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Research Article

Micropropagation and Phytochemical Analysis of Two Medicinal Plants of Western Maharashtra

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ABSTRACT

Micropropagation of important medicinal plants and induction of callus was carried out for *Nyctanthes arbor- tristis Linn.* and *Tinospora cordifolia*. Murashige and Skoog's (MS) media supplemented with 2.5 µM of Benzylaminopurine (BAP) and 4.4 µM of BAP showed best response. Screening of Phytochemical constituents was performed using generally accepted laboratory techniques for qualitative evaluation. The constituents screened for were tannins, cardiac glycosides, saponins, terpenoids, flavanoids, reducing compounds and caratenoids. These plants can be considered as potential source of useful drugs.

Key words: Micropropagation, Callus, Phytochemical constituents

INTRODUCTION

Herbal medicine is among the most respected of the ancient natural therapies and has stood the test of time despite the introduction of modern medical science. Herbs are compatible with the chemistry of the human body, which has adopted over thousands of years to assimilate them. Today there is an enormous resurgence of interest in all herbal products and a rediscovery of the traditional use of medicinal herbs. Production of secondary metabolite by plant is a new horizon for scientists. This includes the discovery of new useful compounds produced by natural plant populations in very small quantities or compounds that may not be produced by the adult plants which are available in cultures.[1]

According to red list of threatened species, 44 plant species are critically endangered, 113 endangered, and 87 vulnerable (IUCN, 2000). Many medicinal plants are also in trouble from over harvesting and destruction of habitat. Population growth, urbanization and unrestricted collection of medicinal plants from wild are resulting in an over-exploitation of natural resources. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Micropropagation therefore, can be used to produce a large number of plants that are genetically identical to parent plant as well as to one another.[2] Considering the potential and promises of plant tissue culture technology, efforts have been directed for implementing this technology to improve the productivity of these plants. Plants used for the present study have lots of medicinal properties. Medicinal value of plants cal observation was done every week for the callus formation as well as growth of the callus and results were noted down.

lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds. With this objective, the present investigations are focused on callus formation and micropropagation from different explants and to find out the phytoconstituents present in those plants.

MATERIALS AND METHODS

The plant materials of *Tinospora cordifolia* and *Nyctanthes arbor-tristis Linn*. were collected randomly from different parts of Western Maharashtra. Different plant parts like young leaves, mature leaves, axillary buds, shoot nodes were taken. For phytochemical analysis, the leaves and the shoots were taken. The plant material was washed thoroughly under running tap water and sun dried. Both the dried leaves and stems were powdered in electric blender separately. Both the powdered materials were stored in air tight containers at room temperature for further use.

Micropropagation: In a laminar flow chamber, three beakers with 70% alcohol, tween 20 and 1% $HgCl_2$ was taken. The plant material were dipped in them for few seconds one by one respectively and then washed twice or thrice with distilled water. The explants were used for inoculation. The sterile explants of *Tinospora cordifolia* (axillary bud, leaves, shoot node,) and *Nyctanthes arbour-tristis Linn.* (leaves, buds and embryo) were inoculated in Murashige and Skoog's (MS) media with (0.63 mg/l and1.0 mg/ml) Benzylaminopurine (BAP) respectively and Kinetin (0.15 mg/l.), 2-4 D (0.5 mg/l) and 3% of sucrose. The pH was adjusted to 5.7. Physi Phytochemical analysis: The plant extracts were subjected to different phytochemical analysis. Tests for tannins, cardiac glycosides, saponins, terpenoids and

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flavanoids were done according to the methods used by Trease and Evans.[3] Tests for reducing compounds and chloride was added to it. The solutions were observed for blue green colour.

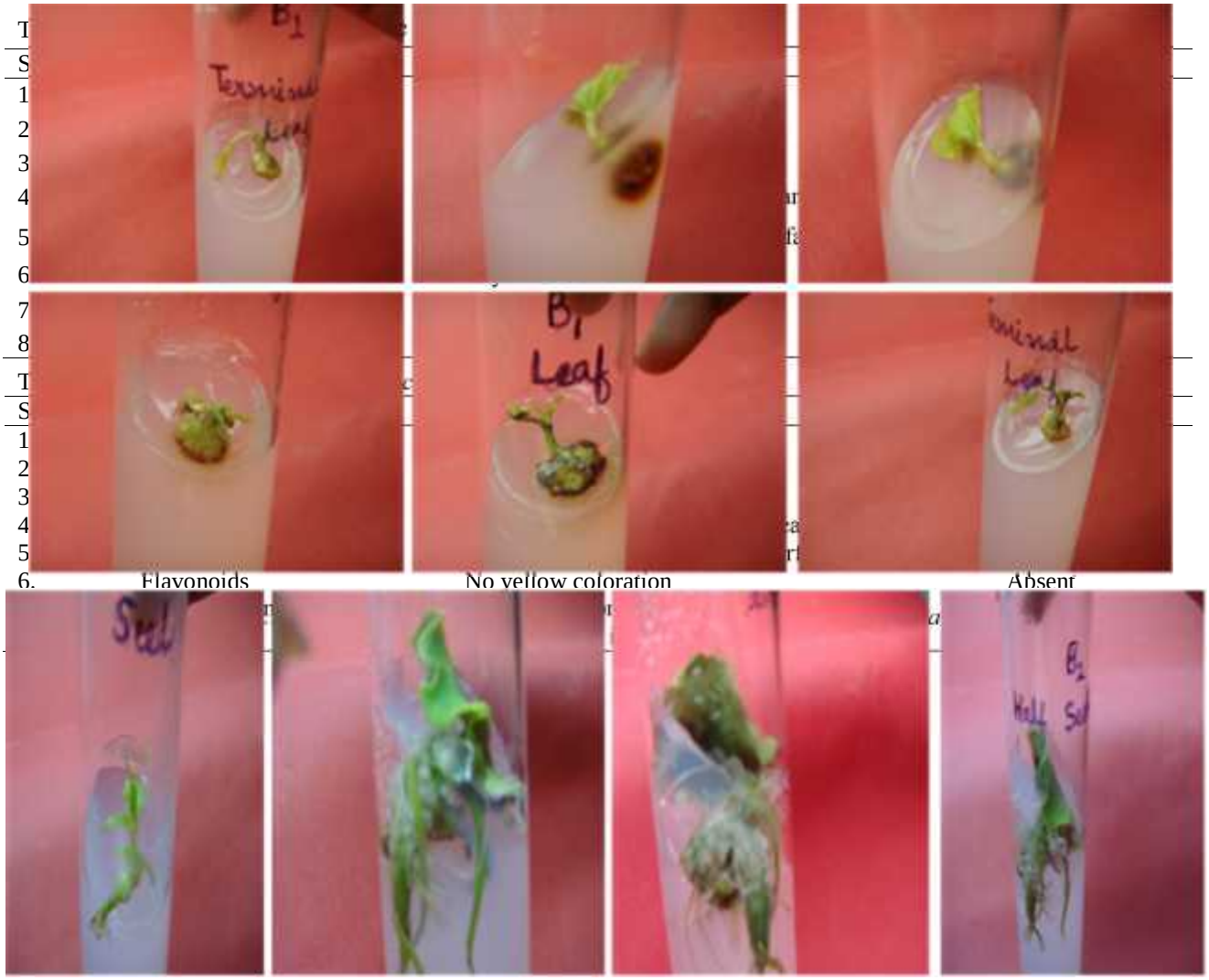

Figure 2- Callus formation and other plant part regeneration of Nyctanthes arbour-tristis Linn. in MS + BAP

Table 1: Callus formation using MS media with different growth regulator combination for *Tinospora cordifolia*.

Sr. No	Explant	MS +(2-4 D)	MS+Kinetin	MS+ BAP
1	Axillary bud	No growth	No growth	Callus formed after 60 days.
2	Young leaves	No growth	No growth	Callus formed after 60 days.
3	Mature leaves	No growth	No growth	No growth
4	Shoot node	No growth	No growth	Callus formed after 60 days.

caratenoids were done according to the method used by Ajayi *et al.*[4]

Test for Cardiac glycosides: 20 mg plant powder was dissolved in 1 ml glacial acetic acid and 1-2 drops of ferric chloride solution was added to it. The mixture was observed for turbidity or precipitation.

Table 2: Callus formation using MS media with different growth regulator combination for *Nyctanthes arbour-tristis Linn*.

Sr. No	Explant	MS + (2-4 D)	MS+Kinetin	MS + BAP
1	Axillary bud	No growth	No growth	No growth
2	Young leaves	No growth	No growth	No growth
3	Mature ovary	No growth	No growth	No growth
4	Young ovary	No growth	No growth	Callus formed after 60 days followed by multiple shoot formation.

Test for Tannins: 20 mg of test sample for both leaves and stems were taken in a test tube and they were dissolved in 1 ml distilled water. 1-3 drops of ferric chloride was added to it. The solutions were observed for blue green colour.

Test for saponins: 40 mg of test sample was dissolved in

5 ml distilled water and they were shaken vigorously for a stable froth. 3 drops of olive oil was added to the froth and again shaken and observed for emulsion formation.
Test for terpenoids: 20 mg test sample was mixed with 1 ml chloroform. 1 ml. of H_2SO_4 was added to it carefully. It was observed for a reddish brown interface.
Test for flavonoids: 1ml of distilled water was added to 20 mg of plant powder. 0.5 ml. of 1% ammonia solution was added to it. To another sample of 20 mg of plant powder and 1ml distilled water, concentrated H_2SO_4 was added. The two mixtures were observed for yellow colouration which tends to disappear after sometime.
Test for reducing compounds: 1 gm of test sample was dissolved in 10 ml. of distilled water and then boiled for 5 minutes. The solution was then filtered and cooled. 20% Sodium hydroxide was added to it in order to make the mixture alkaline. Equal volume of Benedict's solution was added to the mixture and boiled. The solution was observed for brick red precipitation which indicated the presence of reducing compounds.
Test for carotenoids: 1 gm of plant material was taken in a test tube. 10 ml. of chloroform was added to it and it was shaken vigorously. The solution was filtered and 85% H_2SO_4 was added to the mixture. A blue colouration at the interface indicates the presence of caratenoids.

RESULTS AND DISCUSSION

It was observed that callus induction occurs in case of *Tinospora cordifolia* as well as with *Nyctanthes arbour-tristis Linn.* in MS media with BAP but there was no callus formation from explants inoculated in MS media with Kinetin and 2,4-dichlorophenoxyacetic acid (2-4 D). The callus formation was observed after 60 days of inoculation (figure 1, figure 2). In case of *Tinospora,* the calluses were formed from axillary buds, young leaves as well as from shoot node though there was no callus formation from the mature leaf explants. In case of *Nyctanthes,* explants from young ovary showed callus formation which was followed by multiple shoot formation but other explants failed to grow in the same media (table 1, table 2). Present study revealed that in *Tinospora cordifolia* secondary metabolites observed were tannins, cardiac glycosides, saponins, terpenoids, reducing compounds, carotenoids and alkaloids. Flavanoid test showed negative result. In case of *Nyctanthes,* the secondary metabolites present were tannins, cardiac glycosides, saponins, terpenoids, caratenoids and alkaloids. Flavanoids and reducing compounds were absent. The medicinal properties of alkaloids, saponins and tannins were reported by Ayitey Smith and Addoe-Mensah.[5,6] Alkaloids, tannins and saponins used as traditional herbal preparations against various common ailments, have also been reported by Addae-Mensah.[7] *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications has been proposed by Sinha *et al.*[8] Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi) were reported by Singh *et al.*[9] Agarwal *et al* have studied the effect of *Tinospora cordifolia* on learning and memory in normal and memory deficit rats.[10] *Nyctanthes arbor-tristis Linn.* also has immense medicinal & therapeutic properties which can help human kind in its proper survival. It is endemic & grows in particular area only. So its micropropogation is essential for its widespread availability. Studies on immuno-bioactivities of *Nyctanthes arbor-tristis Linn.* (Oleaceae) was done by Kannan *et al.*[11] Depletion of tumor necrosis factor- α in mice by *Nyctanthus abor-tristis Linn.* has been reported by Paul & Saxena.[12]

CONCLUSION

Tinospora cordifolia and *Nyctanthes arbour-trisis Linn.* both are important medicinal plants which should be mass propagated by tissue culture in this region. Phytochemical analysis showed that these two plants can be used as a potential source of drugs against different diseases. Further, quantitative analysis should be done for phytochemical constituents of these plants for antimicrobial and antifungal activity.

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