

In-vitro Antimicrobial, Antispasmodic activity of Methanolic leaf and flower extracts of *Hibiscus rosasinensis*

Narayana Rao Alla*, Raju Kotte, Vamsi Gopi Krishna K, Mrudula Priyanka S, Mounica B, Deva G, Satyanarayana Tanuku

Affiliations: Department of Pharmacology
Mother Teresa Pharmacy College, Sathupally, Telangana, India,
e-mail ID: naraynaraoalla@gmail.com

ABSTRACT :

Hibiscus rosasinensis plant has significant constituents such as Anthocyanin's, flavonoids, cyanidin-3,5-diglucoside, cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, quercetin-3-diglucoside, cyclopeptide alkaloid. The present study was undertaken to evaluate the In vitro anti microbial and antispasmodic activity of methanolic leaf and flower extracts of *Hibiscus rosasinensis*. The anti microbial activity is done with concentrations of 25,50,75,100 mg/ml by agar disc diffusion method ampicillin was used as reference antibiotics, antimicrobial activity was tested against standard micro-organisms. The zone of inhibition of the flower extracts are shown as 7.25,7.5,6.75,7.75mm diameter. and leaf extracts are shown as 6.25,7,6.75,7mm diameter respectively. The zone of inhibition for the standard drug was identified as 6.75 mm diameter. The anti spasmodic activity was evaluated by antagonistic activity of extracts on DRC of acetylcholine by using isolated Chicken Ileum preparation with the doses of 10,20,30,40,80 mg of extracts and the results are recorded as percent inhibition of response. The extracts shown as 25%,31.25%,35%,43.75% for leaf extract 25%,37.55,56.25%,62.5% for flower extracts. Both the extracts are shown significant anti microbial and anti spasmodic activity.

I. INTRODUCTION

Antispasmodics are smooth muscular relaxants that are used to relieve cramps or spasms of the stomach, intestines and bladder. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea and irritable bowel syndrome. Antimicrobial agents act against the microorganisms like bacteria, fungi they can stop the growth and kill the microorganisms. The anti microbial agents are grouped according to the category of microorganism for example the agents act against bacteria are called anti bacterial, an agent which act against the fungi are called anti fungal drugs.

Traditionally several plants and their products have been used in foods as a mode of natural preservative, Flavouring agent as well as a remedy to treat some of the common ailments in human.

The present study was taken for screening for anti microbial and antispasmodic activity of *Hibiscus rosasinensis*, it belongs to the family Malvaceae. Plant has consist Anthocyanin's, flavonoids, cyanidin-3,5-diglucoside, cyanidin-3-sophoroside-5-glucoside, quercetin-3,7-diglucoside, quercetin-3-diglucoside, cyclopeptide alkaloid. The flowers and leaves are used for antifertility, contraceptive, Diuretic, Menorrhagia, Bronchitis, Emmenagogue, Demulcent, Cough, skin diseases and constipation.

II. MATERIALS AND METHODS

Fresh leaves and flowers of *Hibiscus rosasinensis* with no apparent physical, insect or microbial damage were collected from the medicinal garden of Mother Teresa Pharmacy College, Kothuru, Sathupalli. The collected flowers and leaves are dried under shade. After drying it is grind into powder it was authenticated by Dr.N. Dorababu.

2.1 Preparation of Extracts:

The fine powder of flower and leaf powder are extracted with methanol (70%) by soxhlation. A known weight of flower and leaf powder which 50g of each are taken separately in soxhlet apparatus and extracted with 210ml of methanol and 90 ml of water for 9hrs. Final obtained extracts was allowed to dry at room temperature and stored 4°C.

2.2 Evaluation of Antimicrobial activity (disc diffusion method) ⁴:

- The Anti microbial activity was carried out by using Agar disc diffusion Method.
- Plant extracts are made into different concentrations 25mg/ml,50mg/ml,75mg/ml,100mg/ml. Ampicillin was

selected as standard anti bacterial agent.

- 6mm size discs are prepared, and discs are impregnated in standard, test drug concentrations then discs are allowed to dry and dried discs are placed in the petriplates containing sterile agar medium.
- Petri plates are incubated for 24 hours, and recorded the zone of Inhibition.

2.3 Evaluation of Antispasmodic activity by using chicken ileum preparation:

The anti spasmodic activity was evaluated by antagonistic activity of extracts on DRC of acetylcholine by using isolated Chicken Ileum preparation

1. Procurement of Chick ileum Chick ileum was procured from the local market in Sathupally

2. Chemicals: All other chemicals used are of laboratory grade. Sodium Chloride, Potassium Chloride, Calcium Chloride, Magnesium Chloride, Magnesium Sulphate, Sodium bicarbonate, Sodium hydrogen phosphate, Potassium hydrogen phosphate and distilled water. Acetylcholine stock solution (1mg/ml)

3. Preparation of physiological salt solution (PSS) ⁴:

Compound	Tyrode
NaCl	8.0
KCl	0.2
CaCl ₂	0.2
MgCl ₂	0.10
MgSO ₄	-
NaHCO ₃	1.0
NaH ₂ PO ₄	0.05
KH ₂ PO ₄	-
Glucose	1 or 2

Fig:1. Composition of PSS

All values are in g/l. Weighed accurate quantity of the ingredients and dissolved in one liter distilled water. The physiological solution prepared should be clear, and if turbid it is advised to prepare fresh solution before the start of the experiment.

4. Method for evaluation of Antispasmodic activity of *H.rosasinensis* Extract¹⁻²:

1. Fresh entire gastrointestinal tract of healthy cock was obtained from a slaughter house in Sathupally.
2. The caecum was lifted forwards and the ileocaecal junction was identified.
3. A few centimeters of the ileal portion was cut and removed and immediately placed it in the watch glass containing physiological salt solution. The mesentery and adhering tissues were removed with gentle care. Utmost care was taken to avoid any damage to the gut muscle. The ileum was cut into small segments of 2- 3 cm long.
4. Cut the piece of ileum of 1 cm long and gently clean with Tyrode solution.
5. And the ileum is placed in a shallow dish containing Tyrode solution.
6. The ileum is mounted on organ bath by using a long thread on upper side of the ileum and short thread on opposite lower side.
7. Mount the preparation in upright position in the organ bath contain Tyrode solution under a tension of 1 gram.
8. There is no need of maintaining bath temperature.
9. Bubble the organ bath with air.
10. Relax the tissue for 30 min during this period wash the tissue with fresh Tyrode solution for atleast four times.
11. Record the concentrations due to acetylcholine using frontal writing lever.
12. 90 second contact time and a total 5 min time cycle may be used for proper recording of the responses.
13. Record atleast four responses to increasing doses of acetyl choline or till you get the maximum response.
14. Add 1 ml of flower and leaf extracts 10µg/ml of *H.rosasinensis* to the reservoir containing Tyrode solution and irrigate the tissue with Tyrode solution for 30 min.
15. Repeat the concentration response curves of acetylcholine in the presence of flower and leaf extract of *H.rosasinensis* respectively.
16. Label and fix the concentration response curves.
17. Plot both the concentration response curves of acetyl choline i.e one in the absence and other in the presence of flower extract of *H.rosasinensis*.
18. Note the potentiation in the response of acetyl choline and calculate relative E 50 values.

III.RESULTS AND DISCUSSION

3.1 Phytochemical screening of Flower and Leaf Extracts of *H.rosasinensis* ⁵:

Hibiscus rosasinensis methanolic flower and leaf extracts are subjected for phytochemical screening by standard protocol. The results are tabulated.

S.No	Phyto chemical name	Name of the test	Leaf extract	Flower extract
1	Alkaloids	a) Dragendorff's test	+	-
		b) Wagner's test	+	+
		c) Hager's test	+	+
2	Carbohydrates	a) Molish test	+	+
		b) Fehling's test	+	+
		c) Benedict's test	+	-
		d) Barfoed's test	-	-
3	Steroids	a) Salkowski test	+	+
4	Glycosides	a) Legal's test	+	+
		b) Baljet's test	+	+
		c) Bornträger's test	+	+
5	Saponins	a) Foam test	+	+
6	Flavonoids	a) Shinoda test	+	+
7	Fixed oils	a) Spot test	+	-
8	Proteins & Amino acids	a) Ninhydrine test	-	+

Fig:2 for Phytochemical Screening of Leaf and flower extracts

Alkaloids, Carbohydrates, Glycosides, Sterols, Saponins, Flavonoids are the identified Phytoconstituents in both the extracts

3.2 Evaluation of antibacterial activity of *H.rosasinensis* extracts:

Antimicrobial activity of *H.rosasinensis* extracts are evaluated by Disc diffusion method. The results are 25,50,75,100 mg/ml concentrations of Methanolic extract of *Hibiscus rosasinensis* are used for evaluation of antimicrobial activity. The zone of inhibition of the flower extracts are 7.25,7.5,6.75,7.75mm diameter and for leaf extracts 6.25,7,6.75,7mm diameter respectively with the concentrations of 25,50,75,100 mg/ml. The zone of inhibition for the standard drug was identified as 6.75 mm diameter.

S.no	Group	Average zone of inhibition	SEM
1	Control	5.75	0.5
2	Standard	6.75	0.95
3	Flower 25mg/ml	7.25	1.5
4	Flower 50mg/ml	7.5	1.29
5	Flower 75mg/ml	6.75	0.5
6	Flower 100mg/ml	7.75	0.95
7	Leaf 25mg/ml	6.25	0.5
8	Leaf 50mg/ml	7	1.15
9	Leaf 75mg/ml	6.75	0.95
10	Leaf 100mg/ml	7	1.4

Fig:3: Evaluation of antibacterial activity of H.rosasinensis extracts

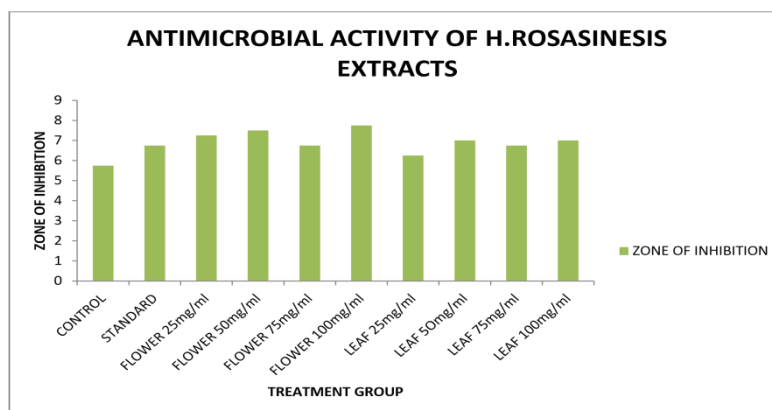


Fig:4.Antimicrobial Activity of H.rosasinensis Extracts

The results are clearly showing that the both the extracts are showing significant difference between the control, standard and test drugs. It is also conforming that the leaf and flower extracts are showing higher response than the standard ampicillin. This is indicating that the extracts are having significant anti bacterial activity.

3.3 Evaluation of Antispasmodic activity of Hibiscus rosasinensis leaf and flower Extracts:

The anti spasmodic activity was evaluated by antagonistic activity of extracts on DRC of acetylcholine by using isolated Chicken Ileum preparation. The effect of extracts on DRC of Acetylcholine are mentioned in the table.

S.No	Concentration of Ach(100µg/ml)	Dose(µg)	log dose	Response (mm)	% response
1	0.1ml	10	1	3	37.5
2	0.2ml	20	1.3	4	50
3	0.4ml	40	1.6	7	87.5
4	0.8ml	80	1.9	8	100

Fig:5: DRC of Acetylcholine by using Chicken Ileum Preparation

S.No	Concentration of Ach(100µg/ml)	Dose(µg)	log dose	Dose of Leaf extract(10µg/ml)	Response	% Response
1	0.1 ml	10	1	1 ml	2	25
2	0.2ml	20	1.3	1 ml	2.5	31.25
3	0.4ml	40	1.6	1 ml	3	37.5
4	0.8ml	80	1.9	1 ml	3.5	43.75

Fig:6: Effect of Hibiscus rosasinensis leaf extract on DRC of ACH

S.No	Concentration of Ach(100µg/ml)	Dose(µg)	log dose	Dose of Flower extract(10µg/ml)	Response	% Response
1	0.1ml	10	1	1 ml	2	25
2	0.2ml	20	1.3	1 ml	3	37.5
3	0.4ml	40	1.6	1 ml	4.5	56.25
4	0.8ml	80	1.9	1 ml	5	62.5

Fig:7: Effect of Hibiscus rosasinensis Flower extract on DRC of ACH

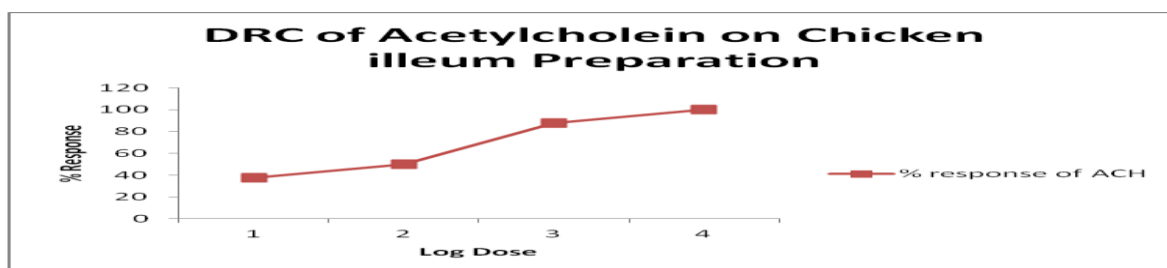


Fig:8: DRC of Acetylcholine on Chicken ileum Preparation

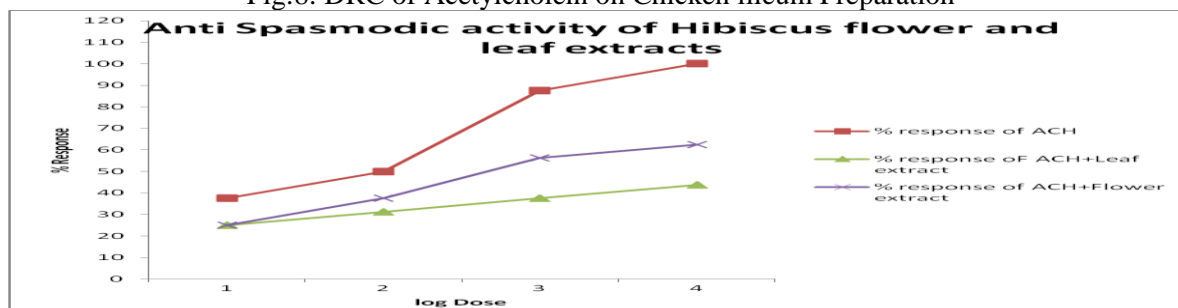


Fig:9: Anti Spasmodic activity of Hibiscus flower and leaf extracts

DRC of acetylcholine was taken with stock concentration of 100 μ g/ml, DRC of acetyl choline was taken for the 10,20,40,80 μ g dose on chicken Ileum preparation. The percent response for the DRC of ACH are noted as 37.5, 50, 87.5, 100 % respectively.

Followed by the effect of Hibiscus rosasinensis leaf and flower extracts on the DRC of ACH are evaluated, The Leaf extract showed the antagonistic action on the ACH.

The response of leaf extracts on DRC of acetyl choline was recorded as 25,31.25,35,43.75 % respectively to the dose 10,20,40,80 μ g of ACH. This indicated that the leaf extract Inhibits 56.25 % of response to the higher dose(80 μ g) of ACH.

The response of Flower extracts on DRC of acetyl choline was recorded as 25,37.5,56.25,62.5 % respectively to the dose 10,20,40,80 μ g of ACH. This indicated that the flower extract Inhibits 37.5% of response to the higher dose(80 μ g) of ACH.

The effect of flower and leaf extracts are inhibits the response of acetyl choline, compared to the both the extracts leaf extract inhibits the response greater than the flower.

The results are clearly indicating that the extracts are showing antagonistic activity on the acetyl choline.

Acetylchoein is the neurotransmitter for the parasympathetic system, it is acting through the nicotinic and muscarnic receptors, nicotinic receptors are located neuro muscular junctions, smooth muscles are contains Muscarnic type acetyl choline receptors. When the acetyl choline binds to the M type reptors it initiates the contraction of smooth muscles.

In our study we are taken the flower and leaf extracts of Hibiscus rosasinensis for evaluation of effect of these extracts on the acetyl cholien. The results are clearly revealed that these extracts are infers the action of acetylcholine and these are reduced the response of acetyl choline, as per the study model our extracts may acting through the muscarnic receptors hence these are may lowered the action of acetyl choline.

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