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EX-VIVO ANTISPASMODIC ACTIVITY OF AQUEOUS LEAF EXTRACT OF *MUNTINGIA CALABURA LINN* ON ISOLATED FROG RECTUM

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ABSTRACT

The present study has been under taken with main objective of evaluating the aqueous leaf extract of Muntingiacalabura for antispasmodic activity on isolated frog rectum.The plant material was authenticated by botanist and extracted with water. Qualitative assay for the presence of plant phytoconstituents were carried out by following standard procedure. The study was carried out on isolated frog rectum preparations. The aqueous leaf extract of Muntingiacalabura was applied in different doses by cumulative manner without washing the tissue. The extract shown positive results for the test of carbohydrates, protein, glycosides, flavonoids, tanins, saponins and phenolic compounds. The spontaneous contraction of isolated frog rectum preparation were abolished by the leaf extract of Muntingiacalabura, in a concentration dependent manner with IC_{100} value of 966.7± 28.87µg/ml and it is compared with the standard drug verapamil which completely abolished (IC_{100} value) the spontaneous contraction of isolated frog's rectum preparation at the concentration of 43.33±5.774 µg/mL.

Key words: Ex-vivo antispasmodic activity, Muntingiacalabura, IC100value, verapamil.

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INTRODUCTION

Antispasmodics are muscle relaxants that are used to relieve cramps or spasms of the stomach, intestines, bladderand other smooth muscles. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea, spastic constipation, colic and irritable bowel syndrome, which affect millions of people. Diarrhea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries (1). Medicinal plants are sources of important therapeutic aid for alleviating human ailments. Approximately 80% of the people in the developing countries all over theworld depend on traditional medicine for their primary health care. Muntingia calabura L. (family-Muntingiaceae), is

known throughout the world as Jamaican cherry. This plant ispopularly known for its antiseptic and antispasmodic properties besidesbeing a proven hypotensive drug (2). Various parts of this tree haveseveral documented medicinal uses. Its leaves, barks and flowers are believed to possess medicinal value and is rich in flavonoids, flavones and flavanones, rendering to its potent antitumor activities (3). Moreover, flowers of M. calabura have been used as anti-septic, antispasmodic, antidyspeptic, diaphoretic, tranquilizer, tonic and for thetreatment of headache, whereas roots are employed as emmenagogue and abortifacient (4). Infusion of the flower of this plant is drunk as a tranquilliser and tonic (4).

MATERIAL AND METHODS

Experimental animal

Frogs belonging to the species of Ranahexadactyla of either sex weighing 100 -150 gms were used.

Preparation of physiological solutions

Frog ringer solution composition: (mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, NaHCO3 11.9, NaH_2PO_4 0.42, glucose 5.6).

Preparation of extract

The fresh leaves of Muntingia calabura were collected from Guntur district of A.P and authenticated by Dr. B. Sandhya, M.Phil., Ph.D. Principal, SIMS College of Life Sciences, Mangaldas Nagar, Guntur. The washed leaves are dried in room temperature (25-35^{0}C), the dried leaves are powdered and passed through sieve number 80.The dried powdered leaves were defatted with petroleum ether and then extracted with water using soxhlet extractor for 22h. The extract was filtered with whatman filter paper and dried at 45^{0}C in an oven and it was used for further phytochemical and pharmacological studies.

Table-1 Preliminary phytochemical tests for plant extract

Phytoconstituents	Test	Observation
Alkaloids (Hager's Test)	2mL extract + few drops of Hager's reagent	Yellow precipitate
Anthraquinones(Borntrager's Test)	3mL extract + 3mL Benzene + 5mL NH_3(10%)	Pink, Violet or Red coloration in ammonical layer
Carbohydrates (Molisch's Test)	2mL extract + 10mL H_2O + 2 drops Ethanolic αnaphthol (20%) +2mL H_2SO_4 (conc.)	Reddish violet ring at the junction
Glycosides (Liebermann's Test)	2mL extract + 2mL $CHCl_3$ + 2mL CH_3COOH	Violet to Blue to Green coloration
Flavonoids	1mL extract + 1mL $Pb(OAc)_4$ (10%)	Yellow coloration
Proteins (Xanthoproteic Test)	1mL extract + 1mL H_2SO_4(conc.)	White precipitate
Saponins (Foam Test)	(a) 5mL extract + 5mL H_2O + heat	Froth appears
	(b) 5mL extract + Olive oil (few drops)	Emulsion forms
Steroids (Salkowski Test)	2mL extract + 2mL $CHCl_3$ + 2mL H_2SO_4 (conc.)	Reddish brown ring at the junction
Tannins (Braymer's Test)	2mL extract + 2mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Terpenoids	2mL extract + 2mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration
Phenol (Ferric chloride test)	2mLextract + 2mL of distilled water + 10 % $FeCl_3$ solution.	Bluish black colour

Experimental design

Isolated frog rectum preparation

The spasmolytic activity of the plant material was studied by using isolated frog rectum preparation (7). Frog was stunned by blow on the head and then pithed. The abdomens of pithed animals were opened and rectum portion of about 2.0cm were 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 frog ringer solution maintained at 37°C and aerated. The tissues were allowed to equilibrate for at least 30 min at preload of 1 gm. Tension changes in the tissue were recorded on kymograph. The smooth muscle relaxant action of test material was observed by administration of leaf extract of Muntingiacalabura in a cumulative fashion and compared with standard drug verapamil.

RESULT AND DISCUSSION

Phytochemical screening

Qualitative phytochemical screening on the Muntingia calaburaleaf extract shows the presence of carbohydrates, protein, glycosides, flavonoids, tanins, saponins and phenolic compounds.

Table-2 Phytochemical analysis of extract of leaves of M. calabura

Phytoconstituents	Test result
Alkaloids (Hager's Test)	–
Anthraquinones (Borntrager's Test)	–
Carbohydrates (Molisch's Test)	+
Glycosides (Liebermann's Test)	+
Flavonoids	+
Proteins (Xanthoproteic Test)	+
phenolic compounds	+
Saponins (Foam Test)	+
Steroids (Salkowski Test)	–
Tannins (Braymer's Test)	+
Terpenoids	–

Effects on frog rectum

In isolated frog rectum preparation, the leaf extract of Muntingia calabura, inhibited the spontaneous contractions in a concentration dependent manner with IC_{100} value of 966.7± 28.87µg/mL as shown in the figure-1. The standard drug verapamil completely abolished (IC_{100} value) the spontaneous contractions of isolated frog's rectum preparation at the concentration of 43.33±5.774 µg/mL.

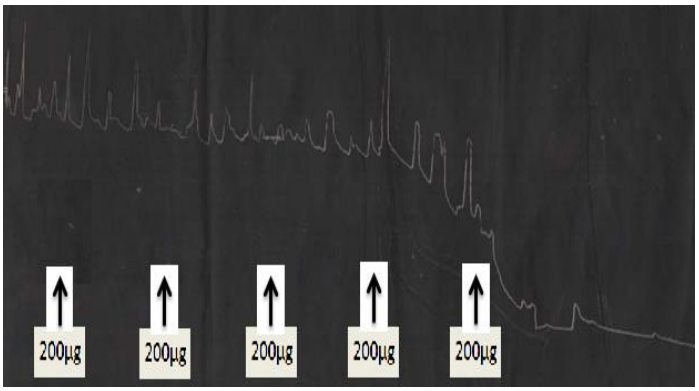

Figure-1 Typical tracing showing the inhibitory effects of crude aqueous extract of Muntingia calabura L. on spontaneous contractions of isolated frog rectum preparation.

The current study was carried out to validate the traditional use of leaves of Muntingiacalabura as anti-spasmodic. 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 influx of calcium into sarcoplasmic reticulum through voltage dependent calcium channel. These events are responsible for spontaneous intestinal responses (8). The aqueous extract of the Muntingia calabura leaves caused a concentration-dependent inhibition of spontaneous contractions in isolated frog rectum preparations by operating some unknown mechanism.

CONCLUSION

The present study on preliminary phytochemical evaluation of aqueous leaf extract of Muntingia calabura produced positive results for carbohydrates, protein, glycosides, flavonoids, tanins, saponins and phenolic compounds. The aqueous extract of leaves of Muntingia calabura exhibit significant dose dependent relaxations of spontaneous contractions in isolated frog rectum preparations. In summary, the findings suggest that Muntingiacalabura has antispasmodic activity. These findings may explain the medicinal use of Muntingia calabura in abdominal colic, diarrhoea and hypertension. However, more detailed studies are in progress to establish the possible mechanism of action, safety, efficacy and isolation of active constituents responsible of this activity.

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