Potential of Ficus religiosa Linn. Flavonoids in Management of Allergic Asthma

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ABSTRACT
Humans have been using medicinal plants as a source of medicine for thousands of years. Truth be told, ancient man was completely reliant on plants for all of his medical needs, including recovery, prevention, and other types of medications, and has done so for millennia. Many plants and herbs hold their prestigious position in the field of medicine among which Ficus religiosa belonging to the family Moraceae is the vital one. Recent studies have reported that Ficus religiosa is used in the traditional medicine to relieve about 50 types of disorders including diabetes, diarrhoea, epilepsy inflammatory disorders, and gastric problems, sexual and infectious disorders. Antihistaminic principles are known to be useful in the treatment of asthma; hence, in the present work, the antihistaminic activity of an ethanol extract of Ficus religiosa was assessed using various methods. The results showed that the ethanol fractions inhibits clonidine-induced catalepsy as well as asthmatic inflammation. This suggests that the inhibition is through an antihistaminic action and that there is no role of dopamine. Hence, we concluded that the ethanol extract has significant antihistaminic activity. The flavonoid derivatives identified from ethanol fractions of leaves of Ficus religiosa may showed potential as a antihistaminic activity. So in future the plant Ficus religiosa isolated these flavonoids may lead significant role in the treatment of asthma.

1. Introduction

Nature provides many things for the well-being of humankind over the years, including the tools for the first attempts at therapeutic intervention. In ancient times, people rely on plants for the treatment of various ailments. Today, plant derived materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemicals.[1] The photochemical is natural bioactive compound(s) found in plants which as act as a defence system against diseases. Based on the functions in plant metabolism, phytochemicals are two kinds viz., primary and secondary constituents. Among the 2, 50,000 - 5, 00,000 plant species in the world, only a small percentage of phytochemicals has been investigated. Medicinal plants are the native heritage with the universal importance. Natural product extracts are very important source of new drugs. In ancient medical system, various parts of plants such as stem bark, root bark, aerial roots, vegetative buds, leaves, fruits and latex are used to cure various ailments. [2]

Therefore, the systematic screening of plant species with the purpose of discovering new bioactive compounds can help us to cure many fungal and bacterial diseases of economically important crops and animals including human being. [3]

Asthma is one of the most common chronic diseases in modern society and there is increasing evidence to suggest that its incidence and severity are increasing. Asthma is widely recognized as a chronic inflammatory lung disease characterized by reversible bronchoconstriction, elevated basal airway tone, eosinophils and lymphocyte accumulation and activation, epithelial cell dysfunction and damage. It is manifested by narrowing of the airways resulting in difficulty in breathing, cough and wheezing. Over the past few decades there have been significant scientific advances leading to better management of asthma, however the current modes of therapy in conventional medicine do not cure disorder but control symptomatology, also the current remedial drugs are having more side effects like hypersensitivity, severe hepatic impairment, cirrhosis and also they are , costlier that a normal man can't afford it and above all it has to be taken under strict medical supervision or in a presence of educator. By considering all these facts, there is need of newer and better therapeutic agents for management of asthma. Hence, the proposed study is an attempt to investigate the phytochemical and pharmacological properties of Ficus religiosa (L.) in the management of asthma. [4-6]

F. religiosa is considered as an herb. Six parts of the trees (i.e., bark, leaves, seeds, fruits, roots and latex) are assessed for their therapeutic qualities. The wood is not used for therapeutic purposes because it is highly porous

in nature. All the parts of the plants have medicinal properties. They are used in different forms and it also gives best results when combined with other medicinal herbs. [5] Traditionally in breast cancer cell lines, the F. religiosa induces apoptosis, wound healing, used as an anti-bacterial, anti-convulsant, anti-viral, anti-protozoal, anti-diarrheal, astringent, anti-cholinergic and also treats gonorrhea, amnesia, anti-diabetic. In other few Asian countries, their bark is used to treat various diseases like cervical cancer, epilepsy, inflammation, ulcers, infectious diseases, acetyl cholinesterase inhibitory activity and anti-anxiety activity and also used for skin diseases resulting from kumkum (bindhi) application. The leaves are testifying for its anti-venom activity, it regulates the menstrual cycle. The tender branches of F. religiosa are used as toothbrush. The latex of this plant is used as a tonic, and can be treated by the powder of its fruits. [6-9] review

The present work was undertaken to evaluate the traditionally recognized antiasthmatic property of the

leaves of Ficus religiosa .

2. Materials and Methods

2.1 Plant Material Collections and Drying:

Fresh matured leaves of Ficus religiosa (L.) plant identified and collected in month of September from Pune district (Maharashtra, India). Dried in the shade at normal room temperature. Crude drug powder stored in sealed bottles away from light and humidity until use for extract preparation.

2.2 Authentication:

Dr. Uday Pawar, Botanist, Amdar Shashikant Shinde Mahavidyalaya, Medha, Satara, authenticated the plant specimen. The Voucher specimen for Plant Ficus religiosa (L.) (Ref. No. 1030/2, Date-21.01.2019)

2.3 Crude Extract preparation: [10-12]

500 gram of dry powdered leaves materials were subjected to successive organic solvent extraction by refluxing in the Soxhlet apparatus each for 12hours. Solvents of graded polarities like Hexane, and ethanol selected for extraction process. Boiling temperature of solvent and all optimum laboratory requirements are maintained during extraction process. The concentrated dried extract were labelled and stored in sterile containers in the refrigerator until further analysis.

2.4 Phyto-chemical Screening and Chromatographic isolation: [2, 3, 21-23, 27]

The concentrated dried ethanol extracts of Ficus religiosa (L.) leaves were observed for their phytochemical characristics and trace out components present as per standard procedures. The gradient elution method with selective mobile phase system as highly nonpolar to polar characristics helps us to separate complex compounds or their derivatives from extracts. It helps to purify desired compounds which will be identify easily and potentiate pharmacological action. 38cm height and 3.5cm diameter column loaded with stationary phase Silica gel for column chromatography (60-120#). 6-8 drops per minute isocratic elution was carried out by using mobile phase solvent n-Hexane, Ethyl acetate, Methanol and water.

2.5 In-vitro evaluation of ethanol fractions for antiasthmatic activity: [13-14]

In-vitro evaluation by Histamine Induced Contraction of Goat Tracheal Chain

From the slaughter house has been collected the goat trachea. Trachea was cut into singular rings and integrated in an arrangement to shape a chain. The trachea was suspended at 37± 0.5OC in a 30 ml organ bath containing the Kreb's solution. The tracheal chain was stabilized for 45 minutes with a load of 400mg. The response of contraction initiated by histamine at dose 0.5µg/ml, without test extracts and with test extracts of 05-40 µg/ml in solution of Kreb's were recorded. The percentage relaxation of histamine contracted goat trachea was measured in the presence of test extracts.

2.6 In-vivo evaluation of ethanol fractions for antiasthmatic activity: [14-24]

Purified and bioactive A5, and A6 fractions evaluated for their anti-asthmatic activity by using following (in vivo animal models).

a. Animals:

Albino Mice (20-30 g), Wistar Rats (170-200 g) of either sex were used during entire time of study. All were

housed at encompassing temperature (22±1°C), relative humidity (55±5%) and 12h/12h light dim cycle. Animals had free admittance to standard pellet diet and dist. water given not indispensable. The convention of the examination was endorsed by the Institutional Animal Ethical Committee of S.G.R. S. College of Pharmacy, Saswad, Dist Pune, according to the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Animals were isolated into various groups for different models. The conveyance of animals in the groups, the arrangement of preliminaries and the treatment apportioned to each group were randomized all through the trial.

b. Drugs and Chemicals:

The drugs used were: Clonidine (Neon Lab. Ltd., India), Chlorpheniramine maleate (Pfizer Ltd.), Dexamethasone (Zydus Heathcare Ltd), Histamine dihydrochloride (HiMedia Lab. Pvt. Ltd), Egg albumin (HiMedia Lab. Pvt. Ltd.), Evans Blue (Loba Chemie), Sodium chromoglycate (Hi-Media Lab. Pvt. Ltd) Carrageenan (Hi-Media Lab. Pvt. Ltd), TLC plate (Merck Germany), Methanol (Research Lab. Industries, India), Solvents (AR grade) all were purchased from commercial source.

3. Antiasthmatic Activity: [14-24]

3.1 Clonidine Induced Catalepsy in Mice

A bar test was carried out to examine the effects of test compound on catalepsy caused by clonidine. Mice were divided into different groups. In every group five mice were separated. Clonidine (1 mg/kg, s.c.) is given to all mice. Before 1 hour of clonidine injection, dist. water 1ml/kg, body weight i.p. administered to control group. Intraperitoneal extract therapy at a dosage of 250 mg/kg body and 500 mg/kg body weight given to the study group. The standard group received Chlorpheniramine maleate 10 mg/kg, body weight. The Forepaws of mice were put on parallel bar and time needed to eliminate the paws for every mice was noted and term of catalepsy were estimated at 15, 30, 60, 90 and 120 min.

3.2 Milk Induced Leukocytosis and Eosinophilia in Mice

Mice were divided into different groups and six animals were added into each group. Animals having a place with control group got dist. water 10ml/kg, body weight p.o. While test group got intraperitoneal treatment of test fractions A5 and A6 at dosages 250 and 500 mg/kg body weight. Standard group got Dexamethasone at a dose of 50 mg/kg, body weight i.p. Boiled and cooled milk at a portion of 4 ml/kg, subcutaneously infused to all the groups 30 minutes after treatment. Blood samples were obtained from every mice from retro orbital plexus, under light ether sedation. Complete leukocyte and eosinophil count was recorded in each group before drug treatment and 24 hrs. after milk infusion. Difference in total leukocyte and eosinophil count before and after 24 hrs. drug administration was determined.

3.3 Passive Cutaneous Anaphylaxis in Rats

Rodents were sensitized by subcutaneous infusion of 100 mg. egg albumin and 12 mg. aluminium hydroxide as adjuvant, on day 1, 3 and 5. On tenth day animals were bled and antiserum containing anti IgE was separated and stored at -20 OC. The rodents were divided into eight groups and five animals were in each group. The rodent homologous antiserum (100µl) was infused to shaved back skin of rodent. After 24hr control group intraperitoneally got dist. water at a portion 1 ml/kg, body weight. Test fraction groups were given intraperitoneal treatment of test extracts at portion 250 and 500mg/kg, body weight while standard group was treated with sodium chromoglycate at a portion of 50mg/kg body weight intraperitoneally. All the groups were infused with 0.5ml of blended solution of 0.5% Evans blue and 1% egg albumin (1:1) through tail vein 30 minutes after the treatment. The region of blue color spillage was estimated, 30 minutes after the infusion of color and expressed as diameter of blue spots in mm2 [135-136].

3.4 Mast Cell Degranulation in Mice

In each group of animals, six animals were taken. The intraperitoneal route of administration preceded three days of treatment plan. Control group received distilled water 10ml/kg, body weight p.o. The test fractions groups were treated with extracts at doses 250 and 500mg/kg, body weight. The standard group treated with sodium chromoglycate at 50mg/kg, body weight. On the fourth day, 0.9% saline solution was given to all mice at a dose of 10 ml/kg, body weight intraperitoneally. The peritoneal cavity has been opened and the mast-cell fluid sucked and collected in the 10-ml RPMI-1640 pH buffer medium pH 7.2-7.4. Centrifugation washed mast cells three times (400 - 500 rpm) and mast cells' pallets were collected and supernatant was discarded. The cell

suspension from all animals challenged with egg albumin (100µg/ml) and incubated at 37OC for 10 minutes. The cell suspension was stained with toludine blue (1 percent) and examined under the microscope. A total of 100 cells are counted from separate visual areas. Degranulated mast cells were shown to be burst instead of unchanged. The average degranulated mast cell number was determined.

3.5 Carrageenan Induced Paw Edema in Rat

Rodents were isolated into various groups, six rodents in each group. Control group treated with dist. water at a portion of 10ml/kg, body weight p.o. Test fractions at dosages of 250 and 500 mg/kg, body weight were directed intraperitoneally and standard group got intraperitoneal treatment of dexamethasone at a portion of 50mg/kg body weight, 30 minutes after the treatment, all groups were infused 0.1ml of 1% (w/v) carageenan to the subplantar region of rear paw. Paw volume was estimated by Plethysmometer (Ugo Basile 4140) at 1, 2 and 3 hours after the infusion of Carrageenan

The increase in PCD onset time were calculated using the following formula,

% increase in PCD time= [1-T1/T2 X 100]

Where, T1= Time required to start symptoms before giving treatment T2= Time required to start symptoms after giving treatment

3.6 Statistical Analysis:

The mean ± SEM values for each category were determined. The methodological research was conducted using a One-way analysis of variance (ANOVA) followed by the Dunnett's test for comparison with the control group. A probability value of less than 0.05 was considered significant.

4. Structural Elucidation:

Thus it was felt necessary to elucidate bioactive fractions for determination of probable structure by spectroscopic analysis. So fractions were screened for Furrier Transfer Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance: H-NMR and Mass spectroscopy (MS).

5. Results and Discussion

5.1 Phyto-chemical Screening and Chromatographic isolation:

Fresh matured leaves of Ficus religiosa (L.) plant extracted with n-Hexane and ethanol. Ethanol extract screened for their phytochemical analysis and based on characristics they were separated by chromatographic isolation. Details of observations are reported in figure no. 01 and table no. 1.

Figure No.1: Column chromatography of Ficus religiosa leaves ethanol extract

Table No. 1: Fractionation of Ficus religiosa leaves ethanol extract:

Solvent used	Ratio	Fraction Code	Colour	% Yield
Hexane	100%	A1	Dark brown	1.4
Hexane: Ethyl acetate	7:3	A2	Brown	0.6
Hexane: Ethyl acetate	5:5	A3	Brown	1.6
Hexane: Ethyl acetate	2:8	A4	Brown	0.3
Ethyl acetate	100%	A5	Brown	2
Ethyl acetate: Methanol	7:3	A6	Yellowish-brown	2.1
Ethyl acetate: Methanol	5:5	A7	Yellowish-brown	0.4
Methanol	100%	A8	Yellow	0.4
Methanol: Water	5:5	A9	Yellow	1.2
Water	100%	A10	Faint yellow	0.5

The separated all fractions are coded differently and characterized with observed colour and percent yield. The used mobile phase solvents and their combined proportion help to isolate components in different fractions. All collected fractions were concentrated and observed their characteristics. After significant yield the five fractions code A1, A3, A5, A6 and A9 was evaluated by in-vitro antiasthmatic method to determine active fraction as well as their isolated components.

5.2 In-vitro evaluation by Histamine Induced Contraction of Goat Tracheal Chain:

All procedure and experimental conditions are same as above method used to evaluate five fractions code A1, A3, A5, A6 and A9.

At a point when isolated goat tracheal chain is treated with histamine; at concentration 0.5µg/ml, demonstrated the contraction. Whereas, goat tracheal chain treated with Ficus religiosa fractions at concentration 05 to 40µg/ml, neither produced contraction nor any othereffects.

The effect of test fractions at concentration 05 to 40µg/ml on histamine-actuated constrictionof goat tracheal chain, were studied and results are expressed in table no. 20 and 21 shows percent restraint of histamine-initiated constriction of goat tracheal chain by test fractions.

Table No.2: Effect of Test Extracts on Histamine Induced Contraction of Goat Tracheal Chain

Sr. No.	Histamineconc. (µg/ml)	Conc. of test fraction (µg/ml)	Height of Response (mm) (Mean ± SEM)				
			A1	A3	A5	A6	A9
1	0.5	-	46.8 ±3.641	46.8 ±3.021	45.2 ±3.102	48.4 ±2.315	45.8 ±3.441
2	0.5	05	43.0 ±3.082	43.0 ±3.112	38.2 ±3.084	39.2 ±1.797	43.0 ±3.086
3	0.5	10	40.3 ± 1.132	42.1 ± 2.101	34.8 ± 1.275	36.4 ± 1.029	41.2 ± 2.154
4	0.5	20	37.4 ± 1.748	37.8 ± 1.701	28.4 ± 1.085	22.0 ± 1.870	38.4 ± 1.078
5	0.5	30	34.6 ±2.079	37.6 ±2.311	22.2 ± 1.828***	21.6 ± 3.367**	36.6 ±2.379
6	0.5	40	32.4 ± 2.017	35.2 ± 2.850	18.6 ± 1.605***	20.7 ± 1.031***	36.4 ± 2.857

*** P<0.001, ** P<0.005

Table No .3: Percent Inhibition of Histamine Induced Contraction of Goat Tracheal Chain

Sr.No.	Histamine conc. (µg/ml)	Conc. of test fraction(µg/ml)	Percent Inhibition of Histamine Induced Contraction of Goat Tracheal Chain				
			A1	A3	A5	A6	A9
1	0.5	-	-	-	-		
2	0.5	05	6.11	6.11	7.66	6.61	5.11
3	0.5	10	10.04	10.14	18.00	10.33	10.04
4	0.5	20	18.34	26.34	35.20	28.41	21.04
5	0.5	30	32.44	18.90	45.97*	40.11*	17.90
6	0.5	40	38.62	27.38	66.15*	58.97*	19.47

In this experiment, we observed that A5 and A6 showed more relaxant effect on histamine induced contraction of goat windpipe than other test extracts.

The relaxation of pre contracted goat windpipe by test extracts might be because of stimulation of β2 adrenergic receptors or hindrances of histamine H1 receptors. Hence, these test fractions have potential to show

bronchodilator activity and may be useful in the treatment of asthma.

In above in-vitro method, we observed that Ficus religiosa A5 and A6 fractions showed significant potential may be due to isolated phytochemicals in extracts. It showed good relaxant effect on histamine induced contraction of goat windpipe than other test fractions. So further need to evaluate selective fraction by in-vivo methods and identify responsible components in the A5 and A6 fractions.

5.3 In-vivo evaluation of ethanol fractions for antiasthmatic activity:

5.3.1 Clonidine Induced Catalepsy in Mice

Catalepsy is a condition where the creature keeps up forced stance for long time prior to recapturing typical stance. Catalepsy is the indication of extrapyramidal impact of medications that hinder dopaminergic transmission or increment histamine discharge in cerebrum. Clonidine, a α2 adrenoreceptor agonist initiates portion subordinate catalepsy in mice, which is restrained by H1 receptor antagonist and not by H2 receptor. It is realized that clonidine discharges histamine from mast cells. Clonidine incited arrival of histamine from mast cells is repressed by α2 adrenoceptor blocker. Subcutaneous administration of clonidine at a dose of 1 mg/kg, body weight produced catalepsy in mice. The maximum duration of catalepsy 142.33 ± 12.387 was observed in the control group, 90 minutes after the subcutaneous administration of clonidine. The pretreatment of test A5 and A6 at doses of 250 and 500 mg/kg, body weight, intraperitoneally and chlorpheniramine maleate at dose of 10mg/kg, body weight, intraperitoneally demonstrated significant ($P<0.05$) decrease in clonidine induced catalepsy in mice. The decrease in duration of catalepsy 90 minutes after the administration of clonidine by A5 at dose of 250 and 500 mg/kg, body weight, was found to be 45.167 ± 7.692 and 35.00 ± 2.530 respectively. The decrease in duration of catalepsy 90 minutes after the administration of clonidine by A6 at dose of 250 and 500 mg/kg, body weight was found to be 38.00 ± 6.19 and 32.00 ± 3.27 respectively. In addition to this decrease in duration of clonidine induced catalepsy 30.00 ± 2.295 was observed in standard group treated with chlorpheniramine maleate at dose 10mg/kg, body weight intraperitoneally table no. 4. In the present study catalepsy inhibited by test extracts at a dose of 500mg/kg, body weight was closely resembled to the standard drug i.e. chlorpheniramine maleate.

Antihistaminic drugs can hinder catalepsy actuated by clonidine. Various phases of the catalepsy are straightforwardly corresponded with histamine substance of cerebrum. Clonidine delivers the histamine from mast cells along these lines to particular savior like compound 48/80. Therefore, the hindrance of clonidine prompted catalepsy by MESML, MEPBL and MECAS affirms the antihistaminic action. Appropriately the antihistaminic property of A5 and A6 might be due to inhibition of histamine discharge.

Figure No.2. Clonidine Induced Catalepsy in Mice

Table No. 4: Effect of Test Extracts on Clonidine Induced Catalepsy in Mice

Treatments	Dose (Per kg,b.w.)	Duration of catalepsy (Sec.), Mean ±SEM				
		15min	30 min	60 min	90 min	120min
Dist. Water	1 ml	62.00 ±4.465	89.33 ±4.828	102.00 ±3.819	142.33 ±12.387	63.00 ±5.190
Chlorpheniramine Maleate Std.	10 mg	4.33 ±0.667*	10.00 ±1.461*	22.00 ±3.367*	30.00 ±2.295*	20.00 ±1.414*
A5	250 mg	6.33 ±1.116*	22.00 ±3.055*	29.00 ±3.215*	45.167 ±7.692*	22.167 ±3.911*
	500 mg	5.00 ±0.856*	16.167 ±2.27*	23.00 ±2.324*	35.00 ±2.530*	17.00 ±2.422*
A6	250 mg	06.16 ± 0.94*	19.83 ± 1.88*	24.00 ± 2.74*	38.00 ± 6.19*	23.66 ±2.10*
	500mg	05.00 ± 0.73*	13.00 ± 1.41*	23.00 ±2.39*	32.00 ±3.27*	23.50 ±4.65*

*p<0.05

5.3.2 Milk Induced Leukocytosis and Eosinophilia in Mice:

During asthmatic aggravation leukocyte discharge cytokines, histamine and significant fundamental protein encourages continuing irritation. An unusual expansion in peripheral eosinophil's count over 4% of whole leukocyte number is named as eosinophilia. During the late stage, of the advancement of unfavorably susceptible asthma, eosinophils assume part as a provocative cell. Eosinophil secretes mediators, for example, eosinophile cationic protein (ECP), eosinophile derived neurotoxin (EDNT), granulocyte macrophage colony stimulating (GM-CSF), tumor necrosis factor (TNF), and Prostaglandin (PG), which brings about epithelial shedding, bronchoconstriction and advancement of irritation in respiratory tract. Eosinophilia is related with respiratory problems; frequently hypersensitive in nature along with aspiratory penetrates that are noticeable on chest films [14-15].

In our investigation of milk prompted leukocytosis and eosinophilia in mice, control group demonstrated most extreme rise in leukocyte tally following 24 hours of the subcutaneous administration of milk at a portion of 4 ml/kg, body weight. The distinction in absolute leukocyte tally when milk administration, in control group was found to be 2936.00 ± 487.13. In a group of mice, treated with A5 at a portion of 250 mg/kg, body weight intraperitoneally diminished the total leukocyte check. The difference in all leukocyte tally before and after milk administration, in group treated with A5 at a dose of 250 mg/kg, body weight was appeared to be 2386.00 ± 374.13 non-significant, when contrasted with control group. Though the group of mice treated with A5L at a dose of 500mg/kg body weight, demonstrated significant (*p<0.004) decline in complete leukocyte check when contrasted with control group. The distinction in complete leukocyte check when milk administration, in group treated with A5 at a portion of 500 mg/kg, body weight was appeared to be 943.00 ± 98.78.

The significant (p <0.04, p<0.003) decline in total leukocyte count 1676 ± 177.83, 760.00 ±

131.71 were observed in groups of mice with intraperitoneal treatment of A6 at a dosages 250 and 500 mg/kg, body weight, respectively when compared with control group.

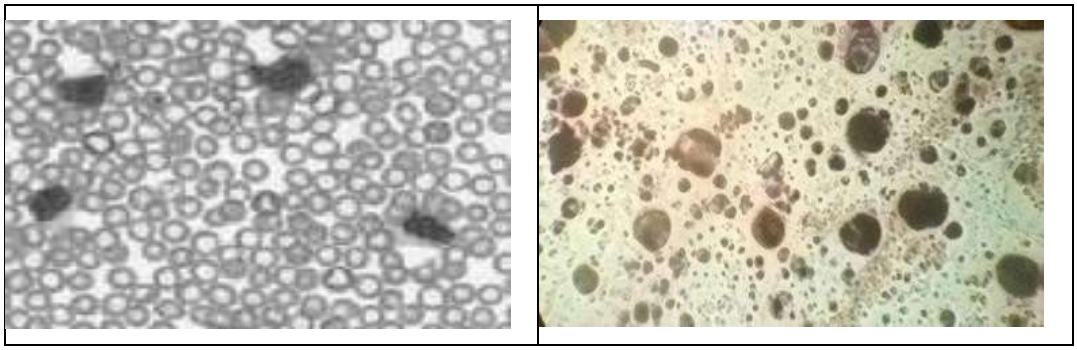
In a group of mice, treated with Dexamethasone at a dose 50 mg/kg body weight intraperitoneally significantly (p <0.001) decreased the total leukocyte count. The difference in total leukocyte count before and after milk administration, in group treated with dexamethasone was found to be 476.00 ± 110.40.

Indications of asthma might be exasperated because of stress brought about by increment in complete leukocyte count [15]. Leucocytosis caused upon subcutaneous administration of milk is reason for various hypersensitive responses. Anyway this hypersensitive condition can be normalized by the treatment with antistress or adaptogenic drugs. Subsequently decline in leukocytes check by test extracts affirms adaptogenic or antiallergic property which proposes the utilization of chose plants in the management of asthma. [15-17]

In milk instigated eosinophilia, control group demonstrated the most rise in eosinophil check after the subcutaneous administration of milk at dose of 4 ml/kg, body weight. The distinction in eosinophil count in control group, before and after administration of milk was appeared to be 214.00 ± 38.64. In a group treated with Dexamethasone at dose 50 mg/kg, body weight caused significant (p<0.025) decline 92.00 ± 22.00 in eosinophils count. Intraperitoneal administration of A5 at a dose of 500 mg/kg, body weight caused significant (P<0.029) decline in eosinophils count 107.00 ± 11.35 when contrasted to control group, while same extract at a dose of 250 mg/kg body weight did not demonstrated any significant effect on eosinophils count.

The significant (p<0.098, P<0.023) decline in eosinophils count 124.00 ± 28.57, 102.00 ± 10.54 were observed in groups of mice intraperitoneally treated with A6 at a dose of 250 and 500 mg/kg, body weight when compared to control group.

During asthma increase in eosinophil count was observed. In our study the treatment of test extract at dose of 500 mg/kg, body weight significantly decreased the milk induced eosinophilia. Comparative decrease in eosinophils counts by test extract were found to be A6>A5. Hence test extracts of selected plants confirms the anti-eosinophil activity and may be useful in the treatment of airway hypersensitivity. Results are given in table no. 24, 25 and figure no.3 and table no.5.



a. Before Milk Treatment b. After Milk Treatment

Figure No.3. Observation for Milk induced leukocytosis in mice

Table No. 5: Effect of fractionated compounds on Milk Induced Leukocytosis

Treatments	Dose (Per kg body weight)	Difference in Total leukocyte count (Before and after treatment)(Per cu mm) (Mean ± SEM)
Control (Distilled water)	10ml	2936.00 ± 487.13
A5	250 mg	2386.00 ± 374.13
	500 mg	943.00 ± 98.78*
A6	250 mg	1676.00 ± 177.83
	500 mg	760.00 ± 131.71***
Dexamethasone	50 mg	476.00 ± 110.40****

*P < 0.004, ***P< 0.003, **** P< 0.001

Table No. 19: Effect of fractionated compound on Milk Induced Eosinophilia

Treatments	Dose (Per kg body weight)	Difference in eosinophil count(Before and after treatment) (Per cu mm) (Mean ± SEM)
Control (Distilled water)	10 ml	214.00 ± 38.64
A5	250 mg	148.00 ± 15.29
	500 mg	107.00 ± 11.35***
A6	250 mg	124.00 ± 28.57
	500 mg	102.00 ± 10.54***
Dexamethasone	50 mg	92.00 ± 22.00***

***P< 0.02

5.3.3 Passive Cutaneous Anaphylaxis In Rats:

Exposure of sensitized animal to an allergen can bring about clinical side effects that differ from minor erythema to perilous anaphylaxis. The responses incited by intradermal infusion of histamine, serotonin or passive cutaneous anaphylaxis (PCA) cause an expansion in fine porousness at the nearby site of the skin. Passive cutaneous anaphylaxis can be actuated by various boosts for example egg albumin and anti IgE. During PCA the infusion of homologous antiserum to the back skin of rodent causes creation of counter acting agents and IgE which ties to the outside of mast cell. After 24 hr, the intravenous infusion of blended solution of Evans blue and 1% egg albumin (1:1) through the tail vein produces blue fix on the skin of rodents where antiserum had infused. Evans blue is utilized for the record of capillary permeability due to its nonpoisonous nature [16].

In present investigation test extracts were assessed on egg albumin actuated PCA in rodents and impact of test

extracts on Evans blue dye spillage were recorded in mm2 after 30 min infusion of blended solution of 0.5% Evans blue and 1% egg albumin (1:1) through tail vein. Control group treated intraperitoneally with dist. water at portion 1 ml/kg, body weight indicated most extreme region 46.80 ± 2.437 mm2 of blue color spillage.

In a group of rodent treated intraperitoneally with A5 at portion 250 mg/kg, body weight demonstrated significant (P<0.03) decline 38.40 ±2.33 in Evans blue color spillage when contrasted with control group whereas group of animal treated with A5 at portion 500 mg/kg body weight indicated significant (P<0.002) decline 33.20 ± 1.65 in blue color spillage when contrasted with control group.

The significant (P<0.001) hindrance of region 29.60 ± 2.24, 13.60 ± 2.70 of Evans blue color spillage were seen in groups of rodents treated intraperitoneally with A6 at portions of 250 and 500 mg/kg, body weight, respectively, when contrasted with control group.

A group of rodent treated intraperitoneally with Sodium chromoglycate at portion 50 mg/kg, body weight demonstrated significant (P<0.001) decline 07.40 ± 1.32 in Evans blue color spillage when contrasted with control group.

Our study uncovered that all extracts at portion of 250 and 500 mg/kg body weight hindered area of Evans blue color spillage dose dependently. Sodium chromoglycate indicated 84.77% of restraint of zone of Evans blue color spillage, while the percent hindrance of 31.68% and 70.94% of blue color spillage were seen after treatment of MESML and MEPBL respectively.

In PCA the appearance of a blue spot has prompted to a capillary enlargement and spillage of liquid from the blood to the intercellular spaces of the skin is effectively perceptible. The release of mediators in PCA results from the conglomeration of explicit IgE receptors on the outside of mast cells by the related antigen which causes the expansion in vascular porousness and spillage of color. The hindrance of blue color spillage by test extracts of chosen plants is perhaps because of restraint of histamine discharge or diminished vascular porousness or restraint of antigen neutralizer responses on the outside of mast cells. The investigation hence affirms the antiallergic action of the test extricates.

Figure no.4: Photograph of Passive cutaneous anaphylaxis in Rat

Table No. 6: Effect of fractionated compound on Passive Cutaneous Anaphylaxis

Treatments	Dose (per kg body weight)	Area of Dye Leakage (mm^2)	% Inhibition
Control Dist. Water	1 ml	46.80 ± 2.437	-
A5	250 mg	38.40 ±2.33*	20.98
	500 mg	33.20 ± 1.65**	31.68
A6	250 mg	29.60 ± 2.24***	36.72
	500 mg	13.60 ± 2.70***	70.94
Sodium chromoglycate	50 mg	07.40 ± 1.32***	84.77

*P<0.03, **P< 0.002, ***P< 0.001

5.3.3 Mast Cell Degranulation in Mice:

Mast cells are broadly disseminated in the connective tissue, with preferential localization adjacent to small blood vessels. The mast cell contain basophil granules in a real sense stacked with dynamic substances which permitted escaping themselves or through enzymatically formed items cause vascular and other tissue responses similar to those attributes of inflammatory process. Sodium cromoglycate a standard mast cell stabilizer forestalls degranulation of mast cells by raising the cyclic adenosine monophosphate. The pharmacological specialist that expands the intracellular degree of cAMP loosens up airway smooth muscle and hinders the release of histamine and basophil.

In our investigation most extreme degranulation 77.6 ± 2.804 of mast cell was seen in control group of mice treated with dist. water at a portion of 10ml/kg, body weight. However the treatment with standard drug sodium chromoglycate at a portion of 50 mg/kg, body weight intraperitoneally, indicated significant (P<0.001) restraint 24.8 ± 2.267 of mast cell degranulation when contrasted with control group.

In a group of mice intraperitoneally treated with A5 at a portion of 250mg/kg, body weight, caused significant (P<0.002) restraint 60.8 ± 2.223 of mast cell degranulation when contrasted with control group. While a group of mice intraperitoneally treated with A5 at a portion of 500mg/kg, body weight caused significant (P<0.001) hindrance 43.8 ± 3.693 of mast cell degranulation when contrasted with control group.

Intraperitoneal treatment of group's mice with A6 at doses of 250 and 500mg/kg, body weight, indicated significant (P<0.001) hindrance 46.4 ± 3.311 and 31.4 ± 2.943 respectively, of mast cell degranulation when contrasted with control group.

The after effects of present investigation uncover that sodium chromoglycate indicated 68.04

% safeguard of mast cells from degranulation. In all test extract dose dependent safeguard of mast cells from degranulation was noticed. The percent protection of mast cells against degranulation, by A5 and A6 fraction at portions of 250 mg/kg body weight was 21.64% and 40.20% respectively. The percent protection of mast cells against degranulation, by A5 and A6 fraction at doses of 500 mg/kg body weight was 43.55% and 59.53% respectively reported at Table no.7 and figure no.5,6.

The degranulation of mast cells happens in light of the immunological stimuli in which antigen –antibody reaction on cell surface is prevails. The likely component of the test fractions to shield mast cell from degranulation might be because of the restraint of antigen- antibody reactions on the outside of mast cells or may be stabilization of mast cell.

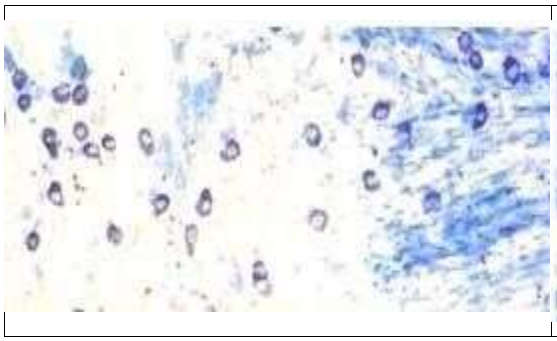


Figure No.5. Intact Mast cells

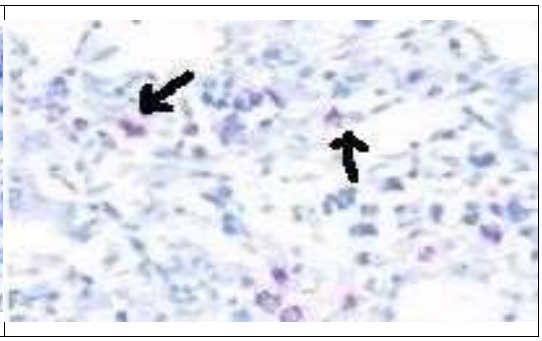


Figure No.6 Degranulated Mast cells

Table No. 7: Effect of fractionated compounds on Mast Cell Degranulation

Treatments	Dose (per kg bw.)	Number of Degranulatedmast cells	Percent protection of mast cells
Control Dist. water	10 ml	77.6 ± 2.804	-
A5	250 mg	60.8 ± 2.223**	21.64
	500 mg	43.8 ± 3.693***	43.55
A6	250 mg	46.4 ± 3.311***	40.20
	500 mg	31.4 ± 2.943***	59.53
Sodium chromoglycate	50 mg	24.8 ± 2.267***	68.04

**P<0.002, ***P<0.001

5.3.4 Carrageenan Induced Paw Edema in Rat:

Inflammation characterized as the local response of living mammalian tissues to injury. Inflammation is described by redness, swelling, pain and warmth. It is a body defence response to wipe out or limit the spread of harmful agent. Carrageenan initiated rodent paw edema is an appropriate test model for assessing the anti-inflammatory effect of natural products [17-18]. Infusion of Carrageenan into the subplantar surface of rodent paw actuates a biphasic edema. During beginning phase, release of histamine, serotonin, bradykinin, and to a less degree prostaglandins created by cyclooxygenase (COX) catalyst, whereas during delayed phase neutrophils infiltration, and prostaglandin is credited 153. Release of the neutrophils-determined free radicals, nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), and interleukin-1 β (IL-1 β) additionally delivered during delayed phase of carrageenan- inacted acute inflammation [19].

In our investigation greatest growing of paw edema 0.82 ± 0.0412, 0.91 ± 0.0244 and 0.99 ± 0.0246 were seen in control group (dist. water, 10ml/kg, body weight) of rats 1, 2 and 3 hours after infusion of carrageenan respectively.

Treatment with Dexamethasone at a portion of 50 mg/kg, body weight indicated significant ($P<0.001$) hindrance 0.51 ± 0.0303, 0.45± 0.0324 and 0.37 ± 0.0273 of rodent paw edema following 1, 2 and 3 hours of infusion of carrageenan respectively when contrasted with control group.

A group of rodent treated with A5 fraction at a portion of 250 mg/kg, body weight, significant ($P<0.001$) decrease 0.71± 0.023 and 0.74 ± 0.0227 toward the finish of 2 and 3 hrs. after the infusion of carrageenan when contrasted with control group. However a group of rodent treated with MESML at a portion of 500 mg/kg, body weight, and significant ($P<0.02$ and $P<0.001$) decrease 0.69 ± 0.0237, 0.65 ± 0.0218 and 0.72 ± 0.0276 towards the finish of 1, 2 and 3 hrs. after the infusion of carrageenan when contrasted with control group. The treatment of rats with A6 fraction at portions of 250 and 500mg/kg, body weight, indicated significant ($P<0.001$) decrease in paw edema towards the finish of 1, 2 and 3 hrs after the infusion of carrageenan when contrasted with control group. Decrease in paw volume by A6 at portion of 250mg/ kg, body weight 0.70 ± 0.0169, 0.63 ± 0.0214 and 0.61 ± 0.0256, at a portion of 500mg/kg, body weight was seen to be 0.59 ± 0.0246, 0.52 ± 0.0335 and 0.47 ± 0.0328 towards the finish of 1, 2 and 3 hrs after the treatment of carrageenan. Results are summed up in table no. 8.

In our study towards the finish of 3 hrs the percent hindrance of rodent paw edema by A5 and A6 at dose of 500mg/kg, body weight was seen to be 27.28% and 52.52% respectively. Results are summed up in table no. 8.

The significant inhibition of rodent paw edema by these extracts may be because of stabilization of mast cell or restraint of proinflammatory mediators for example histamine, serotonin etc. Hence these extracts may possess anti-inflammatory effect, so may be used in the treatment of asthma aggravations.

Figure no.7. Carrageenan Induced Rat Paw Edema

Table No. 8: Effect of fractionated compounds on Carrageenan Induced Rat Paw Edema

Treatments	Dose Per kg body weight	Rat Paw volume (ml) Mean ± SEM		
		1 hr.	2 hr.	3 hr.
Control Dist. water	10 ml	0.82 ± 0.0412	0.91 ± 0.0244	0.99 ± 0.0246
A5	250 mg	0.75 ± 0.028	0.71± 0.023***	0.74± 0.0227***
	500 mg	0.69 ± 0.0237**	0.65± 0.0218***	0.72± 0.0276***
A6	250 mg	0.70 ± 0.0169**	0.63± 0.0214***	0.61± 0.0256***
	500 mg	0.59 ± 0.0246***	0.52± 0.0335***	0.47± 0.0328***
Dexamethasone	50 mg	0.51 ± 0.0303***	0.45± 0.0324***	0.37± 0.0273***

**P<0.002, ***P<0.001

Table No. 9: Percent Inhibition of Carrageenan Induced Rat Paw edema

Treatments	Dose Per kg body weight	% Inhibition of carrageenan induced ratpaw edema		
		1 hr.	2 hr.	3 hr.
Control Dist. water	10ml	-	-	-
A5	250 mg	8.53	21.97	25.25
	500 mg	15.85	28.57	27.28
A6	250 mg	14.63	30.76	38.39
	500 mg	28.04	42.85	52.52
Dexamethasone	50 mg	37.80	50.54	62.62

5.4 Structural Elucidation:

Thus it was felt necessary to elucidate A5 and A6 bioactive column fractions for determination of probable structure by spectroscopic analysis. Spectral analysis of fractions were done by using FTIR, H1 NMR spectroscopy and Mass spectroscopy. The isolated extract after performing TLC, were found that flavonoid is the main constituents showing promising antiasthmatic activity. The spectral characterization is described below,

Fraction A5

Fraction collected (Fraction A5) was Characterized by IR, NMR and MASS spectra. The molecular formula of the compound 1 (Fraction A5) is C16H12O9 with molecular weight of 348.26 The chemical name of the predicted compound is 3,5,6,7-tetrahydroxy-2-(2,3,4-trihydroxy-5-methylphenyl)-4H-1-benzopyran-4-one.

Table 10. Spectroscopic data of isolated Fraction A5.

Spectroscopic techniques	Data interpreted
IR (cm^{-1}) Kbr	3249.47(HC=CH stretching), 2914.88 (C-H stretching), 1764.55 (C=O stretching), 1469.49 (CH from CH_3), 1254.47 (C-0) Ar-OH, 1153.22 (Ar C-O-C stretching),1078.58 (C-C stretch). 754.031 (C-H bending)
^{1}HNMR (DMSO)	δ 9.660 (s, 1H), δ 9.149 (s, 1H), δ 7.348 (m, 1H), δ 7.334 (m, 1H), δ 7.330 (s, 1H), δ 6.84 (s, 1H), δ 6.670 (m, 1H), δ 6.666 (m, 3H), 3.393 (m, 4H), δ 2.513(s, 1H), δ 2.09(s, 1H), δ 2.505(s, 1H), δ 2.502(s, 1H), δ 2.498(s, 1H), δ 1.979 (s, 3H)
EIMS (70 ev): m/z	349.58[M^+] 349.58,339.62,325.61,311.58,297.55,209.25,204.27,181.21,126.05,90.9

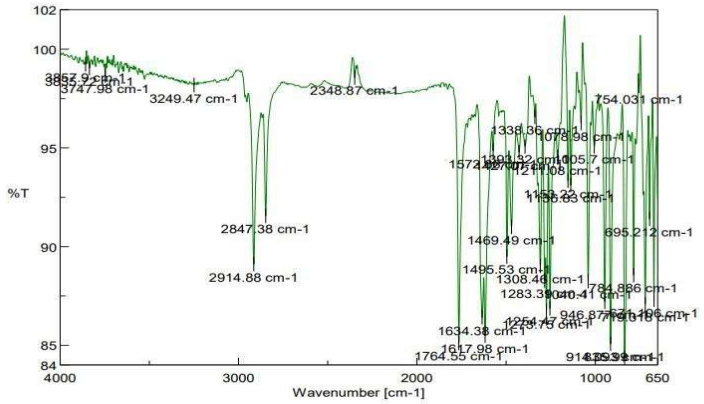


Figure 8. IR spectra of Fraction A5.

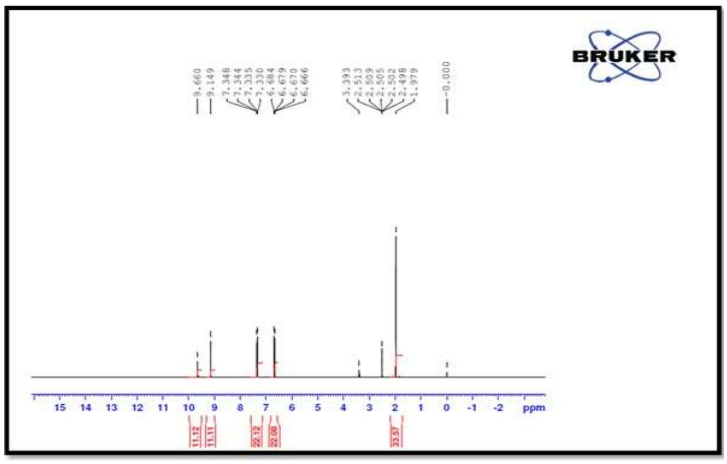


Figure 9. Proton NMR spectrum of Fraction A5

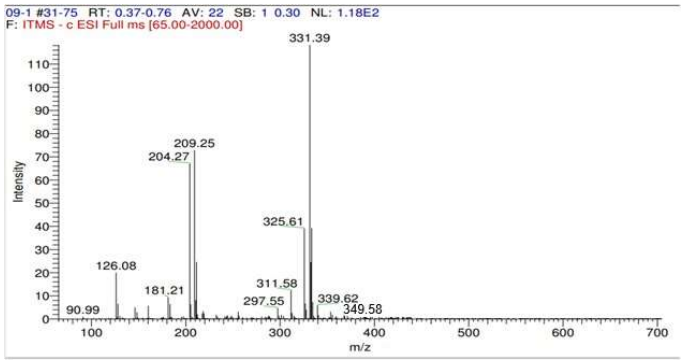


Figure 10. MS spectrum of Fraction A5

On the basis of chromatographic, IR, NMR and MASS spectra data of compound and as per literature elucidated structure is match with spectral data containing steroidal flavonoid and having structural similarity with Rutin.

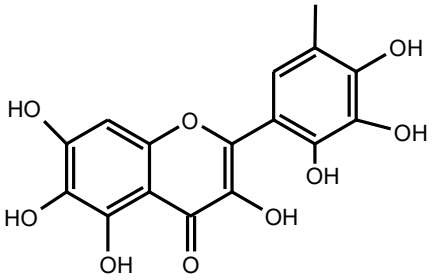


Figure 11. Structure of elucidated Fraction A5.

Fraction A6

Fraction collected (Fraction A6) was Characterized by IR, NMR and MASS spectra. The molecular formula of the compound 2 is C15H10O7 with molecular weight of 302.25 The chemical name of the predicted compound is 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one.

Table 11. Spectroscopic data of isolated Fraction A6.

Spectroscopic techniques	Data interpreted
IR (cm^{-1}) Kbr	3007.44 (C-H stretching), 1636.3(C=C), 1470.46(CH from CH_2), 1370.18[C-H stretching (CH_2 (CH_3)2)], 1172.51(OH-bending) 1070.3 (C-C stretch).
^{1}HNMR (CDCL3)	δ 7.34 (m, 1H), δ 4.12 (s, 1H), δ 4.11 (s, 1H), δ 2.024 (s OH), δ 1.279 (s, 3H), δ 1.264 (s, 3H), δ1.256 (s, 3H), δ 1.242 (s, 3H), δ 1.227 (s, 3H), δ 0.884 (s, 3H),

EIMS (70 ev): m/z	303.25[M^+], 297.55, 246.37,218.34,126.08

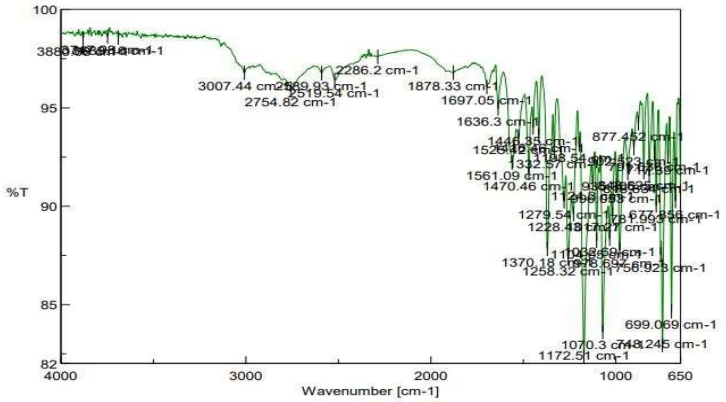


Figure 12. IR spectra of Fraction A6.

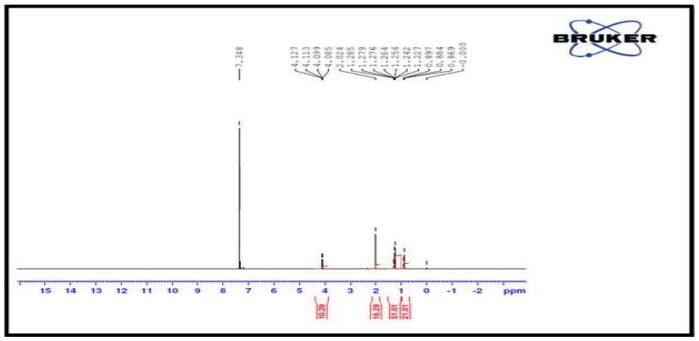


Figure 13. Proton NMR spectrum of Fraction A6.

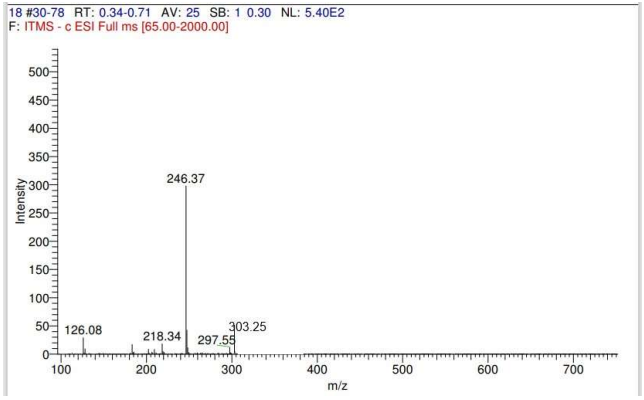


Figure 14. Mass spectrum by MS analysis for Fraction A6.

On the basis of above IR, 1HNMR & GC-MS spectral data and as per literature elucidated structure of compound-5 having steroidal flavonoid nucleus and the predicted compound may be,

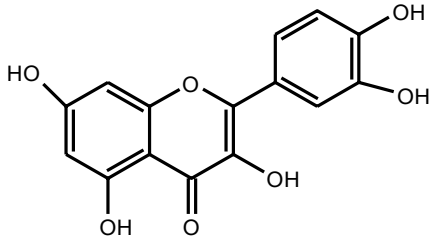


Figure 15. Predicted Structure of Fraction A6

6. Conclusion

All-successive solvent extracts of Ficus religiosa leaves screened for different varieties of phytochemicals. In qualitative analysis primary metabolites like carbohydrates, proteins, fats and secondary metabolites like alkaloids, flavonoids, phenols, alkaloids, tannins, terpenoids and glycosides were showed positive test results in ethanol extract. According to the findings of the study ethanol fractions A5 and A6 of Ficus religiosa leaves can be used as a potential antihistaminic agent. They may become a revolutionary lead medicine source for the prevention of inflammatory response in respiratory system. The ethanol soluble traces of flavonoids and terpenoids have promising activity against asthma. So it can be concluded that Ficus religiosa isolated phytocomponents serve as potential antiasthmatic mediators in the field of pharmaceutical as well as lead developments.

References

[1] Borris RP. Natural products research: perspectives from a major pharmaceutical company. J. Nat Prod Res. 1996; 51:29-35.
[2] Dhasade VV, Komala M., Phytochemical and Pharmacological Potential of Plant Pimenta dioica Linn., International Journal of Pharmaceutical Research, 2020 ,12: 4; 279-286. DOI: https://doi.org/10.31838/ijpr/2020.12.04.070
[3] Dhasade VV, Komala M., Investigation for determination of therapeutic potential for antitubercular activity with special reference to Caesalpinia crista fruits. International Journal of Experimental Research and Review, 2023, 30:321-329. DOI :https://doi.org/10.52756/ijerr.2023.v30.029
[4] Ministry of health and family welfare, department of Ayush. New Delhi. Ayurvedic pharmacopeia of India; pp. 2001;17-20.
[5] Agnivesha. Charaka Samhita. Varanasi: ChoukambaSanskritaSamsthana. Prameha Chikitsa; pp. 2001;446–7.
[6] Haneef J, Parvathy M, Thankayyan RSK, Sithul H, Sreeharshan S. Bax Translocation Mediated Mitochondrial Apoptosis and Caspase Dependent Photosensitizing Effect of Ficus religiosa on Cancer Cells. 2012; PLoS ONE 7.
[7] Nair R, Chanda SV. Anti-bacterial activities of some medicinal plants of the Western Region of India. Turkish Journal of Biology. 2007;31(4):231-6.
[8] Choudhary GP. Evaluation of ethanolic extract of Ficus religiosa bark on incision and excision wounds in rats. Planta Indica. 2006;2(3):17-9.
[9] Damanpreet S, Rajesh KG Anti-convulsant effect of Ficus religiosa: role of sero¬tonergic pathways. J Ethnopharmacol. 2009;123(2):330-4.
[10] Mukherjee PK. Quality Control of Herbal Drugs, Buiness Horizone Pharmaceutical Publushers, New Delhi, 2002; p.380-422.
[11] H arborne JB. Phytochemical Methods. Springer International, London. 1998, p.7
[12] Gokhale SB, Purohit AP, Kokate CK. Pharmacognosy, 57th edn. Nirali Prakashan, Pune. 2020, p.157-166.
[13] Bimakr et al. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Menthaspicata L.) leaves. Food and Bio products processing.89 (1), pp. 67-72. https://doi.org/10.1016/j.fbp.2010.03.002
[14] S. Vidyadhar, M. Saidulu, T.K., Gopal, D. Chamundeeswari, U. Rao, D. Banji(2010). In vitro anthelmintic activity of the whole plant of Enicostemmalittorale by using various extracts. International journal of applied biology and pharmaceutical technology. 1(3) 1119- 1125.
[15] Kapoor M, Jasani N, Acharya N, Acharya S, Kumar V. Phytopharmacological evaluation and anti–asthmatic activity of Ficus religiosa leaves. Asian Pacific journal of tropical medicine. 2011 Aug 1;4(8):642-4.
[16] Poongothai A, Sreena KP, Saradhadevi KM, Santhi R, Annapoorani S. Phytochemicals investigation of Ficus glomerata Linn leaves. (2011): 324-328.
[17] Taur DJ, Patil RY. Some medicinal plants with antiasthmatic potential: a current status. Asian Pacific journal of tropical biomedicine. 2011 Oct 1;1(5):413-8.
[18] Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three Ficus species- an overview. Int J Pharma Bio Sci. 2010;1(4):246-53.
[19] Okpo SO, Adeyemi OO. The antiallergic effects of Crinum glaucum aqueous extract. Phytomedicine, 2002; 9: 438-441.