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PHYTOCHEMICAL SCREENING AND COMPARATIVE STUDY ON ANTIOXIDANT, ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITY OF LEAF AND FLOWER EXTRACTS OF CELOSIA ARGENTEA

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Abstract

The Manuscript deals with the phytochemical screening, antioxidant, antimicrobial and anti-inflammatory activity of Celosia argentea. Flower and leaves using methanolic extract. The phytochemical analysis shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, steroids, tannins and glycosides in the leaf, flower, leaf + flower extracts. Celosia argentea methanolic extract of leaf + flower was most effective at 50µg /ml in terms of DPPH antioxidant activity, with a scavenging capability of roughly 90% compare to standard ascorbic acid 100%. Methanolic extract of Celosia argentea leaf + flower shows the maximum antibacterial activty found at 150µg. Methanolic extract of Celosia argentea leaf + flower extract showed inhibition for S. Aureus (16 mm) and E.Coli (17 mm) respectively. The 50µg of Celosia argentea methanolic extract leaf + flower has demonstrated predominant activity against egg albumin and is almost more or less equivalent to conventional diclofenac medication.

Keywords: Celosia argentea, phytochemicals, antioxidant activity, antimicrobial activity and anti-inflammatory activity.

1. Introduction

Plants in the Amaranthaceae family. The species' blos-soms are frequently described to as cockscombs, brain celosia, wool- flowers, or velvet flowers if the flower heads are crested by fasciation (in Mexico). The herbs, called as in Swahili, are commonly used in the highlands of East Africa. C. argentea is one of the many varieties of plants and is essential for producing nutritious tropical green veggies [3]. The tropical and Indian plant C. argentea is well renowned for its eye-catching hues and age-old use [4].

Other names for C. argentea include wool flower, sil-ver cock's comb, cock's comb and semen.Spirally-arranged leaves, generally pinkish or white blossoms, globular fruits, and black seeds are all characteristics of the plant [5]. Herbs have been used for centuries to treat illness and improve health and account for approximately 80% of medical treatments in the developing world [6,7]. The genetic diversity of 16 populations of Celosia argentea and 6 populations of Celosia argentea in China was investigated using sequence-related amplified polymorphism [8]. There are more than 70 different species, and one of them, C. argentea, is widely used as a leafy vegetable. In India and other tropical nations including Sri Lanka, South Asia, Africa, and America, Celosia ar-gentea (Family: Amaranthaceae) grows as a weed during the rainy season [9]. According to a report by the WHO (World Health Organization) for the medicinal purpose 20,000 plant species are currently used. The usage of plants in the pharmaceutical industries increases because they suggested to use as the remedy of diseases that would have some important ingredient [10]. In India, weeds like Celosia argentea and Daturaalba are typical. This herb has been used medici-nally for a very long time. The plants have antioxidant activity and capable of protective against oxidative stress in biological system. Examining the effects of extracts from celosia argentea leaves, flowers, and mixed extracts of leaves and flowers on various activities is the main goal of the study.

2 Experimental Selections

2.1. Collection of plant material

The leaf and flower of celosia argentea were collected from nearby villages of Tirupattur district Tamil Nadu. The leaf and flower are washed with water and dried carefully in the absence of sunlight to remove the water molecules present in the leaf and flower. The dried leaf and flower are made into fine powder using blender. Then the fine powders are stored properly in an airtight container for future purpose.

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Fig. 1 Celosia argentea plant flower and leaves

2.2 Extraction of sample

About 5gm of the fine powder of the leaf and flower of celosia argentea are taken in a thimble which is placed in an overnight extractor for the purpose of extraction of phytochemicals present in the leaf and flow-er. The extraction is carried out using methanol. The ex-tracts obtained are collected separately and the solvents are evaporated using vacuum distillation and dried. The dried samples are stored in an airtight container for fur-ther analysis. [11].

2.3 Qualitative Phytochemical Screening

Phytochemical screening:

The qualitative tests were carried out in leaf, flower and leaf + flower of Celosia argentea by adopting standard procedure [12,13]. The methanolic extract was screened for the presence of phytochemicals.

1.Test for alkaloids

Mayer's test: small portion of solvent free extract was stirred with few drops of diluted HCl and filtered. The fil-trate was then tested for following colour test. (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. (b) 5gms of potassium iodide was dissolved in 20 ml of distilled water (a) and (b) was mixed and the volume adjusted to 100ml with distilled water. Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

2. Test for flavonoids

Shinoda's test: 5 ml of 20% sodium hydroxide was added to equal volume of the extract. A yellow solution indi-cates the presence of flavonoids.

3.Test for steroids

Liebermann Buchard test: A small amount of sample is treated with 2ml of acetic an-hydride followed by the ad-dition of 3ml of H_2SO_4 Solution. Color changes from vio-let to green or blue indicates the presence of steroids.

4.**Test for terpenoids**

Salkowski Test: To 1ml of extract add 0.5ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids.

5.Test for Saponins

Froth test: 5ml of extract is diluted with 20ml of distilled water and agitated for 10 minutes. Foam is formed which indicates the presence of saponins.

6.Test for Carbohydrates

Fehling test: Two milliliters of each plant extract were hydrolyzed with dilute HCl, neutralized with alkali, and then heated with Fehling's solution A and B. The for-mation of a red precipitate was an indication for the presence of a reducing sugar.

7.Test for tannins and phenolic compounds

Lead Acetate test: 10% lead acetate solution, 0.5g of the extract was added and shaken to dissolved. A white precipitate observed indicate the presence of tannins and phenolic compounds.

8.Test for glycosides:

Keller-Killani test: To 2ml of extract, glacial acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Appearance of reddish-brown color at the junction of the two liquid layers indicates the presence of glycosides.

9.Test for Quinones

Sulfuric acid test: One drop of concentrated sulfuric acid was added to 5 ml of each extract dissolved in isopropyl alcohol. Formation of red color indicates the presence of quinones.

10.Test for Phenols

The sample solution is treated with few drops of 10% ferric chloride. Formation of blue or green col-our indicates the presence of phenols.

11.Test for saponins

To 2ml of distilled water was added with the sample solution and shakes well. Formation of foams indicates the presence of saponins.

2.4 Antioxidant Activity

2,2-Diphenyl-1-Picrylhydrazyl Free radical scavenging activity assay by Brand-Williams et al.[14]

The extracts were prepared in concentrations of 10, 20, 30, 40, and 50 μg/mL for this assay. First, 3 mL of extract of each concentration was mixed with 1 mL of the 0.1 mol/L DPPH solution prepared in methanol. Next, the tubes were incubated in the dark at room temperature for 30 min and then read at 517 nm using a UV-VIS spectrophotometer. Solvent without extract was used as a negative control and AA was used as a positive control. The effect of antioxidant capacity was observed as the color change of purple DPPH to yellow/light-yellow and inhibition values of each extract were calculated using the following equation:

Inhibition (%)= [(control — blank) — (sample — blank] ×100/(control — blank)],

Where A control is the absorbance of the negative control and A sample is the absorbance of AA or extracts. Inhibitory concentration (IC50) values were calculated with inhibition rates using a four-parameter logistic regression model after sigmoidal curves were plotted. Each of the standards and the samples were measured in triplicate and mean values were used for the calculations.

2.5. Antibacterial Activity

Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similar to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then a hole with a diameter of 6mm is punched aseptically with a sterile cork borer or a tip, and a volume (50–150 µl) of the ethanolic extract of Nerium oleander leaf, flower and mixture at desired concentration is introduced into the well. And the positive control tetracycline disc kept in the agar surface. Then agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [2,15]

2.6 Anti-inflammatory Activity

Anti-inflammatory activity by egg albumin denaturation assay

Inhibition of egg albumin denaturation was determined using the method prescribed[16,17].

To prepare phosphate buffer saline (pH 6.4) 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na2HPO4), and 0.24g of potassium dihydrogen phos-phate (KH2PO4) were dissolved in 800 ml of distilled water. The pH was adjusted to 6.4 using 1N hydro-chloric acid (HCl) and made the volume to 1000 ml with distilled water. Control solution (5 ml): 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (pH 6.4), and 2 ml of distilled water. Standard solution (5 ml): 0.2 ml of egg albumin, 2.8 ml of phosphate buffer, and 2 ml of various concentra-tions of the standard drug diclofenac sodium (100-500 µg/ml). Test solution (5ml): 0.2 ml of egg albu-min, 2.8 ml of phosphate buffer and 2 ml of various concentrations of test samples (100- 500 µg/ml).

Procedure

2.8 ml of phosphate buffer (pH 6.4) and 0.2ml of egg albumin were incubated with various concentra-tions (100,200,300,400 and 500 µg/ml) of test sam-ples and standard drug diclofenac sodium (100,200,300,400 and 500 µg/ml) then the samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated us-ing the following formula.

Percentage inhibition = (Abs control − Abs sample)/ Abs control × 100

3. Results and Discussion

3.1 Phytochemical analysis of Celosia argentea

Qualitative phytochemical examination of the leaf, flower, and leaf + flower of Celosia argentea was performed. The methanolic extract of celosia argentea were determine the presence of numerous phyto-chemicals such carbohydrates, alkaloids, flavonoids, glycosides, steroids, tannins, glycosides. And the ab-sence of phenol, terpenoids, and quinones in the leaf, flower, leaf +flower extract.

Table 1: Phytochemical profiling of methanolic extract of Celosia argentea leaf, flower, leaf + flower.

S.No	Phytochemicals	Methanolicextract of *Celosia argentea* LEAF	Methanolicextract of *Celosia argentea* FLOWER	Methanolic extract of *Celosia argentea* LEAF + FLOWER
1.	Carbohydrate	+	+	+
2.	Alkaloids	+	+	+
3.	Glycosides	+	+	+
4.	Phenols	–	–	–
5.	Flavonoids	+	+	+
6.	Saponins	+	–	+
7.	Steroids	+	+	+
8.	Tannins	+	–	+
9.	Terpenoids	–	–	–
10.	Quinones	–	–	–

Symbol (+) indicate positive and (-) indicate negative

3.2 Antioxidant Activity

Power is displayed, possible to look into the antioxidant capacities of bioactive compounds using the stable radical DPPH. By measuring the absorbance of the DPPH radical in the sample at 517 nm, ascorbic acid was used as a control to determine the antioxidant activity of the Celosia argentea methanolic extract. In terms of DPPH antioxidant activity, a methanolic extract of Cel-osia argentea leaf and flower was most efficient at 50µg ml, with a scavenging capacity of around 90% as opposed to normal ascorbic acid's 100%.

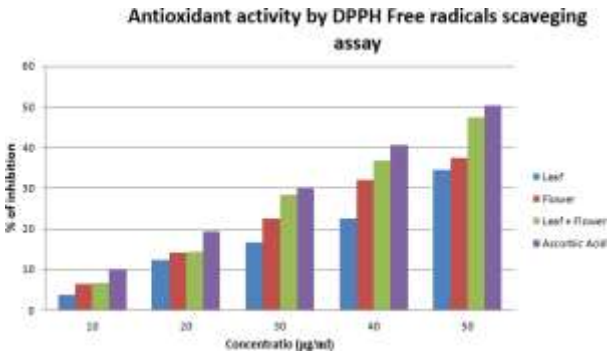


Figure 2: Antioxidant Activity of methanolic Extracts of Celosia argentea leaf, flower, leaf + flower

3.3 Antibacterial Activity

Table 2 shows the Methanolic extract of Celosia argentea that is antibacterial for both gram positive and gram negative bacteria figure 3. Table 2: Zone of inhibition (mm) against gram positive and gram negative bacteria on methanolic extract of leaf, flow-er, leaf + flower of celosia argentea.

Name of the Bacteria	**Methanolic extract of *Celosia Argentea* leaf (mm)**			**Methanolic extract of *Celosia Argentea* flower (mm)**			**Methanolic extract of *Celosia Argentea* leaf + flower(mm)**		
Bacteria	Concentration in µg/ml								
	50	100	150	50	100	150	50	100	150
Zone of inhibition (mm)									
Staphylococcus aureus	10	13	15	8	11	13	10	13	16
Escherichia coli	12	14	15	10	13	15	11	13	17

Table 2: Antibacterial activity of methanolic extracts of Celosia argentea leaf, flower, leaf + flower

150 µg of Celosia argentea leaf + flower methanolic ex-tract exhibits the greatest antibacterial activity. A meth-anolic extract of Celosia argentea leaves and flowers demonstrated zones of inhibition for S. aureus (16mm) and E. coli (17mm), respectively. The methanolic extract of Celosia argentea has a wide range of antibacterial ef-fects. The methanolic extract of Celosia argentea leaf and flower has been proven to have relatively better activity against both gram positive and gram negative bacteria when compared to leaf + flower extracts.

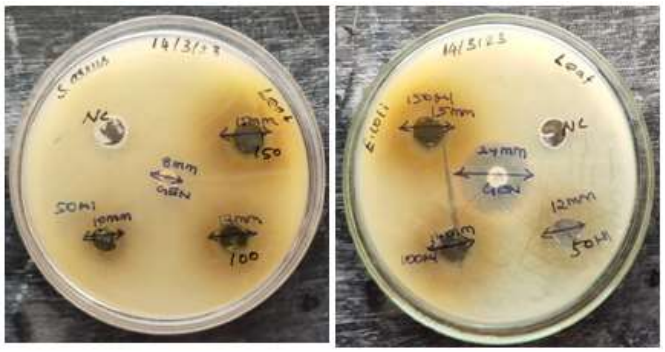


Figure 3 (a)

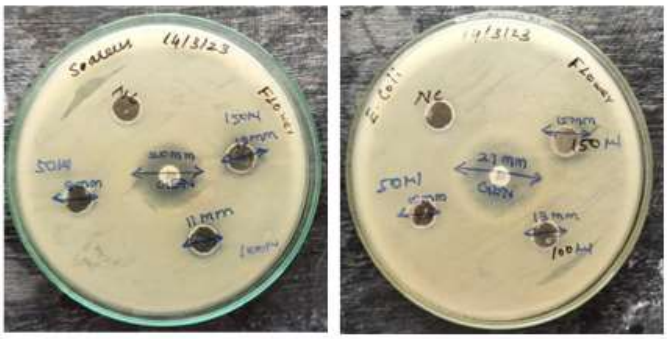
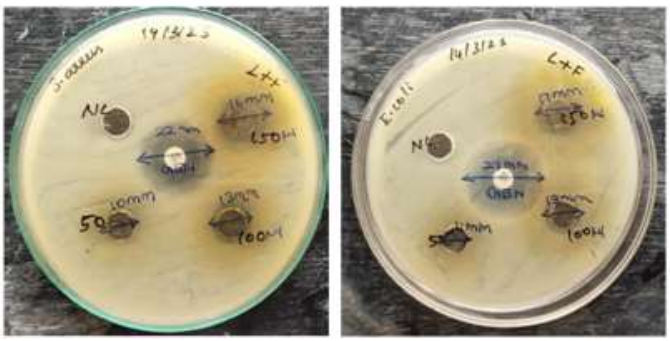


Figure 3 (b)



3.4 Anti-inflammatory Activity

Methanolic extract of Celosia argentea showed anti-inflammatory properties in figure 4.

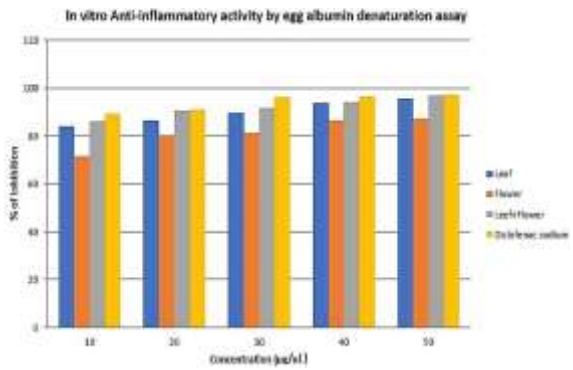


Figure 4: Anti-inflammatory activity of methanolic extracts of *Celosia argentea* leaf, flower, leaf +flower

When compared to methanol extract of *Celosia argentea* leaf and flower in various concentrations, the 50µg of *Celosia argentea* methanolic extract leaf + flower has demonstrated predominant activity against egg albumin and is almost more or less equivalent to conventional diclofenac medication.

4 Conclusions

Alkaloids, tannins, steroids, and carbohydrates and other biologically significant secondary metabolites found in the methanolic extract of *Celosia argentea* in the current study. Using an *in-vitro* technique, methanol extract was found to have significant antioxidant activity. The impact free radical scavenging capability of methanolic extract may account for its *in-vitro* antioxidant potential. According to the results of the antibacterial investigation on the methanolic extract, *Celosia argentea* has high antibacterial activity against a variety of microorganisms, including both gram positive and gram negative bacteria. The many phytochemicals contained in *Celosia argentea* may be primarily responsible for the high antioxidant and antibacterial effects of the methanolic extract of this plant. According to a recent study, *Celosia argentea* methanolic extract may be used as an alternative treatment for a variety of illnesses, including oxidative stress and a wide spectrum of bacterial infections. Leaf + flower mixed extracts contain high amount of biological activity than individual extracts of leaf and flower.

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Conflict of Interest: Nil

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