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PHYTOCHEMICAL AND *IN-VITRO* ASSESSMENT OF ANTIHISTAMINIC AND ANTICHOLINERGIC ACTIVITY OF LEAVES OF *HIBISCUS SABDARIFFA* LINN.

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Keywords:

Hibiscus sabdariffa Linn., Histamine, Acetylcholine, Leaves extract

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ABSTRACT: Objective: The aim of the present study was to find out the herbal drug which has potential antihistaminic properties. Studying the *in-vitro* antagonistic effect of crude extracts on isolated goat tracheal chain preparation for assessment of direct antihistaminic activity using two agonists like histamine and acetylcholine. **Methods:** The ethanolic extract of leaves of *Hibiscus sabdariffa* was found quite useful in showing antihistaminic activities when tested on an experimental isolated goat tracheal. They were divided into five groups. **Results:** PTEE exerted an antagonistic effect on histamine and acetylcholine-induced contraction ($P < 0.05$). Significance is seen at a dose of 2, 4, 10 mg/ml for histamine and acetylcholine Figure 6.2 and 6.4 in a dose-dependent manner. Histamine antagonistic effect seen as (70.12 ± 1.727, 56.09 ± 1.2, 48.17 ± 1.321) similarly the acetylcholine antagonistic effect seen as (85.60 ± 2.489, 60.20 ± 2.456, 44.00 ± 1.141). **Conclusion:** The present study a notable contraction produced by histamine at a dose 1.6µg/ml, as 82 mm taken as 100% while notable contraction was produced by acetylcholine at a dose 1.6 µg/ml, as 92 mm taken as 100% were observed.

INTRODUCTION: *Hibiscus sabdariffa* is a medicinal plant that is consumed for its health benefits, juice/concoction prepared from the plant is taken as a preventive/curative measure against diabetes and hypertension. The antihypertensive and other pharmacological properties such as antibacterial, anti-oxidant, nephro- and hepato-protective, renal/diuretic effect, anti-cholesterol, and anti-diabetic effects of *Hibiscus sabdariffa* have been demonstrated in several studies.

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Constituents of different plant parts of *Hibiscus sabdariffa* include phenolic acids, organic acid, flavonoids, and anthocyanins which may contribute to the pharmacological effects of the plant. There is a growing market for nutraceutical and functional foods, while a study on natural sources of antioxidants and their potential as nutraceutical and functional foods is on the increase [1].

One plant that has attracted much attention over the years for its health benefits is roselle (Hibiscus sabdariffa); many studies on the plant, its numerous preparation, and constituents focused on its antioxidant properties. *Hibiscus sabdariffa* L. (roselle) belongs to the family Malvaceae. It exists as herbs or shrubs, often with fibrous stems [2]. The leaves are deeply three- to five-lobed, 8–15 cm long, arranged alternately on the stems. Vernacular names, in addition to roselle, in English-speaking

regions are rozelle, sorrel, red sorrel, and Florida cranberry. In North Africa and the Near East, *Hibiscus sabdariffa* is called karkadé or carcadé [3]. *Hibiscus sabdariffa* is believed to have originated from India and Malaysia. In India, Africa, and Mexico, all above-ground parts of the *Hibiscus sabdariffa* plant are valued in native medicine. Infusions of the leaves or calyces are regarded as diuretic, cholerectic, febrifugal, and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis. The fresh calyx of *Hibiscus sabdariffa* is eaten raw in salads, is cooked, and used as a flavouring in cakes; presently, it is consumed worldwide as a cold beverage and as a hot drink (sour tea) [4, 5, 6]. The red anthocyanin pigments in the calyces are used as food colouring agents [7]. Seeds of *Hibiscus sabdariffa* are used in oily soups, sauces, and coffee substitute [8, 9]. Root of *Hibiscus sabdariffa* is edible but very fibrousy, mucilaginous, without very much flavor [10]. The aim of the present study was to find out the herbal drug which has potential antihistaminic properties **Fig. 1**.

FIG. 1: *HIBISCUS SABDARIFFA* PLANT

MATERIALS AND METHODS: The plant specimens for the proposed study were collected from the deep forest of Satpuda hills with the help of forest officers of Chopda Tahsil, Dist. Jalgaon, Maharashtra (India) in the month of Dec. 2018, care was taken to select healthy plants and for normal organs. The plant *Hibiscus sabdariffa* was authenticated (Ref. No. BSI/WRC/IDEN.CER/ 2018/H3/115) by Prof. (Dr.) Priyanka A Ingle, scientist, BSI (Botanical Survey of India), Pune (M.S.).

Pharmacological Activity:

Experimental Tissue: Goat trachea was obtained from a slaughterhouse, brought in previously aerated Kreb's solution (concentration in mM/lit: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Glucose, 11.1) maintained at 37±10C.

Standard Drugs: All standard drugs required for the study were purchased, and solvents and chemicals used for physiological salt solution were laboratory grades.

• Histamine	Sigma, USA
• Acetylcholine	Research Lab. INDIA

Preparation of Standard Drug and Extract Solution: Histamine as well as acetylcholine respectively dissolved in physiological saline and stock solution of histamine & acetylcholine was prepared to have a concentration of 0.1 µg/ml and dosed as at 1 ml, 2 ml, 4 ml, 8 ml, 1.6 ml to study the effect of both agonist on tissue contraction at 0.1 µg, 0.2 µg, 0.4 µg, 0.8 µg, 1.6 µg respectively.

Solution of the extract was prepared in 5% Polyethylene glycol, and arbitrarily doses of all extracts were added to the reservoir as 2 mg/ ml, 4 mg/ml, and 10 mg/ml to study the effect of extracts on histamine & acetylcholine-induced contractions for *in-vitro* studies.

Storage of Drug Solution: Fresh drug solutions were prepared for each day's work. The solutions were kept in air-tight amber coloured bottles and stored at room temperature till use.

Routes of Administration: All drugs were administered as per screening model procedures.

Statistical Analysis: The data were presented as mean ± SEM. The statistical significance between the groups has been tested by ANOVA followed by Dunnett's test. A probability value less than 0.05 was considered significant.

***In-vitro* Assessment of Direct Antihistaminic and Anticholinergic Activity:**

Isolated Goat Tracheal Chain Preparation: This method is used to assess the direct antihistaminic activity of plant extract. The pharmacological actions of histamine on trachea-bronchial muscle have been described in many species. In most animal species such as, rodents, dogs, and human trachea, histamine causes bronchoconstriction,

whereas; in cats and sheep, it shows the relaxation of trachea-bronchial smooth muscles. The method is used for the study of action antispasmodic drugs on the tracheal musculature. The method is based upon the findings that the excised trachea will respond to many drugs with the characteristic actions for which the drugs are well known and that with proper magnification, the response can be recorded and measured for comparative purposes. Although the method is known for its suitability in the study of antispasmodic drugs in general, emphasis is given on its use in the testing of bronchodilators. This is because of the close anatomical and physiological association, which exists between tracheal and bronchial musculature. The bronchial muscle of Ox and Pig *in-vitro* has been often utilized for the study of contractile responses of agonists as well as antagonist evaluation. The rodents, dog, and man trachea respond with bronchoconstriction whereas, in cats, histamine has been shown to relax the tracheal smooth muscle [11]. The guinea pig tracheal chain is a classical preparation but requires some skill to prepare and is not very sensitive for many agonists. It is reported that isolated goat trachea contracts in response to acetylcholine (0.1-12.8 µg), histamine (0.1-102.4 µg), and barium chloride (0.1-51.2 µg) in a dose-dependent manner and to 5-HT in a narrow dose range. Pheniramine maleate (H1-receptor antagonist) blocks contractions to histamine, while cimetidine (H2-receptor antagonist) potentiates the contraction. These observations suggest the presence of both H1-excitatory and H2-inhibitory receptors for histamine on the isolated goat trachea. The Observed dose relative contractile responses of different agonists like acetylcholine, histamine, 5-hydroxytryptamine, and bradykinin on isolated goat trachea. With these agonists, the concentration necessary to produce contraction was less with goat tracheal chain than with guinea pig tracheal chain.

They also found that both goat tracheal chain and strip preparation were suitable for screening spasmogenic activity on respiratory smooth muscle, and goat tracheal chain is easier to handle and prepare and is also much more sensitive than a guinea-pig tracheal chain. They also found that both goat tracheal chain and strip preparation were suitable for screening spasmogenic activity on respiratory smooth muscle, and goat tracheal chain is easier to handle and prepare; it is also much more sensitive than guinea-pig tracheal chain [12, 13, 14, 15].

Procedure:

1. Isolated adult goat tracheal tissue was obtained immediately after slaughter of the animals.

2. Trachea was cut into individual rings and tied together in series to form a chain.

3. Trachea was suspended in a bath of Krebs solution of the following composition.

4. NaCl 6.9, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4$ 0.28, $NaHCO_3$ 2.1, KH2PO4 0.16 and Glucose 2.0 gm/litre Which was continusoly aerated and maintained at 37 ± 0.5 ºC.

5. One end of the tracheal chain was attached to an S-shaped aerator tube and the other attached to an isotonic frontal writing lever to smoked drum (magnification 10-12 folds), a stream of 5% CO_2 in oxygen should be bubbled through the organ tube.

6. Tissue was allowed to equilibrate for 45 min. under a load of 400 mg.

7. A dose-response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle was taken in following groups,

Group-I: Control
Group-II: Vehicle (PEG-400, 5% /ml)
Group-III: Test extract (2 mg/ ml)
Group-IV: Test extract (4 mg/ ml)
Group-V: Test extract (10 mg/ ml)

Graph of percentage of maximum contractile response on the ordinate and negative logarithm of the molar concentration of histamine & acetylcholine on abscissa was plotted to record dose-response curve of histamine & acetylcholine, in the absence and in presence test extract to study the possible antihistaminic & anticholinergic ability against histamine and acetylcholine precontracted goat tracheal preparation *in-vitro* (**Table 3-6** and **Fig. 2- 5**)[16-20].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Studies: Pet. Ether and Ethanolic extract of leaves of *Hibiscus sabdariffa* Linn. Showed the presence of various Phytoconstituents such as glycosides, saponins, triterpenoids, tannins and flavanoids **Table 2** and **3**.

TABLE 1: PERCENTAGE YIELD AND PHYSICAL CHARACTERISTICS OF *HIBISCUS SABDARIFFA* EXTRACTS

S. no.	Extract	Description			Yield
		Colour	Odour	Nature	(% w/w)
1.	Petroleum ether	Dark Greenish yellow	None	Oily, sticky	32.20
2.	Ethanol (Absolute)	Dark yellowish brown	Characteristic	Oily thick semisolid	62.50

TABLE 2: PRELIMINARY PHYTOCHEMICAL TEST OF EXTRACTS OF TUBER OF *HIBISCUS SABDARIFFA*

S. no.	Test	PEE	EE*
1	**Test for carbohydrates**		
	Molish's test	-	+
	Fehling's test	-	+
	Benedict's test	-	+
2	**Test for proteins**		
	Biuret test	-	-
	Million's test	-	-
3	Test for amino acids		
	Ninhydrin test	-	-
4	**Test for fats and oil**		
	Solubility test	+	-
	Filter paper test	+	-
5	**Test for steroids**		
	Salkowski reaction	-	+
	Liebermann-Burchard reaction	-	+
6	**Test for glycosides**		
	Legal test	-	+
	Keller-Kellani test	-	+
7	**Test for antraquinone glycoside**		
	Borntrager's test	-	-
8	**Test for saponins**		
	Foam test	+	+
9	Test for flavonoids		
	Shinoda test	-	+
10	**Test for alkaloids**		
	Dragendorff's test	-	+
	Mayer's test	-	+
	Hager's test	-	+
	Wagner's test	-	+
11	**Test for tannins and phenolic compounds**		
	5% $FeCl_2$ solution	+	-
	Lead acetate solution	+	+
	Acetic acid solution	+	+
	Dilute iodine solution	+	+
	Dilute HNO_3	+	-

Remark - (+) Present, (-) Absent, * - bioactive extract (PEE: Petroleum ether extract, EE: Ethanol extract

Pharmacological Activity:

TABLE 3: EFFECT OF VCPEE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

S. no.	Log dose Histamine	Control % Response	Vehicle % Response	PTPEE % Response	PTPEE % Response	PTPEE % Response
1	1.0	7.61±0.584	8.22±0.584	5.48±0.352	5.48±0.352	5.48±0.787
2	0.69	13.41±0.704	13.41±0.704	9.75±0.498	9.75±0.498	8.53±0.498
3	0.39	22.25±0.767	25.60±1.217	19.2±0.584	16.54±0.479	17.07±0.448
4	0.09	49.38±0.607	49.38±1.165	35.36±1.317	30.48±0.771	30.17±0.767
5	0.20	99.08±0.584	97.56±0.996	94.20±1.269	82.92±1.928	85.12±0.787

n=4, values are in Mean ± SEM, Control = % Response of Histamine in the absence of (PTPEE) , Test I, II, III = D.R.C. of Histamine in the presence of (PTPEE) (2, 4, 10 mg/ml respectively) Statistically Non-significant data (90 mm as 100 %)

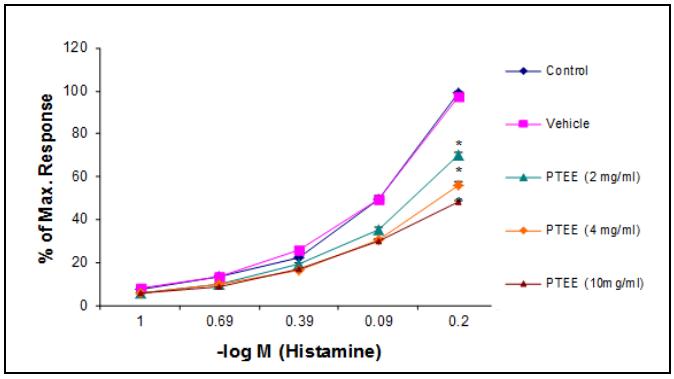

FIG. 2: EFFECT OF PTPEE ON ISOLATED GOAT TRACHEAL CHAIN REPARATION

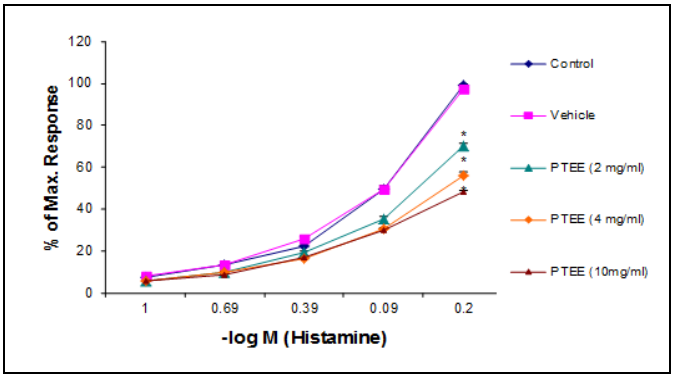

FIG. 3: EFFECT OF PTEE ON ISOLATED GOAT TRACHEAL CHAIN REPARATION

TABLE 4: EFFECT OF PTEE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

S. no.	Log dose Histamine	Control % Response	Vehicle % Response	PTEE % Response	PTEE % Response	PTEE % Response
1	1.0	7.61±0.584	8.22±0.584	5.48±0.277	5.48±0.531	5.48±0.455
2	0.69	13.41±0.704	13.41±0.704	9.75±0.496	9.75±0.716	8.53±0.531
3	0.39	22.25±0.767	25.60±1.217	19.02±0.681	16.54±0.718	17.07±0.719
4	0.09	49.38±0.607	49.38±1.165	35.36± 0.7	30.48±1.47	30.17±0.718
5	0.20	99.08±0.584	97.56±0.996	70.12±1.727*	56.09±1.2*	48.17±1.321*

n=4, values are in Mean ± SEM. Control = % Response of Histamine in absence of (PTEE), Test I, II, III = D.R.C. of Histamine in presence of (PTEE) (2, 4, 10 mg/ml respectively) *p< 0.05 compared with histamine-induced contraction (82 mm as 100 %)

TABLE 5: EFFECT OF PTPEE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

S. No.	Log dose Ach	Percent of maximum response				
		Control Ach	Vehicle	PTPEE (2 mg/ml)	PTPEE (4 mg/ml)	PTPEE(10 mg/ml)
1	1.0	10.25±0.984	9.83±0.982	7.89±0.789	6.87±0.825	6.10±0.498
2	0.69	18.64±1.453	16.54±1.482	14.01±1.035	13.58±1.698	12.58±1.954
3	0.39	30.48±2.040	29.51±2.457	29.57±1.589	26.56±1.458	24.80±2.478
4	0.09	54.32±2.710	49.57±2.410	42.54±3.012	41.20±2.369	38.90±1.256
5	0.20	94.31±2.140	90.24±2.991	86.22±1.865	85.98±2.881	83.40±1.254

n=4, values are expressed in mean ± SEM, Statistically non-significant data (92 mm as 100 %)

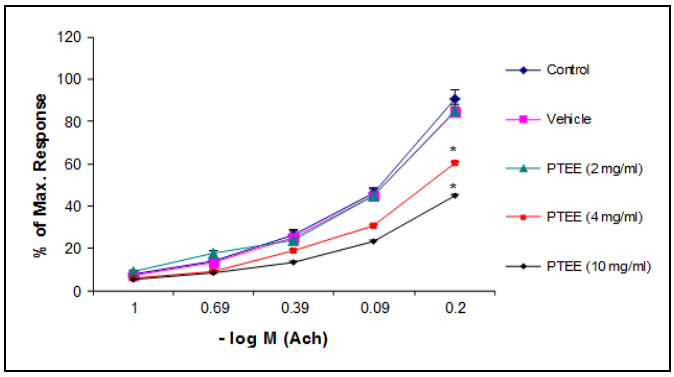

FIG. 4: EFFECT OF PTPEE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

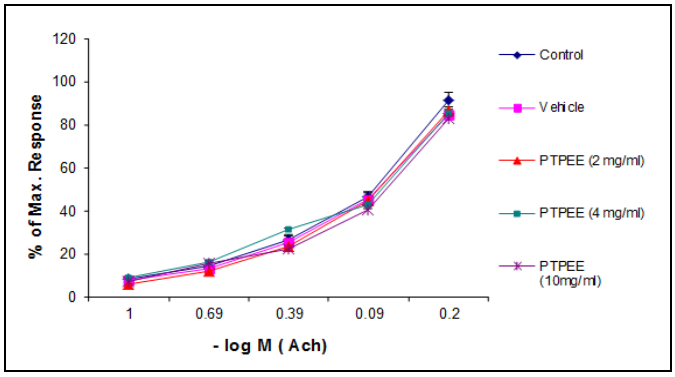

FIG. 5: EFFECT OF PTEE ON ISOLATED GOAT TRACHEAL CHAIN REPARATION

TABLE 6: EFFECT OF PTEE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

S. no.	Log dose Ach	Percent of maximum response				
		Control Ach	Vehicle	PTEE (2 mg/ml)	PTEE (4 mg/ml)	PTEE (10 mg/ml)
1	1.0	10.25±0.984	9.83±0.982	7.51±0.710	8.76±0.748	8.60±0.861
2	0.69	18.64±1.453	16.54±1.482	13.58±1.231	14.89±1.134	15.60±1.629
3	0.39	30.48±2.040	29.51±2.457	29.41±2.688	28.43±2.159	27.45±2.735
4	0.09	54.32±2.710	49.57±2.410	43.60±2.670	41.20±2.357	40.15±2.361
5	0.20	94.31±2.140	90.24±2.991	85.60±2.489*	60.20±2.456*	44.00±2.141*

n=4, values are expressed in mean ± SEM, *p< 0.05 compared with acetylcholine induced contraction (92 mm as 100 %)

DISCUSSION AND CONCLUSION:

Effect of Crude extracts on Isolated Goat Tracheal Chain Preparation: Asthma is a heterogeneous disorder immunologically, physiologically, and biochemically and its etiology is multifactorial.

As different mediators are implicated in asthma, and the precise etiology is not known; also, multiple biochemical processes are triggered by different causative factors, it is difficult to have a single drug, which can effectively and simultaneously act upon different mediators involved in the pathogenesis of asthma. The present study was planned to evaluate the actions of test extracts of leaves of *Hibiscus sabdariffa* on some of the very primary aspects of asthma-like bronchoconstriction may be due to release of histamine and acetylcholine using *in-vitro* models.

Histamine and acetylcholine contract the tracheobronchial muscle of dog, horse, guinea pig and man. Our investigation showed the responses produce by histamine and acetylcholine in goat tracheal chain preparation in the presence of plant extract. PTEE exerted an antagonistic effect on histamine and acetylcholine-induced contraction ($P < 0.05$). Significance was seen at a dose of 2, 4, 10 mg/ml for histamine and acetylcholine Figure 6.2 and 6.4 in a dose-dependent manner.

Histamine antagonistic effect seen as (70.12 ± 1.727*, 56.09 ± 1.2*, 48.17±1.321*) similarly the acetylcholine antagonistic effect seen as (85.60 ± 2.489*, 60.20 ± 2.456*, 44.00 ± 1.141*). A notable contraction produced by histamine at a dose 1.6 µg/ml, as 82 mm taken as 100% while notable contraction produced by acetylcholine at a dose 1.6 µg/ml, as 92 mm taken as 100% as shown in table 6.2 and 6.4.

This primarily justifies the presence of some of the component in ETHANOL extract might be responsible for the antihistaminic and anticholinergic effect. Further investigations are warranted for the necessary development of safer phytopharmaceuticals in the armament of asthma and related respiratory afflictions.

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CONFLICTS OF INTEREST: There is no conflict of interest with this research.

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