Plant Archives Vol. 20 Supplement 1, 2020 pp. 3007-3014 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SALICYLIC ACID ON INCREASING OF SOME PHENOLIC ACID AND FLAVONOIDS IN *CORIANDRUM SATIVUM* CALLUS.

Shaymaa Jawad Kdhim*, Emad Hamdi Jassim and Hawazen H. Salih

Genetic Engineering and Biotechnology Institute for Post Graduate Studies, University of Baghdad, Baghdad, Iraq.

Abstract

Flavonoids and phenolic acid often had pharmacological effects. The goal for project raise several active compound in *C.sativum*. Callus induction occurred on hypocotyl segments 5mm long. Murshige and Skoog medium supplied with 1.5 mg/l from 2,4-dichlorophenoxy acetic acid in addition to 0.6 mg/l of kinetin and maintenance callus in the same concentrations. For a period of thirty days with darkness under the temperature of twenty-five°C±1 then analyzed the Callus utilize high performance liquid chromatography. Methanolic callus excerption appeared altitude in concentrations of some flavonoids and phenolic acid. For growing the value of active substance , SA was supplemented with deferent levels 0,75,125,175,225 mg/L , drive to reduction in both weights fresh and dry of callus with significant increase in all phenolic compound excepted Ferulic acid glucoside compound increased but non-significant .also most of flavonoids gave high significant value at 225 mg/L compared with control treatment While Querecetn-3-O- glucronide significantly increased reached to 303.183µg.ml^{-1} with 125 mg/L SA per 100 mg fresh weight of callus and the lowest concentration recorded was in Hypocotyl (119.994, 79.541, 111.846 and 39.879) µg/ml for each three gram from fresh weight of mother plant respectively.

Key words: *C.sativum*, flavonoids, phenolic acid, salicylic acid.

Introduction

C. sativum L. is medicinal plant belong to Apiaceae family and cultured it for the purpose of obtaining seeds and leaves (Saxena *et al.,* 2015) and it has several previously detected pharmacological activities, such as anxiolytic, antidepressant, sedative-hypnotic, antioxidant, cardiovascular, analgesic, anticonvulsant, memory enhancing, antidiabetic, anticancer, gastrointestinal, dermatological, hypolipidemic, anti-inflammatory, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antimutagenic etc, It is due to the presence of various chemical components such as alkaloids, phenolics, flavonoids, essential oil, terpenoids, sterols, glycosides, reducing sugars, tannins, and fatty acids (Al-Snafi, 2016). About eight thousands phenolic compounds in plants generally found in bound shape as esters or glycosides (Silva *et al.,* 2016). Plant cells have been utilized to produce important pharmaceutical compounds (Giri and Zaheer, 2016). In plant tissue cultures, there are many techniques used to enhance the product yield of these cells (Araybi, 2016). Adding salicylic acid to the nutrient medium is one of the induction methods of plant cells (Perez-Alonso *at el.,* 2014) and it works down a biotic and biotic stress situation like a secondary messenger to excitation of defense genes. Various research appeared that the exogenous implementation of SA ability to excess the evolution to plants through effect on their signaling regulation and prompt enzymes to stimulate forming for defiance compounds, that demonstrate hers function like a elicitor of active compound (Gorni and Pacheco, 2016).

Materials and Methods

That work was done in plant tissue culture Lab., in Genetic Engineering institute, *Coriandrum sativum* L. seeds was acquired from a local market in Baghdad - Iraq.

Seeds Sterilization

The seeds were washed for five min. with tap water. The seeds Were surface sterilized by dipping in 70% of ethanol for sixty seconds and Surface sterilization of seeds were carried out inside a laminar air flow cabinet by immersion in sodium hypochlorite (3%) with 1 drop of tween-20 to ten min., then rinse by sterile distilled water four times. The sterilized seeds culturing in MS medium

Author for correspondence : E-mail : shymalmwsw21@gmail.com

without hormones about (1-2) seeds in each screw glass tube containing 10 ml of MS medium incubated at 25 ±1ºC for 16/8 hours (light/dark) a light intensity of (1000 lux). Two weeks after germination the seedlings attained sufficient growth Fig 1 to be used for callus induction.

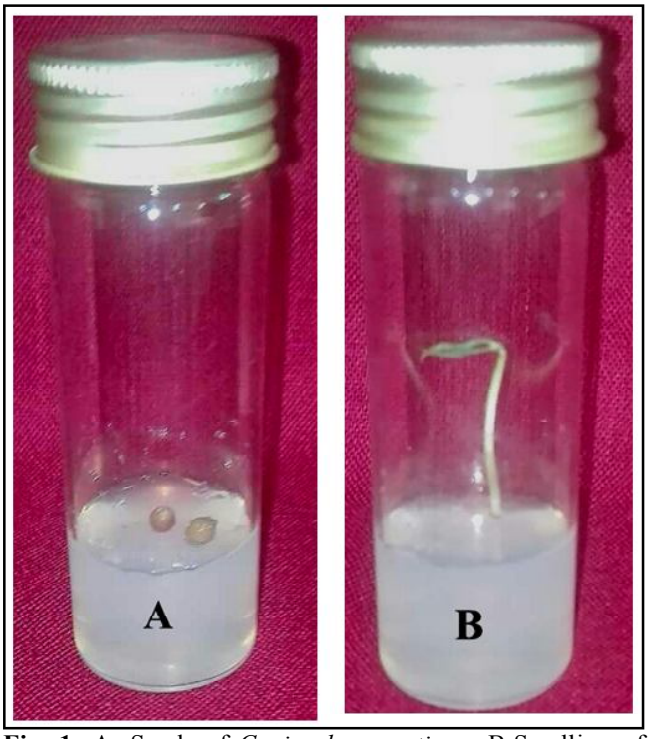


Fig. 1: A. Seeds of *Coriandrum sativum* B.Seedling of *Coriandrum sativum.*

Fig. 2: Callus induction from Hypocotyl explant

Medium Preparation and Callus Induction.

MS medium (Murashige and Skoog, 1962) was prepared, with 30000 mg.L^{-1} sucrose and pH was 5.5 using (0.1 N) NaOH or (0.1 N) HCl, complete volume to 1000 ml and with different levels of auxins (2, 4-D 0.0, 0.5, 1.0, 1.5) mg/L in combination with various concentration of cytokines (Kinetin 0.0, 0.4, 0.6, 0.8) mg/L then (7000 mg.L^{-1}) agar was added to the medium after that all components were put on a hotplate magnetic stirrer till boiling. Then aliquots of (10 ml) were poured in the screw glass tubes (85 x 28) mm then put in autoclaved at 121^{0}C, 1.04 Kg.cm-2 pressures to fifteen minute and leave it to cool at room temperature and became ready for use. The explants Hypocotyls which obtained from seedling transferring to petri dish were cut into (0.5 cm) long inside a laminar air flow cabinate and cultured on

Table 1: Impact of salicylic acid on callus fresh and dry weight to *C. Sativum* after 4 weeks.

SA mg\l	Fresh weight	Dry weight
0	408.00	26.52
75	394.30	25.62
150	386.60	25.12
225	375.40	24.35
300	361.70	23.51
LSD 0.05	17.334 *	1.127 *

* (P<0.05)

Table 2: effect of salicylic acid on increasing of phenolic (µg.ml^{-1}) in *C.Sativum* callus analyzed by HPLC.

SA mg.l^{-1}	Gallic acid	Benzoic acid	Dimethoxy cinnmoyl hexoside	Ferulic acid glucoside	P-comuaroyl quinic acid	3-O-Caffeoyl quinic acid
0	69.624	113.815	193.118	90.693	144.298	183.728
75	87.731	135.254	198.840	94.281	116.313	234.106
125	104.773	139.221	242.265	114.957	191.245	340.060
175	112.577	156.510	275.917	123.078	136.406	336.130
225	123.102	163.675	289.701	124.555	195.027	397.116
LSD 0.05	39.686*	44.63 *	70.83 *	37.23 NS	42.76 *	61.68 *

* (P<0.05).

Table 3: Effect of salicylic acid on increasing of flavonoids (µg.ml^{-1}) in *C.sativum* callus analyzed by HPLC.

SA mg.l^{-1}	Querecetin-3-O-rutinoside	Querecetn-3-O- glucronide	Querecetin-3-O- glucoside	Kaempferol-3-O-rutinoside
0	121.973	126.181	114.735	112.069
75	248.621	178.882	158.554	156.067
125	281.713	303.183	193.006	162.823
175	333.727	174.938	171.119	195.649
225	390.405	286.337	246.975	238.734
LSD 0.05	87.38 **	71.97 *	76.44 *	68.02 *

* (P<0.05).

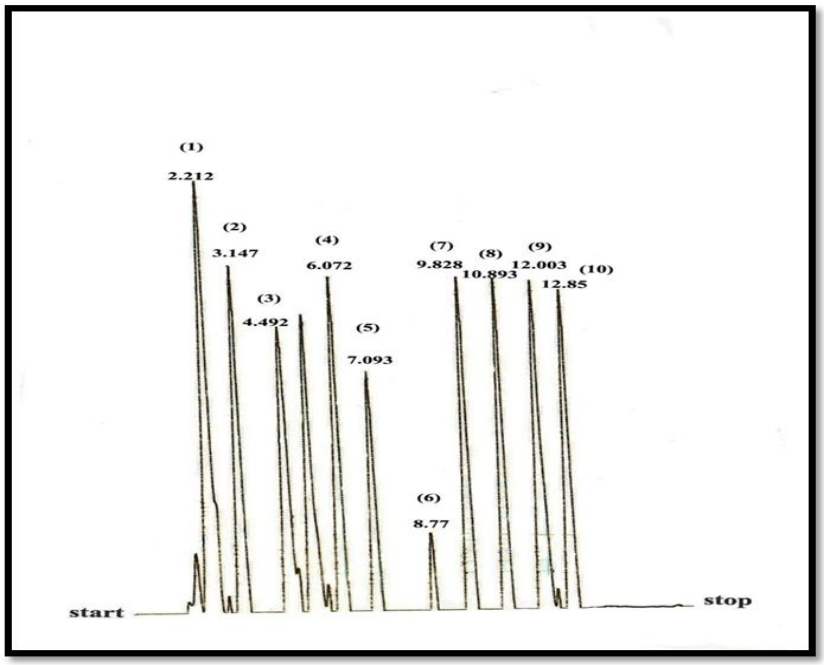
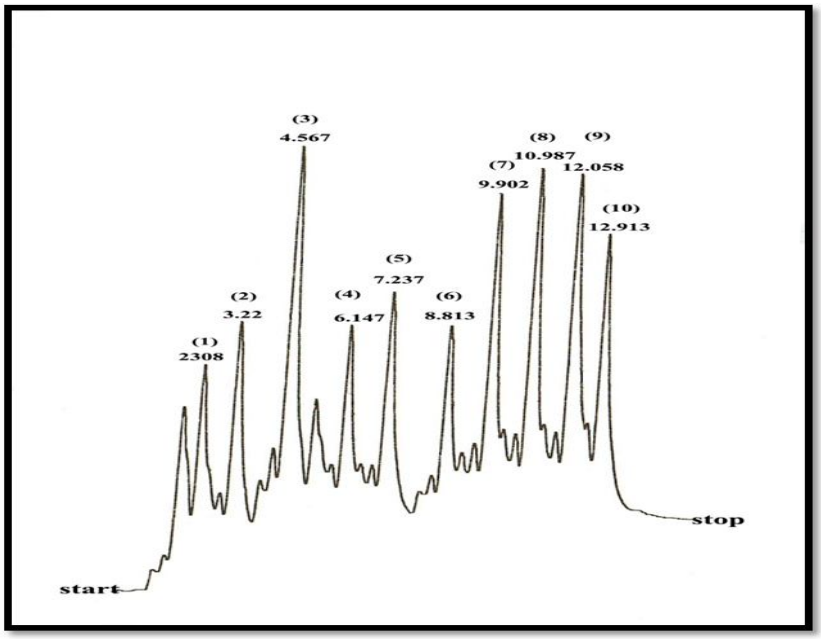


Fig. 3: (A) HPLC analysis of reference standards phenolic acid and flavonoids

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid (µg.ml^{-1})	Gallic acid	1	2.212	153030
	Benzoic acid	2	3.147	112410
	Dimethoxy cinnmoyl hexoside	3	4.492	109330
	Ferulic acid glucoside	4	6.072	113927
	P-comuaroyl quinic acid	5	7.093	101734
	3-O-Caffeoyl quinic acid	6	8.776	51553
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	7	9.828	118364
	Querecetn-3-O- glucronide	8	10.893	119188
	Querecetin-3-O- glucoside	9	12,003	143700
	Kaempferol-3-O-rutinoside	10	12.85	131961



MS medium. Explants were kept in (25±1°C) temperature under dark after 5 weeks of incubation callus formation from explants Fig. 2. Fresh and dry weighing of callus was recorded**.**

Stimulation active compounds of callus

Form the best result (200 mg) of callus that initiated from hypocotyl explant was sub cultured onto MS medium complete with 1.5 2, 4-D and 0.6 Kin that gave high fresh and dry weights for callus induction. With the inclusion of the SA at the concentrations (0, 75, 125, 175, 225 mg/L).

Extraction of phenolic and flavonoids compounds

Three gram of Hypocotyls powder as well as callus 100 mg by paste-motor the samples crushed in tiny segment after that suspending sample in five ml of ethanolic-water (80:20,v/v).and was display for ultra-sonication withe 60% obligation cycles for 15 min to the final sample the pure supernatant of all sample was undergo to treatment of charcoal for tak off pigments before evaporation beneath vacuum desiccated samples in methanolic of one ml HPLC the mixture were re-suspended via vortex and pass into 2.5 µm disposable filter and at 4°C was stored to more analysis , after that the HPLC was inoculated with 20 µl of the sample at most favorable conditions(Suarez, *et al*., 2005).

High Performance Liquid Chromatography (HPLC)

The separation done through liquid chromatography Shimadzu 10 AV-LC equipped by binary delivery pump model LC-10A shimadzu, the eluted peaks were monitored by UV-V is 10A-SPD spectrophotometer. The compounds discrete via, Fast Liquid Chromatographic column.

HPLC Condition

Column: phenomenex C-18um particle size (50 X2.0 mm I.D) Mobile Phase: linear A : HPLC grade 0.1%

Fig. 3(B):HPLC analysis of phenolic acid and flavonoids in callus without treatment.

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid (µg.ml^{-1})	Gallic acid	1	2.308	85236
	Benzoic acid	2	3.22	102352
	Dimethoxy cinnmoyl hexoside	3	4.567	168921
	Ferulic acid glucoside	4	6.147	82659
	P-comuaroyl quinic acid	5	7.237	117440
	3-O-Caffeoyl quinic acid	6	8.813	75774
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	7	9.902	115498
	Querecetn-3-O- glucronide	8	10.987	120314
	Querecetin-3-O- glucoside	9	12.058	131899
	Kaempferol-3-O-rutinoside	10	12.913	118310

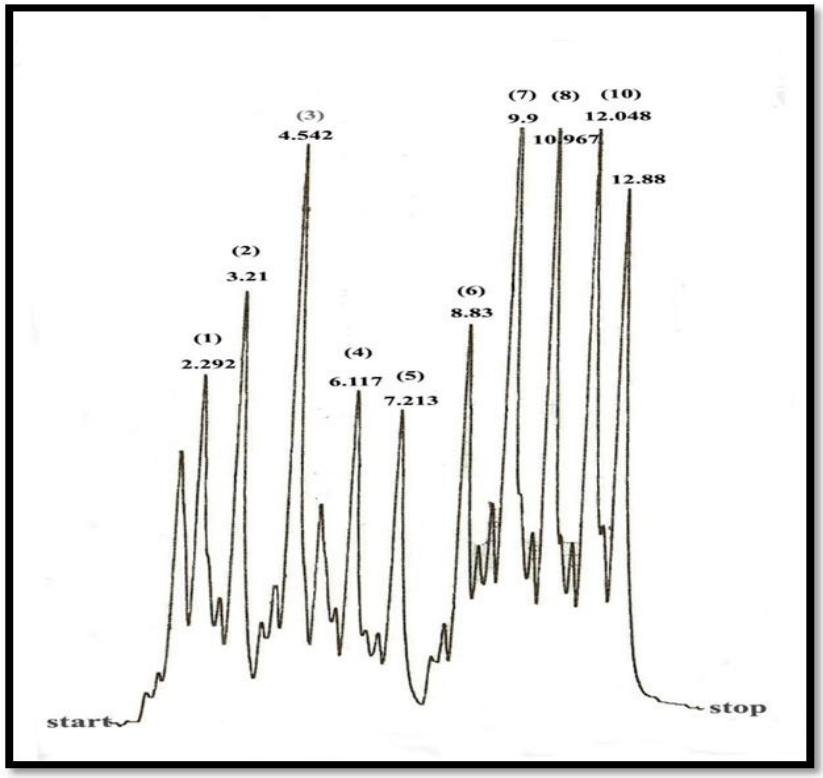


Fig. 3: (C) HPLC analysis of phenolic acid and flavonoids in callus at concentration (75 mg/l) of SA

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid (µg.ml^{-1})	Gallic acid	1	2.292	107404
	Benzoic acid	2	3.21	121631
	Dimethoxy cinnmoyl hexoside	3	4.542	173926
	Ferulic acid glucoside	4	6.117	85929
	P-comuaroyl quinic acid	5	7.213	94664
	3-O-Caffeoyl quinic acid	6	8.83	96551
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	7	9.9	269511
	Querecetn-3-O- glucronide	8	10.967	170565
	Querecetin-3-O- glucoside	9	12.048	182274
	Kaempferol-3-O-rutinoside	10	12.88	164758

formic acid, B : HPLC grade(6:3:1, v/v) of acetonitrile:methanol:0.1% formic acid, gradient program from 0% B to 100% B to ten min. Flow average: 1.2 ml/minute injection volume: 20ul the concentration of active substances was quantified by comparing the area of standard package with an area of the model under the same condition (Budhiraja, 2004). by using the following low:

Concentration of compound = area of sample/ area of standard × concentration of standard × dilution factor.

Conc. For standard: 25µg/ml , Dilution factor: 5µg/ml

Than the results were compared with the mother plant.

Statistical Analysis and Experimental Design

The all experiments executed with ten replicates and utilized ANOVA to statistically analyzed by two-way analysis of variance test using SAS statistical program and completely randomized block design and the differences between group were compared using LSD at ($P<0.05$). (SAS, 2012).

Results and Discussion

All levels of salicylic acid (0, 75, 150, 225 and 300 mg.l^{-1}) due to a significant reduction occurred at mean of callus fresh and dry weight. Control treatment recorded the highest callus fresh and dry weighing (406.30) and (26.40) mg respectively compared with different concentrations of salicylic acid. (Table 1).

Perhaps the difference in the aqueous relationships of cells leads to high pressure on the cells and this requires that the cells reorganize their osmotic stress in a manner consistent with the external conditions that are exposed to the cells and this leads to a decrease in the water readiness and then melts nutrition from the medium where the cells grow and thus negatively effects on growth of callus cells (Yao, 2003) SA suppresses growth of cells by inhibiting the formation of ATP and respiration process, leading to decrease cell size or death, as

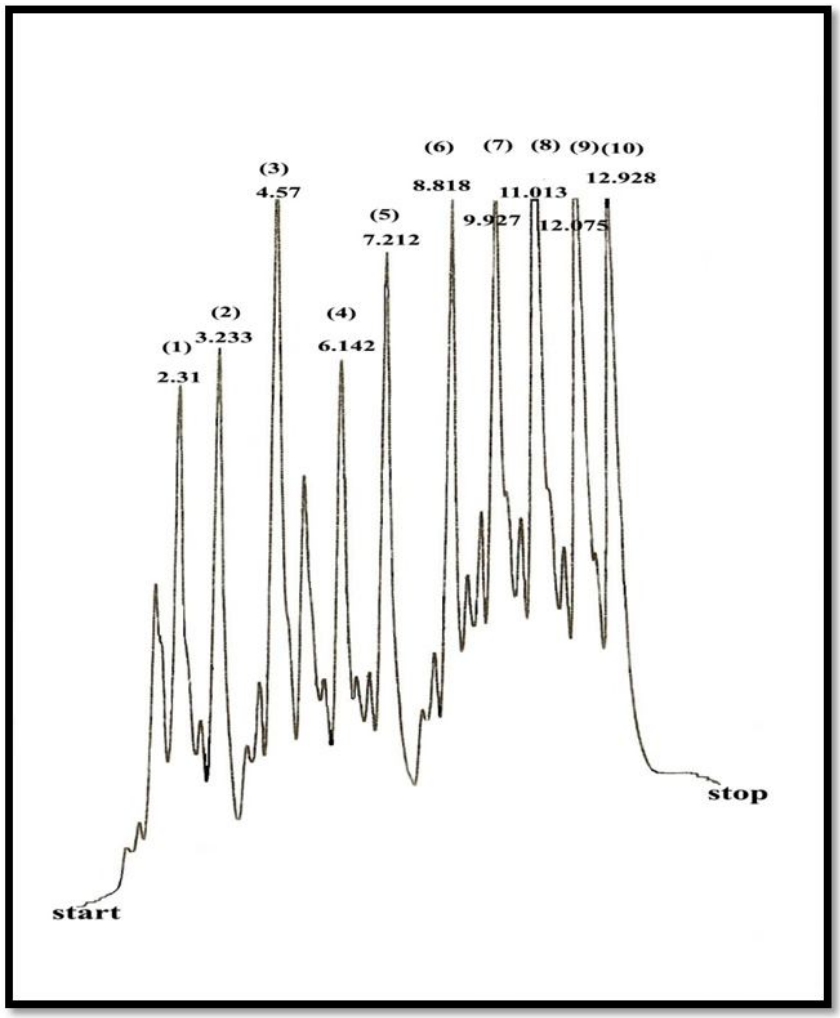


Fig. 3: (D) HPLC analysis of phenolic acid and flavonoids in callus at concentration (125mg/l).

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid ($\mu g.ml^{-1}$)	Gallic acid	1	2.31	128267
	Benzoic acid	2	3.233	125199
	Dimethoxy cinnmoyl hexoside	3	4.57	211910
	Ferulic acid glucoside	4	6.142	104774
	P-comuaroyl quinic acid	5	7.212	155649
	3-O-Caffeoyl quinic acid	6	8.818	140249
Flavonoids ($\mu g.ml^{-1}$)	Querecetin-3-O-rutinoside	7	9.927	266757
	Querecetn-3-O- glucronide	8	11.013	289086
	Querecetin-3-O- glucoside	9	12.075	221880
	Kaempferol-3-O-rutinoside	10	12.928	171890

occurred in plant tissue cultures of tobacco (Vicente and Plasencia, 2011). The results of this study on callus induction of *C. sativum* agree with the studies conducted by Chaichana and Dheeranupattana, (2012). Who reported that SA inhibits callus growth in *Stemona sp*. imposing a strong suppression. Rezaei *et al*., in 2011 observed that the results when high level of SA led to a significant lowering in cell development, Comparison with the treatment free from SA in *Taxus baccata* L.

Impact of salicylic acid on production of phenolic acid in *C. Sativum* callus

The data in table 2 appeared that usage of salicylic acid at 225 $mg.l^{-1}$ concentration gave significantly increased in all phenolic compound Gallic acid, Benzoic acid, Dimethoxy cinnmoyl hexoside, Ferulic acid glucoside, P-comuaroyl quinic acid and 3-O-Caffeoyl quinic acid reached to123.102, 163.675, 289.701, 124.555, 195.027 and 397.116$\mu g.ml^{-1}$ respectively compared with control treatment which recorded of 69.624, 113.815, 193.118, 144.298 and 183.728 µg\l each 100 mg fresh weight from callus except Ferulic acid glucoside gave highe value at 225 $mg.l^{-1}$ concentration reached to 124.555 but non significant differences recorded compared with control . Fig. 3 (A, B, C, D, E, F) the callus tissues extract gave high content of phenolic acid compounds compared with the same compounds in mother plant as declared in table 4 and Fig. 4.

Impact of salicylic acid on production of flavonoids in *C. sativum* callus

The results in the Table 3 Fig. 3 (A, B, C, D, E, F) displayed that, usage of salicylic acid at 225 $mg.l^{-1}$ concentration gave high significant value of Querecetin-3-O-rutinoside, Querecetin-3-O- glucoside and Kaempferol-3-O-rutinoside reached to (390.405, 246.975 and 238.734 $\mu g.ml^{-1}$) respectively compared with control which recorded 121.973, 114.735 and112.069 $\mu g.ml^{-1}$ each 100 mg of callus fresh weight. Whereas Querecetn-3-O- glucronide significantly increased reached to 303.183µg\ml at 125 mg\l concentration of salicylic acid in comparison with control that registered 126.181µg\ml each 100 mg of callus fresh weight.

While Querecetin-3-O-rutinoside, Querecetn-3-O-glucronide, Querecetin-3-O- glucoside and Kaempferol-3-O-rutinoside the lowest concentration recorded was in Hypocotyls (119.994, 79.541, 111.846 and 39.879) µg\ml each

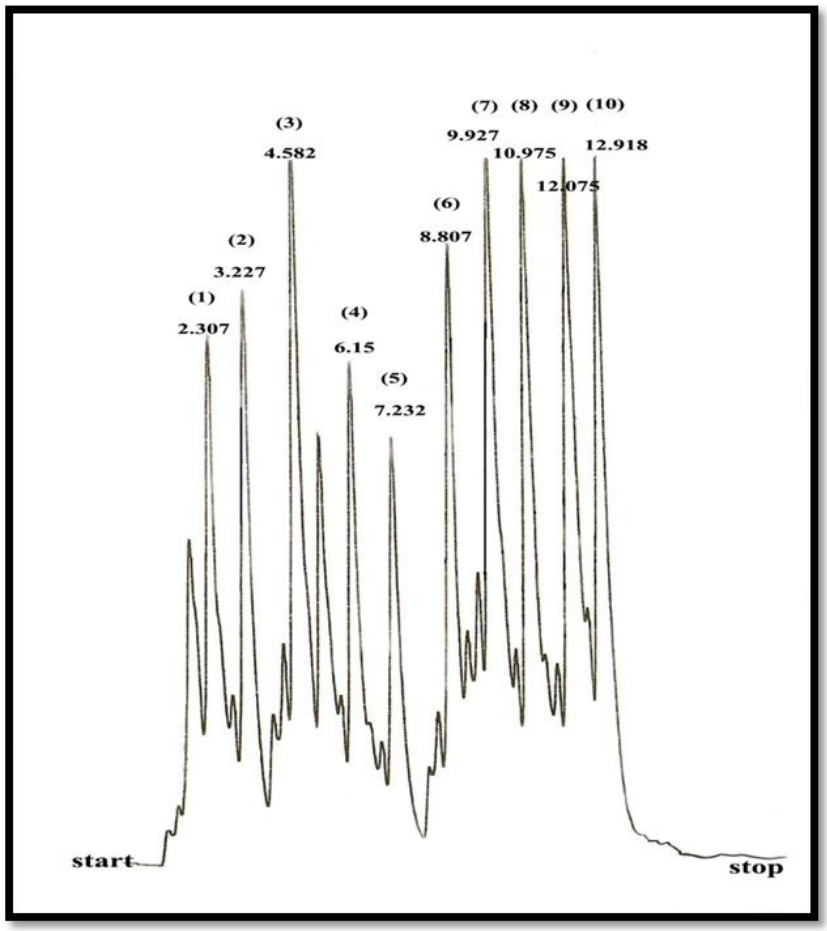


Fig. 3: (E) HPLC analysis of phenolic acid and flavonoids in callus at concentration(175mg/l) of SA

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid ($\mu g.ml^{-1}$)	Gallic acid	1	2.307	137821
	Benzoic acid	2	3.227	140746
	Dimethoxy cinnmoyl hexoside	3	4.582	241346
	Ferulic acid glucoside	4	6.15	112175
	P-comuaroyl quinic acid	5	7.232	111017
	3-O-Caffeoyl quinic acid	6	8.807	138628
Flavonoids ($\mu g.ml^{-1}$)	Querecetin-3-O-rutinoside	7	9.927	316010
	Querecetn-3-O- glucronide	8	10.975	166804
	Querecetin-3-O- glucoside	9	12.075	196718
	Kaempferol-3-O-rutinoside	10	12.918	206544

3g fresh weight of field plant as mentioned in table 4 and Fig. 4.

Discussion

Salicylic acid acts as a biotic elicitor in plants and has ability for increasing produce different compounds of active substance like alkaloids, phenols, flavonoids and terpenes, (Ali et *al.,* 2006; Silva *et al*., 2014). The biosynthesis and increasing production of some different compounds like flavonoids and phenolic acids in response to elicitors' addition; particularly salicylic acid may be clarified through the inducement of a condition of oxidative stress (Perez et *al.,* 2014). The results in table 2 and 3 showed that, high value of phenolic acid and flavonoids accomplished with modified MS medium having 225mg/l of salicylic acid the reason may due the salicylic acid mode of action via its intervention with an antioxidant enzymes where the suppression of the enzyme catalase would give rise to increasing in cellular levels of hydrogen peroxide which have a role in increment of secondary metabolites production in plant like a second messenger (Askari and Ehsanzadeh, 2015). Salicylic acid has been utilized in the inducement of flavonoids and polyphenols in callus, cell suspension, and tissue cultures of diverse plant families (Gadzovska *et al.,* 2013). Some researchers testified that the using of salicylic acid as elicitors caused increment of flavonoid of cultures of cell suspension, (2.1, 1.5) time greater than the control (Wang *et al*., 2015). Also Jassim and Ameen (2014) showed that SA also increased the Serpentine, Vinblastine and Vincristine in the callus of *Catharanthus roseus*. HPLC analysis of Active compounds in mother plants (hypocotyl) of *C. stivum* has not been listed active compounds only after utilizing three gm from fresh Hypocotyl of *C. sativum* in comparison to one hundred mg of free salicylic acid. Such may be consequent to that biosynthesis of effective substances at quite lower amounted to 0.0005% in plants. (Ebrahimzadeh *et al*., 1996).

Conclusion

Salicylic acid is good elicitors for increasing all phenolic acids and flavonoids compounds in *Corindrum sativum* callus, greater than such grown on MS media free of salicylic acid that is beneficial for the pharmacological manufacture.

References

Ali, M., K.W. Yu, E.J. Hahn and K.Y. Paek (2006). Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors.

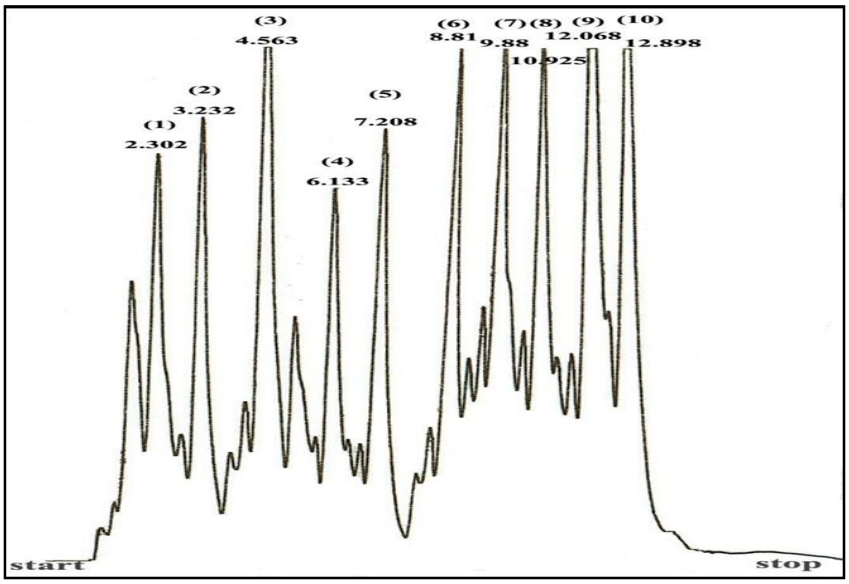


Fig. 3: (F) HPLC analysis of phenolic acid and flavonoids in callus at concentration(225mg/l) of SA

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid (µg.ml^{-1})	Gallic acid	1	2.302	150707
	Benzoic acid	2	3.232	147190
	Dimethoxy cinnmoyl hexoside	3	4.563	253403
	Ferulic acid glucoside	4	6.133	113521
	P-comuaroyl quinic acid	5	7.208	158727
	3-O-Caffeoyl quinic acid	6	8.81	163780
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	7	9.88	369679
	Querecetn-3-O- glucronide	8	10.925	273023
	Querecetin-3-O- glucoside	9	12.068	283923
	Kaempferol-3-O-rutinoside	10	12.898	252029

Plant Cell Rep., **25(6):** 613-620.

Al-Snafi, A.E. (2016). A Review on Chemical Constituents and Pharmacological Activities of *Coriandrum sativum*. *International Organization of Scientific Research Journal of Pharmacy,* **6(7):** 17-42.

Araybi, M. N. Nasr, J. Soorni and D. Sadhu (2016). Enhanced accumulation of scopoletin in cell suspension culture of *Spilanthes acmella* Murr. using precursor feeding. *Braz. Arch. of Biol. and Technol.,* **59:** 1-7.

Askari, E. and P. Ehsanzadeh (2015). Drought stress mitigation by foliar application of salicylic acid and their interactive effects on physiological characteristics of fennel (*Foeniculum vulgare* Mill.) genotypes. *Acta. Physiol. Plant.,* **37(4):** 1- 14.

Budhiraja, R.P. (2004). Separation Chemistry. New Age International Ltd, ublishers, New Delhi, pp.171.239.

Chaichana, N. and S. Dheeranupattana (2012). Effect of methyl jasmonate and salicylic acid on alkaloid production from *in vitro* culture of *Stemona sp. Inter. J. of Biosci. Bioche. and Bioinform,* **2(3):** 146-150.

Ebrahimzadeh, H., A. Ataei-Azimi and M. Noori-Daloi (1996). The distribution of indole alkaloids in different organs of *Catharanthus roseus* L.G. Don. (*Vinca rose* L.). *Daru. J. Sch. Pharm.,* **6(1):** 11-24.

Gadzovska, S., S. Maury, A. Delaunay, M. Spasenoski, D. Hagège, D. Courtois and C. Joseph (2013). The influence of salicylic acid elicitation of shoots, callus, and cell suspension cultures on production of naphtodianthrones and phenylpropanoids in *Hypericum perforatum* L., *Plant Cell Tissue organ Cult., (PCTOC)* **113:** 25–39.

Giri, C.C. and M. Zaheer (2016). Chemical elicitors versus secondary metabolite production in vitro using plant cell, tissue and organ cultures: recent trends and a sky eye view appraisal. *Plant Cell Tis Cult.,* **126:** 1-18.

Gorni, P.H. and A.C. Pacheco (2016). Growth promotion and elicitor activity of salicylic acid on *Achillea millefolium* L. *African Journal of Biotechnology, Kenya,* **15(16):** 657-665.

Jassim, E.H. and S.K.M. Ameen (2014). Influence of L-Tryptophan and salicylic acid on secondary metabolites production from leaves induced callus of *Catharanthus roseus* L.G. Don in vitro. *Jornal of Biotechnology Research Center,* **8(2):** 35- 43.

Perez, MGF., NER. Guzmán, EMS. Silva, GL. Piña and RR. Camacho (2014). Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusions. *Food Chem.,* **156:** 273-278.

Perez-Alonso, N.L., F.A. Labrada, A.C. Pérez, A.P. Pérez, R. Sosa and A. Mollineda (2014). Estimulación de cardenólidos en brotes de *Digitalis purpurea* L. cultivados in vitro mediante elicitores. *Rev. Colomb. Biotecnol.,* **16(1):** 51-61.

Rezaei, A., F. Ghanati and M.A. Dehaghi (2011). Stimulation of taxol production by combined salicylic acid elicitation and sonication in Taxus baccata cell culture, *Int. Conf. Life Sci. Technol.,* **3:** 193-197.

SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Saxena, S.N., Y.K. Sharma, S.S. Rathore, K.K. Singh, P. Barnwal and R. Saxena (2015). Effect of cryogenic grinding on volatile oil, oleoresin content and anti-oxidant properties of coriander (*Coriandrum sativum* L.) genotypes. *Journal of Food Science*

Table 4: the phenolic acid and flavonoids distinguished and counted (µg\ml) in hypocotyls extract and callus without treatment by HPLC.

Active compound		per3000 mgHypocotyl Mother plants	per100 mg callus
phenolic acid (µg.ml^{-1})	Gallic acid	49.108	69.624
	Benzoic acid	108.074	113.815
	Dimethoxy cinnmoyl hexoside	136.352	193.118
	Ferulic acid glucoside	64.060	90.693
	P-comuaroyl quinic acid	68.403	144.298
	3-O-Caffeoyl quinic acid	97.060	183.728
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	119.994	121.973
	Querecetn-3-O- glucronide	79.541	126.181
	Querecetin-3-O- glucoside	111.846	114.735
	Kaempferol-3-O-rutinoside	39.879	112.069

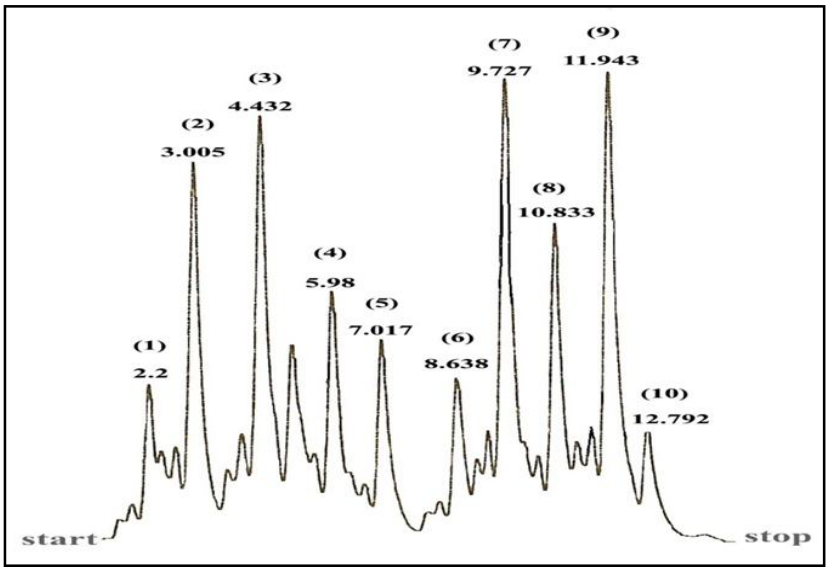


Fig. 4: HPLC analysis of phenolic acid and flavonoids to mother plants (Hypocotyl).

Active compound		N0. 0f compound	retention time (minute)	Area
phenolic acid (µg.ml^{-1})	Gallic acid	1	2.2	60120
	Benzoic acid	2	3.055	97189
	Dimethoxy cinnmoyl hexoside	3	4.432	119268
	Ferulic acid glucoside	4	5.98	58385
	P-comuaroyl quinic acid	5	7.017	55671
	3-O-Caffeoyl quinic acid	6	8.638	40030
Flavonoids (µg.ml^{-1})	Querecetin-3-O-rutinoside	7	9.727	111926
	Querecetn-3-O- glucronide	8	10.833	75843
	Querecetin-3-O- glucoside	9	11.943	128578
	Kaempferol-3-O-rutinoside	10	12.792	42100

and Technology, **52:** 568-573.

Silva, B.V., J.C. Barreira and M.B.P. Oliveira (2016). Natural phytochemicals and probiotics as bioactive ingredients of functional foods: Extraction, biochemistry and protected- delivery technologies. Trends in Food. *Science and Technology,* **50:** 144–158.

Silva, S., C.B. Moreira, M.A. Esquibel, Rass. Gil, Cas. Riehl and A. Sato (2014). Effect of salicylic acid on essential oil compounds of *Melissa officinalis in vitro*. plants. *Tec. Agropecu.,* **35(1):** 178-184.

Suarez, B., N. Palacions, N. Fraga and M.R. Rodriguiz (2005). Liquid chromatographic method for quantifying polyphenols in ciders by direct injection. *Journal of Chromatography A.,* **1066:** 105–110.

Vicente, M. and J. Plasencia (2011). Salicylic acid beyond defense: its role in plant growth and development. *J. of Experi. Bot.,* **62(10):** 3321–3338.

Yao, Z.Z. (2003). Effect of factors on callus biomass and synthetic mass of hypericin in *Hypericum perforatum*. *National Library of Medicine,* **18(10):** 3-921.

Wang, J., J. Qian, L. Yao and Y. Lu (2015). Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of Hypericum perforatum. *Bioresources and Bioprocesses,* 2(5).