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ANTIOXIDANT EFFECT OF THE STEM AND LEAVES OF *HIBISCUS ESCULENTUS* LINN.

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ABSTRACT

The ethanol extract of *Hibiscus esculentus* Linn roots inhibited the formation of oxygen derived free radicals were screened for their *in vitro* antioxidant activity. Stems leaves and root of selected plants were dried at 45°C and powdered for extraction. Extraction was performed with methanol. By employing DPPH (2, 2-diphenyl-1picrylhydrazyl radical) scavenging assays, it was shown that all the ethanol extracts of the samples collected show antioxidant activity. Though, as expected, their potency varied according to the different parts and species. In particular, leaves and stems of *Hibiscus esculentus leaves* displayed the highest activity. The extracts tested ranged from 0.176 mg/mL –2.45mg/mL. It is generally accepted that a diet rich in plants is associated with a reduced incidence of degenerative diseases, such as atherosclerosis and cancer. This study suggests that the *Hibiscus esculentus* plant could be pharmaceutically exploited for antioxidant properties.

INTRODUCTION: Free radicals are potentially important in a number of ailment states that can have severe effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction [1].

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and, in some instances, the need may be several times the RDA.

As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

Although the antioxidant defense systems includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention [2]. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants.

In this context, ethno pharmacology plays a significant part in the search for interesting and therapeutically useful plants. In order to contribute to the knowledge of plants from India in the present study plant parts of *Hibiscus esculentus* Linn. were screened to determine their free radical scavenging and antioxidant activities.

MATERIALS AND METHODS:

Plant Material: The plant materials used in this study, *H. esculentus* roots, stem and leaves of were collected from the field in Khandala Tal. Shrirampur, Dist Ahmednagar identified by Dr. A. K. Mohite, R.B.N.B College, Shrirampur, Maharashtra, India. A voucher specimen of the collected sample was deposited in our institutional herbarium for the reference.

Preparation of plant extracts: 100g of dried and powdered plant material (leaves, root, and stem) were extracted at room temperature with 500 mL of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the biological assays, and then followed by extraction using solvents ethyl acetate, ethanol, chloroform, etc with same procedure.

DPPH Scavenging Test: Quantitative measurement of radical scavenging property was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. Different known antioxidants, vitamin E, and butylatedhydroxytoluene (BHT, Sigma) were used for comparison or as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements was taken at least in triplicate. DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(Ao - A_1) / Ao] \times 100;$$

Where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the sample. The actual decrease in absorption induced by the test compounds was compared with the positive controls. The mean OD 517 results of DPPH scavenging activity was recorded

RESULTS AND DISCUSSION: There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction [1, 4].

The extracts and essential oils of many plants have been investigated for their antioxidant activity [5-7]. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense [8-9]. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes [10-11].

Therefore, in this study, the antioxidant properties of the methanol extracts of leaves and stems of plant like of re examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in **table 1** & **table 2** as comparable with known antioxidant BHT. In terms of antioxidant activity, all the extracts investigated exhibited a rather high degree of activity (more than 40%). In particular, leaves (ethanol extract) of *Hibiscus esculentus* displayed the highest activities as antioxidant activity as removal of the stable radical DPPH and the lowest activity were found in CCl_4 *extract of stem.* As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant.

TABLE 1: ANTIOXIDANT ACTIVITY OF LEAVES

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	20.11	14.53	16.47
0.1	46.91	44.64	20.53	20
0.2	49.24	52.24	28.50	34
0.3	57.57	60.12	40.00	40

TABLE 2: ANTIOXIDANT ACTIVITY OF STEM

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	18	15	14
0.1	46.91	25	22	24
0.2	49.24	27	22	23
0.3	57.57	35	32	28

TABLE 3: ANTIOXIDANT ACTIVITY OF ROOT

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	16	13	10
0.1	46.91	21	20	12
0.2	49.24	22	22	20
0.3	57.57	27	25	22

CONCLUSION: The crude extract of stem, root and leaves show better antioxidant activity as compared

with antioxidant available in market. In all other extract methanolic extract of leaves show erxcellent antioxidant activity.

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