

## NUTRITIONAL EVALUATION AND *IN VITRO* ANTICANCER ACTIVITY ANALYSIS OF *COCCINIA GRANDIS* (L.) VOIGT

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### Abstract

Plants are nature's chemical factories, producing a wide range of compounds that help them grow, protect themselves and interact with their environment. Many of these compounds have valuable medicinal properties and have been used in traditional medicine for generations. Phytochemicals, both primary and secondary plant metabolites, play a key role in the healing properties associated with many plants. Crude methanolic fruit extract of *Coccinia grandis* shows the presence of reducing sugar, alkaloids, flavonoids, steroids, tannins, terpenoids, glycosides and saponins. Quantitative phytochemical analysis showed higher amounts of reducing sugar, total carbohydrates, total proteins, starch, pigments and amino acids. The antinutritional factors such as total phenols, phytic acid and tannic acid were analyzed and found to be present in minimal amounts. Antioxidant activity of the fruit extract has been evaluated for different non-enzymatic and enzymatic antioxidants. Different non-enzymatic antioxidants like proline, lycopene, carotenoids, polyphenols and tocopherol (Vitamin-E) and enzymatic antioxidants like superoxide dismutases (SOD), catalase (CAT), glutathione reductases (GR), peroxidases (POD), amylases, polyphenol oxidases (PPO) and lipoperoxidase (LP<sub>x</sub>) were present in higher amounts, satisfying their use as a potential source of antioxidants. Anticancer analysis of crude methanolic fruit extract in EAC (Ehrlich's Ascites carcinoma) and DLA (Dalton's Lymphoma Ascites) showed higher cytotoxicity in EAC compared to DLA against the standard drug revealing promising anticancer effects of the fruit extract. *In vitro* conservation of different explants on MS medium supplemented with 2 mg/l BAP showed high survival capacity of nodal explant with axillary bud. The present study evaluated various nutritional, medicinal and regenerative aspects of the plant *Coccinia grandis*, exhibiting diverse potentialities of the plant and providing supporting information for its use as an ethnomedicinal plant too.

**Keywords :** *Coccinia grandis*, Cucurbitaceae, DLA, EAC, *In vitro* conservation

### Introduction

Plants play a crucial role as primary producers, harnessing solar energy to synthesize carbohydrates, vitamins, proteins, essential fatty acids and other vital nutrients that are used for human food production. Around 20 major food crops, including cereals, vegetables (such as legumes), fruits and nuts, form the foundation of global diets. In terms of human protein-energy malnutrition (PEM), cereals and food legumes, including oilseed legumes are particularly important (Young and Pellett, 1994).

Plants are a rich source of medicinal compounds, serving as the foundation for modern medicines, nutraceuticals, nutritional supplements and pharmaceuticals. Despite advances in modern medicine, traditional remedies continue to play a vital role in healthcare, owing to the identification and

utilization of medicinal herbs documented in ancient pharmacopeias. The medicinal properties of plants can be attributed to their phytochemicals, which have tangible physiological effects on humans. These plant-derived substances are used to treat various illnesses and promote overall health. The efficacy of medicinal plants in treating numerous ailments has sparked ongoing research into their potential as sources of novel antibacterial agents. Furthermore, spice plants have been employed for centuries in various parts of the world for antiparasitic, anthelmintic, analgesic expectorant, sedative and anti-diabetic compounds (Chowdhury, 2022).

Phytochemicals are the naturally occurring non-nutritive chemical compounds found in fruits, vegetables, herbs, aromatic plants, as well as various plant parts such as roots, leaves,

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flowers and stems that play a crucial role in plant defense and human health, exhibiting antioxidant, anti-inflammatory and potentially anticancer properties, contributing to disease prevention while also contributing to their colour, aroma and flavour (Hafeez, 2024). Common classes of bioactive phytochemicals include alkaloids, flavonoids, tannins, phenolics, saponins, steroids, glycosides and terpenes (Bitwell *et al.*, 2023). Phenols and flavonoids are known for their antioxidant, anti-allergic and antimicrobial properties (Chowdhury, 2022).

In many developing countries, rural communities rely heavily on medicinal plants, which not only provide essential nutrients but also exhibit antimicrobial and antioxidant properties (Beyene and Deribe, 2016). There has been a growing trend towards incorporating natural antioxidants into food products, driven by concerns over potential health risks associated with the consumption of synthetic antioxidants. Fortunately, various plant-based materials have been identified as rich sources of natural antioxidants, including aromatic herbs, spices, seeds, fruits and vegetables (Lourenco *et al.*, 2019).

Members of the family Cucurbitaceae possess various medicinal and pharmacological properties. *Cucurbita pepo* exhibits a range of biological activities, including anti-androgenic, immunomodulatory, antiviral, antibacterial, antifungal, cardiovascular, anti-inflammatory and hepatoprotective effects. *Cucurbita maxima* has been shown to protect against tumor cell-induced liver damage. The seeds of this plant are traditionally used to treat liver and digestive disorders (Rajasree *et al.*, 2016). Plant extracts of *Coccinia grandis* (Ivy gourd) possess a broad spectrum of therapeutic properties, including analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, antitussive and mutagenic effects, making it a valuable plant for various medicinal applications (Pekamwar, 2013).

## Materials and Methods

### Collection of Sample

*Coccinia grandis* was collected fresh from Vembayam, Thiruvananthapuram district of Kerala. The collected plant specimen was processed into a herbarium sheet and deposited in the Herbarium Repository of the Botanical Survey of India (BSI), Southern Regional Centre (SRC), Coimbatore–3, for authentication (Plant Authentication No. 430). For sample preparation, fresh leaves were separated, shade-dried and finely powdered using a mechanical blender. The resulting powder was then stored in airtight containers for subsequent analyses.

### Preparation of plant extract

The dried plant materials were extracted with methanol for 8 hours using Soxhlet apparatus and extracts were obtained as green, black solids respectively. After which, the residues were transferred to a pre-weighted sample container for storage.

### Preliminary phytochemical analysis

The phytochemicals like reducing sugar, alkaloids, flavonoids, steroids, tannins, phlobatannins, iridoids, terpenoids, glycosides, anthraquinones, coumarins and saponins were tested (Harborne, 1977).

### Biochemical analysis

#### Nutritional Analysis

**Reducing Sugar:** Reducing sugar present in the sample was estimated by Dinitrosalicylic acid method (Miller, 1959).

**Total Carbohydrates:** The amount of total carbohydrates present in the sample was estimated by Anthrone method (Hedge and Hofreiter, 1962).

**Total protein:** The amount of total protein present in the sample was estimated by Lowry's method (Lowry *et al.*, 1951).

**Starch:** The amount of starch present in the sample was estimated by Anthrone reagent (Thayumanavan and Sadasivam, 1984).

**Chlorophyll:** The chlorophyll content in the sample was estimated by Arnon's method (Witham *et al.*, 1971).

**Carotene:** The amount of carotene was determined by the method of Lichtenthaler and Wellburn method (1983).

**Amino acids:** The amount of amino acids present in the sample was estimated by Moore and Stein (1948).

#### Antinutritional analysis

**Total phenols:** Total phenol estimation was carried out by Folin-Ciocalteu method (Mayer *et al.*, 1995).

**Phytic acid:** The phytic acid content in the sample was estimated by the method of Wheeler and Ferrel (1971) method.

**Tannic acids/Tannins:** The amount of tannins was determined by the method of Schanderi (1970).

#### Evaluation of Medicinal Properties

##### Non-enzymatic Antioxidants

**Proline:** Proline present in the sample was estimated by the method of Bates *et al.* (1973).

**Lycopene:** The level of lycopene was estimated by the method of Zakaria *et al.* (1979).

**Total Polyphenols (TP):** The total polyphenolic content was determined by the Folin Ciocalteu assay (Eom *et al.*, 2008).

**Carotenoids:** Carotenoids in the sample was estimated by the method of Jagessar (2017).

**$\alpha$ -Tocopherol (Vitamin-E):** The amount of Vitamin-E was determined following the method of Rosenberg (1992).

##### Enzymatic Antioxidants

**Superoxide Dismutase (SOD):** Superoxide dismutase (SOD: EC 1.15.1.1) was assayed by the Nitro Blue Tetrazolium (NBT) method as described by Gong *et al.* (2005).

**Catalase (CAT):** Catalase was assayed by the method of Cakmak *et al.* (1993).

**Glutathione Reductase (GR):** Glutathione Reductase (GR: EC 1.6.4.2) activity was determined by the method of Foyer and

Halliwell (1976).

**Peroxidase (POD):** Determination of peroxidase (POD: EC 1.11.1.7) activity was done as per the method of Putter (1974).

**Amylase:** The amount of Amylase was determined following the method of Miller (1959).

**Polyphenol Oxidase (PPO):** The determination of polyphenol oxidase (PPO: EC 1.14.18.1) was done by the method of Esterbauer *et al.* (1991).

**Lipid Peroxidase (LPx):** Lipid peroxidase activity was determined by the method of Zhang and Kirkham (1996).

##### Antibacterial Activity in *Coccinia grandis*

The extract was tested to detect antibacterial activity against standard strain of pathogenic and industrially important *Escherichia coli* bacterium which was procured from The Microbial Type Culture Collection (MTCC) Chandigarh.

##### Mueller Hinton agar (MHA)

The MHA was used for antibacterial testing. Suspended 38 grams of ready-made media in 1000 ml distilled water and heated gently to 100°C to dissolve the medium completely. It was dispensed to suitable bottles, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and mixed well before pouring. The medium was then poured into sterilized flat-bottomed petriplates in a laminar flow hood. The medium was solidified and then stored at 4°C for later use.

##### Nutrient broth (NB)

The nutrient broth was used for inoculating the bacterial culture. Suspend 25 grams in 1000 ml of distilled water and heat if necessary, to dissolve the medium completely. Distributed in tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

##### Preparation of samples

25  $\mu$ g, 50  $\mu$ g, 100  $\mu$ g and 200  $\mu$ g per disc were used for the screening of the antibacterial activity. Ciprofloxacin (5  $\mu$ g/disc) was used as

the standard drug (positive control).

#### **Agar Diffusion method:**

The Disc Diffusion Method was conducted for antibacterial testing (Bauer *et al.*, 1966). Sterile MHA plates were used for the test. Freshly sub-cultured bacterial strains were suspended in one ml Nutrient broth and incubated at 37°C for 2 hours to obtain log phase cultures, opacity was checked with 0.5 McFarland turbidity standards (approximately 1 to 2 x 10<sup>8</sup> colony forming units per ml). 100 µl of the pure cultures of test strains were swabbed uniformly using a sterile swab on the surface of the MHA plate to obtain an even inoculum. The plates were allowed to dry for 5 minutes. Sterile filter paper discs of 6 mm diameter impregnated with different concentrations of sample were used for conducting testing. The antibacterial activity was observed after incubating the plates for 24 hours at 37°C and the zone of inhibition surrounding the disc was noted in milli meters (mm).

#### **Antifungal Activity in *Coccinia grandis***

The extract was tested to detect antifungal activity against the standard strain of pathogenic and industrially important fungus, *Aspergillus niger* which was procured from the Microbial Type Culture Collection (MTCC) Chandigarh.

#### **Sabouraud Dextrose Agar (SDA)**

The SDA was used for antibacterial testing. Suspended 65 grams of ready-made media in 1000 ml distilled water and heated gently to 100°C to dissolve the medium completely. It was dispensed to suitable bottles, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes and mixed well before pouring. The medium was then poured into sterilized flat-bottomed petriplates in a laminar flow hood. The medium was solidified and then stored at 4°C for later use.

#### **Nutrient broth (NB)**

The nutrient broth was used for inoculating the fungal culture. Suspend 25 grams in 1000 ml distilled water and heat if necessary, to dissolve the medium completely. Distributed in tubes and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

#### **Preparation of samples**

25 µl, 50 µl, 100 µl and 200 µl per disc were used for the screening of the antifungal activity. Fluconazole (10µg/disc) was used as the standard drug (positive control).

#### **Agar Diffusion method**

Disc Diffusion Method was conducted for antifungal testing ((Bauer *et al.*, 1966). Sterile SDA plates were used for the test. Freshly sub-cultured fungal strains were suspended in 1 ml Nutrient broth and incubated at 28°C for 12 hours to obtain log-phase cultures, opacity was checked with 0.5 McFarland turbidity standards (approximately 1 to 2 x 10<sup>8</sup> colony forming units per ml). 100 µl of the pure cultures of test strains were swabbed uniformly using a sterile swab on the surface of the SDA plate to obtain an even inoculum. The plates were allowed to dry for 5 minutes. Sterile filter paper discs of 10 mm diameter impregnated with different concentrations of sample were used for conducting the study. The antifungal activity was observed after incubating the plates for three days at 28°C and the zone of inhibition surrounding the disc was noted in milli meters (mm).

#### **Evaluation of Pharmacological Property**

##### ***In vitro* Anticancer Activity in Crude Methanol Extract**

Anticancer effect of crude methanol extract of *Coccinia grandis* was evaluated by using DLA and EAC cell lines. The crude methanol extract of *Coccinia grandis* at high concentration damaged the cells and make pores on the membrane through which Trypan blue enters. The damaged cells are stained with Trypan blue stain and can be distinguished from viable cells. Since live cells are excluded from staining, this method is also known as dye exclusion method (Pradeesh and Swapna, 2018).

##### **Dalton's lymphoma Ascites Cells (DLA) and Ehrlich Ascites Carcinoma (EAC)**

Varying concentrations (100, 500, 1000 µg/ml) of crude methanol extract of *Coccinia grandis* were prepared. The cancer cells were aspirated from peritoneal cavity of cancer bearing mice and were washed thrice with normal saline.

The cell suspensions ( $1 \times 10^6$  DLA/EAC cells in 0.1 ml) were added to tube containing various concentration of test extract (100, 500, 1000  $\mu\text{g/ml}$ ) and volume was made up to 1 ml using phosphate buffer saline (PBS). Control tube contained only cell suspension. The mixture was incubated for 3 hour at  $37^\circ\text{C}$  and was added with two drops of Trypan blue dye. Dead cells take up the blue colour of Trypan blue while live cells do not. Further percentages of dead cells were evaluated by Trypan Blue Exclusion method. The numbers of stained and unstained cells were counted separately (Pradeesh and Swapna, 2018).

Percentage of Dead cells =  $(\text{Number of Dead cells} / \text{Number of viable cells} + \text{Number of Dead cell}) \times 100$ .

### ***In vitro* Conservation**

*In vitro* conservation of the plant was carried out by using nodal explants like leaf, stem, petiole, node with axillary bud and internodal segments. The explants were pre-sterilized in tap water for 15 minutes, trimmed and washed with tap water and 3 drops of labolene for 10 minutes. Surface sterilization was done using 1% sodium hypochloride solution and 0.1% mercuric chloride solution for 5 minutes followed by washing with distilled water. MS (Murashige and Skoog) medium supplemented with 2 mg/l BAP adjusted to pH 5.6 was used for inoculation. The culture was maintained less than 16 hours photoperiod at a temperature of  $26^\circ\text{C}$ .

Murashige and Skoog (MS) Medium is a commonly utilized nutrient-rich medium in plant tissue culture, designed to promote the growth and development of plant cells, tissues and organs. It comprises essential macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, along with micronutrients like boron, copper, iron, manganese, molybdenum and zinc. Additionally, it includes vitamins (thiamine, nicotinic acid and pyridoxine), amino acids (such as glycine and adenine), a carbon source (typically sucrose) and agar as a gelling agent. MS medium is widely applied in various plant biotechnology practices, including tissue culture, micropropagation, seed germination,

shoot and root development, suspension cultures and genetic transformation techniques (Pradeesh and Swapna, 2018).

## **Results and Dcusson**

### **Qualitative Analysis**

#### **Preliminary Phytochemical Screening**

The preliminary phytochemical analysis of *Coccinia grandis* showed the presence of reducing sugar, alkaloids, flavonoids, steroids, tannins, terpenoids, glycosides and saponins. But the presence of phlobatannins, iridoids, anthraquinones and coumarins were not detected (Table 1).

Reducing sugars are carbohydrates that contain a free aldehyde or ketone group, allowing them to donate electrons and act as reducing agents. This characteristic is especially important in food chemistry, as reducing sugars participate in Maillard reactions with amino acids, contributing to the browning and flavor development in cooked foods. Examples include glucose, fructose and maltose (Nursten, 2005).

Alkaloids are a diverse group of naturally occurring organic compounds that mostly contain nitrogen atoms and are primarily derived from plant sources. These secondary metabolites are known for their wide range of pharmacological effects, including analgesic, antimalarial, antibacterial and anticancer activities. Common examples include morphine, quinine and caffeine. Alkaloids play a crucial role in plant defence and have been extensively studied for their therapeutic potential in modern medicine (Roberts, 2013).

Flavonoids are a large class of polyphenolic compounds widely distributed in fruits, vegetables and other plant-based foods. They function as secondary metabolites with significant antioxidant, anti-inflammatory and cardioprotective properties. Flavonoids play a key role in protecting plants from ultraviolet radiation and pathogens, while in humans; they contribute to disease prevention and overall health promotion. Major types include flavonols, flavones, flavanones and anthocyanins (Panche *et al.*, 2016).

Steroids are a class of lipophilic organic compounds characterized by a core structure of four fused carbon rings. In plants, they function as secondary metabolites known as phytosterols, contributing to membrane stability and defence against pathogens. In humans and animals, steroids play vital roles as hormones, such as cortisol, estrogen and testosterone, regulating numerous physiological processes including metabolism, inflammation and reproductive functions. Plant-derived steroids also exhibit therapeutic potential, including anti-inflammatory and anticancer properties (Piironen *et al.*, 2000).

Tannins are a group of water-soluble polyphenolic compounds found abundantly in various parts of plants, including bark, leaves and fruits. They are known for their ability to bind and precipitate proteins, which contributes to their astringent taste. As secondary metabolites, tannins play an important role in plant defense against herbivores and pathogens. In human health, they exhibit antioxidant, antimicrobial and anti-inflammatory properties and are studied for their potential in treating various diseases (Chung *et al.*, 1998).

Terpenoids, also known as isoprenoids, are a large and diverse class of naturally occurring organic compounds derived from five-carbon isoprene units. They play vital roles in plants by contributing to growth regulation, defense mechanisms and attracting pollinators through their aromatic properties. Terpenoids have extensive applications in medicine, agriculture and industry due to their antimicrobial, anti-inflammatory and anticancer activities (Gershenzon and Dudareva, 2007).

Glycosides are compounds in which a sugar molecule is bonded to a non-sugar moiety, known as the aglycone. These secondary metabolites are widespread in plants and serve various ecological functions, including defense against herbivores and pathogens. In medicine, glycosides have significant therapeutic uses, such as cardiac glycosides that help regulate heart function and others with anti-inflammatory and antimicrobial effects (Hostettmann and Marston, 1995).

Saponins are naturally occurring glycosides found in many plant species, characterized by their soap-like foaming properties when mixed with water. These compounds play a role in plant defense against pathogens and herbivores and have been studied for their diverse pharmacological effects, including cholesterol-lowering, immune-boosting and anticancer activities. Saponins are phyto-protectants in function that take part in plant defense mechanism and are antimicrobial in nature. They can be of triterpenoid, steroid or alkaloid glycoside type based on the sapogenin (aglycone) present in the chemical structure (Sparg *et al.*, 2004).

Phlobatannins are a specific type of condensed tannins, known for their ability to form red precipitates when heated with acids. These compounds are primarily found in the bark and heartwood of certain plants and are involved in plant defense due to their antimicrobial and antifungal activities. In addition to their protective roles in plants, phlobatannins have attracted interest for their potential antioxidant and therapeutic properties in human health (Kumar and Singh, 1984).

Iridoids are a class of monoterpenoid compounds commonly found in medicinal plants, often as glycosides. They play a significant role in plant defense due to their bitter taste and antimicrobial properties. In pharmacology, iridoids have been recognized for their diverse bioactivities, including anti-inflammatory, hepatoprotective, neuroprotective and anticancer effects. These compounds are particularly abundant in plant families such as Rubiaceae, Scrophulariaceae and Lamiaceae (Jensen, 1991).

Anthraquinones are aromatic organic compounds commonly found in various plants, especially in roots, bark and leaves. These secondary metabolites are known for their laxative properties and have been traditionally used in herbal medicine. Additionally, anthraquinones exhibit antimicrobial, anti-inflammatory and anticancer activities, making them valuable in pharmaceutical research and applications (Brien and Fraga, 1991).

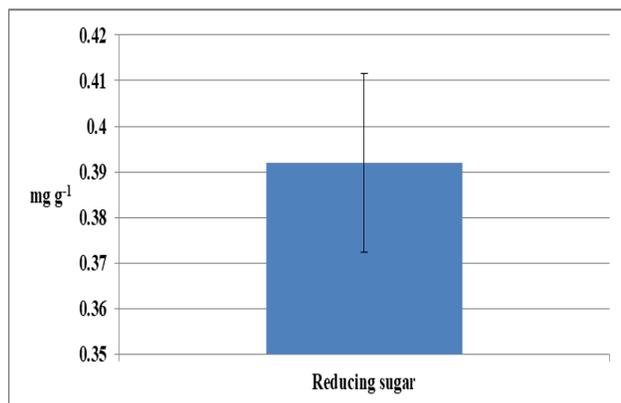
Table 1. Preliminary Phytochemical Evaluation in *Coccinia grandis*

Sl Number	Phytochemicals	Methanol extract of <i>Coccinia grandis</i>
1	Reducing sugar	+
2	Alkaloids	+
3	Flavonoids	+
4	Steroids	+
5	Tannins	+
6	Terpenoids	+
7	Glycosides	+
8	Saponins	+
9	Phlobatannins	-
10	Iridoids	-
11	Anthraquinones	-
12	Coumarins	-

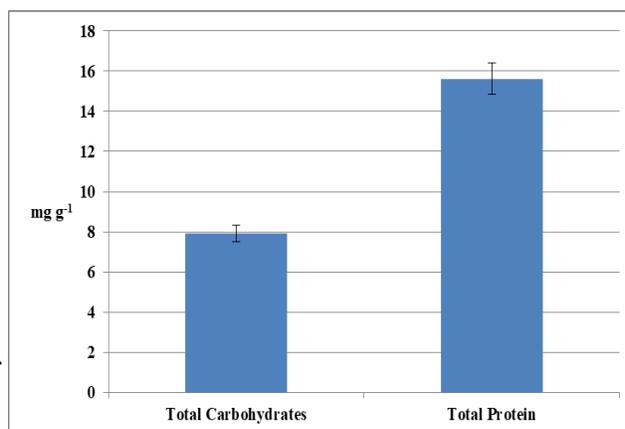
(+ denotes presence, - denotes absence)

### Quantitative Analysis Nutritional Evaluation

Nutritional factors like total reducing sugar, total carbohydrates, total protein, amino acids, pigments and starch were analysed quantitatively in *Coccinia grandis*. The nutritional and biochemical implications of reducing sugars have been critical in food science and health research (Singh *et al.*, 2015). The amount of reducing sugar present in fruit tissue extracted and analysed by the Dinitrosalicylic acid method and the results was found to be higher ( $0.392 \text{ mg g}^{-1}$ ) as shown in figure 1.

Figure 1. Reducing sugar in fruits of *Coccinia grandis*

Carbohydrates are essential macronutrients abundantly found in plants and serve as a major source of energy in the human diet. (Slavin, 2013). The present study estimated the total carbohydrates present in the fruit of *Coccinia grandis* by using the anthrone method. The total carbohydrate amount in *Coccinia grandis* was  $7.916 \text{ mg g}^{-1}$ , which was found to be higher (Fig. 2). Proteins are complex macromolecules that play structural, functional and catalytic roles in living organisms, with amino acids serving as their fundamental building blocks. They are essential for the growth, repair and maintenance of tissues (Friedman, 1996). Estimation of total proteins from the fruits of *Coccinia grandis* was done by Lowry's method and the result was found to be higher ( $15.619 \text{ mg g}^{-1}$ ) as shown in figure 2.

Figure 2. Total carbohydrates and Total protein in fruits of *Coccinia grandis*

Starch is a complex polysaccharide and the primary storage form of carbohydrates in plants. Starch serves as a significant source of dietary energy for humans and is commonly found in plant-based foods such as rice, tubers, legumes and cereals. In addition to its nutritional value, starch also plays a role in determining the texture and quality of various food products (Tester *et al.*, 2004). The amount of starch in *Coccinia grandis* was  $0.592 \text{ mg g}^{-1}$  (figure 3) and was found to be high.

Chlorophyll also has antioxidant properties and contributes to the nutritional value of leafy vegetables consumed by humans. Chlorophyll-a and Chlorophyll-b are the main pigments in

plants that capture light for photosynthesis (Lichtenthaler, 1987). The estimation of different pigments in *Coccinia grandis* was carried out using Arnon's method, with a result of high content of chlorophyll-a ( $0.981 \text{ mg g}^{-1}$ ), chlorophyll-b ( $0.765 \text{ mg g}^{-1}$ ) and total chlorophyll ( $1.632 \text{ mg g}^{-1}$ ) as shown in figure 4.

Carotene is a plant pigment belonging to the group of carotenoids, responsible for the orange, yellow and red colours in many fruits and vegetables. It acts as a precursor to Vitamin -A, which is essential for vision, immune function and skin health. Carotene is abundant in green leafy vegetables and orange-colored plants, making them important dietary sources for maintaining adequate Vitamin-A levels (Britton, 1995). The amount of carotenes present in the fruits of *Coccinia grandis* was estimated and the results were found to be higher ( $1.152 \text{ mg g}^{-1}$ ) as shown in figure 4.

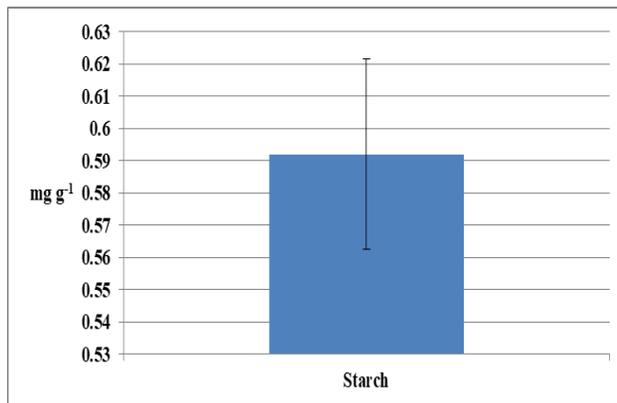


Figure 3. Starch in fruits of *Coccinia grandis*

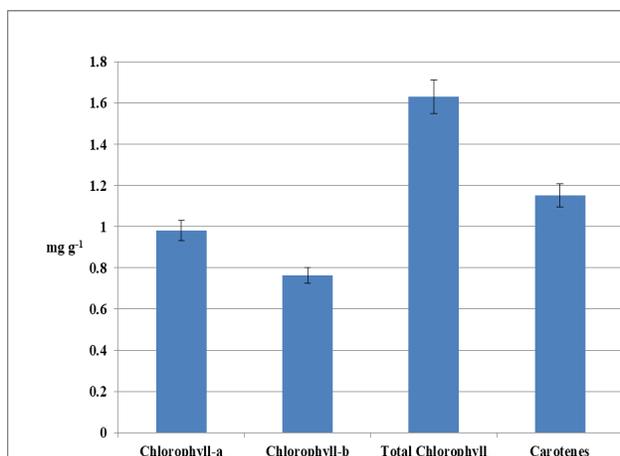


Figure 4. Pigments in fruits of *Coccinia grandis*

Amino acids are the building blocks of proteins and are essential for various biological processes including tissue repair, enzyme production and hormone synthesis. (Wu, 2013). Among the different amino acids, tyrosine ( $0.897 \text{ mg g}^{-1}$ ), phenylalanine ( $0.702 \text{ mg g}^{-1}$ ), serine ( $0.612 \text{ mg g}^{-1}$ ), glycine ( $0.918 \text{ mg g}^{-1}$ ), aspartic acid ( $0.123 \text{ mg g}^{-1}$ ), proline ( $0.529 \text{ mg g}^{-1}$ ), cysteine ( $0.501 \text{ mg g}^{-1}$ ), isoleucine ( $0.492 \text{ mg g}^{-1}$ ) and methionine ( $0.921 \text{ mg g}^{-1}$ ) were estimated and shown in figure 5.

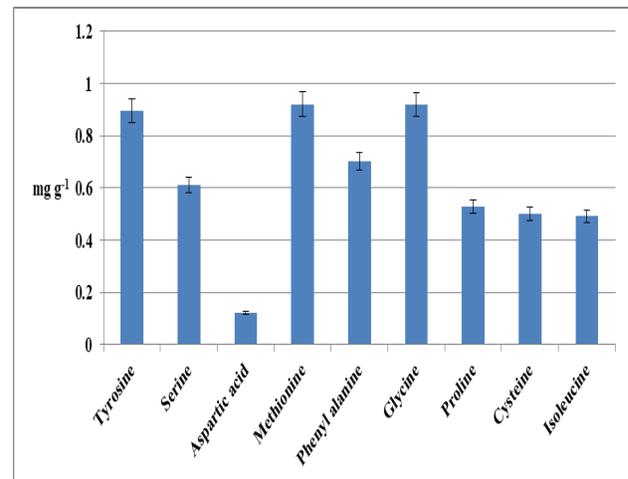


Figure 5. Amino acids in fruits of *Coccinia grandis*

### Antinutritional analysis

Plants contain certain compounds known as anti-nutritional factors that can interfere with the absorption or utilization of nutrients. These compounds such as phytic acids, tannic acid, phenols etc. may reduce the bioavailability of essential minerals, proteins and other nutrients. Although they occur naturally, their presence has led to research into food processing techniques that reduce their levels and enhance the nutritional value of plant-based foods (Kumar *et al.*, 2019). Antinutritional factors like total phenols, phytic acids and tannic acids were estimated in *Coccinia grandis* by standard estimation methods.

The estimation of total phenols in fruits of *Coccinia grandis* was carried out using the Folin-Ciocalteu method and found to be low ( $0.306 \text{ mg g}^{-1}$ ) as shown in figure 6. Phytic acid is a compound found mainly in seeds, grains and legumes, serving as the plant's primary phosphorus storage. Although important for

plants, it acts as an anti-nutritional factor by binding minerals like iron, calcium and zinc, reducing their absorption in humans. This can lead to mineral deficiencies, especially in plant-based diets. Therefore, food processing methods are used to lower phytic acid levels and improve nutrient availability (Gupta *et al.*, 2015). The result showed that very low amount of phytic acid was found in the fruits of *Coccinia grandis* ( $0.019 \text{ mg g}^{-1}$ ) as shown in Fig. 6. Tannic acids are a type of polyphenol found in various plants that can bind and precipitate proteins, reducing protein digestibility and nutrient absorption. As anti-nutritional factors, they may limit the availability of essential nutrients, but also possess antioxidant properties (Price *et al.*, 1978). The amount of tannic acids in *Coccinia grandis* was found to be low ( $0.028 \text{ mg g}^{-1}$ ) as shown in figure 6.

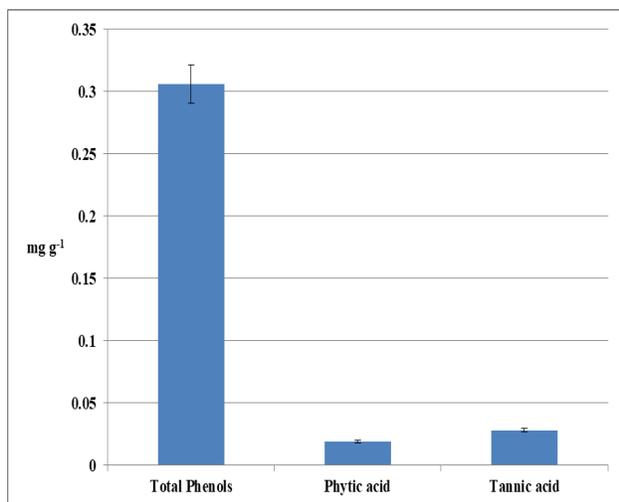


Figure 6. Antinutritional factors in fruits of *Coccinia grandis*

### Evaluation of Antioxidant Properties

Evaluation of non-enzymatic and enzymatic antioxidants in *Coccinia grandis* can help in understanding the therapeutic potential of the plant in terms of its antioxidant properties. The present study evaluated non-enzymatic antioxidants like proline, lycopene, carotenoids, polyphenols and tocopherol (Vitamin-E) and enzymatic antioxidants such as superoxide dismutases (SOD), catalase (CAT), glutathione reductases (GR), peroxidases (POD), amylases, polyphenol oxidases (PPO) and lipoperoxidase ( $\text{LP}_x$ ) by

standard estimation methods.

### Non-Enzymatic Antioxidants

Proline is an amino acid that plays important roles in various physiological and cellular processes in plants, including cell proliferation, programmed cell death and gene expression. It functions as an osmoprotectant under stress conditions like drought or salinity and helps in Reactive oxygen species (ROS) scavenging. As a signaling molecule, proline catabolism in the mitochondria is linked to oxidative respiration, providing energy for growth and recovery after stress (Szabados and Savoure, 2010). The proline content was estimated to be  $0.962 \text{ mg g}^{-1}$  (Fig. 7). Lycopene is a red carotenoid pigment mainly found in tomatoes and other red fruits. It plays a key role in photoprotection and antioxidant defense in plants by neutralizing reactive oxygen species. In humans, lycopene functions as a potent antioxidant that reduces the risk of chronic diseases, including prostate cancer and cardiovascular conditions (Rao and Rao, 2007). The estimated amount of lycopene in the methanolic fruit extract of *Coccinia grandis* was  $1.812 \text{ mg g}^{-1}$  (Fig. 7) and found to be high. Polyphenols are secondary metabolites in plants that play significant roles in stress resistance, defense against pathogens and regulation of growth and development. They act as antioxidants and signaling molecules, modulating processes like cell cycle, apoptosis, and oxidative stress. In human diets, polyphenols are linked to a reduced risk of cardiovascular and neurodegenerative diseases (Pandey and Rizvi, 2009). The phytochemical estimation revealed that the total polyphenol content in *Coccinia grandis* is  $0.824 \text{ mg g}^{-1}$  (figure 7) and found to be higher.

Carotenoids are naturally occurring pigments responsible for yellow, orange and red colors in plants. They are essential for photosynthesis, especially in light harvesting and photoprotection by quenching excess energy and reactive oxygen species. Some carotenoids, such as  $\beta$ -carotene, are also precursors to Vitamin-A, which is vital for vision and immune function (Demmig-Adams *et al.*, 2002). The carotenoid content in the fruits was found to be  $1.109 \text{ mg g}^{-1}$  (figure 7).

Tocopherol (Vitamin-E) is a lipid-soluble antioxidant that protects cell membranes from oxidative damage by scavenging lipid peroxy radicals. In plants, tocopherols are critical for maintaining chloroplast integrity under stress and ensuring seed viability. In humans, they support immune function and help prevent diseases caused by oxidative stress (Munne *et al.*, 2002). The Vitamin-E content was also appreciable, recorded at  $0.692 \text{ mg g}^{-1}$  (figure 7).

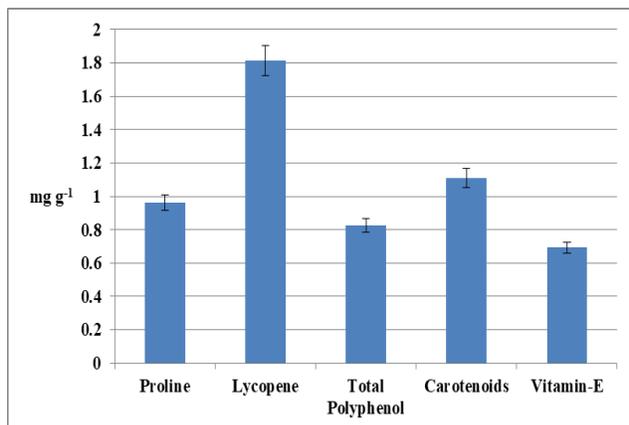


Figure 7. Non-Enzymatic Antioxidants in fruits of *Coccinia grandis*

### Enzymatic Antioxidants

Superoxide dismutase (SOD) are metalloenzymes catalyzing dismutation or reaction of superoxide radicals. On the basis of the cofactor present, they are classified into Fe-SOD (found in mitochondria) and Cu-SOD or Zn-SOD (found in chloroplasts, peroxisomes and cytosol). They are activated under different abiotic stress conditions such as water deficiency, chilling, heat, hypoxia and heavy metals in toxic concentrations. Intracellular SOD may play a key role in protection of cancer cells against reactive oxygen species generated by anticancer drugs and radiation (Yamaguchi *et al.*, 1994). The superoxide dismutase content was found to be  $1.907 \text{ mg g}^{-1}$ , representing the highest among the analyzed enzymes (Fig. 8). Catalase enzyme is an oxidoreductase enzyme as it plays a crucial role in quenching the reactive oxygen species (ROS), i.e. hydrogen peroxide, often produced as a by-product of aerobic respiration (Beers and Sizer, 1952). Hence it acts as an antioxidant and protects the cell against

oxidative stress (Abbott *et al.*, 2009). The catalase content was estimated to be  $1.253 \text{ mg g}^{-1}$ , indicating a high level of activity (figure 8). Glutathione reductase is a flavoprotein enzyme responsible for maintaining the reduced form of glutathione (GSH) by converting oxidized glutathione (GSSG) back to GSH using NADPH. This enzyme is crucial for cellular defense against oxidative stress and is induced under environmental stresses such as heavy metals, drought and pathogen attack. It is found in chloroplasts, mitochondria and the cytosol (Foyer and Halliwell, 1976). The glutathione reductase content was estimated to be  $0.962 \text{ mg g}^{-1}$ , indicating a high level of activity (figure 8).

Peroxidase are heme-containing enzymes that catalyze the reduction of hydrogen peroxide and organic peroxides using various electron donors like phenolic compounds. These enzymes are widely distributed in plant tissues and participate in lignification, suberization and defense responses. POD activity increases under abiotic stresses such as salinity, drought and heavy metal exposure, contributing to ROS scavenging and cell wall reinforcement (Passardi *et al.*, 2005). The peroxidase activity was also high, with an estimated value of  $1.249 \text{ mg g}^{-1}$  (figure 8). Amylase is a hydrolytic enzyme that catalyzes the breakdown of starch into sugars such as maltose and glucose. It exists in two main forms:  $\alpha$ -amylase and  $\beta$ -amylase and plays an important role in seed germination and stress response. Abiotic stresses such as drought, salinity and temperature fluctuations affect amylase activity, influencing carbohydrate metabolism and energy availability (Jacobsen *et al.*, 1986). The amount of amylases is  $0.671 \text{ mg g}^{-1}$  (Fig. 8) and is found to be higher. Polyphenol oxidase is a copper-containing enzyme that catalyzes the oxidation of polyphenols to quinones, leading to the formation of brown pigments. PPO is involved in plant defense mechanisms against pathogens and herbivores. It is localized in plastids and is activated under biotic and abiotic stresses including wounding, drought and UV (Ultraviolet) radiation (Mayer, 2006). The estimated amount of enzymatic antioxidant polyphenol oxidase is  $0.726 \text{ mg g}^{-1}$  and is found to be higher (figure 8). Free radicals such

as H<sub>2</sub>O<sub>2</sub> attacks unsaturated fatty acids producing lipid hydroperoxides and further occurrence of chain reactions changes lipid structure and organization of cell membrane in the process of lipid peroxidation. Peroxidase enzymes (stress enzymes) are haeme-containing enzymes that can oxidize various substrates using H<sub>2</sub>O<sub>2</sub> that prevent its accumulation under metabolism during stress conditions thereby preventing lipid peroxidation (Pradeesh and Swapna, 2018). The result revealed that the amount of lipoperoxide is 0.523 mg g<sup>-1</sup> (figure 8) and is found to be higher.

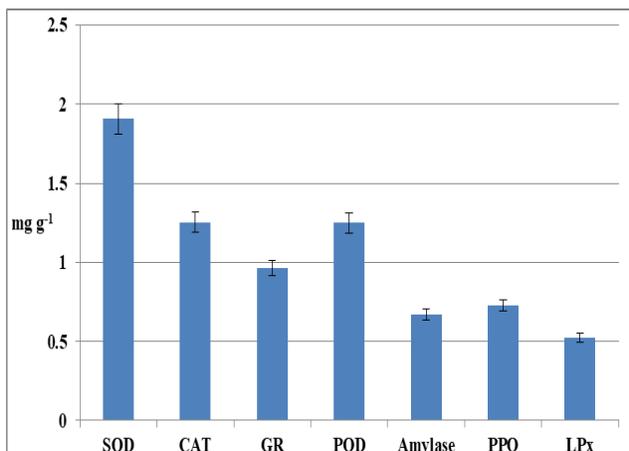


Figure 8. Enzymatic Antioxidants in fruits of *Coccinia grandis*

**Antimicrobial studies by *in vitro* methods**

**Antibacterial activity in *Coccinia grandis***

Antimicrobial activity of *Coccinia grandis* against selected bacterial strain of *Escherichia coli* was evaluated. Results indicated that the methanolic extract of the fruits of *Coccinia grandis* showed a mild action against *E. coli* (Table 2 and Plate 1).

Table 2. Antibacterial activity in the methanolic fruit extract of *Coccinia grandis*

Bacterial Species (Gram -ve)	Zone of inhibition in mm					
	25 µl/ Disc	50 µl/ disc	100 µl/ Disc	200 µl/ disc	Standard (Ciprofloxacin) 5 µg/disc	Control
<i>Escherichia coli</i>	Nil	Nil	7	7	30	Nil

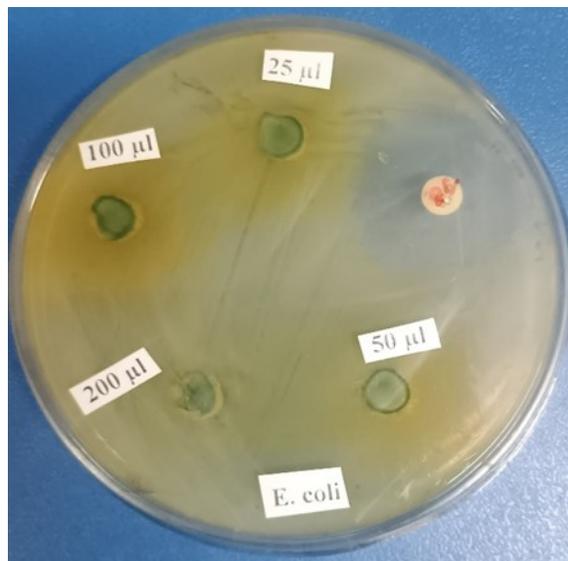


Plate 1. Antibacterial activity against *Escherichia coli*

**Antifungal activity in *Coccinia grandis***

Antimicrobial activity of *Coccinia grandis* against selected fungal strains of *Aspergillus niger* was evaluated. Result revealed that the methanolic extract of *Coccinia grandis* fruits has no zone of inhibition against *Aspergillus niger* (Table 3 and Plate 2).

Table 3. Antifungal activity in the methanolic fruit extract of *Coccinia grandis*

Fungal species	Zone of inhibition in mm					
	25 µl/ Disc	50 µl/ Disc	100 µl/ disc	200 µl/ disc	Standard (Fluconazole) 10 µg/disc	Control
<i>Aspergillus niger</i>	Nil	Nil	Nil	Nil	Nil	Nil

**Evaluation of Pharmacological Property**

***In vitro* Anticancer Activity in Crude Methanolic Extract of *Coccinia grandis***

Plants are known to be effective in treating various diseases since ancient times. Cancer is one of the leading fatal diseases of man. The relevance of active compounds present plants in

treating malignant tumours and preventing cancer is acquiring more popularity in recent years. Present study evaluated *in vitro* anticancer activity in methanolic fruit extract of *Coccinia grandis*. The result obtained from anticancer study revealed that the methanol extract of *Coccinia grandis* showed 38.6, 47.9, 49.2% cytotoxicity in EAC compared to 33.1, 39.9, 42.9% cytotoxicity in DLA at concentrations of 100, 500 and 1000 µg/ml (figure 9 and Table 4). Result obtained in the present study demonstrated that the methanol extract of fruit of *Coccinia grandis* exhibits *in vitro* anticancer activity against DLA and EAC cell lines. The fruit extract showed concentration dependent cytotoxicity which was found to be effective against solid tumor induced by DLA and ascites tumor induced by EAC. Fijesh (2011), reported that the extract treated cells showed membrane blebbing, vacuole formation and nuclear condensation which was absent in untreated cells. Thus the cytotoxic and antitumor effects of the leaf extract can provide possibilities to novel therapeutic findings for treating cancer cells.



Plate 2. Antifungal activity against *Aspergillus niger*

### ***In vitro* Conservation of Nodal Explants of *Coccinia grandis***

*In vitro* conservation of *Coccinia grandis* was carried out with explants such as leaf, petiole, node with axillary bud and internodal segments

in MS medium supplemented with 2 mg/l BAP (Plate 3 and 4). Results revealed that nodal explant with axillary bud possess comparatively higher survival chance (10 days in medium) and direct organogenesis (6 days after inoculation) than other explants used. Node with axillary bud and was less vulnerable to fungal infection in the first week. All the explants were subsequently infected by 11<sup>th</sup> day, node with axillary bud being the last to get infected (Plate 3).

Table 4. *In vitro* anticancer activity in fruits of *Coccinia grandis*

Concentration (µg/ml)	Standard (%)	DLA (%)	EAC (%)
100	60.908	33.108	38.628
500	86.39	39.912	47.901
1000	98.19	42.917	49.205

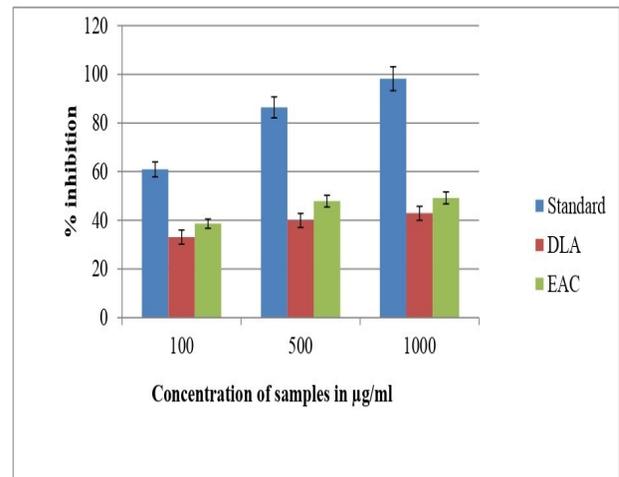


Figure 9. *In vitro* anticancer activity in fruits of *Coccinia grandis*



Plate 3. Inoculated explants of *Coccinia grandis*

### Summary and conclusion

*Coccinia grandis* is commonly called ivy gourd. The present study on *Coccinia grandis* is concerned with preliminary qualitative analysis of phytochemicals, quantitative analysis of nutritional, antinutritional, antioxidant, antimicrobial and anticancer analysis on DLA and EAC cell lines and *in vitro* conservation. Preliminary phytochemical analysis of the methanolic fruit extract of *Coccinia grandis* showed the presence of reducing sugar, alkaloids, flavonoids, steroids, tannins, terpenoids, glycosides and saponins. Quantitative analysis of biochemicals related to nutrition revealed that leaves of the plant contain higher amounts of reducing sugar, carbohydrates, protein, starch, amino acids and pigments. Antinutritional factors like total phenols, phytic acids and tanninic acids were evaluated and found to be present in low concentrations. The enzymatic and non-enzymatic analysis of antioxidant in the sample carried out in the present study. Different non-enzymatic antioxidants such as like proline, lycopene, carotenoids, polyphenols and tocopherol (Vitamin-E) and enzymatic antioxidants like glutathione reductases, superoxide dismutases, peroxidases, amylases, polyphenol oxidases and lipoperoxides were quantified. Both of them were found to be higher in the present study. Anticancer analysis on the crude methanolic fruit extract were carried out. Results obtained in the present study demonstrated the concentration dependent anticancer effect of methanolic fruit extract of *Coccinia grandis* in DLA and EAC cell lines which was found to be moderate to high. Increased concentration of extract showed higher cytotoxicity in EAC than in DLA. The plant can thus provide possibilities of novel findings in the field of cancer therapeutics. The results of cytotoxic and antitumor properties exhibited in this preliminary analysis can serve as base upon which further studies can be carried out to reveal detail profile of anticancer action of the plant extract. *In vitro* conservation of the nodal explants of *Coccinia grandis* was carried out with different explants. Explants were inoculated in MS medium supplemented with BAP and the culture was maintained at 16 hours photoperiod at a temperature of 26°C. Culture was contaminated by fungal infection

10 days after inoculation. Node with axillary bud showed more survival capacity by few days in the culture compared to other explants used. This generated information on phytochemical, nutritional and medicinal characteristics and therapeutic potential of *Coccinia grandis* provide scientific proof for identifying the plant bio resource and its effective utilisation in the future.

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