



IN-VITRO ANTIHELMINTHIC ACTIVITY, PHOTO CHEMICAL SCREENING AND TLC STUDIES OF METHANOL EXTRACTION ON *CELOSIA CRISTATA* FLOWER USING IN - STATE FESTIVAL OF TELANGANA (BATHUKAMMA)

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ABSTRACT

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India being a rich and varied flora of medicinal plants. It's used in traditional festivals of India. the present study deals with the in-vitro Antihelminthic activity phytochemical screening and TLC studies of methanol extraction on *celosia cristata* flower using in - state festival of Telangana (Bathukamma). methanol extraction on *celosia cristata* flower was used evaluation of phytochemical screening determination by some chemical tests and thin layer chromatographic study was carried out by using various solvent system of varying polarity of hexane, ethyl acetate, acetone. Phytochemical screening reflects presence of like carbohydrates, Alkaloids, phenols, tannins, phytosterols, glycosides, flavonoids, tannins shows methanol solvents extracts. thin layer chromatographic studies of the *celosia cristata* flower parts constituted different colored phytochemical compounds

with different R_f values. Methanol extractions of *celosia cristata* flower various concentrations (25, 50, 100mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as a control group. treatment with concluded that the Methanol extract of *celosia cristata* flowers showed potent Anthelmintic activity and was equipotent to standard Anthelmintic drug. The potent Anthelmintic activity could be due to presence of glycosides, flavonoids and sterols. so, from the above findings, it was

concluded that Methanol extract of *celosia cristata* flowers posses's significant wormicide activity property.

KEYWORDS: *Celosia Cristata*, Anthelmintic, Phytochemicals, TLC Profile.

1. INTRODUCTION

The Earth is created with many incredible things which are supporting the life of human beings; we found our life in the nature which gives everything we want like water, food, shelter etc. and everyone pray for these things to remain forever with us. This nature looks more beautiful with different flowers, each flower has a unique fragrance which attracts everyone and these flowers were used for celebrations and other occasions, we also see various plants in the nature and each plant has its own importance.^[1]

Great India is known for its traditions and celebrations of festivals. Every festival has a scientific reason to support its celebration. In this regard, present study is planned to evaluate pharmacological activity of flowers used in Bathukamma festival which has been recently declared as state festival of newly formed Telangana state.^[2]

It is a floral festival in which everyday various colored flowers are arranged row after row in a brass plate, called as Bathukamma, (**figure 1**) placed in front of Diety and daily worshipped for a week. In the evening it is carried to nearby pond or any water body and released in it. (**figure2**).



Figure: 1-floral festival Bathukamma.

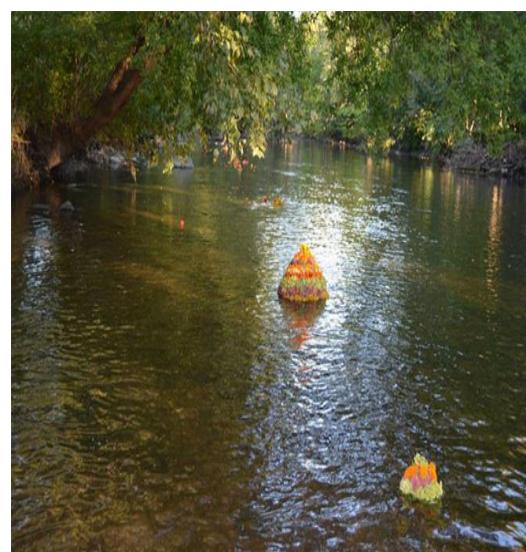


Figure: 2-floral festival Bathukamma in water.

Helminthes parasite infections are global problems with serious social and economic repercussions in the Third World countries. The diseases affect the health status of a large fraction of the human population as well as animals. Some type of dangerous helminthes infections like filariasis has only a few therapeutic modalities at present. The continuous and long-term reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains.^[3] In addition, after treatment with albendazole or mebendazole, several side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness), and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs, such as praziquantel and albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age.^[4] Helminths are the most common infectious agents of humans in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. The disease is highly prevalent particularly in third world countries due to poor management practices. As we know very well, now a days the medicinal preparation available in the market from which most of them either not effective up to the mark or has to develop resistance resulting in reoccurrence again. Plant derived drug serve as a prototype to develop more effective and less toxic medicines.^[4,5,6]

In India many times used in traditional medicine system. It is a common weed throughout India and used in traditional medicine for *Celosia cristata* is most important flower used in this festival. It is commonly known as silver cock's comb, safaid murga, Gunugu in Telugu, Cockscomb flowers are also known as Wool Flowers or Brain Celosia, suggestive of a highly colored brain, it is belongs to family Amaranthaceae.^[7,8,9,10] The recent studies displayed that *Celosia cristata* possessed a wide range of therapeutic activities which were proved that this plant have a potential regenerator capacity of various cells, Antiproliferative activity, Antimicrobial potentiality, adipogenic potentiality, Cytotoxic potential. The wide range of therapeutic potentialities of *Celosia cristata* are mainly due to the presence of various bioactive molecules in flowers, roots, stems, leaves and herbs plant *Celosia cristata* of family Amaranthaceae are all ornamental and colourful.^[11,12] Resent study was shows Flower tops have been used for amenorrhea, dysentery, spitting up blood, haemorrhoids, leucorrhoea, and atypical uterine bleeding activities. To support the significance of immersing these flowers in water reservoirs, their Anthelmintics potential was studied in the present investigation.^[13]

2. MATERIALS AND METHODS

2.1 Plant Material: Fresh flowers of *celosia cristata* were collected from herbal garden of Gunthapally village, near Ramoji film cite in month of October and November-2017. This plant material was identified at Dept. of Botany, Dr. S. Baburaj, Botanist, M.S College of arts and science, kodad. Around 1 kg of fresh flowers were collected and washed with fresh water. The flowers were then dried under shade and homogenized to get a coarse powder. This powder was stored in an air tight container and used for further solvent extraction.

2.2 Preparation of Extract by Maceration

Dried *celosia cristata* flowers powdered (255 gm) materials were of Macerated by 1000 ml of methanol. The solution was occasionally stirred at 25 ± 2 °C for 7 days and then filtered using sterilized cotton and Buchner funnel.^[14] The filtrate was concentrated to evaporate solvent using rotary evaporator at 40 °C and 50 R.p.m. Finally, 16.37 g (yield 8.84 %) of dried extract was obtained and this crude extract was used for phytochemical screening, Thin Layer Chromatography, and Anthelmintica activity studies.

2.3 Qualitative Phytochemical Analysis

The powdered material and extract of the plants were subjected to different kinds of chemical tests to investigate the presence of secondary metabolites such as saponins, tannins, flavonoids, phenol, anthraquinones cyanogenic glycosides, cardiac glycosides and alkaloids etc using standard procedures.^[15,16,17]

2.4 Test for Proteins

2.4.1 Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

2.5 Test for Carbohydrates

2.5.1 Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

2.6 Iodine Test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

2.7 Test for Phenols and Tannins

Crude extract was mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

2.8 Test for Flavonoids

To 1 ml of extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for presence of flavonoids.

Alkaline Reagent Test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

2.9.1 Test for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Concentrated H₂SO₄. Appearance of bluish green colour showed the presence of phytosterol.

2.9.2 Test for triterpenoids

10mg of the extract was dissolved in 1 ml of chloroform, 1 ml of acetic anhydride was added followed by addition of 2 ml of Concentrated H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

2.9.3 Test for tannins

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of tannins.

About 2 ml of the extract was stirred with 2ml of distilled water and few drops of ferric chloride (FeCl₃) solution were added. Formation of green precipitate was indication of presence of tannins.

2.9.4 Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

2.9.5 Test for glycosides

Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour change from violet to blue to green indicated the presence of steroid nucleus, that is, glycone portion of glycoside.

2.9.6 Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides.

2.9.7 Test for steroid

Crude extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. 2ml of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for Steroids a. Salkowski's test

a red color produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids.

Liebermann Burchard test

Development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with concentrated sulphuric acid and acetic acid indicates the presence of steroids.

2.9.8 Test for terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H₂SO₄ was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

2.9.9 Test for Alkaloids

Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

3.0 Thin Layer Chromatography

Thin Layer Chromatography of extracts was done by using standard procedures and is mainly used for the detection of the nature of phytoconstituents present. Thin Layer Chromatography is a very effective technique for the separation of chemical constituents of an extract and for their identification. The history of TLC has been reviewed by various authors. TLC profiles developed for an extract from a defined solvent system and other parameters could be used as a fingerprint in comparative qualitative evaluation of herbal drugs.

The trend of evaluation by this method is becoming popular in view of its simplicity and reproducibility. TLC is an important analytical tool in the separation, identification and estimation of different classes of natural products. In this technique, the different components are separated by the differential migration of solute between two phases – a stationary phase and a mobile phase. Here, the principle of separation is adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phase, its preparation and use with different solvents, separation can be achieved on the basis of partition or a combination of partition and adsorption. In this methods used artificial aluminum oxide TLC plate was using, the plates were activated by heating at 100°C for 10 minutes.^[18]

Solvent systems used in TLC Different solvent system [Hexane: Acetic acid (9:1)] solvent system I, In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), In solvent system III [Hexane: Ethyl acetate: Acetic acid (4:4:2)], In solvent system IV [Hexane: Ethyl acetate: Acetic acid (3:6:1)] used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and TLC plate place the developing jar containing the iodine were used to detect staining process the bands on the TLC plates. The movement

of the active compound was expressed by its retention factor (R_f), values were calculated for different sample.^[18]

R_f = Distance Traveled By Solutes/Distance Traveled By Solvent Front By TLC.

4.0 Anthelmintic Activity

The Anthelmintic activity was performed according to the method of on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblances with the intestinal roundworm parasites of human beings. Five groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released into 50 ml of desired formulation.

Group first serve as control, receive only normal saline, Group second serve as standard, receive standard drug Piperazine citrate (10mg/ml) Group third serve as test Dose-1, receive concentrations of (25 mg/ml) MEIB, Group fourth serve as test Dose -2, receive concentrations of (50 mg/ml) MEIB, Group fifth serve as test Dose -3, receive different concentrations of (100 mg/ml) MEIB, and Observations were made for the time taken to paralyse or death of individual worms. Six worms (same type) in each were placed in it. Time for paralysis was noted when no movement observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Paralysis was said to occur when the worms do not receive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour.^[19,20] Results are shown in (Table-1).

Group I (Normal Control) : Normal saline

Group II (Standard treated) : Piperazine citrate (10mg/ml)

Group III (MEIB Dose-1) : (25 mg/ml) concentrations of MEIB

Group IV (MEIB Dose-2) : (50 mg/ml) concentrations of MEIB

Group V (MEIB Dose-3) : (100 mg/ml) concentrations of MEIB

RESULTS AND DISCUSSION

Table 1: preliminary phytochemical screening of the Methanolic extract of *celosia cristata* flowers.

PLANT CONSTITUENTS TEST	METHANOL EXTRACT
1. Test for Alkaloid	+
2. Test for Carbohydrate	+
3. Test for Flavonoid	+
4. Test for Fixed oils	-
5. Test for Phytosterols	+
6. Test for Glycosides	+
7. Test for Proteins	+
8. Test for Tannins and phenols	+
9. Test for Triterpenoids	-
10. Test for saponins	-

“+” indicates presence

“-” indicates absence

Table 2: Effect of Methanol extract of *celosia cristata* flower on Indian Earth Worms (*Pheritima posthuma*).

GROUP	TREATMENT (DOSE)	TIME FOR PARALYSIS OF WORMS(mins)	TIME FOR DEATH OF WORMS(mins)
I(Normal Control)	Saline	-	-
II(Standard treated)	10mg/ml of Piperazine citrate	37.1±1.50	55.07±0.53
III(MECC Dose-1)	25 mg/ml of MECC	55±1.50	73.20±2.45
IV (MECC Dose-2)	50 mg/ml of MECC	41.12±2.60	67±1.05
V (MECC Dose-3)	100 mg/ml of MECC	22.12±1.63	49.04±1.99

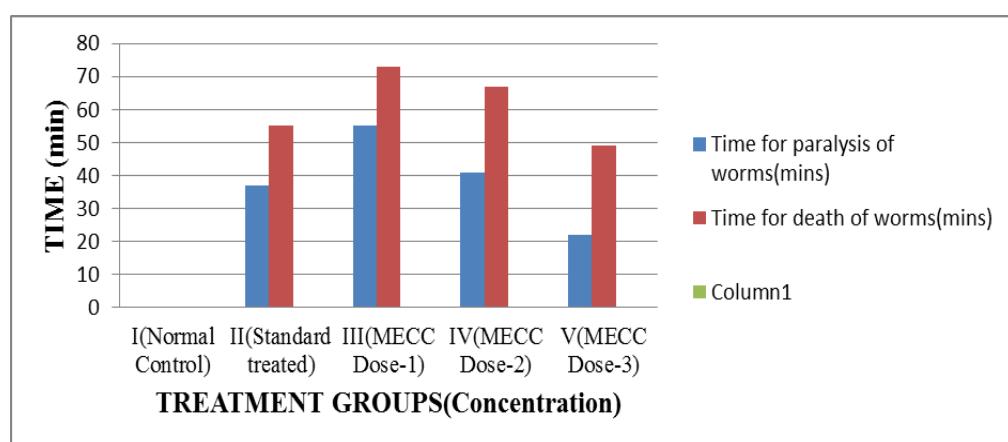
All values represent Mean +SD; n= 6 in each group.

Table 3- R_f values of TLC with respect to Methanol extract of *celosia cristata* flowers Selected using different Mobile Phase solvent systems.

S.NO	SOLVENT SYSTEM	NO OF SPOTS	R _F VALUES
1.	Hexane: Acetic acid (9:1)	0	-
2.	Hexane: Ethyl acetate: Acetic acid (5:4:1)	1	0.67
3.	Hexane: Ethyl acetate: Acetic acid (4:4:2)	1	0.85
4	Hexane: Ethyl acetate: Acetic acid (3:6:1)	2	0.40 & 0.82



Figer-3: preliminary phytochemical screening of the Methanolic extract of *celosia cristata* flowers.



Figer 4: Effect of Methanol extract of *celosia cristata* flower on Indian Earth Wormes (*Pheritima posthuma*).



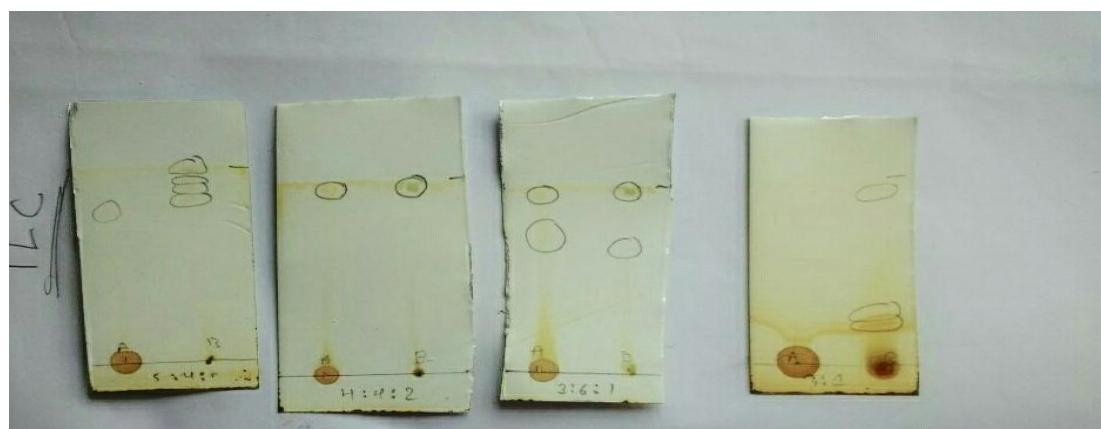


Figure-5: TLC with respect to Methanol extract of *celosia cristata* flowers Selected using different Mobile Phase solvent systems.

Phytochemical screening:

The results of preliminary phytochemical analysis of Methanol extract *celosia cristata* flowers showed the presence of various phytochemical constituents like Carbohydrates, alkolides, phenols, tannins, Phytosterols, glycosides, flavonoids, Tannins (Table-1 & Figure-3).

Anthelmintic activity of Methanol Extract of *celosia cristata* flowers is confirmed by examining the time taken for paralysis (P) and death (D) for *Pheretima posthuma* worms were reported in Table 1. Papaya contains many biologically active compounds. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings, Because of easy availability. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis, As shown in (Table-2 & Figure-4) concentrated Methanol Extract of *celosia cristata* (MECC) exhibited Anthelmintic activity in dose dependent manner taking shortest time for paralysis (P) and death (D) with 100 mg/ml concentration. Hence Methanol Extract of *celosia cristata* (MECC) in its different concentration Shows Anthelmintic activity.

It show shortest time of paralysis ($P=22.12$ min) and death ($D=49.04$ min) in 100mg/ml concentration, while the time of paralysis and death will increase in 50mg/ml concentration ($P=41.12$ min& $D=64.67$ min) and in 25mg/ml concentration ($P=55$ min& $D=73.20$ min) respectively as compare to Piperazine citrate (10mg/ml) used as standard reference ($P=37.1$ min& $D= 55.07$ min) and distilled water as control. The predominant effect of Piperazine

citrate on worm is to cause a flaccid paralysis those results in expulsion of the worm by peristalsis. Thus Methanol Extract of *celosia cristata* (MECC) flowers showed significant Anthelmintic activity as compare to standard reference and control.

Thin layer chromatographic studies A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used. Thin layer chromatographic studies of the hexane extract of Selected mixed herbal powder Solvent system I (Hexane: Acetic acid (9:1), 0 spots (A) were visible. In solvent system II (Hexane: Ethyl acetate: Acetic acid (5:4:1)), 1 spot detected Rf value 0.67. In solvent system III (Hexane: Ethyl acetate: Acetic acid (4:4:2)), 1 spot detected Rf value 0.85. In solvent system IV (Hexane: Ethyl acetate: Acetic acid (3:6:1)), 2 spots were visible Rf values 0.40 and 0.82 (Table -3 & Figer-5).

CONCLUSION

The present study revealed that a result of Methanol Extract of *celosia cristata* flowers contains Phytochemical constituents like flavonoids, glycosides, Carbohydrates, phenolis, Tannins, Alkaloids compounds by phytochemical investigation with respect to chemical tests and TLC chromatographic techniques. Treatment with concluded that the Methanol Extract of *celosia cristata* flowers showed potent anthelmintic activity and was equipotent to standard anthelmintic drug. The potent anthelmintic activity could be due to presence of glycosides, flavonoids and sterols. So, from the above findings, it was concluded that Methanol Extract of *celosia cristata* flowers posse's significant wormicidal activity property. Further carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants and also to evaluate the exact mechanism of action.

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