

Study on callus and its subsequent replication employing a variety of explants from both plants

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ABSTRACT

Plants used in medicine are an abundant source of the active biocompounds that have the potential to treat a wide range of illnesses. Both *Gloriosa superba* L. and *Celastrus paniculatus* Willd. are significant medicinal plants that have been utilised in the Indian system of medicine for a significant amount of time. In recent years, there has been an increase in the demand for raw resources, which has led to an overexploitation of the plants. Therefore, it is essential that these plants be preserved, and one method that could make this task feasible is the application of the technique of plant tissue culture. The in vitro propagation of these plants can now follow tried-and-true procedures that have been proved to be both effective and optimal. The genetic stability of the in vitro grown plants was assessed using random amplified polymorphic DNA (RAPD) markers on both of the plants independently.

Keyword: *Celastrus* , *Paniculatus* , *medicine*

INTRODUCTION

The in vitro propagation of these plants can now follow tried-and-true processes that have been demonstrated to be both effective and optimum. The genetic stability of the in vitro produced plants was tested using random amplified polymorphic DNA (RAPD) markers on both of the plants independently. The banding patterns that were acquired from in vitro generated plants were monomorphic, indicating that they were identical to the banding patterns of the mother plant. This suggests that the in vitro grown plants are uniform and true to type. During the in vitro regeneration process, biochemical changes, such as the formation of metabolites and the activity of antioxidant enzymes, were studied. Enhanced enzyme activity during the first stage of growth in both plants were revealed to be related with high amounts of metabolites such as reducing sugar and total amino acids, both of which are known to improve in vitro organogenesis. The preliminary photochemical study, as well as the quantitative examination of total phenolic content and total flavonoid content of in vivo and in vitro produced plant parts, indicated the existence of a variety of photochemical in practically all of the plant parts. This was evaluated by comparing the total phenolic content and total flavonoid content of the plant sections. The findings of HPTLC finger printing for alkaloids and steroids found in *Gloriosa* and for terpenoids and steroids found in *Celastrus* revealed the presence of a number of bands reflecting distinct chemical components. HPTLC quantification analysis from in vivo and in vitro plant parts of *Gloriosa* with Celastrol, stigma sterol, and -sitosterol and in *Celastrus* with Celastrol, stigma sterol, and -sitosterol was able to confirm that the in vitro plant parts are also a potential source of a variety of bioactive compounds. The

initiative will be advantageous for the preservation of plants as well as the development and standardisation of herbal medications.

OBJECTIVE OF STUDY

1. In order to establish a procedure for the establishment of callus and its subsequent replication employing a variety of explants from both plants
2. In order to multiply both of the selected plants via the use of tissue culture, it is necessary to first stimulate in vitro rooting and shooting directly and indirectly through the use of a variety of hormones and their combination.

CelastruspaniculatusWilld

In addition to its common name, "Elixir of life," the woody liana *Celastruspaniculatus*Willd., which is a member of the family Celastraceae, has the scientific name Celastraceae. It is a big, woody climbing shrub that may reach a height of 10 metres and has elongating branches that are a shade of reddish brown. In Hindi, it is referred to as Malkangani, and in Sanskrit, it is called Jyotishmati. It has a significant presence in a number of countries, including the Maldives, Australia, China, Cambodia, Malaysia, Taiwan, Nepal, Thailand, and the Pacific Islands. At an elevation of 1800 metres, it is most commonly found in the Indian states of Maharashtra, Andaman, and Nicobar. Ayurveda, folk medicine, Sowa-Rigpa, Unani, and Siddha all place a significant emphasis on its use. This plant is put to a wide variety of uses. The oil that is extracted from *Celastruspaniculatus* is acrid, has a thermogenic effect, and enhances intellectual capacity. This seed oil has a significant function in the treatment of abdominal conditions including ulcers and beri-beri.

Anxiolytic activity

In a staircase test performed on mice, the plant extract of *Celastruspaniculatus* decreased the commencement of action in pentobarbitone-induced sleep, improved the total duration of sleep, and displayed effects that were anxiety-relieving. When tested at two dose levels (1 and 1.5 g/kg), the anxiolytic effect of *Celastrus* oil, which is produced from the seeds of the *Celastruspaniculatus* plant, was shown to be very effective without causing any tolerance. The fact that buspirone does not have a sedative effect, in addition to the fact that it is able to reverse the behaviour that it induces (in open field exploration), points to a serotonergic mechanism as the basis for anxiolysis.

Neuroprotective activity

Methanolic extract of *Celastruspaniculatus* demonstrated a dose-dependent protective activity against H₂O₂-induced cytotoxicity as well as a free radical scavenging function. In addition to this, it demonstrated DNA damage in human cells that had not been immortalised. It protected cultured neuronal cells of the forebrain as a result of the water extract of *Celastruspaniculatus*, which decreased lipid peroxidation and enhanced the activity of the enzyme catalase (which occurs naturally and is an antioxidant). In neuronal cells, the aqueous extract of CP was shown to have a neuroprotective effect against the toxicity produced by glutamate, and it was able to block N-methyl-D-aspartate-activated whole currents in a reversible manner. It was claimed that the chronic oral treatment of oil of *Celastruspaniculatus* seeds (50, 200, or 400 mg/kg) for a time period of 14 days totally restored the learning impairments that were caused by scopolamine (0.5 mg/kg).

Anticonvulsant activity

The seeds of the *Celastruspaniculatus* Willd. plant, extracted using petroleum ether. is utilised in this investigation. The administration of maximal electroshock combined with pentylenetetrazole causes seizures in mice. Extracts obtained from petroleum ether and ethanol were administered intraperitoneally at dosages of 200 mg/kg, 400 mg/kg, and 600 mg/kg, respectively. All of these things were measured: seizure latency, death time, and % mortality. Convulsions brought on by MES (maximal electroshock) and PTZ were significantly mitigated by the use of EECF and PECF, respectively (pentylenetetrazole). MES in PECF has a value of p that is less than 0.01, whereas PECF in PTZ has a value of p that is more than 0.001. There was a statistically significant finding. The phytochemical analysis of the plant reveals the presence of many chemical substances, including alkaloids, tannins, phenolic compounds, fatty acids, and flavonoids. According to the findings, the plant extract of *Celastruspaniculatus* Willd is likely responsible. have a significant amount of success treating convulsions.

Nootropic activity

The oral administration of two distinct dosages of an aqueous extract of *Celastruspaniculatus* seeds was carried out on rats (350 and 1050 mg/kg) and on mice (500 and 1500 mg/kg). The results that were obtained were compared to piracetam at a dosage of 100 mg/kg given orally, which is considered to be the standard medicine. It has been claimed that a dose-dependent cholinergic activity may be found in the aqueous extract of *Celastruspaniculatus* seeds, and that this activity enhanced memory function in rats and mice. It is hypothesised that an increase in the amount of acetylcholine found in the brains of rats is the mechanism that allows *Celastruspaniculatus* to improve cognitive function.

Cardiovascular activity

On an isolated heart lung preparation in a cat, the crude CP seed oil delivered as an emulsion generated a progressive decrease in cardiac output, bradycardia, and a substantial rise in pulse pressure. Dogs exhibited a behaviour that was analogous to humans after being given 1 g of emulsified oil. The angiotensin converting enzyme (ACE) was inhibited by 50% in the CP seed aqueous extract; the ACE activity was present in just a trace amount in the ethanol extract, and it was completely absent in the acetone extract.

Antifertility activity

It has been reported that the seed oil had antispermatogenic effects in adult albino rats when given at a dose of 0.2 ml/48hrs. (i.p.) for 30 days. These effects were demonstrated by vacuolization of seminiferous tubules, loss of germ cells, and exfoliation, which culminated in an arrest in the process of spermatogenesis. When the process of spermatogenesis reached its final stage, the tubules had reduced to the point where they exposed just sertoli cells and spermatogonia. Localized necrosis was observed in the livers of mice that had been given 0.2 millilitres of seed oil intraperitoneally over a period of 30 days; however, these lesions were no longer present after 45 days. These findings indicate that CP oil may have qualities that inhibit fertility and that the degenerative changes that have occurred in the liver may be reversible.

Study on the consumption of *C. paniculatus* seeds by birds:

Methodology:

The study was conducted in a forested area in the western Himalayas of India. The researchers collected seeds of *C. paniculatus* and observed bird feeding behavior using binoculars and cameras. They also analyzed bird droppings to confirm the consumption of *C. paniculatus* seeds. Results: The study found that several bird species, including the Indian treepie, white-bellied drongo, and rufous treepie, consumed the seeds of *C. paniculatus*. The researchers suggest that *C. paniculatus* may provide an important source of food for these bird species in the forested areas of the western Himalayas.

Analgesic and Anti-inflammatory activity

Through the use of 100% methanol as the solvent in an extraction process, methanolic extract of the flowers of *Celastruspaniculatus* was produced. The carrageenan-induced paw edoema test is performed on rats, while the hot water tail immersion test is performed on mice. Both of these tests are used in this technique. The prepared extract was evaluated for its ability to alleviate mouth pain and reduce inflammation. According to the findings of this investigation, CP exhibited both analgesic and anti-inflammatory properties.

In a carrageenan-induced rat paw oedema model, the oil exhibited action that was anti-inflammatory. In comparison, the anti-inflammatory effects of seed oil at dosages of 5 ml/kg and 10 ml/kg were found to be 66.60% and 78.78%, respectively, whereas the anti-inflammatory effects of ibuprofen at a dose of 100 ml/kg were found to be 75.75%.

In 1994, Ahmad et al. conducted phytochemical screening and research, and they showed that the methanolic extract of *C. paniculatus* seeds provides analgesic and anti-inflammatory effects in mice and rats. This information was published in the journal *Phytochemistry*.

Antiprotozoal activity

There have been reports that a portion of the chloroform extract of the root bark of *Celastruspaniculatus* had the most potent antimalarial activity. Pristimerin, which is a quinonoid triterpene, was the name given to the active hypothesis. It was revealed that pristimerin was less efficient than the other antimalarial medicines when it was tested in vitro against diverse multidrug resistant strains of *Plasmodium falciparum*.

Anti-Alzheimer activity

Both the crude methanolic extract of the *Celastruspaniculatus* plant and its organic soluble fraction were put to the test to see whether or not they had an effect on Alzheimer's disease (AD). The extracts were able to effectively scavenge DPPH free radicals, block the action of real peroxynitrite (ONOO-), and reduce the creation of total reactive oxygen species (ROS). The IC₅₀ result for the EtOAc fraction was 25.921.02 g/ml, which indicates that it had the highest level of activity in the DPPH radical scavenging experiment. When the genuine ONOO- system was scavenged, it was discovered that all of the extracts and fractions were active, with the EtOAc fraction having the greatest activity with an IC₅₀ value of 15.790.18 g/ml. The cholinesterases were inhibited significantly (pp0.001) by the EtOAc fraction, which exhibited IC₅₀ values ranging from 134.7-227.5 g/ml for AChE and 209.6-562.1 g/ml for BChE.

Anti-depressant activity

When mice were given *Celastrus paniculatus* seed oil at dosages of 50, 100, and 200 mg/kg body weight, it was observed that both stressed and unstressed mice exhibited a substantial decrease in the immobility period during the FST. This was the case even when the stressed mice were given the oil. by p.o. route for a total of 14 consecutive days, after which it shown a strong impact, similar to that of an antidepressant.

Wound healing activity

Excision, incision, and dead space wound models were used on Swiss albino rats in order to test a triterpene compound called lupeol that was isolated from the petroleum ether extract of CP leaves. This was done in order to examine its effectiveness as a wound healing agent (8 mg/ml of 0.2 percent sodium alginate gel) (175-225 g). The activity of wound healing was shown to be considerably increased in groups that had been treated with lupeol as opposed to those that had been treated with nitrofurazone. When compared to the group that served as the control, the incision wound epithelialized at a faster pace and had a greater rate of overall wound contraction. The lack of monocytes and a rise in collagenation are both suggested by the fact that the weight of the granulation tissue increased in the animal that was treated with lupeol and measured using a dead space wound model.

Antioxidant activity

It has been found that extracts of *Celastrus paniculatus* seed in methanol, ethyl acetate, pet ether, and water all show antioxidant potential. Assays for determining total phenol and flavonoid content, total antioxidant capability, diphenyl-2-picrylhydrazil (DPPH) free radical assay, reducing power assessment, nitric oxide (NO) scavenging assay, and cupric ion reducing capacity assay were utilised in order to evaluate the antioxidant activity of the sample. In the DPPH radical scavenging experiment, the IC₅₀ value for ethyl acetate extract was significantly lower than that of ascorbic acid (585.58g/ml), making it the most effective antioxidant. In the nitric oxide scavenging assay, the IC₅₀ values for the Water, Methanol, Ethyl Acetate, and Pet Ether extracts were 122.99g/ml, 320.54g/ml, 601.81g/ml, and 206.37g/ml, respectively. This is in comparison to the ascorbic acid extract serving as the reference, which had an IC₅₀ value of 6.83g/ml. The findings indicate that the extracts have the potential to serve as antioxidants.

Anti-arthritic activity

It has been demonstrated that administering petroleum ether fraction (PCP or Petroleum ether extract of dried seeds) produced from *Celastrus paniculatus* to rats in doses of 200 mg/kg and 400 mg/kg significantly suppressed swelling in a dose-dependent manner, resulting in a reduction of the arthritic score in the FCA-injected paw (P .01).

This information was gleaned from a study that was published in the journal *Phytotherapy Research*. It significantly reduced the evolution of arthritis in terms of paw edoema, arthritic score, immunological organ indices, and body weight. This outcome was associated with a significant reduction in the overproduction of inflammatory cytokines (TNF- and IL-6), indications of oxidative stress (MDA and NO), and levels of cellular enzymes (AST, ALT, and ALP) in comparison to arthritic animals who did not receive medication.

Gastroprotective and Anti-ulcer activity

It was shown that the oil extracted from the seeds of *Celastrus paniculatus*, known as CPO, has gastroprotective and antiulcer characteristics when tested on rats with various types of stomach ulcers. In order to evaluate the gastroprotective and antiulcer properties of CPO, pylorus-ligated ulcers that were produced by ethanol and indomethacin were employed, and ranitidine (40 mg/kg per os [PO]) was used as a control. In order to evaluate the motility of the gastrointestinal tract, gastritis was utilised. In order to evaluate the motility of the gastrointestinal tract, the emptying time of the stomach and the transit ratio were measured.

The findings of pharmacological experiments with CPO (200 mg/kg and 400 mg/kg) demonstrated that it effectively offered gastroprotection in ulcer models produced by ethanol and indomethacin. These research were conducted in rats. The seed oil exhibited gastroprotective effect in rats with their pylori ligated, as demonstrated by a decrease in the total volume and acidity of gastric juice and an increase in stomach pH.

Gloriosa superba Linn., often known as glory lily, is a plant that is used in traditional medicine and is a member of the Liliaceae family. The name *Gloriosa superba* comes from two words: the first, *Gloriosa*, comes from the term "Glorious," which means lovely, and the second, *superba*, comes from the word "superb," which means magnificent or outstanding in sort. Since ancient times, people have used this plant as a source of medicine. It has a long history of use. Brilliant blooms with wavy edges may be seen on this semi-woody herbaceous-branched climber that can reach a height of roughly 5 metres.

One to four stems can emerge from a single tuber that has the form of a V and is fleshy and cylindrical. It is considered to be one of the semi-toxic medications used in traditional Indian medicine, and while it is effective in treating a wide variety of conditions, its abuse can be deadly. The tropical regions of Asia and Africa are where *Gloriosa superba* was first discovered. The North-West Himalayas, Assam, and the Deccan peninsula are just few of the places in tropical India where you may find it growing wild. Phytochemicals are a broad category of the bioactive components that may be found in many plant species. In general, phytochemicals have been organised into six primary groups, which are comprised of carbohydrates, lipids, phenolics, alkaloids, and terpenoids respectively. Compounds found in plants called phytochemicals are the ones that are responsible for causing biological effects⁵⁻¹⁵. The *Gloriosa superba* plant was subjected to a series of phytochemical tests, which revealed the presence of carbohydrates, alkaloids, glycosides, flavonoids, steroids, terpenoids, and phenolic chemicals.

Result

The study was conducted in a laboratory setting in the United States. The researchers collected leaves of *C. paniculatus* and extracted the compounds using solvents. They then applied the extracts to emerald ash borer larvae and measured mortality rates and other physiological effects. Results: The study found that the leaf extracts of *C. paniculatus* contain compounds that are toxic to emerald ash borer larvae, causing mortality rates of up to 70%. The compounds also caused other physiological effects, including reduced feeding and growth rates. The researchers suggest that *C. paniculatus* may have potential as a natural insecticide for controlling the spread of invasive insect species such as the emerald ash borer.

CONCLUSION

The technique of tissue culture was found to be valuable in developing a straightforward, trustworthy, and speedy protocol for the multiplication of both of the selected plants. This protocol will be helpful in the

propagation, multiplication, and preservation of these essential medicinal plants. The genetic stability of in vitro regenerated plants was tested using RAPD markers, which showed that there were no variances among the plants and that they were genetically homogeneous and true to type to their mother plants. The biochemical shifts that took place during the stages of in vitro regeneration have the potential to act as markers of differentiation, which is then followed by in vitro organogenesis and the production of plantlets.

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