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IN-VIVO AND IN-VITRO ANTI-ASTHMATIC STUDIES OF PLANT *MICHELIA CHAMPACA* LINN.

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ABSTRACT: *Michelia champaca* known as champaca is belonging to family of Magnoliaceae. The present work consist of extraction and evaluation of anti-asthmatic activity of flowers of *Michelia champaca* Linn. Flowers was evaluated for anti-histaminic activity using isolated goat tracheal chain preparation and histamine induced Bronchoconstriction in Albino mice. *Michelia champaca* Linn. expressively inhibited dose reliant contraction of goat tracheal chain produced by histamine and also exhibited significant protection by prolonging preconvulsion dyspnea time (PCD) in mice. Thus, *Michelia champaca* Linn. showed anti-allergic activity against histamine and hence possesses potential role in the treatment of asthma.

INTRODUCTION: It consists of 12 genera and 220 species of evergreen trees and shrubs. In recent times there are several reports of medical speciality roles and activities of *Michelia champaca* and its active principles on the circulatory system, antipyretic, diuretic¹. Flowers and the fruit in combination with other drugs are recommended as an antidote to snake and scorpion venoms. Asthma is one of the most common disorder characterized by airway inflammation. It can be various factors like allergens, drugs, respiratory infection, dust, cold air, exercise, emotion, occupational stimuli, chemicals, histamine².

Drugs effective in the Asthma are mostly steroidal in nature. Phytochemical profile of this plant reveals the presences of flavonoids, steroidal nucleus in the form of triterpenoids and various saponin³.

Asthma originates from a Greek term denoting 'panting' or 'breathless'. It is a syndrome of the bronchial tubes that typically presents with wheezing, shortness of breath coughing, particularly in children. Asthma is an allergic reaction triggering inflammation and narrowing of the airways, causing spasm and difficulty in breathing.

Asthma is a chronic lung disorder that occurs commonly in both children and adults in economically developed as well as developing countries. It is increasing in prevalence and severity especially in allergic patients. Asthma prevalence, (the percentage of people who have ever been diagnosed with asthma and still have asthma)

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increased from 7.3% in 2001 to 8.4% in 2010. In 2010, an estimated 25.7 million people had Asthma, 18.7 million adults aged 18 and over, and 7.0 million children aged 0-17 years.

Asthma is characterized by airway inflammatory cells, including eosinophils, macrophages, mast cells, epithelial cells and activated lymphocytes that release various cytokines, adhesion molecules and other mediators. Inflammation results in an acute, sub-acute or chronic process that alters airway tone, modulates vascular permeability, activates neurons, increases secretion of mucus, and alters airway structure reversibly or permanently⁴.

Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anticancer, antimicrobials etc. A large number of plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now be lived that nature has given the cure of every disease in one way or another. Plants have been known to relive various diseases in Ayurveda. Therefore, researcher today are emphasizing on evaluation and characterization of various plants and plants constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plants constituents has always been a challenging task for the reasearchers⁵.

MATERIALS AND METHODS:

Extraction and Phytochemical Analysis:

Collection and Authentication: The plant parts flowers of *Michelia champaca* Linn. was collected in the month of July 2016 from Kolhapur Bhavani Mandap and Shirolu M.I.D.C. district Kolhapur, tehsil Hatkanangale, Maharashtra. After collection the plant material was identified authenticated and, confirmed by Dr. M. Y. Bachulkar, Plant Taxonomist, Principal of Shri. Vijay Singh B. Yadhav Arts and Science College, Peth-Vadgaon. (Reference no-VYMP/03).

Extraction of Plant Material: The powdered material was subjected for extraction with various solvent in Soxhlet apparatus for 24 h based on increasing polarity in order of petroleum ether and chloroform separately. All the extract was concentrated and solvent was evaporated in order

to get rid dry extracts. The % yield of various extract was calculated⁶.

Antiasthmatic Activity:

Isolated Goat Trachea Chain Preparation: (IAEC approval no.- 1290/PO/Re/S/09/CPCSEA).

Isolated adult goat tracheal tissue was obtained immediately after slaughter house of the animals. Trachea was cut into individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37°C + 0.5°C.

DRC of histamine in plane Krebs solution and in 1000 µg/ml extract in Krebs solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract⁷.

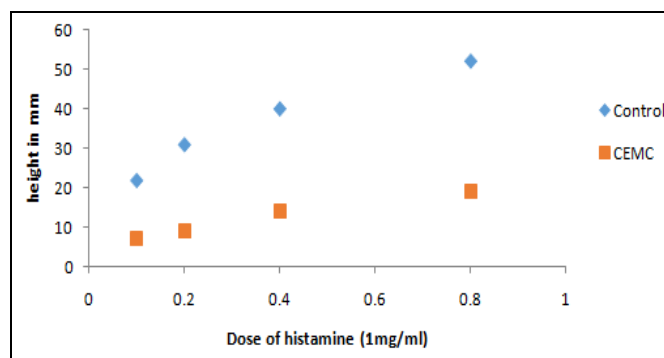


FIG. 1: EFFECT OF PET ETHER EXTRACT OF *MICHELIA CHAMPACA* EXTRACT ON HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

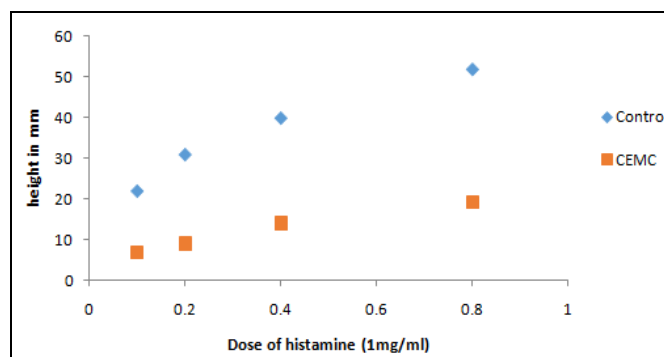


FIG. 2: EFFECT OF CHLOROFORM EXTRACT OF *MICHELIA CHAMPACA* FLOWER ON HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Catalepsy activity (Wood Block Test):

Introduction: Catalepsy is defined as a failure to correct an externally imposed, unusual posture over

a prolonged period of time. Neuroleptics (antipsychotic drugs) which have an inhibitory action on the nigrostriatal dopamine system induce catalepsy, while neuroleptics with little or no nigrostriatal blockade produce relatively little or no cataleptic behavior. Furthermore cataleptic symptoms in rodents have been compared to extrapyramidal side effects seen clinically with administration of antipsychotic drugs. They are dosed intraperitoneally with the test drug or the standard drug (haloperidol: 1 mg/kg, i.p.). Each animal is grasped gently around the shoulders and under the forepaws and placed carefully on the upper edge of the block individually (6 or 4 cm) for mice. An animal is considered cataleptic if it remains on the block for 60 sec. Percentage of cataleptic animals is calculated. Three trials are conducted for each animal at 0, 60, and 120 min. The phenomenon of catalepsy can be used for measuring the efficacy and the potential side effects of neuroleptics. The descent latency (DL) was defined as the time it took the mouse to descend from the block⁸.

Clonidine Induced Catalepsy in Mice: Albino mice were divided into five groups (n = 5). Control group received saline (10ml/kg) and other groups received single dose of extract (200, 400 mg/kg p.o. body weight) respectively. Chlorpheniramine maleate (10 mg/kg, i.p.) was used as standard. All the groups were received clonidine (1 mg/kg s.c.) one hour after the drug administration and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min⁸.

Statistical Analysis: The statistical analysis was performed by using one-way analysis-of-variance (ANOVA) followed by Dunnett's test for individual comparison of groups with control.

Effect of Test Drug on Leukocytosis, Eosinophilia:
Procedure: Mice were divided into six groups, five animals in each group. Animals belonging to group-I received distilled water (DW) 10 mL/kg, (p.o.). Animals belonging to group II, III, IV, V received boiled and cooled milk injection in dose of 4 mL/kg, (s.c.). Animals belonging to groups III, IV and V received test pet ether and chloroform extract of *Michelia champlika* in dose 200 and 400 mg/ kg, p.o. respectively, 1 h before milk injection. Blood samples were collected from each mouse

from the retro orbital plexus, under light ether anaesthesia. Total leukocyte count and total eosinophilia count and was done in each group before drug administration and 24 h after milk injection.

Difference in total leukocyte count and total eosinophilia count before and 24 h after drug administration was calculated⁹.

Result and Phytochemical Screening: In the present study *Michelia champaca* Linn. was used to study antiasthmatic activity. Preliminary qualitative phytochemical screening of petroleum ether, chloroform extract of flowers of *Michelia champaca* Linn. showed the presences of alkaloids, carbohydrates, flavonoids, sterols, sequiterpines and amino acids.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF FLOWER OF M. CHAMPACA LINN. OF PETROLEUM ETHER EXTRACT AND CHLOROFORM EXTRACT SHOWS FOLLOWING PHYTOCHEMICAL CONSTITUENTS PRESENT

S. no.	Phytochemical constituent	Petroleum Ether	Chloroform
1	Alkaloids	+	+
2	Saponins		+
3	Glycosides	+	-
4	Carbohydrates	+	-
5	Amino acids	-	+
6	Flavonoids	+	+
7	Sterols	+	+
8	Sequiterpines	+	+

Isolated Goat Tracheal Chain Preparation: It was observed that *Michelia champaca* petroleum ether extract inhibits contraction produced by histamine in these tissue preparations. Histamine (1mg/ml) was taken in different dose level and DRC was plotted. Study revealed that *Michelia champaca* petroleum extract exhibits significant (p ≤0.01) percentage decreased contraction at concentration 1000µg /ml in goat tracheal chain preparation dose dependent response relationship was seen.

TABLE 2: EFFECT OF HISTAMINE ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Groups	Dose of histamine (1mg/ml)	Control group (mm)
1	0.1 ml	22
2	0.2 ml	31
3	0.4 ml	40
4	0.8 ml	52

TABLE 3: EFFECT OF PET ETHER EXTRACT OF *MICHELIA CHAMPACA* EXTRACTION HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Groups	Dose of histamine (1mg/ml) (ml)	Control group % maximum contraction (Mean \pm SEM)	Test group % maximum contraction (Table Mean \pm SEM)
1	0.1	22	31
2	0.2	31	25
3	0.4	40	22
4	0.8	52	19

TABLE 4: EFFECT OF CHLOROFORM EXTRACT OF *MICHELIA CHAMPACA* FLOWER ON HISTAMINE INDUCED CONTRACTION ON ISOLATED GOAT TRACHEAL CHAIN PREPARATION

Groups	Dose of histamine (1mg/ml) (ml)	Control group % maximum contraction (Mean \pm SEM)	Test group % maximum contraction (Mean \pm SEM)
1	0.1	22	7
2	0.2	31	9
3	0.4	40	14
4	0.8	52	19

N= 6 values are in mean \pm SEM; Control = D.R.C. of histamine in absence of *Michelia champaca* petroleum extract, Test = D.R.C. of histamine in Presences of *Michelia champaca* petroleum extract and chloroform extract each having dose 1000 μ g /ml; Statistical analysis done by using Students 't' -test **p \leq 0.01, significantly different from control.

Clonidine Induced Catalepsy in Mice: Clonidine (1mg/kg, s.c.) produced catalepsy in mice, which remained for 3 h. The vehicle treated group has shown maximum duration of catalepsy (120 \pm 3.5sec.) at 180 min after the administration of clonidine. There was significant inhibition (p<0.05, p<0.01) of Clonidine induced catalepsy in the animal pretreated with *Michelia champaca* extract (200, 400 mg/kg, p.o.) and the duration of catalepsy was found. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed (p<0.01) the Clonidine induced catalepsy in mice ⁸.

TABLE 5: EFFECT OF *MICHELIA CHAMPACA* ON CLONIDINE INDUCED CATALEPSY IN MICE

Group	Duration of catalepsy (sec) at Mean \pm SEM						
	15 min	30 min	60 min	90 min	120 min	150 min	180 min
Group-I Vehicle (10ml/kg, i.p.)	43.66 \pm 0.88	44.00 000 \pm 0.57	64.33 \pm 0.66	67.83 \pm 0.79	80.83 \pm 1.66	110.33 \pm 1.62	64.83 \pm 1.07
Group-II Chlorpheniramine maleate (10 mg/kg, i.p.)	18.83 \pm 0.94	18.50 \pm 0.76	21.50 \pm 0.71	22.5 \pm 0.76	24.5 \pm 0.42	19.16 \pm 1.81	12.83 \pm 1.13
Group-III Pet ether extract of <i>Michelia Champaca</i> (200 mg/kg, p.o.)	42.66 \pm 0.66*	44.00 \pm 0.89*	46.33 \pm 0.49*	48.16 \pm 0.60*	51.5 \pm 0.76*	45.33 \pm 0.88*	44.83 \pm 0.94*
Group-IV Pet ether extract of <i>Michelia Champaca</i> (400 mg/kg, p.o.)	38.5 \pm 0.84*	41.50 \pm 0.76*	42.16 \pm 0.60*	43.83 \pm 0.94*	45.16 \pm 0.70*	43.66 \pm 0.88*	42.66 \pm 0.91*
Group-V Chloroform extract of <i>Michelia champaca</i> (200 mg/kg, p.o.)	41.83 \pm 0.30*	45.00 \pm 0.93*	46.33 \pm 1.03*	51.00 \pm 0.85*	51.16 \pm 0.70*	45.16 \pm 0.94*	44.00 \pm 0.73*
Group-VI Chloroform extract of <i>Michelia champaca</i> (400 mg/kg, p.o.)	39.00 \pm 0.36*	41.66 \pm 0.49*	43.83 \pm 0.47*	44.83 \pm 0.90*	45.66 \pm 0.61*	42.83 \pm 1.10	37.66 \pm 0.88*

Statistical analysis done by ANOVA followed by Dunnett's test. * p<0.05, **p<0.01

Effect of Test Drug on Milk Induced Leukocytosis, Eosinophilia in Mice: In the milk induced leukocyte count of *Michelia champaca* of petroleum ether extract and chloroform extract, dose of 400mg/kg showed significant (p<0.05) activity decrease in number of leucocytosis 1855.50 \pm 20.58, 2165.00 \pm 58.89 as compared to control group 78.00 \pm 2.408. Similarly, eosinophilia count of same plant of both extract dose 200mg/kg, 400mg/kg showed significant (p<0.05) activity decrease in number of eosinophilia 125.00 \pm 1.52**, 82.83 \pm 3.60**, 210.05 \pm 3.18** as compared to control group 21.00 \pm 0.89 **Table 6**.

TABLE 6: EFFECT OF *MICHELIA CHAMPACA* LINN. ON MILK-INDUCED LEUCOCYTOSIS IN MICE

Group	Dose	Difference in no. of leucocytes (per cu mm) (mean \pm SEM)
Control	(Vehicle, 10 mL/kg, p.o.)	78.00 \pm 2.408
Intox.	(milk 4 mL/kg) (SC)	4432.00 \pm 17.821**
Pet ether extract of <i>Michelia Champaca</i> Linn.	200mg/kg	3348.00 \pm 47.51
Pet ether extract of <i>Michelia Champaca</i> Linn.	400mg/kg	1855.50 \pm 20.58
Chloroform extract of <i>Michelia Champaca</i> Linn.	200mg/kg	2602.00 \pm 14.67
Chloroform extract of <i>Michelia Champaca</i> Linn.	400mg/kg	2165.00 \pm 58.89

n=5; values are expressed in mean \pm SEM. *** p <0.001, Intox. group compared with control group using student's t , test and * p <0.05, ** p <0.01, Extract compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnett's test.

TABLE 7: EFFECT OF *MICHELIA CHAMPACA* ON MILK-INDUCED EOSINOPHILIA IN MICE

Group	Dose	Difference in no. of eosinophils (per cu mm) (mean \pm SEM)
Control	(Vehicle, 10 mL/kg, p.o.)	21.00 \pm 0.89
Intox.	(milk 4 mL/kg) (SC)	150.66 \pm 2.33**
Pet ether extract of <i>Michelia Champaca</i>	200mg/kg	125.00 \pm 1.52**
Pet ether extract of <i>Michelia Champaca</i>	400mg/kg	82.83 \pm 3.60**
Chloroform extract of <i>Michelia Champaca</i>	200mg/kg	219.66 \pm 2.20**
Chloroform extract of <i>Michelia Champaca</i>	400mg/kg	210.05 \pm 3.18**

n=5; values are expressed in mean \pm SEM. *** p <0.001, Intox. group compared with control group using student's t , test and * p <0.05, ** p <0.01, Extract compared to intox. Group using Statistical analysis done by ANOVA followed by Dunnett's test.

DISCUSSION: Asthma is a chronic respiratory disease affecting a large proportion of population throughout the world. Bronchial provocation with allergen induces a prompt early phase immunoglobulin E (IgE)-mediated decrease in bronchial airflow (forced expiratory volume in 1 second) followed by late phase IgE-mediated reaction with a decrease in bronchial airflow for 4-8 h. Initially, asthma is characterised by the presence of increased number of various inflammatory mediators that are eosinophils, neutrophils, lymphocytes, bronchial secretions, and mucus.

Antiasthmatic activity using milk induced eosinophilia and leucocytosis model involves release of various types of mediators in pathology. It was reported that subcutaneous administration of milk produces a marked increase in the leukocytes and eosinophil count after 24 h.

Leukocytes during asthmatic inflammation release the inflammatory mediators like cytokines, secondary to adjuvant type I hypersensitivity reactions. Hence, prevention of it by MELA indicates its antiallergic activity⁴.

Histamine contracts the trachea bronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is the easier to handle and to prepare, it is much more sensitive than guinea pig tracheal chain. In the present study the isolated goat tracheal chain,

it was observed that *Michelia champaca* petroleum ether extract inhibits contraction produced by histamine in these tissue preparations. Histamine (1mg/ml) was taken in different dose level and DRC was plotted. Study revealed that *Michelia champaca* petroleum extract exhibits significant ($p \leq 0.01$) percentage decreased contraction at concentration 1000 μ g/ml in goat tracheal chain preparation dose dependent response relationship was seen. Similarly, clonidine induced catalepsy in mice which remained for 3 h. The vehicle treated group has shown maximum duration of catalepsy (120 \pm 3.5sec.) at 180 minute after the administration of clonidine. There was significant inhibition ($p < 0.05$, $p < 0.01$) of Clonidine induced catalepsy in the animal pretreated with *Michelia champaca* extract (200, 400 mg/kg, p.o.) and the duration of catalepsy was found. Chlorpheniramine maleate, (10 mg/kg, i.p.) treated group significantly reversed ($p < 0.01$) the Clonidine induced catalepsy in mice. Petroleum ether and chloroform extract of flower of *Michelia champaca* also carried our milk induced leucocytosis and eosinophilia in mice.

In the milk induced leukocyte count of *Michelia champaca* of petroleum ether extract and chloroform extract, dose of 400mg/kg showed significant ($p < 0.05$) activity decrease in number of leucocytosis 1855.50 \pm 20.58, 2165.00 \pm 58.89 as compared to control group 78.00 \pm 2.408.

Similarly eosinophilia count of same plant of both extract dose 200 mg/kg, 400 mg/kg showed significant ($p < 0.05$) activity decrease in number of eosinophilia $125.00 \pm 1.52^{**}$, $82.83 \pm 3.60^{**}$, $210.05 \pm 3.18^{**}$ as compared to control group 21.00 ± 0.89 **Table 6, 7**^{8,4}.

Herbal formulations used in the treatment of asthma include some antistress herbs to unable adoption to stress excessive stress or nervous debility may aggravate symptoms of asthma

Drugs effective in the asthma are mostly steroidal in nature. Physicochemical profile of the plant *Michelia champaca* reveals the presence of alkaloids, flavonoids steroidal nucleus in the form of triterpenoids. The antiasthmatic activity showed by the plant may be because of these chemical moieties^{10, 4}.

CONCLUSION: In conclusion, the present study confirmed that petroleum ether and chloroform extract of *Michelia champaca* extract significant dose dependent antiasthmatic activity in various *in vitro* and *in vivo* animal models and further support the traditional claim of plant in the treatment of asthma. The plant shows antiasthmatic activity possibly because of presence of flavonoids. This experiment will be helpful in future for scientific evaluation of constituents which shows antiasthmatic activity.

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CONFLICTS OF INTEREST: Nil

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