*Section A-Research paper*
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# Quantitative estimate of phytoconstituents of Glycyrrhizic acid (GA) from Taverniera cuneifolia (Roth) Arn. by HPTLC method and correlated with the standard components

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**Abstract**

*Taverniera* is a Fabaceae genus with twelve species indigenous to Northeast African and Southwestern Asian nations (Stadler et al. 1994). The herb *Taverniera cuneifolia* (Roth) Arn., also known as Indian licorice, grows along the banks of tiny streams. Plant growth regulators are substances, not nutrients, that encourage and impact improved growth and development in little doses. Auxins (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), -naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy-acetic acid (2, 4-D) and cytokinins (Benzylaminopurine (BAP), Kinetin (Kn), 2-isopentyladenine (2-iP), Thidiazuron (TDZ) and auxin for the best outcomes, BAP was used alone or in conjunction with Kin, IBA, IAA, or NAA used as shoot preparation from meristem. The use of plant cell cultures to manufacture secondary metabolites has been a popular method of utilizing plants' biosynthetic capacity to produce desired secondary metabolites. Routian and Nickell (1956) were the first to get a patent for plant tissue culture-based substance synthesis. In callus, suspension cultures, and hairy root cultures, several researchers have reported the generation of valuable chemicals. It can be concluded that Glycyrrhiza acid is widely studied around the world but is limited to extraction by a single plant, licorice roots *(Glycyrrhiza glabra)*. However, the current investigation of *Taverniera cunerifolia* for GA was successfully carried out using the HPTLC method and correlated with the standard component. The findings of the study can be used to better understand the plant's numerous phytochemical features and to establish long-term extraction of the component for a variety of medical and industrial applications.

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## 1. Introduction:

*Taverniera* is a Fabaceae genus with twelve species indigenous to Northeast African and Southwestern Asian nations (Stadler et al. 1994). The herb *Taverniera cuneifolia* (Roth) Arn., also known as Indian licorice, grows along the banks of tiny streams. The tribes utilize the roots of this plant as a substitute for commercial licorice (*Glycyrrhiza glabra*) (Naik 1998). G. *glabra* roots are commonly employed in traditional medical systems all over the world (Grieve 1992). This having antique, anti-inflammatory, antibacterial, antimalarial, antithrombic, antidiuretic,

antitherrosclerotic, antifungal, estrogenic, antiallergic, antidiabetic, and antimutagenic activities (Baltina 2003 ; Fukai et al. 2002 ; Mendes-Silva et al. 2003 ; Rastogi and Mehrotra 1993 ; Sebestain and Thampan 2003 ; Shibat *G. glabra* extract, glycyrrhizin, and its derivatives have been shown to limit the proliferation of viruses such as HIV, SARS, Hepatitis B and C, and Influenza by potentiating the immune system, inhibiting reverse transcriptase, and inducing interferon production (Cinatl et al. 2003). The chemoprofiles of *T. cuneifolia* and *G. glabra* root extracts were compared using chromatography and spectrum analysis. Glycyrrhizin, a sweetening agent, was among the chromatophores that were similar. Both plant species' soluble fractions of ethanol and chloroform had significant in vivo antiinflammatory and protective effect against EMS-induced toxicity in Salmonella typhumurium. Agrobacterium-induced tumors were inhibited by T. cuneifolia extracts (Zore et al.2008). Antifungal activity of T. *cuneifolia* root extract has been demonstrated (Zore et al.2003; 2004 ). Economical licorice is in high demand in the Indian system of medicine Ayurveda, with Indian pharmaceutical businesses requiring 5000 tons a year. It is entirely supplied from Pakistan and Afghanistan at a cost of billions of rupees (Anonymous 2000). Clonal propagation, also known as micropropagation, is a helpful tool for cloning superior genotypes with desirable features quickly utilizing tissue culture techniques.

For the generation of pharmaceutically significant secondary metabolites, plant tissue culture offers an appealing option. Secondary metabolite production is independent of crop quality variation; yield of target phytoconstituents would be consistent and tuned to demand are only a few of the benefits of this technology over traditional agricultural approaches. Good manufacturing practice can be applied, and production can take place under highly controlled conditions, free of pesticides, herbicides, agrochemicals, or fertilizers, and innovative production processes can be patented. The authors focus on the selection of cell lines, optimization of culture conditions, elicitation, in situ product removal, genetic transformation, and metabolic engineering as examples of distinct approaches in plant cells. The biotechnological approaches were reported by Naiva et al. (2016). To improve biomass output, many biotechnological strategies have been developed. Similarly, Kayani et al. (2018) examined biotechnology options for improved plant output (transgenic and heterologous transgenic systems).

## 2. Interventions based on biotechnology

### 2.1. Growth regulators, micropropagation, and hardening effects:

Plant growth regulators are substances, not nutrients, that encourage and impact improved growth and development in little doses (Namdeo, 2010). Auxins (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), -naphthalene acetic acid (NAA), 2, 4-dichlorophenoxy-acetic acid (2, 4-D) and cytokinins (Benzylaminopurine (BAP), Kinetin (Kn), 2-isopentyladenine (2-iP), Thidiazuron (TDZ) and auxin for the best outcomes, BAP was used alone or in conjunction with Kin, IBA, IAA, or NAA used as shoot preparation from meristem. T. moting's reports auxiliary shoot growth, which could be related to its ability to boost endogenous adenine-type cytokinin biosynthesis and accumulation. They noticed the quick initiation and proliferation of shoots inT. *cuneifolia*, however, TDZ overexposure. Shoot elongation is harmed, and

deformed shoots develop, leading to the demise of primary shoots. Aside from plant growth regulators, there are a few more things to consider coconut milk (Ray and Jha, 2001) and other growth enhancers L-glutamine has been shown to increase shoot proliferation (Sivanandhan et al., 2015) in the T. *cuneifolia*.

The plant growth regulators IBA and BAP appear to be the most suited for regrowth and proliferation of shoots In vitro results are shown in Table 3.Explants of several types of plants were used to start the regeneration of shoots and roots. T. *cuneifolia* in MS medium with various growth hormones (Ghimire et al., 2010; Joshi and Padhya, 2010; Logesh et al., 2010; Logesh et al., 2010; Logesh et al., 2010; Logesh et al., 2010; Logesh Udayakumar et al., 2013; Kumar et al., 2011; Kulkarni et al., 2000). The practice of rapidly multiplying stock plants is known as micropropagation employing current technology to create a high number of offspring plants. Methods of plant tissue culture micropropagation is a technique for multiplying small organisms. Plants that have been genetically engineered or developed through crossbreeding conventional plant breeding methods T. in vitro cultures has been *cuneifolia* ltivated from a variety of explants.

It has been reported, explants such as young leaves, nodes, shoot tips, or axillary buds from adult T. are some of the most commonly employed plant parts for micropropagation. Planta when *cuneifolia* compared to other explants, young nodes, axillary buds, and shoot tips produced more shoots. Table 2 covers the plant parts as well as some of the findings of other studies. Murashige and Skoog (MS), 1962; Gamborg et al. (1968) (B5), Schenk and Hildebrandt, (SH) 1972; Lloyd and McCown, 1980 (WPM – Woody Plant Medium) and Nitsch and Nitsch (NN), (1969) have all been explored for T. Several researchers have propagated *cuneifolia*. T. Nitsch and Nitsch (NN), (1969) were investigated for W. Nitsch and Nitsch (NN), (1969). Several researchers have propagated *cuneifolia* MS medium was discovered to be the most suited for T. in vitro study on *cuneifolia* (Table 1).

Plants grow rapidly in vitro under ideal nutritional, moisture, light, and stress-free circumstances; however, the micropropagation success rate is determined by the acclimation of tissue grown plantlets from the laboratory to the field. In vitro produced plants were unable to cope with the external environment, which was full of biotic and abiotic challenges, when they were transferred from the lab to the field (Namdeo, 2010). Hardening of micropropagated plantlets requires a suitable environment (physical and chemical), and attention must be taken to increase growth and decrease mortality in plantlets during the acclimatization stage. Different soil combinations have been promoted for acclimation in vitro cultivated T. *cuneifolia* shoots. A hundred percent dirt or sand did not yield a positive result. Survival rates were better with soil: vermicompost (1:3) (Chakraborty et al., 2013), soil rite (Fatima and Anis, 2011), and soil: leaf manure (3:1) (Chakraborty et al., 2013). (Saema et al., 2015). In the sand: dirt, Kulkarni et al. (2000) observed a 100% survival rate (1:1).

**2.2 Cultures of callus and suspension:**

Under *in vitro* conditions, the growth and proliferation of callus (an unorganized growing and dividing cellular mass) from injured surface-sterilized explant mainly depends on the

selection of plant growth regulators and their combination (Namdeo, 2010). Fig. 3 depicts the influence of plant growth regulators on the fate of explant under *in vitro* conditions. Rani et al. (2016) illustrated callus induction from hypocotyl, root, and cotyledonary leaf segments of T. *cuneifolia.* Their report says the highest callusing (76%) from hypocotyl segments incubated in MS medium containing with 2 mg-1 2,4-D. The frequency of callus initiation increased to 91% on the addition of 0.2 mg L-1 Kin along with 2,4-D. However, the observation reported that 100% callusing from the root and cotyledonary leaf segments of *T. cuneifolia*. When 0.2 mg L-1 Kin was added to 2,4-D, the frequency of callus initiation increased to 91 percent. The root and cotyledonary leaf segments of *T. cuneifolia*, on the other hand, were found to be completely calloused. Shot induction from hypocotyl callus with 2mg L-1 2,4-D and 0.2 mg l-l KN, shoot multiplication in MS + 2mg L-1 BA, and development of multiple shoots after two months in 2mg L-1 BA are also reported by the researchers. We discovered that MS medium containing 2,4 D and Kin in various combinations is the most favorable combination for callus initiation in T. *cuneifolia* and other plants.

For shoot regeneration, BAP alone or in combination with IAA or NAA produced satisfactory results. Explants such as axillary shoots, epicotyls (Udayakumar et al., 2013), hypocotyls (Rani et al., 2003), nodal segments inter-node (Manickam et al., 2000), or leaf segments from in vitro seedlings of T. In certain cases, callus produced by leaf explants turned brown, which could be owing to phenolic component leaching or enzymatic activity (Table 4). Chakraborty et al. (2013) discovered that brown callus can regenerate shoots. Adil et al. (2019) recently reported that the combination of TDZ and NAA was effective. Cell suspension cultures, as opposed to callus cultures, have a homogeneous cell population that allows for rapid and uniform nutrient delivery .growth hormones and assist numerous biotechnological processes precursor feeding, elicitation, biotransformation, or bioconversion, and scale-up for large-scale culture in bioreactors are examples of strategies. Namdeo (2010, Nam W. Suspension cultures *cuneifolia* were produced as a result of utilizing callus cells that are fast-growing and friable in a liquid MS medium that is devoid of added with 2,4-D (0.5, 1, 2, 3, 4, and 5 mg L-1) and gelling agent Kinetin was tested in a shaker spinning at 100 rpm at 25°C and yielded very poor results. the manufacture of *cuneifolia* (Sabir, 2008). Murthy and Nagella (2010) T. suspension cultures have been developed in shaking flasks *cuneifolia*. They looked at how different growth regulators, inoculum density, and other factors affected growth. Various media (MS, B5, NN, and N6), as well as MS medium strength carbohydrate source, sucrose content, and early pH influence on the development of *cuneifolia*. Optimal conditions for biomass accumulation 10 g L-1 inoculum production in full strength MS medium within a four-week culture phase, 3 percent (w/v) sucrose was added to a medium pH of 5.8.

Nagella and Murthy (2011) discovered that macro-element content and composition, as well as the ammonia-nitrate ratio, regulated both biomass accumulation and secondary metabolite generation in T. *cuneifolia* cell suspension cultures. Maximum biomass was found in the media containing 0.5 x NH4NO3, while maximum *cuneifolia* production was found in the medium containing 2.0 x KNO3. However, at a higher concentration of 37.60 mM of NO3 -, a

lower concentration of 7.19 mM of NH4+ combined with a moderate concentration of 18.80 mM of NO3 - supported the maximum increase of biomass and the highest *cuneifolia* synthesis. Sivanandhan et al. (2013a, b) describe the optimal protocol for biomass buildup and *cuneifolia* synthesis in T. niger suspension cultures *cuneifolia*. They looked at the effects of different growth hormone quantities and combinations, glucose sources, agitation speed, and organic additions. They also discovered that seaweed extracts (G. edulis at a 40% concentration) have an important role in biomass enhancement and *cuneifolia* synthesis in cell suspension culture. In a study by Sivanandhan et al. (2014 a, b), increased *cuneifolia* production was attributed to elicitation and precursor feeding in T. *cuneifolia* cell suspension culture.

**3. In vitro conservation and somatic embryogenesis:**

The asexual, bipolar embryos developed from somatic embryos are known as somatic embryos. Hormonal cues cause the explant's somatic cells to divide. This technique of Propagation is the quickest way to produce a big number of plants. Since both the somatic embryos and the clonal plants are contained in the somatic embryos, there are a large number of clonal plants. The root poles and the shoot (Namdeo, 2010). This procedure entails the single somatic cell is used to construct a plant or embryo. A cell of a plant that isn't normally engaged in the embryo's growth leads to Somatic embryogenesis is the creation of a somatic embryo. These are the plants that are in good health. Tissues that do not produce endosperm or seed coat during the somatic development of an embryo.

During in vitro propagation, it is quite important. It is quite a beneficial tool for improving the genetics of medicinal and commercially important plants (Rai et al., 2010). Although there are numerous options, callus-mediated somatic embryogenesis is the most popular. *cuneifolia*. Embryogenic callus was obtained by Rani et al. (2004) cultured on MS supplemented axenic cotyledonary leaf explants had the most somatic embryos, with 3.0 mg l-1.formation.

Sharma et al. (2010) have also tried the somatic method. Wharton's embryogenesis. Two models were used to study *cuneifolia*. It concluded that Leaf explants were found to be more capable of embryogenesis than internodal explants. The elements of the MS medium were varied much more to cause tension in T's culture *cuneifolia* (sleepy) (Swathia et al., 2013).4.0 mg l-1 BAP and 2.0 mg l-1 IAA were supplemented with 4.0 mg l-1 BAP and 2.0 mg l-1 IAA in this study. Decreasing the calcium content, giving the greatest results for differentiating somatic embryos derived from leaf embryogenic callus explants. The conservation of medicinal plants and their long-term use is part of a long-term, comprehensive, scientifically driven action plan that includes the general public. Plant cells, protoplasts, embryos, and enzymes are immobilized by binding them to or within inert, dependable support. Thus, plant cell immobilization is a phenomenon in which plant cells are physically confined in a precisely defined region of space while maintaining their metabolic activities and being used again and continuously. Knowledge of the plant, its protection, preservation, maintenance, and long-term use is necessary for the implementation of such a program. In addition, biotechnological tools are vital for the selection, replication, and conservation of these critical genotypes. Plants that have therapeutic properties. The plant cell tissue and organ culture system is named explicitly. have a lot of promise for

producing high-quality plant-based foods In vitro medicine plant cell tissue, such as synthetic seeds like encapsulating somatic embryos or vegetative propagules (shoot apices, shoot apices, shoot apices, shoot apices, shoot apices, shoot apices, axillary buds, nodes) can help preserve exceptional medicinal germplasm. In vitro, plant-based medicine plant cell tissue, such as synthetic seeds like Encapsulated somatic embryos or vegetative propagules (shoot apices, axillary buds, and nodes) can help preserve exceptional medicinal plant germplasm.

Another work by Singh et al. (2006) shows that synthetic seeds are produced in T. *cuneifolia* by encapsulating shoot tips from 4 weeks old in vitro proliferating shoots with calcium alginate beads made from a mixture of sodium alginate (3.0%) and calcium chloride (75 mM). Furthermore, for in vitro conversion of encapsulated shoot tips into plantlets, the MS medium supplemented with 0.5 mg l-1 IBA demonstrated the best response (87 percent). Under ex vitro conditions, however, soilrite moistened with 1/4 MS salts solution proved to be suitable for conversion. Even if encapsulated shoot tips are stored for up to 60 days at 4°C, their conversion potential will be considerably reduced. In the same way, Fatima et al. (2013) employed axenic nodal segments to make synthetic seeds in T *cuneifolia*. The nodal segments were encapsulated in 3 percent sodium alginate and 100 mM calcium chloride and cultured on MS medium with BAP and NAA for maximum conversion of encapsulated nodal segments into plantlets as well as for shoot sprouting. In contrast to previous research, four weeks of cold storage at 4 °c resulted in the highest (86.2 percent) shoot growth from synthetic seeds.

**3.1 Quantitative estimate of phytoconstituents and their production:**

The use of plant cell cultures to manufacture secondary metabolites has been a popular method of utilizing plants' biosynthetic capacity to produce desired secondary metabolites. Routian and Nickell (1956) were the first to get a patent for plant tissue culture-based substance synthesis. In callus, suspension cultures, and hairy root cultures, several researchers have reported the generation of valuable chemicals. Table 5(A-C) shows several reports on various biotechnological methodologies for the generation of secondary metabolites in T, including tissue and organ culture, cell suspension culture, and hairy root culture *cuneifolia*. The production of secondary metabolites in T. reed tissue/organ culture is depicted in Table 5(A) *cuneifolia*. The production of secondary metabolites by T cells and shoots is influenced by several factors, type of basal media (Ray and Jha, 2001; Praveen and Murthy, 2010), types and concentration of different plant growth regulators (Sivanandhan et al., 2012a, b; Sivanandhan et al., 2013a, b, c), carbon and nitrogen source (Sivanandhan et al., 2015a, b, c), culture medium volume (Sivanandhan et al., 2015a, Sivanandhan et al., 2012a, b; Sivanandhan et al., 2013a, b, c) in vitro cultures of adventitious root or shoot cultures. The most ideal medium for the beginning of cultures and the generation of secondary metabolites in T. *cuneifolia* is MS medium, both full and half strength. Table 5 (A) shows the generation of secondary metabolites in *cuneifolia* tissue/organ culture starting from various explants. Our findings support the findings of many other researchers who have found that liquid MS medium produces more secondary metabolites than semi-solid MS media.

Due to a lack of CO2 in the culture, most plant tissue cultures are incapable of fixing carbon through photosynthesis. Sugar is used as an energy source in the medium, with sucrose being the most common sugar used, though glucose, fructose, and sorbitol have also been observed (Namdeo, 2010). In general, 3 percent sucrose is acceptable for the synthesis of *cuneifolia* in T. *cuneifolia*, as per MS medium and otherwise. According to Sivanandhan et al. (2015)a,b, c, the presence of carbohydrates (e.g. glucose, fructose, sucrose, and maltose) and nitrogen (as NH4NO3, KNO3, NaNO3, L-glutamine, and adenine sulfate) in the medium influences the synthesis of *cuneifolia*. They found that 6 percent sucrose enhanced *cuneifolia* production in shoot cultures, while 2 percent sucrose optimized *cuneifolia* production in adventitious root cultures (Sivanandhan et al., 2012a,b). Furthermore, Sivanandhan and colleagues (2015) found that shoot cultures of T. *cuneifolia* in MS medium with sucrose (6%) and L-glutamine produced the most *cuneifolia* (20 mg l-1). They also suggested that the culture length (5 weeks) and medium volume (20 ml) were important factors in maximizing the generation of *cuneifolia* from shoot cultures (Sivanandhan et al., 2012a, b; Sivanandhan et al., 2013a,b, c). *cuneifolia* is the main chemical component found in plants. HPTLC analysis of withaferin-A from the herbal extract and polyherbal formulations (Mahadevan et al., 2003), and separation, identification, and quantification of selected

Sangwan et al. (2007) found that changing the hormone content of the culture media as well as genotype as the source of the explant resulted in a 10-fold increase in *cuneifolia* productivity in the cultures (0.014 to 0.14 mg per gram fresh weight). In vitro shoot cultures, on the other hand, mirrored *cuneifolia* production. *cuneifolia*, on the other hand, was barely detectable in the aerial sections of field-grown T. *cuneifolia* (explant source), but it accumulated significantly in the plant's in vitro shoot cultures. The roots of *T. cuneifolia*, are biosynthesized from scratch (Sangwan et al., 2008). According to Sabir et al. (2008), when numerous shoots cultures of *cuneifolia* were compared to mother plant shoots, there was an increase in *cuneifolia* accumulation. Root cultures, callus cultures, and suspension cultures all produced *cuneifolia* in vitro, though at lesser amounts. According to Wasnik et al. (2009), the *cuneifolia* content of adventitious root samples was found to be superior to other roots at any stage during the 6-month growing period. In addition, HPTLC analysis of in vitro adventitious roots revealed the existence of a novel chemical.) The growth and chemical constituents of tissue cultured and seed raised *cuneifolia* plants in vitro mass propagation and greenhouse establishment.

**3.2 Elicitation, biotransformation, and precursor feeding:**

For induction of secondary metabolites in suspension cells, calli, and root cultures, many procedures were applied, including elicitation, precursor feeding, and so on. As inducers, many natural or synthesized compounds such as MJ, SA, and fungal-derived products were utilized. However, there are only a few publications that use live microbial cultures as elicitors. Fungal challenged root cultures of *T. cuneifolia* increased GA content in the current investigation (Fig. 2). Furthermore, Mucor hiemalis treated cultures showed the greatest increase in GA (4.900.10 mg/g). A threefold rise in GA was reported in *Fusarium moniliforme* (3.690.09 mg/g) and *Aspergillusniger* (3.180.07 mg/g). GA was discovered when compared to a control root culture

that had not been challenged. In *Penicillium fellutanum* (2.620.38 mg/g) and *Aspergillus tenuis* (1.500.07 mg/g) challenged cultures, however, there was a minor rise in GA content.

When bacterial elicitation was used, the highest GA rise was seen in Rhizobium leguminosarum challenged culture (6.370.41 mg/g) compared to unchallenged control roots (1.480.09 mg/g) (Fig. 3). Furthermore, significant increases in GA content were detected in *Bacillus aminovorans* (2.910.10 mg/g), *Agrobacterium rhizogenes* (2.880.38 mg/g), and Bacillus cereus (2.330.05 mg/g) challenged cultures. However, no significant increase in GA content was seen in root cultures challenged with *Agrobacterium tumefaciens* (1.460.06 mg/g). R. leguminosarum is a nitrogen-fixing gram-negative symbiotic bacteria that colonizes the roots of leguminous plants (Manero et al.,2012). According to Albersheim et al. (1977), symbiotic bacteria do not produce phytoalexins or have a specialized method for escaping the secondary metabolites created by them. The nature of R. leguminosarum's interaction with *T. cuneifolia* root may be worth investigating further to determine the specific molecular process that leads to the elicitation of secondary metabolites.

Roots have a symbiotic and non-symbiotic relationship with the soil microbial flora. Roots primarily release certain secondary compounds to recruit symbionts and keep harmful microorganisms at bay. The sterility of in vitro cultures may explain the reduced output of metabolites. As a result, we inoculated the cultivated roots with a variety of common soil fungi and bacteria. Secondary metabolite production increased several folds, as expected when assessed in terms of GA production. R. leguminosarum, which is a nodule-forming bacteria in nature and a symbiotic microorganism, was shown to accumulate the most GA (Manero et al., 2012). It's worth noting that *T. cuneifolia* is a leguminous plant, and the roots may be involved in a close connection with the bacteria that could cause nodules. Our findings are consistent with those of Manero et al. (2012), who found a progressive increase in hypericin and pseudohypericin after challenging seedlings of Hypericum perforatum with Rhizobacterium. The stimulation of secondary metabolite production was less effective when root cultures were challenged with fungal cultures. This could be owing to their saprophytic nature, which causes poor contact with live roots, particularly in nutrient-rich media.

**3.3 Hairy root cultivation**

Hairy root disease is caused by *Agrobacterium rhizogenes* in plants, which is characterized by neoplastic (cancerous) roots at the infection site. These roots are notable for their genetic and biosynthetic stability, as well as their ability to grow quickly and easily in hormone-free conditions (Namdeo, 2010). A wide range of chemical compounds, including pharmaceuticals, nutraceuticals, pigments, flavors, and other beneficial substances, have been synthesized using this technology.

**3.4 Preparation and Germination of Synthetic Seeds:**

Synthetic seed technology is well known for its contribution to germplasm conservation and sharing, although various researchers have proposed novel applications. The purpose of synthetic envelopes is to shield embryo/meristematic tissue from mechanical and abiotic stressors. Synthetic seeds with 2% sodium alginate and 100mM CaCl2 were robust enough to

handle without compromising germination. Seed germination was further reduced by increasing sodium alginate to 3% and adding 75mM CaCl2 (Data not shown). Up to three months of storage, the frequency of germination was observed. An average of 15.51.87 per 20 synthetic seeds grew into root cultures at the end of the first month, but the germination rate dropped to 13.51.13 per 20 synthetic seeds after three months. The un encapsulated embryos, on the other hand, did not germinate after three months. Finally, after BAP therapy, we present a fast methodology for somatic embryogenesis directly on cultured roots. The ability of these embryos to regenerate into plantlets was established. Synthetic seeds made from these embryos germinated quickly and produced a large number of root cultures.

**3. 5 HPTLC analysis:**

Merck KGaA, Germany, provided precoated TLC plates (Silica Gel 60 F254) with a thickness of 0.2mm and a dimension of 2020cm. Glycyrrhizin standard was bought from Sigma in the United States, and a 1 mg/ml stock solution was produced in ethanol. Using a Linomat IV automatic spotter, ten microliter of crude extracts and standard glycyrrhizin (5 ml) were loaded as 10-mm streaks on HPTLC plates at a 10-mm gap between two streaks (Camag, Pvt. Ltd., Switzerland). Plates were allowed to dry for a few minutes before being developed with a 7:1:2 solvent mixture of n-butanol, acetic acid, and water. Plates were dried, scanned with a Camag Scanner III, and then viewed under UV 254 and 366nm (Switzerland). Rf value, UV Spectra, and each chromatophores' value, percent AUC, and l max were recorded. Plates were derivatized with specified detection reagents (e.g., sulfuric acid reagent for glycyrrhizin, anisaldehyde) and inspected visually and under UV at 254 and 366 nm. Wagner and Bladt were followed for all extractions, chromatographic separations, and analyses. The study discovered that Glycyrrhiza glabra performed best in water: ethanol (70:30 v/v) solvent solution, yielding the greatest results in GA extraction using the HPTLC method. With 8 phytoconstituents, the best extraction was achieved at 254 nm. Figure 1 shows that all sample ingredients were well separated without any diffuseness, resulting in eight peaks that were comparable to standards. The Rf values were in the range of 0.23 to 0.29. The peak with the RF value of 0.28 was the most prominent and comparable to the Rf value of standards (Figure 2). Following correlation analysis, the standard GA as compared to GA extracted from the plant, with the best correlation coefficient r2 of 0.28

**4. Conclusion:**

From all of the above studies, it can be concluded that Glycyrrhiza acid is widely studied around the world but is limited to extraction by a single plant, licorice roots *(Glycyrrhiza glabra)*. However, the current investigation of *Taverniera cunerifolia* for GA was successfully carried out using the HPTLC method and correlated with the standard component. The findings of the study can be used to better understand the plant's numerous phytochemical features and to establish long-term extraction of the component for a variety of medical and industrial applications. It is becoming increasingly vital to standardize the regeneration techniques for such plants. Our technique has the potential to aid in the conservation of the species and serve as an

alternate supply of materials. *T. cuneifolia*, which is widely utilized in traditional medicine by the local people, might benefit from the regeneration strategy detailed here.

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