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PHARMACOGNOSTIC, PHYTOCHEMICAL, PHYSIOCHEMICAL AND ANTISPASMODIC PROPERTIES OF LEAVES OF *CISSAMPELOS PAREIRA* L.

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ABSTRACT

Cissampelos pareira Linn which is commonly known as velvet leaf and pareira brava belongs to the family Menispermaceae. The leaves of *Cissampelos pareira* has traditionally been used for the treatment of abdominal pain and diarrhoea. However, scientific data on the biological activities of plants are limited basis. Therefore, the purpose of the present study was to evaluate the antispasmodic effects of *Cissampelos pareira* leaf constituents on rat ileum. Antispasmodic activity was assessed by the interpolation method on isolated chicken ileum. Effects of acetylcholine, methanolic extract of *Cissampelos pareira* leaves and acetylcholine along with methanolic leaves extract were studied on isolated chicken ileum. The present study results revealed that methanolic leaves extract of *Cissampelos pareira* Linn showed promising antispasmodic action on excised chicken ileum.

KEYWORDS: *Cissampelos pareira*, Pharmacognostic, Phytochemical, Physiochemical, Antispasmodic.

INTRODUCTION

Plant description: *Cissampelos pareira* Linn belongs to family Menispermaceae.[1] It is a perennial climbing herb/shrub with small greenish-yellow flower. It belongs to the

genus *Cissampelos*, of which 30-40 species are distributed in the tropical and subtropical world. One species is found in India.[2] It was first described from Latin America, but actually occurs throughout the tropics. *Cissampelos pareira*, is a perennial climbing shrub with small greenish-yellow flowers, palate or orbicular-reniform, ovate-sub-reniform leaves with truncate cordate base, glabrous, or hairy above up to 3-12 cm long. *Cissampelos pareira* is very widespread and locally common. It is used locally to cure gastrointestinal complaints such as diarrhea, dysentery, ulcers, colic, intestinal worms and digestive complaints, and also urogenital problems such as menstrual problems, venereal diseases, infertility, uterine bleeding, and threatening miscarriage.[3] The plant is reported to have phytoconsituents like cissampeloflavone,[4] cissamparine,[5] pareirubrines A and B,[6] hayatinin,[7] and protoberberine alkaloids.[8] scientific studies revealed its antinociceptive[9], antiarthritic[10], cardiotonic[11] anticancer[12] anti-inflammatory[13] antidiarrheal[14], anti-hemorrhagic, antifertility[15], antioxidant, neuroprotective[16], hepatoprotective[16], antioxidant[17], immunomodulatory[17], anti trypanosomal activities.[1] No pharmacological investigation in the perspective of antispasmodic activity has not yet been reported on *C. pariera*. Therefore, the present study evaluates the Pharmacognostic, Phytochemical and *in vitro* antispasmodic properties of *C. pariera.*

Figure 1: *Cissampelos pareira.*

MATERIAL AND METHOD

Collection of Plant Material

Cissampelos pareira L. fresh leaves were collected from Bhawarnath, Azamgarh District, UP in the month of October 2018. The plant was identified and authenticated by G.P. Sinha, Botanist and Director, Botanical Survey of India, Chatham Lines, Allahabad.

Preparation of plant extracts (cold maceration process)

25 g air dried plant leaf powder of *Cissampelos pareira L* was transferd in air tight container and kept in refrigerator for further use. For the percolation process, the macerated plant powder is soaked in different solvents such as methanol, acetone, aqueous individually. Extraction was done by soaking one part of plant powder to three parts of liquid solvent (1:3) and kept for percolation process for 3-5 days. Occasionally shaking was done for at least 6 hours. Then the crude extracts were filtered using filter paper, evaporated and concentrated into solid extracts under room temperature.[18]

Pharmacognostic Studies

Morphology of leaves

The macroscopic features of the dried powdered root of *Cissampelos pareira* was determined using the method of Evans.[19]

Microscopy of leaves

Microscopic studies were done by preparing a thin hand section of mid rib and the lamina region of *Cissampelos pareira*. The section was cleared with chloral hydrates solution and was stained as per protocol.[19]

Physicochemical Constants

Physico-chemical constants such as percentage of total ash, acid insoluble ash, water insoluble ash, and water, acetone and methanol soluble extractives loss on drying [LOD], Swelling index and foaming index were calculated.[20]

Determination of ash value

A clean crucible was heated for an hour, then cooled it in desiccators and weighed it as (W_1). 1 g sample was placed in the crucible (W_2). The sample was charred over the burner. The crucible was then heated at 55^0C for 6 -8 hours. After the complete ignition crucible was cooled and weighed (W_3). Percent ash was calculated as follow.

$$\% \text{ Ash} = \frac{\text{Wt. of ash } (W_3 - W_1)}{\text{Wt. of sample}} \times 100$$

Acid-insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5ml of hot water

and add this liquid to the crucible. Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral. Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

$$\%\text{Acid insoluble ash} = \frac{\text{Wt.of acid insoluble residue}}{\text{Wt. of total ash}} \times 100$$

Water-insoluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material.

$$\%\text{ Water insoluble ash} = \frac{\text{Wt. of water insoluble residue}}{\text{Wt. of total ash}} \times 100$$

Determination of the moisture

A clean petridish was weight (W_1) and 1-2 gram of sample was added to it. It was placed partially covered with lid in the oven at 105^{o}C, for 4-6 hours, until constant weight was obtained. After which it was then removed and placed in desiccators for 30 minutes in order to cool it. After cooling the dish (W2) was weighted. Percent moisture may be calculated as follow.

$$\%\text{ Moisture} = \frac{W_1 - W_2}{\text{Wt of the sample}} \times 100$$

Determination of swelling index

As per WHO guidelines take 1gm of air dried plant material and transferred it into 25 ml of graduated stoppered measuring cylinder in which each diameter contain 0.2 mm having length of 125mm along with internal diameter 60mm then add 25ml of water and shake it occasionally for atleast 1 hour. Then allow standing for next 3 hours finally measured the length of plant materials which is appear in bottom, middle and upper portion of the solvent. The length of 1gm of air dried plant material which is present in a measuring cylinder. To determine the swelling index substrate the initial reading from final reading.

Swelling index = final reading –initial reading

Extractive value

Take 4gm of air dried plant material transfer it previously tared conical flask then add specified quantity of suitable solvent, occasional shaking is done for at least 6 hrs then allow to stand for next 18hrs makeup the volume up to mark filter it then collect 25ml of filterate and allow to previously tared beaker evaporate it up to dryness at temperature 105^0c for 6hrs then transfer it desiccators for removal of moisture finally calculated the weight of extract in the respect of 100gm of air dried plant material.

$$\% \text{ Extractive value} = \frac{\text{Wt. of extract}}{\text{Wt. of total sample}} \times 100$$

Phytochemical Screening

For preliminary phytochemical study, 25 g powdered material extracted through maceration with several chemical i.e. aqueous, methanol and acetone obtained extract were dried and weigh, the presence of various phytoconstituent alkaloid (Mayer's reagent test), flavonoid (Alkaline regent test), Steroids (direct test), Detection of Resin (H_2O & CH_3COCH_3 test), Detection of Saponin (foam test).[21]

PHARMACOLOGICAL STUDIES

Isolation of chicken ileum

The fresh chicken ileum was collected from local slaughter house in Chatwara in Tyrode solution and cleaned off the mesentry. Respective segments of 2-3 cm long were mounted in a 25 ml tissue organ bath, filled with a mixture of 95% O_2 and 5% CO_2 and maintained at 37^0C. The compostion of Tyrode's solution n (in mM for l lit) was 9 mg KCl, 0.1 mg NaCl, 0.1mg NaHCO3, 0.42mg NaH2PO4, 0.6 mg Glucose and pH value was 7.4.[22]

Antispasmodic activity assay procedure

Firstly concentration dependent responses of acetylcholine were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock-I solution. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution + *Cissampelos pareira* extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.

RESULTS AND DISCUSSION

Macroscopic characters of leaves

Colour: greenish on outer side and grayish underneath.

Size and Shape: 3-9~5-7cm, Cordate.

Apex: Leafs of variable, normally it is obtuse or Emarginated.

Taste: Bitter.

Odour: Slightly aromatic.

Other feature: The leafs shows entire margin, unequal bases, finely palmate venation and peteiolated.

Transverse section of leaf

It is a dorsiventral leaf section passing through the midrib represents convex shape. Midrib shows 5-6 layers of collenchymas, collateral type of vascular bundles. On lower surface anomocytic type stomata present. Upper surface is practically free from stomata. Epidermal cells have wavy walls. A single layer of palisade is present below upper epidermis. Covering trichomes present. The central region is occupied by vascular bundle. The phloem is present on dorsal side and radiating medullary traverses these cells.

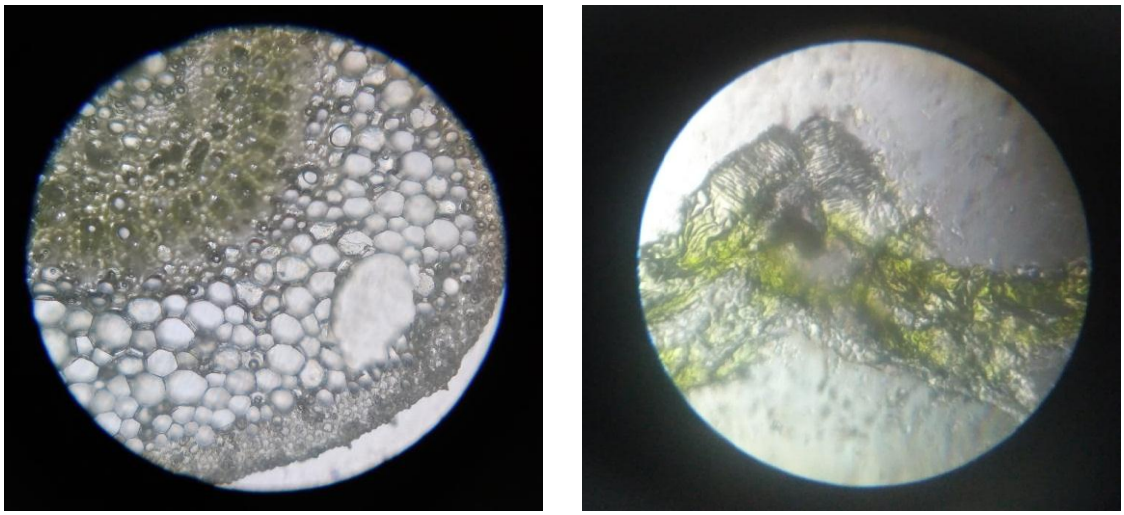

Figure 2: T.S of *Cissampelos pareira*. Leaf.

Powder Characteristic

Fibers are few and lignified. Numerous anisocytic or cruciferous stomata meaning thereby that the cells surrounding the stomatal pores are unequally arranged and cannot be differentiated from other epidermal cells.

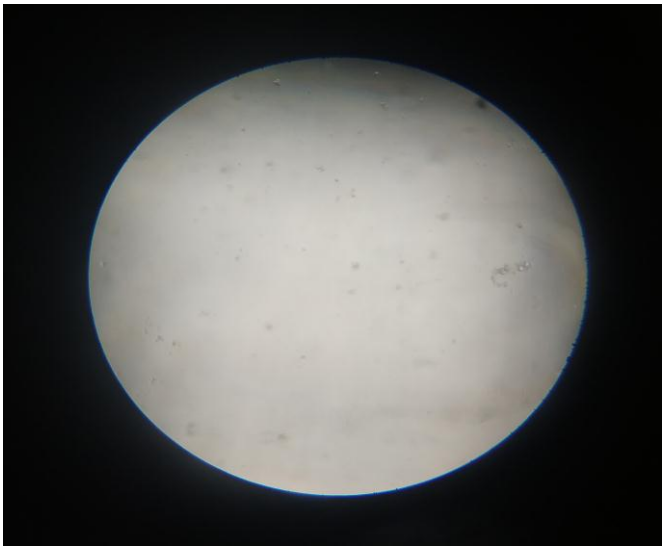

Figure 03: Powder microscopy of *Cissampelos pareira*.

Physicochemical parameter

The percentage of total ash, acid insoluble ash, water insoluble ash, extractive value, loss on drying, swelling index are tabulated formed in table 1 & 2.

Table 01: Physicochemical evalution of the crude drug of *Cissampelos pareira.*

Sr.no	Standardization parameter	%w/w
01	Total ash	30.15
02	Acid insoluble ash	1.60
03	Water insoluble ash	8.79
04	Swelling index	1.1
05	Loss on drying	14.44

Table 02: Extractive value of different extract of powder of leaf of *Cissampelos pareira.*

Sr.no.	Extract	Yield (%w/w)
01	Methanolic extract	23.75%
02	Acetone extract	18.75%
03	Aqueous extract	10%

Phytochemical parameter

Table no. 03: Qualitative phytochemical analysis of extracts of *Cissampelos pareira* leaves in different solvent system.

S.No.	Test	M	Ac	Aq
01	**Detection of alkaloids:** Mayer's test	+	+	_
02	**Detection of flavonoids:** Alkaline reagent test	_	_	_
03	**Detection of saponins :** Foam test	_	_	_
04	Tannins	+	+	_
05	Steroids	+	+	_
06	**Detection of Resins:** Acetone water test	_	_	+
07	Volatile oils	+	+	_

(+), presence. (−), Absence.

Pharmacological Activity

OBSERVATION AND RESULT: Effect of Ach on excised chicken ileum reflected an increase in spasmodic activity (response) with an increase in dose as shown in Fig.7.

Table no. 04: Dose response relationship Observation of Acetylcholine.

S.No.	Drug	Dose (ml)	Response (in mm)
01	Acetylcholine	0.2 ml	2 mm
02		0.4 ml	3 mm
03		0.8 ml	3.5 mm
04		1.6 ml	4 mm
05		3.2 ml	4 mm

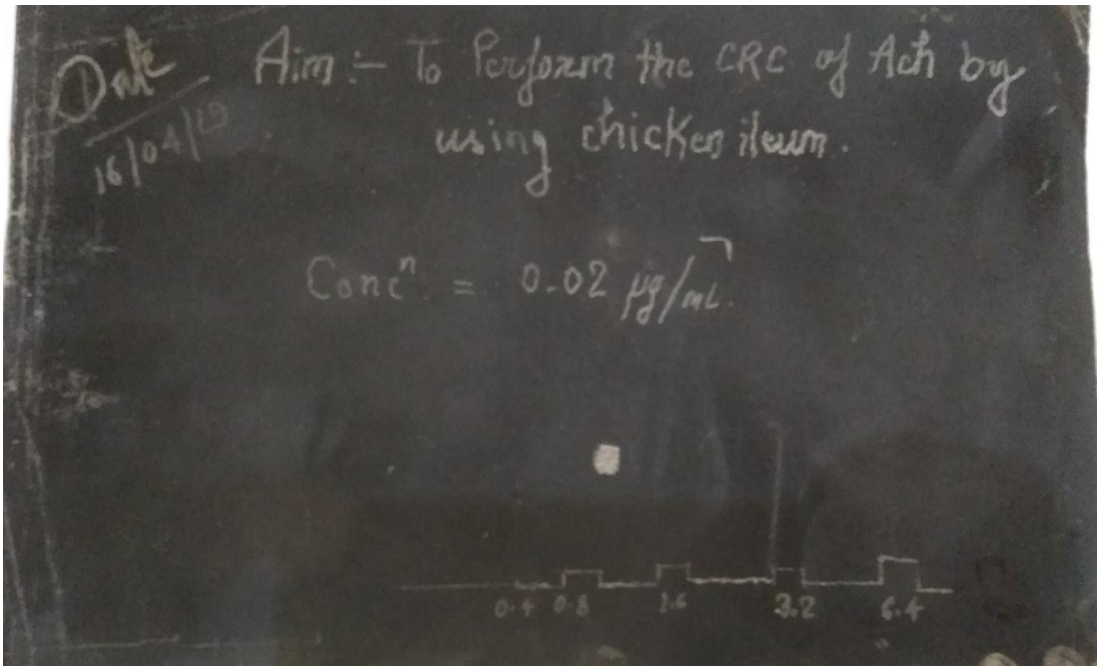

Figure 04: Response Curves of Acetylcholine.

Acetylcholine induced spasm followed by treatment of methanolic extract of *Cissampelos pareira* showed prominent antispasmodic activity as depicted in figure.

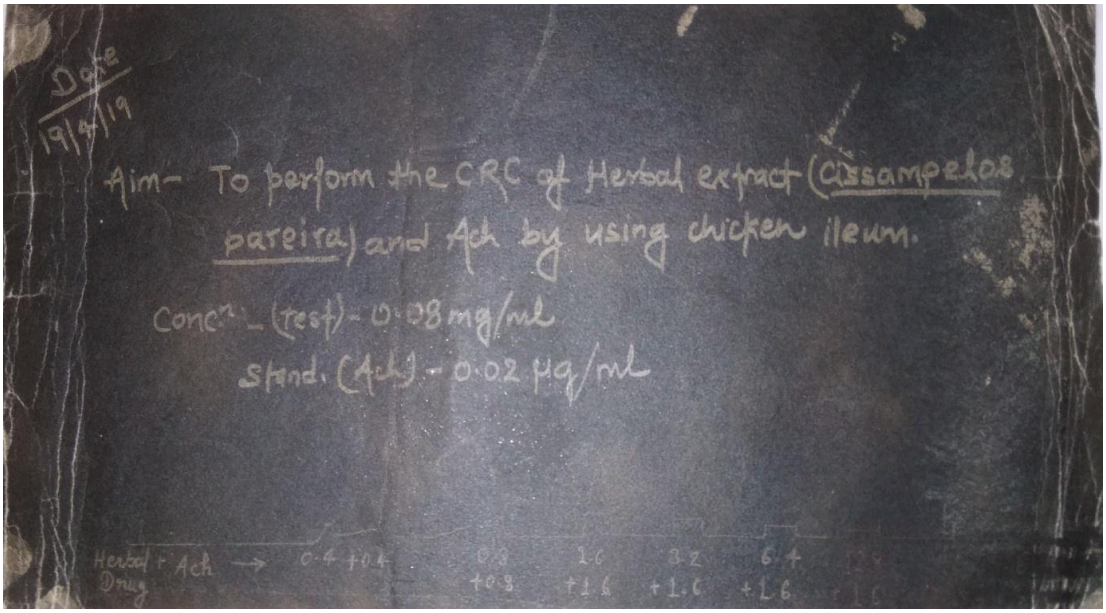

Figure 05: Response Curves of Acetylcholine + Leaves Extract.

Table no. 05: Dose response relationship Observation of *Cissampelos pareira* extract and acetylcholine.

SNo.	Drug	Dose (in ml)	Response (in mm)
01	*Cissampelos pareira* extract + Acetylcholine	0.2 ml +0.2 ml	2mm
02		0.4 ml +0.4 ml	3mm
03		0.8 ml+0.8 ml	3.4 mm
04		1.6 ml +1.6 ml	3 mm
05		3.2 ml+3.2 ml	2 mm

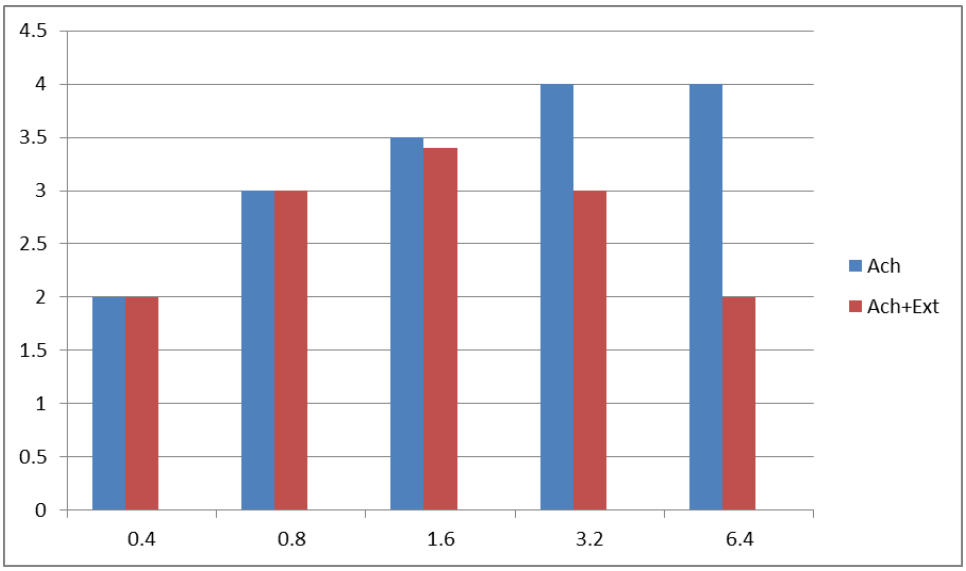

Figure 6: Comparative dose response relationship of acetylcholine and methanolic leaves extract of *Cissampelos pareira* on excised chicken ileum.

DISCUSSION

From the present study results it was observed that acetylcholine (Ach) alone causes contraction of excised chicken ileum but when acetylcholine was given in presence of methanolic leaves extract of plant *Cissampelos pareira L.*, marked decrease in contraction of ileum was observed while increasing dose of plant extract. This revealed that methanolic leaves extract of *Cissampelos pareira L.* possess spasmolytic (anti spasmodic) activity by blocking cholinergic receptors. The antispasmodic activity of the extracts might due to the presence of alkaloids compounds such as alkaloids, tannins, steroid etc.

CONCLUSION

From all observation and results obtained for the present study it was concluded that methanolic leaves extract of *Cissampelos pareira Linn*. exhibits promising antispasmodic activity. It was found that *Cissampelos pareira* has spasmolytic activity. As many antispasmodic drugs available in market and also highly potent drugs but many side effects such as urinary retention, tachycardia etc. *Cissampelos pareira* methanolic leaves extract have showed effect on isolated chicken ileum. Its herbal origin drug with high degree of safety and efficacy compared to other antispasmodic drug.

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