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Studies on phytochemical characteristics of *in vitro* callus development of *Celosia argentea* L.

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Abstract:

Plants contain many chemicals that are used to derive drugs for curing diseases**.** These chemicals are present in almost all parts of plant like bark, leaves, flowers,roots, fruits and seed etc. This property of plant lead to indiscriminate use of plant leading to their depletion. In order to avoid depletion and to conserve the plants *in-vitro* callus development followed by plant regeneration is one the most useful techniques. *in-vitro* callus development can be achieved using Murashig and Skoog medium. *Celosia argentea* L. plants bear simple and spirally arranged leaves, often pinkish or white flowers while fruits are globular and seeds are black in colour. It is used as ornamental plant and it has medicinal value as its different parts are used in Ayurvedic medicines, It contains many phytochemicals that are secondary metabolites having medicinal uses. Callus of *Celosia argentea*was developed on MS medium supplemented with Auxin 0.5 mg/ml and cytokinin o.5 mg/ml **.***Celosia argentea*was extracted by usingvarious solvents viz., methanol,chloroform,and ethanol.Phytochemicalanalysis of these extracts was carried out. This study reports that Celosia argentea callus was found to contain starch, cellulose, flavonoids,saponin,tannin,phenol,terpenoids steroids and alkaloids.

Keywords: *Celosia argentea*, in vitro, Phytochemical, Cellulose, Flavonoids, Saponin, Callus, Murashig and Skoog medium

Introduction:

Celosia argentea (Amaranthaceous), also known ascock's comb, has wide diversity and is widespreadaround tropical Africa, tropical and subtropicalAsia and America. It is a traditional vegetable inWest and Central Africa (Grubben and Denton2004).*Celosia argentea* is used traditionally for the treatment ofjaundice, gonorrhea, wounds and fever. The leaves are usedfor the treatment of inflammations, fever an ditching. TheSeeds are bitter, useful in blood diseases, mouth sores. Theyare efficacious remedy in diarrhea (Kirtikar, 1935). Based onethno botanical practice theplant was investigated for anti-inflammatory (Patil *et al*., 2003), anti–pyretic (Bhujbal *et al*., 2006) anti diabetic,(Thangarasu *et al.,* 2002), anti bacterialand diuretic properties (Patel et al., 1993).Plant is also foundto be useful in cancer. Even though plant is considered asweed but it is seasonal. Looking towards its medicinalproperties it was decided to undertake in vitro studies in*Celosia argentia*.Medicinal plants are very important due to thepresence of secondary metabolites like alkaloids,glycosides, coumarins, flavanoids, steroids, etc. andfind use in a number of pharmaceutical compounds.However, sustained supply of the source materialoften become difficult due to the factors likeenvironmental changes, cultural practices, diversegeographical distribution, labour cost, selection of thesuperior plant stock and over exploitation bypharmaceutical industry. Various technologies havebeen adopted for enhancing bioactive molecules (Skirvin *et al.,* 1990). The compounds found in *C. argentea* contribute topharmacological properties such as antibacterial,antimitotic, antineoplastic, diuretic,hypoglycemic, hepatoprotective,immunomodulatory, cytoprotective, and woundhealing (Hayakawa *et al.,* 1998; Wiart 2000;Vetrichelvan *et al.,* 2002; Gnanamani *et al.,* 2003).Additionally, this plant could produce some usefulchemicals such as antiviral proteins, betalains, andanthocyanin2–4 that can be applied in many beneficialways(Balasubrahmanyam et *al.,* 2000).

MATERIALS AND METHOD:

Collection of Explant: Fresh plant material of *Celosia argentea*L.was collected from the S.M.D Mohekar college campus. Kalamb,Dist.Osmanabad. After collection of plant material firstly wash with clean water to remove sand and dust and surface sterilized by surface sterilizing agent. Like

mercuric chloride kept for some time to dry.The dried plant material crushes in mortar and pestle to get a very fine powder.

Surface sterilization of explants: The leaf which carries the microorganisms on surface so surface sterilization is mandatory. The leaves firstly rinsed with liquid detergent teepol followed by repeated washings with tap water. Rinse the leaves with 70% ethanol and repeatedly wash with distilled water and finally the explants with 0.1% mercuric chloride (surface sterilizing agent) and repeatedly washing with sterile distilled water and finally transfer to sterile Petri plate.

Media preparation and sterilization: Prior to surface sterilization of explants the media preparation and sterilization were done. The MS medium (w/cacl2, viz., sucrose, w/o agar) is used for the culture. The agar concentration was 1.8-2% and the media sterilization which was by autoclaving at 121 psi. The pH of media 5.8 adjusting by ph meter .after autoclaving the addition of growth hormone such as 2,4 D(0.5mg/ml) and cytokines(0.5mg/ml). And then pour into the culture bottle containing approximately 25-30 ml media and then allowing standing for solidification and contamination.

Transfer of explants: After the media preparation and sterilization the sterile explants which is transfer to culture bottle in sterile transfer area that is laminar air flow cabinet.

Culture condition: For callus development cultures were kept at 25+2 0c.in light /dark condition with 16/8 hours light /dark photoperiod.

Data recording: The time for callus induction of *Celosia argentea***L.**was recorded after four week.

Phytochemical Test: The test were do find the presence of active chemical such as alkaloids, glycosides, terpenoids, steroid, flavonoids, saponin and tannin by the following procedure.

Detection of Alkaloid:**Mayer's test:** To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of test tube. A white or creamy precipitate indicates the test as positive.

Wagner's Test**:** To few ml of filtrate, few drops of Wagner's reagent as added by the side of test tube. A reddish- brown precipitate confirms the test as positive.

Hager's Reagent: To 2ml of filtrate add few drops of Hager's reagent .A yellow precipitate indicate the presence of alkaloids.

Detection of Carbohydrates:The extract (100g) is dissolved in 5ml water and filtered. The filtered is subjected to the following test.

Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2min. A characteristic coloured precipitate indicates the presence of sugar.

Molisch Test: To one ml of filtrate 2 drops Molisch reagent was added in a test tube and 2 ml of concentrated H2so4 added carefully along the side of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrate.

Fehling test: To 1 ml of filtrate add 4 ml of Fehling solution in test tube .Then heat for 10 minute in a water bath .formation of red precipitate indicate the presence of reducing sugar.

Protein and Amino Acid:__Biuret Test:__ To 2ml of filtrate add 2ml of 10% NaOH solution .Then heat for 10 minutes. Add a drop of 7% cuso4 solution. A purplish violet colour indicates the presence of protein.

Ninhydrin Test: To 2ml of filtrate add 2ml of Millions reagent. Then heat in waterbath for 5 minute .Then after cooling add few drops of sodium nitrate solution .A white precipitate turn red upon heating that indicates the presence of protein or amino acid.

Test for Flavonoids:To 1ml of extract add 1ml neutral Fecl3.A brown colour indicates the presence of flavonoids.

Test for Tannin: To 1ml of extract add few ml of 5% of Fecl3.A dark blue colour indicates the presence of Tannin.

Test for Phenol: To 1ml of extract add lead acetate solution .A precipitate indicate the presence of phenol.

Test for steroids:**Liebermann-Burchard test**: The extract dissolved in 2ml of chloroform to which 10 drops of acetic acid and 5 drops of concentrated H2so4 were added and mixed .The change of red colour through blue to green.

Test for Terpenoids:**Salkowski Test**: 5ml of each extract was mixed in 2ml of chloroform and 3 ml concentrated H2so4 were added to form a layer .A reddish brown coloration of the interface was formed.

Test for Starch: To 1ml of extract add few drops of iodine solution. Any characteristic colour change indicates the presence of starch.

Test for cellulose*:* To 1ml extract add few drops iodine solution .to this again add the few drop of H2so4.Dark brown or red colour indicates the presence of cellulose.

Detection of Gum and Mucilage*:* About 10ml of aqueous extract is added to 25 ml absolute alcohol with constant stirring .The precipitate is dried in air .Then precipitate is examined for its swelling properties and for the presence of carbohydrates

Results and Discussion:

Table 1: callus induction:

Sr. No	Name of Explants	Concentration of Hormone 2,4-D+Kinetin	Days	Texture	Coloration	Size of callus
1	Stem	500µl+500 µl	4 week	Friable	Friable	Large
2	leaf	500µl+500 µl	4 week	Friable	Friable	Medium

Fig: Callus formation

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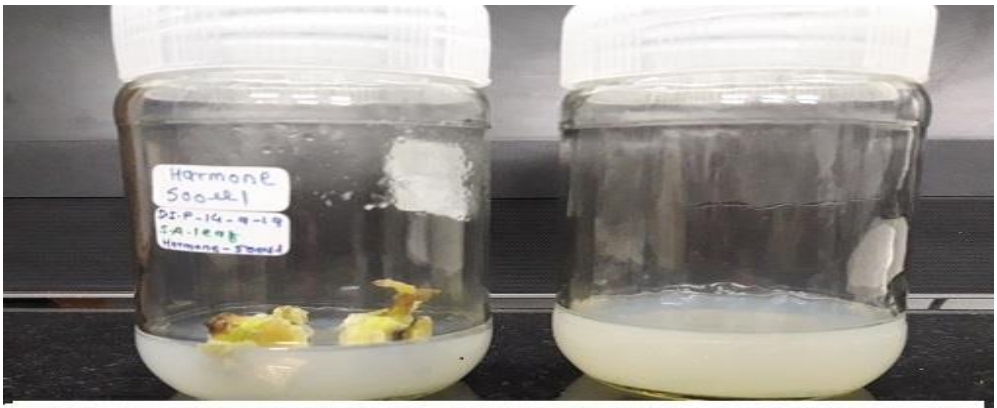

Figure.1.Four week grown callus of Celosia argentea (leaf) on MS media in lfet side and control in right side bottle.

Table 2.Phytochemical Screening

Sr. No	Test	Aqueous Extract	Chloroform Extract	Methanol Extract	Ethanol Extract
1	Wagner's	+	+	+	+
2	Hager's	_	+	_	_
3	Mayer's	_	_	_	_
4	Terpenoid	+	+	+	+
5	Flavonoid	_	+	_	_
6	Steroid	+	+	_	_
7	Phenol	_	+	+	+
8	Starch	_	_	+	_
9	Cellulose	_	+	+	+
10	Tannin	_	_	_	_
11	Carbohydrate	_	_	_	_
12	Biuret	_	_	_	_
13	Quinone	+	+	+	+

Rao *et al*., (2005) are view that all explants can be induced to form calli but Sharma *et al*., (2013) found shoot tip explants to be more amenable to callusing than cotyledonary leaf and hypocotyls. Moreover, they found that cytokinins, in small concentration, play a synergistic role with auxins and induce the callus.Surface sterilization of explants is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue (Conger, 1987). This technique found to be lucrative and quicker in making multiple copies of a plant species. Callus culture is the prerequisite for indirect organogenesis. The technique requires callus production with optimum quantity for transformation and regeneration. Cell suspension culture also utilized for large scale production of secondary metabolites. The callus was induced on MS medium with different concentrations and combination of PGRs using leaf and stems explants and could be differentiated by biomass, color and structure. Suitable callus induction was on MS medium supplemented with 0.5 mg/L NAA and 1.0 mg/L BA for both green compact calluses.

Conclusion: The*Celosia argentea* plant has potential indicating the utility of different plant parts in various disorders but*in vitro* technique denotes the capability to makeprovision of fresh plant material. The propagation of plants parts via *in vitro* method makes this technique more significant.

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