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A study on the antioxidant and antibacterial potential of the mucilage isolated from *Hibiscus rosa-sinensis* Linn. (Malvaceae)

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Abstract

The present study evaluates the *in vitro* antioxidant and antibacterial activity of the mucilage of *Hibiscus rosa-sinensis*, a plant of immense medicinal value in Chinese herbology. The mucilage was isolated from *Hibiscus rosa-sinensis* leaves using solvent precipitation method and the antioxidant activity was tested using different assays such as FRAP, DPPH, hydroxyl, superoxide, nitric oxide, and hydrogen peroxide scavenging assays. The mucilage gave positive results for all the assays but the radical scavenging ability was detected to be lower than those of the corresponding standards. The antibacterial assay carried out using agar well diffusion method revealed positive results against the gram positive *Streptococcus pyogenes* and gram negative *Klebsiella pneumoniae*.

Keywords: *Hibiscus rosa-sinensis*, mucilage, antioxidants, antioxidant assays, antibacterial activity

1. Introduction

Degenerative and pathogenic diseases are two major concerns of medical interest. The development of many degenerative ailments such as dementia, arthritis, mongolism, carcinoma and Parkinson's disease are associated with oxidative stress [1]. It is believed that the chain reaction initiated by reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, nitric oxide, hydroxyl radical, hypochlorite radical, singlet oxygen and various lipid peroxides leads to the onset of such vicious diseases [2].

Plants serve as a rich source of natural antioxidants and have a potential role in preventing human diseases [3]. Several plant chemicals such as phenols and flavonoids inhibit the chain propagation reactions initiated by free radical reactions and hence are said to possess antioxidant property [4]. Polyphenols are reported to have anticarcinogenic, antimutagenic and cardioprotective effects, owing to their free radical scavenging activity [5]. Flavonoids can protect the human body through their antitumor, anti-inflammatory, antimicrobial, antiallergic and antioxidant activities [6, 7].

The present study is concerned with the analysis of the antioxidant and antibacterial potential of the mucilage isolated from *Hibiscus rosa-sinensis*, a very common ornamental, belonging to the family Malvaceae. Mucilages are normal metabolic products formed from sugars and uronic acid units in plant cells [8]. Mucilages are not merely carbohydrate reserves in plant cells, they are reported to possess bioactive properties as well [9, 10]. *Hibiscus rosa-sinensis* is considered to have numerous medical uses in Chinese herbology and has been extensively documented in the literature for its use in the treatment of coughs, venereal diseases, for relieving pain, as laxative, and also for its medicinal properties such as antidiarrhetic, antihypertensive and antiphlogistic [11, 12]. The plant is characterised by the presence of copious amounts of mucilage, however there are no reports on the bioactive properties of its mucilage. So, it would be ideal to explore the free radical scavenging potential and antibacterial activity of *Hibiscus rosa-sinensis* mucilage.

2. Materials and Methods

2.1 Plant Collection

Leaves of *Hibiscus rosa-sinensis* Linn. Were collected from a home garden at Karamana (Kerala, India) in the month of November-December, 2015. The plant was authenticated by the herbarium curator, Department of Botany, Kerala University, Thiruvananthapuram and the voucher specimen was deposited in the Department Herbarium (KUBH 6035). The fresh leaves of *Hibiscus rosa-sinensis* were collected, washed with water to remove dirt and debris, and shade dried for 20 days. The dried leaves were ground to a fine powder and the leaf powder was stored in air tight containers.

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2.2 Chemicals: Methanol, Ascorbic acid, Phosphate buffer, Butylated hydroxyl toluene (BHT), 1, 1- Diphenyl-2-picryl hydrazyl (DPPH), Potassium persulphate, Potassium ferricyanide, Trichloro acetic acid, Sodium nitroprusside, Hydrogen peroxide, Hydrochloric acid, Sulfanilic acid, Glacial acetic acid, Naphthyl ethylenediamine dichloride (NEDD), ferric chloride, Potassium metabisulphite, EDTA, DMSO, Ammonium acetate, Acetone, NADH. All chemicals were of analytical grade.

2.3 Mucilage extraction procedure

The method of extraction of mucilage was adopted from Vignesh and Bindu (2018) [13]. The powdered material was soaked in water for 5 hours at 50 °C. To remove the marc from the solution, it was filtered through an eight layered muslin cloth. Finally, the mucilage was precipitated by adding acetone to the filtrate and oven dried for further analyses.

2.4 Antioxidant assays

2.4.1 Reducing power assay: Different concentrations of the plant mucilage (20-100 µg/ml) was combined with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The combinations were incubated for 20 min at 50 °C. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 2000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the obtained solution was measured at 700 nm [14].

2.4.2 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay: One ml of 0.13 mM DPPH prepared in DMSO was mixed with 1.0 ml of the mucilage ranging from 20-80 µg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517nm [15]. The scavenging ability of the plant extract was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{(*\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \right] \times 100$$

*Abs control is the absorbance of DPPH + DMSO; Abs sample is the absorbance of DPPH radical + sample.

2.4.3 Hydroxyl radical scavenging activity: Various concentrations (20, 40, 60, 80 and 100 µg/ml) of mucilage were taken in different test tubes and evaporated to dryness. One ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 °C for 15 min., the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5% w/v). Three ml of Nash reagent was added to all of the tubes and left at room temperature for 15min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank [16].

2.4.4 Nitric oxide scavenging activity: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions determined by the use of Griess reagent. Two ml of 10 mM Sodium nitroprusside dissolved in 0.5 ml phosphate

buffer saline (pH 7.4) was mixed with 0.5 ml of plant mucilage at various concentrations (20-80 µg/ml). The mixture was incubated at 25 °C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm [17]. The amount of nitric oxide radical was calculated following this equation:

$$\% \text{ of inhibition of NO} = \left[\frac{(*\text{A}_0 - \text{A}_1)}{\text{A}_0} \right] \times 100.$$

*A₀ is the absorbance before reaction and A₁ is the absorbance after reaction has taken place.

2.4.5 Superoxide anion scavenging activity: The reaction mixture consisting of 1ml of plant mucilage (20-80 µg/ml), 1 ml of PMS (60 µM) prepared in phosphate buffer (0.1 M pH 7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25 °C for 5 min. The absorbance was read at 560 nm against blank samples [18].

2.4.6 Hydrogen peroxide scavenging activity: The plant mucilage (4 ml) prepared in distilled water at various concentrations (20-80 µg/ml) were mixed with 0.6 ml of 4mM H₂O₂ solution prepared in phosphate buffer (0.1M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the mucilage without H₂O₂ [19].

2.5 Anti-Bacterial activity

Antibacterial activity of the mucilage was tested using Agar well diffusion method. The activity is tested against *Klebsiella pneumoniae* (a gram negative bacteria) and *Streptococcus pyogenes* (a gram positive bacteria) obtained from MTCC, Chandigarh. Petriplates containing 20 ml Muller-Hinton Agar Medium were seeded with the bacterial cultures (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and sample of 25, 50, 100 µl concentrations were added. The plates were then incubated at 37 °C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin, a standard anti-bacterial agent (20 µg/µl) was used as a positive control [20].

2.6 Statistical analyses

All analyses were done at least in triplicate, and these values were then presented as average values along with their standard deviations. Statistical comparisons were performed with one-way analysis of variance. Data were analyzed using the SPSS software (version: IBM Statistics 22).

3. Results

3.1 Antioxidant activity

The results of the antioxidant assays conducted are presented in tables 1-3.

The reducing capacity of the *Hibiscus rosa-sinensis* mucilage was found to be appreciable.

The results showed that there was an increase in reducing power of the plant mucilage as the mucilage concentration increased (Table 1). The isolated mucilage was found to be a potent scavengers of DPPH, hydroxyl, nitric oxide, superoxide and peroxy radical as there was an increase in inhibition percentages with an increase in the concentration of the mucilage but the values obtained were less by almost half when compared to the corresponding standards (Table 2 -3).

Table 1: Reducing power assay using *Hibiscus rosa-sinensis* mucilage

Concentration of the mucilage µg/ml	Absorbance value of <i>H. rosa-sinensis</i> mucilage	Absorbance value of *BHT
20	0.041±0.12	0.081±0.10
40	0.064±0.11	0.158±0.13
60	0.102±0.07	0.227±0.05
80	0.128±0.02	0.304±0.12
100	0.145±0.08	0.371±0.12

*BHT- Butylated Hydroxyl Toluene

Table 2: DPPH assay and hydroxyl scavenging assay using *Hibiscus rosa-sinensis* mucilage

Concentration of the mucilage (µg/ml)	DPPH scavenging assay		Hydroxyl scavenging assay	
	% of inhibition by <i>H. rosa-sinensis</i> mucilage	% of inhibition by Ascorbic acid	% of inhibition by <i>H. rosa-sinensis</i> mucilage	% of inhibition by Ascorbic acid
20	18.92±0.23	39.50±0.01	10.42±0.17	35.00±0.03
40	23.21±0.19	50.21±0.09	18.16±0.06	43.24±0.10
60	32.23±0.02	75.06±0.03	25.11±0.04	65.27±0.23
80	44.55±0.05	83.73±0.09	34.51±0.12	76.12±0.12

Table 3: Hydrogen peroxide, superoxide and nitric oxide scavenging assay using *Hibiscus rosa-sinensis* mucilage

Concentration of the mucilage (µg/ml)	Hydrogen peroxide scavenging assay		Superoxide scavenging assay		Nitric oxide scavenging assay	
	% inhibition by <i>H. rosa-sinensis</i> mucilage	% inhibition by BHT(*)	% inhibition by <i>H. rosa-sinensis</i> mucilage	% inhibition by BHT(*)	% inhibition by <i>H. rosa-sinensis</i> mucilage	% inhibition by BHT(*)
20	12.31±0.02	39.53±0.07	16.65±0.65	57.18±0.21	12.02±0.18	46.28±0.92
40	19.47±0.21	51.82±0.32	24.23±0.81	65.39±0.39	18.80±0.56	57.45±0.67
60	27.16±0.13	68.34±0.64	32.16±0.23	72.68±0.63	23.95±0.73	68.79±0.56
80	39.51±0.72	76.59±0.32	38.82±0.43	79.05±0.54	36.59±0.87	78.34±0.49

3.2 Anti-Bacterial activity

The isolated mucilage showed activity against both gram positive *Streptococcus pyogenes* and gram negative *Klebsiella pneumoniae*. The activity was more against *Streptococcus* than against *Klebsiella* as evident from the zone values in the table 4.

Table 4: Anti-bacterial activity of *Hibiscus rosa-sinensis* mucilage (HMUC) against *klebsiella pneumoniae* and *streptococcus pyogenes*

Concentration (µg/ml)	Zone of inhibition (cm)	
	<i>Klebsiella pneumoniae</i>	<i>Streptococcus pyogenes</i>
Streptomycin	3.1 ± 0.21	3.3 ± 0.13
HMUC 25	Nil	1.1 ± 0.10
HMUC 50	1.2 ± 0.32	1.4 ± 0.24
HMUC 100	1.5 ± 0.12	1.7 ± 0.21

4. Discussion

Free radicals are generated constantly in the living systems. They cause considerable damage to biomolecules, ultimately leading to tissue damage and associated degenerative diseases. Such tissue damage could be prevented through the mediation of cell signalling modulations by antioxidants [21]. Synthetic and natural antioxidants are known to exist. However, synthetic antioxidants like butyl hydroxyl anisole (BHA) and butyl hydroxyl toluene (BHT) are reported to have specific toxic effects to lungs, foremost stomach and liver [22]. Hence, combating free radicals using natural antioxidant phytochemicals is an area of recent research interest.

Antioxidant assays are used to test the antioxidant potential of plant extracts and pure compounds. The assays carried out in the present study and their efficacies are discussed below:

The principle of reducing power assay is based on the reduction of ferric to ferrous in the presence of antioxidants. The reducing ability depends on the electron donating ability of the antioxidant phytochemical which may reduce the

intermediates in the chain reactions involved in the peroxidation of lipids, thereby act as both primary and secondary antioxidants [23]. DPPH is a stable free radical with a characteristic absorption peak at 517 nm. The degree of reduction in absorbance measurement is indicative of radical scavenging potential of the extract [24]. Hydroxyl radicals are extremely reactive species formed in living systems and has the capacity to break DNA strands leading to cytotoxic and carcinogenic effects [25]. This highly damaging radical is known to be a potential source of active oxygen species that cause lipid peroxidation and subsequent damage to tissues [26]. Nitric oxide radicals are produced in the body during inflammatory reactions which are associated with various chronic disease conditions such as multiple sclerosis, arthritis, juvenile diabetes and ulcerative colitis [27]. Superoxide anion being a strong reactive free radical has damaging effects on cells and DNA leading to associated disorders [28]. Though hydrogen peroxide is not very reactive, it can be toxic once they penetrate the cell membrane as it possibly gets converted to hydroxyl radicals through their probable reaction with ferrous and cuprous ions [29].

Hibiscus leaf mucilage shows comparatively less antioxidant activity compared to the *Hibiscus* leaf extracts as early reports suggest the DPPH scavenging activity of the extract as 84.01% (conc. 60 µg/ml) while the superoxide and hydrogen peroxide radical scavenging activity of the extract was observed to be 68 ±3.4% and 51.9 ±4.5% (conc. 20 µg/ml) [30]. This could be attributed to the presence of antioxidant phytochemicals like tannins (8.5±0.22%), phenols (0.68 ± 0.11%) and flavonoids (0.40 ± 0.15%) in *Hibiscus* leaf extracts [31].

Glucuronic acids and Galacturonic acids are present in significant amounts in *Hibiscus* mucilage [32] and it is reported that uronic acids have high antioxidant potential [33, 34]. The revealed antioxidant property of the mucilage may be due to the hydrogen donating ability of uronic acids.

Mucilage showed activity against both the gram positive and gram negative bacteria studied. The antimicrobial activity was comparatively higher for the mucilage against *Streptococcus pyogenes* than against *Klebsiella pneumonia* (Table 4). The exhibited antibacterial property of the mucilage can be attributed to the presence of antimicrobial components such as uronic acids. Uronic acids and its derivatives are considered to have good antimicrobial activity. A derivative of uronic acid, 4, 5-dihydroxy-2-cyclopentan-1-one (DHCP) is reported to have good antibacterial activity against *Escherichia coli* [35]. The antibacterial activity can also be attributed to the presence of phenolics and flavonoids in the isolated mucilage. Raghavamma *et al.* (2014) [9] reported that the antimicrobial property of the mucilage of *Coccinia grandis* fruit could be due to the presence of quercetin, a flavonoid. The antibacterial activity of the mucilage extracted from the tuber of *Dioscorea esculenta*, on various bacterial strains investigated showed that the mucilage was effective against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but ineffective against *Klebsiella pneumoniae* and *Streptococcus pyogenes* [36]. However, a better antibacterial activity against both *Klebsiella pneumoniae* and *Streptococcus pyogenes* has been shown by the mucilage of *Hibiscus rosa-sinensis* in the present study. This property of the mucilage can make it an important ingredient of many food preservatives, pharmaceuticals and also in cosmetic industry.

5. Conclusion

The present work focuses on the antioxidant and antibacterial activity of the mucilage of *Hibiscus rosa-sinensis*. The mucilage from the leaves of the plant exhibits antioxidant and free radical scavenging activities though lower than that of standards such as BHT and Ascorbic acid. These *in vitro* assays suggest that though small, the antioxidant activity of the mucilage contributes to the medicinal property of the plant. Moreover, the mucilage showed activity against both the tested bacteria, *Klebsiella pneumoniae* and *Streptococcus pyogenes*. Further research is required to understand its synergistic action with other antioxidant phytochemicals having advantageous properties.

6. Conflict of interests

The authors declare that there is no conflict of interest.

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