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***In vitro* Shoot Multiplication from Nodal Explants of *Coccinia grandis* (L.) Voigt. and It's Antidiabetic and Antioxidant Activity**

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ABSTRACT

Coccinia grandis (L.) Voigt. (Cucurbitaceae) is an important vegetable with high food and medicinal value. In this plant major problem is seed germination, seed setting and less number of fruit settings. Plant tissue culture technology may help to overcome these problems to a great extent. Therefore, the present study has been designed to develop an efficient protocol for *in vitro* shoot multiplication, as well as to check its antidiabetic and antioxidant activity. The nodal segments used as explants for cultured. Murashige and Skoog's (MS) agar-gelled medium with different concentration and combination of BAP, KIN, NAA and NB₆ for shoots multiplication and callus. Multiple shoots was seen after 10 days of sub culturing on MS medium. Maximum shoot formed (16±1.5) on MS medium containing 0.5 mg L⁻¹ BAP combination with 0.2 mg L⁻¹ NB₆ after 15 days. Antidiabetic activity of *C. grandis* was also evaluated by using α -amylase inhibition assay and DNS assay. Methanolic extract of fruit, callus (BAP+NB₆) and different combination mixture of callus at 1.0 mg L⁻¹ concentration showed 54.29, 75.72 and 80.00% inhibition of α -amylase activity respectively. The result of agar diffusion amylase inhibition assay indicated that methanolic extract of callus show maximum inhibition (46.66%) compare to the fruit extract and fresh callus showed no inhibition. Antioxidant activity of *C. grandis* was evaluated by using DPPH assay method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. The DPPH radical scavenging activity and significantly decreased the formation of oxygen radicals generated in rat peritoneal macrophages plant extracts. The protocol described here is efficient with high number of shoot multiplication and can be employed for the large scale production and genetic manipulation for antidiabetic medicinal plant *Coccinia grandis*.

Key words: *Coccinia grandis*, shoot multiplication, nodal culture, antidiabetic, antioxidant

INTRODUCTION

In botanical, vegetables refers to “edible part of a plant” mostly collected or cultivated for their nutrition value for human (Hanif *et al.*, 2006). In India, as a large section of people are vegetarian. So, for fulfilling the dietary requirements they depend on vegetables, approximately 400 vegetable crops grown commercially (Nandi and Bhattacharjee, 2005). India is the 2nd largest producer of vegetables in world. In India, 3% of total cropped area is used for crop cultivation. It does not fulfill the requirement of the everyday demand of day/person. This current status not only improves the nutritional requirement but also meet demand of food growing population of day/person is increased. In India, wide range of agro-climatic conditions present which is the maintaining

continues production of vegetable throughout the year. Vegetables gave more yield than any other crops like rice and wheat. They also provide higher quantity of food per unit area. They also give high farm income than any other crops and high export potential compare to any other field crops. Vegetables have given a boon to processing industry as they can be processed to form diverse varieties of food like sauces, chutney, pickles etc.

The maintenance of good health is essential for balance diet including the vegetable. Vegetables provide the vitamin, fiber, protein, carbohydrate and minerals like iron, potassium and sodium. Vegetables have short production cycle which allow for multiple cropping and for that reason large volume of grow worldwide on small area in compare to other crops (Lampe, 1999).

Majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover, many plants propagated by vegetative means contain systemic bacteria, fungi and viruses, which may affect the quality and appearance of selected items. During the last few years tissue culture technique has emerged as a promising technique for rapid and large scale propagation of various plants. Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions, often to produce the clones of plants (Murashige and Skoog, 1962). The control condition provided to culture is required for their growth and multiplication of plant. It includes proper supply of nutrient, pH, temperature and proper gaseous. Research efforts in plant cell and tissue culture have increased dramatically worldwide in recent years including efforts in developing nations.

Digestive system as a key enzyme use pancreatic alpha amylase. It catalyzes the starch hydrolysis and form mixture of oligosaccharides such as maltose. It is calved by glycosidase and degrade to glucose which is on absorption enters the blood stream. The disturbance in this pathway leads to hyperglycemia. When rapidly starch degradation occur, also glucose level increase rapidly in small intestine. The control of glucose level is important aspect for type 2 diabetes, hence control of starch degradation by enzyme such as α amylase and glycosidase play a key role in diabetes. This enzyme is major digestive enzyme and helps in intestinal absorption (Thakur *et al.*, 2011). The treatment of diabetes is the potential targets in the development of lead compound are alpha amylase and glycosidase inhibitors (Nair *et al.*, 2013). The lowering serum glucose level and reduction in glucose absorption because of inhibitors of this enzyme control starch degradation and also slow the process of carbohydrate digestion (Sivaraj *et al.*, 2011; Loizzo *et al.*, 2008).

Antioxidants are substances which are present at low concentration, which search free radical and prevent damage caused by them, significantly delays or inhibits oxidation of that substrate. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. Antioxidant can be classified into two types: enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. However, these compounds have some toxic effects like liver damage and mutagenesis. Nowadays, searching for natural antioxidant sources is more important (Munasinghe *et al.*, 2011).

Coccinia grandis, the ivy gourd, also known as baby watermelon and little gourd. *Coccinia grandis* belong to the Cucurbitaceae family, comprises 960 species. Most of the plants in Cucurbitaceae family are annual vines. It is aggressive vine, can form dense mats on lands that readily cover shrubs and small tree. Leaves are simple, arranged alternately along the stems,

shapes are different from heart to pentagon shaped, up to 10 cm long, upper surface of the leaf is green colour and hairless while lower surface are pale-green colour with hair. Tendrils are simple. Flower is white, solitary, calyx five with recurved lobes, corolla lobe ovate and white, three stamens and ovary inferior. Fruit is slimy in touch, pulpy and ovoid to ellipsoid shaped or cylindrical. It possess about ten white stripes on posterior portion, It is green in color when young which turns to scarlet red when it ripens, 2.5-6.0 cm long, 1.5-3.5 in diameter, glabrous, fruit possesses numerous seeds which are oblong, 6-7 mm (Hussain *et al.*, 2011; Starr *et al.*, 2003; Pekamwar *et al.*, 2013; Yadav *et al.*, 2010).

Chemical composition of *C. grandis* present the carbohydrate-12.62%, total protein-15%, water soluble protein-11.25%, lipid-4.0%, total phenol-61.92 mg/100 g, vitamin C-25.55 mg/100 g, β -carotene-70.05 mg/100 g, potassium-3.38 mg/100 g, phosphorous-1.15 mg/100 g, sodium-0.95 mg/100 g, iron-2.23 mg/100 g and calcium-3.79 mg/100 g. Fruits of *C. grandis* contains steroids, saponins, ellagic acid, terpenoids, lignin, other compound like alkaloids, tannins, flavonoids, glycosides, phenols, B-amyrin acetate, lupeol, taraxerol, B-carotene, lycopene, cryptoxanthin, xyloglucan, carotenoids and β -sitosterol (Khatun *et al.*, 2012).

Coccinia grandis is mostly used due to its hypoglycemic and antidiabetic properties in India. It has been popularly used as an ayurvedic drug for the diabetes mellitus 2. Plant is roborant, emetic and used to treat inflammation, dyspnea, cough, emaciation, fever with burning sensation, convulsion, syphilis pulse and flower are used in jaundice. Fruit is applied to swelling and taken orally for disorder of blood, cure anemia, dried root powder is cathartic, the ash of root is applied for skin disease and also leaves and stems are antiplasmodic and expectorant, also useful in bronchitis. *C. grandis* fruit present the bioactive compound well known for its antimicrobial activity against pathogenic bacteria and inhibited the mycelium growth and sporulation (Satheesh and Murugan, 2011; Shaheen *et al.*, 2009; Sivaraj *et al.*, 2011; Farrukh *et al.*, 2008). Also, it has anticancerous activity, antidyslipidemic, antipyretic (Bhattacharya *et al.*, 2011), hepatoprotective activity (Moideen *et al.*, 2011), antiulcerogenic effect (Mazumder *et al.*, 2008) and anti-inflammatory (Ashwini *et al.*, 2012).

Coccinia grandis is seed germination, seed setting is low and long period of time dormant condition. This reason natural population day by day decline. It is urgent need to conservation for this species. The present study develop a large scale multiplication protocol for *C. grandis* using nodal culture and check their antidiabetic and antioxidant activity.

MATERIAL AND METHODS

Plant tissue culture

Collection of plant material: Mature plants of *C. grandis* (L.) Voigt. (Family-Cucurbitaceae) (Node explants) (Fig. 1a and b), were collected from New Vallabh Vidynagar, District-Anand, Gujarat and surrounding area. The plant was identified by Dr. Kalpesh Ishnava (Plant Taxonomist) at Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidynagar, Gujarat, India.

Culture medium: The basal medium used for the culture was Murashige and Skoog medium (MS medium) with 3% sucrose and 0.8% agar and different growth hormones (Murashige and Skoog, 1962). Basically, a nutrient medium consists of the entire essential major and minor plant nutrient elements, vitamins, plant growth regulator and carbohydrate as carbon source with other organic substance as optimal additives. The concentrated stock solutions of all the ingredients were

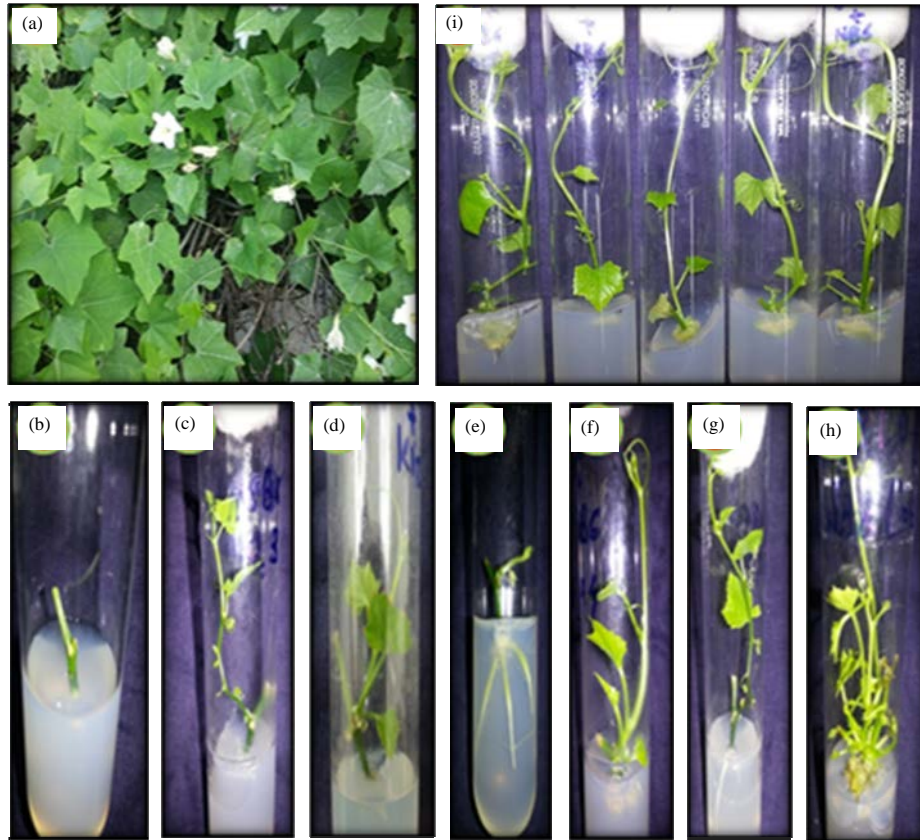


Fig. 1(a-i): *In vitro* shoot multiplication from nodal explants used different hormones in *Coccinia grandis* (a) Whole plants, (b) Nodal explants, (c) Shoot elongation, (d) Shoot produce, (e) Root formation, (f) Shoot with callus, (g) Shoot with root, (h) Shoot multiplication and (i) After shoot multiplication separated plants

prepared and stored under refrigeration. To prepare stock solution of micro salts, all the micro salts, in required quantities were dissolved in 1 L of distilled water and used as stock solution. Likewise stock solutions of all other ingredients were also prepared and kept under refrigeration. Similarly, stock solutions of growth hormones were also prepared.

The medium was prepared by adding required quantities of all the ingredients in the conical flask. After adding all the ingredients in required amounts, the final volume is making up with the help of distilled water. The pH of the medium is adjusted to 5.8 by using 1 M NaOH or 1 M HCl. After adjusting the pH, agar is added to the medium at the rate of 0.8% w/v for solidification of the medium. After pouring media (50 mL/300 mL bottle), bottles are tightly capped and labeled properly. After that media is autoclaved at 121°C for 20 min at 15 psi. These were then left to cool and solid.

Explants sterilization: Nodal segments were excised from the grown plants of *C. grandis*. This segments placed in beaker and covered with net and washed for 30 min under running tap water to remove the all adhering dust particles and microbes from the surface, then sterilized with two to three drops of detergents in required quantity of water and kept for 7-8 min with frequent

swirling and after again kept under running tap water until all detergents are removed from the water and followed by washed with distilled water.

Surface sterilization treatment of explants: There were used many treatment for surface sterilization of *C. grandis* for standardization of treatment. For standardization use the antibiotic (Streptomycin), antifungal (Bavistin) and chemical ($HgCl_2$) of different combinations and time period. In this plant better result in the washing the explants in 1-2 drops of detergent in 100 mL water, followed by 1000 ppm bavistin solution for 10 min and 200 ppm streptomycin solution for 4 min. After the treatments of 0.1% $HgCl_2$ for 3 min as a surface sterilization. After all the treatments, explants were removed from the sterilizing solution and rinsed with sterile distilled water. All treatments were properly followed under laminar air flow unit.

Inoculation of explants: All the experimental manipulations were carried out under strictly aseptic conditions in laminar air flow bench and follow the standard protocol for inoculation of explants (Thiripurasundari and Rao, 2012).

Culture Condition: The tubes and bottles were shifted to culture room with controlled facility of diffused light (2000 lux) for 10 h daily at $28\pm 2^\circ C$ temperatures and 50-60% relative humidity.

Establishment of culture: After approximately 7 days of inoculation, the axillary bud break was seen in the some explants. When the explants attain substantial bud proliferation, these cultures were then transferred to same media containing test tube after 21 days of incubation with a clean and sterilized forceps under laminar air flow unit. The initiated plants were taken out of the test tube, medium adhered to the plants were removed, undesirable/brownish leaves were also removed from the plants and were transferred to the culture test tube containing autoclaved semi-solid media having the same media combination as that for the culture initiation. Then, the all test tubes were placed in growth room under the standard condition. Different medium for establishment of culture are given in Table 1.

Table 1: Effect of different hormones on multiplication of shoots from nodal explants of *Coccinia grandis*

| MS+hormones (mg L ⁻¹) | Average nodal length (cm) | | No. of shoots | |
|-------------------------------------|---------------------------|-----------|---------------|-----------|
| | 10th days | 15th days | 10th days | 15th days |
| MS+BAP (0.1) | 3.5±0.6 | 5.6±0.8 | 1.2±0.8 | 1.5±0.9 |
| MS+BAP (0.5) | 7.7±1.2 | 10.3±0.4 | 1.6±0.5 | 2.21±1.3 |
| MS+KIN (0.4) | 1.1±0.4 | 2.1±0.6 | 2.4±0.5 | 2.8±1.3 |
| MS+NB ₆ (0.1) | 2.6±0.8 | 3.1±1.5 | 3.6±1.2 | 5.0±1.5 |
| MS+NB ₆ (0.3) | 5.1±1.5 | 9.0±1.3 | 2.2±0.8 | 3.2±0.1 |
| MS+NB ₆ (0.5) | 5.7±1.2 | 8.1±1.4 | 3.2±0.8 | 5.0±1.5 |
| MS+BAP(0.3) +NAA(0.3) | 2.4±0.6 | 5.9±1.0 | 1.6±1.3 | 2.3±0.5 |
| MS+NB ₆ (0.2)+NAA(0.2) | 1.4±0.5 | 2.4±1.1 | 1.4±0.5 | 2.4±1.1 |
| MS+NB ₆ (0.5)+NAA(0.5) | 1.83±0.8 | 3.1±1.4 | 3.2±1.3 | 4.0±1.5 |
| MS+NB ₆ (0.4)+KIN(0.4) | 5.3±1.2 | 9.7±1.2 | 3.0±1.5 | 5.4±67 |
| MS+NB ₆ (0.5)+KIN(0.5) | 6.2±1.3 | 7.1±1.5 | 2.2±1.3 | 3.0±1.5 |
| MS+BAP(0.2) + NB ₆ (0.2) | 5.0±0.79 | 9.3±0.9 | 3.7±0.8 | 5.2±0.8 |
| MS+BAP(0.3) + NB ₆ (0.3) | 4.3±0.1 | 6.9±0.1 | 4.4±1.1 | 8.9±0.5 |
| MS+BAP(0.4) + NB ₆ (0.4) | 5.4±0.1 | 9.5±0.3 | 4.0±1.5 | 4.4±0.8 |
| MS+BAP(0.4) + NB ₆ (0.5) | 10.6±0.9 | 12.3±1.3 | 8.2±1.3 | 11.2±1.3 |
| MS+BAP (0.1)+ NB ₆ (0.5) | 9.1±0.7 | 13.9±0.6 | 3.2±1.3 | 4.0±1.5 |
| MS+BAP (0.4)+ NB ₆ (0.2) | 10.0±1.3 | 11.0±1.5 | 2.2±1.3 | 4.2±0.8 |
| MS+BAP (0.5)+ NB ₆ (0.2) | 4.4±0.1 | 8.0±0.2 | 10.8±0.8 | 16.0±1.5 |

MS: Murashige and Skoog's agar gelled medium, BAP: 6-Benzylaminopurine, NAA: α-Naphthalene acetic acid, NB₆: 6-Benzyladenine

Axillary shoots proliferation: Shoots multiplication were repeated subcultures on the multiplication medium. The preparation and sterilization steps for the medium, glassware and chamber were repeated as before. Multiple shoots were transferred from the cultured test tube to a sterile glass plate using sterilized forceps. The brown leaves were removed from the primary shoots and they were sectioned into one node piece after removing the leaves. These nodal segments were then transferred to the multiplication media. All this work was done with extreme care and inside the laminar unit to avoid any possible chance of contamination. This test tube was placed in growth room. Different medium for establishment of culture and multiplication of shoots are given in Table 1.

Antidiabetic activity: The calluses (MS+BAP (0.2)+NB₆ (0.2)) were collected after 30-40 days. Calluses were washed with distilled water to remove all adhering particles. After that calluses were allowed to dry at room temperature. Similarly fruits were also allowed to dry in hot air oven.

Extraction of callus: Dried sample of callus from *in vitro* grown nodal explants and fruits were grounded to powder with mortar and pestle. Five gram of samples was extracted using 20 mL of methanol in Erlenmeyer flask placed on shaker at 100 rpm for overnight at room temperature. The crude extract then filtered with filter paper (Whatman No. 1). The filtrate was collected and allowed to evaporate. After evaporation the remaining material was collected and different stocks were prepared by dissolving in methanol for antidiabetic study.

Alpha-amylase inhibitory activity: The inhibition assay was performed using the chromogenic DNS method. One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions. The 5 mg of methanolic extract was first dissolved in 10 mL of DMSO reagent. Then aliquots of that solution were taken as 0.1, 0.2, 0.3, up to 1.0 mL. Sodium phosphate buffer was added in all the tubes to make up the volume 1 mL. Then 0.5 mL of alpha amylase enzyme was added into each tube. All the tubes were incubated at 37°C for 10 min. After incubation, 0.5 mL of 1% starch was added. Again incubation was done at 37°C for 15 min. After that, 1 mL of 3, 5-dinitrosalicylic acid (DNS) was added in all the tubes. Then, all the tubes were placed in boiling water bath for 10 min and cooled to room temperature. Then, dilute by adding 9 mL distilled water to all the tubes. Control was prepared by taking distilled water instead of methanolic extract solution. The whole procedure was also done for the methanolic extract of callus powder. The 5 mg of extract was dissolved in 10 mL of DMSO reagent and then from that, aliquots were taken. The OD at 540 nm was taken. Percentage inhibition was calculated using following reaction:

$$\text{Inhibition (\%)} = 100 - \text{reaction (\%)}$$

whereby, Reaction (%) = Enzyme activity in test/enzyme activity in control × 100.

Alpha amylase inhibition assay: This alpha amylase inhibition screening assay was performed using cup borer method. In this assay, 160 µL of alpha amylase enzyme and 120 µL of solution were mixed and incubated at 37°C for 45 min. After incubation, the mixture was poured into the well made in the centre of petriplate containing sterile medium containing 1% (w/v) agar and 1% (w/v) starch. Plates were allowed to stand for 3 days at 25°C and then flooded with iodine solution and

allowed to stand for 15 min. The diameter of clear zone of starch hydrolysis was measured. As a control, the enzyme was added into the well of the plate. The whole procedure was also done by using callus powder extract. The % inhibition was calculated by following equation:

$$\text{Amylase inhibition (\%)} = \left(\text{Diameter of control} - \frac{\text{Diameter of test}}{\text{Diameter of control}} \right) \times 100$$

Antioxidant activity

DPPH free radical scavenging activity: Required quantity of ascorbic acid was dissolved in methanol to give the concentration of 5, 10, 20, 30, 40, 50 $\mu\text{g mL}^{-1}$. Stock solution of sample was prepared by dissolving 10 mg of dried methanolic extract in 10 mL of methanol to give concentration of 1 mg mL^{-1} . The 1.5 mL DPPH solution was added to 3 mL methanol and absorbance was taken immediately at 517 nm for control reading. Different volume levels of test sample (0.1, 0.12, 0.14, 0.16, 0.18, 0.2) were added and made 2 mL of each dose level by dilution with methanol. Diluted with methanol with up to 3 and 1.5 mL DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV-visible spectrophotometer after 15 min using methanol as blank. The Free Radical Scavenging Activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\text{Antiradical activity (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Statistical analysis: All the experiments were repeated three times with 10 replicates per treatments. Data on the callus proliferation, percentage of regeneration, number of shoots per culture and shoot length was statistically analyzed using the procedure of SPSS package version X, correlation significance were assessed at 0.05 level.

RESULTS AND DISCUSSION

Coccinia grandis can be propagated through seeds and vegetative cuttings but this conventional propagation of *C. grandis* has limited potential for large scale cultivation. The application of tissue culture techniques might be of great value an alternative method to achieve large scale propagation independent of season and also used for secondary metabolite production. This study highlight the result of micropropagation of *C. grandis* and showed its antidiabetic and antioxidant activity.

The result of the present investigation on shoot multiplication of *Coccinia grandis* (Tindora) are presented under the observation. Development of efficient and reproducible regeneration protocol from cell/tissue is a pre-requisite for the successfully application of recent cellular multiplication techniques for the improvement of crop plants. In this direction the choice of explants is of key role of importance and makes an absolute difference between success and failure in inducing regeneration in vitro condition.

Effect of BAP on shoot proliferation from nodal explants of *Coccinia grandis*: Shoot initiation from nodal segments was mainly a cytokinin effect because the explants in cytokinin free medium did not respond. The role of BAP in bud breaking has been recorded in many medicinal plants such as *Cucumis sativus* (Ugandhar *et al.*, 2011), *Wadelia calendulacea* (Emmanuel *et al.*,

2000), *Vitex trifolia* (Arulanandam and Ghanthikumar, 2011) and *Wattakaka volubilis* (Arulanandam *et al.*, 2011). Ghanthikumar *et al.* (2013) reported the MS augmented with 1.0 mg L^{-1} of BAP in combination with 0.5 mg L^{-1} of Kn showed maximum percentage shoot production (Ghanthikumar *et al.*, 2013). Our results showed the best response on the medium containing 0.5 mg L^{-1} BAP (Table 1). Emergence of shoot bud was seen after 5 days of inoculation and after 10 days elongation of shoots was occurred (Fig. 1c and d). Also, callus formed at the base of the nodal explants.

Effect of KIN on shoot proliferation from nodal explants of *Coccinia grandis*: The MS medium supplemented with 0.2 mg L^{-1} KIN show good response for shoot initiation (Table 1). After 5 days of inoculation shoots formation occurred. Secondary root formed after 20 days of inoculation. Similarly in 0.4 mg L^{-1} KIN also shoot formation occurred (Fig. 1e). Maximum number of shoots regeneration was found at 4.0 mg L^{-1} KIN. Whereas, the shoot buds induction was decreased at high level of KIN (Ugandhar *et al.*, 2011).

Effect of NB₆ on shoot proliferation from nodal explants of *Coccinia grandis*: The MS medium supplemented with various concentration of NB₆ ($0.1\text{-}0.5 \text{ mg L}^{-1}$) produce shoot initiation after 5 days of inoculation. Shoot elongation was observed after 10 days of inoculation. Best response was seen on the MS medium supplemented with 0.5 mg L^{-1} NB₆ (Table 1) (Fig. 1f). Also, root formed in the medium containing 0.3 mg L^{-1} NB₆ growth hormones were observed. 0.1 and 0.4 g L^{-1} concentration are nodal base callus production after 15th days (Table 1). The NB₆ growth hormone is very closed similar to BA. It is very important in the plant tissue culture for shoot initiations are used. Zhang (2009) reported the Phlox Paniculata, with axillary shoots as explants, basal MS media supplemented with 2.0 mg L^{-1} 6-benzyladenine and 0.1 mg L^{-1} α -naphthalene acetic acid was the optimum media with 91% budding; MS plus 1.0 mg L^{-1} 6-benzyladenine and 0.1 mg L^{-1} α -naphthalene acetic acid was highest shoot proliferation efficiency of 3.1 (Zhang, 2009).

Effect of BAP+NAA on shoot proliferation from nodal explants of *Coccinia grandis*: Result indicated that best response for shoot formation was seen on MS medium containing 0.05 mg L^{-1} BAP+ 0.01 mg L^{-1} NAA. Shoot initiation after 7 days of inoculation and elongation of shoot was observed after 15 days (Table 1, Fig. 1f). Karim and Ahmed (2010) reported in teale gourd, different concentrations and combinations of BAP and NAA had significant influence on shoot regeneration from shoot tips, inter-node, leaf and nodal segments. The explants was more suitable of producing multiple shoots compared to other explants, 1.0 mg L^{-1} BAP+ 0.1 mg L^{-1} NAA produced shoots in shortest time (15 days) (Nabi *et al.*, 2002). In our study, lower concentration better response for the shoot initiation and number of the shoot is increasing. After 25th day observe the culture root formation (Table 1). It is constant observed that the old culture shown more response towards root formation. It is advice that the after 20-25th day sub culturing is required.

Effect of NB₆+KIN on shoot proliferation from nodal explants of *Coccinia grandis*: The MS augmented with 1.0 mg L^{-1} of BAP in combination with 0.5 mg L^{-1} of KIN showed maximum shoot formation (Table 1). Callus induction was observed on the cut surface of nodal segments on MS augmented with BAP in combination with KIN. Literatures suggest that BAP is most active at combination of $1.0\text{-}2.0 \text{ mg L}^{-1}$ in many plant worked by the different authors

(Ghanthikumar *et al.*, 2013). Results showing that NB₆ in combination with KIN showed more numbers of shoot formation. 0.4 mg L⁻¹ of NB₆+KIN showed the best response for shoot formation (Table 1, Fig. 1g). Shoot initiated within 5 days of inoculation and elongation of shoot was observed after 10-12 days. Abu-Romman *et al.* (2013) reported in the plant *Cucumis sativus* L. cv. Sultan callusing ability of hypocotyl explants derived from 6-day-old *in vitro* seedlings of cv. Sultan was evaluated on MS medium supplemented with individual treatments of different auxins (2,4-D, IAA and NAA) or cytokinins (BA and Kn). Data was analyzed after four weeks of culture and the analysis showed that callus induction frequency, callus growth rate and nature of callus were affected by the type and concentration of the plant growth regulators (Abu-Romman *et al.*, 2013).

Effect of BAP+NB₆ on shoot multiplication from nodal explants of *Coccinia grandis*: The MS medium supplemented with various concentration of BAP combining with NB₆ gave best response than any other single or combine hormone. Shoot formation after 5 days of inoculation. After 10 days elongation of shoot was seen. Maximum shoot was also produced in BAP combining with NB₆. Result showed that maximum shoot formation was seen on 0.5 mg L⁻¹ BAP with 0.5 NB₆ (Table 1) and also callus was seen at the base of the nodal explants. Mustafa *et al.* (2013) reported the MS medium supplemented with different concentration and combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L⁻¹) and L-glutamic acid (0.5, 1.0, 1.5 and 2.0 mg L⁻¹). After two weeks of culture, explants induced little amount of callus and few shoots from the basal end of the explants. Each adventitious shoot was cut from the basal end and sub cultured again on the M S medium fortified with 2.0 mg L⁻¹ BAP and 0.5 mg L⁻¹ L-glutamic acid. The subcultures were maintained at interval of four weeks. The frequency of multiple shoots was enhanced on the same medium. The results indicated that the nodal explants were more capable of producing multiple shoots compared to internodes. Large numbers of shoots in short time were produced on MS medium.

Effect of BAP+NB₆ on multiplication of shoot from nodal explants of *Coccinia grandis*: High frequencies of multiple shoot regeneration were achieved from the nodal explants. Explants were inoculated on MS basal medium with different concentration and combination of BAP and NB₆ for shoot multiplication. Nodal segments required 7-10 days to initiate shoots. Multiple shoots was seen after 10 days of subculture on MS medium. Maximum 16 numbers of shoot produce on MS medium containing 0.5 mg L⁻¹ BAP combination with 0.2 mg L⁻¹ NB₆ (Table 1, Fig. 1h). Also, after subculture elongation of shoot was observed. Mustafa *et al.* (2013) reported the bud explants of *Momordica balsamina* were cultured on MS media supplemented with different concentration of BAP. 1.0 mg L⁻¹ BAP stimulated the proliferation of bud meristems to form bud clusters and the co-efficient reached 6-8 (Mustafa *et al.*, 2013; Sudha *et al.*, 2011). It is clear that combination of BAP with NB₆ were found to be the best than single application. The MS medium supplemented with BAP with combination of NB₆ was most effective in multiplication of shoots. Also, callus was seen at the cut edges of nodal explants. In our study maximum numbers of the shoot produce in the combination of BAP and NB₆ combination (Table 1). This combination required the maximum 25th days maximum response in the nodal culture (Fig. 1i).

Antidiabetic activity: Diabetes mellitus is a common endocrine system disease that causes metabolic disorders and which leads to multiple organ damage. Clinical diabetes is divided into two types, with more than 90% of patients having type 2 diabetes. Type 2 diabetes is global health challenge and the WHO has recommended research and use of complementary medicines

for the management of this disease. The number of diabetes cases was 171 million in 2000 and is expected to rise to 366 million in 2030 (Gomathi *et al.*, 2012). The goal of treatment is to maintain normal levels of blood glucose and prevent the development of related disorders.

Drugs that reduce post-prandial hyperglycaemia by suppressing hydrolysis of starch, such as PPA inhibitors have been found useful in the control of diabetes mellitus. The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes. Several α -amylase inhibitors including acarbose, voglibose and miglitol are clinically used for treatment but their prices are high and clinical side effects occur (Sama *et al.*, 2012). Many herbal extracts have been reported for their anti-diabetic activities and are currently being used in Ayurveda for the treatment of diabetes. However, such medicinal plants have not gained much importance as medicines due to the lack of sustained scientific evidence (Thakur *et al.*, 2011).

In the study, the α -amylase inhibition by DNS assay revealed that the methanolic extract of callus (BAP+NB₆) of *C. grandis* showed maximum inhibition of α -amylase enzyme activity compared with methanolic extract of fruits and different combine hormones used to produce callus (Fig. 2). Methanolic extract of fruit, callus (BAP+NB₆) and different combination mixture of callus at 1.0 mg L⁻¹ concentration showed 54.29, 75.72 and 80.00% inhibition of α -amylase activity respectively. Percentage of inhibition activity increased as the concentration of sample increased (Fig. 2). The plant has been used since ancient times as an antidiabetic drug by physicians who practice Ayurveda. A double-blind control trial conducted in India, demonstrated significant improvement in glycemic control following 6 weeks' use of powder from locally obtained crushed dried leaves of *C. grandis* in patients with poorly controlled or otherwise untreated type 2 diabetes (Kuriyan *et al.*, 2008; Doss and Dhanabalan, 2008).

Thus, data presented here indicate that methanolic extract of callus of *C. grandis* possesses significant antidiabetic activity. The mechanism by which callus of *C. grandis* exerted antidiabetic action may be due to its action on carbohydrate binding regions of α -1, 4 glucosidic linkage in starch and other related polysaccharides have also been targets for the suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then

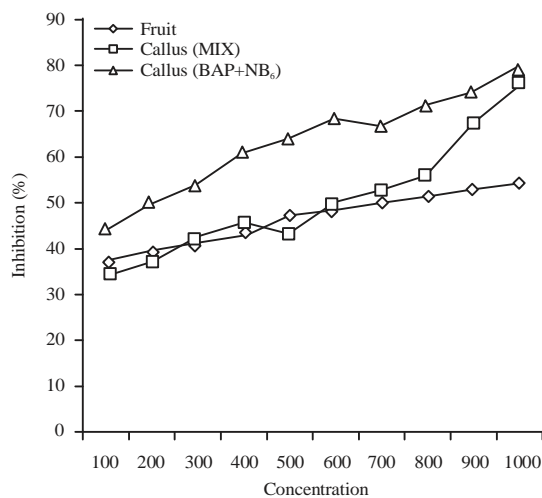


Fig. 2: Mode of inhibition of α -amylase activity by fruit, callus (from different hormone) and callus (BAP+NB₆) methanolic extracts

break down to glucose prior to absorption. Since, α -amylase play as an important role in starch break down in human beings and animals, the presence of such inhibitors in food stuffs may be responsible for impaired starch digestion (Jiju *et al.*, 2013). Food-grade phenolic α -amylase inhibitors from dietary plant extracts are potentially safer and therefore may be a preferred alternative for modulation of carbohydrate digestion and control of glycemia index of food products. This α -amylase inhibitor may be of value as novel therapeutic diabetic agents.

Mode of inhibition of alpha amylase inhibitors: Nonetheless, it is important to mention here that α -amylase breaks down starch into disaccharides that are acted upon by isomaltases, especially α -glucosidase to release glucose. The presence of potent α -glucosidase inhibitory activity therefore, appears more important in controlling the release of glucose from disaccharides in the gut than α -amylase inhibition. However, moderate α -amylase inhibition with potent α -glucosidase inhibitory activity may offer better therapeutic strategy that could slowdown the availability of dietary carbohydrate substrate for glucose production in gut. Food-grade phenolic α -amylase inhibitors from dietary plant extracts are potentially safer and therefore may be a preferred alternative for modulation of carbohydrate digestion and control of glycemic index of food products. Furthermore, the present results demonstrate that the methanolic extract from *C. grandis* fruit, methanolic extracts of callus and methanolic extract of Callus-BAP+NB₆ contained potent α -glucosidase, α -amylase inhibitors and were effective for suppressing post-prandial hyperglycemia (Fig. 2).

Amylase inhibition assay: The result of agar diffusion amylase inhibition assay indicated that methanolic extract of callus show maximum inhibition compare to the fruit extract and fresh callus showed no inhibition (Table 2). Gulati *et al.* (2012) reported the preliminary agar diffusion amylase inhibition assays indicated that all of the Australian aboriginal plant extracts showed complete inhibition of α -amylase enzyme such that no hydrolysis of starch was evident.

Antioxidant activity

Antioxidant activity of methanolic extract of *Coccinia grandis*

DPPH Method (1, 1 diphenyl 2, picryl hydrazyl): DPPH assay method is based on the reduction of methanolic solution of color free radical DPPH by free radical scavenger. The procedure involves the measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC₅₀. Based on the data it can be concluded that methanolic extract of callus powder showed greater activity compared to the methanolic extract of fruits powder (Fig. 3). The activity of these two extracts is compared with standard ascorbic acids. The activity of methanolic extract is comparatively similar with standard ascorbic acid. The methanolic extract of callus showed more activity than the methanolic extract (Fig. 3).

Table 2: Amylase inhibition assay of *Coccinia grandis*

| Extracts | Zone of inhibition (cm) | Inhibition (%) |
|--------------|-------------------------|----------------|
| Control | 3.0 | 0.00 |
| Fruit | 2.1 | 30.00 |
| Fresh callus | 2.9 | 3.30 |
| Callus | 1.6 | 46.66 |

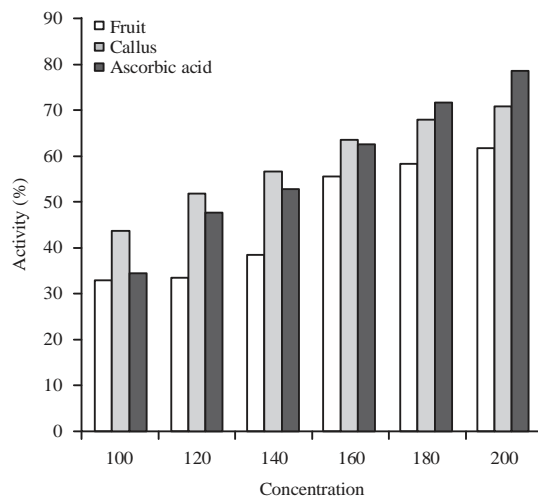


Fig. 3: DPPH activity of methanolic extract of fruit, callus and ascorbic acid

Rawri *et al.* (2013) reported the alcoholic leaves extract of *C. grandis* dose dependently demonstrated antioxidant potentials by DPPH scavenging activity. The DPPH scavenging potential of extract might be due to its reducing actions, which might donate hydrogen to a free radical, reducing it to non-reactive species. The presence of reeducates are responsible for reducing capacity, which involved in prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging (Rawri *et al.*, 2013). *Coccinia grandis* also showed the DPPH radical scavenging activity and significantly decreased the formation of oxygen radicals generated in rat peritoneal macrophages. The DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts (Nanjo *et al.*, 1996). The DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the fractions in a concentration-dependent manner. The reduction in the number of DPPH molecules can be correlated with the number of available hydroxyl groups. All the fractions showed significantly higher inhibition percentage (stronger hydrogen donating ability) and positively correlated with total phenolic content.

CONCLUSION

The result of the present investigation on *Coccinia grandis* is a perennial creeper, described as 'Indian substitute for Insulin' for *in vitro* shoot multiplication and assessment of antidiabetic and antioxidant activity. Maximum shoot formed on MS medium containing 0.5 mg L⁻¹ BAP combination with 0.2 mg L⁻¹ NB₆. Also, after sub culturing elongation of shoot was observed. Thus, present protocol showed more efficient for more number of shoot produce with short time and more survival rate.

Methanolic extract of callus of *C. grandis* possesses significant antidiabetic activity. Methanolic extract from *C. grandis* fruit, methanolic extracts of callus and methanolic extract of callus BAP+NB₆ contained potent α -glucosidase, α -amylase inhibitors and were effective for suppressing post-prandial hyperglycemia. The DPPH assay is one of the most widely used methods for screening antioxidant activity of *C. grandis* also showed the DPPH radical scavenging activity and

significantly decreased the formation of oxygen radicals generated in rat peritoneal macrophages plant extracts. The protocol described here is simple efficient with high regeneration frequency and can be employed for the large scale production and genetic manipulation for antidiabetic medicinal plant of *C. grandis*.

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REFERENCES

- Abu-Romman, S., M. Suwwan and E.A.D. Al-Ramamneh, 2013. The influence of plant growth regulators on callus induction from hypocotyls of cucumber (*Cucumis sativus* L.). Adv. Environ. Biol., 7: 339-343.
- Arulanandam, L.J.P. and S. Ghanthikumar, 2011. Short somnunications: Indirect organogenesis of *Vitex trifolia* L-An important medicinal plant. Indian J. Nat. Prod. Resour., 2: 261-264.
- Arulanandam, L.J.P., S.G. Kumar and M. Sowmini, 2011. Micropropagation and conservation of rare medicinal plant *Wattakaka volubilis* (Linn.) stapf. Indian J. Biotechnol., 10: 238-241.
- Ashwini, M., N. Lather, S. Bole, A.B. Vedamutrhy and S.A.M. Balu, 2012. *In vitro* antioxidant and anti inflammatory activity of *Coccinia grandis*. Int. J. Pharm. Pharmaceut. Sci., 4: 239-242.
- Bhattacharya, P. Pal, A. Lalee, D.K. Mal and A. Samanta, 2011. *In vivo* and *in vitro* anticancer activity of *Coccinia grandis* (L.) Voigt. (Family: Cucurbitaceae) on Swiss albino mice. J. Pharm. Res., 4: 567-569.
- Doss, A. and R. Dhanabalan, 2008. Anti-hyperglycaemic and insulin release effects of *Coccinia grandis* (L.) voigt leaves in normal and alloxan diabetic rats. Ethnobotanical Leafli., 12: 1172-1175.
- Emmanuel, S., S. Ignacimuthu and K. Kathiravan, 2000. Micropropagation of *Wedelia calendulacea* Less., a medicinal plant. Phytomorphology, 50: 195-200.
- Farrukh, U., H. Shareef, S. Mahmud, S.A. Ali and G.H. Rizwani, 2008. Antibacterial activities of *Coccinia grandis* L. Pak. J. Bot., 40: 1259-1262.
- Ghanthikumar, S., L.J.P. Arulanandam and L.H. Joseph, 2013. Micropropagation of *Coccinia indica* wight and Arn: A medicinal plant. Int. J. Applied Bioresearch, 18: 1-4.
- Gomathi, D., M. Kalaiselvi and C. Uma, 2012. *In vitro* α -amylase and α -glucosidase inhibitory effects of ethanolic extract of *Evolvulus alsinoides*. Int. Res. J. Pharm., 3: 226-229.
- Gulati, V., I.H. Harding and E.A. Palombo, 2012. Enzyme inhibitory and antioxidant activities of traditional medicinal plants: Potential application in the management of hyperglycemia. BMC Complement. Altern. Med., Vol. 12. 10.1186/1472-6882-12-77
- Hanif, R., Z. Iqbal, M. Iqbal, S. Hanif and M. Rasheed, 2006. Use of vegetables as nutritional food: Role in human health. J. Agric. Biol. Sci., 1: 18-20.
- Hussain, A., S. Wahab, A. Rizvi and M.S. Hussain, 2011. Macroscopical, anatomical and physico-chemical studies on leaves of *Coccinia Indica* Wight and Arn., growing wildly in eastern Uttar Pradesh region of India. Indian J. Nat. Prod. Resour., 2: 74-80.
- Jiju, V.I., C. Samuel, N.S. Thomas, M.M. Sabu and D.T. Vasudevan, 2013. The inhibitory effect of *Carica papaya* leaf extracts on alpha amylase. Universal J. Pharm., 2: 135-139.

- Karim, M.A. and S.U. Ahmed, 2010. Somatic embryogenesis and micropropagation in teasle gourd. *Int. J. Environ. Sci. Dev.*, 1: 10-14.
- Khatun, S., F. Pervin, M.R. Karim, M. Ashraduzzaman and A. Rosma, 2012. Phytochemical screening and antimicrobial activity of *Coccinia cordifolia* L. *Plant. Pak. J. Pharm. Sci.*, 25: 757-761.
- Kuriyan, R., R. Rajendran, G. Bantwal and A.V. Kurpad, 2008. Effect of supplementation of *Coccinia cordifolia* extract on newly detected diabetic patients. *Diabetes Care*, 31: 216-220.
- Lampe, J.W., 1999. Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nutr.*, 70: 475s-490s.
- Loizzo, M.R., A.M. Saab, R. Tundis, F. Menichini and M. Bonesi et al., 2008. *In vitro* inhibitory activities of plants used in Lebanon traditional medicine against Angiotensin Converting Enzyme (ACE) and digestive enzymes related to diabetes. *J. Ethnopharmacol.*, 119: 109-116.
- Mazumder, P.M., D. Sasmal and R.A. Nambi, 2008. Antiulcerogenic and antioxidant effects of *Coccinia grandis* (Linn.) Voigt leaves on aspirin-induced gastric ulcer in rats. *Nat. Prod. Radiance*, 7: 15-18.
- Moideen, K., S.H. Sherief, S. Sengottuvelu and T. Sivakumar, 2011. Hepatoprotective and antioxidant activity of *Coccinia grandis* root extract against paracetamol induced hepatic oxidative stress in wistar albino rats. *Int. J. Res. Ayurveda Pharm.*, 2: 858-863.
- Munasinghe, M.A.A.K., C. Abeysena, I.S. Yaddehige, T. Vidanapathirana and K.P.B. Piyumal, 2011. Blood sugar lowering effect of *Coccinia grandis* (L.) J. Voigt: Path for a new drug for diabetes mellitus. *Exp. Diabetes Res.* 10.1155/2011/978762
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*, 15: 473-497.
- Mustafa, M., T.N. Swamy, S. Raju and S.K.P. Mohammad, 2013. Multiple shoot induction from the nodal cultures of teasle gourd (*Momordica dioica* Roxb.). *Int. J. Biosci.*, 3: 8-12.
- Nabi, S.A., M.M. Rashid, M. Al-Amin and M.G. Rasul, 2002. Organogenesis in teasle gourd (*Momordica dioica* Roxb.). *Plant Tissue Cult.*, 12: 173-180.
- Nair, S.S., V. Kavrekar and A. Mishra, 2013. *In vitro* studies on α amylase and α glucosidase inhibitory activities of selected plant extracts. *Eur. J. Exp. Biol.*, 3: 128-132.
- Nandi, B.K. and L. Bhattacharjee, 2005. Why fruits and vegetables? Their contribution to improving nutrition in developing countries. *FAO Regional Office for Asia and the Pacific*, March 2005, Bangkok, Thailand, pp: 1-14.
- Nanjo, F., K. Goto, R. Seto, M. Suzuki, M. Sakai and Y. Hara, 1996. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radic. Biol. Med.*, 21: 895-902.
- Pekamwar, S.S., T.M. Kalyankar and S.S. Kokate, 2013. Pharmacological activities of *Coccinia grandis*: Review. *J. Applied Pharmaceut. Sci.*, 3: 114-119.
- Rawri, R.K., K. Bharathi, K.N. Jayaveera and S.M.B. Asdaq, 2013. In vitro antioxidant profile of alcoholic extract of *Coccinia grandis* leaves. *Int. J. Pharm. Technol.*, 5: 5434-5440.
- Sama, K., K. Murugesan and R. Sivaraj, 2012. *In vitro* α amylase and α -glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. *Asian J. Plant Sci. Res.*, 2: 550-553.
- Satheesh, S.L. and K. Murugan, 2011. Antimicrobial activity of protease inhibitor from leaves of *Coccinia grandis* (L.) Voigt. *Indian J. Exp. Biol.*, 49: 366-374.
- Shaheen, S.Z., K. Bolla, K. Vasu and M.A.S. Charya, 2009. Antimicrobial activity of the fruit extracts of *Coccinia indica*. *Afr. J. Biotechnol.*, 8: 7073-7076.

- Sivaraj, A., B.P. Jenifa, M. Kavitha, P. Inbasekar, B. Senthilkumar and A. Panneerselvam, 2011. Antibacterial activity of *Coccinia grandis* leaf extract on selective bacterial strains. *J. Applied Pharm. Sci.*, 1: 120-123.
- Starr, F., K. Starr and L. Loope, 2003. *Coccinia grandis*. United States Geological Survey-Biological Resources Division Haleakala Field Station, Maui, Hawaii, pp: 4-10.
- Sudha, P., S.S. Zinjarde, S.Y. Bhargava and A.R. Kumar, 2011. Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants. *Complementary Altern. Med.*, Vol. 11. 10.1186/1472-6882-11-5
- Thakur, G.S., M. Pandey, R. Sharma, B.S. Sanodiya, G.B.K.S. Prasad and P.S. Bisen, 2011. Factors affecting in vitro propagation of *Momordica balsamina*: A medicinal and nutritional climber. *Physiol. Mol. Biol. Plants*, 17: 193-197.
- Thiripurasundari, U. and M.V. Rao, 2012. Indirect organogenesis from nodal explants of *Coccinia grandis* (L.) Voigt. *Indian J. Biotechnol.*, 11: 352-354.
- Ugandhar, T., M. Venkateshwarlu, G. Begum, T. Srilatha and K. Jaganmohanreddy, 2011. In vitro plant regeneration of Cucumber (*Cucumis sativum* (L.) from cotyledon and hypocotyl explants. *Sci. Res. Rep.*, 1: 164-169.
- Yadav, G., A. Mishra and A. Tiwari, 2010. Medical properties of ivy gourd (*Cephalandra indica*): A review. *Int. J. Pharm. Res. Dev.*, 9: 92-98.
- Zhang, W., 2009. Establishment of tissue culture regeneration and receptor regeneration system of two species Phlox. Master Thesis, University of China.