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Quality control study and standardization of *Hibiscus rosasinensis* l. flowers and leaves as per WHO guidelines

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Abstract

Hibiscus rosasinensis flowers and leaves are available all over India and well known for its Antidiabetic property. In the present investigation aqueous extract and ethanolic extract of the flowers and leaves were made using soxhlet apparatus, water extract and maceration. The qualitative Phytochemical screening procedure was performed on each extract. Phytochemical study reveals that alkaloids, tannins, saponins, triterpenoids, coumarins, steroids, flavonoids were present in the three extracts. An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of flowers and leaves anatomically and physico chemically. TLC finger printing and fluorescence analysis of powdered flowers and leaves has been conducted and reported. The antidiabetic activity was performed by enzyme inhibition (α -glycosidase) in *in vitro* method on each extract and ethanolic extract showed significant inhibition.

Keywords: *Hibiscus rosasinensis* flowers and leaves, Anti-diabetic activity, Extracts.

1. Introduction**1.1 Plant profile** ^[1, 2]**(a) Morphology**

The plant *Hibiscus rosasinensis* is a perennial shrub with tap root. The leaves are 3.5-12 cm. In length and 2-5.5 cm wide. Leaves are simple ovate or ovate-lanceolate. Leaves are entire at the base and coarsely toothed at the apex. Taste is mucilaginous. Flowers are pedicellate, Actinomorphic, pentamerous and complete. Corolla consists of 5 petals, red in colour and about 3 inches in diameter, generally available in many areas within its hardiness range.

(b) Origin and distribution

Hibiscus rosasinensis are native to Tropical Asia. A native of south eastern Asia (China), the plant is commonly found throughout the tropics and as a house plant throughout the world. Most ornamental varieties are hybrids. The present wide range of cultivars is considered to be a complex of inter specific hybrids, between 8 or more different species originating from the African East Coast and islands in the Indian and Pacific Ocean.



Botanical Name	:	<i>Hibiscus rosasinensis</i> L.
Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Dilleniidae
Order	:	Malvales
Family	:	Malvaceae
Genus	:	Hibiscus
Species	:	rosasinensis

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Fig 1: *Hibiscus rosa-sinensis* flower and leaves

Vernacular name

Sanskrit	-	Japa,
Hindi	-	Jasum, Gulhar,
Bengali	-	Jaba,
English	-	Chinese hibiscus, Shoe flower, China rose,
Malayalam	-	Bunga Raya,
Tamil	-	Sembaruthi,
Telugu	-	Mandara,
Tribal name	-	HinduMa-pangi (Marma), Raktajaba (Chakma).

(c) Chemical constituents

Leaves and stems contain β -sitosterol, stigma sterol, taraxeryl acetate and three cyclo propane compounds and their derivatives. Flowers contain cyaniding diglucoside, flavonoids and vitamins, thiamine, riboflavin, niacin and ascorbic acid (Ghani, 2003). Quercetin-3-diglucoside, 3,7-diglucoside, cyanidin-3,5-diglucoside and cyanidin-3-sophoroside-5-glucoside have been isolated from deep yellow flowers; all above compounds and kaempferol-3-xylosylglucoside have been isolated from ovary white flowers.

(d) Pharmacological uses

Abortifacient effect, Acid phosphatase stimulation, Alkaline phosphatase inhibition, Analgesic activity, Androgenic effect, Anticonvulsive activity, Anti-FSH activity, Antiestrogenic effect, Antifertility effect, Antifungal activity, Antigonadotropin effect, Antihypertensive activity, Anti-implantation effect, Anti-inflammatory activity, Anti-ovulatory effect, Antipyretic activity, Antispasmodic activity, Antispermatogetic effect, Antiviral activity, Barbiturate potentiation, Beta-glucuronidase inhibition, Beta-glucuronidase stimulation, CNS depressant activity, Contraceptive agent, Embryo toxic effect., Estrogenic effect, Estrous cycle disruption effect, Gonadotropin synthesis inhibition, Hypoglycemic effect, Hypotensive activity, Hypothermic activity, Inotropic effect positive. Juvenile hormone activity, Plant germination inhibition, Lactate-dehydrogenase-X inhibition, Luteotropic effect, Menstruation induction effect, Radical scavenging effect, Teratogenic effect, Toxicity assessment (quantitative).

2. Materials and Methods**2.1 Morphological study**

The drug was evaluated by its colour, odour, taste, size, shape and special features, like texture, touch, etc. evaluation was carried based on the morphological and sensory profiles of whole drug.

Table 1: Measurement of length and width of phloem fibres of *Hibiscus rosasinensis* leaf

Range	Length of fibers (μ)	Width of fibers (μ)
Highest range	1900	30
Lowest range	400	40
Avg	1150	35

2.2 Microscopy and Microscopical measurements ^[3]

The microscopic measurements were performed for the

important characters like fibres the experiment was performed as per standard reference books.

Measurement of length and width of fibres were determined and mentioned in table.1

2.3 Determination of Stomatal Index ^[3]

They were carried out using standard procedures and mentioned in results.

2.4 Determination of Vein-Islet Number and vein termination number ^[3]

They were carried out using standard procedures and mentioned in results.

2.5 Determination of Palisade Ratio ^[3]

They were carried out using standard procedures and mentioned in results.

2.6 Determination of Stomatal Number ^[4]

They were carried out using standard procedures and mentioned in results.

2.7 Extraction ^[4]

Solvent extraction (Alcohol) using soxhlet

Extraction by water & Extraction by maceration with alcohol were carried out using standard procedures. The % yield is given table. 2 for flowers. The extracts were subjected to physical examination and given in table.3 for leaves.

Table 2: Colour, Consistency and Yield of *Hibiscus rosasinensis* flowers

Extracts	Colour	Consistency	% Yield
Flower maceration extract	Reddish brown	Oily	1.8
Flower soxhlation extract	Reddish brown	Oily	2.98
Flower water extract	Black	Oily	10.3

Table 3: Colour, Consistency and Yield of *Hibiscus rosasinensis* leaves

Extracts	Colour	Consistency	% Yield
Leaf maceration extract	Reddish	Oily	3.56
Leaf soxhlation extract	Greenish black	Oily	8.3
Leaf water extract	Brownish black	Oily	2.1

2.8 Determination of foreign matter ^[4]

They were carried out using standard procedures and mentioned in results.

2.9 Proximate analysis & LOD ^[4]

Proximate analysis was carried out for the flowers and leaves of *Hibiscus rosasinensis*.

Determinations of total ash value, acid-insoluble ash value, Water soluble ash value, Sulphated ash were performed. Data (s) were presented in Table 4 & 5.

Table 4: Proximate analysis of *Hibiscus rosasinensis* L. flowers

S. no.	Parameters	Values % (w/w)
1	Ash values	
	Total ash value of crude drug	5.5
	Acid insoluble ash	2
	Water soluble ash	1.5
	Sulphated ash	13
2	Loss on drying	78
3	Extractive values	
	Water soluble extractives	33.6
	Alcohol soluble extractives	18.4

Table 5: Proximate analysis of *Hibiscus rosasinensis* L. leaves

S.no	Parameters	Values % (w/w)
1	Ash values	
	Total ash value of crude drug	14
	Acid insoluble ash	5.5
	Water soluble ash	0.5
	Sulphated ash	9
2	Loss on drying	86
3	Extractive values	
	Water soluble extractives	36
	Alcohol soluble extractives	9.6

Table 6: Phytochemical screening of *Hibiscus rosasinensis* L flower ethanol extracts, water extract

Chemical constituents	Tests	Flower maceration extract	Flower soxhlation extract	Flower water extract
1. Alkaloids	Wagner test	present	present	present
	Hager test	present	present	present
	Dragendorff's test	present	present	present
2. Carbohydrates	Fehling's test	absent	absent	absent
	Barfoed's test	absent	absent	absent
	Molisch test	absent	absent	absent
3. Triterpenoids	Salkowski test	present	present	absent
	Liebermann test	present	present	absent
4. Coumarins	10% NAOH	present	present	present
5. Steroids	Liebermann test	present	absent	absent
6. Tannins	5% FeCl ₃	present	present	present
7. Saponins	Water	absent	present	present
8. Flavones	Schinoda test	present	present	present
9. Chalcones	Conc. HNO ₃ & H ₂ SO ₄	absent	absent	absent
	Acetic acid & conc. H ₂ SO ₄	absent	absent	absent
10. Amino acids	Ninhydrin	absent	absent	absent
11. Glycosides	Keller kiliani test	absent	absent	absent
	Antraquinone test	absent	absent	absent
12. Proteins	Biuret test	absent	absent	absent
	Million's test	absent	absent	absent
	Xanthoprotein test	absent	absent	absent
13. Phenols	10% FeCl ₃	absent	absent	absent
	Dil. HNO ₃	absent	absent	absent

Table 7: Phytochemical screening of *Hibiscus rosasinensis* L leaves ethanol extracts, water extract

Chemical constituents	Tests	Leaves maceration extract	Leaves soxhlation extract	Leaves water extract
1. Alkaloids	Wagner test	present	present	present
	Hager test	present	present	present
	Dragendorff's test	present	present	present
2. Carbohydrates	Fehling's test	absent	absent	absent
	Barfoed's test	absent	absent	absent
	Molisch test	absent	absent	absent
3. Triterpenoids	Salkowski test	present	absent	absent
	Liebermann test	present	absent	absent
4. Coumarins	10% NAOH	present	absent	present
5. Steroids	Liebermann test	present	present	absent
6. Tannins	5% FeCl ₃	absent	absent	absent

2.10 Determination of extractive values

Extractive values of flowers and leaves of *Hibiscus rosasinensis* L. were determined separately. Determination of alcohol-soluble extractives, Determination of water-soluble extractives.

2.11 Determination of moisture content (loss on drying)

They were carried out using standard procedures. Results were mentioned in table. 6 & 7.

2.12 Preliminary phytochemical screening ^[4]

After extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery. These extracts were used in testing for various phytoconstituents as per standard tests. The results were mentioned in table. 8 & 9.

2.13 Determination of volatile oil in drugs ^[10]

Volatile oil procedure carried out for flowers and leaves. Results were noted.

2.14 Foaming index ^[4]

Foaming index was carried out using standard procedures for flowers and leaves. Results were mentioned in the table. 10

7. Saponins	Water	absent	absent	present
8. Flavones	Shinoda test	absent	absent	present
9. Chalcones	Conc. HNO ₃ & H ₂ SO ₄	absent	present	absent
	Acetic acid & conc. H ₂ SO ₄	absent	present	absent
10. Amino acids	Ninhydrin	absent	absent	absent
11. Glycosides	Keller kiliani test	absent	absent	absent
	Antraquinone test	absent	absent	absent
12. Proteins	Biuret test	absent	absent	absent
	Million's test	absent	absent	absent
	Xanthoprotein test	absent	absent	absent
13. Phenols	10% FeCl ₃	absent	absent	absent
	Dil. HNO ₃	absent	absent	absent

Table 8: determination of foaming index of *Hibiscus rosasinensis* flowers and leaves

S. no	Concentration	Leaves	Flowers
1	1 ml conc.	0.2 ml	-
2	2 ml conc.	0.5 ml	-
3	3 ml conc.	0.5 ml	0.2 ml
4	4 ml conc.	1 ml	0.5 ml
5	5 ml conc.	0.5 ml	0.5 ml
6	6 ml conc.	0.5 ml	1 ml
7	7 ml conc.	0.7 ml	1 ml
8	8 ml conc.	0.5 ml	0.5 ml
9	9 ml conc.	0.5 ml	0.5 ml
10	10 ml conc.	1 ml	1 ml

Table 9: determination of swelling index of *Hibiscus rosasinensis* flowers and leaves

S. No	Swelling index	For leaves	For flowers
1	1 gm drug make up to 20ml with water	4.5 ml	6 ml

Table 10: determination of % Inhibition of FMA of *Hibiscus rosasinensis*

Concentration (µg/ml)	% Inhibition
250 µg/ml	8.3%
500 µg/ml	33%
750 µg/ml	48.3%

2.15 Swelling index^[4]

Swelling index carried out for flowers and leaves. Results were mentioned in the table. 11.

Table 11: Determination of % Inhibition of FSA of *Hibiscus rosasinensis*

Concentration (µg/ml)	% Inhibition
250 µg/ml	6.6%
500 µg/ml	21.6%
750 µg/ml	60%

2.16 In vitro Anti-diabetic screening of various extracts of *Hibiscus rosasinensis* flowers and leaves^[5,6]

Procedure for inhibition of α- glycosidase enzyme method
The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of

plant extract for 5 min at 37 °C. The reaction was initiated by adding 1 ml of alpha-glycosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35 °C. Then the reaction was terminated by the addition of 2 ml of 6N HCL. Then the intensity of the colour was measured at 540 nm. Calculation of 50% Inhibitory Concentration (IC₅₀). The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I \% = (Ab - As) / Ac \times 100$$

Where, Ab is the absorbance of the blank and as is the absorbance of the sample.

Tables and figures were mentioned in results.

2.17 Microbial load^[7]

They were carried out using standard procedures. Results were noted.

2.18 Aflatoxins^[8]

Solvent system: Chloroform, acetone and isopropyl alcohol
Aflatoxins carried out using standard procedures for flowers and leaves. TLC is performed to the Aflatoxins test. In the TLC blue fluorescent spots are not observed and noted in results.

2.19 Pesticide residues^[9]

Solvent system: n-hexane: diethyl ether (1:2)

Detection: P- dimethyl amino Benzaldehyde: ethanol: HCL (0.15 gm: 47.5 ml: 2.5 ml)

TLC is performed. In the TLC yellow or Rose colour spots

are not observed. Pesticide residue analyses were carried out using standard procedures for flowers and leaves. Results were noted.

2.20 Heavy metals ^[10]

1. Limit Test for Iron
2. Limit Test for Lead
3. Limit test for Arsenic

They were carried out using standard procedures and mentioned in results.

2.21 Bulk density and Tapped density ^[11]

Bulk density and Tapped density standard procedures carried out for flowers and leaves. Results were noted.

2.22 PH of extracts ^[12]

PH was carried out using standard procedures for flowers and leaves. Results were mentioned in the table. 12.

Table 12: determination of % Inhibition of FWE of *Hibiscus rosasinensis*

Concentration ($\mu\text{g/ml}$)	% Inhibition
250 $\mu\text{g/ml}$	5%
500 $\mu\text{g/ml}$	58.3%
750 $\mu\text{g/ml}$	65%

2.23 Determination of Total Anthocyanin Content ^[13]

They were carried out using standard procedures. Results were noted.

2.24 Determination of Total Tannin Content ^[14]

They were carried out using standard procedures. Results were noted

2.25 Determination of Total Flavonoid Content ^[14]

They were carried out using standard procedures. Results were noted

2.26 Thin Layer Chromatography ^[15, 16]

Solvent systems and spot colours mentioned in table 19 as per standard books and mentioned in results.

2.27 UV fluorescence analysis

Powdered flowers and leaves of *Hibiscus rosasinensis* was subjected to analysis under ultra violet light after treatment with various chemicals and organic reagents. Three parameters were taken into account i.e., observation under long wave length U.V (365 nm), short wave length U.V (256nm) and normal day light. The results were given in table 13 & 14

Table 13: Fluorescence analysis of *Hibiscus rosasinensis* flower powder

Flower powder treated with different chemical reagents	Long wave length			Short wave length			Day light		
	0 min	30 min	24 hr	0 min	30 min	24 hr	0 min	30 min	24 hr
Conc. HCL	black	brown	brown	brown	brown	brown	pink	pink	pink
Dil. HCL	black	brown	brown	brown	brown	brown	orange	pink	pink
Conc. H ₂ SO ₄	black	black	black	brown	brown	brown	pink	pink	pink
Dil. H ₂ SO ₄	orange	orange	orange	pink	orange	brown	orange	orange	orange
Conc. HNO ₃	black	black	black	green	green	green	green	yellow	yellow
Barfoed's reagent	black	black	black	blue	green	green	blue	green	green
Glacial acetic acid	black	black	black	pink	pink	brown	pink	pink	pink
Glacial acetic acid+ conc. HNO ₃	black	black	black	green	green	green	green	yellow	yellow
5% CuSO ₄	black	black	black	blue	blue	blue	blue	blue	blue
Dragendorff's reagent	black	black	black	white	white	white	white	pink	pink
Mayer reagent	black	color less	black	color less	green	green	color less	brown	brown
Wagner reagent	black	black	black	reddish black	green	reddish brown	red	rose pink	rose pink
Hager reagent	black	black	black	yellow	reddish brown	reddish brown	yellow	orange	orange
Picric acid	brown	brown	brown	orange	brown	brown	pink	pink	pink
5% FeCl ₃	black	black	black	black	black	black	black	black	black
10% NaOH	black	black	black	green	green	green	green	brown	brown
Ethanol	brown	brown	brown	green	green	green	pink	pink	pink
Dil. NH ₃	green	green	green	green	green	green	green	green	green
Chloroform	black	black	color less	green	green	green	orange	orange	reddish brown
Ethanolic KOH	black	black	black	green	green	green	orange	orange	orange
Ninhydrin solution	color less	color less	color less	color less	color less	color less	color less	color less	brown
Cold water	black	black	black	green	green	green	red	red	red

Table 14: Fluorescence analysis of *Hibiscus rosasinensis* L leaf powder

Flower powder treated with different chemical reagents	Long wave length			Short wave length			Day light		
	0 min	30 min	24 hr	0 min	30 min	24 hr	0 min	30 min	24 hr
Conc. HCL	black	Black	black	green	green	green	green	green	green
Dil. HCL	green	Green	green	green	green	green	green	green	green
Conc. H ₂ SO ₄	black	Black	black	green	green	green	green	green	green
Dil. H ₂ SO ₄	green	Green	green	green	green	green	green	green	green
Conc. HNO ₃	black	Black	black	green	green	green	green	green	green
Barfoed's reagent	black	Black	black	blue	blue	blue	Blue	blue	blue
Glacial acetic acid	green	Green	green	green	green	green	green	green	green
Glacial acetic acid+ conc. HNO ₃	black	Black	black	green	green	green	green	green	green
5% CuSO ₄	black	Black	black	blue	blue	blue	Blue	blue	blue
Dragendorff's reagent	black	Black	black	color less	color less	color less	color less	color less	color less
Mayer reagent	color less	color less	color less	color less	color less	color less	color less	color less	color less
Wagner reagent	black	Black	black	reddish brown	reddish brown	green	reddish brown	reddish brown	reddish brown
Hager reagent	black	Black	black	yellow	green	green	yellow	yellow	yellow
Picric acid	black	Black	black	green	green	green	brown	brown	brown
5% FeCl ₃	black	Black	black	reddish	green	green	Red	red	red
10% NaOH	color less	Pink	Pink	color less	green	green	color less	color less	green
Ethanol	black	Black	black	green	green	green	green	green	green
Dil. NH ₃	black	Black	black	green	green	green	green	green	green
Chloroform	black	Black	black	green	green	green	brown	brown	brown
Ethanolic KOH	black	Black	black	orange	green	green	orange	orange	orange
Ninhydrin solution	black	color less	color less	color less	color less	color less	color less	color less	color less
Cold water	black	Black	Black	green	green	green	green	green	green

3. Results and Discussion

3.1 Morphological study

For flowers

Colour : Pink
Odour : Odourless
Taste : Tasteless

For leaves

Colour : Green
Odour : Odourless
Taste : Mucilaginous

3.2 Microscopy

Transverse section of leaf

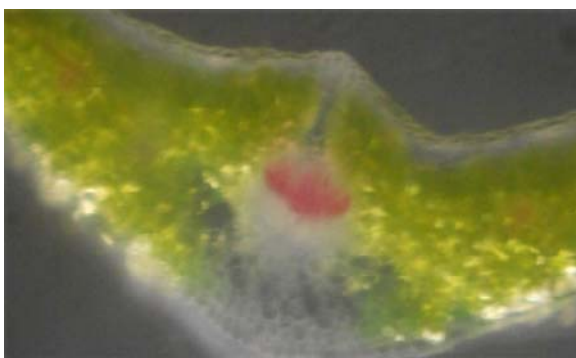


Fig 2: Transverse section of leaf

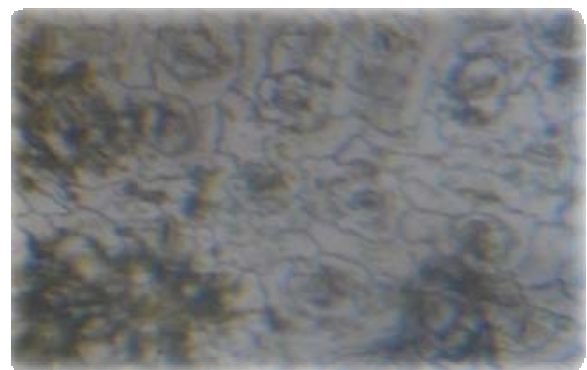


Fig 3: Anisocytic stomata of leaf

3.3 Measurement of length and width of phloem fibres

This helps in identification of adulteration and mentioned table 1.

3.3 Stomatal Index

Stomatal index of leaves of *Hibiscus rosasinensis* was found to be 14.28.

3.5 Vein-Islet Number and vein termination number

Vein islet number and Vein termination number of leaves of *Hibiscus rosasinensis* was found to be 10 and 14.

3.6 Palisade Ratio

Palisade ratio was found to be in *Hibiscus rosa-sinensis*

leaves was 5.

3.7 Stomatal number

Stomatal number of leaves of *Hibiscus rosasinensis* was found to be 10.

3.8 Extraction

Colour and consistency and yield were noted in the table.2 and 3.

3.9 Foreign matter

No foreign matter is observed in the flower powder and leaf powder of *Hibiscus rosasinensis*.

3.10 Proximate analysis

Total ash value and Acid insoluble ash value more in leaves than flowers. Water soluble ash value and Sulphated ash value more in flowers. Water soluble extractive value more in leaves than leaves. Alcohol soluble extractive value more in flowers than leaves. LOD was more in leaves compare than flowers. Loss on Drying (Moisture content) were determined by standard method and the values were tabulated in 4 and 5.

3.11 Preliminary Phyto chemical screening of various extracts

The extracts were subjected to phytochemical screening for the presences of type of phytoconstituents. The flower and leaves both extracts consist of alkaloids, flavones, coumarins, saponins and quinones. Additional were present in flowers was triterpenoids, saponins, tannins and the leaves consist of steroids. The presence of phytochemical constituents present in flower extracts were given in table.6 and for leaves in table 7.

3.12 Volatile oil in drugs

5 ml aromatic water is present in flowers. No oil is present in leaves.

3.13 Foaming index

In the leaves higher foaming index was found to be in 1ml at 4 ml concentration then decreases the foaming index and again increased in 10 ml concentration. In the flowers higher foaming index was found to be 1ml at 6 ml and 7 ml concentration then decreases the foaming index and again higher in the 10 ml concentration. Values were tabulated in table 8.

3.14 Swelling index

Swelling index was found to be more in flowers than leaves of *Hibiscus rosasinensis*. Values noted in table 9.

3.15 In vitro Anti-diabetic screening of various extracts of Hibiscus rosasinensis flowers and leaves



Fig 4: Extracts

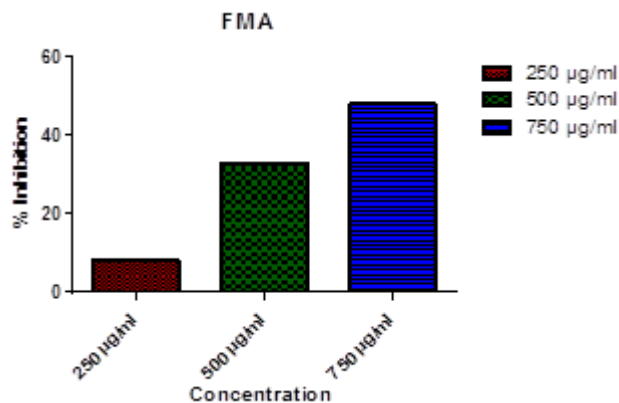


Fig 5: Comparative graph of % inhibition for FMA concentrations of *Hibiscus rosasinensis*

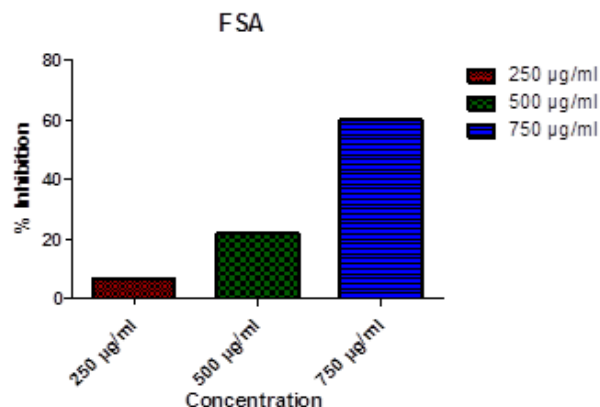


Fig 6: Comparative graph of % inhibition for FSA concentrations of *Hibiscus*

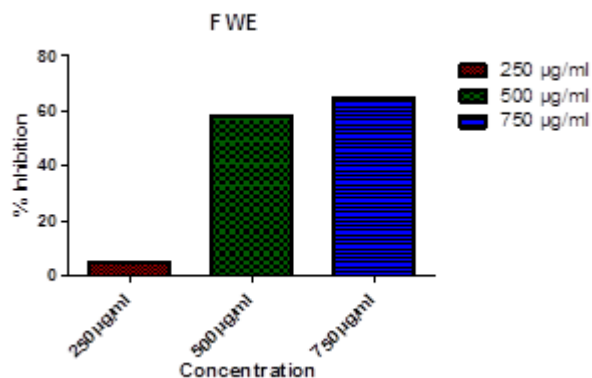


Fig 7: Comparative graph of % inhibition for FWE concentrations of *Hibiscus rosasinensis*

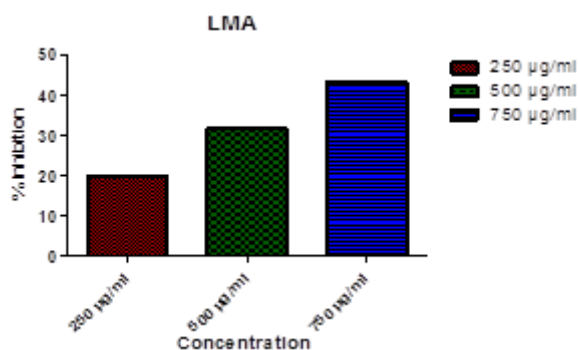


Fig 8: Comparative graph of % inhibition for LMA concentrations

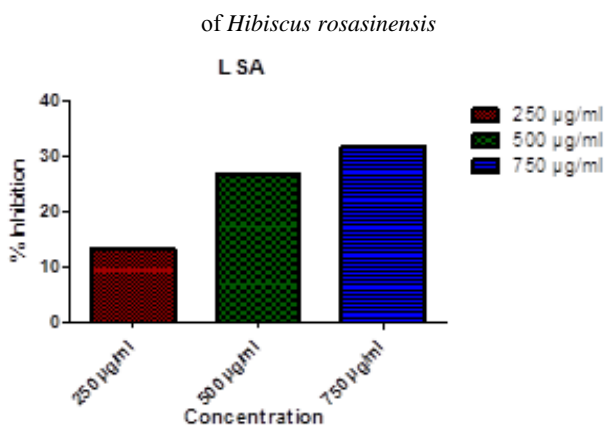


Fig 9: Comparative graph of % inhibition for LSA concentrations of *Hibiscus rosasinensis*

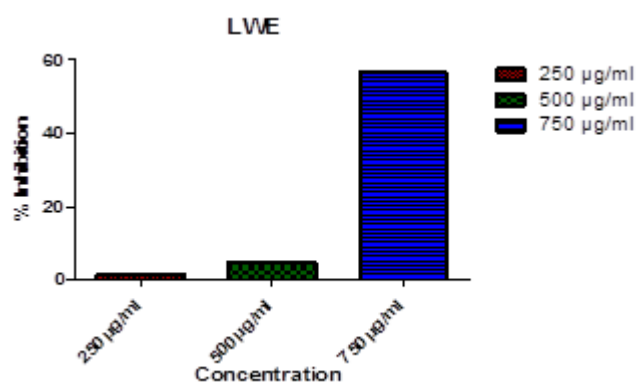


Fig 10: Comparative graph of %inhibition for LWE concentrations of *Hibiscus rosasinensis*

Table 15: determination of % Inhibition of LWE of *Hibiscus rosasinensis*

Concentration (µg/ml)	% Inhibition
250 µg/ml	1.6%
500 µg/ml	5%
750 µg/ml	56.6%

3.16 Microbial load

No microbial growth was observed in the streak plate method.

3.17 Aflatoxins

Aflatoxins were absent in flowers and leaves of *Hibiscus rosasinensis*

3.18 Pesticide residue

Pesticide residues were absent in flowers and leaves of *Hibiscus rosasinensis*.

3.19 Heavy metals

Heavy metals (Lead, iron, arsenic) were absent.

3.20 Bulk density and Tapped density

For flowers

Bulk density = 0.125 gm/ml

Tapped density = 0.167 gm/ml

For leaves

Bulk density = 0.142 gm/ml

Tapped density = 0.2 gm/ml

pH of extracts

Mentioned in the table 16.

Table 16: Determination of pH of extracts of *Hibiscus rosasinensis* flowers and leaves

Extracts	pH
Flower maceration	5.1
Flower soxhlation	4.1
Flower water	5.4
Leaf maceration	5.0
Leaf soxhlation	5.2
Leaf water	5.8

3.21 Total Anthocyanin Content [TAC]

TAC in the flowers was found to be 32.18%. Figures mentioned 11

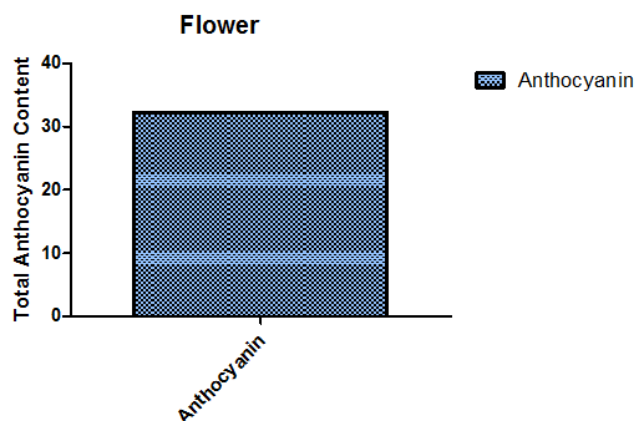


Fig 11: Graph of TAC for extract of flower of *Hibiscus rosasinensis*

3.22 Thin Layer Chromatography

The TLC to identify phytoconstituents with different solvent systems showed different spots in different colours under UV light in three different wavelengths with Rf values which are tabulated in table 17.

3.23 UV fluorescence analysis

Powder of flowers and leaves were subjected to analysis under UV light after treatment with various chemical and organic reagents. The findings were tabulated in the tables. 13 for the flowers and table 14 for the leaves.

Table 17: Thin layer chromatography of *Hibiscus rosasinensis* flowers and leaves

Phytoconstituents	Solvent system	Spot colour	R _f value
Flavones	Ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6)	FMA- dark green	0.30
		FSA- yellow green	0.26
		FWE- orange colour	0.5
		LWE- yellow green	0.29
Steroidal saponin	Ethyl acetate: Ethanol: water: ammonia (6.5:2.5:0.9:0.1)	FMA- blue violet	0.16
Triterpenoids	Ethyl acetate: glacial acetic acid: water: formic acid (10:1.1:1.1:2.6)	FMA- light blue	0.37
		FSA- bright blue	0.43
		LMA- light blue	0.19
Anthocyanin	Ethyl acetate: glacial acetic acid: water: formic acid (10:1.1:1.1:2.6)	Flower extract- red to violet colour	0.28
Tannins	Chloroform: ethyl acetate: ethanol (6:4:4)	FMA- green colour	0.34
		FSA- green colour	0.4
		FWE- green colour	0.27
Alkaloids	Toluene : ethyl acetate: diethyl amine (7:2:1)	FMA- orange	0.25
		FSA	0.49
		FWE	0.23
		LMA	0.59
		LSA	0.77
		LWE	0.70

4. Conclusion

Based on literature review *Hibiscus rosasinensis* belongs to the family Malvaceae, flowers and leaves of this plant traditionally used in the anti-diabetic activity and cosmetics. The macro and microscopical characters along with physicochemical and fluorescence characters of flower and leaf powder and sections of *Hibiscus rosasinensis* L., is used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guidelines. The in vitro Anti-diabetic screening α -glucoamylase enzyme is dose dependent. Maximum inhibition was observed in flower alcoholic extract prepared by Maceration. In leaf maximum inhibition was observed in leaf alcoholic extract prepared by soxhlet.

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