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Evaluation of Antioxidant Potential and Formulation of Nanosuspension of *Celosia argentea* Extract

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Abstract

It is a well-known undisputed truth that plants play a significant role in health and are frequently used in the food and medicinal industries. Among plant parts, leaves, stems, roots, and bark are being extensively researched for their biological characteristics. However, flowers are overall neglected and significant activities were not explored. *Celosia argentea*, Family: Amaranthaceae is wide used as ancient drugs with long history. More than 79 chemicals, mostly saponins, peptides, phenols, fatty acids, and amino acids, were extracted and identified from this plant. Saponins are considered to be the distinctive and active components of the herb. Experimental data showed that the active ingredients in this herb have broad biological effects, including the ability to treat number of eye conditions. The flowers of *Celosia argentea* were collected and extracted using methyl alcohol and water as solvent. Preliminary phytochemical analysis of extracts were carried out by chemical tests. Antioxidant activity was evaluated for the hydroalcoholic extract by DPPH and H_2O_2 technique. Formulation of nanosuspension of *Celosia argentea* was carried out by Nanoprecipitation technique. Nanosuspensions of *Celosia argentea* potentiates the antioxidant potential and bioavailability.

Keywords: *Celosia argentea*, Nanosuspension, DPPH and H_2O_2 method, Nanoprecipitation method.

INTRODUCTION

It is a renowned undeniable fact that plants area unit wide utilized in pharmaceutical and food industries because of their biological importance. Since ancient times, herbal treatments have been widely used around the world. Due to either their large molecular size or poor water solubility, the bulk of an extract's active ingredients are unable to cross the lipid membrane of cells, which results in limited absorption and poor bioavailability.[1] Combining herbal medicines with nanotechnology, since nanostructure systems may be able to enhance the effects of herbal extracts while lowering dosage requirements, reducing side effects, and enhancing bioactivity.[2] A fascinating area of research in the field of plant engineering focuses on the creation of nanoparticles of various sizes and shapes, as well as their potential use in clinical treatment. [3, 4] Because engineering and science are integrated at the 10^{-9} nanoscale.[5] Techniques like bottom-up technology and top-down technology are used to transform the drug microparticles/micronized drug powder into drug nanoparticles. Nanosuspensions are surfactant -stabilized unit submicron mixture dispersions of drug particles.

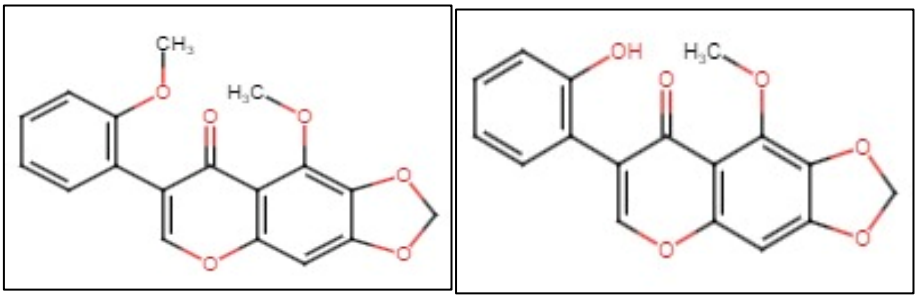
A poorly soluble drug without any matrix material is suspended in dispersion in a nanosuspension. These will be accustomed enhance the solubility of medicine that area unit poorly water soluble in water in addition as lipid media. [6,7] Due to the active compound's increased flooding rate as a result of its twofold solubility, the maximum plasma level is attained sooner. Molecules having weak solubility, poor permeability, or both benefit from this method. Due to the smaller particle size, poorly soluble medications are more likely to be administered through blood vessels without blocking blood capillaries. [5,4] E.g.: Griseofulvin nanosuspension is prepared by the microemulsion technique.[8]

When present in low concentrations compared to those of an oxidizable substance, antioxidants significantly slow down or stop the chemical reaction of that substance.[9] Free radical production naturally regulated by a variety of advantageous substances known as antioxidants [10] ·and their activities include free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity.[11] Reactive oxygen species corresponds to various neutralising antioxidants Eg: Hydroxyl

radicals-Vitamin C, Folic acid, [12,13] Hydrogen peroxide-Vitamin C, folic acid, glutathione, lipoic acid, β-carotene[14,4]

Parts like leaves, stems, roots and bark area unit being widely studied for his or her biological properties. However, flowers area unit nearly neglected and don't seem to be abundant probed for his or her importance. *Celosia argentea* L. is widely used as an old medication with a long and rich history. [15] Over seventy-nine compounds from this plant were isolated and known, Eg: saponins, peptides, phenols, fatty acids and amino acids, of that saponins are active constituents of *Celosia argentea*. Experimental data showed that *Celosia argentea's* active chemicals have broad biological effects, including the ability to treat a number of eye conditions. They have single electron in their outermost and become more reactive.[15] Free radicals can be stabilised, rendered inactive, and scavenged by antioxidants before they damage cells.[16] The optimal maintenance of cellular and systemic health and wellbeing depends on antioxidants.

The *Celosia argentea* L. aerial portion. includes Tlatlancuayin and Betavulgarin, two flavonoids. Aspartic acid, Threonine, Glutamic Acid, Moroidin, Celogenamide A, and Celogentin A are among the amino acids found in the seed. Citrusin C, an indicator glycoside, is present in the leaf. It also contains alkaloids, tannins, saponins, carbohydrates, proteins, steroids.[15]



Tlatlancuayin Betavulgarin

Figure 1: Chemical structures of Tlatlancuayin and Betavulgarin

The flower and seed are having specific uses like parasiticide and poultice, astringent, haemostatic, Ophthalmic. Bloody stools, diarrhoea, uterine bleeding, haemorrhoids, Hypertension, cataracts, and bloodshot eyes and other conditions are all treated with it. The seed also contains antimicrobial properties that prevent pseudomonas from growing. [13]

MATERIAL AND METHODS

Collection of plant material

Celosia argentea were collected from nearby area of Ratnagiri district. The inflorescence of plant was dried under shade away from direct sunlight. Dried part were cleaned and coarsely powdered in grinder and coarse powder used for extraction.

Identification

Celosia argentea plant was collected and herbarium sheet was prepared and submitted to Dr. Rajendra D. Shinde, St. Xavier's College, Mumbai, Maharashtra, India. A voucher specimen number (P. D. 1391), authenticated plant and deposited in Department of Pharmacognosy.

Chemicals and Drugs

Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), Phosphate Buffer(pH-7.4), Hydrogen peroxide, Ascorbic acid all are of AR grade.

Extraction:

Dried flowers of *Celosia argentea* (50gm) was taken and extracted with solvent methanol and water (1:1) by cold maceration for 3-4 days. The filtrate was filtered by Whatman Filter paper for any impurities. The extract was concentrated under the reduced pressure on water bath at a temperature below 50^0C to form a syrupy like consistency. These extracts are to be observed for color,

consistency including yield. Extract of *Celosia argentea* was found to be brownish black color with semisolid consistency and yield was found to be 7.6 gm.

Preliminary Phytochemical Investigation [17]

To check for the presence of various chemical components, a preliminary phytochemical examination of the hydroalcoholic extract of *Celosia argentea* was conducted.

Table 1: Preliminary Phytochemical Investigation of hydroalcoholic extract

Sr.No:	Compound	Test	Result
1	Carbohydrates	Molisch test	+
2	Alkaloids	Dragendroff test	+
3	Tannins	Alkaline reagent test	+
4	Flavonoids	Detection of flavonoids	+
5	Saponins	Foam test	+
6	Proteins and amino acids	Million's test	+
7	Steroids	Steroid test	+

+ = shows Positive test

Formulation of Nanosuspension extract of *Celosia argentea* by Nanoprecipitation Method:

For the creation of nanosuspensions, the nano-precipitation process was used with a few modifications. Plant extract (2.5g) was sonicated for 60 seconds after being diluted in 15 ml of acetone and ethanol (3:1). The resultant solution was injected gradually (1 mlmin-1) into 25 ml of water containing 1.5 percent PVA by weight while being continuously magnetically stirred at 1000 rpm. The created emulsion was generated and then diluted in 50 ml of PVA solution (0.2 percent w/v in water) to limit coalescence. The resultant mixture was then continuously stirred (at 500 rpm) for 6 hours at room temperature to promote the evaporation of the solvent and the formation of nanoparticles. The created nanosuspension was lyophilized after that, and it was cooled to -180 degrees C.[18]

Evaluation of Antioxidant potential of *Celosia argentea*

The antioxidant activity of the methanolic extract of *Celosia argentea* was evaluated by

DPPH (1,1-diphenyl-2-picrylhydrazyl) method

A technique that is widely used to assess the ability of plant-based medicines to function as antioxidants is DPPH method. The main component of the DPPH test method is the reduction of coloured DPPH free radicals in methanolic solution by free radical scavengers. The process involves measuring the DPPH absorbance at 516 nm, which is proportional to the concentration of free radical scavengers added to the reagent solution. The activity is given as effective concentration

By using 1,1-diphenyl-2-picrylhydrazyl (DPPH) to assess the free radical scavenging capacity of plant extracts from *Celosia argentea*. Method involves preparation of, 0.1 mM solution of DPPH in methanol solution. Three ml of variously prepared extracts in methanol were combined with one ml of the prepared solution at aliquot concentrations (0, 2, 4, 6, 8, and 10 g/ml). Only extracts used are solubilized in methanol and their various concentrations were prepared by dilution method. After giving the combination a good shake, the mixture was let to stand at room temperature for 30 minutes. Additional absorbance was measured using a spectrophotometer at 517 nm (UV-VIS Shimadzu). Ascorbic acid was the reference standard substance utilised in the experiment. Using a log dosage inhibition curve, the sample's IC_{50} value−the concentration of the sample needed to inhibit 50% of the DPPH free radical−was determined. The reaction mixture's lower absorbance was determined using the equation below. Effect on scavenging DPPH (%) or Inhibition (%). [5,6]

%Inhibition =(A0-A1)/A0*100

Where, A0=absorbance of the control

A1=absorbance in the presence of the sample of extract and standard

Hydrogen peroxide method

Various concentrations of plant extract (4 ml) were made in distilled water, and they were combined with 0.6 ml of 40 mM H_2O_2 prepared in phosphate buffer (0.1 M pH 7.4). The mixture was then incubated for 10 mins. The aforementioned solution's absorbance should be measured at 230 nm. A positive control was ascorbic acid. By comparing the absorbance values of the control and test samples using the provided formulae, the percentage of inhibition was determined.[7]

$S\%=[(A_{control}-A_{sample})/A_{control}]*100$

Where, $A_{control}$= absorbance of the blank control (containing all reagents except the extract solution) and A_{sample}=absorbance of the test sample

RESULTS

Evaluation of Invitro antioxidant potential

Evaluation of antioxidant activity of *Celosia argentea* by DPPH scavenging activity:

Evaluation of antioxidant activity done by using DPPH scavenging method was found at concentration of 7.31µg/ml and 7.75µg/ml, the scavenging effect of methanolic extract and ascorbic acid reached 54.7% and 51.61%. The extract was chosen for further study. Based on the DPPH radical scavenging method using methanolic plant extracts of *Celosia argentea's* dose-dependent response curve are as follows.

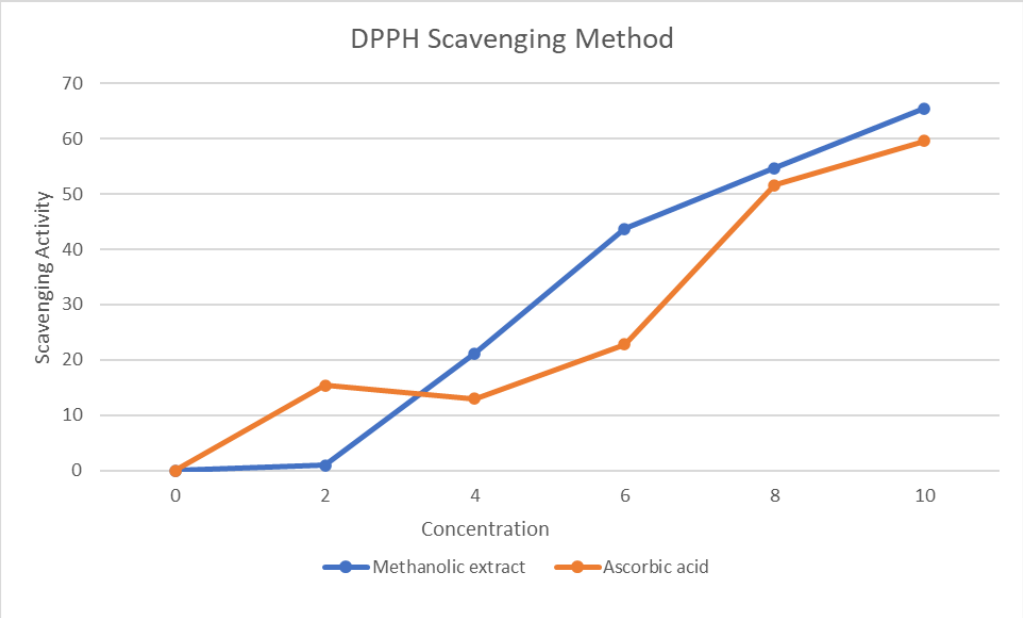


Figure 2: Antioxidant activity of *C. argentea* by DPPH scavenging method

In terms of IC_{50} value (µg/ml), the scavenging effects of methanolic extract of *Celosia argentea* and ascorbic acid were seen as 7.31 µg/ml (54.7% inhibition) and 7.75 µg/ml (51.61% inhibition).

Evaluation of antioxidant activity of *Celosia argentea* by Hydrogen peroxide scavenging assay

Evaluation of antioxidant activity done by using H_2O_2 scavenging method was found at concentration of 29.26µg/mol and 29.18µg/ml, the scavenging effect of methanolic extract and ascorbic acid reached 51.5%, and 51.4%. The extract was chosen for further study.

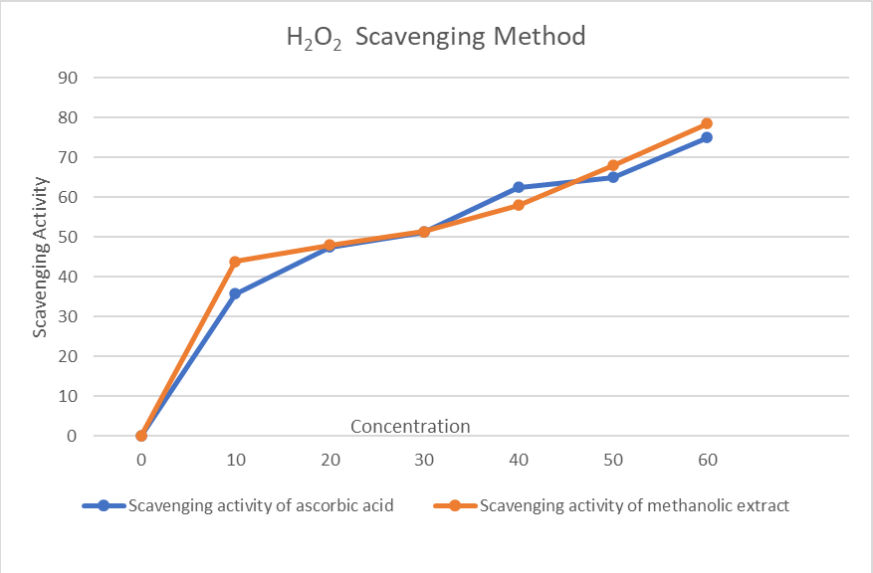


Figure 3: Antioxidant activity of *C. argentea* by Hydrogen peroxide scavenging method

In terms of IC_{50} value (µg/ml), the scavenging effects of methanolic extract of *Celosia argentea* and ascorbic acid were seen as 29.26 µg/ml (51.5 % inhibition) and 29.18 µg/ml (51.4 % inhibition).

DISCUSSION

Over the past few decades, there has been an increase in the screening of food and medicine plants for antioxidant capabilities in the hopes of discovering a cure for various modern ailments and a way to delay the signs of ageing. Type II diabetes, neurological conditions, and various cancers are among the ailments connected to the excessive oxidation of cellular substrates i.e. oxidative stress. In addition, there is a big market for natural antioxidants in the food industry and other allied industries to replace synthetic preservatives.

Antioxidants are extremely significant compounds that have the power to protect the body from harm brought on by oxidative stress put on by free radicals. In an effort to discover new bioactive chemicals from natural sources, the antioxidant activity of *Celosia argentea* hydroalcoholic extracts was examined. The extract of *Celosia argentea* showed good antioxidant potential as compared to standard drug ascorbic acid.

Preliminary phytochemical investigation revealed the presence of polyphenolic components. Due to their hydroxyl groups' ability to donate hydrogen, plant polyphenols have reducing and antioxidant properties. We could therefore draw the conclusion that these polyphenols are to credit for the antioxidant activity found in this investigation.

Antioxidants are hypothesised to affect DPPH because of their capacity to donate hydrogen. It's necessary to engage in radical scavenging activities to stop free radicals from acting a harmful role in a variety of disorders, including cancer, diabetes etc. The considerable reduction of the stable DPPH radical to the yellow diphenyl picryl hydrazine complex by the methanolic extract of *Celosia argentea* suggests that it may have some anti-free radical properties. Increasing concentration of the methanolic extract of *Celosia argentea* revealed the better percentage of inhibition. In terms of IC_{50} value (µg/ml), the scavenging effects of methanolic extract of *Celosia argentea* and ascorbic acid were seen as 7.31 µg/ml (54.7% inhibition) and 7.75µg/ml (51.61% inhibition). The findings of this investigation indicate that the *Celosia argentea* extract demonstrated radical scavenging activity through their ability to donate hydrogen or transfer electrons. Presence of polyphenolic components and antioxidant activity that scavenges free radicals are closely connected.

The hydrogen peroxide scavenging of the *Celosia argentea* extract may be hypothesized to its polyphenolic components like flavonoids and tannins etc. Phytoconstituents can donate electrons to hydrogen peroxide, leads to neutralization of water. The findings of this investigation indicate that the *Celosia argentea* extract demonstrated radical scavenging activity through their ability to donate hydrogen or transfer electrons. In terms of IC_{50} value (µg/ml), the scavenging effects of methanolic extract of *Celosia argentea* and ascorbic acid were seen as 29.26 µg/ml (51.5 % inhibition) and 29.18 µg/ml (51.4 % inhibition). The extract was capable of scavenging hydrogen peroxide in a concentration-dependent manner. Presence of polyphenolic components and antioxidant activity that scavenges free radicals are closely connected.

Further work is going on related to evaluation of antioxidant activity of nanosuspensions of *Celosia argentea* flower extracts.

CONCLUSION

The results of this study provide credence to the idea that plant hydroalcoholic extracts are promising sources of prospective antioxidants and may be effective as preventatives for a number of ailments. To classify them as biological antioxidants, additional thorough research on the isolation, identification, and *in vivo* evaluation of phytoconstituents of this plant extract are required.

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Conflict of Interest

None declared.

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