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# EFFECT OF DIFFERENT PLANT GROWTH REGULATORS AND ELICITORS ON SECONDARY METABOLITE PRODUCTION IN *OLDENLANDIA CORYMBOSA* L. (RUBIACEAE)

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## ABSTRACT
*Oldenlandia corymbosa* L. is a potential medicinal herb belongs to the family Rubiaceae. *In vitro* culture studies along with elicitor induced secondary metabolite production were analyzed in *O. corymbosa* through the present study. The nodal explants of *Oldenlandia corymbosa* L. were cultured on MS media supplemented with different concentrations and combinations of cytokinins and auxins and observed at regular intervals. Similarly biochemical analysis reveals that, various combinations of growth regulators along with elicitors were used to examine the occurrence of potential secondary metabolites which are present in *O. corymbosa*.

**KEY WORDS:** Growth regulators, Elicitors, Secondary metabolites, *O. corymbosa*

## INTRODUCTION
Medicinal plants have been the subject of man's curiosity since time immemorial.[1] Medicinal plants are being used by about 80 % of the world population for primary health care.[2] The practice of using medicinal plants for the treatment of various diseases started since the dawn of civilization.[3] In fact ancient man was totally dependent on medicinal plants for the treatment of various ailments.[4]

A drug obtained from such medicinal plants consists either from entire plant or their parts like leaves, roots, fruits, seeds etc. The dried plants or plant parts are been widely used for the preparation of phytomedicines in ayurvedic and folk medicines. The disease curing properties of plants are associated with their chemical constituents. However, presently there has been an increasing interest for the plant based drugs because of the ready acceptance to local population due to relative inexpensiveness and minimal side effects.[5]

The WHO estimates that 80 % of more than 4000 million inhabitants of the world rely on traditional medicine for their primary health care need, and it can be safely be presumed that the majority of the traditional therapy involves the use of plant extracts or their principles.[6] In addition to essential primary metabolites, higher plants are able to synthesize a wide variety of low molecular weight compounds called secondary metabolites. They have no recognized role in the maintenance of fundamental life process in the plants that synthesize them, but they do have an important role in the interaction of plant with its environment. The production of these compounds is often low (less than 1% dry weight) and depends greatly on the physiological and developmental stages of the plant.[7] Plant tissue culture is an example of a biotechnology with a multitude of applications to medicinal plant improvement programs, as a result the tissue culture will serve as an indispensable tool for the identification of useful medicinal plant varieties for the production of plant secondary metabolites.[8] Plants produce secondary metabolites in nature as a part of their defense mechanismagainst attack by pathogens. Elicitors are signals triggering the formation of secondarymetabolites. Elicitation, has been one ofthe most effective strategies for improving the productivity of bioactive secondary metabolites. Biotic and abiotic elicitors which are classified on their origin are used to stimulate secondary metabolite formation in plant cell cultures.[9]

Through the present study an attempt was made for the studies on multiplication of shoot in different MS media composition and effect of different elicitors in varying concentrations for *in vitro* production of secondary metabolites by *Oldenlandia corymbosa* L.

## MATERIALS AND METHODS
**Selected Plant: *Oldenlandia corymbosa* L.** (Rubiaceae).[10] (Fig. 1).

**Synonym:** *Hedyotis corymbosa* L.

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

**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

**The *in Vitro* Culture Methods:** Excised plant tissues and organs will only grow *in vitro* on a suitable artificially prepared nutrient medium, which is composed of inorganic salts in the form of macro and micro salts, an iron source, vitamins, amino acids and growth regulators. For the present study Murashige and Skoog's (MS) medium is being used and Agar is used for solidifying the medium, the medium contain all the growth regulating factors along with $CaCl_2$ etc.[11]

Sodium alginate, calcium chloride and salicylic acid were used as elicitors in the present study. Out of the above mentioned elicitors, sodium alginate and calcium chloride were added in solid form and concentrations suh as 0.5 %, 1 %,1.5 %, 2 % and 2.5 % of both were used for studying the effect on secondary metabolite production. Salicylic acid was used in the liquid form and the respective concentrations 0.05 Mm,0.5mM,1mM,1.5mM and 2mM.

The explants such as nodal portions and shoot tip were collected from mature healthy plants of *Oldenlandia corymbosa* L., these are also treated with elicitors and surface sterilized. The surface sterilized explants were cut in to small pieces and were inoculated to sterile petri plates using flame sterilized forceps. The cultures were periodically observed and their morphogenetic responses were recorded. Callus that was initiated from shoot tip and nodal explants were maintained using MS medium supplemented with 3 mg/l BAP, 1mg/l NAA+2 mg/l Kn and varying concentrations of elicitors. Responded cultures were sub cultured into MS medium supplemented.

**Analysis of Secondary Metabolites:** To analyze the effect of growth regulators and elicitors on metabolite production were screened through biochemical studies were conducted. The qualitative tests for carbohydrates, phenolics, flavones and flavonones, Terpenoids and alkaloids were conducted**.** Qualitative analyses of carbohydrates were conducted using Molisch's test and Fehling's test. Similarly for phenolic compounds were using Ferric chloride test, Lead acetate test, flavones and flavonones were conducted by Shinoda's test, Terpenoids were conducted by Salkowski's test and Alkaloids were conducted by Mayer's test, Dragendroff's test, Hager's test and Hager's test.[12]

## OBSERVATION AND RESULTS

The present study shows the effect of plant growth regulators and elicitors on morphogenetic responses and the secondary metabolite production in an important medicinal plant *Oldenlandia corymbosa* L. through direct and indirect shoot induction from nodal explants.

### Importance of the Selected Plant

*Oldenlandia corymbosa* L. belongs to Rubiaceae family which is commonly known as 'diamond flower' and '*parpadagam* or *parppatakapullu'* in Traditional Medicine of Kerala. It is a medicinal herb, widely distributed throughout India, Philippines, Common in and about towns, in open waste places, grass lands etc.

The whole plant is used in medicinal purposes. The plant is cooling, bitter, febrifugal, and pectoral, digestive, diuretic, laxative, stomachic and vermifuge. It is used to cure hepatopathy and also used for treating venomous bites. Decoction of plant is used for fevers, stomach aches, and skin diseases and also given internally to cure heat eruptions.Tincture of roots is used as vermifuge. It is also used to cure morbid *pitta* and*kapha*, jaundice, liver diseases, thirst and burning sensation.It has antioxidant, antitumor, antimalarial, anti inflammatory, antithelmintic, digestive and antimicrobial, hepatoprotective.[13] The *In vitro* responses of nodal explants in MS medium supplemented with various plant growth regulators.

**Direct Shoot Regeneration:** For the induction of direct multiple shoots from nodes, explants were cultured on MS medium supplemented with various concentration and combinations of growth regulators. Multiple shoots were formed directly from nodal and shoot tip explants on MS medium supplemented with different plant growth regulators such as BAP (3 mg/l), NAA (1 mg/l) +BAP (2 mg/ l ) and kin (3mg/l) along withvarious concentrations of elicitors (Table-1-9 &Fig.2-10).

**Table. 1: Effect of Sodium alginate in MS+3 mg/l BAP on shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of SA IN mM | % OF Response | NO.OF Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.05 | 50 | 2.5±0.40 | 1.5±0.02 |
| 0.5 | 50 | 3.2±0.47 | 2.2±0.30 |
| 1 | 50 | 3.3±0.30 | 2.1±0.02 |
| 1.5 | 30 | 2.2±0.31 | 2.0±0.2 |
| 2 | 30 | 1.5±0.2 | 1±0.1 |

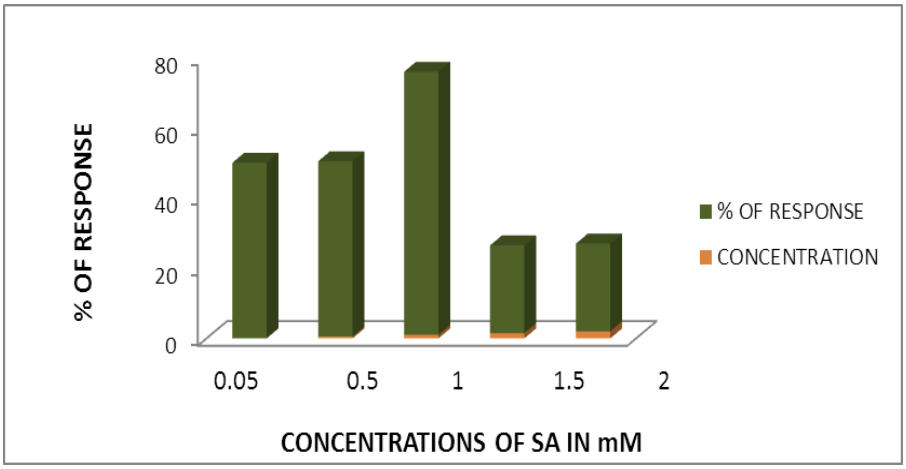


**Figure. 2: Effect of Sodium alginate in MS+3 mg/l BAP on nodal explants of *Oldenlandia corymbosa* L.**

**Table. 2: Effect of Sodium alginate in MS+1 mg/l NAA+2 mg/1 BAP on shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of Sodium Alginate in Grams | % of Responses | No. of Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.5 | 50 | 2.4±0.02 | 2±0.01 |
| 1 | 50 | 3.6±0.38 | 3±0.05 |
| 1.5 | 30 | 2.5±0.2 | 3±0.21 |
| 2 | 0 | - | - |
| 2.5 | 0 | - | - |

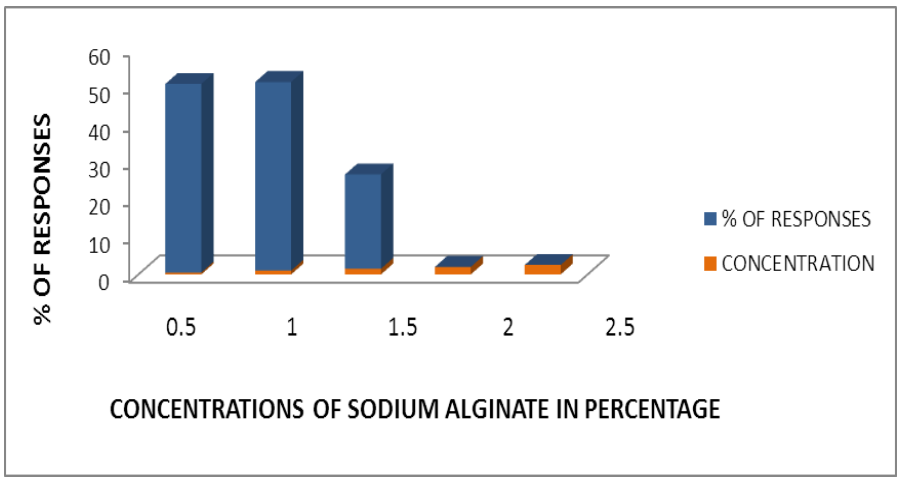


**Figure. 3: Effect of Sodium alginate in MS+1 mg/l NAA+2 mg/1 BAP on nodal explants of *Oldenlandia corymbosa* L.**

**Table. 3: Effect of $CaCl_2$in MS+1 mg/l NAA+2 mg/1 BAP on shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of Calcium Chloride in Percentage | % of Response | No. of Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.5 | - | - | - |
| 1 | - | - | - |
| 1.5 | 50 | 3.4±0.15 | 1.88±0.18 |
| 2 | - | - | - |
| 2.5 | - | - | - |

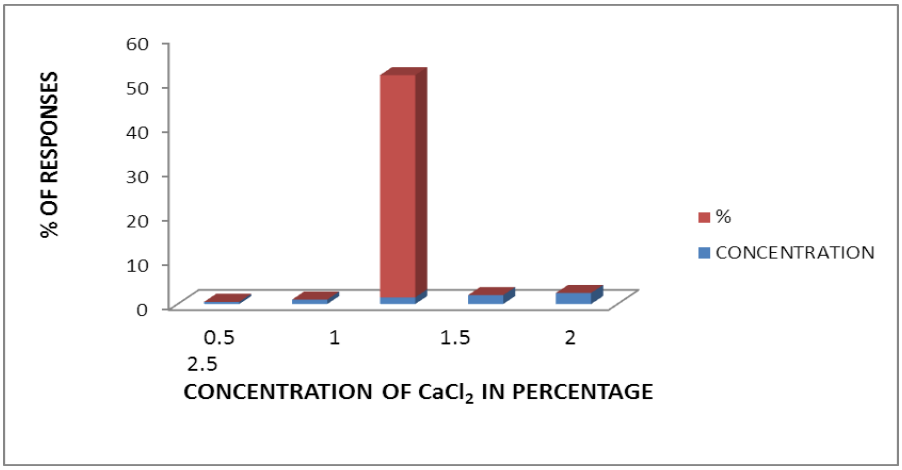


**Figure. 4: Effect of $CaCl_2$in MS+1 mg/l NAA+2 mg/1 BAP on nodal explants of of *Oldenlandia corymbosa* L.**

**Table. 4: Effect of SA IN MS+1 mg/l NAA+2 mg/l BAP on shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of Sa In mM | % of Response | No. of Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.05 | 0 | - | - |
| 0,5 | 0 | - | - |
| 1 | 60 | 1.71±0.14 | 1.32±0.12 |
| 1.5 | 50 | 1.53±0.13 | 1.42±0.1 |
| 2 | 50 | 1.4±0.02 | 1.2±0.01 |

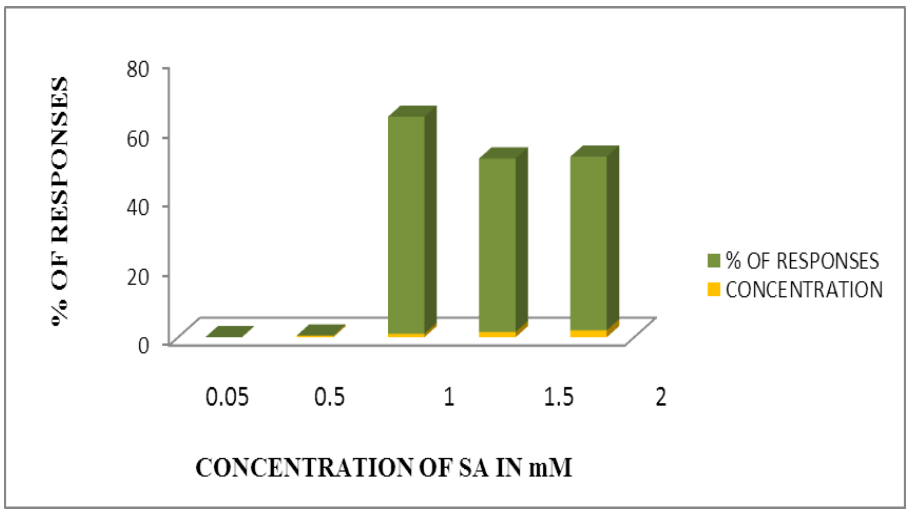


**Figure-5: Effect of SA IN MS+1 mg/l NAA+2 mg/l BAP on nodal explants of of *Oldenlandia corymbosa* L.**

**Table. 5: Effect of SA IN MS+3 mg/l KINon shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of Sodium Alginate in Percentage | % of Response | No. of Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.5 | 80 | 6±0.8 | 2.04±0.37 |
| 1 | 50 | 3±0.54 | 5.75±0.89 |
| 1.5 | 3 0 | 3±0.42 | 4±0.35 |
| 2 | 0 | - | - |
| 2.5 | 0 | - | - |

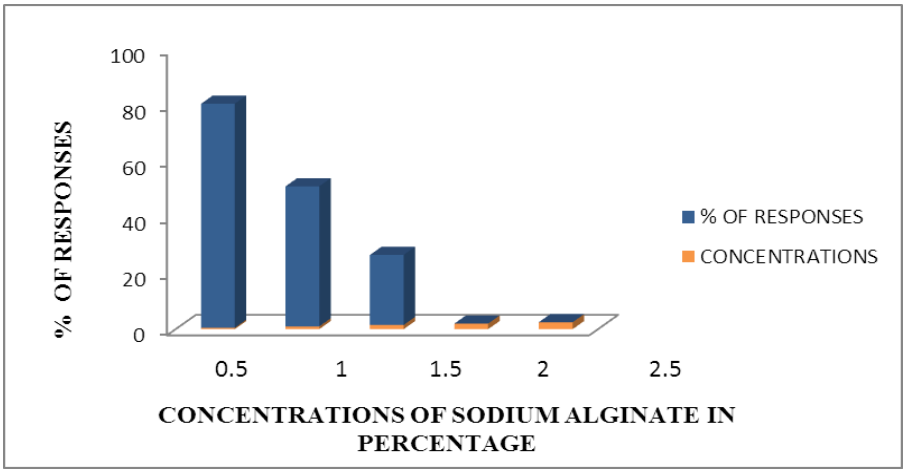


**Figure. 6: Effect of SA IN MS+3 mg/l KINon nodal explants of of *Oldenlandia corymbosa* L.**

**Table. 6: Effect of $CaCl_2$in MS+3 mg/l KINon shoot induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of in Cacl2 Percentage | % of Response | No. Of Shoots Per Explants | Length of Shoots |
|---|---|---|---|
| 0.5 | 20 | 2.2±0.2 | 1.5±0.03 |
| 1 | 0 | - | - |
| 1.5 | 0 | - | - |
| 2 | 40 | 2.2±0.1 | 1.2±0.2 |
| 2.5 | 0 | - | - |

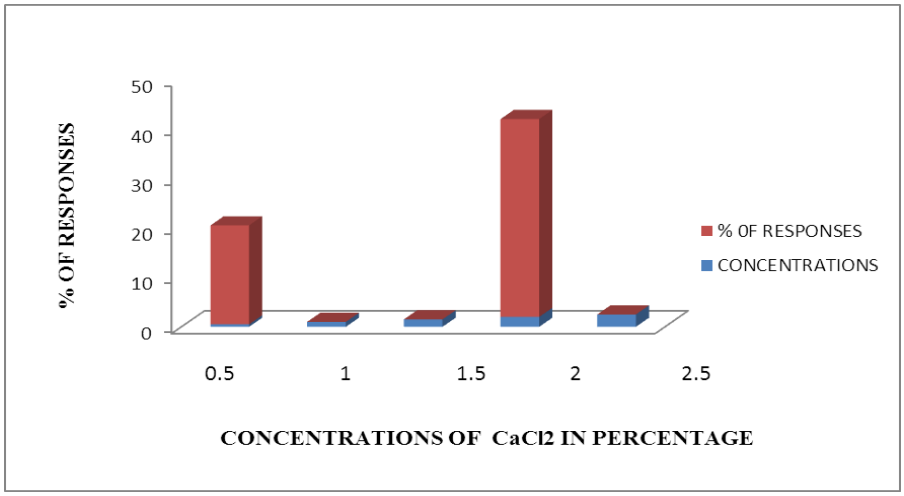


**Figure. 7: Effect of $CaCl_2$in MS+3 mg/l KINon nodal explants of of *Oldenlandia corymbosa* L.**

**Table. 7: Effect of Sodium alginate MS+1 mg/NAA+2mg/1KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L.**

| Concentration of Sodium Alginate in Percentage | % of Responses |
|---|---|
| 0.5 | 60 |
| 1.5 | 80 |
| 1 | 60 |
| 2 | 80 |
| 2.5 | 80 |

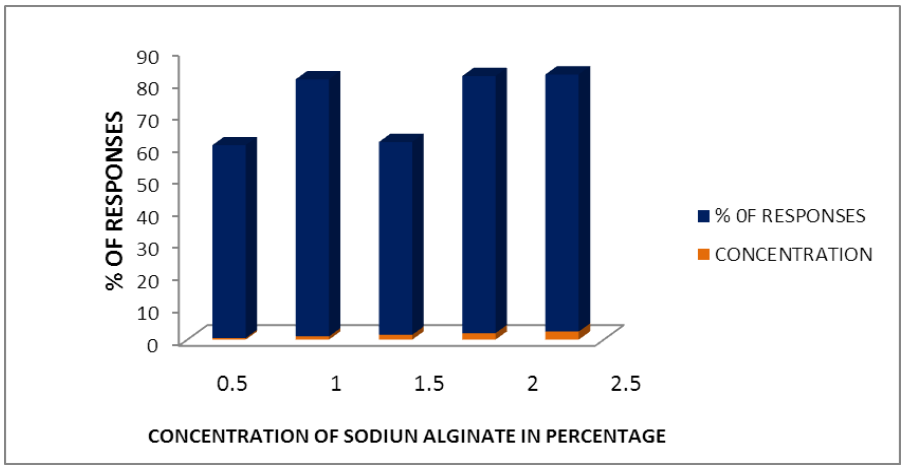


**Figure. 8: Effect of Sodium alginate MS+1 mg/NAA+2mg/1KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L.**

**Table. 8: Effect of $CaCl_2$in MS+1 mg/l NAA+2 mg/1 KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L.**

| concentration of cacl2 in percentage | % of Responses |
|---|---|
| 0.5 | 80 |
| 1 | 80 |
| 1.5 | 60 |
| 2 | 80 |
| 2.5 | 80 |

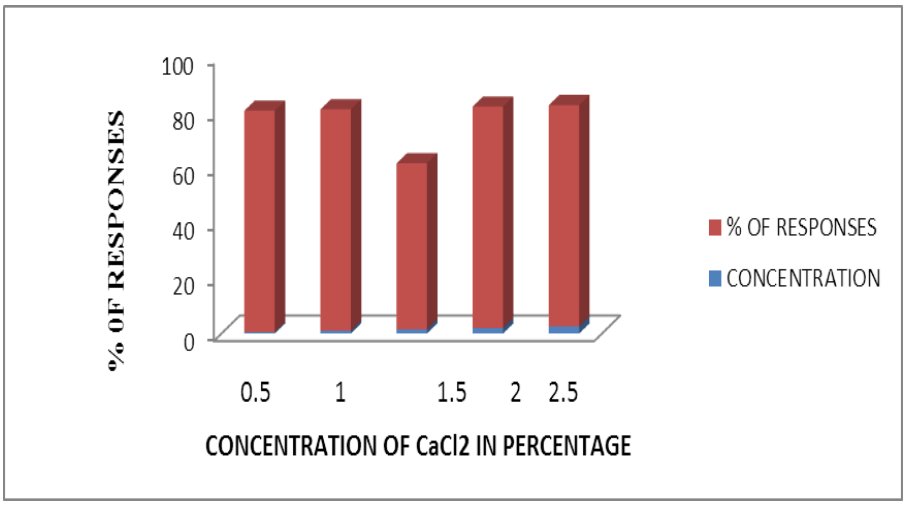


**Figure. 9: Effect of $CaCl_2$in MS+1 mg/l NAA+2 mg/1 KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L.**

**Table. 9: Effect of SA in MS+1 mg/l NAA+2 mg/1 KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L..**

| Concentration of SA In mM | % 0F Responses |
|---|---|
| 0.05 | 75 |
| 0.5 | 50 |
| 1 | 75 |
| 1.5 | 50 |
| 2 | 50 |

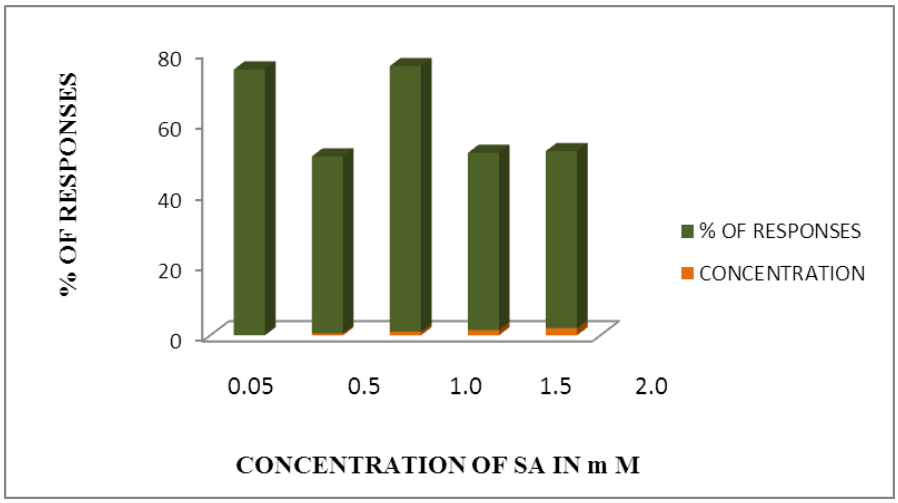


**Figure-10: Effect of SA in MS+1 mg/l NAA+2 mg/1 KIN on callus induction from nodal explants of *Oldenlandia corymbosa* L.**

**Biochemical Studies**

To analyze the effect of growth regulators and elicitors on metabolite production were studies through biochemical profiling. The qualitative for the analysis of carbohydrates, phenolics, flavones and flavonones, Terpenoids and alkaloids were conducted and their results were given in below mentioned table (Table-10).

**Table. 10: Effect of elicitors and plant growth regulators on metabolite production in *In vitro* cultures of *Oldenlandia corymbosa* L.**

| Growth Regulator+Elicitor | Carbohydrates | Flavonoids | Phenolics | Terpenoids | Alkaloids |
|---|---|---|---|---|---|
| MS +3mg/l BAP+2mM Salicylic acid | + | – | – | – | – |
| MS +1mg/l NAA+2mg/l BAP+0.5% Sodium Alginate | + | – | – | – | – |
| MS +3mg/l Kin +0.5% $CaCl_2$ | + | – | – | – | – |
| MS +3mg/l Kin +1% Sodium Alginate | + | – | – | – | – |
| MS +1mg/l NAA+2mg/l Kin+0.05mM Salicylic acid | + | – | – | – | – |
| MS +3mg/l BAP | + | – | – | – | – |
| MS +3mg/l BAP+0.5mM Salicylic acid | + | + | + | + | + |
| MS +3mg/l BAP+1mM Salicylicacid | + | + | + | + | + |

The present work was mainly concentrated on *in vitro* culture studies (direct regeneration of shoot from nodal explants and analysis of the effect of plant growth regulators and elicitors on secondary metabolite production) of the important medicinal plant *Oldenlandia corymbosa* L. Medicinal plants are of great

interest into the researchers in the field of Biotechnology as most of the drug industries depend in part, on plants for the production of pharmaceutical compounds. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and industrially important biochemical.[14] *In vitro* plant materials are one of the good sources for the production of secondary metabolite and elicitation can be used as one of the important tool in order to improve the synthesis of these compounds.[15]

In the present study on *Oldenlandia corymbosa* variations were observed in metabolite production with elicitors used in the media. Of the various elicitors tested Salicylic acid was found to be highly effective in promoting the production of various secondary metabolites in the cultures of *O. corymbosa*. Similarly elicitor induced secondary metabolite production was reported in several plant species like *Hibiscus* species.[16] and *Beta vulgaris.*[17]

## CONCLUSION

The present study was on *in vitro* shoot regeneration from the nodal explants and analysis of the effect of different plant growth regulators and elicitors on secondary metabolite production in *O. corymbosa*. The nodal explants of *Oldenlandia corymbosa* L. were cultured on MS media supplemented with different concentrations and combinations of cytokinins and auxins and observed at regular intervals. Similarly biochemical analysis reveals that, various combinations of growth regulators along with elicitors were used to examine the occurrence of potential secondary metabolites which are present in *O. corymbosa*. Secondary metabolites have played a significant role in medicine since ancient time period. The extensive literature survey reveals that *O. corymbosa* L. has a long history of traditional uses and that have been proved by many research works. Further studies also recommended in *O. corymbosa* revealing various active principles behind many prospective medicinal properties.

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