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IN VITRO CULTURE STUDIES ON MEDICINAL PLANT: *OLDENLANDIA CORYMBOSA* L. (RUBIACEAE)

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ABSTRACT
The present study was to standardize *in vitro* culture of *Oldenlandia corymbosa* L. on MS medium supplemented with different growth regulators. Present study resulted in the identification of a suitable media with proper propositions of growth regulators to induceshoot, root and flower bud formation of O. *corymbosa*. Moreover *O. corymbosa* is the one of the medicinal plant with varied medico-potential activities. The further studies are required to compare the production of essential phytochemical constituents in both *in vitro* and other conditions.

KEYWORDS: *In vitro* Culture; Medicinal Plant; *O. corymbosa*; Rubiaceae.

INTRODUCTION
Medicinal plants have been the subject of man's curiosity since times immemorial. Almost every civilization has a history of medicinal plant use. Approximately 80% of the world's developing countries rely on traditional medicines for their primary health needs and about 85% of the traditional medicine involves the use of plant extracts.[1] Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans and can be used to effectively treat human diseases. Plant tissue culture studies were carried out for the preservation of medicinal plant resources and efficient production of pharmaceutically important secondary metabolites.[2]

Plant tissue culture provides excellent opportunities of mass propagation of plants in test tubes. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutritive medium of known proposition.[3] The cells or tissues are obtained from any part of the plant like stem, root, leaf etc. which encouraged producing more cells in culture and to express their totipotency, where totipotency is the genetic ability to produce more plants.[4] Cells or tissues are grown in different types of glass vials containing a medium with minerals, vitamins and plant growth regulators. In recent years there has been a large increase in the number of research laboratories using tissue culture techniques to investigate many fundamental and applied aspects of plants, even many horticultural companies are setting up small units to multiply plants which are difficult to propagate by conventional methods.[5]

Oldenlandia is a genus of flowering plants belonging to the family Rubiaceae. It is pantropical in distribution. It is an important medicinal plantand it become bitter, febrifuge, diaphoretic, stomachic, laxative, anthelmintic, diuretic, depurative, expectorant and liver tonic. It also used for the treatment of ailments like dysentery, jaundice, depression, leprosy, coughs, skin diseases and bronchitis.[6]

MATERIALS AND METHODS
Selected Plant: *Oldenlandia corymbosa* L. (Rubiaceae).[7] (Fig. 1).

Synonym: *Hedyotis corymbosa* (L.) Lam.

Description: Diffuse or spreading prostrate herbs; stem 4-angled. Leaves simple, opposite, 1-2.5 x 0.2-0.4 cm, linear-lanceolate, acute at apex, base attenuate, scabrid on margins; stipules sheathing. Flowers 2-6 in a corymb; 4-merous; peduncles 4-8 mm long. Calyx tube c. 1 mm long, lobes minute. Corolla white; tube c. 1 mm long with a ring of hairs at throat. Stamens 4 inserted at corolla base. Capsules c. 2 mm across, subglobose,

loculicidal, dehiscing at top only; seeds minute, trigonous.

Local Name: Parpadakapullu.
Habit: Herb.
Habitat: Plains.
Fl. & Fr.: Apr.-Sept.
Distribution: Pantropical.

Fig. 1 Image of *Oldenlandia corymbosa* L. (Rubiaceae).

THE *IN VITRO* METHODS

Culture medium

Excised plant tissues and organs will only grow *in vitro* on a suitable artificially prepared nutrient medium which is known as culture medium. A culture medium is composed of inorganic salts in the form of macro and micro salts, an iron source, vitamins, amino acids, growth hormones and carbohydrate supply. For the present study Murashige and Skoog's (MS) medium was being used and agar was used for solidifying the medium, the medium contain growth regulators.[8]

RESULTS AND DISCUSSION

Nodal regions of *Oldenlandia corymbosa* were taken as the explants for the study. The culture tubes with explants were incubated under controlled conditions and observation were taken at regular intervals. Thevaried responses were recorded from the culture media with MS medium and different compositions of growth regulators and results are presented as following.

Media for shooting, rooting and flower bud formation are given in Table-1,2 & 3; Fig. 2 & 3; Pl.1

1. MS medium supplemented with BA 1mg/l

The explants inoculated in this concentration were seen to produce callus after 7-10 days. After callus induction it was found to produce multiple shoots by 18-20th day. About 60% of culture tubes showed callus induction.

2. MS medium supplemented with BA 2mg/l 70% of the culture tubes showed callus formation within 7-10 days. Multiple shoots were formed, more in number than in the earlier concentration.

3. MS medium supplemented with KN 1mg/l

In this medium, the explants again showed multiple shoot formation. The 70% of the culture tubes gave a positive result. Callus induction was found after the 7th day and it continued till the 18th day after which shoot was initiated ultimately leading to the formation of the multiple shoots. The growth is still continuing.

4. MS medium supplemented with KN 2mg/l

The medium supplemented with this concentration was found to be the most vigorous medium. The explant was found to produce multiple shoots within the time period of 13 to 16 days. The callus induction started with a swelling at the base of the explant after which the shoot initiation occurred.

5. MS medium supplemented with BA1mg/L+ KN 1mg/l

This medium was mainly used for callus regeneration. The callus formed in the media supplemented with NAA 1mg/l+ BA4 mg/l was sub cultured into the media with BA1mg/L+ KIN 1mg/l and was incubated. After the 20th day it was observed that there was a shoot initiation from the callus and by the 26th day root was also found to grow in this medium. The growth is still continuing in the medium.

6. MS medium supplemented with BA2mg/l+ KN 2mg/l

The response in this medium was about 80% but there was no shoot initiation in this medium. The callus was found to grow indefinitely in this medium with many minute roots. The roots were formed in 24-27 days after inoculation.

7. MS medium supplemented with NAA 1mg/l + BA 4mg/l

In this medium about 80% of the culture tubes gave a positive response in callus formation but there was no organogenesis obtained from this medium. The callus was seen to multiply indefinitely in this concentration.

8. MS medium supplemented with NAA 1mg/l + BA 6mg/l

In this concentration the shoot was initiated from a small callus mass after the 15th day. It was also found that in one of the culture tubes 2 flower buds were initiated. About 90% of the culture tubes responded to the concentration.

9. MS medium supplemented with NAA 1mg/l+ KN 4mg/l

There was a response of 90% in this culture media. There was a direct organogenesis in this concentration. Shoots were formed by 6-9days and direct roots were formed by the 16th day of inoculation. The shoot was initiated from a swelling on the explant.

10. ¼ MS medium supplemented with NAA 0.5mg/l

In the medium, callus was formed on the 7th day. The shoot and roots were produced in 15-23 days. The response in the medium was about 50%.

11. MS medium supplemented with NAA 0.2mg/l
The medium with this concentration was observed to produce callus and shoot initiation in 15-18 days. Another important finding was the formation of flower buds in some of the culture tubes. Roots were also formed in the medium in 20-26 days.

12. MS medium supplemented with IBA 1mg/l +KIN 2mg/l
The explant showed a response of about 80% in this concentration. In the medium the callus was induced in 6-9 days. Callusshowed indefinite growth. Many minute roots were also formed into the medium.

Table. 1 *In vitro* response of nodal explants of *Oldenlandia corymbosa* on MS medium supplemented with varying concentrations of growth regulators.

Medium	*Invitro* response noticed at various time intervals						
	5 Days	10 Days	15 Days	20 Days	25 Days	No: of shoots	3O Days
BA 1mg/l	-	Callus initiation	Callus growth	Shoot iniation	Multiple shoot formation	2	Growth continues
BA 2mg/l	-	Callus initiation	Callus growth	Shoot iniation	Multiple shoot formation	3	Growth continues
KIN 1mg/l	-	Callus initiation	Shoot iniation	Multiple shoot formation	Growth continues	3	Growth continues
KIN 2mg/l	-	Callus initiation	Shoot iniation	Multiple shoot formation	Growth continues	3	Growth continues
KIN 1mg/l + BA 1mg/l	-	Callus initiation	Callus growth	Shoot formation	Root formation	3	Growth continues
KIN 2mg/l + BA 2mg/l	-	Callus initiation	Callus growth	Callus growth	Root formation	-	Growth continues

Table. 2 *In vitro* response of nodes of *Oldenlandia corymbosa* on MS medium supplemented with varying growth regulators.

Medium	*Invitro* response noticed at various time intervals					
	5 Days	10 Days	15 Days	20 Days	25 Days	30 Days
NAA 1mg/l + BA 6mg/l	-	Callus initiation	Shoot initiation and flower bud formation	Growth continues	Growth continues	Growth continues
NAA 1mg/l + BA 4mg/l	-	Callus initiation	Callus growth	Growth continues	Growth continues	Growth continues
NAA 1mg/l + KN 4mg/l	-	Shoot formation	Root formation	Callus initiation	Callus growth	Growth continues
IBA 1mg/l + KN 2mg/l	-	Callus initiation	Callus growth	Callus growth	Root formation	Growth continues
NAA 0.2mg/l	-	Callus initiation	Shoot formation	Flower bud initiation	Root formation	Growth continues
¼ MS + NAA 0.5mg/l	–	Callus initiation	Shoot formation	Root formation	Growth continues	Growth continues

Table- 3 Effect of growth regulators on flower bud formation.

Concentration	% Response	Number of flower buds
NAA 1mg/l + BA 6mg/l	10	2
NAA 1mg/l + BA 4mg/l	_	
NAA 0.2mg/l	40	2±1

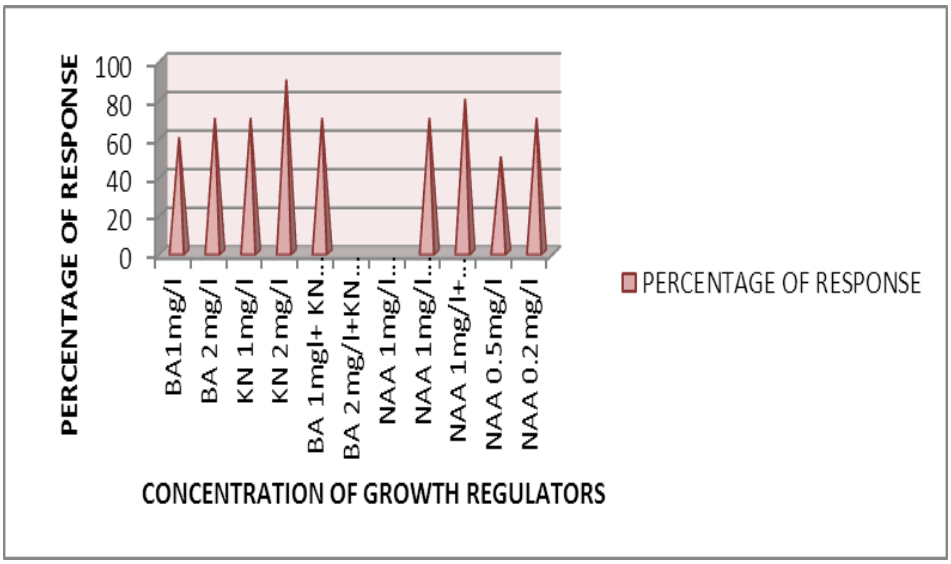


Fig. 2 Response rate of explants *O. corymbosa* in different shooting medium.

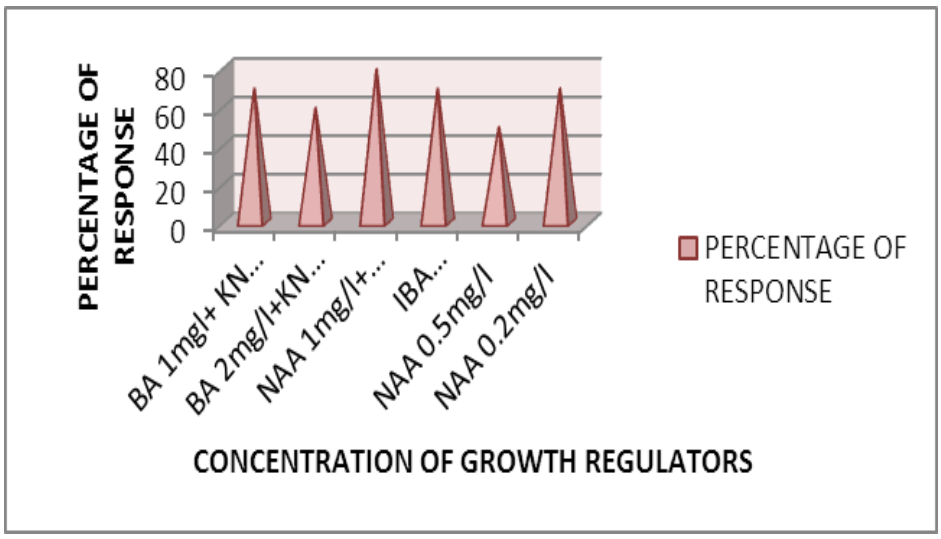


Fig. 3 Response rate of explants *O. corymbosa* in different rooting medium.

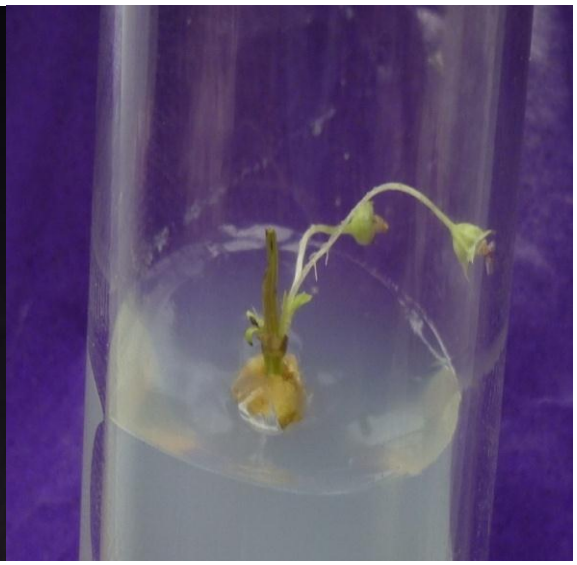
Plate-1.

Direct organogenesis (NAA 1.0mg/l + KN 4.0mg/l). **Multiple shoot formation (KN 2.0mg/l).**

Multiple shoot formation (BA 2.0mg/l).

Indirect organ ogenesis (KN1.0mg/l+ BA 1.0mg/l).

Root initiation (¼ MS + NAA 0.5mg/l).



***In vitro* flowering (NAA 1mg/l + BA1mg/l).**

The growth and organogenesis of explants in tissue culture is influenced by genotype of explants, environment and the tissue development factors. In the present study the effect of cytokinin (BA and KN) in combination with auxin (NAA and IBA) and cytokinin (BA+KIN) and auxins (NAA) alone were studied. Cytokinins have an effective role in shoot induction and cell division whereas auxins are needed for root induction. The reagent such as 0.1% $HgCl_2$ was used for surface sterilizing the explant in the present study. For the present study the nodal explants of *Oldenlandia corymbosa* were used. Many works have been done in plants belonging to the family Rubiaceae. Similar studies were conducted in some species like *Cephaelis ipecacuanha*[9], *Morinda* spp[10], *Neolamarckia cadamba*[11] and *Ixor acoccinea*,[12]

In the present study the media supplemented with KN was found to be the most effective than other growth regulators like BA and the combination of the same for shoot induction and callus formation. The effective growth of various other plant species was also reported in the KN medium. Some of them are *Sinocalamus latiflora*.[13], *Cucumis melo*.[14], *Zingiber officinale* and *Curcuma longa*.[15], *Tinospora cordiflora*.[16], *Stevia rebaudiana*.[17] The growth in this medium was seen to be prominent among all the other culture media but the degree of response of the explant varied slightly in different concentrations of the same.

The MS medium supplemented with NAA+ KN exhibited root induction. The same results were observed in plant species like *Stylosanthus guyanensis*.[18], *Cephaelis ipecacuanha*.[19], *Uncaria elliptica*.[20], *Morinda elliptica*[21], and *Curcuma zedoaria* (Loc et.al. 2005). Due to variation in the genotype of the explants within the same species the response of the explant in different medium was different.

CONCLUSION

The present study was to standardize*in vitro* culture of *Oldenlandia corymbosa* on MS medium supplemented with different growth regulators. With the help of the present study, a suitable media with proper propositions of growth regulators were used to induced shoot, root and flower bud formation in the plant. From the observations made at regular intervals, it can be concluded that the shoots were initiated in medium supplemented with cytokinins alone and in combination of auxins and cytokinin. Of all the media the highest rate of shoot proliferation was seen in the MS medium supplemented with KN. The maximum root formation was obtained from MS medium supplemented with NAA+ KN. About 80% of the culture showed root initiation. Even though the maximum root induction was shown in NAA+ KN, all other combinations of growth regulators also exhibited the formation of minute roots of varying lengths. Similarly the flower buds were also initiated in varying concentration of growth regulators.

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