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In Vitro Assessment Of The Effectiveness Of Hibiscus Rosa-Sinensis Linn Flower Extract For The Treatment Of PCOS

Shahada K[1], Mrs. Anaswara R Nath[2]
Grace College Of Pharmacy, Palakkad
Assistant Professor, Department Of Pharmacology, Grace College Of Pharmacy, Palakkad

Abstract
This study explores the antioxidant and enzyme inhibitory effects of Hibiscus rosa-sinensis Linn flower aqueous extract in the management of PCOS. Using DPPH and FRAP assays, and alpha-amylase and alpha-glucosidase inhibition assays, the extract showed moderate antioxidant properties and weak enzyme inhibition compared to standard drugs. These results suggest potential, though further studies are needed to isolate active compounds and improve efficacy.

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I. Introduction

This is PCOS condition, which, according to medical study, afflicts up to 5-10% of reproductive women with symptoms of insulin resistance, excess and excess androgen production and ovarian dysfunction. Metformin and hormonal therapists for the management of this sickness are often associated with side effects; this has lead to the need to turn to natural remedies. Scientifically, this is Hibiscus rosa-sinensis Linn and it had been found in folklore medical use for many diseases due to its antioxidant, inflammation suppressor, hypoglycemic effects.Some recent clinical studies have proposed that aspects of oxidative stress and the change in the activities of certain enzymes are involved in the development of PCOS .Therefore, The Hibiscus rosa sinensis flower extract contains enough antioxidants and enzyme inhibitions, which can be substituted for PCOS remedy. This research aims at evaluating the antioxidant activity of Hibiscus rosa sinensis flowers aqueous extract in DPPH and FRAPS assays and studying the extract's effect on alpha amylase and alpha glucosidase enzyme inhibition.

II. Materials And Methods

Preparation of Hibiscus rosa-sinensis Aqueous Extract:

The plants that were used were Hibiscus rosa sinensis flowers, used fresh flowers, washed appropriately and allowed to dry for 3 to 5 days and then grinded into the powdered form. The powder was dissolved in about 20 g in 1 L distilled water for 5 minutes, filtered and then the mixture was concentrated in a rotary evaporator. It was kept under 20 °C.

Antioxidant Activity:

1. **DPPH Assay:** The extract was diluted 10, 20, 50 and 100 micro gram/mL and 2ml of 0.1mM DPPH to each tube and incubated in another dark room for 30 minutes. The concentration of the samples was calculated using absorbance obtained from the percent scavenging activity was determined by UV-Vis spectrophotometer at 517nm.
2. **FRAP Assay:** The extract (10, 20, 50, 100 µg/mL) was prepared from each sample and 2.5 mL of FRAP reagent is added and both were allowed to incubate for 30-minutes at 37ºC.Ferric-reducing power was determined at amount of absorbance at 593 nm .

Enzyme Inhibition Assays:

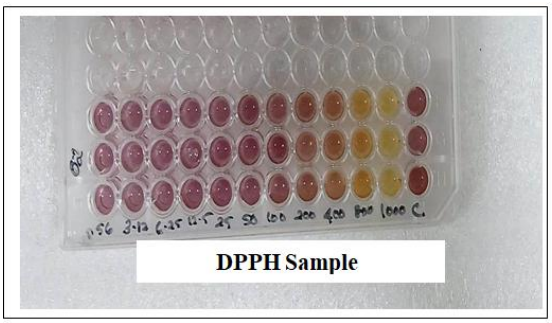
1. **Alpha-Amylase Inhibition Assay:** The extract (10, 20, 50, 100 µg/mL was mixed with 500µL of starch (1% w/v) and 500µL of alpha-amylase (0.5 mg/mL) and incubated at 37°C for 10- minutes. The reaction was then terminated with 1mL of DNS reagent and heated at 90°C for 5-minutes. Absorbance is measured at 540 nm.
2. **Alpha-Glucosidase Inhibition Assay:** Thus, the extract concentrations were 10, 20, 50 and 100 micrograms/mL, to which 500 µL of p-NPG solution at 5mM, and 500µL of the enzyme alpha-glucosidase at

0.5 U/mL was added into, and then incubated at 37°C for 30minutes.The Absorbance had been measured at 405 nm.

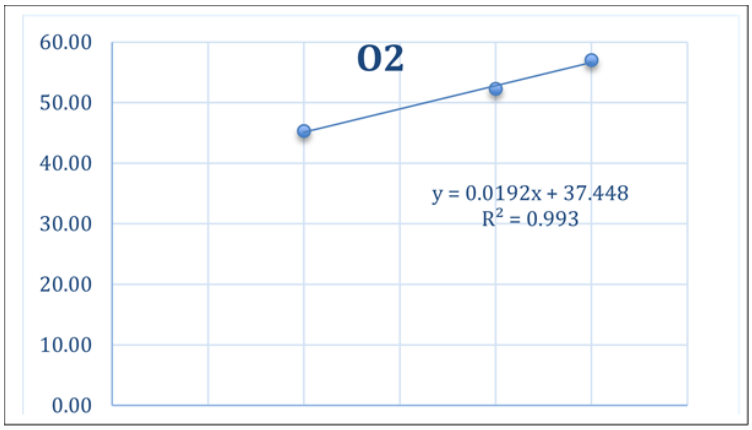
III. Results

The antioxidant and enzyme inhibition activities of Hibiscus rosa-sinensis aqueous extract were evaluated using DPPH, FRAP, alpha-glucosidase inhibition assays and Alpha amylase inhibition assay.

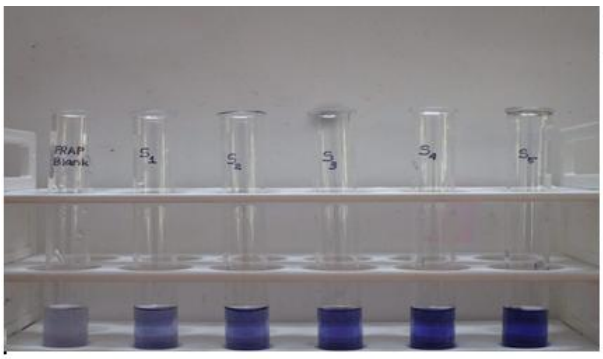
DPPH assay: The extract showed an IC_{50} of 661.05 µg/mL, much higher than ascorbic acid (28.19µg/mL), indicating weaker radical scavenging activity.



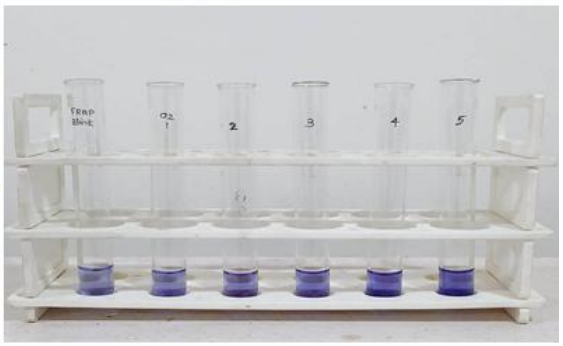
Standard	Concentration (µg/ml)	Absorbance at 515nm			Triplicate Average	Percentage Inhibition (%)
		OD1	OD2	OD3		
Control	-	0.8231	0.8226	0.8229	0.8229	-
Ascorbic acid	1.56	0.7987	0.7989	0.7881	0.7952	3.36
	3.12	0.7272	0.7274	0.7275	0.7274	11.61
	6.25	0.6813	0.6810	0.6813	0.6812	17.22
	12.5	0.5518	0.5514	0.5514	0.5515	32.98
	25	0.4585	0.4588	0.4588	0.4587	44.26
	50	0.1465	0.1466	0.1466	0.1466	82.19
	100	0.0926	0.0929	0.0922	0.0926	88.75
	200	0.0841	0.0848	0.0842	0.0844	89.75
	400	0.0654	0.0648	0.0647	0.0650	92.11
	800	0.0418	0.0411	0.0417	0.0415	94.95
	IC 50					**28.19**



FRAP assay: At 100 µg/mL, the extract's absorbance was 0.696 at 593 nm, lower than ferrous sulphate (2.174), reflecting reduced ferric-reducing potential**.**



FRAP Standard

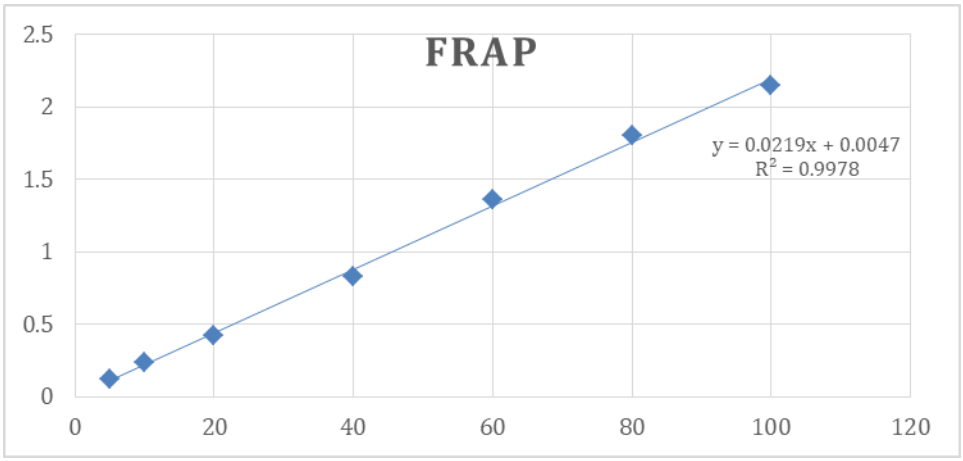


FRAP Sample

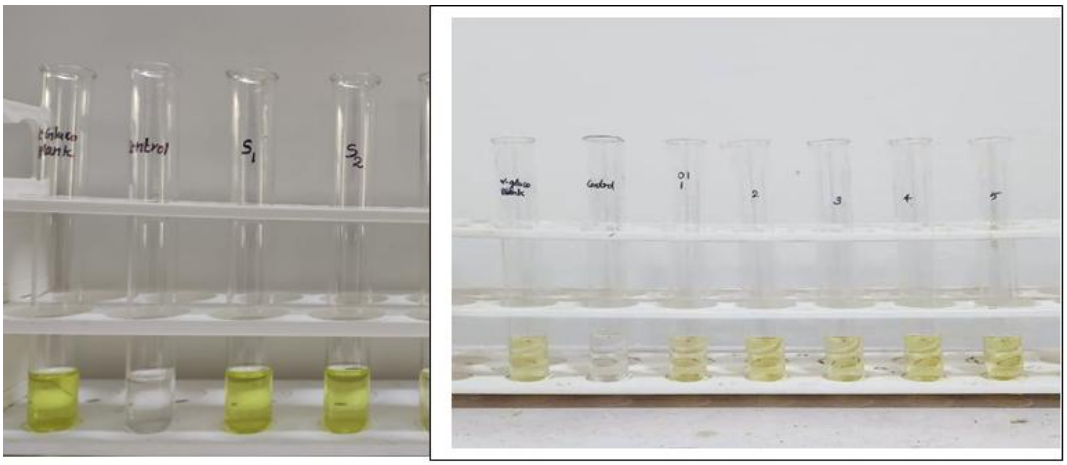
Standard	Concentration (ng/ml)	Absorbance at 593nm
FeSO4 (3.6µM/ ml)	5	0.111
	10	0.256
	25	0.562
	50	1.117
	100	2.174

Sample code	Concentration (ng/ml)	Absorbance at 593nm	Fe^{2+}(ng/ml)
O2	6.25	0.051	1.29
	12.5	0.134	5.24
	25	0.313	13.76
	50	0.447	20.14
	100	0.696	32.00

Sample code	Concentration (ng/ml)	Avg.OD at 593nm	Fe2+(ng/ml)
SWCM	6.25	0.07	2.98
	12.5	0.19	8.46
	25	0.369	16.63
	50	0.458	20.70
	100	0.599	27.14
SWIM	6.25	0.196	8.74
	12.5	0.433	19.56
	25	0.604	27.37
	50	0.951	43.21
	100	1.139	51.79



Alpha-glucosidase inhibition assay: The extract showed 33.17% inhibition at 100 µg/mL with an IC_{50}>100 µg/mL, compared to acarbose's 97.64% inhibition and IC_{50} of 16.72 µg/mL, indicating weaker enzyme inhibition.



Alpha-glucosidase inhibition assay-test

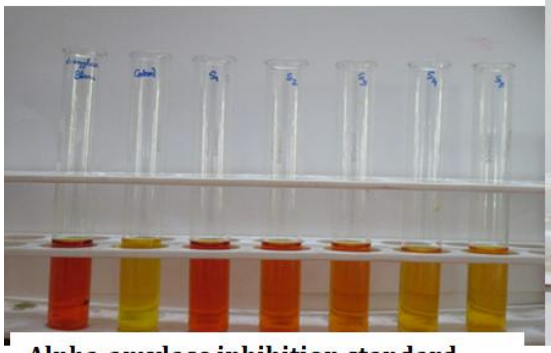
Sample code	Concentration (µg/ml)	Absorbance at 400nm	Percentage of Inhibition (%)
Blank	-	0.704	-
Control	-	0.026	-
Acarbose (standard)	6.25	0.567	20.20
	12.5	0.442	38.64
	25	0.208	73.15
	50	0.050	96.46
	100	0.042	97.64
IC 50	**16.72**		

Alpha-glucosidase inhibition standard

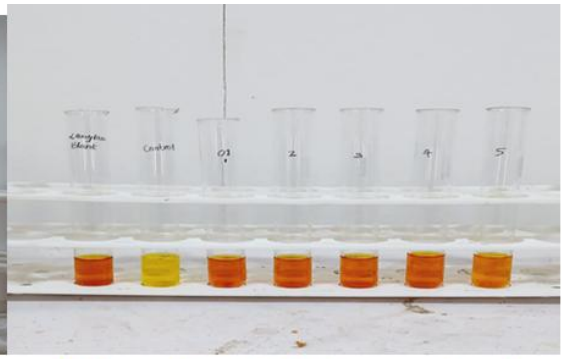
Sample code	Concentration (µg/ml)	Absorbance at 400nm	Percentage of Inhibition (%)
Blank	-	1.228	-
Control	-	0.034	-
O1	6.25	1.218	0.84
	12.5	1.116	9.38
	25	1.012	18.09
	50	0.914	26.30
	100	0.832	33.17
	IC 50		**>100 µg/ml**

IC 50	**>100µg/mL**

Alpha-amylase inhibition assay: The extract exhibited weak inhibition with an IC_{50}>100 µg/mL and less than 50% inhibition at 100 µg/mL, compared to acarbose's stronger effect with an IC_{50} of 36.44 µg/mL at 540 nm.



Alpha-amylase inhibition standard



Alpha-amylase inhibition assay-Sample

Sample code	Concentration (µg/ml)	Absorbance at 540 nm			Percentage Inhibition (%)
Blank	-	**1.421**			
Control	-	**0.052**			
			B-A	**B-C**	
	6.25	**1.392**	**0.029**	**1.369**	**2.12**
	12.5	**1.201**	**0.22**	**1.369**	**16.07**
	25	**1.099**	**0.322**	**1.369**	**23.52**
O1	**50**	**0.918**	**0.503**	**1.369**	**36.74**
	100	**0.864**	**0.557**	**1.369**	**40.69**
	IC 50				**Above 100**

Sample code	Concentration (µg/ml)	Absorbance at 540 nm	Percentage of Inhibition (%)
Blank	-	1.421	-
Control	-	0.052	-
O1	6.25	1.392	2.12
	12.5	1.201	16.07
	25	1.099	23.52
	50	0.918	36.74
	100	0.864	40.69
	IC 50		**>100 µg/ml**

Standard	Concentration (µg/ml)	Absorbance at 540 nm	Percentage of Inhibition (%)
Blank	-	0.998	
Control	-	0.034	
Acarbose	6.25	0.911	9.02
	12.5	0.852	15.15
	25	0.639	37.24
	50	0.346	67.63
	100	0.139	89.11
IC 50	**36.44**		

Percentage of Inhibition = $\frac{\textbf{(B -A) x 100}}{\textbf{(B - C)}}$

IV. Discussion

The extract displayed moderate levels of antioxidant and enzyme inhibition activities significantly less than standards. These results indicate the appearance of bioactive compounds that would benefit from further studies to increase the therapeutic potential of this drug. Having high IC_{50} values and low inhibition percentages it is assumed that DPPH scavenging free radicals and inhibition of carbohydrate digestive enzymes might be weak for the extract's bioactive compounds. The weak alpha glucosidase and alpha amylase inhibition implies that higher concentrations of the extract would be needed to produce significant effects. Given these results, further study is needed to isolate the active compounds from the extract and discover ways to heighten the extract's therapeutic potential.These results highlight the need for further investigation to isolate the active compounds and explore their potential in managing PCOD, where antioxidant support and enzyme inhibition could help regulate oxidative stress and glucose metabolism.

V. Conclusion

Moderate antioxidant and alpha glucosidase inhibitory activity of the aqueous extract of Hibiscus rosa-sinensis suggest possible role in oxidative stress and insulin resistance management as major factors of PCOS. This therefore suggests that the extract may aid the regulation of glucose metabolism and decrease oxidative damage associated with treating PCOS symptoms. Isolation of active compounds, investigation of their mechanisms, and determination of the extract's role in the management of PCOS, need further studies.

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