



SCIENTIFIC EVENTS GATE

The International Innovations Journal of Applied Science

Journal homepage: <https://ijas.eventsagate.org/ijas>

ISSN: 3009-1853 (Online)



Treatments of Coleus (Plectranthus Barbatus) that increase rooting to get larger crude forskolin extract

Abo- El-Enin H. I ^{1*} and El-Yamany A. O. ²

^{1,2} Plant physiology branch, Agric. Botany Dept., Fac.Agric., Al-Azhar University, Cairo -Egypt
<https://orcid.org/0000-0001-6079-9414>

ARTICLE INFO

ABSTRACT

Article history:

Received 18 Nov. 2023

Revised 26 Jan 2024,

Accepted 8 Feb 2024,

Available online 15 Mar. 2024

Keywords:

Forskolin

Tissue culture

Phytohormones

Microelements

The aim is study of effect of some phytohormones and microelements in a tissue culture system to extract forskolin by different solvents from hairy roots in *Plectranthus barbatus* Andr. (Syn.*Coleus forskohlii* Briq.). Our results indicated that, the best parameters for increasing the number and length of roots were by indole butyric acid. Low concentrations of boron and silicon also promote rooting of *Coleus* cuttings. Methanol is a more efficient solvent than ethanol and ethyl acetate in forskolin extraction. Root hairs of the terminal cuttings of *Coleus forskohlii* appear after treatment with bacterial inoculum *Agrobacterium rhizogenes*. Forskolin levels increased to 13 and 5.3 mg/L in hair roots after 4 weeks of incubation. Forskolin extraction from tissue culture media is more efficient (40 mg/L per g wet weight) compared to extraction from root cuttings (13 mg/10 g wet weight).

1- Introduction

Plectranthus barbatus Andrews (syn. *Coleus forskohlii* Briq.), a genus of over 500 worldwide species, belongs to the Lamiaceae family (Paton et al., 2018). Its ornamental value comes from its gorgeous speckled leaves. *Coleus forskohlii*, a perennial plant that originated in India and spread to Saudi Arabia, East Africa, Ethiopia, Brazil, and the subtropical regions of Asia, including Nepal, Thailand, and Myanmar, is one of these species (Lock & Gaur, 2001).

The majority of this plant's crop consists of its thick, tuberous, radially spreading roots, which have a golden hue

and measure 20 cm in length and 0.5–2.5 cm in diameter. After roughly 160 days after sowing, they are gathered in the autumn (Mastiholi, 2013). Only root cork contains the diterpene forskolin (Ammon & Müller, 1985), which is used in Ayurvedic medicine to treat a variety of ailments like heart disease, respiratory illnesses, and (Mir, 2015). In Ayurvedic medicine, the roots have long been used to treat intestinal spasms, convulsions, heart and lung diseases, and insomnia. Forskolin stimulates the release of adenosine monophosphate (cAMP), which is an intracellular signaling transmitter that regulates a number of biological processes, including increasing basal metabolic rate and promoting weight reduction (Mitra et al., 2020).

* Corresponding author.

E-mail address: abdelrhmanelyamany@azhar.edu.eg
(A. EL-Yamany)

Forskolin concentrations in the sample varied from 0.01% to 1% per gram of dry weight (Misra et al., 2016). The blooms are a rich source of nectar for bee honey, the roots can be pickled when combined with mustard (Valdes et al., 1987), the powdered root is used as a spice in India, and the paste can be applied externally to wounds (Kotia et al., 2014)

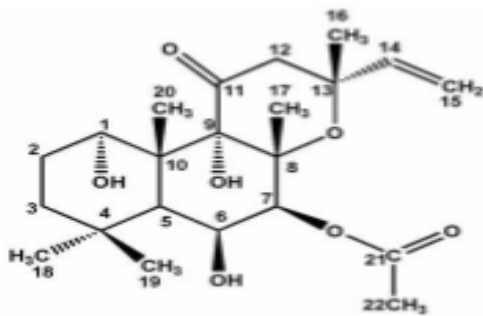


Figure 1: *Coleus forskohlii* Briq plant and forskolin structure (This figure was uploaded by Arti Kumari. Content may be subject to copyright).

Auxin treatment of cuttings encourages the production of the longest and most roots possible (Blakesley et al., 1991). Plant roots that are subjected to stress produce more secondary metabolites (Chandran et al., 2020). One example of this is when bacteria or endophytic fungi are introduced into *Coleus* roots (Das et al., 2012; Mastan et al., 2020). Hairy roots high in forskolin are produced by *Agrobacterium rhizogenes* or *Agrobacterium tumefaciens* -infected roots (Shanks & Morgan, 1999). Over the past 20 years, researchers have become interested in studying hairy roots in an effort to produce significant amounts of secondary metabolites (Choudhary et al., 2019). When treated with auxins, tissue cultures produce more roots and are a good way to extract secondary metabolites commercially (Mersinger et al., 1988).

Forskolin is present in very small amounts in the stem and is absent from the leaves (Abdul Shukkoor et al., 2005); on the other hand, it is abundant in flavonoids and phenolic chemicals (Awasthi et al., 2016). According to Malleswari et al. (2013), Forskolin contains anti-fungal, anti-bacterial, and anti-insect properties (Krishnamoorthy & Ramaswamy, 2015; Nayini et al., 2020). Plant breeders strive to develop the largest crop from the roots in order to extract the largest quantity of forskolin because *Coleus* is one of the herbaceous plants whose stem or leaf cuttings are easily rooted (Tiwari & Bilaspur, 2010).

The Egyptian Desert Research Center at Mataria, Cairo brought *Coleus forskohlii* seedlings in June 2023 AD from Thailand (Veeresham et al., 2012). The research aims to conduct a local study on the treatments that encourage the rooting of the plant cuttings in order to obtain the largest crude extract of forskolin.

2- Material and Methods

The experiments conducted on *Coleus* cuttings in this paper with tissue culture design were using some phytohormones and microelements to encourage the emergence and growth of roots on *Coleus* cuttings. Then treated it with bacterial inoculum to increase the growth of root hairs and cultivating them using different media to obtain the largest amount of forskolin and extracted by some different solvents.

2.1 Effect of phytohormones on rooting amount of *Coleus forskohlii*

The viable shoot tips of *Coleus* cuttings (as explants from 4-month-old were acquired from the Egyptian Desert Research Centre in Mataria, Cairo.) were with length between 3-6 inches (or long enough) to stand straight up in water, and two leaves and a nodular tip. Following surface sterilization, three rounds of sterilising with water and

clorox 10% at 10-20 minutes according to (Belniaki et al., 2018). The cuttings were treated with solutions containing four distinct hormones: gibberellic acid (GA₃/USA), naphthalene acetic acid (NAA/USA), indole butyric acid (IBA/India), and indole acetic acid (IAA/ Thailand). The solutions were applied in two different doses (1000 and 5000 mg/l). The cuttings are put into 250 ml sterile glass vials (Ks-Tek) that have plastic closures on them and contain 30 ml of a hormonal solution, pH of each solution was adjusted to 5.7. The sterile hormonal solutions are filtered into the sterile bottles using a double 0.2 µm filter unit (blue). In addition to a hormone-free group as a control treatment, eight hormone administration groups were established. Three replicates of each treatment were made, with one cut in each copy. Every glass container was stored in a laboratory setting. After 30 days of treatments, by extracting roots immediately and measuring numbers and length of root by core method to measure rooting.

2.2- Effect of microelements on rooting amount of *Coleus forskohlii*

Coleus cuttings are cultivated similarly to the preceding experiment. Using Murashige & Skoog (MS-Netherlands) salts as the basal media, the effects of microelements on the in vitro growth of *Coleus* cuttings were examined. Three doses (20, 40, and 80 g/l) of silicon (as potassium silicate) and three concentrations (100, 150, and 200 mg/l) of boric acid were employed. Every glass container was stored in a laboratory setting. The core method, which involves extracting roots and measuring root length immediately after 15 and 30 days of planting, has been used to measure roots.

2.3 Production of hairy roots in *Coleus forskohlii* tip cuttings

The approach of involves incubating *Coleus* explants (tip cuttings)

on MS-based solid medium at half-power (MS/2) two days prior to inoculation with *Agrobacterium tumefaciens* bacteria (Gai et al., 2015) The bacterial strain cultivates in Luria-Bertani (LB/ Sigma-Aldrich-USA) liquid medium over night at 28±1°C. Then it shaken (Table orbital laboratory shaker 600x480mm 250 rpm) at 180 rpm. Centrifugation (Centrifuge MSLZL43- 4000 rpm) at 2000 rpm for 10 minutes is used to collect the bacterial cells, which are then suspended in a liquid medium based on MS/2 contains 3.0% vitamins and 1 mM arginine and 125 µmol acetosyringone (this compound allows for higher conversion efficiency in epigenetic transformation procedures) for inoculation (Schrammeijer et al., 2000). Then dried on sterile filter paper for 6 min. After two days of co-cultivation, the bacteria were transferred to a solid, hormone-free medium containing a broad-spectrum antibiotic Cefotaxime sodium (300 mg/L) to eliminate residual bacteria, and incubated in the dark at 25±1°C. Explants were immersed for overnight and incubated in the dark at 25±1°C into the suspension of *A. tumefaciens*. Subsequently, the initials of the root are isolated from the cuttings and subcultures every two weeks. *Coleus forskohlii* hairy root sample (10 g fresh weight) is taken to extract forskolin by different solvents used in the study.

2.4 *Coleus* explant tissue cultures

According to (Praveena et al., 2012). *Coleus forskohlii* cuttings are used to form the callus, because it is richer in parenchyma cells than others. Superficially sterilize with 0.5 % sodium hypochlorite for 2 minutes. Then wash it several times with sterile distilled water, and immerse in alcohol 2 minutes, then

wash it again with sterile distilled water several times. MS medium was prepared and adjust the pH at 6.5. Sterilized the medium in an autoclave and the molten medium is poured into tubes (25 x 125 mm) at a rate of 15 ml per tube. The plugged culture tubes were sterilized in an autoclave at 121°C for 20 minutes and cooled to room temperature. Indole butyric acid (1mg /l) and benzyl amino purine (0.5 mg /l) were added to the basal medium. The tubes are inoculated with superficially sterilized *Coleus* tissues and incubate it for 4 weeks at laboratory temperature. The formation of the callus and root growth is monitored.

2.5 Evaluation of the use of different solvents for extraction of forskolin from *Coleus* roots

10g fresh weight of *Coleus forskohlii* roots is obtained, after giving the roots a thorough water wash, and sliced into 0.5 cm thick pieces and placed within a polyethylene-lined hessian bag. To preserve the maximum quantity of forskolin, slides are mechanically dried at 40 °C and then stored with less than 12% humidity (Rajagam, 2005). To lower the moisture content, use a grinder to grind the dried roots. Solvents are applied to the roots in a 1:4 or 1:6 (raw material: solvent) ratio in accordance with established protocols given by (Weiser, 1959; Harborne, 1984). A half-hour at room temperature (20 ± 1 °C) is spent extracting 5 g of the homogenized sample using 30 ml of the suitable solvent in an Erlenmeyer flask (Rocwing Borosilicate Glass Conical Flask Erlenmeyer Graduated Boro 3.3 Lab – UK) with a magnetic stirrer (JOAN LAB HS-5C- 200-1500 rpm, Co., Ltd.) at 700 rpm, then filter the samples using filter paper (WHATMAN Brand No. 1 filter papers 89 pcs, with box 125 mm diameter) the extract. Three times the extraction procedure was carried out. The six pure solvents that were purchased from Misr Chemical Industries Co. for trading drugs,

chemicals & medical supplies-Egypt. represented the three different polar groups: the polar group (ethanol, methanol, and water), the intermediate polar group (ethyl acetate and chloroform), and the non-polar group (petroleum ether). The solvent is used to wash the wet cake multiple times. The extract is then divided. The crude forskolin is weighed after the solvent has evaporated.

2.6 Comparison of hairy root and tissue culture treatments in crude forskolin production

Ten grams fresh weight of *Coleus* roots from each of tissue cultures and hairy roots were prepared to forskolin extraction. The biomass is treated with solvents in a ratio of 1:4 (raw material: solvent) according to standard protocols provided by Harborne, (1984) and Sofowora, (1993). The solvent is evaporated and the crude forskolin weighed.

3- Results and Discussion

3.1 Effect of auxins on rooting amount of *Coleus forskohlii*

The roots that grew on *Coleus* cuttings were noted and shown in Figure 1 following a course of treatment lasting two to four weeks. The roots were more numerous when auxins were present than when they weren't. As auxin concentration increases, roots become longer and more numerous (except for IAA). The best possible care was given when IBA, IAA, and NAA were present.

According to Belniaki, (2018) apical leaves are essential for *Coleus* stem cuttings to root in this regard. According to Villar & Gaget-Faurobert, (1997) the following processes might lead to the creation of roots in plant cuttings treated with auxins: the elongation and division of cells, the production of new protein, the accumulation of metabolites in lieu of

addition, and the formation of the callus.

Hartmann et al., (1997) reported that IBA's activity is widely known because it has long been the preferred auxin for root formation on cuttings, stimulating the growth of lateral roots while inhibiting primary root elongation. Several studies have offered explanations for the rise in rooting when IBA is present. For instance, IBA might be boosting the vascular cambium's activity, which would raise the amount of RNA in cuttings and promote cell division when the rooting process is at its peak (Al-Samurai, 2009). The low level of auxin in cuttings combined with an increase in inhibitory substances may be the cause (Karakurt et al., 2009). Additionally, IBA treatment promotes

Furthermore, compared to intermediate or peripheral cuttings, the best rooting rate (97%) occurs when Bougainvillea basal stem cuttings are treated with IBA (2000 ppm) (Eed et al., 2015). Lower auxin concentrations cause the IAA effect and lead to the expansion of Coleus callus, whereas greater auxin concentrations cause adventitious root regeneration (Balasubramanya et al., 2012). They also said that roots were discovered by Brian et al., (1960) after IBA encouraged rhizogenesis.

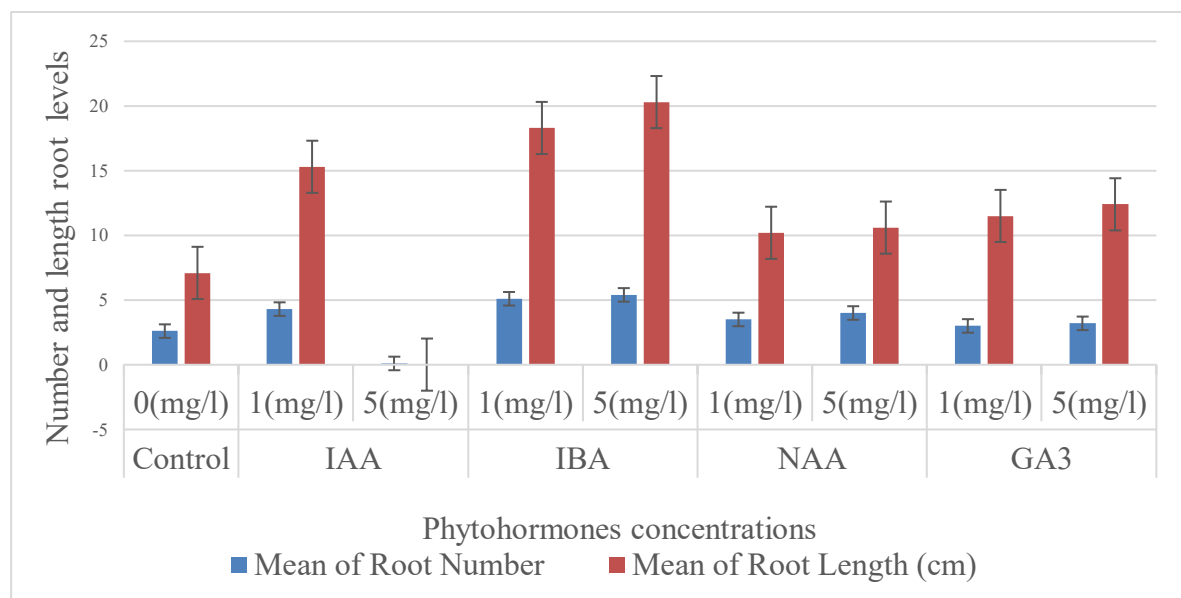


Figure 2:Effect of phytohormones on rooting amount of *Coleus forskohlii*

IAA and NAA. IAA's capacity to regulate the rate of cell expansion is what initially drew attention to it, auxin encourages cell elongation in stems and coleoptiles, although it predominantly inhibits it in roots (Jiang et al., 2017). Regarding the effect of gibberellic acid to promote the extension of pea and bean stem cuttings while inhibiting

3.2 Effect of microelements on rooting amount of *Coleus forskohlii*

Coleus cutting rooting is enhanced by low concentrations of both silicon and boron (Figure 3), although boron is more effective. For cutting roots, the high quantities of silicon (200 mg/l) were not appropriate. These findings are in line with those of Al-Yasari, (2014), who found that plants are hazardous to 200–400 mg/l of boron. Additionally, wheat seedlings were negatively affected by silicon oxide concentrations exceeding 200 mg/l (Karimi & Mohsenzadeh, 2016). Boron may have

an impact on the regulation of the following processes: (1) development and differentiation of the entire plant; (2) membrane permeability and sugar transport; and (3) enzymes that are involved in the metabolism of sugars, lignin, auxins, phenolics, and nucleic acids (Lewis, 1980). He continued by saying that inadequate lignification and xylem differentiation are caused by boron deficit. Many researchers have discussed the function of boron in cutting rooting; boron stimulates the rooting of *Clematis* cuttings in two recent investigations (Weiser, 1959). The incorporation of boron into IBA rooting powder yielded a significant and useful advantage by augmenting the quantity and calibre of rooted cuttings. Additionally, boron can control

endogenous auxin levels during root formation, and providing boric acid to *Aloysia* cuttings is essential for the growth and development of main roots (Jarvis et al., 2006; Stefanini et al., 2004). According to Muhammad et al., (2013) boron is involved in controlling the levels of some antioxidants such phenols and auxins.

Adding silicate fertilizer at a mean of 40 g/L produced the longest root length, the highest plant height, and the fresh and dry root weights in both kalanchoe and carnation cuttings, indicating the role of silicon in cutting rooting (Bae et al., 2010). According to Hu et al., (2019), all poinsettia cultivars benefited

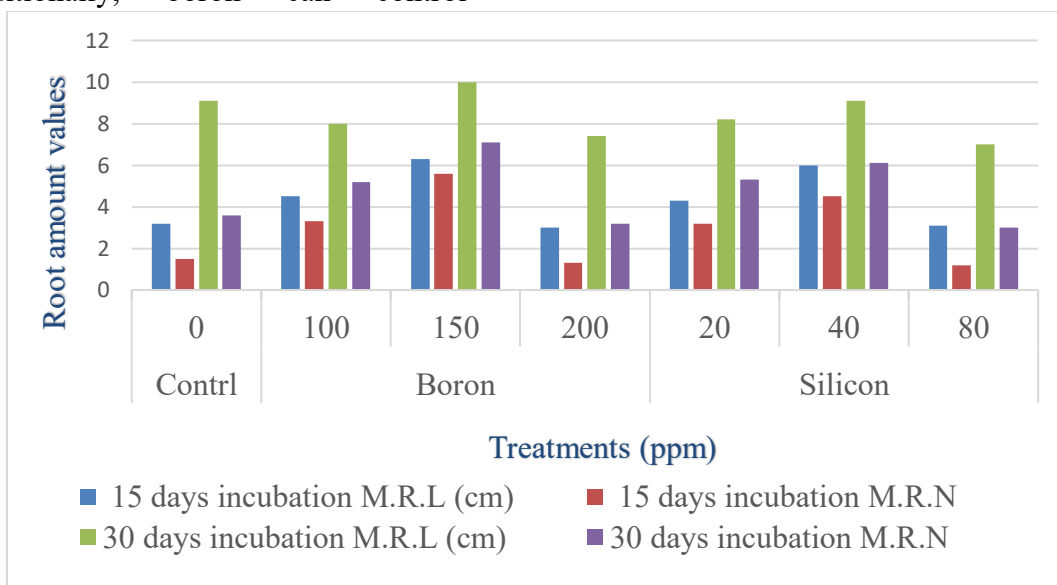


Figure 3: Effect of three concentrations of boron and silicon on root number (M.R.N) and root length (M.R.L) of *Coleus* cuttings after 15 and 30 days incubation under laboratory conditions.

from silicon treatment (50 ppm as potassium silicate) in terms of increased root count, longest root length, fresh root weight, and dried root weight.

According to Hu et al., (2019), silicon has been shown to encourage the growth of roots in a variety of plant species.

3.3 Evaluation of the use of different solvents for extraction of forskolin from *Coleus* roots

Figure 4 shows how well forskolin can be extracted using different solvents. The results clarified the use of several solvents (0.15–2.5 mg/l) for the extraction of forskolin. Methanol is a more efficient solvent than ethanol and ethyl acetate for the extraction of forskolin. Forskolin concentrations in water, petroleum ether, and chloroform were 0.15, 0.25, and 0.31 mg/l, respectively. This result is consistent with the findings of Singh & Suryanarayana, (2019), who discovered that methanol is more effective than ethanol and water in extracting

forskolin, and the majority of terpenoids are hydrophobic and difficult to dissolve in water (Pandey et al., 2014; Singh & Suryanarayana, 2019), also reported that ethanol is superior to chloroform in this regard. When 2.0 mol/dm³ aqueous sodium cumenesulfonate solutions were utilized, *Coleus* roots gave over 80% of the 50% pure forskolin; shaking extraction is preferable than reflux extraction in this regard (Sultana et al., 2009).

3.4- Production of hairy roots in *Coleus forskohlii* tip cuttings

Within 2-4 weeks of an *Agrobacterium rhizogenes* infection, hairy roots of *C. forskohlii* tip cuttings

emerge at wound sites. After the first 3 to 4 cm long shoot emerged from the nodal region, roots began to sprout from the node's base within 12 days. After four weeks, the hairy roots showed a high concentration of forskolin (13 mg/10g f.w.). *C. forskohlii* hairy roots were caused by an *Agrobacterium rhizogenes* strain infection. Week 2 marked the beginning of rapid growth, which lasted until week 5 (Sasaki et al., 1998). The fifth week produced the highest forskolin yield (1.45 mg/g f.w.), while the sixth week saw a decrease in forskolin content.

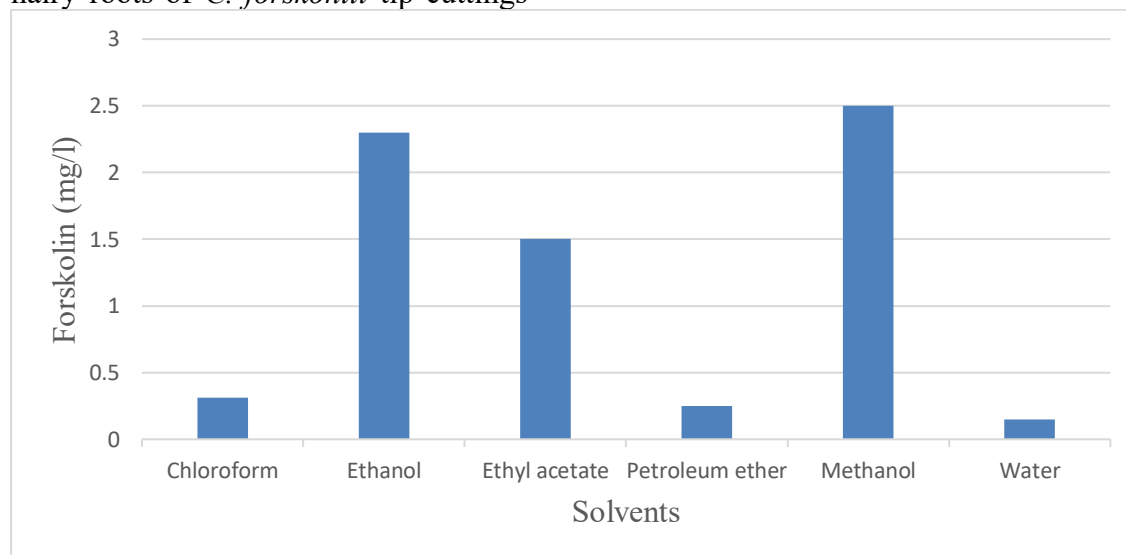


Figure4: Efficiency of different solvents in extracting forskolin from *Coleus*

additionally, by Pandey et al., (2014) who reported that the nodal stem portion of *C. forskohlii* infected with *Agrobacterium* responded with enormous amounts of root production, and by Veeresham & Chitti, (2013), who enhanced the production of forskolin from the hairy roots by keeping them in a medium containing antibiotic Cefatoxine (500 mg/l). According to Shanks & Morgan, (1999) hairy roots have a high consistent productivity under hormone-free circumstances, making them ideal for producing essential metabolites that are hard to synthesise in natural conditions.

Additionally, hairy roots have been studied for the past 20 years for the synthesis of significant secondary metabolites, which has opened the door for their economic utilisation, according to Guillon et al., (2016).

3.5- *Coleus* explant tissue cultures

Upon incorporating IBA and BAP into the (MS) basal medium, each *Coleus* explant demonstrated its maximal growth potential. After incubating for four weeks, forskolin reached 5.3 mg/g f.w. This result is in line with that of Tripathi et al. (1995), who obtained the maximum forskolin concentration (7.5 mg/g d.w.) from a

medium containing 0.5 ppm IBA and 0.5 ppm glycine, 200 ppm casein hydrolysate, 0.7 ppm sucrose, and 5 ppm glycine. Accordingly, plant hormones have a major impact on the synthesis of forskolin in *Coleus* tissue cultures (Choudhary et al., 2019). Additionally, tissue cultures are an appropriate technique for the commercial generation of secondary metabolites, according to (Veeresham & Chitti, 2013).

3.6- Comparison of hairy root and tissue culture treatments in crude forskolin production

Forskolin production from tissue cultures is higher than that from hairy root cultures (13 mg/ 10 g fresh biomass) at 40 mg/10 g. The results of Sasaki et al., (1998) and Choudhary et al., (2019) support this result, showing that *C. forskohlii* tissue cultures yield higher levels of forskolin (5.60 mg / g f.w.) than hairy root cultures (1.45 mg / g f.w.).

4. Conclusions

The speckled leaves of *Coleus forskohlii* are what give it its decorative significance. This plant's crop is primarily its roots, and the only place to find the diterpene forskolin (which has antibacterial properties) is in the root cork. Ayurvedic medication for treating a range of conditions, such as respiratory issues, heart problems, and sleeplessness. In two to four weeks, the roots developed on *Coleus* cuttings. With the exception of IAA, roots grow longer and more numerous as auxin concentration rises. When IBA, IAA, and NAA were present, the optimal treatment was provided. Low quantities of silicon and boron both improve the rooting of *Coleus* cuttings. In terms of extraction, methanol is a more effective solvent than ethanol and ethyl acetate. Forskolin extraction concentrations ranged from 0.15 to 2.5 mg/l. Within 2-4 weeks of an *Agrobacterium rhizogenes*

infection, hairy roots of *C. forskohlii* tip cuttings emerge at wound sites.

5. References

- Abdul Shukkoor, M., Dhasan, P., & Rafiullah, M. (2005). Isolation of forskolin from stem of *Coleus forskohlii*. *Pharmacognosy Magazine*, 1(3). DOI: [10.1007/978-3-642-22144-6-177](https://doi.org/10.1007/978-3-642-22144-6-177)
- Al-Samurai, S. M. (2009). Effect of the date of implantation and the acid of the indole butyric acid (IBA) in the rooting of the mind of henna plant, *Lawsoina inermis* L. *Basra Research Journal (Operations)*, 35 (6): 85-91. (In Arabic).
- Al-Yasari, K.A., (2014). Physiological, Biochemical and Anatomical study of plants differing in their tolerance to boron toxicity and its recovery by zinc salts, in terms of rooting response. PhD thesis. Kerbala University, 210 pp. <https://doi.org/10.1063/1.5123111>
- Ammon, H. & Müller, A. (1985). Forskolin: from an ayurvedic remedy to a modern agent. *Planta Med.*, 51(6):473-477. DOI: [10.1055/s-2007-969566](https://doi.org/10.1055/s-2007-969566)
- Awasthi, P., Mahajan, V., Jamwal, V., Kapoor, N., Rasoo, S., Bedi, Y., Sumit, G., & Gandhi, S. (2016). Cloning and expression analysis of chalcone synthase gene from *Coleus forskohlii*. *J. of Genetics*, 95 (3): 647- 657. DOI: [10.1007/s12041-016-0680-8](https://doi.org/10.1007/s12041-016-0680-8)
- Bae, M., Park, Y. & Jeong, B. (2010). Rooting and subsequent growth of potted plants. *Horticulture, Environment and Biotechnology*, 51(5): 355-359.
- Balasubramanya, S., Rajanna, L., & Anuradha, M. (2012). Effect of plant growth regulators on morphogenesis and forskolin production in *Coleus forskolin* (*Plectranthus barbatus*). *In vitro cell Dev. Biol. Plant*, (48): 208-215. DOI: [10.1007/s11627-011-9417-9](https://doi.org/10.1007/s11627-011-9417-9).
- Belniaki, A., Rabel, L., Gomes, E., & Ribas, K. (2018). Does the presence of leaves on *Coleus* stem cuttings influence their rooting? *Ornamental Horticulture*, 24(3): 206. DOI: [10.14295/oh.v24i3.1204](https://doi.org/10.14295/oh.v24i3.1204)
- Blakesley, D., Weston, G., & Hall, J. (1991). The role of endogenous auxin in root

- initiation. *Plant Growth Regul.*, 10:341-353. DOI: [10.1007/BF00207595](https://doi.org/10.1007/BF00207595)
- Brian, P., Hemming, H., & Lowe, D. (1960). Inhibition of rooting of cuttings by gibberellic acid. *Ann. Bot.*, N.S. 24, 407–419.
<https://doi.org/10.1093/oxfordjournals.aob.a083714>
- Chandran, H, Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology Reports*, 26: e00450. DOI: [10.1016/j.btre.2020.e00450](https://doi.org/10.1016/j.btre.2020.e00450)
- Choudhary, A., Upadhayaya, S., Sharma, A., & Raidas, D. (2019). Effect of plant growth hormones on growth, tuber character and forskolin content of patherchur (*Coleus forskohlii* Briq). *J. of Pharmacognosy and Phytochemistry*, 8 (2): 686-688.
- Das, A., Kamal, S., Shakil, N., Sherameti, I., Oelmüller, R., Dua, M., Tuteja, N., Johri, K., & Varma, A. (2012). The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. *Plant Signal Behav.*, 7(1):103-112.
DOI: [10.4161/psb.7.1.18472](https://doi.org/10.4161/psb.7.1.18472)
- Eed, A., Albana'a, B., & Almaqtari, S. (2015). The effect of growing media and stem cutting type on rooting and growth of *Bougainvillea spectabilis* plants. *Univ. Aden J. Nat. and Appl. Sc.*,19(1):141-146.
- Gai, Q., Jiao, J., & Luo, M. (2015). Establishment of hairy root cultures by *Agrobacterium rhizogenes* mediated transformation of *Isatis tinctoria* L. For the efficient production of flavonoids and evaluation of antioxidant activities. *PLoS One*, 10(3): e0119022. DOI: [10.1371/journal.pone.0119022](https://doi.org/10.1371/journal.pone.0119022)
- Guillon, S., Guiller, J., Pati, P., & Rideau, M. (2006). Hairy root research: Recent scenario and exciting prospects. *Current Opinion in Plant Biology*, 9(3):341-346.
<https://doi.org/10.1016/j.pbi.2006.03.008>
- Harborne, J. (1984). *Methods of Plant Analysis*. In: *Phytochemical Methods*, Springer, Berlin, pp. 1-36.
- Hartmann, H.; Kester, D., & Davies, F. (1997). *Plant propagation, principles and practice*. 6th ed. p770. Prentice Hall, Under Saddle river, NJ.
- Hu, J., Cai, X., & Jeong, B. (2019). Silicon affects root development, tissue mineral content, and expression of silicon transporter genes in poinsettia (*Euphorbia pulcherrima*) cultivars. *Plants (Basel)*, 8(6):180. DOI: [10.3390/plants8060180](https://doi.org/10.3390/plants8060180)
- Hu, J., Li, Y., & Jeong, B. (2020). Silicon promotes root development by modulating polar transport of auxin during cutting propagation of poinsettia. *Acta Hort.*,1291(32). DOI: [10.17660/ActaHortic.2020.1291.32](https://doi.org/10.17660/ActaHortic.2020.1291.32)
- Jarvis, B., Ali, A., & Shaheed, A. (2006). Auxin and boron in relation to the rooting response and ageing of mung bean cuttings. *New Phytologist*, 95(4) :509 - 518. <https://doi.org/10.1111/j.1469-8137.1983.tb03516.x>
- Jiang, Z., Li, J., & Qu, L. (2017). Auxins, in book (*Hormone Metabolism and Signaling in Plants*), pp. 39-76.
- Karakurt, Y., Unlu, H., & Padem, H. (2009). The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, 59(3):233-237.
<https://doi.org/10.1080/09064710802022952>
- Karimi, J., & Mohsenzadeh, S. (2016). Effects of silicon oxide nano particles on growth and physiology of wheat seedlings. *Russian Journal of Plant Physiology*, (63):119-123.
DOI: [10.7868/S0015330316010103](https://doi.org/10.7868/S0015330316010103)
- Kotia, A., Adhikari, B., Rawat, G., & Pasha, M. (2014). Status and distribution of *Coleus barbatus* Benth. in Tehri Garhwal District, Uttarakhand. *J. Biodivers Endanger Species*, 2: 127. DOI: [10.4172/2332-2543.1000127](https://doi.org/10.4172/2332-2543.1000127)
- Krishnamoorthy, R. and Ramaswamy, M., 2015. phytochemical investigation Gc – MS analysis and In vitro antimicrobial activity of *Coleus forskohlii*J. of Bangladesh Pharmacological Society, (10): 924 – 930. DOI: [10.3329/bjp.v10i4.24406](https://doi.org/10.3329/bjp.v10i4.24406)
- Lewis, D.H. (1980). Boron, lignification and the origin of vascular plants - a unified hypothesis. *The New Phytologist*, 84: 209-229. DOI: [10.1111/j.1469-8137.1980.tb04423.x](https://doi.org/10.1111/j.1469-8137.1980.tb04423.x)
- Li, W., Koike, K., Asada, Y., Yoshikawa, T., & Nikaido, T. (2005). Rosmarinic acid production by *Coleus forskohlii* hairy root cultures. *Plant Cell, Tissue and Organ Culture* , (80) :151–155. DOI: [10.1007/s11240-004-9541-x](https://doi.org/10.1007/s11240-004-9541-x)
- Lock, J. & Gaur, R. (2001). *Flora of the*

- District Garhwal North west Himalaya (With Ethnobotanical Notes). Kew Bulletin, 56(1):251. DOI:[10.2307/4119449](https://doi.org/10.2307/4119449)
- Mastan, A., Rane, D., Dastager, S., & Babu, C. (2020). Plant probiotic bacterial endophyte, *Alcaligenes faecalis*, modulates plant growth and forskolin biosynthesis in *Coleus forskohlii*. Probiotics and Antimicrobial Proteins, (12) :481–493. DOI: [10.1007/s12602-019-09582-1](https://doi.org/10.1007/s12602-019-09582-1) Mastiholi, A., Chavan, M., Guranna, P. (2013). Biomass production and its accumulation in *Coleus forskohlii* Briq.: Impact of spacing and time of harvest. J. of Root Crops, 39 (2): 131-213.
- Malleswari, D., Bagyanarayana, G., & Hindumathi, A. (2013). Anti-bacterial activity of *Coleus forskohlii* extracts against some pathogenic bacteria. J. Nat. Prod. Plant Resour., 3 (4):75-78.
- Mersinger, R., Dornauer, H., & Reinhard, E. (1988). Formation of forskolin by suspension cultures of *Coleus forskohlii*. Planta Med., 54(3): 200-204. DOI: [10.1055/s-2006-962403](https://doi.org/10.1055/s-2006-962403)
- Mir, R., Kaushik, P., Chowdery, R., & Anuradha, M. (2015). Elicitation of forskolin in cultures of *Rhizoctonia bataticola*-a phytochemical synthesizing endophytic fungi. International Journal of Pharmacy and Pharmaceutical Sciences, 7(10):185-189.
- Misra, A., Srivastava, S., Srivastava, P., & Shukla, P. (2016). Chemotaxonomic variation in forskolin content and its correlation with ecogeographical factors in natural habitat of *Coleus forskohlii* Briq. collected from Vidarbha (Maharashtra, India). Industrial Crops and Products, (84):50-58. DOI:[10.1016/j.indcrop.2016.01.034](https://doi.org/10.1016/j.indcrop.2016.01.034)
- Mitra, M., Viswavidyalaya, B., Gantait, S., Viswavidyalaya, B., & Mandal, N. (2020). *Coleus forskohlii*: advancements and prospects of *in vitro* biotechnology. Applied Microbiology and Biotechnology, 104(6). DOI: [10.1007/s00253-020-10377-6](https://doi.org/10.1007/s00253-020-10377-6)
- Muhammad, B., Alsousow, M., & Batt, H. (2013). The effect of foliar spray of nitrogen, boron and zinc on the growth indicators and the content of the leaves of elements of two olive cultivars (Nebaly mohasan and Dan). Damascus University Journal of Agricultural Sciences, 29(3):139-212. Doi: [10.21608/ajas.2019.41249](https://doi.org/10.21608/ajas.2019.41249)
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15(3): 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Nayini, S., Nalagoni, C., & Raju, S. (2020). Forskolin induced morphological and ovarian deformities in the *Exorista bombycis* (Uzi fly) and anti-feedent activity of forskolin. IJPSR, 11(10): 4940-4944. [http://dx.doi.org/10.13040/IJPSR.0975-8232.11\(10\).4940-44](http://dx.doi.org/10.13040/IJPSR.0975-8232.11(10).4940-44)
- Ngomuo, M., Mnerney, E., & Ndakidem, P. (2013). The effects of auxins and cytokinin on growth and development of *Musa sp.* var. Yangambi explants in tissue culture. American J. of Plant Sciences, 4(11): 2174. DOI: [10.4236/ajps.2013.411269](https://doi.org/10.4236/ajps.2013.411269)
- Pandey, R., Krishnasamy, V., Kumaravadivel, N., & Rajamani, K. (2014). Establishment of hairy root culture and production of secondary metabolites in *Coleus* (*Coleus forskohlii*). Journal of Medicinal Plants Research, 8(1): 58-62. DOI:[10.5897/JMPR12.1182](https://doi.org/10.5897/JMPR12.1182)
- Paton, A., Mwanyambo, M., & Culham, A. (2018). Phylogenetic study of *Plectranthus*, *Coleus* and allies (*Lamiaceae*): Taxonomy, distribution and medicinal use" (PDF). Botanical Journal of the Linnean Society, 188 (4): 355-376. <https://doi.org/10.1093/botlinnean/boy064>
- Praveena, R., Pandian, S., & Jegadeesan, M. (2012). *In Vitro* culture studies on medicinal herb, *Coleus forskohlii* Briq. Libyan Agric. Res. Cen. J. Intl., 3 (1): 30-35.
- Rajagam, J. (2005). Studies on the effect of planting methods and growth regulators on growth, tuber development, yield and quality, and standardization of postharvest technology of *Coleus forskohlii*. PhD Thesis, Tamil Nadu Agricultural University, Coimbatore ,180 pp.
- Rajkumar, K., & Malathi, R. (2015). Phytochemical investigation GC-MS analysis and *in vitro* antimicrobial activity of *Coleus forskohlii*. Bangladesh J. Pharmacol., (10): 924-930. DOI:[10.3329/bjp.v10i4.24406](https://doi.org/10.3329/bjp.v10i4.24406)
- Rivas, F., & Parra, A. (2013). Enzymatic glycosylation of terpenoides. Phytochem. Rev., (12):327-339. DOI:[10.1007/s11101-013-9301-9](https://doi.org/10.1007/s11101-013-9301-9)
- Sasaki, K., Udagawa, A., & Shimomura, K. (1998). High forskolin production in hairy roots of *Coleus forskohlii*. Plant

- Cell Reports, (17): 457–459. DOI: [10.1007/s002990050425](https://doi.org/10.1007/s002990050425)
- Schrammeijer, B., Beijersbergen, A., Idler, KB, Melchers, L., Thompson, D., & Hooykaas, P. (2000). Sequence analysis of the vir-region from *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Journal of Experimental Botany*, 51 (347): 1167–1169. DOI: [10.1093/jexbot/51.347.1167](https://doi.org/10.1093/jexbot/51.347.1167)
- Shanks, J., & Morgan, J. (1999). Plant hairy root culture. *Current Opinion Biotechnology*, (10): 151-155. [https://doi.org/10.1016/S0958-1669\(99\)80026-3](https://doi.org/10.1016/S0958-1669(99)80026-3)
- Shoaib, R., Ali, S., Metwally, S., Ibrahim, M., & Aboud, K. (2020). Phytochemical and molecular analyses of some *Coleus* cultivars cultivated in Egypt. *Bulletin of the National Research Centre*, 44: 105. DOI: [10.1186/s42269-020-00358-0](https://doi.org/10.1186/s42269-020-00358-0)
- Singh, P., & Suryanarayana, M. (2019). Effect of solvents and extraction methods on forskolin content from *Coleus forskohlii* roots. *Indian Journal of Pharmaceutical Sciences*, 81(6). DOI: [10.36468/pharmaceutical-sciences.614](https://doi.org/10.36468/pharmaceutical-sciences.614)
- Sofowora, A. (1993). *Phytochemical screening of medicinal plants and traditional medicine in Africa* edition. Spectrum Books Ltd., Nigeria, pp. 150-156.
- Stefanini, M., Ming, L., & Uesugi, G. (2004). Influence of IBA and boric acid on rooting of stem- cuttings of *Aloysia triphylla*. *Acta Horticulturae*, 629(629): 329-332. DOI: [10.17660/ActaHortic.2004.629.42](https://doi.org/10.17660/ActaHortic.2004.629.42)
- Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of the selected medicinal plant extracts. *Molecules*, 14(6):2167-80. DOI: [10.3390/molecules14062167](https://doi.org/10.3390/molecules14062167)
- Tiwari, R., & Bilaspur, B. (2010). Sprouting and survival of *Coleus forskohlii* stem cuttings by hormonal pretreatment. *J. of Medicinal and Aromatic Plant Sciences*, 32 (3):239-244.
- Tripathi, C., Basu, S., Jain, S., & Tandon, J. (1995). Production of coleonol (forskolin) by root callus cells of plant *Coleus forskohlii*. *Biotechnology Letters*, (17): 423–426. DOI: [10.1007/BF00130801](https://doi.org/10.1007/BF00130801)
- Valdes, L., Mislankar, S., & Paul, A. (1987). *Coleus barbatus* (*C. forskohlii*) (*Lamiaceae*) and the potential new drug forskohlin. *Econ. Bot.*, (44): 474-483.
- Veeresham, C. and Chitti, p., 2013. Therapeutic agents from tissue cultures of medicinal plants. *Research Article*, 1(4) :5 pp. DOI: [10.4172/2329-6836.1000118](https://doi.org/10.4172/2329-6836.1000118)
- Veeresham, C., Reddy, C., & Praveena, C. (2012). Strategies to improve the production of forskolin from hairy root cultures of *Coleus forskohlii*. *International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)*,5(2). DOI: <https://doi.org/10.37285/ijpsn.2012.5.27>
- Villar, M., & Gaget-Faurobert, M. (1997). *Mentor Effects in pistil-mediated pollen-pollen interactions*. p. 315– 332. Shivanna, K. and V. Swhney, V. (ed.) *Pollen biotechnology for crop production and improvement*. Cambridge Univ., Press, New York, 468 pp. DOI: <https://doi.org/10.1017/CBO9780511525469.017>
- Weiser, C. J. (1959). Effect of boron on the rooting of *Clematis* cuttings. *Nature*, (183): 559 - 560. DOI: [10.1038/183559a0](https://doi.org/10.1038/183559a0).