate was diluted with water to 100ml. The solution was kept in the dark in a well-stoppered bottle. With most alkaloids the solution gives a reddish yellow flocculent precipitate.

#### **Wagner's reagent (Chemical Abstracts, 1909)**

This reagent was also known as "Iodo potassium iodide reagent". 2gm of iodine and 6gm of potassium iodide were dissolved in 100ml of water. This reagent causes precipitations with many of the alkaloids.

### **Mayer's Solution (American Journal of Pharmacy, 1863)**

Mercuric Iodide (13.55 gm) was dissolved with 50gm of potassium iodide reagent". This reagent yields white precipitate in acid medium with alkaloids, if present.

### **Antioxidant Activity**

#### **Ferric thiocyanate (FTC) Method**

The method of mitsuda etal (1967) osawa Namiki(1981) which was slightly modified by kikuzaki and Nakatani (1993) was used as the FTC method. A mixture containing 4gm of the sample in 4ml of 99.5% ethanol (final concentration, 0.02%), 4%ml of 2.52% of Linoleic acied in 99%, ethanol, 8ml of 0.05 ml phosphate buffer (p<sup>H</sup> 7.0) and 3.9 ml of water was placed in a vial with a screw cap and the placed in an incubator at 40°C in the dark. To 0.1ml of this mixture, 9.7ml of 75% ethanol (v/v) and 10 ml of 30% ammonium thiocyancte were added precisely 3 min after the addition of 0.1 ml of 0.02m ferrous chloride in 3.5% hydrochloric acid was placed to the reaction mixture : the absorbance of the red color indicated the antioxidant activity was measured at 500nm for every 24 hours until the absorbance of the control related maximum. The control and the standard were subjected to the same procedure as the sample except that for the control, only solvent was used and for the standard 4mg of the sample was replaced by 4mg of vitamin E.

#### **Thiobarbituric acid (TBA) Method**

The method of Ottolenghi,A. (1959) was used to determine the TBA values of the samples. The formation of melanaldehyde is the basis for the well- known TBA method used for evaluating the extent of lipid peroxidation. At low pH. and high temperature( 100°C), melanaldehyde bindsTBA to form a red color that can be measured at 532 nm. 2ml of 20% trichloro acetic acid and 2ml of 0.67% TBA solutions were added to 2 ml of the mixture containing the sample prepared in the FTC method. This mixture was kept in a water bath (100°C) for 10 minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 mm on the final day of the assay.

### Statistical Analysis

The percentage of antioxidant activity (AO) was calculated by the following formula for both FTC and TBA methods

$$\% \text{ of A O activity} = 100 - \frac{\text{Test OD}}{\text{Control OD}} \times 100$$

### RESULTS AND DISCUSSION

Alkaloids are group of organic molecule that are showing excellent therapeutic activity against variety of diseases. The choser Hibiscus rosa sinensis Linn plant contains quantitatively 12.5% w/w of total alcoholic extract which was confirmed quantitative chemical tests by Dragendorff's reagent, wagner's reagent and mayer's solution from the total alkaloid extract, 0.2mg/ml was used the studying the effect on FTC and TBA methods.

FTC method was used to measure the amount of peroxide formed of the primary stage of Linoleic acid peroxidation. The total antioxidant activity produced by the extract is shown in the Figure 1 in terms of absorbance at 500 nm. The percentage of antioxidant activity was shown in Figure.2 at a concentration of 0.02%. The total alcholid extract (TK) produced strong antioxidant activity. which is shown in Fig.3 and in terms of percentage of antioxidant activity in figure 4.

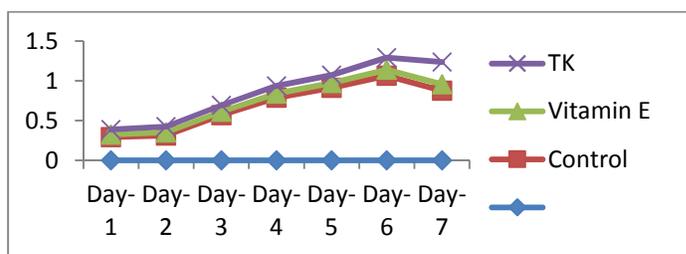
The total antioxidant activity of the various extracts from the flowers of Hibiscus Rosa-Sinensis Linn were estimated by both FTC and TBA method and were compared with vitamin E. In FTC method, the control showed increased in absorbance values from day 1 and reached maximum on day 7 and dropped on day 8. This reduction is due to the formation of increased level of methanol dehyde compounds from linleic acid oxidation, which is not static (Maznal Ismail, 2000). The total alkolidal extract TK elicited strong antioxidant activity which is close or more the vitamin E, a well known chain breaking antioxidant (Burton Give etal 1982). The pattern of activity has very similar for both TBA and FTC method.

The strong antioxidant activity produced by total alkaloid extract could be due to the presence of antioxidant molecules which need further investigation of isolation of individual molecule (SankarSubramanian. S and AGR Nair. 1972). The extracts from the flower of Hibiscus Rosa—Sinensis Linn. were reported to contain cardiac stimulant active molecule against the heart preparation (S.kumarguru et al 2002). The assay is conducted for seven days as chronic period of evolution of total antioxidant activity from the red flowers of Hibiscus Rosa – Sinensis Linn. Each day, amount of peroxide formed is detected in terms of its optical density using spectrophotometer. Every day, free radical is generated inside the test tube was determined. Here Linoleic acid is a lipid molecule considered to be present in the lipid part of the cell membrane which undergo lipid peroxidation. The yield of malonaldehyde is determined from day 1 to day 7.

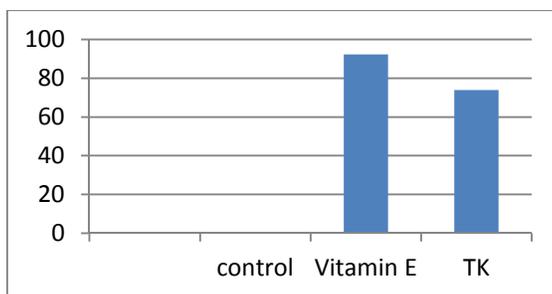
In TBA method, total peroxide formed due to the linoleic acid oxidation was estimated. The higher the optical density, the lower the level of antioxidant activity. The control produced highest absorbance value (0.7851) followed by Drug vitamin E (0.0424) and total alkaloid TK (0.1784) the neuro protective (Kasture et al 2000). Therefore, the antioxidant characteristics of the flowers of Hibiscus rosa-sinensis Linn total alkaloid extracts relevant their use in traditional medicine to cure various diseases. Accordingly, this confirms the inhibition of linoleic acid peroxidation may be useful for the treatment of cardiological and neurological diseases.

## CONCLUSION

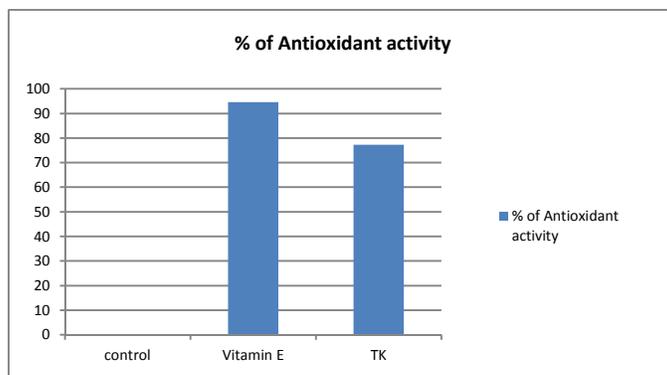
Based on the results of the present study, we conclude that the plant extract contains alkaloids which exerted significant antioxidant activity. However, further studies are essential to study the underlying mechanisms of antioxidant effects and to isolate the active compounds responsible for these pharmacological activities.



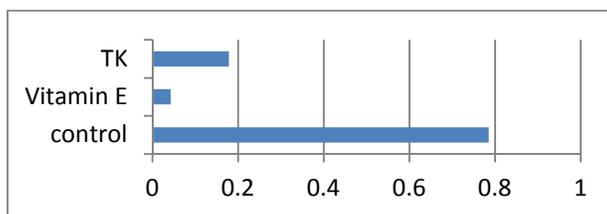
**Figure 1** Absorbance value of the Hibiscus Rosa – sinensis Linn extracts using FTC method.



**Figure 2** Antioxidant activity of Hibiscus Rosa - Sinensis Linn flower extracts using FTC method.



**Figure 3** Antioxidant activity of the Hibiscus Rosa – Sinensis Linn flower extract using TBA method.



Absorbance value

**Figure 4** Absorbance values of the Hibiscus Rosa-Sinensis Linn flower extracts using TBA method on day 7.

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