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The Phytochemical and Antioxidant Property of Ethereal Extract of *Hibiscus rosasinensis* Leaves Extract.

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ABSTRACT

The plant *Hibiscus rosasinensis* commonly known as Jaswant in India has many uses in Ayurvedic treatment related to the different types of diseases. The present study about *Hibiscus rosasineneis* leaves are selected for the Phytochemical investigation and antioxidant properties. The Phytochemical ingredient like alkaloids, carbohydrate and phenols etc. are responsible to capture an electron and reduces itself and act as good antioxidant. That's why in the present study we have given the documentary evidence according to the presence of Phytochemicals in the ethanolic extract. For the comparison of the antioxidant property of the leaves extract ascorbic acid is used as the standard. The sample shows good antioxidant property. The $IC_{50}$of the ascorbic acid 14.97 and for the diethyl ether extract of leave is 46.85

Keywords: *Hibiscus rosasinensis*, Phytochemical, antioxidant property, ether extract.

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INTRODUCTION

From the past decades the traditional system of medicine become important, due to their safety and less side effect as compare to the synthetic drugs. [1]The present study is *Hibiscus rosasinensis* leaves. The plant of Hibiscus rosasinensis widely distributed throughout the world and commonly known as Jaswant in India.[2]The antioxidant property of plant extract depends on various factors like solvent used for extraction, methods of extraction, in case of supercritical extraction external conditions like temperature, pressure, solvent etc.[3] Flavonoids are good antioxidant. It is also reported that flavonoids and tannins are the major group of compounds that act as primary antioxidant or free radical scavengers.[4].The antioxidant property of flavonoids is due to simultaneous hydrogen atom donation to free radicals, electron transfer and metal chelation. In the case of antioxidant using DPPH, the suggested mechanism include hydrogen atom donation. [5]It is reported that the leaves of Bacopamannieri (L) has high extractive value for methanol (0.1%) than ethanol (8.6%), water (7.6%), chloroform(2%), acetone(1.5%) and dichloromethane (0. 6%).[6]It is reported that when antioxidant activity of amaranthusspinosus leaves extract is evaluated, the extract in 100% methanol exhibit high DPPH activity.[7]Free radicals are the molecule with unpaired single electron. Hence highly reactive. Such species react with DNA, proteins, and lipid cells. These causes oxidative trets . There are various extract such as aqueous stem bark extract schotialatifolia which shows considerable antioxidant property because of presence of phenolic ingredients.[8]Basis of the traditional medicinal systems are plants which exist thousands of years. Nowadays also for health system plants play the essential role. It has been proved by the world health organization that near about 80% of the world population of development country uses medicine based on herbal plants. [9] By the experimental studies shows that oxidation products of lipids called free radicals, can harmful to the healthy cells, it helps to create harmfull molecules and also helps to the degenerative process related to the aging and diseases, e.g. cancer, cardiovascular disease and neurodegenerative disorders like Alzheimer's disease. [10] The presence of antioxidant and antimicrobial constituents in plant spices or herbs can be suggested by the preservative effect. [11] Leaves extract of petridiumaquilinum Kuhn shows the good amount of antioxidant activity as compared with the ascorbic acid. [12] BHT and BHA are synthetic antioxidants which need to be replaced with natural antioxidants because they were toxic and carcinogenic in animal model. [13] Environmental variations can be significantly influenced plants internal organization. Ali Noman shows that as self defense system develops in plants under stress condition, plants experience changes like increase in the number of stomata and trichromes per unit area which prove to be a support to the plant for their survival in contaminated environment. [14]Phytochemical like phytosteroids, saponins and polyphenolic compounds can be caused anti-fertility effects of the test plantsand it was observed that anti-fertility effect of these components was sufficiently substantiated in animal models. [15]

MATERIAL AND METHODS

The leaves of *Hibiscus rosasinensis* were collected from the local area of Aurangabad. It was finely washed and dried under shade for 6 days. The dried leaves were grind using kitchen grinder and used for the further analysis. The chemicals used for the present research were purchased from S.D fine laboratory. Distilled again and used.

Sohxlate: 30 gms of the sample was subjected for the multiple extraction in Soxhlet apparatus. The solvent was used as Diethyl ether. Extraction was repeated till the colorless solution obtained in the soxhlet apparatus. Diethyl ether was distilled off after extraction.

Qualitative Phytochemical Screening:

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative Phytochemical analysis were done using the following procedures.[16]

Detection of alkaloids: Solvent free extract, 50 mg was stirred with 5 ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

i) **Wagner's test:** To a few ml of filtrate, few drops of Wager's reagents were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent: Iodine (1.27 g) and potassium iodide (2 g) was dissolved in 5 ml of water and made up to the 100 ml with distilled water**.**

ii) **Hager's test:** To a few ml of filtrate 1 or 2 of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive.

iii) **Dragendroff's test:** To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent were added. A prominent yellow precipitate indicates the test as positive.

Dragendroff's reagent:

Stock solution: Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few minutes with 50 ml glacial acetic acid. After 12 hrs, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, reddish brown filtrate, 40 ml was mixed with 160 ml ethyl acetate and 1 ml water and stored in amber color bottle.

Working solution: stock solution of 9.0 ml was mixed with 20 ml of acetic acid and made up to 100 ml with water.

Detection of Carbohydrate:

The extract 100 mg was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

i) **Molisch's tests:** To 2 ml of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

ii) **Fehling's test:** One ml of filtrate was boiled on water bath with 1 ml each Fehling's solutions A and B. Red precipitate indicates the presence of sugar.

Fehling's solution: Solution A ; copper sulphate (34.66 g) was dissolved in distilled water made up to 500 ml with distilled water.

Solution B: potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 ml.

iii) **Barfoed's test:** To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. red precipitate indicates the presence of sugar.

Barfoed's reagents: copper acetate 30.5 g was dissolved in 1.8 ml of glacial acetic acid.

iv) **Benedict's test:** To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristics colored precipitate indicates the presence of sugar.

Benedict's reagent : Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water and boiled to make it clear solution. Copper sulphate(17.3 g) dissolved in 100 ml distilled water.

Detection of glycosides: For the detection of glycosides, 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on water bath, filtered and the hydrolysate was subjected to the following test.

i) **Borntrager's test:** To 2 ml of the filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicates the presence of glycosides.

ii) **Legal's test:** Fifty mg of extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside was indicating the pink co**lor.**

Detection of proteins and Amino acids:

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through whatman filter paper no. 41 and filtrate was subjected to test of proteins and amino acids.

i) **Biuret test:** An aliqot of 2 ml of filtrate was treated withone drop of 2 % copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicates the presence of proteins.

ii) **Ninhydrine test:** two drops of ninhydrine solution (10 mg of ninhydrine in 200 ml of acetone) were added to two ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of phytosterols:

i) **Libermann-Burchard's test:** The extract (50 mg) was dissolved in 2 ml acetic anhydride. To this one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube. An array of color changes showed the presence of the phytosterols.

Detection of phenolic compounds and tannins:

i) **Ferric chloride test:** The extract (50 mg) was dissolved in 5 ml distilled water. To this few drops of neutral 5% ferric chloride solutions were added. A dark green color indicates the presence of phenolic compounds.
ii) **Gelatin test:** The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% solutions of gelatin containing 10% sodium chloride was added to it. White precipitate indicates the presence of tannins.
iii) **Leads acetate tests:** the extract (50 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of flavonoids compounds.
iv) **Alkaline reagent test:** an aqueous solution of the extract was treated with the 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.
v) **Antioxidant property:**
vi) Antioxidant properties of the samples were measured by DPPH (2,2-diphenyl picrylhydrazyl) method. It is very stable free radical commeriacally available. DPPH reacts with antioxidant molecules presents in the herbal extract and convert into diphenyl hydrazine. The intensity was messured at 517 nm. The different concentration of the extract was prepared. To that 1 ml of extract 1 ml DPPH and 3 ml ethanol were added. And the intensity of the color was measured at 517 nm. The DPPH radical scavenging capacity was calculated as the percentage inhibition.
vii) % Inhibition of DPPH radical = $\frac{Control-test}{Control} X\ 100$

RESULT AND DISCUSSION

Phytochemical test: Phytochemicals present in the plants are responsible for the antioxidant properties of the any plant. The ethereal extract of *Hibiscus rosasinensis* leaves shows the positive results for the alkaloids, carbohydrate, proteins and phenols (table 1).

Table 1: Phytochemical screening of diethyl ether extract of *Hibiscus rosasinenesis* leaves

Sr. No.	Reagent	DEE
1.	**Detection of Alkaloids**	
A.	Mayer's test	-ve
B.	Wagner's test	+ve
C.	Hager's test	-ve
2.	**Detection of carbohydrate**	
A.	Molish test	+ve
B.	Fehling's test	+ve
C.	Benedic test	-ve
D.	Barfoad's test	-ve
3.	**Detection of Glycosides**	
A.	Borntrager's test	-ve
B.	Legal's test	-ve
4.	**Foam test**	-ve
5.	**Detection of proteins and amino acid**	

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A.	Millon's test	-ve
B.	Nitric acid test	+ve
C.	Biuret test	-ve
D.	Ninhydrine test	+ve
6.	**Detection of phenolic compound and tannins**	
A.	Ferricchlorid test	+ve
B.	Gelatin test	-ve
C.	Lead acetate test	-ve
D.	Alkaline reagent test	+ve

Antioxidant property: The plants contain vitamins, terpenoids, phenolic acids, lignin, stilbenes, tannins, flavonoids, quinines, coumarins, alkaloids, etc. was free radical scavenging properties and these are presets Longley in plants. By absorbing free electron they themselves get reduced. Hence act as antioxidant[17].Phenolic compounds are responsible for the antioxidant properties of any herbs. [18]**.** The antioxidant property of ethereal extract of *Hibiscus rosasinensis* leaves were measured by DPPH method. (Table: 2 and 3) Ascorbic acid was used as the standard and it was observed that the sample shows less antioxidant property when low in concentration but as concentration increases, increase in % inhibition. The IC_{50} value of the ether extract is 46.85.

Table 2: DPPH free radical inhibition activity ofAscorbic acid (standard).

Absorbance of the sample at 517 nm Absorbance of control = 0.825

Sr. No.	Concentration µg/ml	Absorbance	% Inhibition	IC_{50} value µg/ml
1.	5	0.491	40.48	14.97
2.	10	0.453	45.09	
3.	20	0.370	55.15	
4.	30	0.335	59.39	
5.	40	0.271	67.15	
6.	50	0.264	68.00	

Table 3: Anti oxidant activity of *Hibiscus rosasinensis*leaves extract.

Absorbance of the sample at 517 nm Absorbance of control = 0.825

Extracts	Concentration µg/ml	Absorbance	% Inhibition	IC_{50} value µg/ml
Diethyl ether	5	0.504	38.90	46.85
	10	0.494	40.12	
	20	0.464	43.75	
	30	0.442	46.42	
	40	0.419	49.21	
	50	0.408	50.54	

CONCLUSION

The result of this preliminary study shows the presence of alkaloids, carbohydrates, proteins, amino acids and phenols. The antioxidant property of phenols is due to presence of phenols. Ethanol extract of *Hibiscus rosasinensis* shows good antioxidant properties. The present study can be expand by the investigation of other anti-microbial study.

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