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THE EFFECT OF $CACL_2$ INDUCED STRESS ON CALLUS OF IN-VITRO PROPAGATED *CISSUS QUADRANGULARIS*. LINN

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Abstract-

The current research focused on *Cissus quadrangularis* Linn (Family -Vitaceae). It has medicinal properties such as anti-inflammatory, anti-obesity, antioxidant, analgesic, antiulcer, anti-osteoporotic, antiviral, antimicrobial, and bone healing. Callus is obtained by inoculating nodal sectors and leaves on MS + NAA (1.5mg/L) + BAP (0.5mg/L). Callus was subjected to treatment with a medium containing various concentrations of $CaCl_2$. The callus was allowed to grow on the above medium for three weeks and then used for phytochemical analysis.

Key Words: Elicitation, Hadjod, Micropropagation, Phytochemical, *Cissus quadrangularis* Linn.

Introduction:-

The *Cissus quadrangularis* Linn. is a valuable herbal climber belonging to the family Vitaceae. It's called "Hadjod" in Hindi because of its bone-healing properties. The plant is a succulent, perennial climber with a 1.00 meter to 2.00-meter height and a quadrangular jointed cactus-like thick stem. It is native to India's hotter and drier regions, including the Deccan Plateau. It is also found in the Western Ghats and is common in drier regions like India, South Africa, Thailand, Sri Lanka, and Malaysia [1]. *Cissus quadrangularis* is a medicinal plant with a variety of pharmacological benefits, including anti-osteoporotic, bone healing, antibacterial, anti-inflammatory, anti-obesity, antiviral, antiulcer, and antioxidant characteristics [2]–[5]. In India, *Cissus quadrangularis* Linn is used to aid in the treatment of fractures. Alkaloids, carotene, Ascorbic acid, palidol, resveratrol, piceatannol, quadrangularins, nicotinic acid, phytosterol compounds, flavonoids, enzymes, calcium, tyrosine, vitamins, and triterpenoids are all found in *Cissus quadrangularis* [6]. β-sitosterol is an active principle present in *Cissus* responsible for enhancement of bone healing process. The plant is available in various formulas, comprising dry powder, syrup, and capsules. "Laksha Gogglu," an Ayurvedic medicine, is used to relieve pain, reduce swelling, and promote the healing of minor fractures [7].

MATERIALS AND METHODS: -

MS medium supplemented with 30% sucrose and NAA (1.5mg/L) + BAP (0.5mg/L) was used for callus induction. The pH of the medium was maintained between 5.8 and 6.0; 0.8% is used as solidifying agent [8]. The medium and other essential glassware were sterilized by autoclaving at a temperature of 121ºC (Degree Celsius) and 15lbs. pressure for 20 minutes. Explants consisting of tendrils, shoot apices, and nodal segments were procured from a plant growing in the Botanical Garden of College. These explants were cut into 6-10 cm pieces and cleaned for 30 minutes under running tap water followed by washing with Teepol and Twin 20. The final washing is done with sterile distilled water for 2-3 times. Surface sterilization was carried out in Laminar Air Flow Cabinet with 0.1% HgCl2, inoculated for 5-6 minutes followed by 4-5 times washing with sterile distilled water. The explants were cut from both the edges to remove dead tissues and inoculated on the culture medium. The cultures were kept at 25±2°C with a 16/8 dark/light period. Each treatment had three replicates, and all experiments were carried out three times. After 15 days of inoculation, callus formation was observed. After 25 days of inoculation, a subculture was carried out on the same medium composition to obtain a sufficient amount of callus. Further, about 2gm of callus was subjected to elicitation treatment with a medium containing the following concentration of $CaCl_2$ along with control.

MS+ NAA (1.5mg/L) + BAP (0.5mg/L) + 0.1mM $CaCl_2$.

MS+ 1.5mg/L NAA+0.5mg/L BAP + 0.2mM $CaCl_2$

MS+ 1.5mg/L NAA+0.5mg/L BAP + 0.3 mM $CaCl_2$

The callus was allowed to grow on the above medium for three weeks and then used for phytochemical analysis.

RESULTS AND DISCUSSION

Cissus quadrangularis have been of interest for phytochemical analysis by many researchers, but the callus of ***In-Vitro*** **Propagated** ***Cissus quadrangularis*** L. has been less studied. So, the current study targeted the analysis of phytochemicals from the callus of ***In-Vitro*** **Propagated** ***Cissus quadrangularis*** L. For micropropagation, the medium was supplemented NAA (1.5mg/L) + BAP (0.5mg/L). It was observed that the nodal segments and internodal segments could be used for callus induction in *Cissus quadrangularis* L. Tendrils and shoot apical meristem also showed callus formation, but the callus did not survive for longer period. It was found that nodal segments show maximum callus induction compared to other explants. Nodal segments were determined to be the best for callus tissue culture. [9], [10] The callus was seen to be whitish-green and expanding rapidly. After 15 days, Callus induction was observed. A sufficient amount of callus for subculture was obtained after 25 days of the first inoculation.

Table 1: Results of the qualitative phytochemical analysis of *C. quadrangularis L.*						
S.N.	**Plant Sample**	**Alkaloid**	**Flavonoids**	**Tannins**	**Phenols**	**Steroid**
1.	0.0mM CaCl2	+++	++	++	++	+
2.	0.1mM CaCl2	+++	+	++	+	+
3.	0.2mM CaCl2	++	+	+	+	+
4.	0.3mM CaCl2	+	+	+	+	_

+++ = shows abundantly presence of phytochemicals, ++ = shows moderate concentration of phytochemicals, + = indicates presence of phytochemicals, and - = indicates absence of phytochemicals.

After 20 days of subculture, sufficient amount of callus was obtained for elicitor ($CaCl_2$) treatment. (2 gm callus/per tube/per treatment). Various concentrations of $CaCl_2$ were added to the culture medium. After 21 days of treatment, phytochemical analysis was performed using the methods of Harborne (1973) and, Trease and Evans (2002), Edeoga *et al*. (2005). [11]–[13] The following results were obtained (Table 1).

A quantitative phytochemical analysis of alkaloids, flavonoids, and Sterols was performed on the callus. Changes in phytochemical concentrations were analyzed, and the results are presented in Figure 1. $CaCl_2$ concentrations had a significant effect on Alkaloids content. Callus treated with 0.1 mM $CaCl_2$ had the highest levels of total Alkaloids (2.495 mg/10 g). As the concentration of $CaCl_2$ increases, the Alkaloids concentration decreases presented in Figure 1. Flavanoids content was also affected by CaCl2 concentrations. Increasing $CaCl_2$ concentrations shows a negative effect on the amount of Flavanoids. Callus with 0.0 mM $CaCl_2$ (control) shows the maximum amount of Flavonoids when we add CaCl2 to the callus; as the concentration of $CaCl_2$ increases, the Flavanoids concentration decreases; similar types of the result were recorded by [14] while working on *Polygonum hydropiper*. Callus with 0.0 mM $CaCl_2$ (control) and 0.1 mM $CaCl_2$ shows maximum amount Sterols. The concentration above 0.1 mM $CaCl_2$ shows a significant decrease in sterols content.

Table 2: Mean results of the quantitative phytochemicals analysis on the extracts of *C. quadrangularis L.*				
Sr. No	**Concentration of Cacl2**	**Alkaloid (mg/10gm)**	**Flavonoids (mg/10gm)**	**Sterols (mg/10gm)**
1.	0.0mM CaCl2	2.153	1.882	0.243
2.	0.1mM CaCl2	2.495	1.588	0.252
3.	0.2mM CaCl2	1.712	1.486	0.092
4.	0.3mM CaCl2	1.2	1.242	0.048

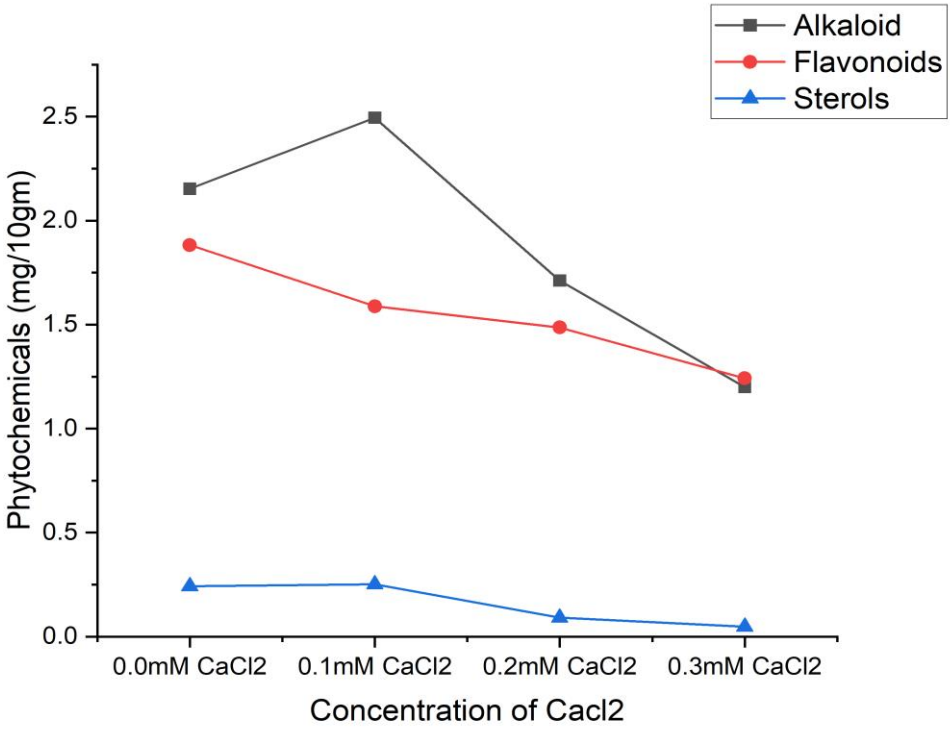

Fig 1- Mean results of the quantitative phytochemicals analysis on the extracts of *C. quadrangularis L.*

CONCLUSION

According to the results of this study, M.S. media supplemented with NAA (1.5mg/L) and BAP (0.5mg/L) was the optimal media for callus induction. It was discovered that nodal segments induce the most callus compared to other explants. The lower concentration of $CaCl_2$ enhances the production of alkaloids, flavonoids, and Sterols. The present study also revealed that $CaCl_2$ treatment can be used for enhancing the production of alkaloids and sterols. This information can be used to scale-up the production of important phytochemicals.

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