# *In vitro*, callus induction and estimation of some active constituents in three different medicinal plants

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**ABSTRACT:**

*Euphorbia peplus, Sisymbrium irio* and *Malva parviflora* are medicinal plants and found in many parts of the world. They have a large number of secondary metabolites that are utilized to treat numerous illnesses. The current study deals with examining the influence of plant growth regulators on callus induction by applying different explants of each of the studied plants. A comparative study was done between the ethanolic extracts of roots, stems and leaves of the mother plants and those regenerated from *in vitro* to analyze the amount of alkaloids, tannins and flavonoids. The obtained data disclosed that the maximum callus induction %, fresh and dry weights were recorded by root segments of *E. peplus* on Murashige and Skoog's medium containing 2 mg / l benzyl adenine and 0.5 mg / l 2,4-dichlorophenoxyacetic acid. Stem explants of *S. irio* achieved the obvious percentage of callus initiation on medium augmented with 0.5 mg / l Kinetin and 2mg/l 2,4-dichlorophenoxyacetic acid. In the case of *M. parviflora* the best callus initiation as well as fresh dry weight were recorded by root explant on Murashige and Skoog's medium including 2,4-dichlorophenoxyacetic acid alone. The study also showed that ethanolic callus extracts have better synthesis for investigated alkaloids, tannins, and flavonoids. The results demonstrated that different explant types may differ in accordance with species, as a result of different responses of their microenvironments to media ingredients.

**Keywords**: *Sisymbrium irio; Euphorbia peplus* and *Malva parviflora*; callus induction Benzyl Adenine,2,4-dichlorophenoxyacetic acid, Kinetin, secondary metabolites.

## INTRODUCTION

*Euphorbia peplus* (Euphorbiaceae) is an annual herb common in Egypt. It is used as a purgative and to treat warts, waxy growths, corns, asthma, catarrh, stomach, liver, uterus, diarrhea, dysentery and decreasing blood pressure (Tchinda, 2008), skin cancers (Tchinda, 2008 and Ramsay *et al*., 2011), skin diseases, migraine, and intestinal parasites (Ozbilgin and Citoglu, 2012). *E. peplus* includes many active compounds like diterpenoids that are used as anti-inflammatories (Wan *et al*., 2016). The latex in this plant is abundant with terpenoids, alkaloids and cardenolides, which have a protective role against infections by pathogens or herbivorous insects (Frezza *et al*., 2018). In addition, it has anticancer, cytotoxic, antimicrobial activities, wart cures and insecticidal properties (Hamed *et al*., 2019). It plays an important role in breast cancer treatment (Al-Emam *et al*., 2019). Jatrophane diterpenes that were isolated from the seeds of *E. peplus* were used for the lysosomal-autophagy pathway (Chen *et al*.,2021).

*Sisymbrium irio* (Brassicaceae) distributes in many regions of the world. It is used to treat rheumatism, coughs, chest congestion, spleen, detoxify the liver, clean wounds and reduce swelling (Lev, 2003), main alkaloids were extracted from the aerial parts (Alsaffar *et al*., 2016). *S. irio* is containing various secondary metabolites such as flavonoids, triterpenoids, steroids, tannins, carbohydrates and saponins (Khalil *et al*., 2017) and has antimicrobial, antifungal, anticancer and anti-inflammatory (Temesgen, 2019). The seeds' elemental analysis revealed the presence of lead, phosphorus, sodium and potassium (Hailu *et al*., 2021). Biosynthesized AgNPs from the seed extracts of *S. irio* indicated that it has fungistatic properties against the chosen plant pathogenic fungi and it has cytotoxic activity against HeLa cells (Rizwana *et al*., 2022).

*Malva parviflora* (Malvaceae) has anti-inflammatory, antimicrobial and cytotoxic activities (Abdel-Ghani *et al*., 2017) it is used to treat many diseases; gastrointestinal, dermatological, urological, haemorrhoidal, menstrual and vaginal disorders (Gasparetto *et al*., 2011), stomach pain, edema and fever (Gutierrez, 2017). The plant was used also as an antidiabetic, antifungal, hepatoprotective, antioxidant, anti-ulcerogenic, analgesic (Navneet, 2017), anti-irritant, antiulcer, wound healing properties, neuroprotective (Munir *et al*.,2021). Fruits and leaves mucilage of *M. parviflora are* used as biological sources of

antitussive and gastro-protective agents (Altyar *et al*.,2022).

In particular, a high auxin-cytokinin or cytokinin-auxin ratio promotes the regeneration of root and shoot, individually, while a mediate ratio of cytokinin and auxin aids in callus induction (Skoog and Miller, 1957). According to several studies the culture medium's auxin and cytokinin hormone levels must be balanced for callus induction because they work together synergistically to promote cell division and elongation, which is a crucial step in the callus induction process (Coenen and Lomax, 1997&Roy and Banerjee ,2003). As reported by (Loredo-Carrilo *et al.,* 2013) 2,4-Dichlorophenoxyacetic acid (2,4-D) can be utilized singly or in conjunction with cytokinin, particularly 6-benzyl amino purine (BAP), to induce the formation of bioactive chemicals *in vitro* and callus induction owing to the species variation,

Plant tissue culture considers an important tool to produce active compounds involving secondary metabolites (Castro *et al*., 2016). Callus culture is a quicker and farther trustworthy means of obtaining medicinal metabolites compared to the collection of plant materials from the wild (Efferth, 2019) since *in vitro* callus production is a direct and quick method of cell multiplication (Cardoso *et al*., 2019). The purpose of the present work is to identify the most effective treatment and explant for callus induction and to estimate active compounds that are produced from *in vitro* callus of the tested species (*E. peplus, S. irio* and *M. parviflora*).

## MATERIAL AND METHODS

This study was carried in the Plant Tissue Culture Unit in the Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt. Seeds for studied species were collected from Cairo (waste land habitats).

### Surface sterilization of seeds

plants were soaked in 70 % ethanol for 1 minute and then rinsed with sterile distilled water to eliminate all traces of the alcohol. Secondly, seeds were immersed in 10 %, 15 %, and 20 % sodium hypochlorite solution with 1 drop of tween 20 for different periods of time (10, 15, 20, and 25 minutes) for each treatment, followed by five rinses with sterile distilled water to remove all traces of disinfected and detergent agents.

### Germination and explant preparation

Seeds plants sterilized for germinating on growth regulator free Murashige and Skoog's (1962) medium, basal salts fortified with 30 g / l sucrose (half- strength MS and full-strength MS medium). About 5 seeds were put in each jar containing 35 ml of culture media. For each duration, 3 jars were used for each treatment. The cultures were kept at room temperature (25 ± 2 °C), illuminated by cool fluorescent lambs with a photoperiod of 16 hours. The obtained seedlings were used as sources of explants (leaf, stem, and root).

### Preparation of growth regulator hormones

Benzyl adenine (BA) and kinetin (Kin) were dissolved in HCl, and 2,4 dichlorophenoxy acetic acid (2,4-D) was dissolved in alcohol. A concentration of 1 mg/ml stock solutions of each hormone was prepared individually by dissolving 100 mg of each hormone in 2 ml of 1 M HCl, and distilled water was then added to make a final volume of 100 ml of stock solution of each hormone. Stock solutions were stored at 2- 4ºC.

### Treatments for calli induction:

Calli induction of *E. peplus*, explants (leaf, stem and root segments) were separately cultured on MS culture medium supplemented with BA (0, 1, 2 and 3 mg / l) in combination with 2,4-D (0.0, 0.25, 0.5, and 0.75 mg / l),while for calli induction of *S. irio*, explants were separately cultured on MS culture medium supplemented with 2,4-D (0, 1, 2 and 3 mg / l) in combination with Kin (0.0, 0.25, 0.5 mg / l). In case of calli induction of *M. parviflora*, explants were separately cultured on MS culture medium supplemented with 2,4-D (0, 1, 3 and 5 mg / l) in combination with Kin (0.0 and 1 mg / l). The conical containers were incubated at 25 ±2 ºC with a photoperiod of 16 h light / 8h dark every day. The data for callus induction from tested explants of *E. peplus* were recorded after two weeks. For calli induction of *S. irio* and *M. parviflora* the data were recorded after four weeks.

### Measurement of fresh and dry weight

Fresh weights of inducted callus were measured after 6 weeks of culturing and the dry weights of callus measured after the treatment at 50 ºC for 48h.

### Determination of secondary metabolites

One gram of the (*in vivo* plant and *in vitro* callus extracts) was used for determining total alkaloids according to Harborne (1984), 0.5 gram of (mother plant or callus) was used for determining total flavonoids according to

Bohm and Kocipai-Abyazan, (1974) and 0.1 gram of (mother plant or callus) was used for determining total tannins according to Ali *et al.,* (1991).

**Statistical Analyses**

The means of three analytical replications are used to calculate all analytic values. Analysis of variance (ANOVA) was used to determine significance by using SPSS software (version 18.0), where P< 0.05 is considered significant.

Sterilized seeds of *E. peplus* using 10 % sodium hypochlorite (NaClO) for 20 minutes and generated on full strength MS medium for 2-3 months showed the highest percentage of several seedlings, while sterilization of *S. irio* seeds by 10 % (NaClO) for 25 minutes produced 80 % of seedlings on full strength MS medium after 3 weeks. On the other hand, sterilization of *M. parviflora* seeds using 20 % of (NaClO) intended for 25 minutes produced 66.7 % of seedlings on half strength MS medium but10 % of (NaClO) intended for 25 minutes produced 66.7 % on full strength Ms medium through 3-6 months.

**Callus induction and morphological characters of species studied**

Explants (leaf, stem and root) were removed from *in vitro* seedlings that were aseptic.

***Euphorbia peplus***

2,4-dichloro phenoxy acetic acid (2,4-D) alone or accompanied by Benzyl Adeni ne (BA) at numerous concentrations was added to the MS culture medium to stimulate callus formation from different *E. peplus* explants (leaf, root, and stem segments). The results are listed in Tables 1,2 and 3 after three weeks of growth. The explants of the stem and root sections produced the most calli among all treatments(100%), while explants of the leaf produced the least callus.

With regard to the morphological features of the gained calli, the findings show the calli induction frequency, the calli surface was smooth or nodular, the texture was spongy or compact, uniformity was uniform or patch, and colour varied from white, green, light green, and brownish green. Calli from the three sorts of explants were observed in (Figure1).

Yang *et al*., (2009) found that the maximum rates of callus production of *E. helioscopia* were noted on MS medium containing 3.0 mg / l 2,4-D and morphological characters of callus were yellow, loose and granular**.** Malayaman *et al*., (2014) realized that the maximum induction of callus (82.5 %) produced from the leaf segments of *Phyllanthus debilis* on MS medium containing 2, 4-D (0.5 mg / l) as well as BAP (3.5 mg / l) and NAA (2.5 mg / l). Hegazi *et al*., (2020) suggested that supplemented 0.45 or 4.54 μM of thidiazuron (TDZ) into the medium was the most suitable for producing callus induction (100 %) from cotyledonary leaves of *Jatropha curcas*, which was green and nodular. Fufa and Daksa, (2020) revealed that medium supplemented with a mixture of 4.52 μM 2,4-D and 4.44 μM BA formed the highest percentage of callus (100 %) for three accessions of *Jatropha* by using leaf explants.

***Sisymbrium irio***

After four weeks of growth, Kin either unaccompanied or accompanied by 2,4-D at numerous concentrations was further added to the MS culture medium to stimulate callus formation from different *S. irio* explants (leaf, root, and stem segments) (Tables 4,5 and 6). Amongst the several cultures medium tested the highest 100 % of callus induction was developed from explants that were cut from root segments on MS medium containing 2,4-D (1mg / l) alone and containing Kin with concentrations (0.25 mg / l and 0.5 mg / l). Table 5, the best induction of callus was gained on MS medium including 0.25 mg / l Kin combined with 1mg / l 2,4-D by stem explants. By using leaf explants of *S. irio*, the highest ratio of callus induction (100 %) was obtained on MS amended with 1mg / l 2,4-D alone. Anegligable (0 %) callus induction was recorded from explants excised from the leaf of *S. irio* on MS supplemented with 1 mg / l, 0.5 mg / l and 0.25 mg / l Kin alone. In general, Kin combined with 2,4-D generated pale yellow, light green, yellow and white calli obtained on same the treatments.

Concerning the morphological features of calli achieved from *S. irio* explants, the results demonstrate the percentage of calli induction, with a spongy or compact texture. Moreover, the calli surfaces were nodular and uniformity for calli was uniform in all treatments except one treatment patch was recorded with stem segments. Figure (2) shows calli from the three different types of explants employed in the study by Osman *et al*., (2016) reported that 2,4-D is among the best auxins efficient for callus development, and several varieties respond positively when it is present in a mixture with a proper concentration of cytokinin as a distinct auxin. With respect to explants Bodede *et al*., (2022), reported that cotyledon root and

stem explants of *Senegalia nigrescens* were more efficient at producing calluses than the leaves explants.

***Malva parviflora***

To stimulate callus development from different *M. parviflora* explants, Kin alone or in combination with 2,4-D in MS culture medium augmented with various concentrations was added and the obtained results were recorded after four weeks of growth. Explants from stems and roots of *M. parviflora* seedlings yielded the highest callus formation percentage. The proportion of callus induction from leaf explants was least in comparison with those obtained from the root and stem.2,4-D alone or combined with Kin had a positive effect on callus induction from all explants tested. The 100 % callus initiation was noted by stem explants on MS medium having 0.1 Kin and 3 mg / l 2.4-D. Root explants on MS culture medium involving 5 mg / l 2.4-D alone yielded 100 % callus induction.

According to the results shown that Tables 7, 8, and 9, the calli that were produced varied in colour from white to green to whiteish green to whitish brown and yellow. Calli also varied in texture from spongy to compact, with smooth or nodular surfaces and uniform or patchy uniformity. Figure(3) showed calli the three different types of explants employed. There were two types of calli: friable and compact. As stated by (Bhatia ,2015 and Bodede *et al*.,(2022), friable calli are typically flexible and need lower force to break than compact calli, while compact calli are usually solid and comprise distinct constructions that might be split into individual components and occasionally reflect various phases of somatic embryogenesis.

The reasons for the detected morphological variations in the calli remain unknown. Xie and Hong, (2001) illustrated *Acacia mangium*, explants excised from seedlings, leaves, embryo axes, cotyledons, petioles and stems, each recorded 100 % of callus growth when cultured on media containing 2.0 mg / l 2,4 D and 3.0 mg / l kin.

**Measurement of fresh and dry weight**

***Euphorbia peplus***

The results obtained recorded in Table 10 showed that the variation in the weight of fresh and dry calli that formed from different explants of *E. peplus* with respect to the type and concentration of the BA and 2,4-D hormones. The highest fresh and dry weight were recorded (4.88 g and 0.117 g) by root explants calli on MS medium supplemented with 2 mg / l BA in a mixture with 0.5 mg / l 2,4-D, followed by the leaf explants calli recorded (4.22 g and 0.113 g) on MS medium supplemented with 0 .25 mg / l BA alone and finally, stem segments explants calli recorded (3.97 g and 0.08 g) respectively.

Li *et al*., (2012) reported that a mixture of Kinetin (0.1 mg / l) and (1 mg / l) Naphthalene acetic acid (NAA) was the most suitable medium for inducing callus formation and growth from *Jatropha curcas*.. Aljibouri *et al*., (2014) indicated that the maximum callus production of nodules of *E. peplus* callus reached 100 % on MS medium containing 0.5 and 2 mg / l 2,4-D under dark conditions but under light conditions reached 75 % on MS containing 2, 1.5 and 1 mg / l 2,4-D.`

***Sisymbrium irio***

From the data recorded in Table 11 generally, the medium without Kin and 2,4-D had remarkable effects on the formation of calli using leaf, root and stem explants. Kin and 2,4-D alone or in combination significantly affected callus initiation from leaf and stem explants. The maximum fresh and dry weight of calli was identified in medium comprise 2,4-D alone, a combination between 0.25 Kin and 1mg / l 2,4-D was favorable for the root segments to produce calli with the maximum fresh and dry weight. Moreover, 0.5 Kin in combinate with 2 mg / l 2,4-D was the most favorable for calli formation with the maximum fresh and dry weight.

Amin *et al*., (2009) clarified that the highest callus fresh weight was documented by hypocotyl explants of *S. irio* that inoculated on MS increased with 1 mg / l 2,4-D and 0.5 mg / l Kin. Memon and Memon, (2021) reported that the highest callus formation frequency (100 %) of nodal explants of *Brassica nigra* was documented on MS medium including 0.5 mg / l + (NAA) 0.1 mg / l (BAP) and 0.2 mg / l NAA + 0.6 mg / l BAP. Li *et al*., (2022), in their study, showed that cotyledon leaves of Radish (*Raphanus sativus*) had the best callus induction (91.01 %) on MS medium complemented with 8 g / l agar, and 3 g / l sugar in addition to 4 mg / l 6-BA, 0.1 mg / l kinetin, 0.2 mg / l 2,4-D, 0.5 mg / l TDZ and 0.2 mg / l NAA with a callus formation percentage of (94.77 %).

***Malva parviflora***

The findings illustrated in Table 12 showed that, root segment explants recorded the largest production of calli. Root segments had the highest fresh and dry weight (3.07 g and

0.12 g, respectively), trailed by stem segments (2.75 and 0.11 g), and then leaf segments (2.16 g and 0.07 g, respectively). It is to be taken into consideration also that the growth regulator concentrations required to produce the largest amount of callus from all kinds of explant differ. It was obvious that 0.1 mg / l Kin hasn't achieved any response for three explants.

Shaikh *et al*., (2018) recorded the maximum (%) of callus initiation and fresh weight 0.82 g from nodal explants of *Helicteres isora* (100 % and 0.82 g) respectively, by supplementing 0.5 mg / l of 2,4-D to media. The obtained results disagree with the study Hosseini *et al*., (2017) on *Althaea digitata*, where showed shoot tip explants achieved the highest results for callus induction (82.98 %) on MS with 2,4- D(5 mg / l) and Kin (0.1 mg / l), the leaf explants produced calli about (81.39 %) of with the combination of 2,4-D (10 mg / l) and Kin (0.1 mg / l) but callus initiation by root explants(72.50 %) on MS with(5 mg / l) and Kin (0.1 mg / l which was low as compared to the leaf and shoot tip explants. Sobrinho *et al*., (2022) showed that callus formation of hypocotyls of *Hibiscus sabdariffa* was produced on MS supplemented with 0.1 mg / l of 2,4- D and 0.1 mg / l. Benzyl Amino Purin (BAP). Diverse plants, even varying explants of the same plants, have different plant tissue culture (PCT) conditions. Therefore, when creating a novel culture system, it is crucial to choose a suitable medium that includes a suitable basic media and a growth-regulating concentration percentage (Gulzar *et al*., 2020; Sharma and Sharma 2021& Shukla ,2020). Conserving natural resources is very important the data obtained from the present study can help to conserve valuable plant species by the production of bioactive components via callus induction Bodede *et al*., (2022).

**Phytochemical analysis of mother plant and calli**

***Euphorbia peplus***

Figure (4) presented that total alkaloids, total flavonoids and total tannins in ethanolic extract of leaves, stems and roots of the mother plant and their induced calli *in vitro.* It appears that calli achieved from root segments stored the sixfold amount of total alkaloids produced from the root mother plant (120 mg / g dry wt.). With respect to total flavonoids the highest amount recorded in the calli was obtained from leaf, stem and root (280,240 and 280 mg/g dry wt.) respectively, in comparison with those extracted from mother plant tissue.

With regard to the total tannin content of the investigated plant materials, the results explained in Figure 4 shows that the calli produced from leaf, root and stem segments accomplished the maximum levels of total tannins (190,70 and 60 mg/g dry wt.) respectively, compared to those of the mother plant, leaf, stem and root (20,40 and 30 mg/g dry wt.) respectively. In addition, calli attained from stem segments had approximately nine folds of whole tannin (190 mg/g dry wt.) than from mother plant tissues.

***Sisymbrium irio***

The phytochemical analysis were illustrated in Figure (5). The amount of total alkaloids ranged between 20 and 100 mg / g plant dry wt. The calli made from root segments have the most total alkaloids (100 mg / g dry wt.), while total alkaloids extracted from the powder of root for the mother plant contained only 20 mg / g dry wt. respectively. Total alkaloids of the leaf mother plant were five folds than recorded in leaf segments calli. The highest amount of total flavonoids was recorded in the leaf mother plant tissues (280 mg / g dry wt.) and the lowest amount was obtained from calli induced from stem and leaf segments (140 mg / g dry wt.). The amount obtained from root calli (260 mg / g dry wt.) was greater than the amount obtained from the root mother tissue. The amount of total flavonoids recorded in calli formed from stem segments was higher than the amount obtained from the stem mother.

The results of Figure 5 revealed that the calli produced from leaf and root segments contained the highest levels of total tannins (110 mg/g dry wt.), which were observed in the mother plant's leaves and roots, respectively, at 70 and 50 mg/g dry wt. Contrarily, calli originated from segments of the stem including the lowest value of total tannin (30 mg/g dry wt.) compared to that of the mother plant's stem.

***Malva parviflora***

Based on the data obtained in the present study and illustrated in Figure (6) the amount of total alkaloids ranged between 180 and 80 mg / g plant dry wt. It appears that calli produced from leaf segments and stem segments showed the maximum amount of total alkaloids (90 and 100 mg/g dry wt.) respectively. However, total alkaloids in root mother plant were two folds than recorded in root segments calli.

With respect to total flavonoids Fig 6, the highest amount was recorded from calli obtained from leaf, stem and root segments (360,200 and 220) mg / g dry wt. while those obtained from leaf, stem and root of mother plant tissues (140,180 and 100) mg / g dry wt. respectively.

The total tannin content existing in the extract of the tested plant materials, the results showed in Figure 6 showing that the calli obtained from leaf segment, stem segments and root segments were recorded the greatest amounts of total tannins (40,50 and 40) mg / g dry wt.) as compared to those were recorded from leaf, stem, and root of mother plant (20,40 and 30 mg /g dry wt.) respectively.

Sobrinho *et al*., (2022) reported that some active compounds were produced by the callus of *Hibiscus sabdariffa* not produced by the leaves from the mother plant and reported that callus culture facilitates the generation of bioactive chemicals constantly under a controlled environment and free of contamination.

Commonly, the generation or accumulation of different secondary metabolites is influenced by the anatomical, biological and biochemical properties of the cells that formed the culture.

Many studies had observed that callus cultures are capable of synthesizing and accumulating different bioactive compounds with large amounts than mother plants such as Sativoside (Janarthanam *et al*., 2010), flavonoids and stilbenes (Maneechai *et al*.,2012), sterols (Loredo-Carrilo *et al*., 2013), cardenolides (Sahin *et al*., 2013) and phenolic acids (Szora and Ekiert, 2014). Significant amounts of tannins, flavonoids and phenolics were detected in callus cultures of *Byrsonima verbascifolia* Castro *et al*., (2016). The findings of this study may, at least in part, concur with earlier findings that indicated increased production of certain plant secondary metabolites from callus (Ebad *et al*., 2017; Bodede *et al*.,2022& Vignesh *et al*., 2022).

The tissue culture technique in the present study can help to minimize the stress on wild cultivated populations and help to conserve the valuable flora and to improve their medical and pharmaceutical importance.

## CONCLUSION

The results obtained recorded in the present study show that different types of explants may vary according to the species due to the variance in reactivity of their microenvironments to media components.

It is to be taken into consideration that the most percent for callus initiation, fresh and dry weight were observed through root segments (100 %, 4.88 g, and 0.117 g) respectively2 mg / l BA mixed with 0.5 mg / l 2,4-D for *E. peplus*, stem segments explants achieved callus induction %, fresh and dry weight for callus production (0.75 g, and .07 g) respectively, on MS medium 0. 5 mg / l Kin mixed 2 mg / l 2,4-D for *S. irio* and percentage of callus initiation, fresh and dry weight were gained by root segments (100 %, 3.07 g, and .0.12 g) respectively for *M. parviflora* on MS medium containing 2,4-D 5 mg / l alone. Generally, amount of secondary metabolites increased in the callus than in mother plants. This study will be considered background for the next studies that will be carried out on those plants in the plant tissue culture field. Consequently, it is crucial to choose a suitable medium, ratio and concentration of growth-regulating substances as well as explant type in the production of the bioactive components via *in vitro* system.

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**Table 1:** Some morphological characteristics of calli obtained from leaves explants of *E. peplus in vitro*

| BA(mg/l) | 2,4-D(mg/l) | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | - | - | - | - | - | - |
| | 0.25 | 30% | ++ | Green | Smooth | Compact | Uniform |
| | 0.50 | 0 | - | - | - | - | - |
| | 0.75 | 0 | - | - | - | - | - |
| 1 | 0 | 80% | ++ | White green | Nodular | Compact | Patch |
| | 0.25 | 70% | +++ | White | Smooth | Compact | Uniform |
| | 0.50 | 70% | ++ | White green | Smooth | Compact | Patch |
| | 0.75 | 100% | ++ | White | Smooth | Compact | Uniform |
| 2 | 0 | 0 | - | - | - | - | - |
| | 0.25 | 50% | + | green | Smooth | Compact | Uniform |
| | 0.50 | 40% | + | Whit green | Nodular | Compact | Patch |
| | 0.75 | 40% | ++ | Green | Smooth | Compact | Uniform |
| 3 | 0 | 0 | - | - | - | - | - |
| | 0.25 | 30% | ± | Green | Smooth | Compact | Uniform |
| | 0.50 | 50% | ++ | Green | Smooth | Compact | Uniform |
| | 0.75 | 90% | ++ | Green | Smooth | Compact | Uniform |

**Table 2:** Some morphological characteristics of calli obtained from stem segments explants of *E. peplus in vitro*

| BA(mg/l) | 2,4-D(mg/l) | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | - | - | - | - | - | - |
| | 0.25 | 100% | +++ | Green | Nodular | Compact | Uniform |
| | 0.50 | 60% | +++ | White, green | Nodular | Compact | Patch |
| | 0.75 | 80% | + | Light green | Nodular | Compact | Uniform |
| 1 | 0 | 80% | ++ | Brownish white | Nodular | Compact | Patch |
| | 0.25 | 100% | ++ | green | Nodular | Compact | Uniform |
| | 0.50 | 70% | +++ | White green | Nodular | Compact | Patch |
| | 0.75 | 100% | ++ | Light green | Smooth | Compact | Uniform |
| 2 | 0 | 80% | ++ | green | Nodular | Spongy | Uniform |
| | 0.25 | 100% | +++ | White green | Nodular | Compact | Patch |
| | 0.50 | 100% | ++ | green | Nodular | Compact | Uniform |
| | 0.75 | 100% | +++ | Green | Nodular | Compact | Uniform |
| 3 | 0 | 100% | + | Green | Smooth | Compact | Uniform |
| | 0.25 | 80% | ++ | Green | Smooth | Compact | Uniform |
| | 0.50 | 90% | ++ | light green | Nodular | Compact | Uniform |
| | 0.75 | 100 | ++ | White green | Smooth | Compact | Patch |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 3:** Some morphological characteristics of calli obtained from root segments explants of *E. peplus in vitro*

| BA(mg/l) | 2,4-D(mg/l) | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | - | - | - | - | - | - |
| | 0.25 | 100% | ++ | White green | Nodular | Spongy | Patch |
| | 0.50 | 100% | + | White | Nodular | Spongy | Uniform |
| | 0.75 | 80% | + | White | Nodular | Compact | Uniform |
| 1 | 0 | 0 | - | - | - | - | - |
| | 0.25 | 100% | +++ | Light green | Nodular | Spongy | Patch |
| | 0.50 | 100% | +++ | White green | Nodular | Spongy | Patch |
| | 0.75 | 100% | +++ | White green | Nodular | Spongy | Patch |
| 2 | 0 | 0 | - | - | - | - | - |
| | 0.25 | 100% | +++ | Green | Nodular | Compact | Uniform |
| | 0.50 | 100% | +++ | White green | Nodular | Spongy | Patch |
| | 0.75 | 100% | +++ | White green | Nodular | Spongy | Patch |
| 3 | 0 | 0 | - | - | - | - | - |
| | 0.25 | 100% | ++ | White green | Smooth | Spongy | Patch |
| | 0.50 | 100% | ++ | White green | Smooth | Spongy | Patch |
| | 0.75 | 100% | +++ | White | Nodular | Spongy | Uniform |

No callus (-), Doubt (±), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 4:** Some morphological characteristics of calli obtained from leaf segments explants of *S. irio in vitro*

| Kin (mg/l) | 2,4- D (mg/l) | Callus induction (%) | Growth | Color | Surface | Texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | - | - | - | - | - |
| | 1 | 100% | ++ | light green | Nodular | Spongy | Uniform |
| | 2 | 40% | +++ | pale yellow | Nodular | Spongy | Uniform |
| | 3 | 90% | +++ | yellow | Nodular | Compact | Uniform |
| 0.25 | 0 | 0 | - | - | - | - | - |
| | 1 | 90% | ++ | light green | Nodular | Spongy | Uniform |
| | 2 | 80% | +++ | yellow | Nodular | Spongy | Uniform |
| | 3 | 20% | +++ | pale yellow | Nodular | Compact | Uniform |
| 0.50 | 0 | 0 | - | - | - | - | - |
| | 1 | 30% | ++ | pale yellow | Nodular | Compact | Uniform |
| | 2 | 10% | ++ | pale yellow | Nodular | Compact | Uniform |
| | 3 | 50% | +++ | pale yellow | Smooth | Spongy | Uniform |
| 1 | 0 | 0 | - | - | - | - | - |
| | 1 | 90% | +++ | white | Nodular | Spongy | Uniform |
| | 2 | 100% | +++ | pale yellow | Nodular | Spongy | Uniform |
| | 3 | 80% | +++ | light green | Nodular | Spongy | Uniform |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 5:** Some morphological characteristics of calli obtained from stem segments explants of *S. irio in vitro*

| Kin (mg/l) | 2,4- D (mg/l) | Callus induction(%) | Growth | Color | Surface | Texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | - | - | - | - | - |
| | 1 | 70% | +++ | yellow | Nodular | Spongy | Uniform |
| | 2 | 70% | ++ | yellow | Nodular | Spongy | Uniform |
| | 3 | 70% | ++ | light green | Nodular | Compact | Uniform |
| 0.25 | 0 | 30% | +++ | green | Smooth | Compact | Patch |
| | 1 | 100% | +++ | light green | Nodular | Spongy | Uniform |
| | 2 | 90% | ++ | light green | Nodular | Spongy | Uniform |
| | 3 | 40% | + | pale yellow | Nodular | Compact | Uniform |
| 0.5 | 0 | 20% | ++ | pale yellow | Nodular | Compact | Uniform |
| | 1 | 70% | ++ | pale yellow | Nodular | Spongy | Uniform |
| | 2 | 90% | ++ | palye yellow | Nodular | Compact | Uniform |
| | 3 | 70% | +++ | pale yellow | Nodular | Compact | Uniform |
| 1 | 0 | 0 | - | - | - | - | - |
| | 1 | 40% | ++ | white | Nodular | Spongy | Uniform |
| | 2 | 70% | +++ | light green | Nodular | Compact | Uniform |
| | 3 | 90% | +++ | light green | Nodular | Spongy | Uniform |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 6:** Some morphological characteristics of calli obtained from root segments explants of *S. irio in vitro*

| Kin (mg/l) | 2,4- D (mg/l) | Callus induction (%) | Growth | Color | Surface | Texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | - | - | - | - | - |
| | 1 | 100% | + | pale yellow | Nodular | Spongy | Uniform |
| | 2 | 100% | + | yellow | Nodular | Spongy | Uniform |
| | 3 | 70% | + | yellow | Nodular | Compact | Uniform |
| 0.25 | 0 | 0 | - | - | - | - | - |
| | 1 | 100% | ++ | yellow | Nodular | Compact | uniform |
| | 2 | 70% | +++ | yellow | Nodular | Spongy | uniform |
| | 3 | 80% | ++ | light green | Nodular | Compact | Uniform |
| 0.5 | 0 | 0 | - | - | - | - | - |
| | 1 | 100% | ++ | pale yellow | Nodular | Compact | Uniform |
| | 2 | 80% | + | pale yellow | Nodular | Spongy | Uniform |
| | 3 | 100% | +++ | pale yellow | Nodular | Compact | Uniform |
| 1 | 0 | 0 | - | - | - | - | - |
| | 1 | 50% | +++ | white | Nodular | Spongy | Uniform |
| | 2 | 30% | ++ | white | Nodular | Compact | Uniform |
| | 3 | 60% | ++ | pale yellow | Nodular | Compact | Uniform |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 7:** Some morphological characteristics of calli obtained from leaf segments explants of *Malva parviflora in vitro*

| Kin mg/l | 2,4-D mg/l | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0% | - | - | - | - | - |
| | 1 | 30% | ++ | Light green | Nodular | Compact | Uniform |
| | 3 | 30% | +++ | White | Nodular | Compact | Uniform |
| | 5 | 90% | + | White | Smooth | Compact | Uniform |
| 0.1 | 0 | 0% | - | - | - | - | - |
| | 1 | 70% | ++ | Light green | Smooth | Compact | Uniform |
| | 3 | 60% | ++ | Whitish brown | Smooth | Compact | Patch |
| | 5 | 60% | +++ | Whitish brown | Smooth | Compact | Patch |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 8:** Some morphological characteristics of calli obtained from stem segments explants of *M. parviflora in vitro*

| Kin mg/l | 2,4-D mg/l | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0% | - | - | - | - | - |
| | 1 | 90% | +++ | Green | Nodular | Compact | Uniform |
| | 3 | 70% | +++ | Yellow | Smooth | Compact | Uniform |
| | 5 | 80% | ++++ | Whitish brown | Smooth | Compact | Patch |
| 0.1 | 0 | 0% | - | - | - | - | - |
| | 1 | 90% | +++ | Yellow | Smooth | Compact | Uniform |
| | 3 | 100% | ++++ | White | Smooth | Compact | Uniform |
| | 5 | 90% | +++ | White | Smooth | Compact | Uniform |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 9:** Some morphological characteristics of calli obtained from root segments explants of *M. parviflora in vitro*

| Kin mg/l | 2,4-D mg/l | Callus induction (%) | Growth | Color | Surface | texture | Uniformity |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0% | - | - | - | - | - |
| | 1 | 80% | +++ | White | Nodular | Compact | Uniform |
| | 3 | 70% | +++ | Whitish green | Nodular | Spongy | Patch |
| | 5 | 100% | +++ | Light green | Nodular | Compact | Uniform |
| 0.1 | 0 | 0% | - | - | - | - | - |
| | 1 | 60% | ++ | Whitish brown | Smooth | Compact | Patch |
| | 3 | 90% | ++ | White | Nodular | Compact | Uniform |
| | 5 | 90% | + | White | Nodular | Compact | Uniform |

No callus (-), Week growth (+), medium growth (++), vigorous growth (+++).

**Table 10:** Effect of type of explant and various growth regulator treatments on fresh and dry weights of calli induced from various explants of *E.peplus* after six weeks.

| Treatments (mg/l) | | Origin of callus (Type of explant) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leaf | | Stem segments | | Root segments | |
| BA | 2,4-D | Fresh weight(g) | Dry weight(g) | Fresh weight(g) | Dry weight(g) | Fresh weight(g) | Dry weight(g) |
| 0 | 0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 0.25 | 4.22* | 0.113* | 2.16* | 0.070* | 1.84* | 0.070* |
| | 0. 5 | 2.35* | 0.043* | 3.197* | 0.08* | 0.87 | 0.043* |
| | 0.75 | 0.50 | 0.033* | 0.157 | 0.010 | 0.61 | 0.033 |
| 1 | 0.0 | 0.19* | 0.027 | 01.79* | 0.043 | 0.00 | 0.00 |
| | 0.25 | 0.153* | 0.030* | 0.14 | 0.010 | 3.947* | 0.117* |
| | 0. 5 | 0.367 | 0.023 | 3.03* | 0.053* | *2.91* | 0.077* |
| | 0.75 | 0.10 | 0.015 | 0.413 | 0.027 | 3.75* | 0.160* |
| 2 | 0.0 | 0.00 | 0.00 | 0.380 | 0.037 | 0.00 | 0.00 |
| | 0.25 | 0.69 | 0.04* | 2.813* | 0.077* | 3.67* | 0.077* |
| | 0. 5 | 0.237 | 0.033* | 0.433 | 0.133* | 4.887* | 0.117* |
| | 0.75 | 0.263 | 0.017 | 1.40* | 0.053* | 4.01* | 0.123* |
| 3 | 0.0 | 0.00 | 0.00 | 0.170 | 0.030 | 0.00 | 0.00 |
| | 0.25 | 0.187 | 0.033* | 0.563 | 0.073* | 1.387* | 0.057* |
| | 0. 5 | 0.587 | 0.053* | 0.637 | 0.027 | 1.51* | 0.034 |
| | 0.75 | 0.473 | 0.033* | 0.517 | 0.050 | *1.95 | *0.05 |

Each value is a mean of three replicates. * = significant at P < 0.05 and other values not significant.

**Table 11**: Effect of type of explant and various growth regulator treatments on fresh and dry weights of calli induced from various explants of *S. irio* after six weeks.

| Treatment (mg/l) | | Origin of callus (Type of explant) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leaf segments | | Root segments | | Stem segments | |
| Kin | 2,4-D | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) |
| 0. 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1 | 0.330* | 0.030* | 0.420* | 0.031* | 0.103* | 0.010* |
| | 2 | 0.240* | 0.020* | 0.157* | 0.020* | 0.417* | 0.03 |
| | 3 | 0.427* | 0.040* | 0.103* | 0.010* | 0.833* | 0.06* |
| 0.25 | 0 | 0.00 | 0.00 | 0.330* | 0.030* | 0.0 | 0.00 |
| | 1 | 0.247* | 0.030* | 0.460* | 0.045* | 0.107* | 0.012* |
| | 2 | 0.270* | 0.30* | 0.137* | 0.010* | 0.100* | 0.010* |
| | 3 | 0.140* | 0.10* | 0.323* | 0.020* | 0.110* | 0.010* |
| 0.5 | 0 | 0.00 | 0.00 | 0.130* | 0.010* | 0.00 | 0.00 |
| | 1 | 0.143* | 0.10* | 0.117* | 0.010* | 0.113* | 0.010* |
| | 2 | 0.130* | 0.10* | 0.130* | 0.010* | 0.750* | 0.07* |
| | 3 | 0.130* | 0.10* | 0.130* | 0.010* | 0.443* | 0.040* |
| 1 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1 | 0.153* | .0117* | 0.130* | 0.017* | 0.333* | 0.023* |
| | 2 | 0.320* | 0.030* | 0.157* | 0.017* | 0.143* | 0.010* |
| | 3 | 0.205* | .020* | 0.217* | 0.020* | 0.733 * | 0.07* |

Each value is a mean of three replicates. * = significant at P < 0.05 and

**Table 12:** Effect of type of explant and various growth regulator treatments on fresh and dry weights of calli induced from various explants of *M. parviflora* after six weeks.

| Treatment (mg/l) | | Origin of callus (Type of explant) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leaf segments | | Root segments | | Stem segments | |
| Kin | 2,4-D | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) | Fresh weight (g) | Dry weight (g) |
| 0. 0 | 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | 1 | *1.437 | *0.053 | *1.270 | *0.063 | 0.860 | 0.043 |
| | 3 | *1.667 | *0.057 | *1.347 | *0.063 | *2.307 | *0.083 |
| | 5 | *1.403 | *0.067 | *3.073 | *0.127 | *1.757 | *0.077 |
| 0.1 | 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | 1 | 0.957 | *0.047 | 0.677 | *0.050 | *2.100 | *0.113 |
| | 3 | *2.167 | *0.070 | *2.367 | *0.100 | *2.547 | *0.120 |
| | 5 | *1.710 | *0.113 | *1.083 | *0.073 | *2.757 | *0.113 |

Each value is a mean of three replicates. * = significant at P < 0.05 and other values not significant.

**Figure 1:** Callus formation from Leaf (a), Stem segments (b) and Root segments (c) of *E. peplus* using BA + 2,4-D.

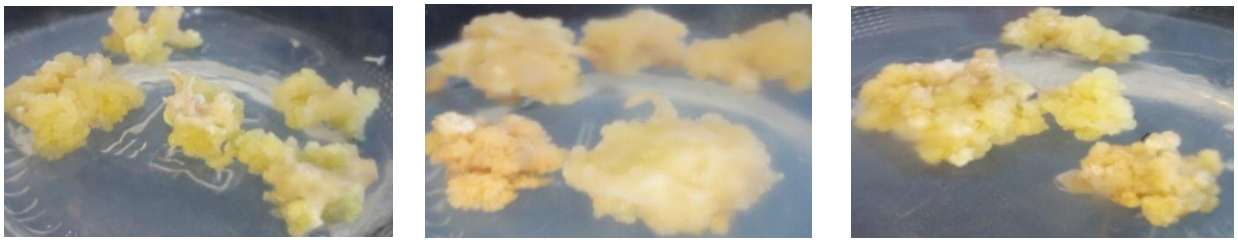

**Figure 2:** Callus formation from leaf segments (a), stem segments(b) and root segments (c) of *S. irio*

**Figure 3:** Callus formation from leaf segments (a), stem segments(b) and root segments (c) of *M. parviflora*

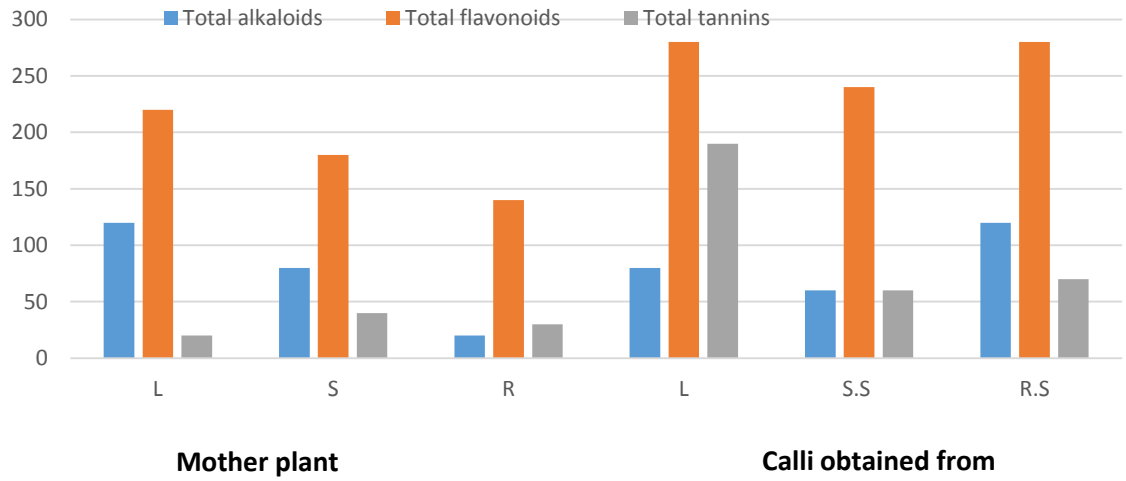


**Figure 4:** Amount of some total secondary metabolites (mg/g plant dry wt) of mother plant and calli obtained from various explants of *E. peplus in vitro.* L=Leaf, S= stem, L= Leaf ,S.S= Stem segments, R.S = Root segments.

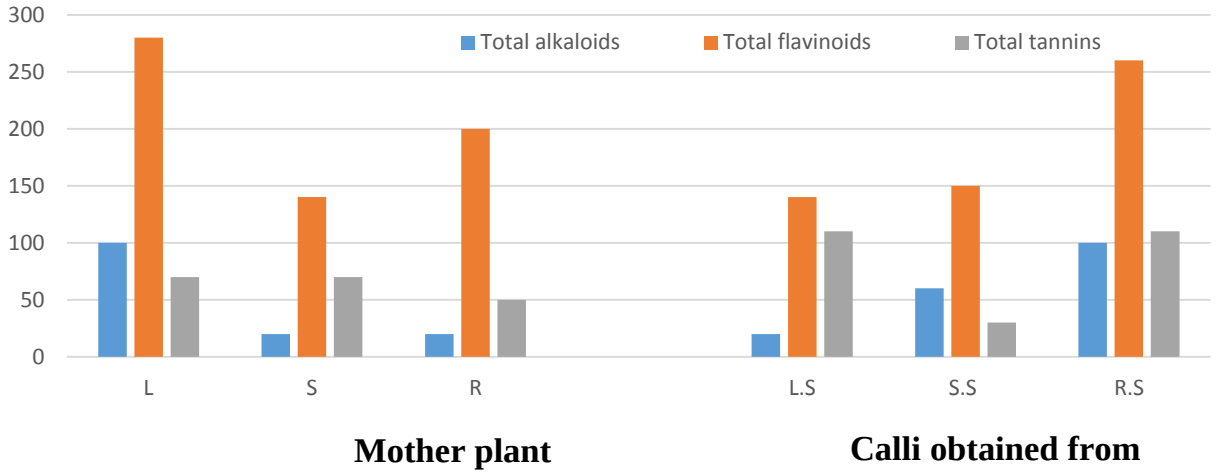


**Figure 5:** Amount of some total secondary metabolites (mg/g plant dry wt) of mother plant and calli obtained from various explants of *S. irio in vitro.* L=Leaf, S= stem, L= Leaf ,S.S= Stem segments, R.S = Root segments.

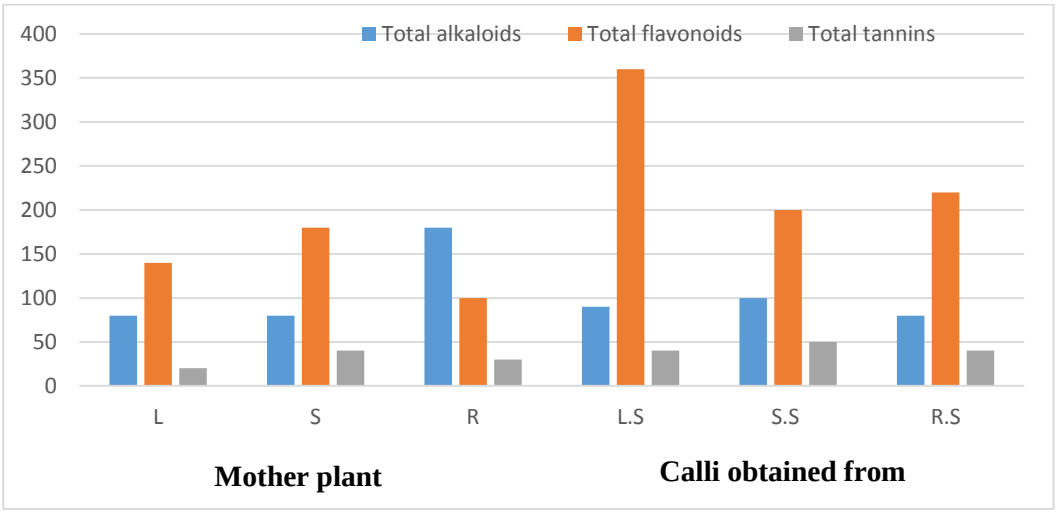


**Figure 6**: Amount of some total secondary metabolites (mg/g plant dry wt) of mother plant and calli obtained from various explants of *M. parviflora in vitro.* L=Leaf, S= stem, L= Leaf ,S.S= Stem segments, R.S = Root segments.

# استحثاث الكالس وتقدير بعض المركبات الفعاله الناتجه من الكالس لثلاث نباتات مختلفه معمليا.

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## الملخص العربي

تعتبر الودينه و فجل الجمل و الخبيزه نباتات طبية تنتشر على نطاق واسع في جميع أنحاء العالم و تحتوي على العديد من المركبات الثانوية التي تستخدم في علاج العديد من الأمراض. أجريت هذه الدراسة للتعرف علي تأثير منظمات النمو على استحثاث تكون الكالس باستخدام منفصلات نباتيه مختلفه لكل من النباتات المختبرة.كما أجريت مقارنة بين المستخلصات الإيثانولية لجذور وسيقان وأوراق نباتات الأم والكالسات التي تم الحصول عليها معمليا لتحليل كمية القلويات و االتانينات والفلافونويدات لكل منها. تم تسجيل أفضل استحثاث للكالس والوزن الطازج والوزن الجاف للكالس من منفصلات جذورنبات الودينه (100٪ و 4.88 جم و 0.711 جم) المنزرعه علي وسط ميرشيج وسكوج المزود ب 2 مجم / لتر من البنزيل ادنين مع 0.5 مجم / لتر من ثنائي كلوروفينوكسي حمض الخليك (2, 4- D) و المنفصلات المأخوذه من ساق نبات فجل الجمل حققت نسبة استحثاث الكالس ، الوزن الطازج والوزن الجاف لإنتاج الكالس (90% و 0.75جم و 0.07جم) المنزرعه علي وسط ميرشيج وسكوج المزوده ب 0.5 مجم / لتر من الكانتين (kin) مع 2 مجم / لتر من ثنائي كلوروفينوكسي حمض الخليك (2, 4- D) كما تم تسجيل نسبة استحثاث الكالس و الوزن الطازج والوزن الجاف بواسطة منفصلات جذور نبات الخبيزه (100٪ ، 3.07 جم ، 0.12 جم) المنزرعه علي وسط ميرشيج وسكوج المزوده ب5 مجم / لتر من ثنائي كلوروفينوكسي حمض الخليك (2, 4- D) بمفرده . أظهرت الدراسة أيضًا أن المستخلصات الإيثانولية للكالس حققت أفضل انتاج للقلويدات و االتانينات والفلافونويدات.كما أظهرت النتائج أن الأنواع المختلفة من المنفصلات ربما تختلف وفقًا لأنواع النبات بسبب استجابتهم المختلفه لمكونات الوسط الغذائي.

**الكلمات الاسترشادية:** نباتات الودينه و فجل الجمل و الخبيزه., استحثاث الكالس,البنزيل ادنين, ثنائي كلوروفينوكسي حمض الخليك, الكانتين, المركبات الثانوية