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A COMPREHENSIVE ANALYSIS OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND VITAMIN C FROM *HIBISCUS ROSA SINENSIS*

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ABSTRACT

Hibiscus rosa sinensis is shrub of the Malvacace family, native of tropical Asia. Due to its ethnomedicinal properties hibiscus flowers are used in many ways to prevent many diseases. Hibiscus also contains phytonutrients that include vitamins, minerals, flavonoids, terpenoids, anthocyanins, saponins, glycosides, tannins and vitamin c. The study was aimed at evaluating the antioxidant activity and Total phenolic content of the Methanolic extract of Hibiscus rosa sinensis flowers. The extract was found to contain excellent amounts of antioxidants, phenolic compounds and fair amounts of vitamin c. The results showed that DPPH Antioxidant Activity exhibited 89.49 % of inhibition against free radicals. Antioxidant activity by FRAP method showed 47597.03 for µg/g, Total phenolic content was 3146 of mg/100g and 16.49 mg /100g of vitamin c was present. All these phytonutrients exhibit pharmacological properties such as Antifertility activity, Hypotensive properties, wound healing properties, antimicrobial activity etc. Hibiscus flowers showed rich in poly phenols, Antioxidant and vitamin c content have the property of protecting cells from damage and malicious diseases. Dry hibiscus flowers rated highest organoleptic properties when compared to fresh hibiscus flowers during the sensory evaluation.

Key words: Hibiscus rosa sinensis, antioxidants activity, phenolic content, vitamin c , Sensory Evaluation.

INTRODUCTION

People have always looked to nature for drugs when they wanted to get better from a disease. The use of medicinal plants gradually became based on explicatory facts rather than an empiric framework [1]. Most medicinal plants have a mixture of different chemicals that can work together, separately, or together to improve health. For instance, a single plant may contain phenolic compounds that can serve as antioxidants, anti-bacterial and anti-fungal, tannins that serve as natural antibiotics, diuretic, substances that improve the elimination of waste products and toxins, alkaloids that improve mood and provide a sense of well-being, and bitter substances that encourage digestion [2].Hibiscus rosa-sinensis, a decorative plant; possesses a variety of pharmacological activities and is used to treat a variety of diseases [3] The medicinal plant *Hibiscus rosa-sinensis* is a member of the family Malvaceae. The tropical region of Asia is home to the hibiscus rosa-sinensis. The plant is a houseplant that can be found all over the world and is a native of China in southeastern Asia. Various illnesses, including alopecia, can be treated with *hibiscus rosa-sinensis*. In addition, it has traditionally been used as an anti-dandruff agent. *H. rosa-sinensis* flower contains the flavonoid quercetin. In addition, it improves the blood flow to the hair and stimulates hair follicles, both of which are useful for promoting hair growth [4]. *Hibiscus rosa-sinensis* (Malvaceae) is an enduring decorative bush accessible all through India. *Hibiscus rosa sinensis* is a medicinal plant with numerous pharmacological and therapeutic properties. It is known that the flowers, leaves, and roots of this plant have medicinal properties like oral contraception, laxative, aphrodisiac, menstrual cramping, and so on [5].

Fig: 1, Hibiscus Flower

Hibiscus rosa sinensis certain to emerge soon as a major player in the growing field of herbal health, nutritional and medicines both in daily self-care and in health care field. The principal constituents of Hibiscus rosa sinensis are flavonoids, Flavones contain quercetin-3-diglucoside, quercetin-3,7-diglucoside, cyaniding-3,5-diglucoside, quercetin-3-sophootrioside, kaempferol-3xylosylglucoside, cyaniding-3-sophoroside-5-glucoside and vitamin B1,vitamin B2, vitamin C. All the parts of Hibiscus rosa sinensis Linn and chemical constituents contains many ethnomedicinal values and pharmacological properties [6]. Bangladeshi women make a tea made of hibiscus flower petals. The Chinese use flower extract in hot water as a tonic and emmenagogue. East Indies use flower extract

to regulate menstruation and induce abortion. In Fiji, the infusion of dried flowers is used to aid digestion and hot water extract of flowers and leaves is taken orally to ease labors. In Haiti, flowers are used to treat the flu and cough, and in Hawaii, flowers are eaten to induce lactation [7].

Hibiscus rosa-sinensis L. (Malvaceae) is traditionally used as a natural diuretic in tea in West India. This plant's vitamin C-rich extract is traditionally used as a mild medicine [8]. Reports indicate that hibiscus flowers extracts can inhibit the growth of cancer cell types such as mammary carcinoma, leukemia, and melanoma. For instance, recent studies found that Hibiscus polyphenols inhibit melanoma cell, in this study, they investigated how melanoma cell growth was affected by aqueous *H. rosa-sinensis* flower extract. These studies show that this extract has components that stop melanoma cells from growing [9].

Hibiscus rosa-sinensis contains tannins, quinines, phenols, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, proteins, free amino acids, reducing sugar, mucilage, essential oils, and steroids, according to a phytochemical analysis. The all-out phenol, tannin, alkaloid, and flavonoid items in hibiscus tiliaceus L. Wood separates from petrol ether, ethyl acetic acid derivation, and methanol were analyzed in their study. Contrasting ethanol separate with petrol ether and methanolic separate, ethanol extricates uncovered higher phenol, alkaloid, flavonoid, and tannin contents [10]. Phenolic compounds and flavonoids were found to be abundant in the hibiscus extract. Also, the diminishing power (ability to decrease Fe3+ to Fe2+), the ability to rummage hydrogen peroxide, superoxide extremists and nitric oxide were assessed. Concentration-dependent scavenging activity was demonstrated by the extract [11]. when intact human RBCs were incubated with polyphenol rich extract, a strong protective effect against hydrogen peroxide induced hemolysis and lipid peroxidation was observed. It can be used for minimizing or preventing lipid oxidation, slow down the formation of toxic oxidation products and prolonging the shelf life of food and pharmaceuticals. *H. rosa sinensis* contained phenolic and flavonoid components and had superior antioxidant properties. The isolated compound can be used to scavenge free radicals, stop toxic products from forming, and extend the shelf life of food and drugs [12]. Hence the present study framed to investigate ‘The antioxidant activity and polyphenolic activity of *hibiscus rosa sinensis.*

2 MATERIALS AND METHODOLOGY

2.1 Collection of flowers

The flower of Hibiscus rosa-sinensis Linn were collected in the month of April 2023 from the home plants in Gajwel, Siddipet district, Telangana. The petals were removed from the collected flowers, washed with distilled water and were shade dried. The dried petals were ground into fine powder using a mortar and pestle. The powdered shade dried petals were further used for the extraction process and stored in airtight bottles.

2.2 Solvent extraction (Methanol extraction)

10 grams of flower powder were weighed and added to 100 ml of organic solvent (methanol) in a conical flask. After 24 hours, it was filtered using a thin muslin cloth and centrifuged at 5000 rpm for 15 minutes. The

precipitate was then gathered in a round base jar and the dissolvable was separated to make the last volume of one-fourth of the first volume, giving a convergence of 40_µg/0.1ml. It was put away at 40°C in water/air proof jugs for additional studies

2.3 Determination of Antioxidant Activity by DPPH method

The methanol extracts with various fractions (10, 50, 100, 200, 400, 600, µg/ml) were arranged utilizing methanol. In a solution of 1-100 g/ml, ascorbic acid served as the standard. 5 ml of the DPPH solution was mixed separately with 5 ml of the extract solution and standard solution after being prepared in ethanol to 0.004 percent. let the solutions in dark for 30 mins. The extent to which DPPH purple changed color to DPPH yellow indicated the extract's capacity for scavenging. The absorbance of the mix was resolved at 517 nm utilizing UV-Noticeable Spectrophotometer and ascorbic corrosive was filled in as a positive control. Lower absorbance of the response blend showed higher free extremist searching activity. (Debasis nayak,2012).
% DPPH scavenging effect = A0 – A1/A0 x 100

2.4 Determination of Antioxidant Activity by FRAP method

The FRAP reagent was prepared with acetate buffer (300 mM, pH 3.6),TPTZ (10 mM in HCl, 40 mM) and FeCl3 (20 mM). The ratio was 10:1 to 1 (v: v:v),individually. The FRAP reagent was diluted appropriately with the extract (1:30, v: v) were added, and the assay was carried out with 96-well plates and an automated microplate reader. The absorbance is taken at 593 nm and recorded. Each triplicate extract underwent the analysis in triplicate, and the calibration curve of Trolox was used to calculate the values, which ranged from 2.5 to 33 M. Per gram of dry weight, the results are expressed as mmol or g of Trolox equivalent (Monika skowyra,2014).

2.5 Determination of Total phenolic content

Total phenolics were resolved utilizing the Folin-Ciocalteau reagent Tests (2 g) were homogenized in 80% fluid ethanol at room temperature and centrifuged in cold at 10 000 g for 15 min and the supernatant was saved. The supernatants were pooled, placed in evaporating dishes, and evaporated to dryness at room temperature after the residue was re-extracted twice with 80% ethanol. Then it is dissolved with 5ml of distilled water. This extract was diluted to 3 milliliters with water and 0.5 ml of Folin–Ciocalteau reagent. Two ml of 20 percent sodium carbonate were added after three minutes, and the mixture was thoroughly mixed. The variety was created and absorbance estimated at 650 nm in a Bausch and Lomb spectronic-21 UVD spectrometer after 60 min utilizing catechol as a norm, the results were expressed as mg catechol/100 g of fresh weight material.(Biochemical methods,1996).

2.6 Determination of vitamin c

Around 100 g of the sample powder was thoroughly separated with ethanol. A residue was formed from the concentrated extract. For future use, the crude extract was kept in a refrigerator in a sterilized bottle. In a standard

flask that was made up to 100 milliliters with distilled water, 10 milliliters of each filtrate were combined with 20 percent glacial acetic acid.

Preparation of dye: The standard color arrangement was ready by dissolving 50mg of blue color in 50 ml of refined water. Filtered and kept, the mixture was diluted to 200 milliliters.

standard ascorbic arrangement: This was ready by dissolving 100mg glasslike ascorbic corrosive in 50 ml of 20% frosty acidic corrosive and weakened to 100 ml with refined water.

Methods for titration: 10 ml of the ascorbic corrosive arrangement was titrated with the color arrangement. Each drop of the color in touch with the arrangement becomes pink. The end point was reached when the pink variety goes on for 10 seconds. In a similar manner, 10 milliliters of each prepared sample were subsequently titrated with the due, and the titre values were recorded (Biochemical methods, 1996).

2.7 Sensory Evaluation

Sensory evaluation is done between the fresh hibiscus flowers and dried hibiscus flowers after making tea with dry and fresh flowers. Where panelists are asked to rate the acceptability of the samples by 5 point hedonic for the evaluation. The parameters are aroma, taste, colour, consistency, mouth feel and overall acceptability.

2.8 Statistical Analysis

Statistical analysis performed by T test to compare between the samples of Hibiscus flower dry and fresh.

3. RESULTS AND DISCUSSION

3.1. Antioxidant and Total Phenolic Activity of Hibiscus flower

The preliminary phytochemical investigation of the methanolic extracts of Hibiscus flowers showed the presence of Antioxidants, total phenols, and vitamin c (Table 1). Quantitative estimation of phytochemicals and vitamin c in methanolic flower extracts of Hibiscus flowers

Table 1: Antioxidant and total phenolic activity of Hibiscus flower

S. No	Parameters	Method of Analysis	Result
1	Antioxidant activity (% Inhibition)	DPPH method	89.49
2	Total Antioxidant Activity (µg/g)	FRAP method	47597.03
3	Vitamin c (mg/100g)	Biochemical methods, 2nd Edn. 1996	16.49
4	Total phenols (mg/100g)	Biochemical methods, 2nd Edn. 1996	3146.00

Methanolic concentrate of hibiscus flower powder showed that, Hibiscus rosa sinensis displayed brilliant Antioxidant Activity in the DPPH Technique, that is 89.49 % of Inhibition against free radicals for 100gms.Total

Antioxidant Activity of hibiscus flowers was likewise satisfying with the after effect of 47597.03 µg/g of Antioxidant capacity (Table 1). 16.49 mg/100g of L-ascorbic acid was available in hibiscus rosa sinensis subsequently helps in adding the worth of day-to-day intake of L-ascorbic acid. Total phenolic content was around 3146.00 of mg/100g, revealed higher amount of phenolic content. Antioxidants helps to protect cells from damage caused by free radicals, which are molecules produced when the body breaks down food or when exposed to radiation, tobacco smoke, or pollution. Thus, protecting the body from malicious diseases. Antioxidants present in the body are insufficient to subside the free radical damaging thus, dietary intake of antioxidants from hibiscus flowers helps in prevention of cell damage.

Similarly, when compared with (Yin Wei Mak et al., 2013) Antioxidant by DPPH method got a result of 83.08% which is lesser than the results of present research study. Antioxidant activity by FRAP method is 2349.06 µmoles/100g, which is also lesser than the present research study, Total phenolic content was 4598.16 GAE/100g was greater than the present research study. When compared with (Sumanta Mondal et al., 2020) Antioxidant Activity by DPPH method was reported as 91.15 % of free radical inhibition which little greater than the present research study. When compared to (Anusha Bhaskar et al., 2011) Antioxidant Activity by DPPH method was 49.7 % of free radical inhibition which is very less when compared with the present research work, where Total phenolic content was 259 GAE which is also less than the present research work. (Pankaj K. Tyagi, and Shruti Tyagi) got a result of 26.48 mg/100g of vitamin c, which is greater than than current work. (Zulfiqar Ali Khan et al) studies said that DPPH Antioxidant Activity represent 75.46 % of Inhibition against free radical scavenging activity, where Total phenolic content was 61.45±3.23 mg / 100gm which lesser the value of present research study.

3.2 Sensory Evaluation

Mean and standard deviation of the samples fresh flowers and dry flowers are represented in Table 2.

Table 2: Sensory Evaluation of Wet and Dry Hibiscus Flowers.

Parameter	Sample A (Fresh flowers)	Sample B (Dry Flowers)
Aroma	3.1 ±1.43	3.9±1.25
Taste	2.95 ±1.05	2.55±1.23
Colour	3.5±1.10	4.45±0.69
Consistency	3.35±0.88	4.15±0.59
Mouth feel	2.7±1.30	2.45±1.29
Overall acceptability	3.3±1.0	3.5±0.6

According to the sensory analysis results, sample B (dry) scored the highest on the hedonic scale with aroma (3.9), color (4.45), consistency (4.15) and overall acceptability (3.5) compared to sample A (wet) with aroma (3.1), color (3.5), consistency (3.35), and overall acceptability (3.3). Sample A (wet) scored the highest on the hedonic scale with taste (2.95) mouthfeel (2.7), and sample B was rated as taste (2.55) and mouthfeel (2.45). the above comparison states that sample B has better aroma, colour, consistency, overall acceptability whereas sample A has better taste and mouthfeel respectively.

3.3 STATISTICAL ANALYSIS

The significance p value (p = 0.05), compared with the other sensory parameters (Table 3)

Table 3: statistical analysis of hibiscus rosa sinensis

Parameters	P (T<= t) two
Aroma	0.013991
Taste	0.118933
Colour	0.002733
Consistency	0.000393
Mouth feel	0.3298
Overall acceptability	0.50614

The above Table 3 represents that aroma, colour, and consistency is significant, where as Taste, mouth feel and overall acceptability is not significant.

CONCLUSION

This research study Summarize that *Hibiscus rosa sinensis had* many medicinal and nutritional and phytochemicals properties that had beneficiary effect on human body. It is used for curing many diseases and also posses many pharmacological properties. Hibiscus rosa sinensis showed high in Antioxidant activity, total phenolic content along with vitamin c protect body against free radicals that causes damage to the body and causes lethal diseases such as cancer. The sensory properties shown excellent score for *Hibiscus rosa sinensis* Dry flower when compared to fresh flower sample.

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