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STUDY OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF LEAVES EXTRACT OF *CRINUM SOLAPURENSE*

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ABSTRACT:

The widely used indigenous medicinal plant *Crinum solapurense* is the subject of the current inquiry. According to the study and pertinent literature, the plant *Crinum solapurense* is the most well-known for its use in traditional medicines, while different portions of the plant have received less attention. According to the findings of the present thorough analysis, *Crinum solapurense* leaves have high phenolic and flavonoids contents as well as potential antioxidant activity. The antioxidant activity was studied using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP). The present study is to evaluate the antioxidant activity. The leaves extract of *Crinum solapurense* was evaluated for the antioxidant activity. Extraction was carried out by using solvents aqueous extract, ethanol, ethyl acetate, chloroform; petroleum ether was also used for the evaluation of the activity. The antioxidant activity was studied using DPPH and FRAP. In the evaluation of all extracts, the Ethanolic extract showed significant DPPH radical scavenging activity and ferric reducing potential. From all the extracts, Ethanolic extract showed a maximum of DPPH free radical scavenging activity (57.61µg/ml IC_{50} value) and the ferric reducing power (54.48 µg/ml IC_{50} value). The plant *Crinum solapurense* might be a brand-new source of biologically active, stable antioxidants that could provide a solid scientific foundation for its usage in contemporary medicine.

Keywords: *Crinum solapurense*, Antioxidant activity, *In-vitro* antioxidant activity, DPPH free radical scavenging activity, Ferric reducing potential etc.

INTRODUCTION:

Medicinal plants are used to treat many diseases from ancient times [1]. Free radicals induced tissue injury contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS. Therefore currently, there is a world-wide trend in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury and use of them as antioxidants in foods and drugs. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, and anti-mutagenic, anti carcinogenic, antibacterial or antiviral activities. Medicinal value of plants is related in their phytochemical components and their secondary metabolites such as: Phenolic compounds, flavonoids, alkaloids and tannins and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [2]. The antioxidants have a wide range of biochemical activities

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including, inhibition of reactive oxygen species generation, direct or indirect scavenging of free radicals, metal chelating ability and alteration of intracellular redox potential. Plants present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Therefore, to prevent oxidative damage, the search for natural antioxidants, especially of plant origin, has notably increase in recent years. [3] The pan tropical genus *Crinum L.* comprises about 112 species distributed in tropical Africa, America, Asia and Australia. The genus is most diverse in Southern Africa. In India, the first detailed taxonomic treatment of Crinum is that of William Roxburgh's Flora Indica, in which he recorded 14 species from British India. Subsequently, in his classical work on Amaryllidaceae included six species and four varieties of Crinum from British India. In the Handbook of the Amaryllidaceae gave detailed insight into the genus. Out of 79 species of Crinum listed by him, 12 species and 2 varieties were from British India. At the beginning, he gives a sub generic classification scheme of Stenaster, Platyaster and Codonocrinum in reduced form. Hooke recognized 17 species from British India of which 7 species were treated as imperfectly known [4] Recent studies have shown that antioxidants work best for removing free radicals that produce oxidative stress and may act as protective agents to shield cells from reactive oxygen species and halt the progression of various diseases as well as lipid per oxidation [5-7]. Amaryllidaceae J.St.-Hil. Comprise some 1100 species from 75 genera found mainly throughout the tropics [8] Amaryllidaceae alkaloids are specific type of isoquinoline alkaloids exclusive to the family. They are structurally diverse, biogenetically related and classified into nine basic skeleton groups: norbelladine-, lycorine-, tazettine-, homolycorine-, crinine-, haemanthamine-, arciclasine-, montanine- and galanthamine-type alkaloids [9] Amaryllidaceae alkaloids are of great interest because of their wide range of biological activities. Lycorine (LY) and galanthamine (GAL) are potential plant growth-inhibitors [10] and exhibit cytotoxic [11] anti-inflammatory [12] antitumor [13] anti-viral, anti-bacterial, anti-malarial and acetyl cholinesterase inhibitory properties [14] Species of the genus are economically important and known for their ornamental and medicinal properties. They exhibit various medicinal properties such as anti-diabetic, anti-tumor, immune-stimulating, analgesic, cytotoxic, antiviral, anti-inflammatory, antioxidant, antimalarial and antimicrobial due to the presence of pharmacologically active principles [15].

MATERIALS AND METHODS:

Plant materials:

We collected a *Crinum solapurense* plant from swamp bordering the Bhima River in Solapur district of Maharashtra, India. The plant material was shade dried, without exposing the material to direct sunlight. After drying the leaves were powdered separately in mixer. The powder was then passed through sieves No: 40. This fine leaf powders were stored separately in a cool and dry place until its use.

Plant identification:

The *Crinum Solapurense* plant was identified and authenticated by plant taxonomist Dr. D. L. Shirodkar, Botanist, BSI, WRC, Pune-1 is done from the Botanical Survey of India Pune.

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(Government of India, Ministry of Environment, Forests & Climate Change Botanical Survey of India, Western Regional Centre, 7- Koregaon Road, Pune-411001)

Preparation of leaf extracts:

For the preparation of various leaf extracts 250 g of plant materials were separately extracted using ethanol, using soxhlet extractor at a temperature not exceeding 45°C. The extracts were concentrated and dried by using rotary evaporator and was stored in a refrigerator at 4°C until use.

Chemicals and instruments:

All the chemicals and solvents used in the present study were of analytical grade.

***IN-VITRO* ANTIOXIDANT ACTIVITY:**

DPPH free radical scavenging activity:

The free radical scavenging potential of *Crinum solapurense* leaves extracts were tested by using the methanolic solution of DPPH. DPPH is a stable free radical. Antioxidants reduce DPPH to the 2, 2-diphenyl 1-picryl hydrazine which is measured at 517 nm. Ascorbic acid is used as standard antioxidant. According to the method of brand Williams *et al.* the assay is performed. The reaction mixture used to evaluate this activity contains 0.1 ml of methanolic solution of different extracts (containing 25, 50, 100, 150, 200 and 400µg /ml) and 3.9 ml methanolic solution of DPPH. For standard 0.1 ml of ascorbic acid was used instead of extract. And for blank preparation, 0.1 ml of methanol was used. This mixture was incubated for 30 minutes in dark at room temperature. Absorbance measured at 517 nm. Percentage of DPPH radical scavenging activity of all extracts was measured and compared with the standard ascorbic acid [1, 4]. Percentage of the DPPH radical scavenging activity as measured as:

$$\text{Percent inhibition} = \frac{\text{Abs Control} - \text{Abs treated}}{\text{Abs Control}} \times 100$$

Ferric (Fe 3+) Reducing power assay:

In this assay, Tannic acid was used as standard. In this method, antioxidant compounds forms colored complex with potassium ferric cyanide in the presence of trichloroacetic acid and ferric chloride. In this assay, reaction mixture contain 1 ml of methanolic solution of different extracts (25, 50, 100, 150, 200 and 400 µg /ml) of *Crinum solapurense* leaves, 2.5 ml of phosphate buffer of pH 6.6 and 2.5 ml of 1 % potassium ferric cyanide. This mixture was incubated for 20 min at 50°C, cooled rapidly. Add 2.5 ml of 10 % trichloroacetic acid and 0.5 ml of 0.1 % ferric chloride to each. Blank is prepared by using 1ml of methanol instead of extract solution. Then absorbance was measured at 700 nm. Percentage of Ferric reducing power of all extracts was measured and compared with the standard tannic acid [1]. Percentage inhibition of the ferric reducing power was measured as:

$$\text{Percent inhibition} = \frac{\text{Abs Control} - \text{Abs treated}}{\text{Abs Control}} \times 100$$

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Abs Control

RESULTS AND DISCUSSION:

DPPH free radical scavenging activity:

Percentage of DPPH free radical scavenging activity of different leaves extracts of *Crinum solapurense* at different concentrations is shown in table no. 1. Percentage inhibition of all leaves extracts of *Crinum solapurense* is then compared with Percentage inhibition of standard ascorbic acid shown in Fig. 1, Fig. 2, and the Fig. 3 shows the IC_{50} value of each extract.

Table No. 1: Percentage of DPPH free radical scavenging activity of different leaf extracts of *Crinum solapurense*.

Sr. No.	Concentration in µg/ml	Percentage of DPPH free radical scavenging activity					
		Standard Ascorbic acid	Aqueous Extract	Ethanolic Extract	Ethyl Acetate Extract	Chloroform Extract	Petroleum Ether Extract
1	25	26.47 ± 0.174	25.18 ± 0.156	26.21± 0.113	21.17± 0.121	23.69± 0.114	20.76± 0.129
2	50	45.59 ± 0.116	44.52 ± 0.163	44.99± 0.147	41.37± 0.142	43.53± 0.137	38.85± 0.113
3	100	68.41 ± 0.123	66.8 ± 0.182	67.8± 0.123	60.28± 0.178	65.7± 0.165	60.17± 0.128
4	150	74.79 ± 0.159	71.92 ± 0.119	73.18± 0.139	66.94± 0.148	69.94± 0.187	67.39± 0.198
5	200	80.94 ± 0.158	79.34 ± 0.137	79.89± 0.154	72.84± 0.166	74.59± 0.193	73.83± 0.136
6	400	85.69 ± 0.149	82.23 ± 0.194	83.45± 0.168	78.49± 0.170	80.32± 0.120	79.23± 0.165
7	**IC_{50}**	**53.35**	**64.05**	**57.61**	**101.28**	**77.74**	**104.62**

Ferric (Fe 3+) Reducing power assay:

Percentage of ferric reducing power of different leaves extracts of *Crinum solapurense* at different concentration are shown in Table No. 2. Percentage inhibition of all leaves extracts of *Crinum solapurense* is then compared with Percentage inhibition of standard tannic acid shown in Fig. 4, Fig. 5, and the Fig. 6 shows the IC_{50} Value of each extracts.

Table No. 2: Percentage of ferric reducing power of different leaves extracts of *Crinum solapurense*.

Sr. No.	Concentration in µg/ml	Percentage of ferric reducing power					
		Standard Tannic acid	Aqueous Extract	Ethanolic Extract	Ethyl Acetate Extract	Chloroform Extract	Petroleum Ether Extract
1	25	33.97± 0.123	31.48± 0.112	32.56± 0.119	25.68± 0.179	26.74± 0.149	30.65± 0.123

2	50	49.42± 0.105	46.78± 0.137	47.8± 0.121	37.49± 0.139	40.84± 0.104	42.48± 0.154
3	100	61.88± 0.167	58.96± 0.127	60.87± 0.164	55.6± 0.162	53.29± 0.112	55.39± 0.187
4	150	75.87± 0.143	72.49± 0.175	73.49± 0.186	69.4± 0.149	62.65± 0.195	66.89± 0.147
5	200	82.34± 0.165	81.2± 0.189	80.37± 0.194	76.58± 0.158	77.5± 0.136	73.65± 0.154
6	400	94.57± 0.140	92.36± 0.148	92.38± 0.169	88.39± 0.195	85.59± 0.127	85.86± 0.136
7	**IC_{50}**	**44.15**	**61.91**	**54.48**	**98.19**	**101.55**	**87.76**

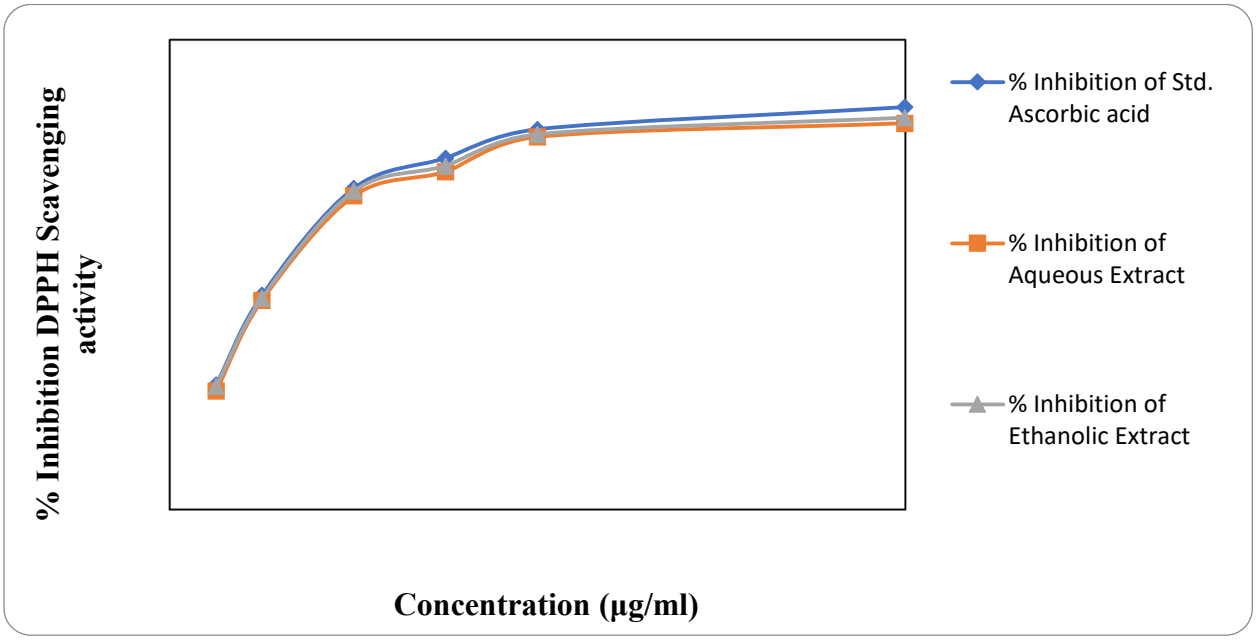

Fig. 1: DPPH free radical scavenging activity of Standard Ascorbic acid, Aqueous Extract, and Ethanolic extract of *Crinum solapurense*

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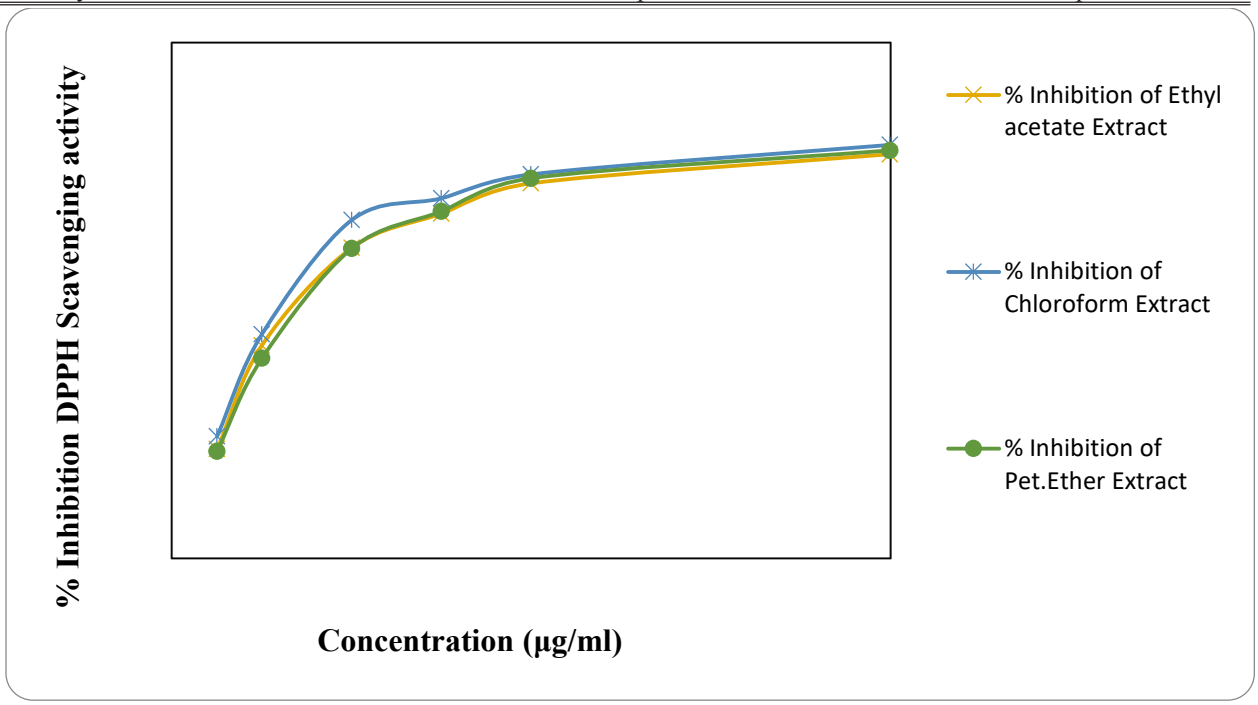

Fig. 2: DPPH free radical scavenging activity of Ethyl Acetate Extract, Chloroform Extract and Petroleum Ether Extract of *Crinum solapurense*

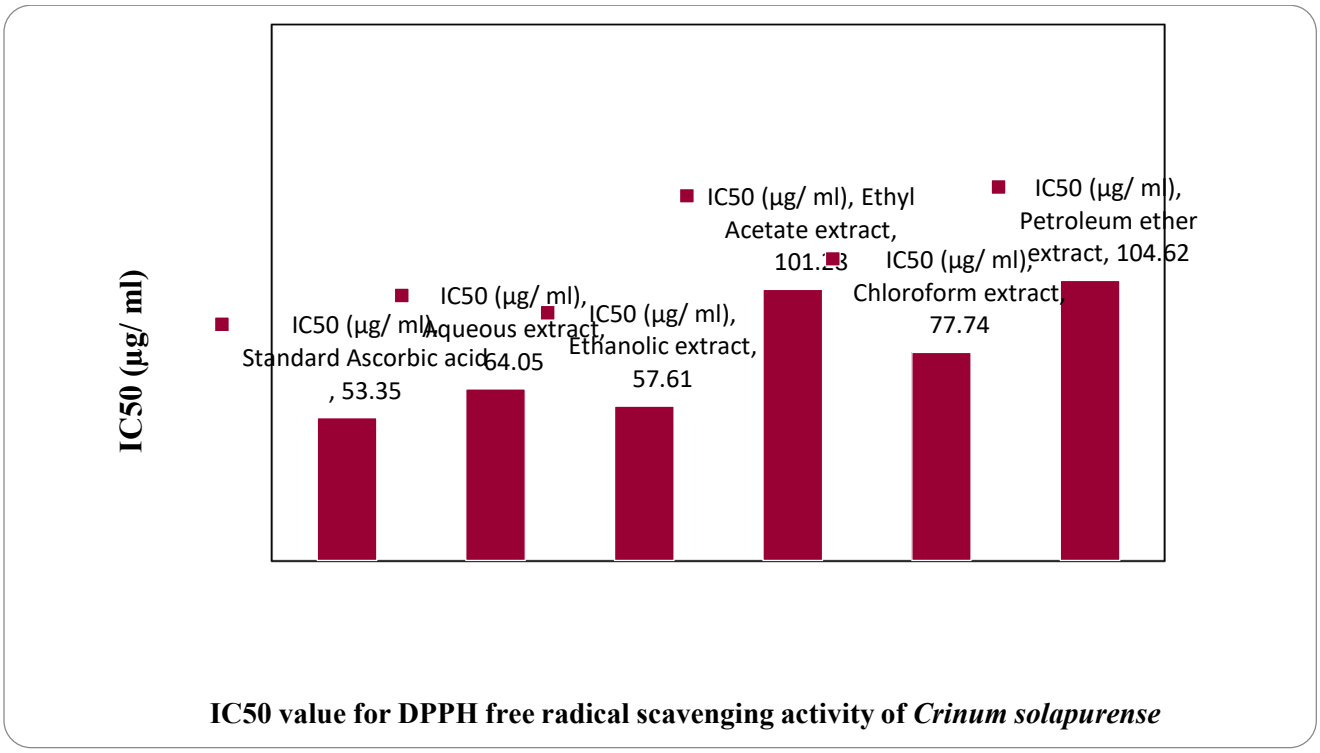

Fig. 3: DPPH free radical scavenging activity for IC_{50} of *Crinum solapurense leaves extract*

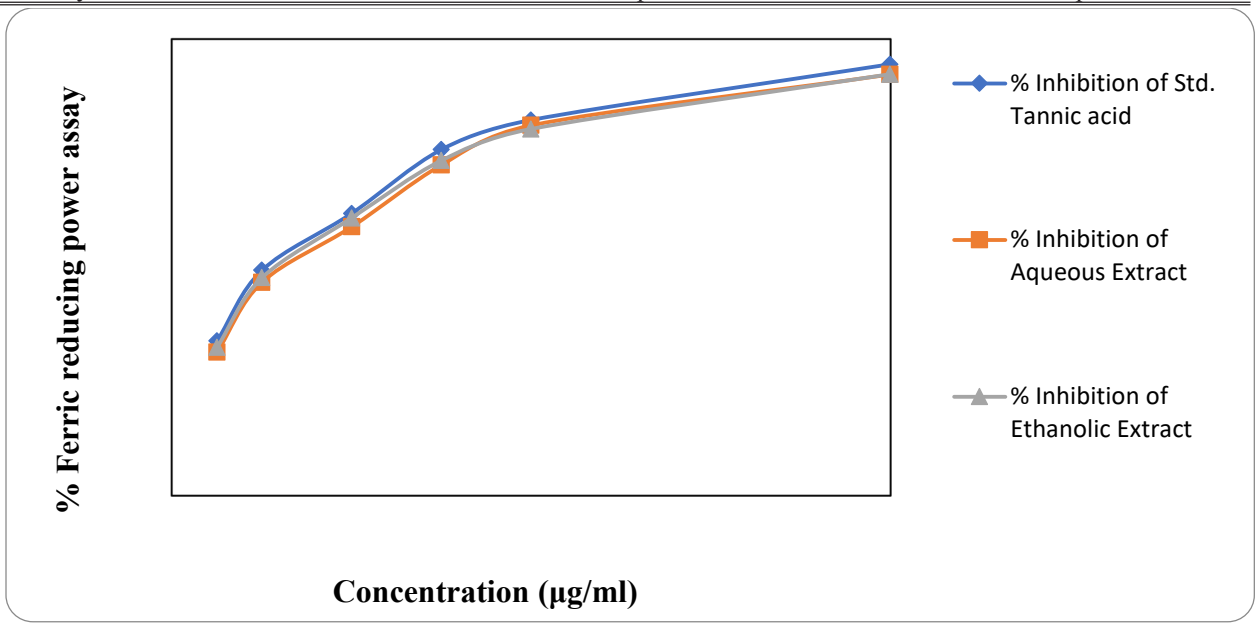

Fig. 4: Ferric reducing power assay of Standard Tannic acid, Aqueous Extract, and Ethanolic extract of *Crinum solapurense*

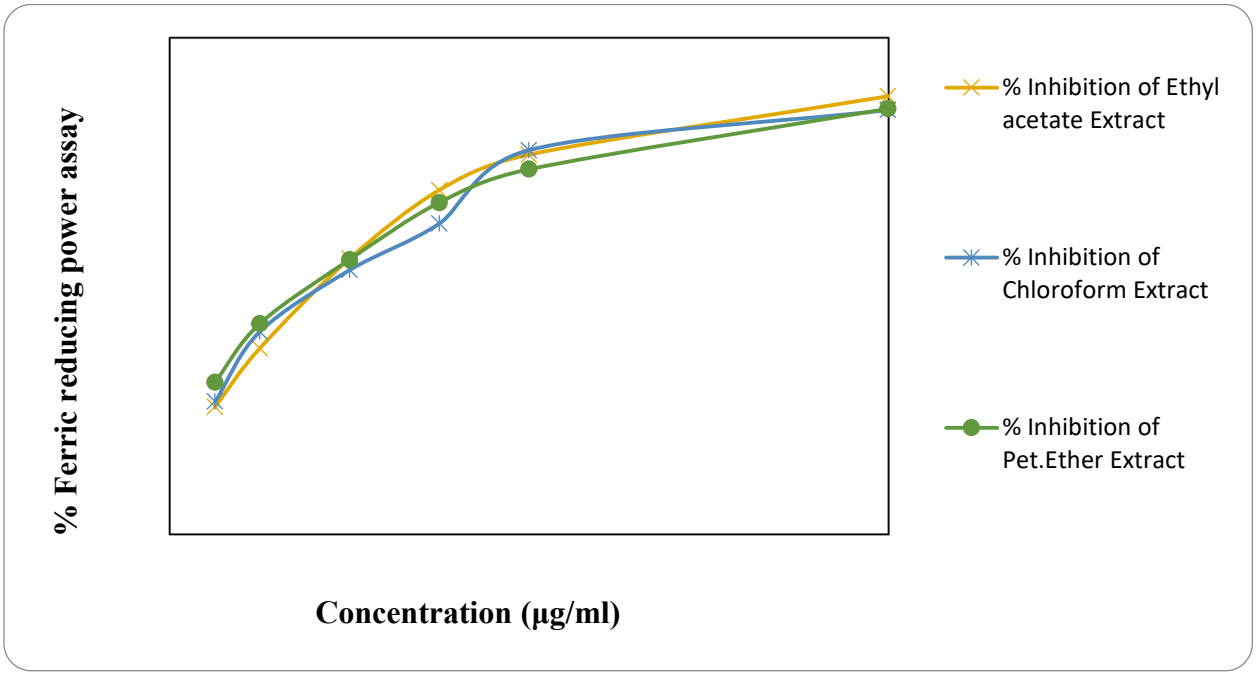

Fig. 5: Ferric reducing power assay of Ethyl Acetate Extract, Chloroform Extract and Petroleum Ether Extract of *Crinum solapurense*

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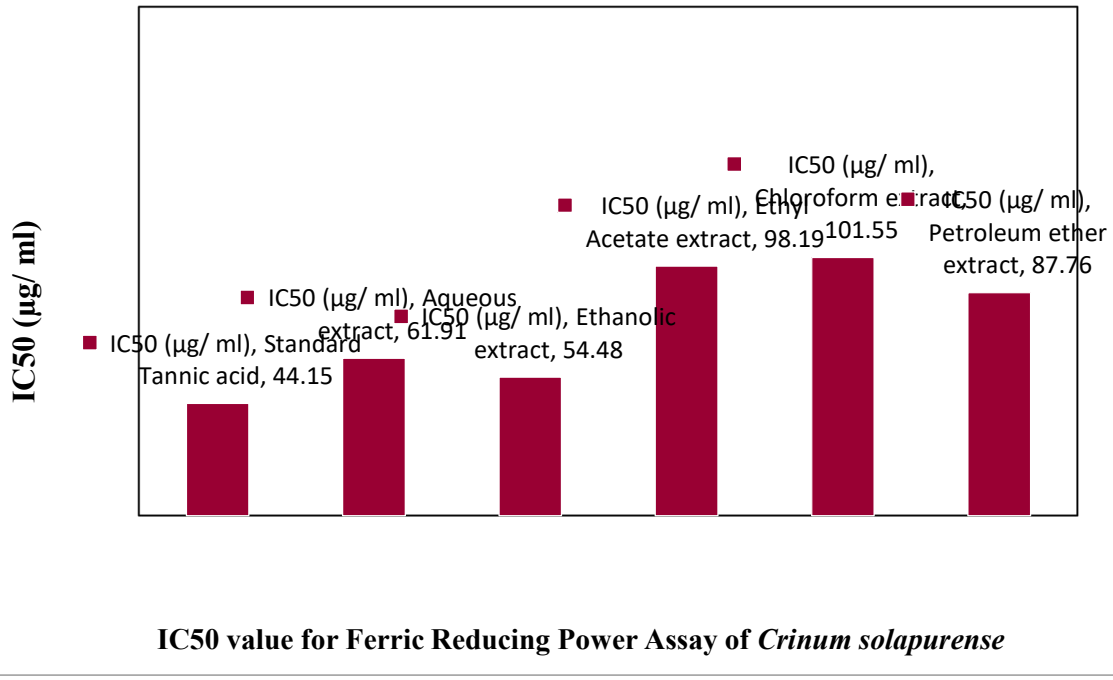

Fig. 6: Ferric Reducing Power Assay for IC_{50} of *Crinum solapurense leaves extract*

CONCLUSION:

From the present investigation of *in-vitro* antioxidant activity, it is concluded that the various leaves extracts of *Crinum solapurense* shows potential for DPPH radical scavenging activity and ferric reducing potential. The plant *Crinum solapurense* may represent a new source of antioxidants with stable, biologically active compounds that can establish a scientific base for use in modern medicine. In the study of all extracts ethanolic extract shows significant DPPH scavenging activity and ferric reducing power. This result is compared with the standard ascorbic acid and standard tannic acid respectively.

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