In vivo bioactivity– Guided isolation of antiasthmatic fraction of Celosia argenteae Linn. leaves in rodents

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ABSTRACT

Celosia argentea is leafy herb commonly known as quail grass, feather cockscomb, and Lagos spinach belongs to the family Amaranthaceae. The Celosia argentea has great medicinal value, used in the treatment of fatigue, leucorrhoea, atherosclerosis and osteoporosis and the seeds have been used for reducing the “liver heat”, improving the eye sight, clearing wind heat and as an anti-inflammatory agent. In present study the flavonoids fraction from alcoholic extract of Celosia argentea was investigated for its anti-asthmatic potential by in vitro and in vivo techniques. Phytochemical evaluation detected methanolic fraction to be enriched in flavonoids. Hence, methanolic fraction was concentrated and loaded on a column of Sephadex using a step gradient of chloroform: methanol mixture (95:5, 90:10, 80:20,….100:0). The first fraction 95:5 gave distinct single band with the selected solvent system were further purified on HPLC column of MCI HP20. Fraction was collected using an automatic fraction collector and monitored by HPLC. Chromatographically pure compound were pooled, concentrated, and freeze-dried. Then the isolated compound was screened for antiasthmatic activity in rodents. Data were analyzed using ANOVA at p ≤ 0.05. Isolated compound from celosia argentea (10mg/kg) significantly reduces paw volume at dose 10 mg/kg showed (0.180±0.005**) reduction of paw volume was comparable to standard drug Dexamethasone (0.160±0.005**). Also it significantly protect degranulation of mast cell at dose (10mg/kg) it gives 63.6% and Ketotifen fumarate at dose (1mg/kg, p.o.) gives 74.1% protection to mast cells. In case of histamine induced contraction in goat tracheal chain at concentration 30 μg/ml isolated compound of celosia argentea at 10μg/ml significantly inhibited the tracheal contraction which was compared with Chlorpheniramine maleate (1 μg/ml). This indicates that there was competitive antagonism between histamine and isolated compound of celosia argentea for H1 receptors present on the smooth muscle. The result indicates the isolated bioactive fraction of Celosia argentea responsible for its antiasthmatic effect which may be produce due to presence of quercetin flavonoid.

Keywords: Anti-asthmatic Activity, Celosia Argentea, Flavonoid Fraction.

1. BACKGROUND

Asthma is a multifaceted inflammatory disorder which results into narrowing of airway with broncho constriction and spasm. As the complex pathophysiology of asthma, various animal models are generally used to investigate the immunological and physiological pathogenesis of disease. Celosia argentea is leafy herb commonly known as quail grass, feather cockscomb, and Lagos spinach belongs to the family Amaranthaceae. Plant show simple and spirally arranged leaves, flowers are often pinkish or white colour, fruits are in globular shape and seeds are black. Traditionally it is used for prophylaxes of various disorders like jaundice, inflammation, fever and itching. Blood disorders and mouth sores treated with the help of seeds of Celosia argentea which have bitter taste. They are effective remedy for diarrhoea [1]. The Celosia argentea contains a variety of species some of them includes spicata, crista, c. argentea. This is also known as kardu. This is the earliest classical herbal in China, and is frequently used in traditional Chinese medicine for treating eye diseases, ulcer, to serve as anthelmintic, to treat trauma to blood, hygro-paralysis etc [2]. The treatment with Celosia argentea also significantly improved the weight loss which was observed in diabetic rats by restoring the urinary glucose and protein release. Hypertiglyceridemia and hypercholesterolemia are the common lipid abnormalities in diabetes [3]. Based on ethno botanical practice the plant was investigated for anti inflammatory, anti – pyretic [4] anti diabetic [5], anti bacterial and diuretic properties [6]. The stems and leaves bruised and applied as poultice is used for infected...
sores, wounds and skin eruptions [7]. Poultrie of leaves, smeared in honey, is used as cooling application to inflamed areas and painful affections such as buboes and abscesses. Leave concussions are used to relieve gastrointestinal disorders and are antipyretic. Seeds when in decoction or finely powdered, are considered antidiarhoeal or aphrodisiac. Whole plant is used for antidote in snake poison while root is used for abdominal colic, gonorrhoea and eczema [8]. The seeds are prescribed for haemorrhagic conditions including menorrhagia, haematuria, haematemesis, haemoptysis, epistaxis, acute retinal haemorrhage and bleeding haemorrhoids [9]. *Celosia argentea* L. has sometimes been useful for treatment of disorders like excessive menstruation and leucorrhoea. *Celosia argentea* is also used in traditional medicine for sores, ulcers, and skin eruptions [10]. Leave concussions are used to relieve gastrointestinal disorders and are antipyretic. Whole plant is used for antidote in snake poison while root is used for abdominal colic, gonorrhoea and eczema. On the basis of this and on account of alleged usefulness of *Celosia argentea* in the traditional treatment this current study was aimed to investigate anti-asthmatic potential of the flavonoid fraction from alcoholic extract of the leaves of *Celosia argentea* in animal models i.e. Passive cutaneous anaphylaxis test, Compound 48/80- induced mast cell degranulation in rats and histamine induced contraction of goat tracheal chain. *Celosia argentea* is an erect annual herb up to 2 metres tall. The stem is ridged, glabrous and branches up to 25 per plant. The leaves are alternate, simple, without stipules; petiole indistinctly demarcated; blade ovate to lanceolate-oblong or narrowly linear, up to 15 centimetres x 7 centimetres, tapering at base, acute to obtuse and shortly mucronate at apex, entire, glabrous and pinnately veined. Inflorescence a dense, many flowered spike at first conical but becoming cylindrical up to 20 centimetres long, bracteate, slivery to pink in ornamental forms completely or partly sterile and in many colours. Flowers are small, bisexual, regular five merous, tepal free, narrowly elliptical-oblong, 6-10 millimetres long, stamen fused at base, ovary superior, 1- celled, style filiform up to 7 millimetres long, stigma 2-3, very short. Fruit is an ovoid to globose capsule 3-4 millimetres long circumsissile, few seeded with seeds being lenticular, 1-1.5 millimetres long, black, shining, shallowly reticulate [11].

2. METHODS

Collection, preparation of plant material and crude extract
The plant was collected from the fields located in outskirt of Bharati Vidyapeeth, morewadi, Kolhapur. Routine pharmacognostic studies were carried out to confirm identity of material. The Plant was authenticated by the Botany Department (Shivaji, University, Kolhapur, and Maharashtra); Plant authentication voucher specimen number was (GGK-01).

Sequential extraction of crude methanol extract of *Celosia argentea* leaves
The leaves were shade dried and 1.5 kg coarsely powdered leaves were subjected to hot continuous extraction in soxhlet apparatus with methanol (95%). The extracts were concentrated and dried for further studies at reduced temperature and pressure in rotary evaporator. Yield obtained was 300 g (20%) [4].

Column chromatographic separation of flavonoid fractions
Phytochemical evaluation detected methanolic fraction to be enriched in flavonoids. Hence, methanolic fraction was concentrated and loaded on a column of Sephadex using a step gradient of chloroform: methanol mixture (95:5, 90:10, 80:20,…100:0) [12]. The first fraction 95:5 gave distinct single band with the selected solvent system were further purified on HPLC column of MCI HP20. Fraction was collected using an automatic fraction collector and monitored by HPLC. Chromatographically pure compound were pooled, concentrated, and freeze-dried. The protocol followed was as below:

- Sample preparation: Each dried fraction was dissolved in HPLC grade methanol. Following centrifugation supernatant was injected
- Column: MCI HP20; 20 × 6 cm
- Mobile phase gradient: Methanol: water (35:65) followed by methanol:water:acetic acid (30:70:1)
- Flow rate: 7.48 ml/min
- Injection volume: 2 Ml
- Ultra violet (UV) detection: 280 nm
- Fraction collection: 0.5 min/fraction with 6 ml/tube fraction
- Yield: Compound 2 - 21 mg.

Separation of column fractions by TLC
A line of about 1.5 cm from the bottom of the silica coated plate was drawn. The samples obtained from the column chromatographic separations were spotted (using a capillary tube filled with the fraction) on this line, equidistant from each other. The plates were placed in a TLC chamber saturated with Toluene: ethyl acetic acid derivation: formic acid, in the ratio (5:4:0:2) as mobile phase. The plate was thereafter removed from the chamber when the solvent had risen towards the end of the plate. The position of the solvent front was marked. The TLC plate was thereafter examined under ultra violet light. Fractions with similar retardation factor (RI) were pooled together. Melting point of isolated active constituents by capillary method and digital melting point apparatus which was given to be range of 120-125°C, similar in MP range of Quercetine 120-130°C.

Characterization
The isolated compounds were further subjected to various spectroscopic studies like: UV (V-530; Perkin Elmer Lambda-35), Fourier transform infrared Spectroscopy (FT-IR) (FTIR 460 Plus; Jasco Corporation), Confirmation of identity was performed by comparing with the marker Compounds on HPTLC (Linomat 5-140435; CAMAG) [13,14].

Experimental animals and dosing protocol
Wistar albino rats weighing 150-250g and Swiss albino mice weighing 20-25g were housed in standard cages at room temperature 25 ± 2°C and 50±5% relative humidity, under a light/dark cycle of 12/12 h, for 1 week before the experiments. Animals were provided with standard rodent pellet diet (Amrut laboratory animal feed, Sangli, Maharashtra, India) and water ad libitum.
Toxicity study
For acute toxicity studies the mice were divided into four groups each containing 10 mice and they were treated with following doses viz., 25, 50, 100,200 and 400 mg/kg orally by isolated compound. After treatment the animals were observed for behaviour changes and their mortality. From the study it was revealed that the isolated compound was found to be safe up to 200 mg/kg i.p since there was no mortality. Further work was carried out by selecting 10 mg/kg dose orally (5% of LD50)

3. BIOLOGICAL ACTIVITY BY IN VIVO TECHNIQUE
Passive cutaneous anaphylaxis test [13,14]
Wistar rats were divided into five groups (n=5). Antiserum to egg albumin was raised in rats by using aluminum hydroxide gel as an adjuvant. On 1st, 3rd and 5th day, animals were given three doses of 250 µg of egg albumin (s.c.) adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline. On 10th day of sensitization, the blood of each animal was collected from the retro orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum is separated by centrifugation at 1500 rpm. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The right hind paw received an equal volume of saline. Animals belonging to group I served as control and was administrated with only distilled water (10 ml/kg, p.o.). Animals belonging to group II served as standard and were administrated by Dexamethasone (0.5 mg/kg, i.p.); whereas animals belonging to group III served as test group and were administrated with respective dose of isolated flavonoid from Celosia argentea (10mg/kg) 24 hr after sensitization. One hr after drug administration, animals were challenged by giving 10 µg of egg albumin in 0.1 ml of saline in the left hind paw and the paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140). The difference in the reading prior to and after antigen challenge represents the edema volume and the percent inhibition of edema was calculated by using the formula,

% Inhibition = \left[1 - \frac{T}{C}\right] \times 100

Where, T - Mean relative change in paw volume in test group, C- Mean relative change in paw volume in control group.

Compound 48/80- induced mast cell degranulation in rats [15]
Rats were divided into five groups (n=5). On the 1st day of sensitization, all the animals from each group were injected with Compound 48/80 (1mg/kg, s.c.). Animals belonging to group II served as standard and were administrated with Ketotifen fumarate (1mg/kg, p.o.). While animals belonging to group III served as test group and were administrated with isolated flavonoid from Celosia argentea at dose of (10mg/kg) for 15days. On day 15th, 2 hour after the assigned treatment, mast cells were collected from the peritoneal cavity. Ten ml of normal saline solution was injected into peritoneal cavity and abdomen was gently massaged for 90 second. The peritoneal cavity was carefully opened and the fluid containing mast cells were aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4).The mast cells were then washed thrice by centrifugation at low speed (400-500 rpm) and the pallets of mast cells were taken in the RPMI-1640 medium. The mast cell suspension (approximatelly 1 x 10 6 cells/ml) was challenged with 5 µg/ml of compound 48/80 solution and stained with 0.1 % toluidine blue and observed under high power microscope (45 X). Total 100 cells were counted from different visual areas. The numbers of intact and degranulated cells was counted and the percent protection was calculated using the formula,

% Protection = \left[1 - \frac{T}{C}\right] \times 100

Where,
T- No. of degranulated cells of test.
C-No. of degranulated cells of control.

4. BIOLOGICAL ACTIVITY BY IN VITRO TECHNIQUE
Histamine induced contraction of isolated goat trachea preparation [16, 17]
The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb’s solution (Composition mM: NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; Mg2SO4, 1.19; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb’s solution and maintained at 37 ± 0.5 °C, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 1g. The contractile responses of tracheal strip to histamine (30µg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.8mland 1.6ml were recorded in absence and presence of isolated compound of celosia argentea (10µg /ml) by using Sherrington’s Recording Drum with a frontal writing lever. The similar concentration-effect curve was taken in presence of standard drug Chlorpheniramine Maleate (1µg/ml). The height of response curve was measured to express percentage inhibition. The graph was plotted by taking log dose verses height of response curve.

5. STATISTICAL ANALYSIS
The data obtained were expressed as mean ± standard deviation (Mean ± SD). The data were subjected to one way analysis of variance (ANOVA), and differences between the control and treatment groups were determined by Dunnett’s multiple comparison test.
test using Graph Pad Prism® (Version 5.0, San Diego, CA). P values= 5% were regarded as significant

6. RESULT
Preliminary phytochemical screening
Preliminary phytochemical investigation of *Celosia argentea* leaves observed the presence of steroids, saponin, alkaloids, flavonoids, and glycosides

Isolation and characterisation
UV spectra of extracts and standard
The UV spectroscopy analysis is important characterisation of the drug by using wavelength maximum absorbance (λ max). The UV spectra of extract were in methanol. The λ max of extract *celosia argentea* leaves was observed at 256nm which shows Figure 2. The UV spectra for Standard quercetin was executed out by UV spectroscopy. The quercetin shows strong absorption band at around 256nm and 372nm of wavelength which shows Figure 3. UV Spectra of extract shows presence of quercetin flavonoid at the same wavelength this was the first confirmation regarding presence of quercetin.

![Fig. 1: UV spectra of CA leave extract](image1)

![Fig. 2: UV spectra of standard Quercetin](image2)

Isolation of active constituents
The methanolic fraction was concentrated and loaded on a column of Sephadex using a step gradient of chloroform: methanol mixture (95:5, 90:10, 80:20,...,100:0) [12]. The first fraction 95:5 gave distinct single band with the selected solvent system were further purified on HPLC column of MCI HP20. Fraction was collected using an automatic fraction collector and monitored by HPLC. Chromatographically pure compound were pooled, concentrated, and freeze-dried. The isolated compound further used for TLC study by comparing with standard drug quercetin. After analysis using TLC, it was confirmed that band 1 shows quercetin. (TLC) of active ingredients of extract by using Toluene: ethyl acetate derivations: FA (5:4:0.2) as mobile phase. Retention factor (Rf) was execute to be 0.75 and 0.76 from the reported work it approves the presence of Quercetine.

![Fig. 3: TLC of isolated compound](image3)

**Table 1:** TLC analysis of sample
The UV study of isolated active constituents showed maxima with peak 210nm, which is characteristic of unsaturated hydrocarbon containing $\pi$ electrons and transition $\pi-\pi^*$ indicating hetero atoms.

**Passive paw anaphylaxis in rats**

Isolated compound from *celosia argentea* (10mg/kg) showed significantly ($P<0.001$) reduction in paw volume in dose dependent manner when compared with control group as shown in Graph 1 at dose 10 mg/kg showed (0.180±0.005) reduction of paw volume was comparable to standard drug Dexamethasone (0.160±0.005).

### Table 2: Effect of isolated flavonoid on Passive paw edema volume (ml)

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Paw Edema Volume (ml) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 hr</td>
</tr>
<tr>
<td>I (Control)</td>
<td>0.490±0.005</td>
</tr>
<tr>
<td>II (Std) (Dexamethasone) (0.5 mg/kg, i.p.)</td>
<td>0.270±0.005**</td>
</tr>
<tr>
<td>III (Test) (Isolated Flavonoid) (10mg/kg)</td>
<td>0.450±0.005**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean± S.E.M. where n= 5, Statistical analysis done by using ANOVA followed by Dunnett’s test where **$p<0.01$ Group II, III, compared with Group I.
Isolated flavonoids from *Celosia argentea* (10mg/kg) showed significant inhibition of paw edema when compared with standard group as shown in Table 3.

### Table 3: Percentage Inhibition of Paw Edema

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Inhibition of Paw Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 hr</td>
</tr>
<tr>
<td>II (Standard)</td>
<td>44 ±0.22</td>
</tr>
<tr>
<td>III (Isolated flavonoid)</td>
<td>09.00±</td>
</tr>
</tbody>
</table>

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**Graph 2: Effect of isolated flavonoid on % inhibition of paw edema**

### Compound 48/80- induced mast cell degranulation in rats

**Table 4: Effect of isolated flavonoid in Compound 48/80- induced mast cell degranulation in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mast cells (Mean ± SEM)</th>
<th>Percent protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Degranulated</td>
</tr>
<tr>
<td>I (Control)</td>
<td>15.2 ± 0.58</td>
<td>87.2 ± 0.58</td>
</tr>
<tr>
<td>II (Std)</td>
<td>76.6 ± 0.50**</td>
<td>22.6 ± 0.50 **</td>
</tr>
<tr>
<td>III (Isolated flavonoid)</td>
<td>68.2 ± 0.86**</td>
<td>31.8 ± 0.86 **</td>
</tr>
</tbody>
</table>

**p<0.01 when Group II, III compared with Group I.**

The control group showed degranulation of mast cell while groups treated with isolated flavonoid (10mg/kg) and Ketotifen fumarate (1mg/kg, p.o.) Significantly ***(P<0.001) protect degranulation of mast cells. Also the percent protection provided by isolated flavonoid was seems to equivalent to standard drug. Table 4**

### Graph 3: Effect of isolated flavonoid on compound 48/80-induced mast cell degranulation in rats

**Histamine induced contraction of isolated goat trachea preparation**

Histamine produced dose dependant contraction in goat tracheal chain preparation at the concentration 30 μg/ml. This was significantly inhibited by modified PSS into which the preparation was incubated with isolated compound of *celosia argentea* at
10μg/ml as well as with Chlorphenamine maleate (1 μg/ml). This indicates that there was competitive antagonism between histamine and isolated compound of *celosia argentea* for H1 receptors present on the smooth muscle. (Graph 4)

Control = D.R.C. of histamine (30 μg/ml) in absence of test drug Isolated flavonoid = D.R.C. of histamine (30 μg/ml) in presence isolated compound of *celosia argentea* (10μg/ml). CPM= D.R.C. of histamine (30 μg/ml) in presence Chlorpheniramine maleate (1 μg/m)

7. DISCUSSION
Several flavonoids isolated from the medicinal plants have been discovered to possess significant anti inflammatory activity [18]. The toxicity studies of the plant suggest that it has reasonable safety margin justifying its wide application in various communities and lack of any reported side effects with traditional use of this plant. Phytochemical screening of isolated extract showed the presence of flavonoids, saponin, alkaloids and glycosides. The literature survey of plant reported the presence of flavonoids and phytochemical screening of ethanolic extract revealed that ethanolic extract of the leaves of *Celosia argentea* contains various classes of phytoconstituents such as Alkaloids, Sterols and flavonoids [19]. Flavonoids are reported to possess mast cell stabilizing, antiallergic and antihistaminic activities [20, 21]. Our results revealed that administration of isolated flavonoid compound inhibited the edema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. In Passive paw anaphylaxis in rats, the flavonoid fraction produced significant anti-asthmatic activity at the dose of 10 mg/kg. The degranulation of mast cell occurs in response to the immunological stimuli in which antigen antibody reactions are predominant. Isolated flavonoid compound (10mg/kg) significantly protect compound 48/80 induced degranulation of mast cell in a dose dependent manner. Isolated flavonoid compound at 10 mg/kg protect mast cell comparable to Ketotifen fumarate (1mg/kg, p.o.). Hence antiasthmatic activity isolated flavonoid compound of *Celosia argentea* is due to the presence of flavonoid fraction.

It is reported that activation of α-adrenergic and H1- histaminergic receptors causes activation of VIP (Vasoactive Intestinal Polypeptide) in cerebral cortex, which is responsible for release of histamine from sensory neurons [23]. This leads to activation of IP3 and DAG pathway. This increased IP3 is responsible for releasing the microsomal calcium, leads to phosphorylation of actin-myosin fibers of goat trachea causing the contraction. Thus, the contraction of tracheal or bronchial smooth muscle in vitro has often been utilized for the study of contractile / dilator responses of agonists as well as antagonist [24].

In the present study, histamine showed maximum contraction while isolated flavonoid of *celosia argentea* significantly inhibited the histamine induced contraction of isolated goat tracheal chain preparation. The parallel shift of graph towards right side in histamine concentration-response curves in the presence of increasing concentrations of while isolated flavonoid of *celosia argentea* indicating that there was competitive antagonism between histamine and isolated flavonoid of *celosia argentea* for H1 receptors present on the smooth muscle. This effect may be due to its antihistaminic or antispasmodic activity.

In conclusion, the result of the study supports the traditional use of this plant in some painful inflammatory conditions. Hence antiasthmatic activity of isolated flavonoid of *celosia argentea* may be due to presence of quercetin flavonoid. Further studies are currently, in fact, underway to characterize the active principles responsible for its anti asthmatic activity

8. ABBREVIATIONS
CL- *Celosia argentea*, SD-Standard deviation, ANOVA- Analysis of variance, TLC- Thin layer chromatography, RF- Retardation factor, DRC-Dose response curve, CPM-Chlorpheniramine maleate, COX- Cyclooxygenase.

9. ACKNOWLEDGEMENT
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10. AUTHOR CONTRIBUTION
AJ-Drafted the work, designed it, and analyzed the results. ABP and VHT revised the proposed draft and study design and did substantial contribution in manuscript preparation. TVC made substantial contribution in manuscript preparation and revision of the
study. DB analyzed part of the results. The authors have read and approved the manuscript.

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**Declaration**

The ethical approval for this work was obtained from BVCP, Kolhapur IAEC. (CPCSEA Approval No:BVCPK/CPCSEA/IAEC/01/25/2020)

**BIBLIOGRAPHY**


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