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Effect of colchicine on different growth parameters and seconday metabolite (Mangiferin, scopoletin and quercetin) evaluated by HPTLC analysis in ethanolic micro extracts of *Canscora decurrens* (Dalz)

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Abstract
In the present study, dry seeds of *C. decurrens* were treated with different concentration of colchicine(0.5,1 and 2%), to increase the synthesis and accumulation of secondary metabolite as well as changing the genetic activity which may lead to increased chance of obtaining high yielding plant variety. In the study highest doses of colchicine exhibited enhanced shoot and root length, no. of leaves and node. In contrast to that fresh weight was significant decreased in 2% colchicine as against in control. Chlorophyll content (chl a, b and total chl) showed great variability as well as uneven distribution of chlorophyll pigment irrespective of colchicine doses was found. In quantitative HPTLC analysis of seconday metabolite (mangiferin, scopoletin and quercetin) in ethanolic micro extracts all doses of colchicines was found to effective increasing the active phytochemical, quantity of scopoletin was found highest in 1% than control.

Keywords: *C. decurrens*, colchicine, HPTLC, mangiferin, scopoletin, quercetin

Introduction
Canscora decurrens (syn. *C.diffusa*) is such a potential medicinal plant known to cure large number of disorders of central nervous system [6]. Whole plants of *Canscora* species are used in large number of Ayurvedic herbal preparations of nervous disorders like epilepsy, insanity, nervous debility and as nervine tonic [13]. In the present study approaches was to optimize the synthesis and accumulation of secondary metabolite are either growing plants should be exposed to stressful condition using colchicine or changing the genetic activity which may lead to increased chance of obtaining high yielding plant variety, further different solution of colchicine were worn on biomass production in terms of shoot and root length, number of leaves, fresh weight, dry weight, chlorophyll content as well as on Phytochemistry [28].
Colchicine (C22H25O6N), a product extracted from the seeds and bulbs of *Colchicum autumnale* L., is probably the most widely used chemical for induction of polyploidy. Colchicine inhibit formation of spindle fibres which result in polyploid cells [1]. These cells are often larger than diploid counter parts and greater cell volume frequently develops into thicker tissues, thus resulting in large size plant organs. It also affects stomata size, stomata frequency, pollen grain diameter and other plant morphological characteristics in new higher ploidy levels [24].
In *C. decurrens* extraction was done through Microwave assisted extraction method to extract the secondary metabolite from plant material. In Microwave assisted extraction heating of mixture (Powder +solvent) occurs in targeted and selective manner as no heat is lost, this method is most suitable for polar mixtures [17]. The extraction is governed by ionic conduction and dipole rotation of both solid plant matrix and solvent and hence unlike sohxlet (conductive heating) microwave heat the whole sample simultaneously microwave extraction cutback cost and time with very less solvent consumption [27]. It is also simple, rapid and safe. Further effect different concentration of colchicine on secondary metabolite [9]. Effect of different doses of colchicine on secondary metabolite Mangiferin (Xanthone), Scopoletin (Coumarin) and Quercetin (Flavonoid) was evaluated by HPTLC analysis. HPTLC has emerged as an efficient and powerful analytical technique for fingerprinting and quantification of marker compounds in herbal drugs due to its merits of reliability, simplicity, sensitivity, accuracy, suitability for high throughput screening and speed of estimation of the content of phytomarkers in herbs [13, 5]. It is more accurate, reliable, cost effective and user friendly than other methods like, NMR, GC-MS, LC-MS and IR [26].
No attempts have been done to study the effect of different concentration on growth and phytochemisty. The present study bridges this gap and has come out with interesting results.

Material and Methods
1. a. Growth and Germination
Mature and dry seeds of *C.decurrens* (7mg for every treatment) were soaked in aqueous solution of Colchicine (0.5, 1 and 2%) for 6hrs. At the end of treatment the seeds were surface sterilized (1 wash of mercuric chloride and 3 times with distilled water) and seeds were inoculated on MS basal medium and kept under controlled condition for 3 months for further germination and growth. Effect of colchicine was studied on various growth parameters like, shoot length, root length, no.of node, no. of leaves, fresh weight and dry weight and chlorophyll content.

b. Chlorophyll Content
500mg of fresh leaves were ground in 80% acetone(10ml),

filtered and centrifuged (5000rpm) for 5 min. This procedure was repeated for three times The supernatant were analyzed spectrophotometrically (ELICO BL-198) by recording absorbance at 663 and 645nm wavelength.

Quantity of chlorophyll was calculated by following formula [19]:

mg chlorophyll a\g tissue=12.7(A_{663})-2.69(A_{645}) ×v\1000×w

mg chlorophyll b\g tissue=22.9 (A_{645})-4.68(A_{663}) ×v\1000×w

Total chlorophyll\g=20.2(A_{645}) +8.02(A_{663}) ×v\1000×w.

2. Micro-Extraction

The plants grown from the seeds treated with aqueous colchicine (0.5%, 1% and 2%) were shade dried and powdered. 500 mg powder was taken in absolute ethanol and microextracted in waterbath in microwave (Kenstar3D power and set at60%) in 8 cycle of 30sec of each.

3. HPTLC Analysis

Phytochemical groups namely xanthones, coumarin and flavonoid were selected. HPTLC procedure involved laoding of 4µlethanolicmicroextracts (obtained from microwave) using mangiferin (Xanthones), scopoletin (Coumarin) and quercetin (flavonoids) as reference standard. Plates were developed using a mobile phase Ethylacetate: Glacial acetate: Formic acid: Water (100:11:11:26) for Xanthones and Toluene-diethylether (1:1) saturated with 10% acetic acid for Coumarin and flavonoids. Densitometric analysis was done at258nm for mangiferin, for scopoletin and quercetin at 366nm. Derivatization was done by NP\PEG and 10% KOH at UV254 and UV 366nm for Xanthone, Coumarin and flavonoids UV 365nm.

4. Quantification

Quantity of mangiferin, scopoletin and quercetin in all the treated and control plants was obtained by the following formula:

Amount of secondary metabolite present in sample (µg) = Concentration of standard in 4µl× peak area of sample\ peak area of Standard

The Concentration of sample is 0.25µg\4µl in ethanol and for standard (Mangiferin, Scopoletin) is 0.25µg\4µl and 0.5µg\4µl for (quercetin) in ethanol, width of band were -6mm.

Results and Discussion

1. Effect of Colchicine on the Shoot and Root Length, No. Of Leaves and Node

In *C. decurrens*, colchicine was found to have negative impact on seed germination and survival of plants at maturity [18]. There was stimulatory effect of all doses on shoot growth as compared to that in control. Among all doses 2% colchicine exhibited highest shoot length (6.5 cm) and it decreased with decrease in colchicine concentration (fig,1).Root length also showed promising increase with better root growth and highest root length (11.6 cm) in 2% colchicine. In other doses it was 6.55 cm (0.5%) and 8.0cm in (1%) which was more than that of control [15]. Similar enhancing trend was found with respect to no. of nodes and leaves after colchicine treatment. It was highest again in 2% colchicine with 6.5 number of nodes with 15 leaves. In 0.5 and 1% both the parameters showed increased. Value that of control (3.84-no.of nodes and 8.9 - no. of leaves (Table.1). increase in plant height, no. of branches, no. of leaves and leaf dry weight in *Datura stramonium* [3].

In contrast to that fresh weight was significant decreased in 2% (5.87gm) as against in control (11.07 gm). The probable reason for this might be low survival of plants at higher dose. When the fresh weight biomass was dried lowest dry weight was encountered in control (0.53gm). The difference in weights gradually increases from highest dose to control (Table. 1). Interestingly as the dose increased stimulation increased. Early reports indicated that colchicine has capacity to affect certain metabolic process increasing the rate of enzymatic reactions in proportion to its concentration [7].

Table 1: Effect of Colchicine on different growth parameters and biomass of *C. decurrens*.

Concentration\ Parameters	Shoot length (cm)	Root length (cm)	No. of nodes	No. of leaves	Fresh weight (g)	Dry weight (g)
0.5%	4.70±0.03^{b}	6.55±0.06^{b}	4.46±0.04^{b}	11.01±0.08^{b}	10.46±0.15^{c}	0.79±0.01^{c}
1	5.50±0.09^{c}	8.04±0.11^{c}	5.16±0.08^{c}	12.27±0.17^{c}	7.277±0.11^{b}	0.68±0.008^{b}
2	6.5±0.12^{d}	11.60±0.29^{d}	6.50±0.10^{d}	15.01±0.20^{d}	5.87±0.12^{a}	0.49±0.03^{a}
Control	3.78±0.04^{a}	3.71±0.06^{a}	3.84±0.08^{a}	8.91±0.08^{a}	11.07±0.10^{d}	0.53±0.01^{a}

The data shown are means±SD of three replicates. Mean with in coloumn followed by same letter are not significantly different at p≤ 0.05. Different lettera a,b,c and d denote significant

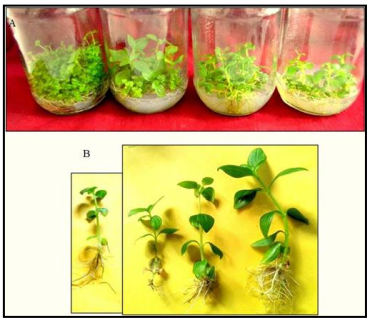

Fig 1: Effect of colchicine treatment Growth paorameters of *C. decurrens* A (R to L) – shootegth in control, 2,1 and 0.5% colchicine, B no. of node leaves in control, 0.5,1 and 2% colchicine

The fundamental significance of photosynthesis in any commercial plants is its direct impact on biomass production (Fw\ Dw) and economical viability. High rates of photosynthesis maintain the plants against stressful eventualities [11].Chlorophyll content (chl a, b and total chl) shows great variability. In general chl a was found to be higher than chl b in control and chlorophyll treatment except 2% dose where chl b is much higher than chl a, chl b content, on the other hand, show linear decrease from higher to lower doses [4, 6]. These result are in agreement with those obtained in *Hibiscus sabdariffa* [22] (Table. 2).

Table 2: Effect of Colchicine on chlorophyll content

Concentration	Chlorophyll a	Chlorophyll b	Total chlorophyll
%	(mg)	(mg)	(mg)
0.5	12.78	5.38	18.2
1	17.39	6.55	23.9
2	0.04	12.05	12
Control	13	5.5	18.5

2. Quantitative analysis of seconday metabolite (mangiferin, scopoletin and quercetin) in *C. decurrens*

- Colchicine is a potent chemical which can be used to get vigorous, giant plants. However the main problem in colchicine treatment in delayed or inhibited cell division. due to inhibited spindle apparatus formation.[12, 21] It leads to severe lethality in the treated plant which is evident from to results of present investigations increase in phytochemical were envised in *C. decurrens.* The data of HPTLC quantification of xanthones (with reference to standard mangiferin), coumarin (with reference to standard scopoletin) and flavonoid (with respect to standard quercetin) in control and treated plants, In *C. decussate* quantification is done for mangifrine by HPLC method [10, 25]. Similarly spectrofluorometeric determination of scopoletin and mangiferin in *C.decussata*.[20]

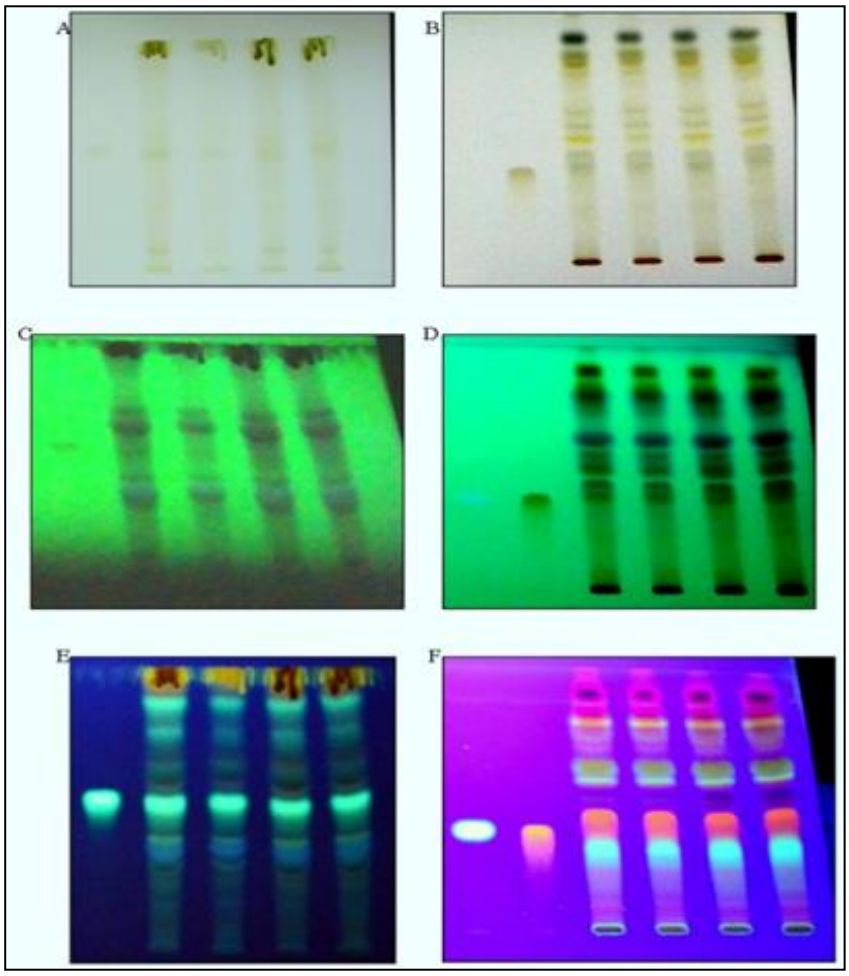

Fig 2: Finger printing profiling of colchicine treated plant extafct and control (L-R) photo Amangiferin, 0.5,12% and control b-standard, (scopoletin and quercetin), 0.5,12% and control in visible c and d- in 254nm and e,f in 366nm after derivatision

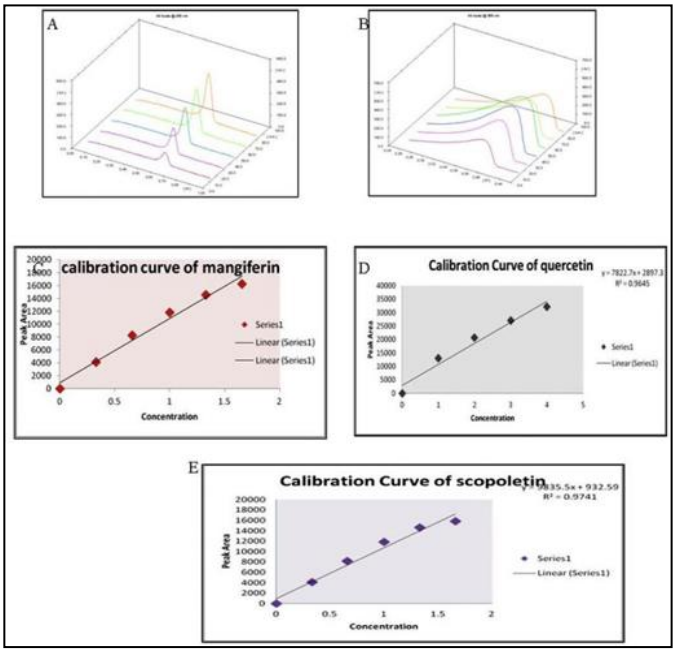

Fig 3: spectra and callbration curve of standard (mangiferin, scopoletin and querceyin). A-mangiferin;B-qyercetin; C,D,E-callbration curve

In colchicine pure standard mangiferin, scopolein and quercetin showed Rf and peak area (0.64, 12466.1AU), (0.43, 9363AU) and (0.41, 23139.5) respectively. In colchicine treated extract (0.5, 1 and 2%) mangiferin Rf ranged from 0.59- 0.73 and peak area found to be 11127.0, 9797.0, 11697.1 AU respectively. In control peak area for mangiferin was 8492.8 AU for scopoletin and quercetin Rf ranged between 0.42-0.47 .0.5% treatment showed peak area 8868.4 and 7135.9 AU was observed in1%, 11858.8 and 6496.0AU and in highest dose 2% showed 8655.3 and 6341.9.In control 6914.9 and 4727.2AU. All the colchicine doses showed increase in peak area for mangiferin, scopoletin and quercetin. Maximum peak area for mangiferin was observed in 2% **(**11697.1 AU), in scopoletin it was observed in 1% (11858.8 AU) and in quercetin (7135.9 AU) in 0.5% (Table. 3).

- **For quantification of Mangiferin**. **(fig. 2,3)**

The developed HPTLC plates of standard mangiferin was scanned at 258 nm wavelength and indicated Rf value ranging between 0.53(start position) to 0.66 (end position).

The estimation of mangiferin by standard calibration curve, showed a good correlation coefficient (r^2= 0.9803) in the selected concentration range per spot with respect peak area. The equation of standard curve y=9973.9x + 888.11. The plot has slop (m) y = mx + c (fig C) (fig. 3).

At 258nm spectra of all track indicated presence of 7 peaks in *C. decurrens* sample corresponding to 7 xanthones derivatives (fig.2 E,F).However the peak corresponding to that of standard mangiferin (Rf 0.65) in each track was selected for quantification. The peak area of mangiferin in each sample (each track) is designated by densitogram (fig. 4).

- **For quantification of Quercetin and Scopoletin**

Both quercetin and scopoletin were resolved in the same mobile phase (Toulene: diethylether 1:1 saturated with 10% glacial acetic acid) and detected in 254 nm as dark absorbing (quenching) bands and in 365nm as fluorescent blue bands (scopoletin) and orange fluorescence quercetin (fig. 2).

The standard calibration curve of quercetin showed colinerity between different concentration and their respective peak area (fig3.D) with r2= 0.9645.correlarion coefficient. The equation of best felting line was y= 7822.6x+2897.3, similar for scopoletin good colinerity was observed having R2=0.9741(fig.3.E).The corresponding Rf for quercetin was found to be in the range 0.26(start position) to 0.42 (end position) while for scopoletin it was 0.34 (start position) and 0.43 (end position. The spectra at selected Rf indicated presence of 2 closely situated peaks.

Table 3: Rf range and area of peak in colchicine treated plant extracts

Peak	start position	end Position	Area	Area%
I. Mangiferin-				
	Track1-Std Mangiferin			
1	0.47 Rf	0.64 Rf	12466.1	100.00%
	Track 2-0.5% colchicine			
1	0.59 Rf	0.72 Rf	11127.0 AU	70.91%
	Track 3-1%colchicine			
1	0.63 Rf	0.73 Rf	9797.0 AU	66.05%
	Track 4- 2%colchicine			
1	0.57 Rf	0.71 Rf	11697.1 AU	78.34%
	Track5 –control			
1	0.60 Rf	0.73 Rf	8492.8 AU	75.53%
II. Scopoletin and Querctin				
	Track1- Std Scopoletin			
1	0.34 Rf	0.43 Rf	9363.0 AU	100.00%
	Track2-Std Quercetin			
1	0.28 Rf	0.41 Rf	23139.5 AU	100.00%
	Track3-0.5% colchicine			
1	0.34 Rf	0.42 Rf	8868.4 AU	50.85%
2	0.42 Rf	0.47 Rf	7135.9 AU	40.91%
	Track4-1%colchicine			
1	0.28 Rf	0.42 Rf	11858.8 AU	64.61%

2	0.42 Rf	0.47 Rf	6496.0 AU	35.39%
	Track5-2%colchicine			
1	0.34 Rf	0.42 Rf	8655.3 AU	52.79%
2	0.42 Rf	0.46 Rf	6341.9 AU	38.68%
	Track6-Control			
1	0.35 Rf	0.42 Rf	6914.9 AU	52.56%
2	0.42 Rf	0.47Rf	4727.2	35.93%

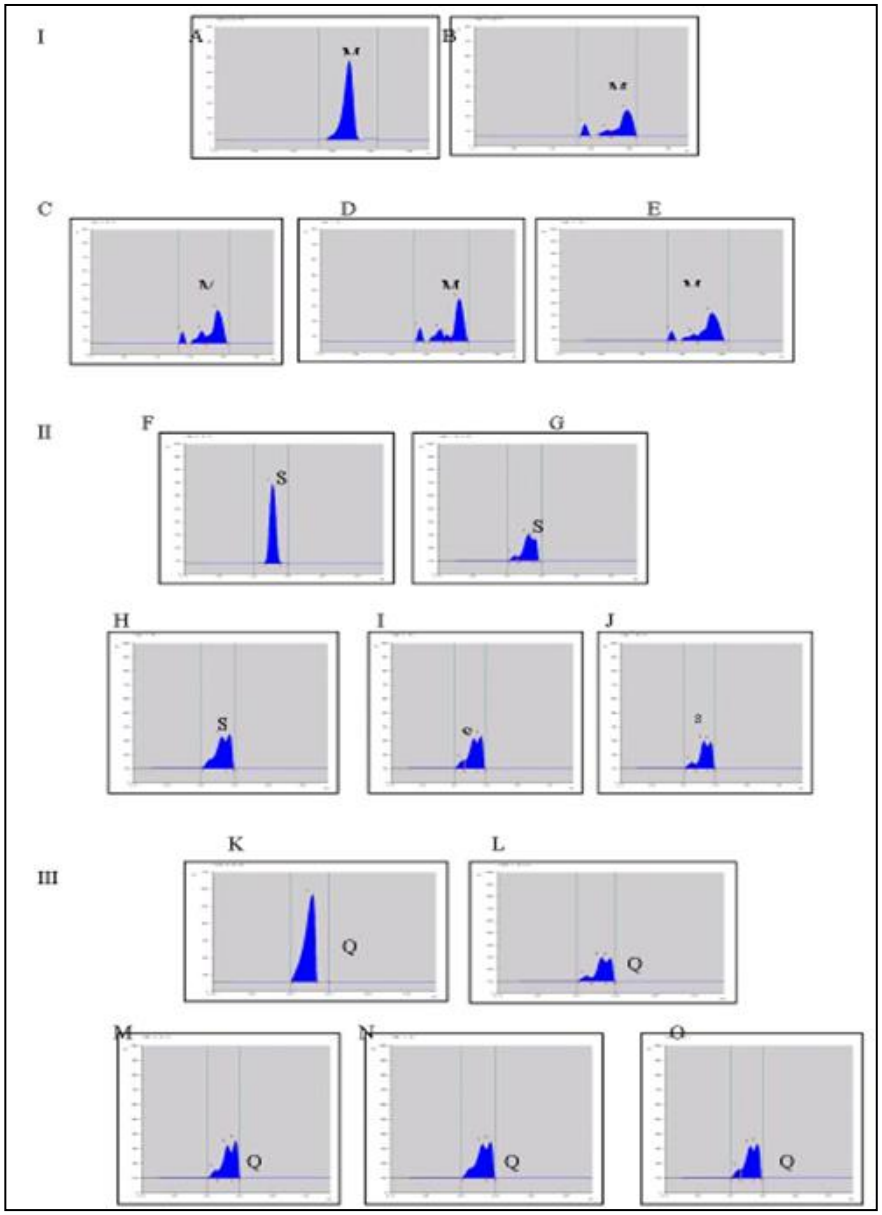

Fig 4: Densitogram showing mangiferin, scopoletin aqnd queercetin in control, colchicine treated plant extract I mangiferin A- standard B-control, C-0.5%, D-1%E-2%, II scopoleti F standard G control, H 0.5% I-0.55;J-2%;III Quercetin K-standard L control, m 0.5%;N-1%;o-2%;

Table 4: Effect of Colchicine on quantity of mangiferin, scpoletin and quercetin in *C.decurrens*

Doses	**Quantity of secondary metabolite**		
	Mangiferin	**Scopoletin**	**Quercetin**
	(µg/ml)		
0.5%	147.5	155	152.5
1	130	207.5	140
2	152.5	152.5	137.5
Control	110	120	105

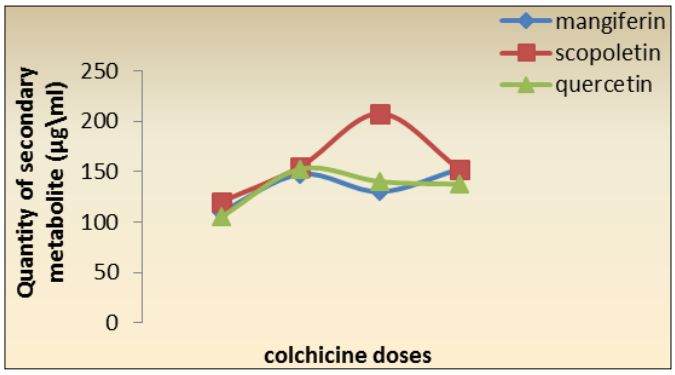

Fig 5

Colchicine was found to be most effective in enhancing the quantity of all phytoconstituents mangiferin concentration was increased in all doses of colchicine viz 0.5% (147.5), 1% (130) and 2% (152.5µg/ml. In control mangiferin was found in 110 µg/ml concentration. All doses of colchicine showed induced scopoletin production as compared to that in control. Quercetin on the other h and was promising increased but as the dose increased the quercetin quantity decreased i,e higher dose lower quantity of quercetin. (Table.4). In the present investigation, very high stimulation was observed in all the three phytoconstituents. Interestingly the phytochemical increase is positively correlated with increased in biomass similar report found in phytoconstituent Camptothecin and shikonin after treated with mutagen [8].

Conclusion

- The establish method of extraction detection and identification in futuristic commercial exloitation of this potent medicinal plant.
- Colchicine at all doses were found to be positively interacting with *C. decurrens* genome as all the growth parameters showed positive growth with subsequently led to higher fresh weight\ dry weight.
- In colchicine the quantity of scopoletin increased than control at 1% but higher doses was found to be detrimental.

References

1. Adaniya S, Shira D. In vitro induction of tetraploid ginger (*Zingiber officinalis* Roscoe) and its pollen fertility and germinability. Scientia Horticulturae. 2001; 88:277-287.
2. Akshatha Chandrashekar KR, Effect of gamma irradiation on germination growth and biochemicalparameters of *Pterocarpus santalinus*, an endangered species of Eastern. Ghats. Pelagia Research Library European Journal of Experimental Biology. 2012; 3(2):266-270.
3. Amiri S, Kazemitabaar SK, Ranjbar G, Azadbakht M, The effect of trifluralin and colchicine treatments on morphological characteristics of Jimsonweed (*Datura stramonium*). Trakia J. Sci. 2010; 8(4):47-61.
4. Azoush S, Kazemitabar SK, Heidari P. Evaluation of polyploidy induced in terms of chlorophyll content and catalase activity in Borage (Echium amoenum Glowacka Acta Advances in Agricultural Sciences. 2014; 2(2):8-12.
5. Dhapte V, Kadami S, Pokharkar V. Development and validation of HPTLC method for analysis aqueous Bacopa monnieri extract. Int J Pharm Pharm Sci. 2013; 5(2):490-494.
6. Dikshit SK, Tiwari PVand Dixit SP, Anticonvulsant activity of C. decussate. 1972; 12-18.
7. Eigsti OJ, Dustin P. Colchicine in agriculture, medicine, biolog and Chemistry. Ames: Iowa State College Press, 1955.
8. Fulzele TB, Satdive RK, Pol BB, Untransformed root cultures of Nothapodytes foetida and production of camptothecin. Plant Cell Tissue Organ Culture. 2002; 69:285-288.
9. Hao J, Han W, Huang S, Xue B, Deng X, Microwave-assisted extraction of artemisinin. 2005; 134(1):31-37.
10. Hostettmann K, Hostettmann M, Xanthones, Methods in plant biochemistry. Vol. 1, Plant phenolics. Dey PM, Harborne JB (edi). London: Academic Press. 1989; 493-508.
11. Idrees M, Naeem M, Khan MA. The superiority of cv "rosea" over cv "alba" of periwinkle (Catharanthus roseus L.) in alkaloid production and other physiological attributes, Turkish Journal of Biology. 2010; 34(1):81-88.
12. Kadota M, Niimi Y. In vitro induction of tetraploid plants from a diploid Japanese pear cultivar (*Pyrus pyrifolia* N. cv. Hosui). Plant Cell Rep. 2002; 21:282-286.
13. Karthika R, Subba, Lakshmi B. Effect of gamma rays and EMS on two varieties of soybean. Asian J Plant Sci. 2006; 5:721-724.
14. Kirtikar KR, Basu BD. Indian medicinal plants. 1918; 3.
15. Mensah JK, Obadoni BO, Akomeah PA, Ikhajiagbe B Ajibolu J. The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (Sesame indica L). Afr. J Biotechnol. 2007; 6(5):534-538.
16. Omar R, Abdullah MA, Hasan M, Marziah. Development of growth medium for *Centella Asiatica* cell culture via response surface methodology. Am. J Applied Sci. 2004; 1:215-219. DOI: 10.3844/ajassp.215.21.
17. Pan X, Niu G, Liu H. Comparison of microwave-assisted extraction and conventionalparameters of *Pterocarpus santalinus*, an endangered species of Eastern Ghats, 2003.
18. Ramdas D, Pokhriyal PG, Prerna, Kumar M. A study of foliar epidermal pattern in colchiploid of *Zanthoxylum armatum*. Internat. J Plant Sci. 2012; 7(2):208-215.
19. Sadasivam S, Manickam. A Biochemical methods. 2nd Edn., New Age International Publishers. 1996; 107-109.
20. Sethiya NK, Thakore SG, Mishra SH. Comparative evalution of commercial analysis of medicinal Shankhpushpi for antistress potential a priliminary study Pharmacologyonline. 2009; 2:460-7.
21. Shao J, Chen C, Deng X, In vitro induction of tetraploid in pomegranate (*Punica granatum*). Plant Cell Tissue Org Cult. 2003; 75:241-246.
22. Sherif MH, Sarwat MI. Physiological and chemical variations in producing roselle plant (*Hibiscus sabdariffa L.*) by using some organic farmyard manure. World Journal of Agricultural Sciences. 2007; 3(5):609-616.
23. Shu YY, Ko MY, Chang YS. Microwave-assisted extraction of ginsenosides from ginseng, 2012.
24. Tiwari AK, Mishra SK. Effect of colchicine on polyplodization and morphological chractersitics of *phlox*

drummondi. African Jour. Of biotechnology. 2012; 11(39):9336-9342.
25. Upadhyay V, Sharma N, Tiwari KA, Malik A, Singh B. Standardization of HPLC Method of Scopoletin in Different Extracts of Convolvulus pluricaulis International Journal of Pharmaceutical Sciences and Drug Research. 2013; 5.1:28-31
26. Wagner S, Urena A, Reich E, Merfort I, Validated HPTLC methods forthe determination of salicin in Salix sp. and of harpagoside in Harpogophytumprocumbens. J Pharm. Biomed. Anal. 2008; 48:587-591.
27. Yashwant M, Hadkar UB, Dhruv N, Chavan B. Microwave assisted extraction of phytoconstituent. *Asian* Journal of Phytomedicine and Clinical Research. 2012; (3):73-86.
28. Yadav V. Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* DalzJournal of Pharmacognosy and Phytochemistry. 2017; 6(1):123-129.