

Research Article

Immunomodulatory profile of *Celosia argentea*-Activity of Isolated compounds I and II

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[Received-11/05/2015, Accepted-23/05/2015, Published-05/06/2015]

ABSTRACT:

Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Immunomodulators not only boost immunity, but normalize it. Being safe, effective and pocket friendly, plant based immunomodulators can provide as alternatives or adjuvants to conventional drugs. The aerial parts of *Celosia argentea* Linn, a common weed found in Maharashtra are reported to be rich in flavonoids that are believed to play a significant role in body's defense mechanism. Hence the present study explores the effectivity of the compound I-Luteolin 7-O glycoside and compound II-1-(4-hydroxy-2-methoxybenzofuran-5-yl)-3-phenylpropane-1,3-dione obtained from the aerial parts of *Celosia argentea* Linn. Fresh whole plants of *Celosia argentea* Linn. were collected from Bhor, district-Pune, Maharashtra and dried in the shade at room temperature. The herbarium of the plant specimen was deposited and authenticated at Botanical Survey of India, Pune. Proximate analysis was done and Physicochemical constants such as moisture content, ash value, extractive value and foreign organic matter were studied using standard methods. When both the isolated compounds I and II were tested for in vivo immunomodulatory activity using Mice Paw Sensitivity test, Phagocytic Index and HA Titre parameters, it was found that Compound I exhibited impressive immunomodulatory activity (**p<0.001) followed by compound II (**p<0.01), for all the three assays. Immunosuppressive state is involved in the etiology as well as pathophysiology of many neoplastic, inflammatory and autoimmune diseases. Thus, the present research work suggests that compounds I and II of aerial parts have a significant potential for immunoregulation and may be administered as alternatives or adjuvants to therapies requiring immunomodulation, especially when the host defense mechanism has to be activated under the condition of impaired immune response in degenerative diseases.

Keywords: *Celosia argentea* Linn, Immunomodulator, Compound I, Compound II, Flavonoid

INTRODUCTION:

Immunomodulator is a substance that alters the immune response by augmenting or reducing the ability of the immune system. The substances

showing such property are called as Immunomodulatory agents. Modulator given to someone with a healthy immune system will have

little effect in the body and modulator given to someone with an overactive immune system can help to normalize that overactive immune system. Immunomodulators do not tend to boost immunity, but to normalize it. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning [4].

Immunosuppressive state is involved in the etiology as well as pathophysiology of many neoplastic, inflammatory and autoimmune diseases. Immunomodulators help circumvent the immune lowering pathogens, boost the immunity and have a broad spectrum of anti-infective effect also shielding the reoccurrence. The healthy state is believed to be based on a sophisticated fine tuning of immunoregulatory mechanisms [6]. *Celosia argentea* Linn. (Amaranthaceae), is a herbaceous annual plant growing as a weed post monsoon throughout



Figure 1: *Celosia argentea* Linn Plant

India and other tropical regions of the world such as Sri Lanka, South Asia and America Nigeria, Benin and Congo [9]. The entire plant is used in treatment of ulcers, piles, bleeding nose, inflammation, gynecological disorders, mouth sores, eye diseases, glandular swellings, eczema, and constipation as an aphrodisiac. The seeds are used in the treatment of blood diseases, diarrhea etc. and the roots are well known for their anti-diabetic activity [13]. Since the plant is also

reported to be rich in flavonoids [8], having a significant role to play in body's defense mechanism, the present research work is aimed at evaluation of immunomodulatory effect of vital components of aerial parts of *Celosia argentea* Linn.

Plant-derived immunomodulatory & anti-tumor phytoconstituents:

Immunomodulation using plant material can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the condition of impaired immune response. Immunotherapy involves the treatment of cancer by modification of the host-tumor relationship. It is now known that this relationship is quite complex and only some of the interactions have been elucidated. With a better understanding of the immunology of this anticancer response, recent trials have focused on certain aspects of the process to stimulate an antitumor response. Some of the novel biological response modifiers work as general stimulants of the immune system, through varied mechanisms including induction of stimulatory cytokines (such as IFN-alpha, TNF-alpha and IL-12) and activation of T cells and the antigen-presenting dendritic cells. These compounds include Toll-like receptor agonists, several of which are in clinical trials at present. Some of these compounds have single-agent activity in clinical trials, while others such as DMXAA have shown promise in combination with chemotherapy without increasing toxicity (Pandey *et al.*, 2009). More lipophilic flavonoids may disrupt microbial membranes. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifranchaside (*Swertia chirata*) and glycyrrhizin (*Glycyrrhiza glabra*) against HIV. These compounds include Toll-like receptor agonists, several of which are in clinical trials [15].

MATERIALS AND METHODS:

Collection, drying and authentication: Fresh whole plants of *Celosia argentea* Linn., were collected from Bhor, district-Pune, Maharashtra and authenticated at Botanical Survey of India, Pune; SVDAC1 (Reference number- BSI/WC/Tech/2011). Dried aerial parts of *Celosia argentea* Linn were coarsely powdered in grinder, passed through 180 mesh and were used for extraction.

Proximate analysis: Physicochemical constants such as moisture content, ash value, extractive value and foreign organic matter were studied using standard methods to determine its purity and stability (Khandelwal, 2005).

The cleaned, dried and powdered aerial parts excluding seeds were subjected to defatting using N-Hexane followed by exhaustive successive solvent extraction using solvents with ascending order of polarity; Pet. ether → Chloroform → Ethylacetate → Methanol → Water. The completion of extraction was indicated by taking sample from the siphon tube on the TLC plate and placing it in iodine chamber. Absence of colored spot on plate indicated complete extraction (Mukherjee, 2002). The extracts obtained were concentrated by using Rotary vacuum evaporator and preserved in vacuum desiccator for further phytochemical and pharmacological screening. [13]. These extracts were concentrated and subjected to phytochemical screening for the presence of flavonoids, sugars, tannins, glycosides and steroids. A phytochemical research can be recognized and valued only if efforts are made towards isolation of the bioactive compound responsible for the significant pharmacological activity. Considering this fact the bioactive methanolic extract was further subjected to column chromatography using a step gradient of Chloroform:methanol mixture (95:5, 90:10, 80:20, 100:0). The isolated fractions were further purified on a column of MCI HP20 leading to the isolation of two compounds (Lee *et al*, 2007, Delazaret *et al*, 2006).

Acute Toxicity Studies: The dose levels 10 and 20 mg/kg of the test extract were selected on the basis of the toxicity studies performed by (Bhujbalet *al*, 2008) revealing that the dose is safe up to 2000 mg/kg i.p. without any mortality.

In vivo Immunomodulatory Activity:

Grouping of animals: Control-1ml of distilled water (vehicle); Negative Control-Cyclosporin (50 mg/kg); Standard immunosuppressant agent - Sheep RBC (SRBC) (concentration of 20% prepared in 0.49% EDTA and PBS solution in the ratio of 1:1) was used for immunization and 1% for challenge 2.4.2 Mice paw sensitivity test-Cell mediated immunity [12] On 0 day of study the groups I to IV were immunized with 0.1ml 20% SRBCs in normal saline. From day 1 to 7 animals from all the groups were administered with test drugs and Cyclosporin; a negative control drug.

Cellular immune response: On the 7th day, the thickness of right hind footpad was measured using digital vernier. Foot pad reaction was assessed after 24hrs and 48hrs i.e. on 8th and 9th day respectively in terms of increase in the thickness of footpad due to oedema caused as a result of hypersensitivity reaction [12]. The immune response was calculated by measuring the difference in the thickness (mm) between the pre- and post- treatment of right hind footpad injected with SRBCs, while the untreated left hind paw served as the control (Bafna *et al*, 2004).

Bacterial clearance test:

Phagocytic Index: Blood samples were collected by puncturing the retro-orbital plexus into heparinised vials before commencement of the treatment with the extracts. Test drug was fed to animals at the dose of 50 and 100mg/kg body weight for a period of 7 days. After completion of 7 days, blood was again collected in heparinised tubes and challenged with 0.1 ml of pure culture of *Streptococcus pyogenes* and incubated for 1 hour at 37°C, after 1 hour blood smears were prepared by fixing and staining with Leishman's stain and

number of cells containing phagocitized bacteria were counted under microscope (Yadav *et al.*, 2011).

Haemagglutination antibody (HA) Titre-Humoral immune response: On day 0 all the animals were immunized with 20% SRBC (0.1 ml/100 gm body weight i.p.), including control. On the 7th day blood samples were collected from retro-orbital plexus from all the antigen sensitized mice and serum was separated by centrifuging at 200 rpm for 15min 96-well microtitre plates i.e. 12 wells in each row and each well having a 'U' bottom were used for the estimation of antibody Titre. Two rows i.e. 24 wells were used for the dilution of each serum sample. Normal saline was used as a vehicle to make serial dilutions. Each well of a microtitre plate was filled initially with 25 µl of saline, 25 µl of serum was then added to this well. Subsequently 25 µl of dilute serum was removed from the first well and transferred to the next subsequent well to get two-fold dilution of the antibodies present in the serum. Further two-fold dilutions of this diluted serum were similarly carried out till the last well of the second row (24th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 25 µl SRBC (20%) was added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for hemagglutination [14]. The antibody Titres were expressed in graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were examined visually for agglutination and compared with the control for statistical significance [15]. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody Titre (Bafna *et al.*, 2006).

Statistical Analysis: Data was collected and presented in the form of tables and graphs using MSEXCEL. Further analysis was done using SPSS IBM software-- version 20. The results were analyzed by One-way ANOVA followed by Dunnett's Multiple Comparison Test, T-

test, Wilcoxon ranked test. The value of * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$ were considered statistically significant.

Results and Discussion:

Acute toxicity studies the dose levels were safe up to 2000mg/kg i.p., with no signs of mortality.

In vivo Immunomodulatory Activity:

Mice Paw sensitivity test: The Mice Paw sensitivity test is a direct correlate of cell mediated immunity (CMI). During CMI responses, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblast and secrete lymphokines, attracting more scavenger cells to the site of reaction leading to delayed type hypersensitivity (DTH).

As observed in Figure no. 2, both the compound treated groups showed rise in the footpad thickness; the highest being observed in compound 1 (20 mg/Kg BW) treated group with footpad thickness of 156.2 ± 2.07 *** (** $p < 0.001$) followed by compound 1 (10 mg/Kg BW) treated group $\{142.0 \pm 2.13$ ** (** $p < 0.01$)}. Compound 2 in a dose of 20 mg/Kg BW also exhibited significant immunostimulant activity with foot pad thickness $141. \pm 4.62$ ** (** $p < 0.01$) though slightly lesser than compound 1. This could probably be due to the active immune system that released large number of cytokines, interleukins and TNF- α factors at the site of injection, resulting in pain, inflammation and edema.

Phagocytic Index Test: Ability of neutrophils to adhere to endothelial surface and migrate into inflammatory site is critical for their ability to control bacterial infections. The number of bacteria phagocytized by host cells after drug treatment is indicative of the immunomodulatory potential of the drug. Compound 1 (20mg/kg) body weight treated group showed the highest level of phagocytosis (17.25 ± 1.06 ***; *** $p < 0.001$) followed by Compound 2 (13.75 ± 1.51 ***; *** $p < 0.001$) at 20mg/kg (9.0 ± 1.41 ; ** $p < 0.01$), when compared with the control (2.5 ± 1.21). In Figure 3 the haematological

slide (b) and (c) clearly show neutrophils containing engulfed bacterial cells (red arrow). Slide (a) of the control group shows intact neutrophil cells without bacteria.

Haemagglutination antibody (HA) Titre
 Highest dilution of the aerial extracts showed formation of complete haemagglutinates indicating the drug to be capable of activating immunoglobulins which in turn activates pre β cells and /or dendritic cells resulting in activation of antibodies leading to higher agglutination Titer against SRBC's antigens. The Titre is expressed as a reciprocal of the final dilution of serum associated.

The specific antibodies against SRBCs were found to be more effectively formed in case of Compound 1 with 20 mg/kg bw having average Agglutination titer of $1:256 \pm 0.0^{***}$ ($***p < 0.001$) followed by compound 1 with 10mg/kg bw ($1:246 \pm 00$; ($***p < 0.001$.(Table 6.36). The HA Titre for compound at 20 mg/kg bw was also significant ($1:242 \pm 3.30^{**}$; $**p < 0.01$). This indicates that both the compounds resulted in complete agglutination of the treated blood sample, as a result of activation of the cytokines and interleukins in the blood sample indicating its immunostimulant potential.

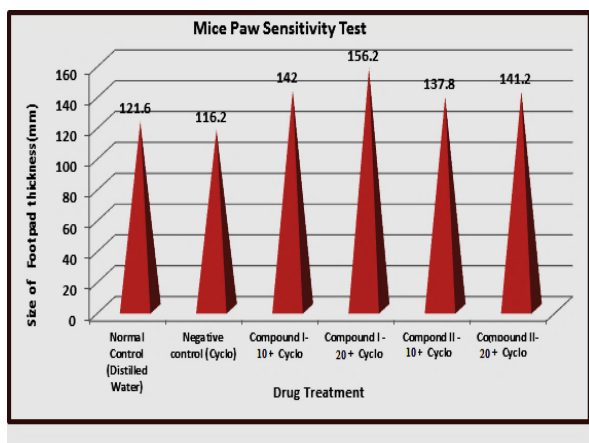


Fig. 2: Effect of Compound I and Compound II on Mice Paw Volume in Mice Paw sensitivity test

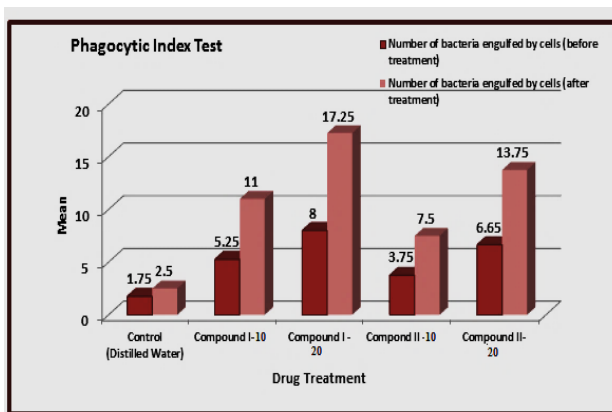
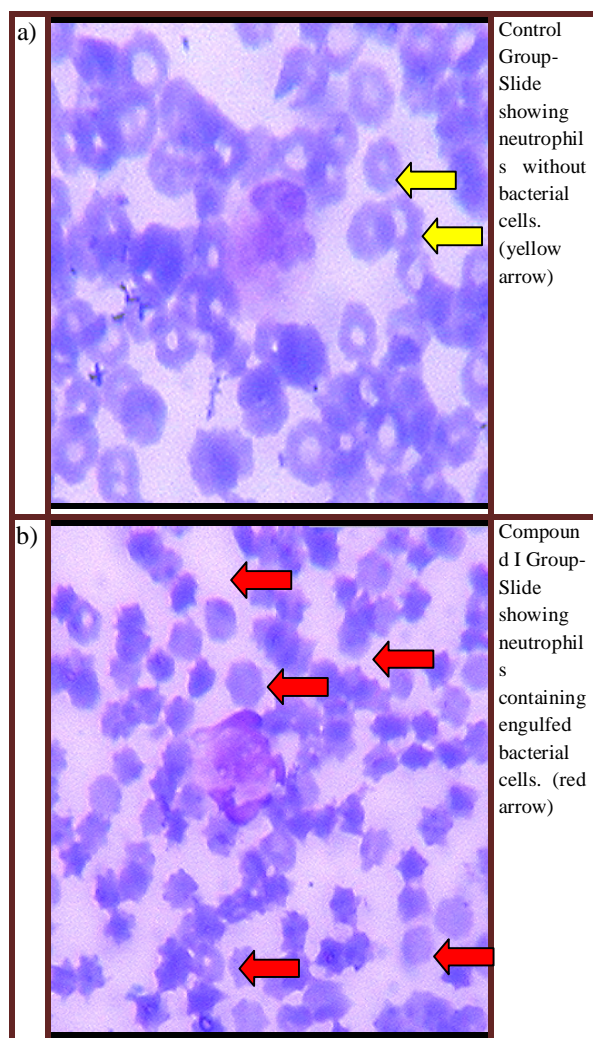


Fig. 3.Effect of,Compound I and Compound II on Phagocytic Index



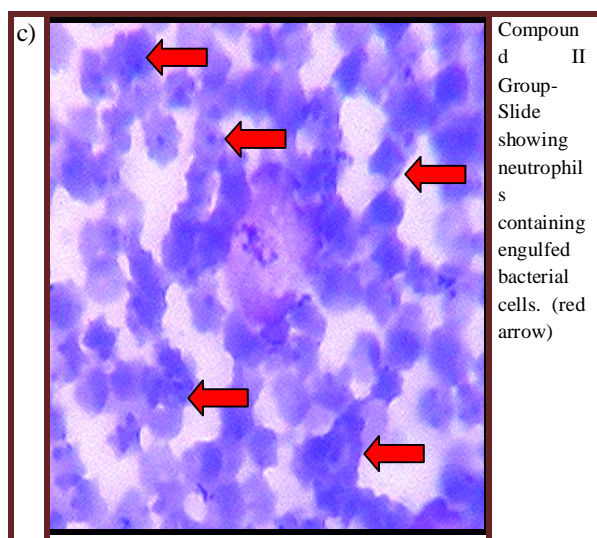


Fig. no. 4. of bacteria engulfed by host cells after treatment with (a) Control Group (b) Methanolic fraction treated Group (c) Compound I Treated Group and (d) Compound II Treated Group

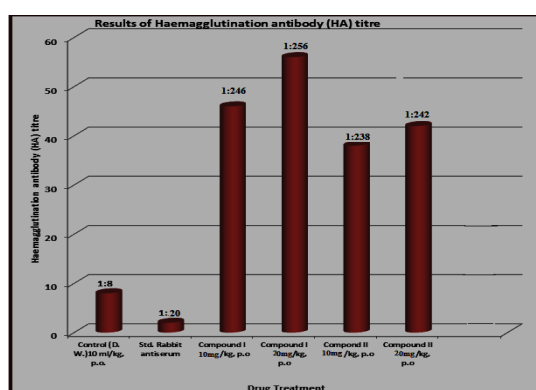


Fig.5. Effect of Compound I and Compound II on (HA) Titre.

Structural elucidation of

Compounds: (Agarwal; Paridhavi)

Compound 1 was obtained as a yellow amorphous solid with elemental analysis, C- 56.10; H- 4.90; N- Nil. It gave a melting point in the range of 266-268°C. The compound also gave positive color reactions for hydroxyflavones with several reagents. Its UV spectrum in methanol showed a peak at λ_{max} 344 and 263 nm.

FT-IR spectra of the compound showed bands at ν_{max} 3389 cm^{-1} (Strong and broad) suggesting -OH group; 2991 cm^{-1} (Strong) suggesting

aliphatic -C-H stretch; 3150 cm^{-1} (Strong) suggesting aromatic -C-H stretch; 1715 cm^{-1} (very strong suggesting aromatic -C=O); 1624 cm^{-1} (Strong) suggesting -C=C stretch and 1370 cm^{-1} (strong) suggests -C-O stretch of pyran ring; 1523 cm^{-1} (strong) suggesting -C=C stretch of aromatic ring; 1446 and 722 cm^{-1} (Medium) suggest aliphatic and aromatic C-H bend respectively. The results of 1H -NMR data summarized show presence of aromatic and benzopyran ring that suggests possibility of a flavonoid structure. GC-MS exhibited the $[M+H]^+$ at m/z 450.09 dissociated primarily by the loss of glucose residue (Glu) (-162) resulting in the peak at 287.9871. The results of the above physical, chemical, elemental and spectral analysis confirmed that the isolated compound is Luteolin - 7-O-glucoside (C₂₁H₂₀O₁₁) (Figure 6.71),

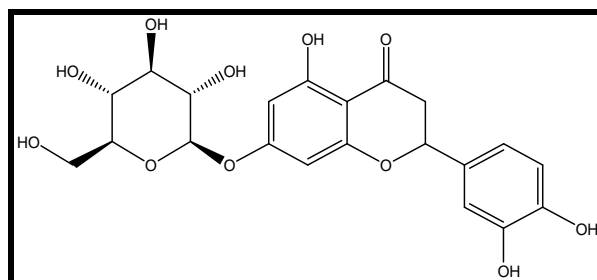


Figure No.6. Luteolin - 7-O-glucoside (C₂₁H₂₀O₁₁)

IUPAC Name: 2-(3,4-dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxochromen-4-one
Compound 2 was obtained as yellowish brown amorphous solid having melting point in the range of 1870 to 1890°C, the compound gave positive color reactions for phenolic compounds (blue green colour with FeCl₃). Its elemental analysis was C- 56.10; H- 4.90; N- Nil. Its UV spectrum in methanol showed a peak at λ_{max} 427 nm. FT-IR spectra of the compound showed bands at 3504 cm^{-1} (Strong and broad) suggesting phenolic -OH group; 2889 cm^{-1} (Strong) suggesting aliphatic -C-H stretch; 3150 cm^{-1} (Strong) suggesting aromatic -C-H stretch; 1640 cm^{-1} (very strong) suggesting aliphatic -C=O; 1610 cm^{-1} (Strong)

suggesting aromatic $-C=C$ stretch; peaks between 1050 cm^{-1} and 1250 cm^{-1} (strong) suggests $-c-o$ stretch of furan ring; $-c-o$ stretch of phenolic ether is predicted between $1080-1120$; bands at 1446 and 722 cm^{-1} (Medium) suggest aliphatic and aromatic $C-H$ bend respectively. The results of ^1H-NMR data showed resemblance to curcumin with the addition of benzofuran ring; $\delta-6.810$ and $\delta-6.972$, that suggests possibility of a novel phenolic compound with benzofuran ring.

GC-MS exhibited the $[M+H]^+$ at m/z 310.3216.

The results of the above physical, chemical, elemental and spectral analysis confirmed that the isolated compound is a novel phenolic compound; 1-(4-hydroxy-2-methoxybenzofuran-5-yl)-3-phenylpropane-1,3-dione, ($C_{18}H_{16}O_5$).

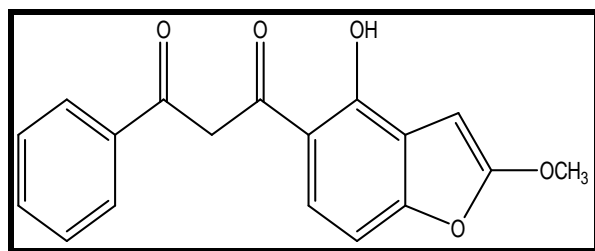


Figure No. 7. (4-hydroxy-2-methoxybenzofuran -5-yl)-3-phenylpropane-1,3-dione

CONCLUSION:

The isolated compounds of aerial parts of *Celosia argentea* Linn; Luteolin - 7-O-glucoside ($C_{21}H_{20}O_{11}$) and 1-(4-hydroxy-2-methoxybenzofuran-5-yl)-3-phenylpropane-1,3-dione ($C_{18}H_{16}O_5$) exhibited significant in vivo immunomodulatory activity. When both the compounds were tested using Mice Paw Sensitivity, Phagocytic Index and HA Titre parameters, it was observed that Compound I exhibited impressive immunomodulatory activity followed by compound II in all the three models. Immunosuppressive state is involved in the etiology as well as pathophysiology of many neoplastic, inflammatory and autoimmune diseases. Immunomodulators help circumvent the immune lowering pathogens, boost the immunity and have a broad spectrum of anti-infective effect

also shielding the reoccurrence. The healthy state is believed to be based on a sophisticated fine tuning of immunoregulatory mechanisms. Thus, the present research work suggests that compounds I and II of aerial parts of *Celosia argentea* Linn have a tremendous potential for immunoregulation and may be administered as an adjuvant or alternative to therapies requiring immunomodulation.

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