

Ex vivo Antispasmodic Activity of Aqueous Extract of Flowers of *Muntingia calabura* Linn. on Excised Rabbit's Jejunum

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ABSTRACT

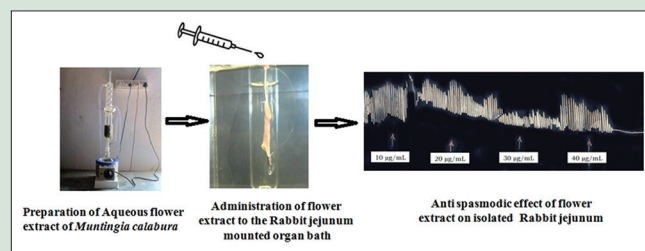
Objective: The present study has been undertaken with the main objective of evaluating the aqueous extract of flowers of *Muntingia calabura* for antispasmodic activity on isolated rabbit's jejunum. **Materials and Methods:** The study was carried out on isolated rabbit's jejunum preparations. The aqueous extract of flowers of *M. calabura* was applied in different doses by cumulative manner without washing the tissue. Spontaneous contractions abolished by the extract were recorded. **Results:** In isolated rabbit's jejunum preparation, the flower extract of *M. calabura* inhibited the spontaneous contractions in a concentration-dependent manner with IC_{100} value of $36 \pm 3.02 \mu\text{g/mL}$, which is potent than the standard drug verapamil with IC_{100} value of $40 \pm 1.02 \mu\text{g/mL}$ on the rabbit's jejunum. **Conclusion:** The aqueous extract of flowers of *M. calabura* exhibits significant dose-dependent relaxations of spontaneous contractions in isolated rabbit's jejunum preparations.

Key words: Ex vivo antispasmodic activity, IC_{100} values, *Muntingia calabura*, verapamil

SUMMARY

- Flowers of *Muntingia calabura* L. were collected, authenticated and extracted by water using soxhlet apparatus. Crude extract was qualitatively tested for the presence of phytochemical constituents and it shows the presence of flavonoids, carbohydrates, glycosides, saponins, and phenolic compounds. Crude extract was quantitatively tested for its antispasmodic potential. It showed a dose-dependent relaxation of spontaneous contractions in isolated rabbit's jejunum preparations and it was concluded

that the aqueous flower extract of *Muntingia calabura* L. has potent antispasmodic activity.



Abbreviations Used: IC_{100} : Maximal inhibitory concentration, IAC: Institutional animal ethics committee, SIMS: Southern institute of medical sciences, h: Hour, kg: Kilogram, μg : Microgram, mL: Milliliter, mM: Millimol, $^{\circ}\text{C}$: Degree Celsius.

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INTRODUCTION

Medicinal plants are sources of important therapeutic aid for alleviating human ailments. Approximately 80% of the people in the developing countries worldwide depend on traditional medicine for their primary healthcare. *Muntingia calabura* L. (family *Muntingiaceae*) is known throughout the world as Jamaican cherry. *M. calabura* is one of the most common roadside trees widely cultivated in warm areas of the Asian region and Southeast Asia such as Malaysia, Indonesia, and the Philippines. Several medicinal uses have been documented on various parts of this tree. *M. calabura* leaves, barks, flowers, and roots have been used as a folk remedy.^[1] In Peruvian folklore medicine, the flowers and barks are used as antiseptic and anti-inflammatory. In Colombia, the infusion of the flowers is used as a tranquilizer and tonic. The leaves, boiled in water, are used to reduce gastric ulcer and swelling of the prostate gland and to alleviate headache and cold.^[2] Moreover, the boiled barks can be used as a wash to reduce swelling in the lower extremities. In Mexico, the plant is used to treat measles, mouth pimples, and stomach ache. In the Philippines, the flowers are also used to treat headache and incipient cold or as tranquilizers, antispasmodics, and antidyspeptics. Other than that, the roots of *M. calabura* have been used as an emmenagogue in Vietnam and as an abortifacient in Malaysia.^[3]

MATERIALS AND METHODS

Drug and solution used were verapamil (Nicholas Piramal India Ltd.) and Tyrode's solution.

Preparation of physiological solutions

Tyrode's solution was prepared carefully using analytical grade reagents and distilled water. pH of the final solution was adjusted to 7.4. Composition of Tyrode's solution is given in mmol/L: NaCl, 137; KCl, 2.7; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.8; NaH_2PO_4 , 0.4; NaHCO_3 , 12; glucose, 5.5.

Experimental animal

Either sex of New Zealand White Rabbits weighing 1.5–2.0 kg were used in the experiments. The animals were housed at a constant room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$) with 12:12 h light and dark cycle and they were maintained on a standard pellet diet and water *ad libitum*.

Preparation of extract

The fresh flowers of *M. calabura* were collected from Guntur district of Andhra Pradesh and authenticated by Dr. S. M. Khasim M.Sc.,

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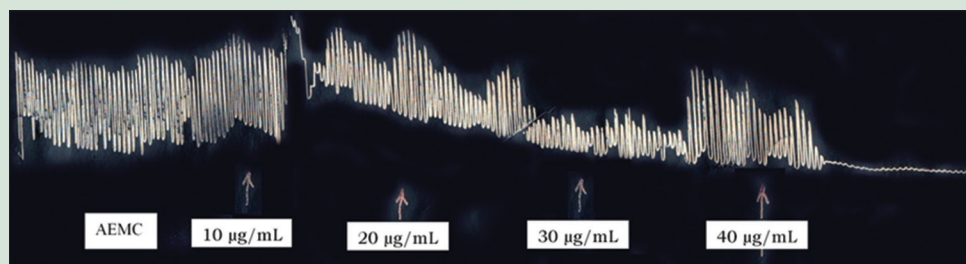


Figure 1: Typical tracing showing the inhibitory effects of crude aqueous extract of *Muntingia calabura* L. on spontaneous contractions of isolated rabbit jejunum preparation

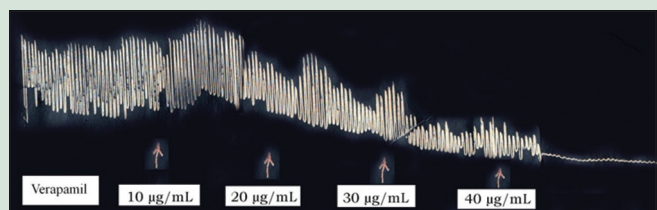


Figure 2: Typical tracing showing the inhibitory effects of verapamil on spontaneous contractions of isolated rabbit jejunum preparation

Ph.D., Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh. The washed flowers were dried at room temperature (25°C–35°C), the dried flowers were powdered and passed through sieve number 80, the dried powdered flowers were defatted with petroleum ether and then extracted with water using Soxhlet extractor and dried at 45°C in an oven, and it was used for further phytochemical and pharmacological studies.^[4-6]

Qualitative phytochemical screening

Crude extract was qualitatively tested for the presence of chemical constituents by performing various tests such as Mayer's test for alkaloid, steroids with Salkowski's test, glycosides with Keller–Killiani test, carbohydrates with Molisch's test, flavonoids with aluminum chloride test, phenolic/tannins with ferric chloride solution, and saponins with water. These were identified by characteristic color change using standard tests.^[7,8]

Experimental design

The experimental protocol was approved by the Institutional Animal Ethics Committee which follows the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The protocol approval number is IAEC/SIMS/2013/006 dated 07/May/2013.

Rabbit jejunum preparation

The spasmolytic activity of the plant materials was studied using isolated rabbit jejunum.^[9] Rabbits were starved for 24 h before the experiment and they were stunned by a blow on the head and then pithed. The abdomens of pithed animals were opened, and the jejunum portion of about 2.0 cm was removed. Each preparation was cleared off the mesentery so that it could freely give spontaneous contractions. Each segment of about 2 cm lengths was suspended in a 10 mL tissue bath containing Tyrode's solution maintained at 37°C and aerated. The tissues were allowed to equilibrate for at least 30 min at preload of 1 g. Tension changes in the tissue were recorded on kymograph. The smooth muscles relaxant action of test material was observed by administration of flower extract of *M. calabura* in a cumulative fashion directly without the use of an agonist.

RESULTS

Phytochemical screening

Qualitative phytochemical screening on the *M. calabura* flower extract shows the presence of flavonoids, carbohydrates, glycosides, saponins, and phenolic compounds.

Effects on rabbit jejunum

In isolated rabbit's jejunum preparation, the flower extract of *M. calabura* inhibited the spontaneous contractions in a concentration-dependent manner with IC_{100} value of $36 \pm 3.02 \mu\text{g/mL}$ [Figure 1], similar to that of verapamil with IC_{100} value of $40 \pm 1.02 \mu\text{g/mL}$ on the rabbit's jejunum [Figure 2].

DISCUSSION

The current study was carried out to validate the traditional use of flowers of *M. calabura* as antispasmodic. Contractile effects of the intestine are due to the cytosolic-free calcium levels. Moreover, intracellular and extracellular calcium stores exchange with each other. Periodic depolarization and repolarization of the tissues are due to the influx of calcium into sarcoplasmic reticulum through voltage-dependent calcium channel. These events are responsible for spontaneous intestinal responses.^[10] The aqueous extract of *M. calabura* flowers caused a concentration-dependent inhibition of spontaneous contractions in isolated rabbit jejunum preparations, thus showing an antispasmodic action which is equipotent to that of verapamil.

CONCLUSION

The present study on preliminary phytochemical evaluation of aqueous flower extract of *M. calabura* produced positive results for alkaloids, glycosides, carbohydrates, saponins, and phenols. The aqueous extract of flowers of *M. calabura* exhibited a significant dose-dependent relaxation of spontaneous contractions in isolated rabbit's jejunum preparations.

In summary, the findings suggest that *M. calabura* has antispasmodic activity. These findings may explain the medicinal use of *M. calabura* in abdominal colic, diarrhea, and hypertension. However, more detailed studies are in progress to establish the possible mechanism of action, safety, efficacy, and isolation of active constituents responsible for this activity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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