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Phytochemical and Pharmacological Evaluation of *Hibiscus mutabilis* leaves

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Abstract

Hibiscus mutabilis ,Shalapara,Sthalapadma is widely used medicinal plant throughout India and in various systems of medicine like Ayurveda and Siddha.In the traditional system of medicine, the various parts such as leaves ,flowers,seeds,stems are used as emollient, in pectoral and pulmonary complaints, stimulant and leaves applied to swelling. The present review is therefore an effort to give detailed survey of the literature on Pharmacognosy,Phytochemistry and Pharmacological activities of Hibiscus mutabilis. Family: Malvaceae.

Key words: Hibiscus mutabilis, pharmacognosy, phytochemistry, pharmacological activities, review.

Introduction

The curative natures of herb and plants have always shown the way to medicinal discoveries and the fact can hardly be denied that any effort to alienate man from nature will make his body system a play ground for diseases. Hence the treatment of diseases in man with plants, plant extracts and pure compounds have been practiced since dawn of human intellect. Exploitation of plants have lead to the search of many medicinally useful components and their precursors. Medicinal plants and the active principals isolated from them are and will be of immense importance to humanity in their fight against diseases and disorders.

Hibiscus mutabilis (Malvaceae)is large bushy shrub or small tree about 8 ft.high.It is said to be native to China. It is cultivated in Indian gardens as an ornamental plant for its beautiful flowers, which may be single or double. The double flower type is more common. The propagated type plant is more common. The plant is propagated by cuttings or seeds. Flowering takes place in profusion in constant succession during September and October. Annual pruning (in April) induces profuse flowering.

Materials and Methods

A] Pharmacognostic Studies [1,2]

I] Macroscopical characteristics:

Leaves ovate to broad ovate, 10-22.5 cm in length and 6-10 cm in width, margin is irregularly crenate dentate.Colour of upper surface of leaf is fresh green and lower surface is pale green. midrib is prominent on both surfaces. Texture of leaf is hairy and rough. Apex is pointed, base is cordate, petiole long about 6-8 cm, taste is bitter and odour is characteristic.

II] Microscopical and powder characteristics:

Transverse section of leaf through midrib shows upper and lower epidermis made up of single layer of rectangular cells, covered with cuticle.Mesophyll is differentiated into palisade and spongy parenchyma cells. A single layer of palisade cells is observed in the lamina beneath the epidermis. The epidermal cells are seen clearly. A spongy parenchyma cell contains coloring pigments. Numerous unicellular and multicellular covering trichomes are present on upper and lower epidermis.

Numerous calcium oxalate crystals are present in clusters.Multicellular parenchyma was observed below the vascular bundle. Vascular bundle is collateral type and surrounded by pericyclic fibres.It also contains many calcium oxalate crystals in clusters.

Surface character showed the presence of star shaped trichomes and anomocytic stomata. Powder characters revealed the presence of spongy parenchyma with brown colored matter and calcium oxalate crystals, trichomes and anomocytic stomata.

B] Phytochemical Studies [3,4,5]

For phytochemical studies,leaves were extracted with different solvents .Thereby several components were extracted into different fractions.At first the leaves were extracted with petroleum ether which extract out fatty substance sterols,triterpene type of compounds. Then the defatted marc was treated with chloroform which extracts out chlorophyll.Furthur marc was extracted with methanol which extracts out phenolic compounds,alkaloids,saponins,glycosides,etc and the extractive values were calculated.[Table 2].

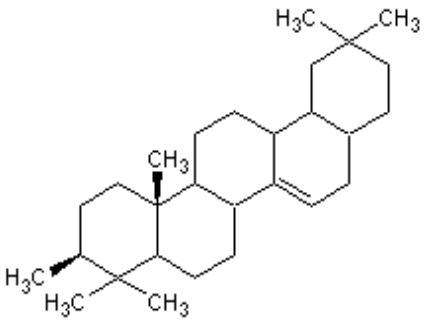
Table No.1 : Physical constants of leaves

Total ash	[%w/w]	11.22
Acid insoluble ash	[%w/w]	2.3760
Acid soluble ash	[%w/w]	7.227
Loss on drying (moisture content including volatile matters) [%]		10.90

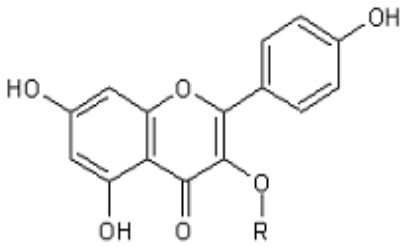
Table No.2 : Extractive values of extracts obtained from leaves :

Petroleum ether	1.6937 %w/w
Chloroform	1.5 %w/w
Methanol	2.585 % w/w

The structures of compounds isolated from the plant is given below.[6,7]



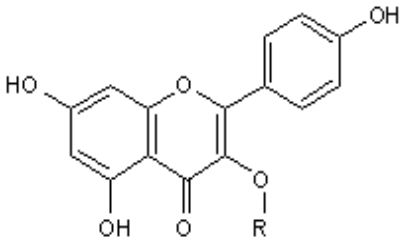
(I) Teraxeryl acetate



Kaempferol-3-rhamnoglucoside R= Rhamno glucose

Kaempferol-3-glucoside R= Glucose

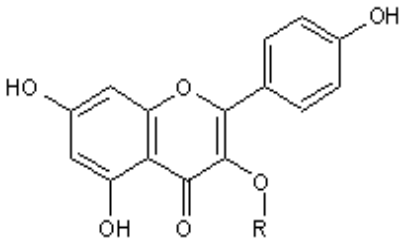
(II) & (III)



Kaempferol-3-rhamnoglucoside R= Rhamno glucose

Kaempferol-3-glucoside R= Glucose

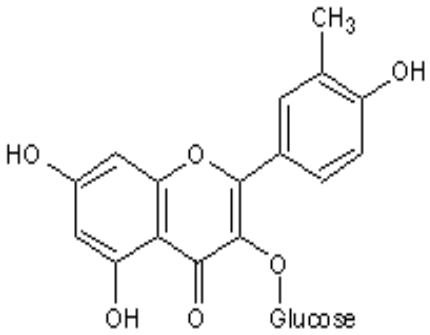
(IV) & (V)



Kaempferol-3-rhamnoglucoside R= Rhamno glucose

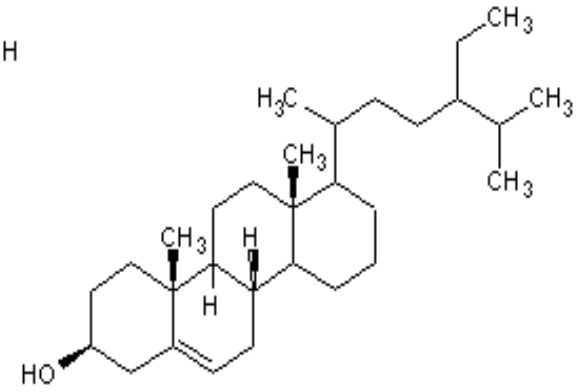
Kaempferol-3-glucoside R= Glucose

(VI) & (VII)



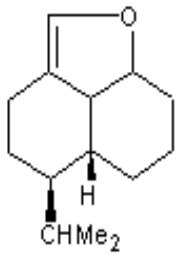
Isorhamnetin glucoside

(VIII)



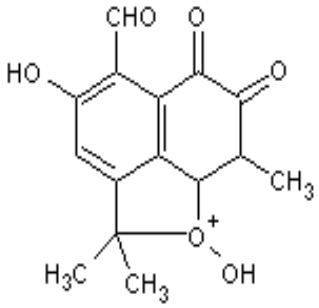
β– Sitosterol

(IX)



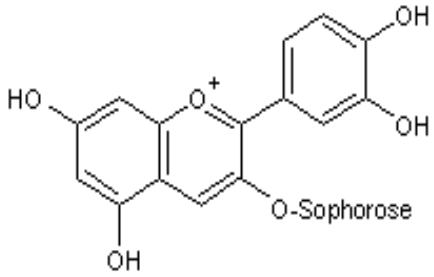
Hibiscones

(X)



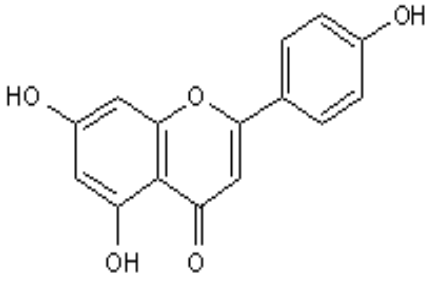
Hibiscoquinone

(XI)



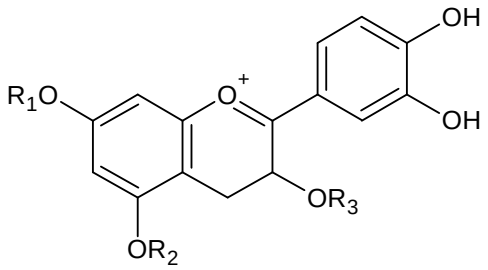
Cyanidine-3-Sophoroside

(XII)

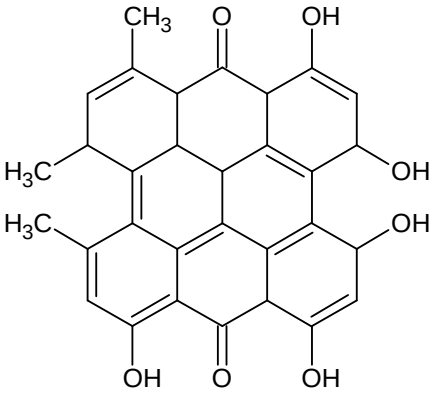


Kaempferol

(XIII)

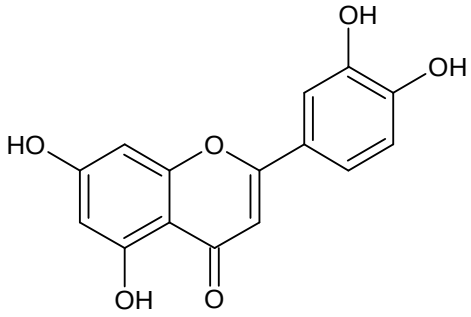


Sr. No.	Name of Compound	R_1	R_2	R_3
XIV	Quercetin	H	H	H
XV	Quercimeritrin	Glucose	H	H
XVI	Meratrin	H	H	Diglucose
XVII	Quercetin-3-Xyloside	H	H	Xylose
XVIII	Quercetin-3-sambubioside	H	H	Sambubiose
XIX	Hybridin	H	H	0-β-D-xylopyranose-β-D-galacto-furanoside



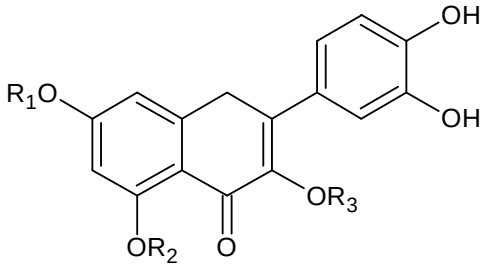
Kaempferol

(XX)

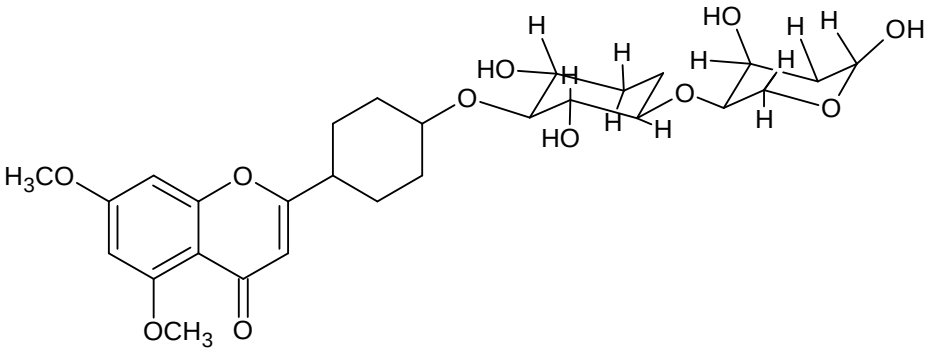


Isoquercetin

(XXI)

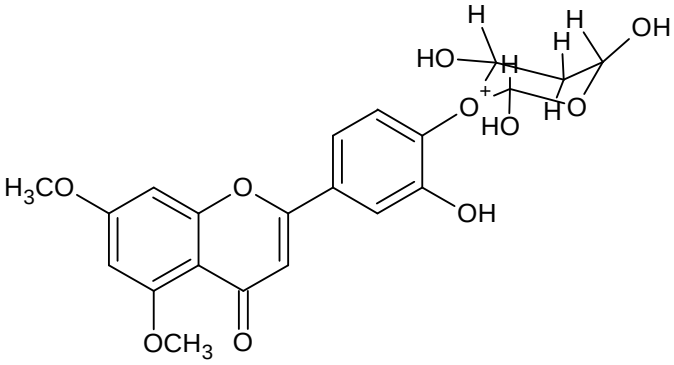


Sr.No.	Name of Compound	R_1	R_2	R_3
XXII	Cyanidin-3-xylosyl glucoside	H	H	Xylose Glucoside
XXIII	Cyanidin-3-xylosyl glucoside	H	H	Glucose

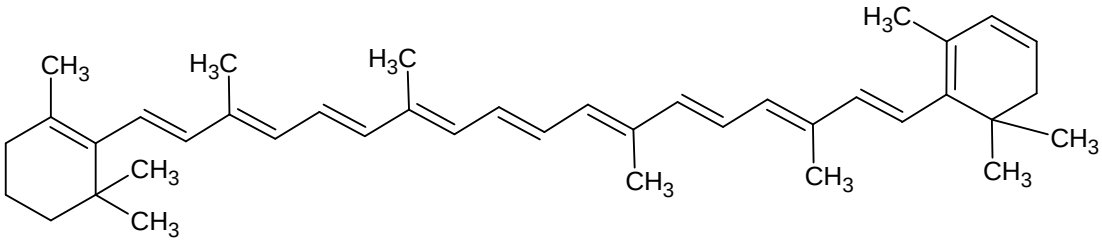


Naringenin-5,7-dimethyl ether-4'-o- β -D-arabinopyranoside

(XXIV)



Eriodictyol-5,7-dimethyl ether-4’-β-D-arabinopyranoside
(XXV)



β-Carotene(XXVI)

Following are the compounds isolated from different parts of plant.

Stem: Naringenin-5,7-dimethyl ether,4'-β-D-xylopyranosyl- β-D-arabinopyranoside, Eriodictyol-5,7-dimethyl ether-4'-β-D- arabinopyranoside.

Flowers: Quercetin , Quercemeritrine , Quercetin-3-D-Xyloside , Quercetin-3-sambubioside , Isoquercetin , Meratrin , Hybridin, Kaempferol ,Hyperin , Guaijaverin , Cyanidine-3-xlosyl glucose,Cyanidin-3-monoglucoside , Hibiscones , Hibiscoquinones.

Leaves : β-Sitosterol , β-Carotene , Quercetin ,for other isolated compounds structures are not established .

Quantitative studies [8,9]

All extracts were subjected to preparative TLC from which further compounds were isolated. Different solvent systems were tried for development of chromatographic separation performed on silica gel plates with Toluene : Ethyl acetate : Formic acid (5 : 4 : 1), Ethyl acetate : Ethyl methyl ketone : Formic acid (5 : 3 : 1 : 1) ,Benzene : Methanol (9 : 1) .The plates were scanned densitomertically at 254 nm and 200 nm.

Isolated compounds were charctacterized by physical method, chemical method and spectroscopical techniques. Compounds isolated are listed in Table.3

Table No.3 : Rf value for isolated compounds and their percentage determined by HPTLC.

Sr.No.	Compounds	Rf value		% w/w
		Std	Sample	
1	β -Sitosterol	0.39	0.38	1.160
2	β -Carotene	0.84	0.84	0.116
3	Quercetin	0.65	0.62	0.170

Quantitative studies were performed by using modern thin layer chromatography i.e.HPTLC. Samples and standard solutions were dosaged on different plates in its respective mobile phases, plates were scanned using TLC scanner III and area under curve were determined using integration software ,CATS 4.05.

Rf values for isolated compounds and their percentage quantity reported.[Table 3]

C] PHARMACOLOGICAL STUDY:[10,11]

Anti-inflammatory activity:

Anti-inflammatory activity was determined for ethyl acetate extract.Method used for it was carrageenan induced hind paw oedema in rats.The reading of paw volume displaced was noted at 0,15,30,45,60,90,120,180 minutes.

The percentage inhibition of paw oedema was calculated as :

(1-Vt/Vc)*100

Where Vt = Volume of paw oedema in animals treated.
Vc = Volume of paw oedema in carrageenan treated.

Table.No. 4 : Effect of Ethyl Acetate extract on rat paw oedema.

Sr. No.	Treatment	Dose (mg/kg)	Time (min)	Avg. Paw volume displacement	Vt	% inhibition
1	Carrageenan	0.1 ml of 1% w/v	0	0.18 ± 0.004		
			15	0.32 ± 0.009		
			30	0.53 ± 0.008		
			60	0.59 ± 0.009	0.592	
			90	0.67 ± 0.009		
			120	0.77 ± 0.011		
			150	0.81 ± 0.004		
			180	0.87 ± 0.014		
2	Nimuselide	10mg	0	0.21 ± 0.009		
			15	0.22 ± 0.004*		
			30	0.21 ± 0.008*		
			60	0.18 ± 0.004*		
			90	0.17 ± 0.004*	0.176	70.22%
			120	0.15 ± 0.004*		
			150	0.14 ± 0.014*		
			180	0.14 ± 0.004*		
3	Ethyl acetate extract	100 mg	0	0.23 ± 0.009*		
			15	0.24 ± 0.008*		
			30	0.23 ± 0.008*		
			60	0.22 ± 0.004*	0.210	64.52%
			90	0.21 ± 0.004*		
			120	0.2 ± 0.008*		
			150	0.18 ± 0.009*		
			180	0.17 ± 0.009*		

*ANOVA is applied which is followed by Donnet test.
[Permission of CPCSEA is taken to conduct the activity in Pravara Rural College Of Pharmacy,Loni,M.S,India. as per CPCSEA 21]

D] MICROBIOLOGICAL STUDY :[12]

Anti-microbial Activity :
Antimicrobial activity for methanolic extract and ethyl acetate obtained were determined on microbial cultures viz; *Staphylococcus aureus* (NCIM 2079 ATCC 6538P) , *Salmonella typhi*

(NCIM 2501 ATCC 23564),*Proteus vulgaris* (NCIM 2857),*Bacillus subtillus*(NCIM 2063 ATCC 6633),*Klebsiella pneumoniae* (NCIM 2957)and *Escherichia coli* (NCIM 2931 ATCC 25922).Activity was determined by using "Agar Diffusion method ".Zone of inhibition of the extracts showed that the extracts have sufficient activity against Bacillus subtilis when compared with standard drug.Zone of inhibition for methanolic extract (8mg) and ethyl acetate extract (8mg)was found 15mm and 13mm respectively as compared to standard Trimethoprim-Sulfamethoxazole combination, which had 14 mm zone of inhibition.

Table 5 : Zone of inhibition of extracts

Sr.No.	Name of micro-organisms	Zone of inhibition of extracts in mm		
		M	**E**	**S**
1	*Staphylococcus aureus*	13	13	16
2	*Proteus vulgaris*	12	13	13
3	*Bacillus subtilis*	15	13	14
4	*Salmonella typhi*	13	13	20
5	*Escherichia coli*	12	10	18
6	*Klebsiella pneumoniae*	12	13	20

Results and Discussion

The present article is an attempt to study the plant H.mutabilis in detail. The experimental work was mainly divided into four parts, Pharmacognostic,Phytochemical, pharmacological and microbiological studies of leaves. Pharmacognostic studies emphasized on macroscopy, microscopy, powder characteristics, loss on drying and ash values of leaves. Macroscopical studies helped for confirmation of plant. Microscopy of leaves showed dorsiventral characters. Numerous unicellular and multicellular trichomes were found in both epidermis. Numerous calcium oxalate crystals in veins ,Anomocytic stomata in surface preparation and star shaped trichomes are characteristic features of the plant. For phytochemical studies leaves were extracted with different solvents like Petroleum ether, Chloroform, Methanol and extractive values reported. Further the extracts were purified and separated by using different solvents ,techniques like column chromatography and preparative TLC. Finally five different compounds were isolated. and subjected for chemical and spectroscopical analysis like UV Spectroscopy,IR spectroscopy, and HPTLC analysis. HPTLC analysis has shown that all three isolated compounds are comparable with respective standards according to their Rf values and β – Sitosterol is obtained in quite higher percentage. Plant was also evaluated using Antimicrobial activity and Anti-inflammatory activity. Results of anti-inflammatory activity revealed that ethyl acetate extract has comparable anti-inflammatory activity when compared with standard drug Nimuselide. Ethyl acetate extract has 64.52 % inhibition of paw edema as compared to standard which showed 70.22 %.

Anti microbial activity was performed on various organisms which showed satisfactory results . Values showed that methanolic extract has shown good activity against *Bacillus subtilis* and with other extract activity is around similar for Gram+ve and Gram–ve organisms.

Conclusion

The plant is having anti-inflammatory, antibacterial activity. A literature survey showed that although it is used in number of diseases but its therapeutic efficacy has not been assessed yet. Therefore it is imperative that more clinical and pharmacological studies should be conducted to investigate unexploited potential of this plant. Plant is having a vitamin like β –Carotene in a small amount, but still for getting in large quantity it needs further sophisticated isolation and purification techniques.

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