# 8. SUMMARY

Plant secondary metabolites provide a wealth of medicinally useful compounds and play a crucial role in modern and traditional medicine. In some cases the compound of interest is easily identifiable, and can often be synthetically produced. However, in many cases it is not easy to determine whether the medicinal benefits are produced by one or several compounds or if they have additive or synergistic effects. In these instances a synthetic product cannot be produced and medicinal preparations will rely on plant material collected from the wild, grown using conventional cultivation techniques, or produced using *in vitro* methods. *Echinacea spp.* (family Asteraceae) herbal medicines and dietary supplements are traditionally used as immunostimulants in the treatment of inflammatory and viral diseases. *Echinacea purpurea (L.)* is an important commercial species. A great deal of research has indicated the chemical composition of *Echinacea spp.*, including alkamides, caffeic acid derivatives (chicoric acid, caftaric acid and chlorogenic acid), and polysaccharides attracting claims of beneficial pharmacological activity. *Echinacea* is an example of plant species with medicinal activity that cannot be attributed to a single chemical, necessitating plant production to obtain the full spectrum of metabolite. Several compounds are thought to play significant roles in the efficacy of *Echinacea* products and are often used to ensure and test for quality. Thus developing artificial (controlled) culture systems with the aims of standardizing and improving production and marketing of medicinal species allows rapid propagation of plants selected for their active principles and improves the quality and quantity of natural pharmaceutical compounds. Accordingly, the current study aimed to establish plant cell lines capable of producing high yields of secondary compounds in cell suspension cultures as an alternative to conventional whole plant production to improve the productivity of locally cultivated *Echinacea purpurea* and to ensure that produced compounds are effective. The targeted group of compounds to be studied was the polyphenolics (caffeic acid derivatives).The long-term goal of our research is to aid in the development of effective therapeutic preparations of *Echinacea,* the goals of this particular project are

**1. Establishment of callus cultures from explants isolated from plant material:**

Callus induction is necessary, as the first step, in many tissue culture experiments. Callus is produced when the initial response of the tissues to a wound is followed by the external addition of growth regulators in an aseptic medium in order to maintain the rapid cell division response and sustain it indefinitely. Calli can be obtained from almost any part of the plant; here the root was chosen to be the explant. Phytohormones, such as cytokinin and auxin, are necessary ingredients in any plant cell culture environment. Generally speaking, an equal proportion of auxin to cytokinin hormones in solution will impact only cell proliferation and result in the formation of callus masses. In our study callus induction was achieved using MS media supplemented with 1.5mg/L BA and 0.5mg/L NAA.

**2. Establishment of liquid cultures (suspension cultures) from static cultures:**

To initiate mother cell cultures, pieces of established undifferentiated, friable calli were subdivided into small sections and transferred from solid media to liquid media supplemented with 1.5mg/L BA and 0.5mg/L NAA. Periodic addition of fresh media and draining out the exhausted media ensures maintenance of suspension cultures under steady state of growth for long periods. Cell suspension cultures were sub-cultured for several weeks to expand the total biomass to be collected.

**3. Study of the influence of elicitors and precursor feeding on the accumulation of active principles in suspension cell cultures:**

Production of secondary metabolites can be enhanced by the treatment of the undifferentiated cells with elicitors. An 'elicitor' may be defined as a substance which, when introduced in small concentrations to a Living cell system, initiates or improves the biosynthesis of specific compounds. Elicitation is the induced or enhanced biosynthesis of metabolites due to addition of trace amounts of elicitors. The elicitors used in this study were: MeJA, chitosan, SNP, $CuSO_4$ and. MeJA solution was used with concentrations 0, 5, 10 and 20 mg/L whereas chitosan used at concentrations 0, 5, 25 and 50 mg/L, SNP used concentrations were 0, 50, 100 and 250 µM and $CuSO_4$ concentrations were 0, 2, 4 and 8 µM, Also, the impact of 1, 2, 3 mM phenylalanine precursor on the accumulation of polyphenolics was studied.

**4. Evaluating the calli extracts from chemical and biological points of view and comparing it to that of the locally cultivated plant extracts:**

All elicited (treated) calli hydro-alcoholic extracts were compared with extracts from untreated callus and both were compared with the cultivated intact plant. Total polyphenolic conent was measured using follin-Ciocalteau method, followed by caffeic acids derivatives analysis and quantitation using LC/MS/MS.

The highest total polyphenolic content was achieved and detected in hydro-alcoholic extracts of calli treated with **10 mg/L MeJA,** with a statistically significant **1.81-fold** increase as compared to the cultivated plant, followed by calli treated with **2 mM Phenylalanine** that showed a statistically significant **1.5-fold** increase in polyphenoilc content in comparison with the cultivated plant. There was no significant increase was observed in caffeic acid derivatives yield in the elicited calli extracts.Also, the efficacy of the extracts of treated and untreated calli were compared with the cultivated plant extracts, all *Echinacea* extracts were examined for their cytotoxicity, anti-oxidant activity, immunostimmulatory activity, enhancement of macrophage and neutrophil phagocytic activity and in-vitro anti-inflammatory activity. It was observed that the hydro-alcoholic extracts of calli treated with **10 mg/L MeJA, 2 mM Phe and 2 µM CuSO4** exhibited the most potent activities among all the tested extracts.

# 9. APPENDIX

All Experimental results were presented as Mean ± SEM of three parallel measurements. Statistical evaluation was carried out by one-way analysis of variance (ANOVA). Statistical significance is expressed as $p < 0.05$ where,

**a**: Effective concentration value is statistically significant compared to its value for Callus extract

**b**: Effective concentration value is statistically significant compared to its value for Cultivated extract

**c**: Effective concentration value is statistically significant compared to its value for Phe 2 extract

**d**: Effective concentration value is statistically significant compared to its value for MeJA 10 extract

**e**: Effective concentration value is statistically significant compared to its value for SNP 50 extract

**f**: Effective concentration value is statistically significant compared to its value for MeJA 5 extract

**g**: Effective concentration value is statistically significant compared to its value for EtOH 0.5 extract

**h**: Effective concentration value is statistically significant compared to its value for $CuSO_4$ 2 extract

**i**: Effective concentration value is statistically significant compared to its value for EtOH 1 extract

**j**: Effective concentration value is statistically significant compared to its value for Control acetic acid extract

**k**: Effective concentration value is statistically significant compared to its value for $CuSO_4$ 4 extract

**l**: Effective concentration value is statistically significant compared to its value for Chitosan 5 extract

**m**: Effective concentration value is statistically significant compared to its value for Chitosan 50 extract

**n**: Effective concentration value is statistically significant compared to its value for SNP 250 extract

**o**: Effective concentration value is statistically significant compared to its value for Phe 1 extract

**p**: Effective concentration value is statistically significant compared to its value for MeJA 20 extract

**q**: Effective concentration value is statistically significant compared to its value for Phe 3 extract

**r**: Effective concentration value is statistically significant compared to its value for SNP 100 extract

**s**: Effective concentration value is statistically significant compared to its value for Chitosan 25 extract

**t**: Effective concentration value is statistically significant compared to its value for EtOH 2 extract

**u**: Effective concentration value is statistically significant compared to its value for $CuSO_4$ 8 extract

**Table 4:** Total polyphenolic content results

| Extract Identity | Concentration in (µg GAE/2 mg DW) |
|---|---|
| Callus | 21.4200±.40<br>c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Cultivated | 22.0200±.00<br>c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Phe 2 | 33.2200±.00<br>a,b,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| MeJA 10 | 40.0200±.00<br>a,b,c,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| SNP 50 | 9.7200±.900<br>a,b,c,d,h,n,o,p,s,u |
| MeJA 5 | 12.1200±1.70<br>a,b,c,d,g,h,i,j,k,l,n,o,p,q,s,u |
| EtOH 0.5 | 8.8200±.6000<br>a,b,c,d,f,h,m,n,o,p,s,t,u |
| $CuSO_4$ 2 | 17.6200±.400<br>a,b,c,d,e,f,g,i,j,k,l,m,n,o,p,q,r,t |
| EtOH 1 | 9.6200±.400<br>a,b,c,d,f,h,n,o,p,s,u |
| Control acetic acid | 9.5200±.500<br>a,b,c,d,f,h,n,o,p,s,u |
| $CuSO_4$ 4 | 8.1200±1.10<br>a,b,c,d,f,h,m,n,o,p,r,s,t,u |
| Chitosan 5 | 9.5200±.7000<br>a,b,c,d,f,h,n,o,p,s,u |
| Chitosan 50 | 11.4200±.400<br>a,b,c,d,g,h,k,n,o,p,q,s,u |
| SNP 250 | 21.9200±.900<br>c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Phe 1 | 4.3200±.1000<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,q,r,s,t,u |
| MeJA 20 | 4.3200±.1000<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,q,r,s,t,u |
| Phe 3 | 8.1200±.3000<br>a,b,c,d,f,h,m,n,o,p,r,s,t,u |
| SNP 100 | 11.1200±.90<br>a,b,c,d,h,k,n,o,p,q,s,u |
| Chitosan 25 | 18.8200±.800<br>a,b,c,d,e,f,g,i,j,k,l,m,n,o,p,q,r,t |
| EtOH 2 | 11.5200±.100<br>a,b,c,d,f,g,h,k,n,o,p,q,s,u |
| $CuSO_4$ 8 | 17.4200±2.4<br>a,b,c,d,e,f,g,i,j,k,l,m,n,o,p,q,r,t |

**Table 5:** Total antioxidant activity

| Extract Identity | IC50 values (µg/ml) of ABTS scavenging activity |
|---|---|
| Callus | 39.6367±1.06529<br>c,f,i,j,k,m,o,t,u |
| Cultivated | 30.8748±1.10652<br>f,i,j,k,m,o,t,u |
| Phe 2 | 7.1797±1.39872<br>a,e,f,i,j,k,l,m,o,q,t,u |
| MeJA 10 | 26.6113±.01798<br>f,i,j,k,m,o,q,t,u |
| SNP 50 | 47.6856±3.03857<br>c,f,g,i,j,m,o,s,t,u |
| MeJA 5 | 77.3569±1.87402<br>a,b,c,d,e,g,h,i,j,l,m,n,o,p,r,s,t,u |
| EtOH 0.5 | 16.7225±2.27150<br>e,f,i,j,k,m,o,q,t,u |
| $CuSO_4$ 2 | 18.8335±2.11050<br>f,i,j,k,m,o,q,t,u |
| EtOH 1 | 1.1501E2±1.88550<br>a,b,c,d,e,f,g,h,j,k,l,m,n,o,p,q,r,s,u |
| Control acetic acid | 1.6665E2±1.66150<br>a,b,c,d,e,f,g,h,i,k,l,m,n,o,p,q,r,s,t,u |
| $CuSO_4$ 4 | 72.2770±1.89400<br>a,b,c,d,e,f,g,h,i,j,m,n,o,p,q,r,s,t,u |
| Chitosan 5 | 45.055±5.83250<br>c,f,i,j,m,o,t,u |
| Chitosan 50 | 4.8301E2±19.45350<br>a,b,c,d,e,f,g,h,i,j,k,l,n,o,p,q,r,s,t,u |
| SNP 250 | 23.6320±.02800<br>f,i,j,k,m,o,q,t,u |
| Phe 1 | 2.0989E2±1.11750<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,p,q,r,s,t |
| MeJA 20 | 27.4600±1.37501<br>f,i,j,k,m,o,q,t,u |
| Phe 3 | 57.9615±1.62950<br>c,d,g,h,i,j,m,n,o,p,r,s,t,u |
| SNP 100 | 19.4496±.05920<br>f,i,j,k,m,o,q,t,u |
| Chitosan 25 | 15.9717±3.88189<br>e,f,i,j,k,m,o,q,t,u |
| EtOH 2 | 1.2182E2±1.09152<br>a,b,c,d,e,f,g,h,j,k,l,m,n,o,p,q,r,s,u |
| $CuSO_4$ 8 | 2.0805E2±40.68719<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,p,q,r,s,t |

**Table 6:** *In vitro* phagocytic activity assay using Polymorphonuclear neutrophils

| Extract Identity | $EC_{50}$ values (µg/ml)of neutrophil phagocytosis activity | $EC_{50}$ value (µg/ml) of neutrophil yeast killing |
|---|---|---|
| Callus | 2.0131E2±5.1315<br>b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u | 1.1112E2±1.30550<br>e,g,j,k,m,n,q,r,s |
| Cultivated | 40.9805±1.46650<br>a,c,d,e,g,h,i,j,k,l,m,n,o,p,t,u | 56.2915±2.05450<br>e,j,m,n,q,s |
| Phe 2 | 7.7215±.31450<br>a,b,e,f,i,j,k,l,m,n,o,p,q,r,s,t,u | 50.2625±.04650<br>e,j,m,n,q,s |
| MeJA 10 | 6.2045±.1155<br>a,b,e,f,i,j,k,l,m,n,o,p,q,r,s,t,u | 22.8230±1.34000<br>e,f,j,m,n,q,s |
| SNP 50 | 85.8410±5.49400<br>a,b,c,d,f,g,h,i,j,k,l,m,n,q,r,s,u | 3.0549E2±2.12450<br>a,b,c,d,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| MeJA 5 | 48.5935±2.50050<br>a,c,d,e,g,h,i,j,k,l,m,n,o,p,r,t,u | 1.4006E2±1.95950<br>d,e,g,h,j,k,m,n,p,q,r,s,t |
| EtOH 0.5 | 1.0985±.0525<br>a,b,e,f,i,j,k,l,m,n,o,p,q,r,s,t,u | 3.2750±.27200<br>a,e,f,i,j,l,m,n,o,q,s |
| $CuSO_4$ 2 | 4.9390±.7850<br>a,b,e,f,i,j,k,l,m,n,o,p,q,r,s,t,u | 33.7615±.08150<br>e,f,i,j,m,n,q,s |
| EtOH 1 | 3.0596E2±5.8315<br>a,b,c,d,e,f,g,h,j,k,l,m,n,o,p,q,r,s,t | 1.3991E2±4.25050<br>d,e,g,h,j,k,m,n,p,q,r,s,t |
| Control acetic acid | 1.7534E2±2.9100<br>a,b,c,d,e,f,g,h,i,k,l,m,n,o,p,q,r,s,t,u | 1.4820E3±1.39218E2<br>a,b,c,d,e,f,g,h,i,k,l,m,n,o,p,q,r,s,t,u |
| $CuSO_4$ 4 | 1.2662E3±.0000<br>a,b,c,d,e,f,g,h,i,j,l,m,n,o,p,q,r,s,t,u | 6.9285±2.07350<br>a,e,f,i,j,l,m,n,o,q,s |
| Chitosan 5 | 7.6259E2±26.635<br>a,b,c,d,e,f,g,h,i,j,k,m,n,o,p,q,r,s,t,u | 1.0254E2±8.14700<br>e,g,j,k,m,n,q,r,s |
| Chitosan 50 | 3.5878E2±.13200<br>a,b,c,d,e,f,g,h,i,j,k,l,n,o,p,q,r,s,t,u | 5.0473E2±13.21000<br>a,b,c,d,e,f,g,h,I,j,k,l,n,o,p,q,r,s,t,u |
| SNP 250 | 3.3584E2±1.6965<br>a,b,c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u | 1.2933E3±15.55250<br>a,b,c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Phe 1 | 68.8815±1.99350<br>a,b,c,d,f,g,h,i,j,k,l,m,n,r,u | 1.0554E2±5.66050<br>e,g,j,k,m,n,q,r,s |
| MeJA 20 | 70.8180±.33000<br>a,b,c,d,f,g,h,i,j,k,l,m,n,q,r,u | 32.4735±.06050<br>e,f,i,j,m,n,q,s |
| Phe 3 | 50.8900±.93600<br>a,c,d,e,g,h,i,j,k,l,m,n,p,r,t,u | 6.5797E2±4.99500<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,r,t,u |
| SNP 100 | 27.2250±2.03500<br>a,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,s,t0,u | 1.8505±.00150<br>a,e,f,i,j,l,m,n,o,q,s |
| Chitosan 25 | 52.0435±2.66250<br>a,c,d,e,g,h,i,j,k,l,m,n,r,t,u | 7.1812E2±.99300<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,r,t,u |
| EtOH 2 | 75.5715±2.86350<br>a,b,c,d,f,g,h,i,j,k,l,m,n,q,r,s,u | 35.8020±4.38000<br>e,f,i,j,m,n,q,s |
| $CuSO_4$ 8 | 3.1603E2±4.2850<br>a,b,c,d,e,f,g,h,j,k,l,m,n,o,p,q,r,s,t | 80.2885±2.8945<br>e,j,m,n,q,s |

**Table 7:** *In vitro* phagocytic activity assay using peritoneal macrophages

| Extract Identity | Macrophage phagocytosis | Macrophage killing |
|---|---|---|
| Callus | 1.5526E5±4.20072E2<br>d,e,k,l,q | 1.4331E2±4.31800<br>e,i,p |
| Cultivated | 5.5813E4±3.76028E3<br>d,e,k,l,q | 37.9610±2.33100<br>e,i,p |
| Phe 2 | 5.4885E4±3.00251E3<br>d,e,k,l,q | 58.9275±2.80250<br>e,i,p |
| MeJA 10 | 4.8736E5±3.97574E5<br>a,b,c,g,h,j,k,l,m,n,o,p,r,s,t,u | 58.7630±5.48600<br>e,i,p |
| SNP 50 | 4.6772E5±7.29453E3<br>a,b,c,g,h,k,l,m,n,o,p,q,r,s,t,u | 4.6325E3±2.23653E2<br>a,b,c,d,f,g,h,I,j,k,l,m,n,o,p,q,r,s,t,u |
| MeJA 5 | 2.2444E5±1.82088E5<br>k,l,q | 70.1625±.96550<br>e,i,p |
| EtOH 0.5 | 2.7048E3±8.4645<br>d,e,k,l,q | 2.1475±.19650<br>e,i,j,p |
| $CuSO_4$ 2 | 7.2694E3±28.99450<br>d,e,k,l,q | 16.1375±1.64950<br>e,i,j,p |
| EtOH 1 | 2.6454E5±3.98021E3<br>k,l,q | 1.4741E3±1.42694E2<br>a,b,c,d,e,f,g,h,j,k,l,m,n,o,p,q,r,s,t,u |
| Control acetic acid | 1.9051E5±6.10151E3<br>d,k,lq | 1.9845E2±12.8725<br>e,g,h,i,q,r,s,t |
| $CuSO_4$ 4 | 2.0523E6±3.92084E4<br>a,b,c,d,e,f,g,h,i,j,l,m,n,o,p,q,r,s,t,u | 1.0148E2±2.23450<br>e,i,p |
| Chitosan 5 | 9.3297E5±8.32530E3<br>a,b,c,d,e,f,g,h,i,j,k,m,n,o,p,r,s,t,u | 1.5555E2±8.04800<br>e,i,p |
| Chitosan 50 | 1.6014E5±9.08919E3<br>d,e,k,l,q | 1.0554E2±3.71850<br>e,i,p |
| SNP 250 | 7.2168E4±3.27062E3<br>d,e,k,l,q | 1.5612E2±.27150<br>e,i,p |
| Phe 1 | 6.1102E4±5.02436E3<br>d,e,k,l,q | 1.3009E2±9.08700<br>e,i,p |
| MeJA 20 | 1.6900E5±1.72142E4<br>d,e,k,l,q | 3.3915E2±23.0725<br>a,b,c,d,e,f,g,h,I,k,l,m,n,o,q,r,s,t,u |
| Phe 3 | 7.5254E5±1.52674E4<br>a,b,c,e,f,g,h,I,j,k,m,n,o,p,r,s,t,u | 6.0430±.29700<br>e,i,j,p |
| SNP 100 | 1.1170E4±2.70671E2<br>d,e,k,l,q | 4.7955±.12950<br>e,i,j,p |
| Chitosan 25 | 5.2126E4±2.33536E2<br>d,e,k,l,q | 7.1830±.11900<br>e,i,j,p |
| EtOH 2 | 6.4927E4±1.63950E3<br>d,e,k,l,q | 5.9160±.49100<br>e,i,j,p |
| $CuSO_4$ 8 | 1.5357E5±4.99336E3<br>k,l,q | 37.3645±1.55050<br>e,i,p |

**Table 8:** *In-vitro* immunostimulatory Activity

| Extract Identity | $EC_{50}$ values (expressed in in µg/ml) causing a lymphocyte stimulation index of 3 |
|---|---|
| Callus | 1.5865E3±1.44479E2<br>b,c,d,e,f,g,h,i,j,k,l,m,n,p,q,r,s,t,u |
| Cultivated | 1.6366E2±8.67650<br>a,c,e,i,j,k,l,n,o,q,s,u |
| Phe 2 | 2.5439E2±19.31100<br>a,d,e,g,i,j,k,l,n,o,p,q,s,t,u |
| MeJA 10 | 1.3332E2±.15900<br>a,c,e,i,j,k,l,m,n,o,q,s,u |
| SNP 50 | 5.3718E2±45.80950<br>a,b,c,d,f,g,h,j,k,l,m,n,o,p,q,r,t,u |
| MeJA 5 | 1.8513E2±5.04800<br>a,e,g,I,j,k,l,n,o,q,s,t,u |
| EtOH 0.5 | 72.5350±2.24300<br>a,c,e,f,g,h,i,j,k,l,m,n,o,q,s,u |
| $CuSO_4$ 2 | 2.1224E2±24.92800<br>a,e,g,i,j,k,l,n,o,q,s,t,u |
| EtOH 1 | 5.6548E2±19.65100<br>a,b,c,d,f,g,h,j,k,l,m,n,o,p,q,r,s,t,u |
| Control acetic acid | 4.1465E2±1.71600<br>a,b,c,d,e,f,g,h,i,k,m,n,o,p,r,t,u |
| $CuSO_4$ 4 | 4.7508E3±.00000<br>a,b,c,d,e,f,g,h,i,j,l,m,n,o,p,q,r,s,t,u |
| Chitosan 5 | 4.0998E2±3.31750<br>a,b,c,d,e,f,g,h,i,k,l,m,n,o,p,r,t,u |
| Chitosan 50 | 2.6307E2±6.02400<br>a,d,e,g,i,j,k,l,m,n,o,p,q,s,t,u |
| SNP 250 | 1.9565E3±29.60300<br>a,b,c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Phe 1 | 1.5191E3±28.98850<br>b,c,d,e,f,g,h,i,j,k,l,m,n,p,q,r,s,t,u |
| MeJA 20 | 1.3752E2±11.78350<br>a,c,e,i,j,k,l,m,n,o,q,s,u |
| Phe 3 | 3.8066E2±3.55250<br>a,b,c,d,e,f,g,h,i,k,m,n,o,p,r,t,u |
| SNP 100 | 1.6861E2±4.41050<br>a,c,e,i,j,k,l,n,o,q,s,u |
| Chitosan 25 | 4.3339E2±31.08000<br>a,b,c,d,e,f,g,h,i,k,m,n,o,p,r,t,u |
| EtOH 2 | 74.1580±2.36600<br>a,c,e,f,h,I,j,k,l,m,n,o,q,s,u |
| $CuSO_4$ 8 | 3.2551E3±34.89100<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t |

**Table 9:** *In vitro* anti-inflammatory activity

| Extract Identity | Effective concentration in µg/ml causing a lymphocyte stimulation index of 3 |
|---|---|
| Callus | 1.5903E2±6.70950<br>b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| Cultivated | 18.0095±.13850<br>a,d,e,g,h,i,j,k,l,m,n,q,r,s,t,u |
| Phe 2 | 18.3355±.09050<br>a,d,e,f,g,h,i,j,k,l,m,n,q,r,s,t,u |
| MeJA 10 | 6.3525±.21450<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| SNP 50 | 46.0320±1.53800<br>a,b,c,d,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| MeJA 5 | 12.8005±1.74150<br>a,d,e,g,h,i,j,k,l,m,n,o,p,q,r,t,u |
| EtOH 0.5 | 3.8630±.00300<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| $CuSO_4$ 2 | 1.3525±.31450<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| EtOH 1 | 60.4690±.57000<br>a,b,c,d,e,f,g,h,k,l,m,n,o,p,q,r,s,t,u |
| Control acetic acid | 64.8825±1.36750<br>a,b,c,d,e,f,g,h,k,l,m,n,o,p,q,r,s,t,u |
| $CuSO_4$ 4 | 79.5135±2.01350<br>a,b,c,d,e,f,g,h,i,j,l,m,n,o,p,q,r,s,t,u |
| Chitosan 5 | 92.7165±2.85350<br>a,b,c,d,e,f,g,h,i,j,k,m,n,o,p,q,r,s,t |
| Chitosan 50 | 1.0072E2±3.14150<br>a,b,c,d,e,f,g,h,i,j,k,l,n,o,p,q,r,s,t |
| SNP 250 | 2.0270±.42000<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| Phe 1 | 22.4015±2.23650<br>a,d,e,f,g,h,i,j,k,l,m,n,q,r,s,t,u |
| MeJA 20 | 20.3855±1.37350<br>a,d,e,f,g,h,i,j,k,l,m,n,q,r,s,t,u |
| Phe 3 | 5.1790±.85500<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| SNP 100 | 4.4785±.29650<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| Chitosan 25 | 7.3335±.59850<br>a,b,c,e,i,j,k,l,m,o,p,u |
| EtOH 2 | 4.1375±.01250<br>a,b,c,e,f,i,j,k,l,m,o,p,u |
| $CuSO_4$ 8 | 97.5910±3.03600<br>a,b,c,d,e,f,g,h,i,j,k,n,o,p,q,r,s,t |

**Table 10:** Nitric oxide scavenging activity

| Extract Identity | EC50 in µg/ml |
|---|---|
| Callus | 3.3939E2±15.81800<br>b,c,d,e,f,g,h,j,l,m,n,o,p,q,r,s,u |
| Cultivated | 1.3030E2±.241<br>a,d,e,g,h,i,k,l,m,n,o,q,s,t |
| Phe 2 | 28.9375±.0175<br>a,b,e,i,k,l,m,n,o,q,t |
| MeJA 10 | 6.3730±.49800<br>a,b,e,i,k,l,m,n,o,q,t |
| SNP 50 | 5.3849E2±2.55200<br>a,b,c,d,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u |
| MeJA 5 | 51.7450±2.66800<br>a,e,i,k,l,m,n,o,q,t |
| EtOH 0.5 | 5.2175±.08050<br>a,b,e,i,k,l,m,n,o,q,t |
| $CuSO_4$ 2 | 22.4880±2.882<br>a,b,e,i,k,l,m,n,o,q,t |
| EtOH 1 | 2.9588E2±6.96750<br>b,c,d,e,f,g,h,j,l,m,n,o,p,q,r,s,u |
| Control acetic acid | 61.6670±8.180<br>a,e,i,k,l,m,n,o,q,t |
| $CuSO_4$ 4 | 2.8318E2±14.4275<br>b,c,d,e,f,g,h,j,l,m,n,o,p,q,r,s,u |
| Chitosan 5 | 7.7059E2±41.86150<br>a,b,c,d,e,f,g,h,i,j,k,m,n,p,q,r,s,t,u |
| Chitosan 50 | 1.1503E3±1.05772E2<br>a,b,c,d,e,f,g,h,i,j,k,l,n,o,p,q,r,s,t,u |
| SNP 250 | 1.3146E3±56.44400<br>a,b,c,d,e,f,g,h,i,j,k,l,m,o,p,q,r,s,t,u |
| Phe 1 | 8.3263E2±18.92000<br>a,b,c,d,e,f,g,h,i,j,k,m,n,p,q,r,s,t,u |
| MeJA 20 | 68.373±3.53750<br>a,e,i,k,l,m,n,o,q,t |
| Phe 3 | 6.7502E2±000<br>a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,r,s,t,u |
| SNP 100 | 87.8045±3.93250<br>a,e,i,k,l,m,n,o,q,t |
| Chitosan 25 | 32.7320±.09800<br>a,b,e,i,k,l,m,n,o,q,t |
| EtOH 2 | 3.2755E2±3.30100<br>b,c,d,e,f,g,h,j,l,m,n,o,p,q,r,s,u |
| $CuSO_4$ 8 | 49.9875±8.25650<br>a,b,e,i,k,l,m,n,o,q,t |

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# دراسة بالتقنية الحيوية لنبات الاشنيسيا المنزرع محليا

رسالة مقدمة إلى

كلية الصيدلة– جامعة الإسكندرية

كمتطلب جزئى للحصول على

## درجة الماجستير في العلوم الصيدلية (عقاقير)


**مقدمة من**

**نيڤين ممدوح عبد الرحمن على**

بكالوريوس فى العلوم الصيدلية

كلية الصيدلة

جامعة الإسكندرية

٢٠٠٨

قسم العقاقير

كلية الصيدلة

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**٢٠١٤**

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